Biogeochemistry (2015) 122:113–129
DOI 10.1007/s10533-014-0034-7

Eastern oyster (*Crassostrea virginica*) filtration, biodeposition, and sediment nitrogen cycling at two oyster reefs with contrasting water quality in Great Bay Estuary (New Hampshire, USA)

Timothy J. Hoellein · Chester B. Zarnoch · Raymond E. Grizzle

Received: 29 January 2014 / Accepted: 4 September 2014 / Published online: 17 September 2014

Abstract Benthic deposition of carbon (C) and nitrogen (N)-rich oyster biodeposits may increase denitrification, or anaerobic respiration of nitrate (NO_3^-) to di-nitrogen gas (N_2). However, environmental drivers of C and N dynamics in oyster biodeposits and reef-adjacent sediments require clarification. In July 2012, we collected intact sediment cores adjacent to and 15–20 m away from two oyster reefs (*Crassostrea virginica*) in Great Bay, New Hampshire, USA: one reference site and one site with cultural eutrophication. We also measured seston, chlorophyll *a*, and in situ oyster feeding and biodeposition. Cores were incubated in continuous-flow chambers where inflow water received ^{15}N-ammonium ($^{15}NH_4^+$), $^{15}NO_3^-$, or no isotopes (control). We quantified fluxes of dissolved nutrients and gasses (oxygen, $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, and argon) after 24 h. Finally, we measured size-fractionated sediment organic matter. At the eutrophic site, abundant phytoplankton in the 5–28 μm size range was correlated with enhanced oyster feeding rates and biodeposit quality (lower C:N). This site had greater denitrification rates in reef-adjacent cores relative to distal cores. Low production of $^{29,30}N_2$ in $^{15}NH_4^+$ amended cores suggested water column or biodeposit NH_4^+ were unlikely to be converted to N_2. At both sites, reef-adjacent cores had more shell and higher $^{29,30}N_2$ production with $^{15}NO_3^-$ addition relative to distal cores, suggesting direct denitrification enhancement near reefs. Oysters likely increased sediment N_2 production via high quality biodeposits (eutrophic site), and NO_3^- diffusion via structural complexity of reef-adjacent sediment (both sites). Overall, results suggest oyster-mediated ecosystems services may be expected to vary with environmental conditions.

Keywords Biodeposits · Clearance rate · Denitrification · Eutrophication · Phytoplankton

Responsible Editor: Leila J. Hamdan.

Electronic supplementary material The online version of this article (doi:10.1007/s10533-014-0034-7) contains supplementary material, which is available to authorized users.

T. J. Hoellein (✉)
Department of Biology, Loyola University Chicago, 1032 W Sheridan Rd, Chicago, IL 60660, USA
e-mail: thoellein@luc.edu

C. B. Zarnoch
Department of Natural Sciences, Baruch College, City University of New York, Box A-0506, 17 Lexington Ave, New York, NY 10010, USA
e-mail: chester.zarnoch@baruch.cuny.edu

R. E. Grizzle
Jackson Estuarine Laboratory and Department of Biological Sciences, University of New Hampshire, 85 Adams Point Road, Durham, NH 03824, USA
e-mail: ray.grizzle@unh.edu

Introduction

Oyster reefs have suffered >80 % reduction from global historic ranges (Beck et al. 2011), attributed to harvest, disease, and environmental degradations (Beck et al. 2011). In New Hampshire, similar declines have occurred with the most dramatic recent losses since the mid-1990s (Barber et al. 1997; Piscataqua Region Estuaries Partnership 2013). Healthy oyster reefs provide valuable ecosystem services (Coen et al. 2007; Grabowski and Peterson 2007) including fish habitat (Coen et al. 1999), water filtration (Grizzle et al. 2008b; Plutchak et al. 2010), and increased denitrification, or the anaerobic microbial respiration of nitrate (NO_3^-) to dinitrogen gas (N_2) (Kellogg et al. 2013). Although historically oyster restoration programs have focused on oysters as a harvestable resource, documentation of their contribution to ecosystem services and ecological communities has caused a shift in emphasis in many areas (Brumbaugh and Coen 2009; Coen et al. 2007; Luckenbach et al. 1999; Zu Ermgassen et al. 2013). Developing best practices for restoration and conservation of oyster reefs as a tool to address cultural eutrophication requires documentation of multiple oyster-mediated ecosystem services (Cerco and Noel 2007), across variable environmental conditions (Hoellein and Zarnoch 2014).

Oysters couple benthic and pelagic components of ecosystems through filtration (i.e., clearance) and excretion (Dame 2012; Newell and Langdon 1996). Particles captured by oysters are sorted to maximize the ingestion of nutritious material. Excess and non-nutritious particles are rejected as pseudofeces. Oysters ingest the palatable component of the filtrate, digest the food, and release unabsorbed material as feces. Body size and temperature are well studied drivers of oyster feeding behavior (Cranford et al. 2011; Newell and Langdon 1996). Seston concentration and nutritional quality can also affect oyster filtration and relative production of fecal and pseudofecal biodeposits (Newell 2004). For example, Ryther (1954) demonstrated that oyster stocks in Great South Bay (NY, USA) were negatively affected by extensive blooms of picoplankton algae which were stimulated by high levels of ammonia and uric acid from agricultural runoff. Oysters retain and digest fewer phytoplankton from these smallest size classes (i.e., below 5 to 6 μm) (Riisgård 1988). Conversely, eutrophic conditions in the Peconic Estuary (NY, USA) favored diatoms and autotrophic nanoflagellates which were positively correlated with oyster growth (Wall et al. 2013). The influence of eutrophication on oyster filtration and biodeposition via changes in seston is important to study as many reef conservation and restoration projects occur in sites with cultural eutrophication (Hoellein and Zarnoch 2014).

In addition to water filtration, oysters may provide an ecosystem service through nutrient assimilation in soft tissues and shells, and can promote conditions required for production of N_2 through denitrification and anaerobic ammonium oxidation (anammox) by sediment microbes (Beseres Pollack et al. 2013; Kellogg et al. 2013; Smyth et al. 2013). The latter processes represent the permanent removal of N from the aquatic environment (Seitzinger et al. 2006), but anammox is considered less prevalent than denitrification in estuaries (Engström et al. 2005; Koop-Jakobsen and Giblin 2009). Oysters may enhance microbial denitrification by affecting 1 or more the required conditions: (1) organic carbon (C), (2) favorable redox conditions, or (3) increased NO_3^- availability through nitrification. Oysters can increase sediment C, either via biodeposition or changes in hydrodynamics (Hoellein and Zarnoch 2014). Enhanced organic matter can stimulate microbial respiration and promote sediment anoxia. Finally, oyster biodeposits contain organic N and ammonium (NH_4^+), which can be converted through sequential N transformations into N_2. Organic N would be first mineralized to NH_4^+, then nitrified to NO_3^-, and finally denitrified to N_2 (Kellogg et al. 2013; Newell 2004; Newell et al. 2002). Nitrification occurs under aerobic conditions, so coupled nitrification–denitrification requires adjacent oxic and anoxic microsites (Newell et al. 2005; Seitzinger et al. 2006) and may be inhibited in conditions where oyster biodeposits enhance sediment organic matter.

Some recent evidence suggests oysters increase sediment N_2 production within or adjacent to oyster reefs (Kellogg et al. 2013; Smyth et al. 2013), although other studies have found little effect of oysters on sediment N dynamics, even at high densities when sampling below experimental enclosures or floating aquaculture cages (Higgins et al. 2013; Hoellein and Zarnoch 2014). Other bivalve taxa have been shown to affect sediment N transformations. For example, zebra

mussels (*Dreissena polymorpha*) enhanced coupled nitrification–denitrification (Bruesewitz et al. 2008) and reduced NO_3^- limitation of denitrification (Bruesewitz et al. 2009), and the cockle *Austrovenus stutchbury* increased denitrification potential in sandy sites in New Zealand estuaries through bioturbation and NH_4^+ excretion (Jones et al. 2011). Understanding the pathways through which oysters affect N_2 production and the influence of environmental conditions such as eutrophication is critical for predicting ecosystem services provided through reef conservation and restoration.

