INTRODUCTION

A major consequence of anthropogenic changes to the global nitrogen (N) cycle is the accumulation of reactive N in estuaries and coastal marine ecosystems (Vitousek et al. 1997, Howarth et al. 2000, NRC 2000). Estuarine watersheds that drain large agricultural areas and are in close proximity to dense population centers receive substantial N loads through a variety of point and non-point sources, leading to a complex array of ecosystem-scale conservation and management issues resulting from eutrophication.
Chesapeake Bay (USA) is a highly impacted estuary in which N pollution has contributed to chronic hypoxia−anoxia, reduced water clarity, and loss of biodiversity (Boesch et al. 2001, Diaz 2001, Kemp et al. 2005). In addition to limiting N inputs, finding ways to enhance natural N sinks within the Bay is a promising approach being investigated to manage this ecosystem.

Denitrification (DNF) and anaerobic ammonium oxidation (AMX) are microbially-mediated biogeochemical processes that permanently remove N in the form of inert N2 gas. DNF is a natural N sink that removes an estimated 20 to 50% of N inputs to estuaries (Seitzinger 1988). Sediment DNF rates have been measured across a number of coastal marine ecosystems and generally represent N removal on the order of 1 to 2 mmol N m−2 d−1 (Seitzinger et al. 2006, Fennel et al. 2009). DNF may occur as direct DNF of water column NO3− fueled by labile organic carbon, or through the coupled mineralization, nitrification, and DNF of organic N. There is evidence that moderate organic matter (OM) loading may drive higher DNF rates compared to high OM loading, which can inhibit coupled nitrification−denitrification (Sloth et al. 1995, Laursen & Seitzinger 2002, Eyre & Ferguson 2009). AMX is less well characterized, typically constituting a smaller contribution to N removal, but can be a non-trivial contributor to total N2 production (Devol 2003, Francis et al. 2007). If these biogeochemical N2 removal pathways have the potential to be expanded in the Bay, they could serve as tools for mitigating N pollution in impacted marine and estuarine ecosystems.

The potential of suspension-feeding bivalves to mitigate eutrophication in coastal marine ecosystems has attracted interest in understanding their impact on microbe-mediated nutrient removal mechanisms. Within Chesapeake Bay, sharp increases in N loads concurrent with loss of over 99% of the native eastern oyster Crassostrea virginica population have occurred simultaneously over the last century, making it difficult to disentangle the impact of these 2 factors and understand how they are interrelated. Suspension-feeding bivalves affect the distribution and cycling of nutrients and energy, particularly in shallow waters, by filtering the water column and depositing a mixture of feces and pseudofeces (collectively referred to as biodeposits) to sediments, thereby coupling benthic and pelagic processes (Officer et al. 1982, Dame et al. 1984). Feces and pseudofeces combine into mucus-coated aggregates that have a higher sinking velocity than the surrounding seston (Giles & Pilditch 2004), increasing sedimentation rates of nutrient-enriched OM and promoting benthic−pelagic coupling. Loss of suspension feeders has arguably shifted the trophic structure of such ecosystems from ones dominated by benthic−pelagic coupling to ones that are almost entirely dominated in the pelagic zone by nutrient-accelerated primary production (Jonas 1997, Kemp et al. 2005, Newell et al. 2005, Dame 2012).

A plausible hypothesis is that suspension-feeding bivalves (e.g. oysters) stimulate increased sediment N2 production rates by accelerating OM sedimentation via biodeposition, thereby expanding a N sink resource by facilitating accelerated permanent N removal (Haven & Morales-Alamo 1966, Kaspar et al. 1985, Kautsky & Evans 1987, Deslous-Paoli et al. 1992, Newell et al. 2002, Newell 2004). Experimental laboratory evidence has indicated that coupled nitrification−denitrification rates increase with the addition of pelletized algal cells (used as a biodeposit analog), yielding estimates that 0.5 g N oyster−1 yr−1 may be removed in Chesapeake Bay via oyster biodeposit stimulation of increased coupled nitrification−denitrification rates (Newell et al. 2002). Field measurements of DNF rates in sediments associated with bivalve cultivation (oysters, clams, and mussels) in other marine systems (Gilbert et al. 1997, Christensen et al. 2003, Nizzoli et al. 2006, Minjeaud et al. 2009) have yielded results that are equivocal or indicate inhibition of DNF. Alternatively, rather than promoting net N removal, bivalve systems may facilitate N retention (Dame & Libbes 1993). Studies of both natural reefs and aquaculture systems (clams and mussels) have indicated significant increases in water column NH4+ as the deposited organic N is remineralized and returned to the water column as reactive N (Porter et al. 2004, Gibbs et al. 2005, Dame 2012). It is therefore equally plausible that biodeposition by aquaculture may reduce DNF by enhancing reducing conditions (as a consequence of enhanced OM), thereby reducing nitrification which fuels coupled nitrification−denitrification.