Our objectives were to measure oyster (*Crassostrea virginica*) feeding rates, biodeposition, and denitrification pathways in sediments directly adjacent to and 15–20 m away from an oyster reef. We conducted this study at 2 reefs in Great Bay Estuary, New Hampshire, USA, which have contrasting levels of cultural eutrophication. We define eutrophication as an increase in the rate of supply of organic matter to a system (Nixon 1995), which in this case originates from a combination of wastewater effluent and non-point agricultural and urban sources in the watershed upstream of the estuary. In contrast, our reference site was removed from terrestrial and riverine inputs and maintained lower organic matter and water column nutrients.

We recently presented a conceptual model to predict how the role of oysters on sediment N cycling may change under a gradient of cultural eutrophication (Fig. 7 in Hoellein and Zarnoch 2014), which we used to guide our hypotheses in this study. At the study site with greater cultural eutrophication (hereafter referred to as 'enriched'), we expected higher seston quantity, but lower seston quality (i.e., non-nutritious phytoplankton) would generate lower oyster clearance rates and lower quality of biodeposits relative to the site with no cultural eutrophication (hereafter referred to as 'reference'). We made this prediction following the suggestion in Newell (2004) that changes in nutrient regimes associated with enrichment may favor the growth of non-nutritious or harmful picoplankton that may negatively influence bivalve feeding (Gobler et al. 2002; Malone 1992; Ryther 1954). For sediment biogeochemistry, we predicted the enriched site would have higher sediment C than the reference site, regardless of oyster presence. Therefore, C added to the sediment by oyster biodeposits would be less likely to affect total organic matter at the enriched site, and N in oyster biodeposits would be less likely to complete coupled nitrification–denitrification in C-rich (i.e., low oxygen) sediments.

Methods

Study sites

The two study sites are in Great Bay Estuary, New Hampshire. Nannie Island (reference) is in the central portion of the bay (latitude 43.070643, longitude −70.863547), largely removed from riverine inputs of nutrients or organic matter. For maps of Great Bay see Grizzle and Ward (2013) and Grizzle et al. (2008a). The Squamscott River (enriched) is the name of the estuarine portion of the Exeter River as it enters Great Bay (latitude 43.051752, longitude −70.911031). The Exeter/Squamscott River receives effluent from the City of Exeter's wastewater treatment facility as well as runoff from various urban and agriculture sources. Like coastal waters worldwide, Great Bay has lost approximately 90 % of historic oyster reef coverage (Odell et al. 2006). Although the oyster reefs we studied have persisted for decades, oyster densities and other reef characteristics have varied widely since the early 1990s. Areal extent of both study reefs were measured in 2013 using towed, underwater videography (Grizzle et al. 2008a). The Squamscott River reef covered 3.1 ha, and the reef lying just north of Nannie Island (also called Woodman Point reef) covered 6.2 ha (Grizzle and Ward 2013). Total live oyster densities on the Squamscott and Nannie reefs, respectively, in summer 2012 were 85.6 and 59.2 oysters m^{-2} (NH Fish and Game Department, unpublished data). Water quality monitoring stations are maintained near each site as part of the system wide monitoring program of the Great Bay National Estuarine Research Reserve System (NERRS). In addition, we measured mid-day temperature and DO at the oyster reefs using a DO probe (Pro20, YSI, Inc., Yellow Springs, Ohio), and salinity was measured using a refractometer.

Oyster feeding and biodeposition

Oysters with similar mean (±SE) shell heights (Nannie Island = 85.4 (±2.7) mm, Squamscott River = 81.0 (±2.1) mm; n = 25 $site^{-1}$) were collected from the reefs by hand or scratch rake. Oysters were transported

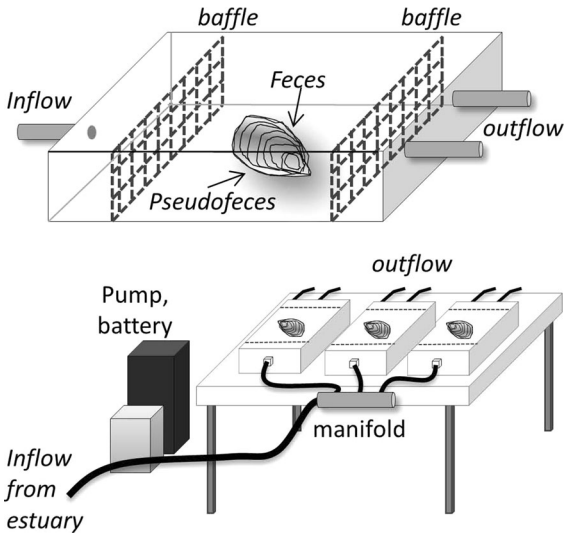

Fig. 1 Illustration of the materials used to measure oyster feeding and biodeposition. The *top panel* shows a feeding tray with one inflow, two baffles, and two outflows. The oyster was placed between the two baffles. Feces and pseudofeces were collected separately on opposite sides of the oyster. The *lower panel* shows the water pumped from the estuary through manifolds that control the flow of water going into each feeding tray. Tables were shaded under a large pop-up canopy

on ice to the adjacent shoreline at each site to perform feeding measurements following the "biodeposition method" as described by Iglesias et al. (1998), and (Bayne 2002; Bayne et al. 1999). This method integrates the quantitative characterization of the seston with measures of feeding and biodeposition (Iglesias et al. 1998). Tables were setup under a pop-up canopy in the high intertidal zone at each site. We deployed a submersible pump in the bay with plastic tubing leading to manifolds that continuously distributed ambient seawater (450 ± 50 ml min^{-1}) into specially designed feeding trays ($n = 16$) on the tables (Fig. 1). The pump was suspended in the middle of the water column using a plastic float. Feeding trays (30 cm × 22 cm × 7 cm) had a single inflow, mesh baffles to reduce turbulence, and two separate outflows (Fig. 1). Biodeposits were not suspended by water flow, but settled near the oysters. Oysters were completely submerged in the trays and the flow rate ensured there was no refiltration of the seston. A single oyster was placed in each tray and left undisturbed until it consistently produced biodeposits (i.e., feces or pseudofeces). During 30–60 min timed trials, all feces and pseudofeces from each oyster were collected by pipette without disturbing oyster feeding activity, and placed in separate containers. If an oyster did not produce biodeposits within 60 min it was replaced. Biodeposits were collected continuously throughout the trials. Continuous collection and tray design minimized chances for biodeposits loss before collection.

Feces and pseudofeces produced by each oyster were immediately filtered onto separate (ashed and preweighed) glass fiber filters (GF/F). Filters with biodeposits were washed with isotonic ammonium formate, placed in 47 mm petri dishes, and stored at −20 °C until processed in the laboratory. Two sequential collections of feces and pseudofeces were performed for each individual oyster. The filters from the first collection were dried for >24 h at 70 °C, weighed, ashed for 4 h at 450 °C, and weighed again to measure organic content. For the second collection, filters were dried for >24 h at 70 °C, weighed, suspended over fuming hydrochloric acid for 20 min to remove carbonates, dried again, and then analyzed for C and N content using a Series II 2400 CHN Analyzer (Perkin Elmer Life and Analytical Sciences, Shelton, CT) using acetanilide as the standard. Oysters from feeding trials were stored at −20 °C until processed. Oysters were measured for shell height (longest axis), width, and depth. Tissue was removed from the shell, and tissue and shell were dried separately at 70 °C for >48 h to obtain dry weights. The C and N content of the dried tissue was determined with the CHN analyzer.

We used biodeposit measurements to calculate total production (mg h^{-1}), organic content, inorganic content, and C and N content of feces and pseudofeces. Feeding rates and efficiencies were derived from these data and seston measurements (Bayne 2002; Bayne et al. 1999) (Supplementary Material 1). Feeding rates were corrected for size differences among individuals by relating to a standard body size of 1 g tissue dry weight following Bayne and Newell (1983). The constant b applied in the equation was 0.58 (Cranford et al. 2011).

Seston analyses

To characterize the seston, we collected triplicate water samples (500 ml) during the feeding trials, and filtered through ashed and preweighed GF/F filters. Seston filters were treated the same way as described

for biodeposits. For C and N determination of the seston filters, filtered seawater (0.45 μm) was passed through the GF/F filters and analyzed as blanks on the CHN analyzer. We determined total particulate matter (TPM, mg l^{-1}), particulate inorganic matter (PIM, mg l^{-1}), particulate organic matter (POM, mg l^{-1}), and seston C:N mass ratio. Triplicate water samples (100–200 ml) were also used for total and size fractionated chlorophyll *a* content (5–28 and <5 μm). We filtered samples through a 0.45 μm nitrocellulose membrane for total chlorophyll *a*. Size-fractionated samples were filtered through a 28 μm Nitex® screen and particles captured on a 5.0 μm nitrocellulose membrane. The filtrate was then passed through a 0.45 μm nitrocellulose membrane to determine the <5 μm fraction. Membranes were stored in the dark at −20 °C until analyzed. Samples were extracted overnight at 4 °C in 90 % acetone and measured spectrophotometrically (Parsons et al. 1984).

Sediment cores and flow-through analyses

At each reef, we collected 18 sediment cores (acrylic, 30 cm length × 7.6 cm width) using a corer with a one-way valve designed to minimize disturbance of the sediment–water interface (Gardner et al. 2006). We collected 9 cores immediately adjacent to oyster reefs (i.e., <0.5 m), and 9 cores at sites 15–20 m away from the reefs. Sediment depth in the cores was 10–15 cm. There were no live oysters in the reef-adjacent cores. Cores were sealed with black rubber caps and placed in a dark cooler for transport to the laboratory. We collected 6, 20 l carboys of site water to be used in continuous-flow measurements. There was no evidence for DO stratification, attributed to shallow depth (∼1 m) and tidal flow at each site.

Continuous-flow measurements were set up within 2–3 h according to Gardner and McCarthy (2009) to measure nutrient and gas fluxes. A plunger was placed inside each core, which contained a rubber O-ring on the sides and polyetheretherketone inlet and outlet tubing (PEEK; Zeus. Inc., Branchburg, NJ, USA) plumbed through the plunger surface. Approximately 230 ml of water was maintained above the sediment water interface in each core, and all cores were incubated in the dark to avoid photosynthesis and bubble formation. Three treatments were established for inflow water: control (no isotope added), $+^{15}NH_4^+$ (+10 μM $^{15}NH_4^+$-N), and $+^{15}NO_3^-$ (+10 μM $^{15}NO_3^-$-N) (N = 3 cores per treatment). Aerated site water was gently passed over the intact cores for 24 h at a rate of 1.1 ml min^{-1} at 23 °C (2–3 °C below ambient water temperature). After 24 h, water was collected from each in-flow carboy and from each of the core outflows and filtered (0.2 μm nylon syringe filters, Thermo Scientific, Rockwood, TN, USA) into 3, 20 ml scintillation vials and frozen for until measurement NH_4^+, NO_3^-, and soluble reactive phosphorus (SRP). In addition to inflow samples, we collected water from the inflows and outflows into triplicate 15 ml ground glass stoppered test tubes (Chemglass, Vineland, NJ, USA) for measuring dissolved gasses. We filled each tube slowly from the bottom and allowed them to overflow for several volumes. We added 200 μl of 50 % zinc chloride (McCarthy et al. 2007b), capped the vials ensuring no air in the headspace, and stored them underwater at room temperature or below until measurement of dissolved gasses. Inflow water was measured directly from the reservoir, which does not account for tubing or pump effects. Those effects were minimized because the experiment was run at room temperature and the inflow and outflow tubing is chemically inert. Finally, we based our 24 h sampling period on previous studies using identical equipment (Bruesewitz et al. 2013; Gardner and McCarthy 2009; McCarthy and Gardner 2003; McCarthy et al. 2007b), but did not measure steady state conditions directly.

Dissolved inorganic nutrients were measured on an Autoanalyzer III (Seal Analytical, Inc., Mequon, WI) using the phenol hypochlorite technique for NH_4^+ (Solorzano 1969), the antimonyl tartrate method for SRP (Murphy and Riley 1962), and the cadmium reduction method for NO_3^- (APHA 1998). We measured dissolved gasses with membrane inlet mass spectrometry (MIMS; Bay Instruments, Easton, MD, USA; Kana et al. 1998). On the MIMS, a peristaltic pump pulled water from the glass tubes and dissolved gasses were extracted across a membrane under vacuum. The mass spectrometer measured abundance of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, $^{32}O_2$, and ^{40}Ar. Standards consisted of artificial seawater maintained at constant temperature (24.5 °C; Circulating Bath, VWR International, Radnor, PA, USA), equilibrated to atmospheric gasses by stirring at low speed (Lab Egg

RW11 Basic, IKA Works, Inc., Wilmington, NC, USA). Samples were corrected for instrument drift with standard water throughout the run. Finally, we note that the quadrupole mass spectrometer in the MIMS ionizes gasses, and can produce O+ ions that form nitric oxide (NO) in the presence of N_2, thereby affecting $^{28}N_2$ and $^{30}N_2$ measurements (Eyre et al. 2002; Kana and Weiss 2004). The error magnitude is machine-specific (McCarthy and Gardner 2003), but has not yet been quantified for the MIMS used in this study. Because no mass 30 production was detected in our control cores (representing NO), we assumed this effect was minimal (McCarthy et al. 2008).

Nutrient and gas fluxes were equal to the difference between concentration in the outflow minus concentration in the inflow, and corrected for surface area of the core and pump flow rate (flux units = μmol element m^{-2} h^{-1}). A negative value indicates net retention and a positive value net production or flux out of the sediment (i.e., efflux). We used control cores to report net N_2 flux and sediment oxygen demand (i.e., respiration), rather than net N_2 flux or respiration from the $+^{15}NH_4^+$ or $+^{15}NO_3^-$ cores, which are less likely to reflect in situ rates. We used equations in An et al. (2001) to calculate simultaneous N-fixation and denitrification in $+^{15}NO_3^-$ cores. Potential denitrification was measured as the sum of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$ in the $+^{15}NO_3^-$ treatment (McCarthy et al. 2007a), after accounting for N-fixation. We considered $^{29}N_2 + {}^{30}N_2$ production in the $+^{15}NO_3^-$ treatment an index of direct denitrification of NO_3^-, and $^{29}N_2 + {}^{30}N_2$ production in the $+^{15}NH_4^+$ treatment an index of coupled nitrification–denitrification. Anammox can also contribute to isotope labeled N_2, but calculation of anammox via isotope pairing requires measurement of final $^{15}NH_4^+$ and $^{15}NO_3^-$ atom % in the cores, which we did not measure. We note sulfide can inhibit the last step in denitrification, and N_2 values do not account for incomplete denitrification to nitrous oxide. Mean (±std deviation) final O_2 concentration was 99.8 (±32.7) μM O_2, within the range of other studies (Kellogg et al. 2013). Finally, we calculated the proportion of ^{15}N in the $^{29,30}N_2$ pool relative to the total amount of ^{15}N added in the $^{15}NH_4^+$ and $^{15}NO_3^-$ cores. For all rates, we calculated flux in each core after 24 h and averaged three replicate cores for each treatment (Bruesewitz et al. 2013; Gardner and McCarthy 2009). We consider the detection limits of flux measurements as the rate at which SE did not overlap with zero (after Gardner and McCarthy 2009).

After completing the continuous-flow measurements, we collected the top 5 cm of sediment from 3 reef-adjacent cores and 3 cores from 15 to 20 m away from the reef at each site for measurement of sediment organic carbon. We acknowledge that heterotrophic metabolism occurring during the incubations could reduce sediment organic matter in the cores relative to the amount prior to the incubation. However, loss of organic matter through metabolism was likely minimized given the relatively short incubation period (24 h), and any error is equal across replicates from a collection site. Sediment was frozen until analyses. Sediment was sieved (1 mm^2; Humboldt Manufacturing Company, Chicago, IL, USA), placed into preweighed and ashed tins, and sediment was dried at 60 °C for a minimum of 4 days and re-weighed for dry mass. The samples were then combusted at 550 °C for 3 h for ash-free dry mass (AFDM).