In this study, we tested the hypothesis that biodeposition from aquacultured oysters significantly alters sediment N2 production rates, having either an inhibitory or stimulatory effect, in estuarine sediments at 2 commercial-scale floating-raft oyster aquaculture sites (80 000 to 120 000 oysters) and 2 accompanying reference (no aquaculture) sites in Chesapeake Bay. Over the period of study, we used 2 independent methods, 15N tracer and N2:Ar ratio measured via membrane inlet mass spectrometry (MIMS), to estimate the effect of varying levels of oyster biodeposition treatments on sediment N2 production, consider-
Higgins et al.: Oyster biodeposition effect on sediment N₂ ing spatial and temporal variability. We collected oyster biodeposits at each site to estimate biodeposition and total nitrogen delivery rates to sediments at a density of 286 oysters m⁻² (similar to wild oyster reef densities of 100 to 350 m⁻², Schulte et al. 2009). In a series of field experiments, we forced the accumulation of oyster biodeposits in sediments directly underneath aquaculture rafts in the field, controlling for the effect of hydrodynamic flow conditions, and measured the effect on sediment N₂ production rates. In laboratory tests, we collected reference sediment cores in the field and inoculated them with freshly collected biodeposits in increasing quantities, to test for a biodeposit effect on N₂ production rates.

**MATERIALS AND METHODS**

**Experimental design**

The experimental approach was designed to measure the effect of oyster biodeposition on sediment N₂ production rates taking into account spatial, temporal, and methodological variations. To accomplish this goal, we examined (1) whether aquacultured oyster biodeposition impacts N removal (i.e. induces increased or decreased rates) under 2 different study site conditions seasonally; (2) the quantitative relationship between biodeposit N-load delivered to sediments and N removal; and (3) effects of field and laboratory manipulations of biodeposit amendments to sediments. Two widely accepted state-of-the-art techniques for measuring sediment N₂ production (¹⁵N tracer and MIMS; Groffman et al. 2006) were used to investigate the impact of oyster biodeposition on rates of sediment N₂ production at both aquaculture sites and comparable reference sites in Chesapeake Bay. The ¹⁵N tracer method is a measure of the sediment N₂ production potential rate that allows for distinction between DNF and AMX, particularly effective for comparisons among treatments. In contrast, MIMS is an in situ method that measures total sediment N₂ production that includes coupled nitrification-denitrification but does not distinguish between DNF and AMX. These methodological approaches were applied in a series of experiments over a 2 yr period categorized into 2 trials by method of sediment N₂ production measurement and included 1 overlapping set of samples at St. Jerome Creek (see below for details of study sites) where both methods were used for cross-validation analysis (see Table S1 in the supplement at www.int-res.com/articles/suppl/m473p007_supp.pdf). In Trial 1, the ¹⁵N tracer method was used to measure sediment DNF and AMX (combined N₂ production) at both Spencer’s Creek and St. Jerome Creek in June 2008 and October 2008, and at St. Jerome Creek in May 2009. In Trial 2, MIMS was used to measure N₂ production at St. Jerome Creek in May 2009 and at Spencer’s Creek and St. Jerome Creek in August 2009. Within the study trials, sediment transect samples were collected at the study sites according to the following descriptions, referred to hereafter as treatment types: sediments directly underneath an oyster aquaculture array (OY), sediments 350 to 500 m away from an oyster aquaculture array (REF), sediments 5 to 10 m outside an oyster aquaculture array (NOY), sediments exposed to forced accumulation of oyster biodeposits in the field (FNC), isolated oyster biodeposits (BIO), and biodeposit additions to sediment cores in the lab (BA).