Statistical analyses

We used a *t* test to compare water chemistry, chlorophyll *a*, seston, oyster size, and oyster feeding and biodeposition between sites. Data were transformed to meet the assumptions of equal variance and normal distribution when needed. Data that did not meet these assumptions after transformation were analyzed using the nonparametric Kruskal–Wallis test. We used a 2-way ANOVA by study site (Nannie Island and Squamscott River) and reef proximity (reef-adjacent and 15–20 m away from reef) to compare all dissolved nutrient fluxes, gas fluxes, and sediment AFDM. Finally, Pearson's product-moment correlation was used to compare sediment organic matter characteristics to dissolved gas fluxes. All statistics were completed with SYSTAT 13 (Systat, Inc., Chicago, IL) and IBM SPSS 20 (Armonk, NY).

Results

Study sites and physiochemistry

Dissolved nutrients, sediment organic matter, chlorophyll *a*, and seston measurements confirm conditions

Table 1 Mean (±SE) values for water column nutrients, chlorophyll *a*, and seston characteristics at Nannie Island and Squamscott River study sites

	Nannie	Squamscott	*t*-test		
			df	Test statistic	*p* value
Nutrients					
SRP (μM)	2.9 (0.1)	2.4 (0.4)	4	1.184	0.302
NH_4^+-N (μM)	11.6 (1.4)	20.9 (0.9)	4	−4.992	**0.008**
NO_3^--N (μM)	4.7 (0.8)	10.5 (0.8)	4	−4.612	**0.019**
Chlorophyll a					
Total (μg l^{-1})	5.5 (1.2)	13.3 (0.6)	4	−6.407	**0.004**
5–28 μm (μg l^{-1})	2.4 (<0.1)	7.5 (1.1)	4	−4.655	**0.010**
<5 μm (μg l^{-1})	3.6 (0.3)	2.8 (0.6)	4	1.136	0.319
<5 μm: Total (fraction)	0.7 (0.1)	0.2 (0.1)	4	3.812	**0.019**
Seston					
Total particulate (mg l^{-1})	10.8 (1.5)	15.2 (1.2)	9	−2.310	**0.046**
Organic content (%)	37.6 (1.1)	38.7 (2.5)	9	−0.421	0.684
C:N	4.0 (0.01)	5.6 (0.3)	9	−2.682	**0.025**

We used *t*-test to compare between sites, and *p* values < 0.05 are in bold

SRP soluble reactive phosphorus NH_4^+ ammonium, NO_3^- nitrate, *C:N* carbon/nitrogen mass ratio

consistent with greater cultural eutrophication at the Squamscott River relative to Nannie Island (Table 1). There was no difference in SRP (*t* test, $p = 0.302$), but NH_4^+ and NO_3^- concentrations were approximately two times higher at the Squamscott River (*t* test, $p = 0.008$ and 0.019, respectively). Values during our sampling in July 2012 were similar to the NERRS record for measurements of inorganic nutrient concentrations. Water temperature was 25 and 26 °C at Nannie Island and the Squamscott River, respectively. Salinity at the Squamscott River was 24 psu, while salinity at Nannie Island was 29 psu. Dissolved oxygen was also lower at the Squamscott River (5.4 mg l^{-1}) compared to Nannie Island (7.2 mg l^{-1}).

Chlorophyll *a* and seston measurements suggested differences in quality and quantity of food resources available to oysters at each site (Table 1). Total chlorophyll *a* at the Squamscott River was significantly higher than Nannie Island (*t* test, $p = 0.004$). The <5 μm chlorophyll *a* fraction was the most dominant size class (71 %) at Nannie Island (*t* test, $p = 0.019$; Table 1), while 5–28 μm size class was more abundant at the Squamscott River (*t* test $p = 0.01$; Table 1). Finally, total particulate matter was higher at Squamscott River than Nannie Island (*t* test, $p = 0.046$), although there were no differences in % seston organic content (*t* test, $p = 0.684$). The C:N mass ratio of the seston particulates was higher at the Squamscott River (*t* test, $p = 0.025$).

Oyster feeding and biodeposition

Feeding and biodeposition data (Table 2) were collected on eight oysters at Nannie Island and nine oysters at the Squamscott River. The shell height of oysters used in the measurements were similar at both sites (*t* test, $p = 0.277$), however, tissue biomass was greater at Nannie Island (*t* test, $p = 0.001$). Oyster tissue N content was 7.2 % at both sites, and tissue C content was 40.3 % in Nannie Island oysters and 28.3 % in the Squamscott River oysters (*t* test, $p < 0.001$).

Most oyster feeding rates were greater at the Squamscott River. The clearance rate of oysters at the Squamscott River was 7.31 l g^{-1} h^{-1}, double the rate at Nannie Island (3.51 l g^{-1} h^{-1}). The particle filtration rate at Squamscott River was nearly 3 times greater than Nannie Island. However, the percentage of rejected particles was the same at both sites (*t* test, $p = 0.801$).

There were few differences in oyster selection and absorption efficiencies between sites. For example, selection and absorption efficiency for organic particles was not statistically different between the sites (Kruskal–Wallis, $p = 0.191$ and 0.123, respectively). The pattern for N absorption efficiency was the same (Kruskal–Wallis, $p = 0.652$), as was N absorption rate ($p = 0.173$). N selection efficiency was higher at Nannie Island (Kruskal–Wallis, $p = 0.047$).

Table 2 Mean (±SE) values for oyster feeding measurements for organic matter and nitrogen (N) at Nannie Island and Squamscott River sites in Great Bay, NH

Oyster feeding and biodeposition	Nannie	Squamscott	*p* value
Organics feeding measurements			
Clearance rate (l g^{-1} h^{-1})	3.51 (0.25)	7.31 (1.02)	**0.001**
Filtration rate (mg g^{-1} h^{-1})	38.01 (2.68)	111.21 (15.5)	**<0.001**
Rejection rate (%)	47.02 (5.32)	45.41 (3.34)	0.801
Selection efficiency (fraction)	0.30 (0.15)	0.60 (0.05)	0.191
Ingestion rate (mg g^{-1} h^{-1})	7.27 (2.12)	34.52 (4.49)	**0.001**
Organic content of ingested matter (fraction)	0.30 (0.09)	0.55 (0.03)	**0.027**
Absorption rate (mg g^{-1} h^{-1})	5.27 (1.84)	26.69 (4.21)	**<0.001**
Absorption efficiency (fraction)	0.45 (0.14)	0.76 (0.04)	0.123
N-specific feeding measurements			
N filtration rate (mg g^{-1} h^{-1})	0.43 (0.03)	1.05 (0.15)	**0.001**
Pseudofeces N production (mg g^{-1} h^{-1})	0.09 (0.01)	0.28 (0.08)	**0.012**
N selection efficiency (fraction)	0.56 (0.03)	0.44 (0.04)	**0.047**
N ingestion rate	0.34 (0.03)	0.78 (0.08)	**0.001**
N absorption rate (mg g^{-1} h^{-1})	0.05 (0.02)	0.17 (0.07)	0.173
N absorption efficiency (fraction)	0.16 (0.07)	0.21 (0.07)	0.652

We used *t*-test or Kruskal–Wallis to compare between sites, and p values < 0.05 are in bold. N = 8 at Nannie Island and n = 9 at Squamscott River

Like with feeding rates, biodeposition rates were higher for oysters at the Squamscott River. The total rate of biodeposition as well as the rate of organic C and N deposition of oysters at the Squamscott River was more than double the rates at Nannie Island (Fig. 2a, b). The C:N ratio for feces and pseudofeces was significantly higher at Nannie Island ($p < 0.01$; Fig. 2c).

Nutrient and gas fluxes

Reef proximity and study site had no effect on flux of inorganic nutrients from control cores (i.e., cores with no added $^{15}NH_4^+$ or $^{15}NO_3^-$). Results indicated net SRP and NO_3^- uptake for cores collected adjacent to and 15–20 m away from reefs at both study sites (Table 3). There was net NH_4^+ release from all cores at Nannie Island, but at the Squamscott River, reef-adjacent sediment cores showed net NH_4^+ release and more distant cores showed net NH_4^+ uptake. We note that fluxes were highly variable among replicate cores, and standard error was higher than mean flux in several instances (e.g., NH_4^+ flux from reef adjacent sediments; Table 3).

Study site and reef proximity had no effect on denitrification from control cores (Fig. 3a). However, there was a significant interaction between reef proximity and study site (2-way ANOVA, Interaction $p = 0.038$), indicating the effect of reef proximity was different at each site (i.e., no difference between sites at Nannie Island, but higher rates at reef-adjacent cores at the Squamscott River). Reef proximity had no effect on respiration, but rates were higher at the Squamscott River relative to Nannie Island (2-way ANOVA, $p = 0.049$; Fig. 3b). We measured no N-fixation except in Nannie Island cores taken 15–20 m away from the oyster reef (mean = 4.9 μmol N m^{-2} h^{-1}).

Addition of $^{15}NH_4^+$ and $^{15}NO_3^-$ specified the transformation rate for each N species into $^{29,30}N_2$ (Fig. 4). There was very little production of isotope-labeled N_2 from sediments exposed to $^{15}NH_4^+$ (Fig. 4a), and no difference between sites or in reef-adjacent cores relative to more distant cores. The mean (±SE) amount of added $^{15}NH_4^+$–N denitrified was 0.11 (±0.03) and 0.08 (±0.05) % in reef adjacent and distal cores, respectively, at Nannie Island, and 0.30 (±0.15) and 0.19 (±0.11) % in reef-adjacent and distal cores, respectively, at the Squamscott River. In contrast, reef-adjacent sediment had significantly greater production of isotope-labeled N_2 with added $^{15}NO_3^-$ than cores collected 15–20 m away from the reef, and the pattern was the same at both sites (2-way ANOVA $p = 0.019$; Fig. 4b). The mean (±SE) amount of added $^{15}NO_3^-$–N denitrified was 2.26 (±1.12) and 0.97 (±0.19) % in reef adjacent and distal

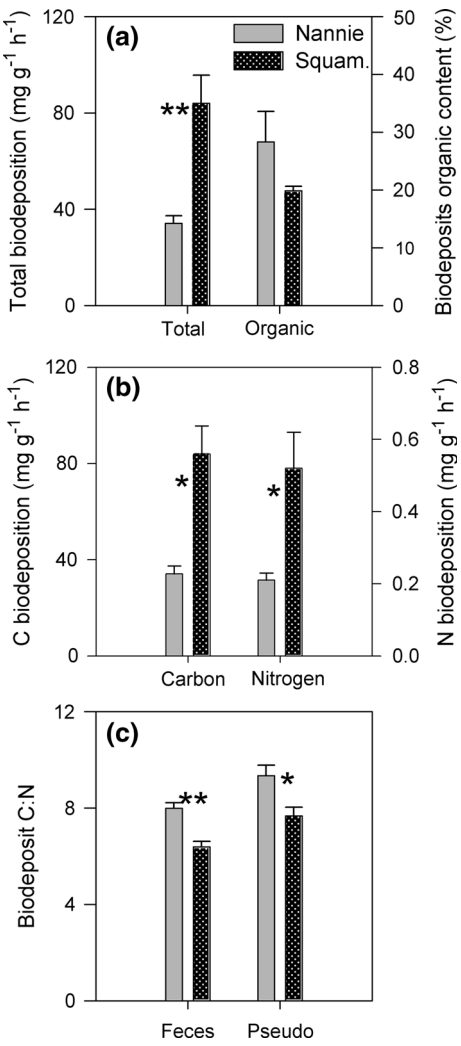

Fig. 2 Mean (±SE) **a** total biodeposition rate (mg g^{-1} h^{-1}) and organic content (%) of the biodeposits (feces + pseudofeces) **b** Carbon (C) and nitrogen (N) biodeposition rates (mg g^{-1} h^{-1}), and **c** C:N ratios of the feces and pseudofeces at 2 reefs in Great Bay, NH. Nannie = Nannie Island, Squam = Squamscott River, and pseudo = pseudofeces. n = 8 at Nannie Island and n = 9 at Squamscott River. *T*-test results compare the 2 sites for each metric, where * indicates $p \leq 0.01$ and ** indicates $p \leq 0.001$

cores, respectively, at Nannie Island, and 1.99 (±1.11) and 0.81 (±0.18) % in reef adjacent and distal cores, respectively, at the Squamscott River.

Sediment characteristics and relationship to gas fluxes

Sediment AFDM was variable by study site and reef proximity. Squamscott River cores had higher total AFDM than Nannie Island cores (2-way ANOVA, $p = 0.002$; Fig. 5a). The pattern was repeated for AFDM < 1 mm^2 (small particles; 2-way ANOVA, $p = 0.002$; Fig. 5b). However, AFDM of sediment >1 mm^2 (large particles) was greater at reef-adjacent cores than 15–20 m reef distant cores for both sites (2-way ANOVA, $p = 0.009$; Fig. 5c).

Denitrification and respiration were correlated with separate components of the sediment AFDM fraction. Total denitrification in control cores was unrelated to either sediment AFDM > 1 mm^2 (correlation, $p = 0.160$) or AFDM < 1 mm^2 (correlation $p = 0.504$; data not shown). However, production of isotope-labeled N_2 following addition of $^{15}NO_3^-$ was positively correlated to abundance of large sediment particles (AFDM >1 mm^2; correlation, $p = 0.068$; Fig. 6a) which was significantly higher in sediment adjacent to both oyster reefs (Fig. 5c). In contrast, respiration was positively correlated with the mass of small sediment particles (AFDM < 1 mm^2), which was significantly higher at the Squamscott River relative to Nannie Island (correlation, $p = 0.053$; Figs. 6d, 5b). We note *p* values of 0.068 and 0.053 are marginally significant, however, the values are lower than any of the other correlations and represent ecologically meaningful patterns (Fig. 6).

Discussion

Relative to the reference site, we predicted the enriched site would have reduced oyster clearance rates, lower quality of biodeposits, and oyster reef proximity would have little influence on sediment biogeochemistry. However, we found the opposite pattern for almost all completed measurements. The discrepancy between our predictions and results appears to be explained by the high quality of food and more labile biodeposits at the more enriched site, and modest differences in oyster density.

Contrast in physicochemical conditions between study sites

As expected, physicochemical measurements confirmed that the Squamscott River exhibited characteristics of cultural eutrophication, including water column nutrients and sediment organic matter. In addition, our measurements for the Squamscott River

Table 3 Mean (±SE) flux of dissolved inorganic nutrients (μmol N or P m^{-2} h^{-1}) from control cores (no added nitrogen), collected adjacent to and 15–20 m away from oyster reefs at Nannie Island and the Squamscott River in Great Bay, NH (N = 3 cores from each proximity at both sites)

Flux	Nannie island		Squamscott River		ANOVA *p* values		
	Adjacent	15–20 m	Adjacent	15–20 m	Site	Reef	Interaction
SRP	−15.6 (4.9)	−25.6 (4.4)	−13.9 (13.3)	−23.0 (8.2)	0.667	0.084	0.924
NH_4^+	6.2 (21.2)	22.4 (9.2)	7.50 (117.9)	−121.6 (14.2)	0.113	0.189	0.108
NO_3^-	−41.9 (14.4)	−40.5 (9.8)	−21.2 (18.6)	−31.1 (15.5)	0.142	0.652	0.551

2-way ANOVA by site (Nannie Island or Squamscott River) and reef proximity showed no significant effects on nutrient flux ($p > 0.1$ in all cases)