**Study sites and oyster cultivation**

We conducted trials in 2 shallow (1.5 to 2.5 m mean low tide) mesohaline tributaries in separate sub-watersheds of Chesapeake Bay (Fig. 1) where oysters were cultivated in floating rafts as described by Higgins et al. (2011). Both study sites had a residence time of ~5 d (modeled regional estimate from Thomann et al. 1994) and were selected from areas where the depth range (1 to 3 m) and distinct estuarine environments were representative of sites commonly utilized for oyster aquaculture in mid- to lower Chesapeake Bay. Spencer’s Creek, Virginia (37° 54’ 22”N, 76° 17’ 27”W; HUC 02070011), within Little Wicomico River, is a low wave energy site approximately 60 m wide, with poor hydrodynamic exchange, loamy sediment high in OM (Table S2 in the supplement), a benthic index of biotic integrity (B-IBI) of 1 (determined using a modification of the method of Llansó et al. 2002), and salinity ranging from 5 to 15‰. St. Jerome Creek, Maryland (38° 07’ 13”N, 76° 20’ 53” W; HUC 02070011), located north of the mouth of the Potomac River, is a high wave energy site that is approximately 800 m wide with a low OM sandy bottom (Table S2), a B-IBI of 1, and salinity ranging from 12 to 15‰.

At both sites, an oyster aquaculture station (OY treatment) was used to raise oysters to harvest size (≥76 mm), whereas no oysters were cultivated at comparable reference stations (REF treatment). Oysters were reared in floating rafts at both sites as described by Brown et al. (1998) and Higgins et al. (2011). Floating rafts consisted of a rectangular PVC
frame approximately 1.8 × 0.9 m with 3 polyethylene mesh bags (2 cm diamond mesh) suspended across the long edges, each bag containing 200 oysters, such that each raft contained ~600 oysters. Oysters were maintained 1 to 2 deep to maximize access to seston and reduce access to pre-filtered water from neighboring oysters. When tied together in an array, the floating rafts were ~0.3 m apart, equating to a maximum oyster density of 286 oysters m⁻², covering a total bottom area of approximately 250 m² at Spencer’s Creek and 350 m² at St. Jerome Creek, with each raft covering 2.1 m² of sediment.

At Spencer’s Creek, an array of oyster rafts containing 80,000 oysters was deployed overlying sediments not previously exposed to oyster biodeposition. During the period of study, the maximum shell dimension of oysters at this site ranged from 50 to 85 mm. At St. Jerome Creek in 2008, an oyster raft array containing 100,000 to 120,000 oysters was deployed overlying sediments that were not previously exposed to oyster biodeposition. During the period of study, oysters ranged from 40 to 85 mm. Oyster sediment samples collected from St. Jerome Creek in May and August 2009 were from a location within the commercial site in operation since 1991 containing ~200,000 oysters (oysters ranging from 15 to 130 mm).