SRP soluble reactive phosphorus, NH_4^+ ammonium, NO_3^- nitrate

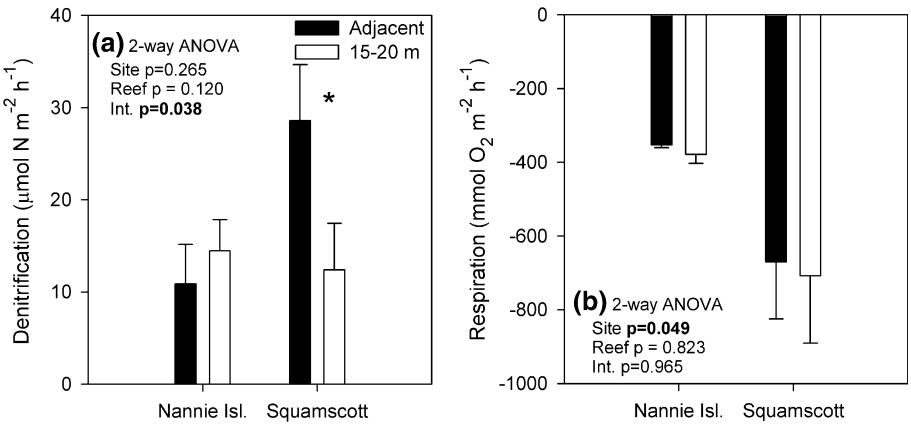

Fig. 3 Mean (±SE) **a** denitrification and **b** respiration rates from control cores (i.e., no nutrients added; N = 3 cores per mean). 2-way ANOVA by study site (Site) and reef proximity (Reef) indicate no difference in denitrification rates, but a significant interaction (Int) between reef and site. There was no effect of oyster reef proximity on respiration, but rates were higher at the Squamscott River site. *Bolded values* indicate *p* values < 0.05

were consistent with the longer-term record from NERRS, suggesting that eutrophication has been ongoing for at least 5 years. Based on these comparisons, we refer to Squamscott River as enriched. However, we acknowledge that defining parameters for the terms eutrophic, oligotrophic, and mesotrophic is a challenge in aquatic ecology (Cloern 2001), as there are no threshold values for DIN, chlorophyll *a*, or sediment C for classification of sites as eutrophic. Elevated values for DIN, dissolved P, chlorophyll *a*, and sediment C are among the well-established characteristics of more eutrophic ecosystems (Nixon 1995). For the purposes of this study, the 2 sites demonstrate a clear contrast in water quality and sediment C, which has consequences for seston biogeochemistry, oyster biodeposits, and sediment N transformations.

Environmental drivers of oyster feeding and biodeposition

Oyster feeding and biodeposition rates are influenced by a number of factors including temperature, salinity, seston characteristics, and oyster tissue biomass (Newell and Langdon 1996; Shumway 1996). Expressed on a standard soft tissue dry weight basis (an individual of 1 g DW), the clearance rates we measured at both sites were similar to clearance rates reported by others in both laboratory (Riisgård 1988) and field studies (Grizzle et al. 2008b). However,

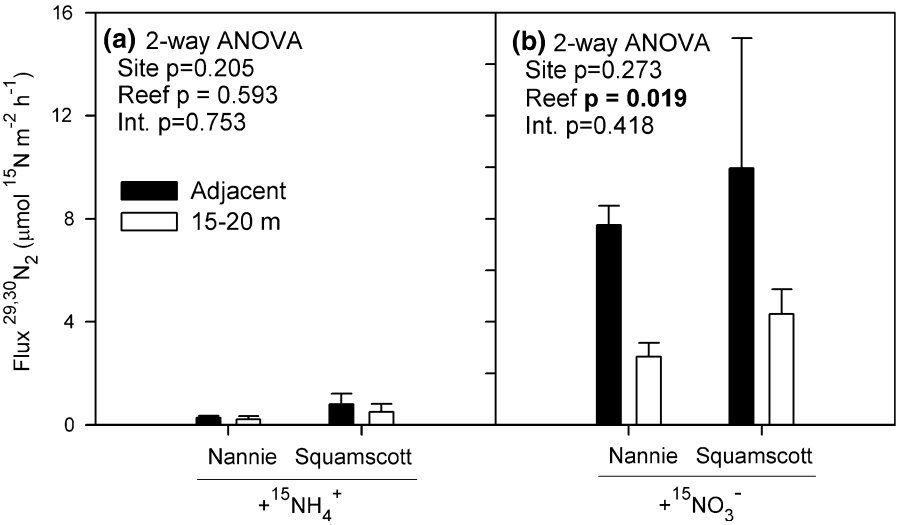

Fig. 4 Mean (±SE) production of $^{29,30}N_2$ for sediment cores with added **a** $^{15}NH_4^+$ or **b** $^{15}NO_3^-$ (N = 3 cores per mean). 2-way ANOVA *p* values by study site (Site) and reef proximity (Reef) show no effects on production of isotope-labeled N_2 in $+^{15}NH_4^+$ cores. However, at both study sites, reef adjacent sediment had greater production of isotope-labeled N_2 $+^{15}NO_3^-$ cores. *Bolded values* indicate *p* values < 0.05, *Int* interaction between site and reef

several feeding rates (i.e., clearance, filtration ingestion, and absorption) were significantly higher at the Squamscott River than at Nannie Island (Table 2). Water temperature and salinity are unlikely explanatory variables as the differences between sites were low. Instead, the higher rates of feeding and biodeposition at the Squamscott River could be attributed to tissue biomass or phytoplankton composition. Nannie Island oysters had greater tissue biomass despite having similar shell lengths as Squamscott River oysters. Oysters with greater biomass are generally expected to have greater feeding rates (Newell and Langdon 1996), however, rates were lower at Nannie Island (Table 2). Oysters may not filter at their maximum rates when a larger proportion of their total biomass is comprised of energy reserves and reproductive tissue due to a reduction in gill area per unit body weight (Newell and Langdon 1996). We did not quantify tissue-specific biomass of oysters (i.e. gonadal tissue) from feeding trials, but Nannie Island oysters had a greater percentage of organic C, suggesting they may have been in a different physiological state (e.g., more energy reserves) than oysters at Squamscott River. Along with site-specific seston differences, this may explain reduced feeding and biodeposition rates at Nannie Island.

Total chlorophyll *a* was significantly higher at the Squamscott River and the phytoplankton community was dominated by 5–28 µm phytoplankton cells, which was correlated with enhanced feeding and biodeposits quality. Conversely, the phytoplankton community at the Nannie Island site was composed mostly of phytoplankton cells <5 µm (71 %). The efficiency of cell capture by oysters is reduced when cells are <5 µm, although retention can still be high for cells >3 µm (Bayne and Newell 1983; Riisgård 1988). Smaller phytoplankton forms may also be less nutritious due to the abundance of structural compounds (e.g., thick cell wall) relative to more digestible intracellular material (Langdon and Newell 1996). Other evidence from feeding measurements suggests that the phytoplankton community was less nutritious at Nannie Island. For example, the organic content of the biodeposits was significantly higher at Nannie Island (Fig. 2) which could suggest that phytoplankton cells were only partially digested. Oyster absorption efficiency was lower at Nannie Island (45 %) compared to the Squamscott River (76 %). This difference was not significant ($p = 0.123$) due to high variation at Nannie Island (14 %; Table 2). Lastly, the C:N ratios of the feces and pseudofeces were significantly higher at Nannie Island, even though seston C:N was lower (Fig. 2, Table 1), also suggesting that a greater proportion of organic C was rejected or released partially digested in feces (Fig. 2). The higher seston quality and greater representation of

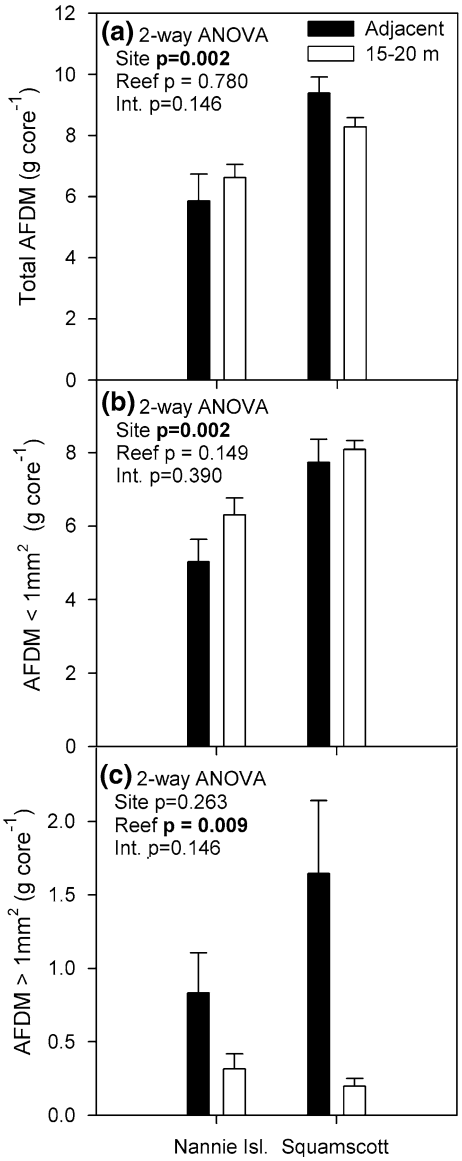

Fig. 5 Mean (±SE) ash-free dry mass (AFDM) in sediment cores, **a** total AFDM, **b** AFDM < 1 mm^2, and **c** AFDM > 1 mm^2. 2-way ANOVA by study site (Site) and reef proximity (Reef) show no effect of reef proximity on total AFDM or AFDM < 1 mm^2, which was higher at Squamscott River than Nannie Island. AFDM of sediment >1 mm^2 was higher near oyster reefs at both sites. *Bolded values* indicate p values < 0.05. *Int* interaction between site and reef

larger phytoplankton size-classes at the Squamscott River was positively correlated with oyster feeding, biodeposition rate, and biodeposition quality, which can have significant effects on sediment C and N dynamics.

Organic matter quality can drive multiple rates of C and N transformations in marine sediments such as decomposition, respiration, denitrification, and N-fixation (Eyre and Ferguson 2005; Fulweiler et al. 2013; Seitzinger et al. 2006). C:N of the oyster biodeposits was higher at Nannie Island (attributed to less nutritious seston), therefore, the organic matter directed to the benthos from oyster biodeposits at Nannie Island was of lower quality (i.e., less labile) for sediment microbes than biodeposits at the Squamscott River (Fig. 2). Along with slightly higher oyster density at the Squamscott River reef, greater biodeposits quality may account in part for higher denitrification of sediment microbes at reef-adjacent cores in the Squamscott River (see below). However, biodeposit accumulation is also affected by reef macrofauna and local hydrodynamics, which are affected by sediment structure (i.e., shells vs. organic matter) and vary by reef proximity and study site. Nevertheless, feeding and biodeposition measurements illustrate clear differences between sites, which have the potential to influence biogeochemical cycling in reef-adjacent sediments. These data suggest seston characteristics could be an important factor driving the role of bivalves on sediment C and N biogeochemistry.

The biodeposition method, although not technically conducted in situ, is often referred to as an in situ approach because the measurements are conducted on site using natural seston (Galimany et al. 2013; Iglesias et al. 1998; Petersen et al. 2004). There are only a few studies that describe oyster filtration rates under field conditions (Grizzle et al. 2008b; Zu Ermgassen et al. 2013). Our data add to the modest set of field measurements. Recent studies have called for further development of these datasets to inform modeling studies which depend upon in situ measurements (Cerco and Noel 2007; Zu Ermgassen et al. 2013).

Linking water chemistry, oyster biodeposition, and pathways for N_2 production

Combining our results for water quality, biodeposition, and sediment biogeochemistry clarifies pathways whereby oysters can influence N_2 production, and which pathways are less likely. Across sites, $\sim$0.1–0.3 % of the added $^{15}NH_4^+$ was detected in the $^{29,30}N_2$ pool, while 0.8–2.0 % of added $^{15}NO_3^-$ was recovered in the $^{29,30}N_2$ pool, suggesting that

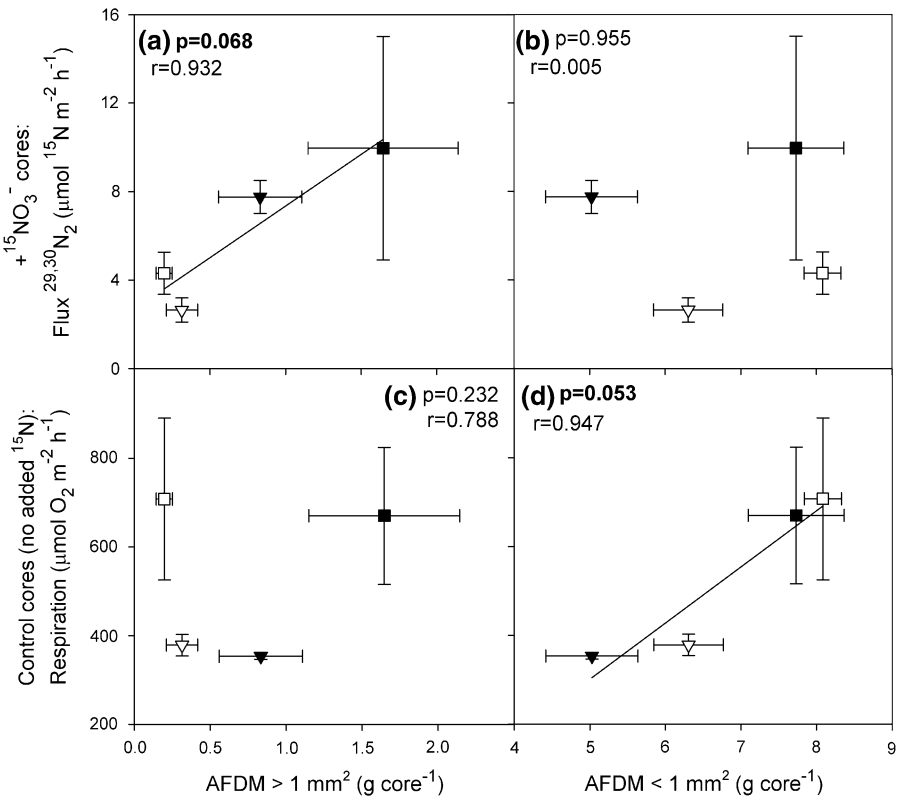

Fig. 6 Flux of $^{29,30}N_2$ in + $^{15}NO_3^-$ cores was correlated with **a** AFDM > 1 mm^2 (large particles), but not **b** AFDM < 1 mm^2. Sediment respiration was not correlated with **c** AFDM > 1 mm^2, but was positively related to **d** AFDM < 1 mm^2 (small particles) which was higher at the Squamscott River relative to Nannie Island. *Triangles* indicate Nannie Island and *squares* indicate Squamscott River. *Black* shows reef-adjacent sediment and *white* shows sediment collected 15–20 m from oyster reefs. *p* and *r* values are from Pearson's product moment correlation

NH_4^+ is less likely to be converted to N_2 via coupled nitrification–denitrification or anammox than NO_3^- via denitrification. This was contrary to our expectations, as we predicted oxidized NH_4^+ to be an important substrate for N_2 production at Nannie Island, where water column nutrients were lower. Other studies have found coupled nitrification–denitrification to be the primary N_2 production pathway in coastal marine sediments (Risgaard-Petersen 2003). However, the importance of coupled nitrification–denitrification is reduced with high water column NO_3^- and low sediment oxygen (Eyre and Ferguson 2005; Newell et al. 2002; Seitzinger et al. 2006). Our lack of evidence for coupled nitrification–denitrification is consistent with results showing oysters do not affect sediment nitrification in eutrophic Jamaica Bay, New York City, which was attributed to high sediment organic matter and water column NO_3^- (Hoellein and Zarnoch 2014). We did not budget the added ^{15}N into pools other than N_2, but the remainder was likely either assimilated, adsorbed ($^{15}NH_4^+$), transported out of the cores in the same form, or recycled via dissimilatory processes (e.g., nitrification and dissimilatory NO_3^- reduction to NH_4^+).

While there was little evidence to suggest N in oyster biodeposits could affect coupled nitrification–denitrification, enhanced sediment C from oyster biodeposition could increase denitrification of water column NO_3^-. Water column NO_3^-, sediment organic matter, and biodeposit quantity and quality were higher at the Squamscott River (Fig. 2), and the significant interaction between site and reef proximity indicated enhanced N_2 production in reef-adjacent sites at the Squamscott River only (Fig. 3a). In contrast, Nannie Island had lower water column NO_3^-, sediment organic matter, and biodeposit quantity and quality than Squamscott River, and denitrification rates were lower than Squamscott River with no evidence for an effect of oyster reef proximity. Oyster density was also modestly higher at Squamscott River (85.6 oysters m^{-2}) than Nannie Island (59.5 oysters m^{-2}). These data suggest a possible link between oyster biodeposits and increased N_2 production in reef-adjacent cores at the Squamscott River. However, if biodeposits increased sediment C quantity for denitrification, we expected more sediment AFDM at the reef-adjacent cores than those collected 15–20 m away from the Squamscott River reef. There

was a trend of higher total AFDM at the Squamscott River reef-adjacent sites, but it was not significant (Fig. 5a). Oyster biodeposition could increase organic matter quality without an increase in quantity, as biodeposits can have lower C:N ratio than sediments composed of settled phytoplankton or terrestrial C. Unfortunately, we do not have data for C:N ratio of sediments from reef-adjacent and 15–20 m away cores.

The potential for oysters to alleviate C-limitation of denitrification in coastal sediments has been shown in other studies. Smyth et al. (2013) found high sediment oxygen demand (SOD) at oyster-reef adjacent sediments, and speculated oyster biodeposits enhanced microbial respiration at this site, leading to more denitrification. We found no relationship between SOD and N_2 fluxes, but a positive relationship between SOD and N_2 production has suggested C-limitation of denitrification in past research (Piehler and Smyth 2011; Seitzinger et al. 2006). High SOD can also be associated with increased dissimilatory NO_3^- reduction to NH_4^+ (DNRA; Gardner and McCarthy 2009), but DNRA only accounted for 11.2 % of the NO_3^- flux in summer oyster reef sediments from Bogue Sound, North Carolina (Smyth et al. 2013). In contrast, Kellogg et al. (2013) showed sediment cores with intact oyster reef segments had greater abundance of organic C than control sediment, which was one factor that increased N_2 production from reef cores (along with diffusion and biofilm surface area; see below). In addition, abundance of denitrification genes for nitrite reductase (i.e., *nir*S and *nir*K) is positively correlated with temperature, inorganic N concentrations, and sediment C in estuaries (Dang et al. 2009). Sediment C may increase sediment denitrifier abundance and favorable redox conditions, but there may be a threshold above which sediment C no longer increases denitrification. In Jamaica Bay, New York City, Hoellein and Zarnoch (2014) found oysters increased sediment C, but there was little effect of oysters on denitrification potential because it was not C-limited.

Positive correlations between denitrification and size-fractionated sediment organic matter suggest diffusion may be an important driver of N_2 production. Denitrification of $^{15}NO_3^-$ to $^{29,30}N_2$ was positively related to sediment AFDM > 1 mm^2 (Fig. 6), and both were higher in reef-adjacent sediment at each site (Figs. 4b, 5c). The reef-adjacent cores had greater shell hash, but those collected 15–20 m away from the reefs were consolidated sand and fine sediment. The 3-dimensional complexity of oyster reef structure has significant impacts on many reef-associated organisms (Coen et al. 1999). Kellogg et al. (2013) found high surface area of intact reef segments was correlated with increased respiration and N transformation rates, relative to cores without oyster reefs (i.e., sand and fine organic sediment). The authors speculated that reef structural complexity allowed for diffusion of water column NH_4^+ and organic matter to reef biofilms, which resulted in exceptionally high rates of coupled nitrification–denitrification. Denitrification was also higher in estuarine habitats with greater structural complexity in an oligotrophic estuary in North Carolina, including oyster reef-adjacent sediments, submerged aquatic vegetation, and cores with intact salt marsh plants (Piehler and Smyth 2011).

We collected measurements in summer because temperature and food availability suggested this was the period of peak oyster activity, however, the patterns recorded here may not apply to other times of year. For example, seasonal variation in denitrification from estuary sediments is commonly recorded, including higher rates in summer (Eyre et al. 2013; Fulweiler et al. 2013; Piehler and Smyth 2011; Smyth et al. 2013). In addition, Smyth et al. (2013) found higher denitrification near oyster reefs in summer than in winter, and Kellogg et al. (2013) found oysters enhanced denitrification the most in August and the least in November. We expect a similar pattern would occur in Great Bay, but to our knowledge no simultaneous measurements of oyster feeding and sediment biogeochemistry have been completed on a seasonal basis.

Denitrification has been the primary subject of research for oyster effects on N cycling, while other N transformations receive less attention. For example, anammox is a significant source of N_2 in some marine ecosystems (Thamdrup and Dalsgaard 2002) and may be stimulated by oyster biodeposits. We were unable to calculate anammox rates, but studies from estuaries near Great Bay suggest it is a relatively minor contributor of total N_2 production (i.e., 2–9 % of denitrification; Engström et al. 2005; Koop-Jakobsen and Giblin 2009). In addition, the greenhouse gas N_2O is produced via nitrification and denitrification, and is emitted from marine invertebrate guts, biofilms on

shells, and sediments (Heisterkamp et al. 2010), but has not been measured in association with oyster reefs. DNRA is a sometimes overlooked as source of NH_4^+, and may be preferred to denitrification under some conditions (Gardner and McCarthy 2009). Smyth et al. (2013) found DNRA represented a maximum of 11.2 % of NO_3^- flux near oyster reefs in summer. DNRA and mineralization of NH_4^+ from anoxic sediments serve as a positive feedback for maintaining eutrophic conditions (Newell et al. 2002). Oysters could affect anammox, N_2O production, and DNRA differently in eutrophic and oligotrophic waters, but oysters' influence on these rates across enrichment gradients is unknown.

Overall, our data showed oyster reef-adjacent sediment had significantly higher rates of direct denitrification than sediments taken further from oyster reefs, and suggested the mechanism for increased N_2 production was either (1) oyster biodeposits increased C quality (Squamscott River only), or (2) enhanced diffusion of water column NO_3^- in reef-adjacent sediments (both sites). Although our predictions that oyster-mediated changes in sediment biogeochemistry would depend upon water quality conditions were incorrect, our previous conceptual model did not account for higher phytoplankton and biodeposit quality in eutrophic habitats (Hoellein and Zarnoch 2014), which appeared to drive patterns between sites. Future research on how oysters' role in sediment biogeochemistry changes with eutrophication can address these complications by uniting analytical approaches from disparate studies, including simultaneous use of cores with intact reefs (Kellogg et al. 2013), reef-adjacent cores (this study; Smyth et al. 2013), cores from multiple habitats (Piehler and Smyth 2011; Smyth et al. 2013), experimental manipulations of oyster densities (Hoellein and Zarnoch 2014), and concurrent measures of biodeposits and seston characteristics (this study). In addition, isotope-labeled oyster biodeposits (^{15}N and ^{13}C) could trace the environmental fate of elements in feces and pseudofeces (Atkinson et al. 2014). Results from the this study contribute to the growing body of research on oysters' role in sediment biogeochemistry by narrowing the pathways whereby oysters affect N_2 production in Great Bay Estuary and similar environments. These data will inform N budgets and best practices for management of oyster reefs and cultural eutrophication.

Acknowledgments Funding was provided by the Hudson River Foundation. For field assistance we thank Krystin Ward and Deb Lamson. For assistance in the lab we thank Michael Hassett, Matthew Girard, and Ashley Cook from Loyola University Chicago and Doris Law, Andrew Liang, and Erika Fusco from Baruch College. Thanks to Denise Bruesewitz for comments on an early version of this manuscript. The manuscript was also greatly improved by insightful comments from 3 anonymous reviewers and Associate Editor Leila J. Hamdan.

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