**Collection of biodeposits and determination of biodeposition rates**

Replicate samples of biodeposits were collected as they settled beneath floating oyster rafts using replicate 100 µm nylon mesh devices secured under representative oyster rafts (Fig. 2A). These devices were deployed for similar time periods to Jaramillo et al. (1992), McKindsey et al. (2009), and Clavier & Chauvaud (2010), but differed from typical settling tubes by promoting the flow of ambient water through the nylon mesh collection device while providing for collection from hundreds of oysters simultaneously. The continuous tidal and water movement also was observed to minimize entanglement of falling biodeposits and served to effectively move them toward the jar. Collected biodeposits were used to quantify the total mass load of the nutrients N and carbon (C) that oysters delivered to sediments and were conducted in conjunction with direct measures of sediment N₂ production. Nutrient content of the collected biodeposits per unit of time was used to estimate the nutrient content of materials processed by oysters and made available for biogeochemical N₂ removal by sediments. Quantification of the properties and amounts of oyster biodeposition was accomplished...
by making replicate collections of biodeposits (n = 5) using separate biodeposit catchment devices underneath replicate oyster rafts during sampling events at both sites. Deployment periods reflected seasonal variation in filtration and were determined by preliminary analyses (data not shown), resulting in empirically determined periods that provided sufficient material for analysis, captured variability of deposition across several 24 h cycles, and minimized the possibility of loss of some particulate organic N (PON) and particulate organic C due to microbial metabolism over longer periods. Oyster biodeposits collected at both aquaculture sites were captured as they settled and before they reached the sediment for a 2 to 3 d period in June 2008 and 3 to 5 d period in October 2008 and May 2009, when biodeposition rates decreased. Over the same time period as oyster biodeposits were collected, seston samples were collected using sham rafts without oysters deployed at the reference stations to determine the background particulate OM settling out of the water column. Biodeposits and settled seston samples were rinsed using ambient water, sieved to remove epibenthic fauna and terrestrial debris, and dewatered through 100 µm mesh; then, both wet and dry weights were recorded to quantify isolated oyster biodeposit N loads to sediments, excluding allochthanoous and autochthanoous inputs. Biodeposits were placed at 4°C immediately upon collection and stored for <12 h prior to analysis.

To account for the dispersal of biodeposits outside of the oyster aquaculture array due to the effect of hydrological flow conditions, a funnel-shaped 100 µm nylon mesh fence (FNC) was deployed beneath representative oyster rafts to force the accumulation of biodeposits in the sediments underneath a raft (Fig. 2B). The mesh walls were sized to maximize biodeposition accumulation in sediments beneath a raft and provide ~0.5 m slack to accommodate the height of tidal exchange. During the period of deployment, continuous vertical and horizontal water movement served to transport materials that periodically collected on the fabric down to the sediment surface. The biodeposit fence was deployed at both the Spencer’s Creek and St. Jerome Creek aquaculture sites in Trial 1 and only at St. Jerome Creek during Trial 2. These field trials were designed to force the accumulation of biodeposits upon sediments directly underneath oyster rafts to facilitate measurement of the microbial community N₂ production response to concentrated inputs of biodeposits to the system while maintaining realistic environmental conditions that are difficult to reproduce in biodeposit addition experiments in a laboratory setting.

**Sediment, biodeposit, and pore water analyses**

Triplicate sediment cores for each treatment were collected in Trial 1 using 7 cm diameter × 19 cm high metal corers and in Trial 2 using 6.4 cm diameter × 17 cm high plexiglass tubes. To determine the extent to which sediment conditions were conducive to sediment N removal, oxygen consumption, nutrient analyses, and chlorophyll a (chl a, used as a proxy for benthic microalgae and an indicator of pseudofeces production) were measured at each site for both trials. Pore water analyses were performed on subsamples of OY, REF, NOY, and FNC in June 2008 for both sites, OY and REF in October 2008 for both sites,
and OY and REF in May 2009 for St. Jerome Creek. Well-mixed (0–3 cm) subsamples were analyzed for total nitrogen (TN) and total carbon (TC) by combustion using a Perkin-Elmer 2400 CHN elemental analyzer. This depth interval was reported by Sørensen (1978) to encompass peak denitrification activity and was confirmed appropriate based on preliminary sediment analyses of 3 cm increments down to 20 cm. Nutrient content was determined by US Environmental Protection Agency (EPA) approved standard methods: NOx (SM 4500-NO3F), NH4+ (EPA 350.2), total Kjehldahl nitrogen (TKN; EPA 351.3), total organic nitrogen (TON; calculated), biochemical oxygen demand (BOD; 5210B), chemical oxygen demand (COD; 5520C), chl a (EPA 445.0 using 1 g sediment), OM (SM 2540G), and sediment oxygen demand (SOD; calculated as the flux of O2 as per Kana et al. 1994, Smith et al. 2006). Pore water in the 0–3 cm section of cores was obtained from subsamples by centrifugation at 7000 × g (20 min at 4°C), followed by analysis for NH4+ (350.1) and NO3− (353.2). For MIMS analyses, water was filtered through Whatman GF/F filters (25 mm diameter, 0.7 µm nominal pore size) and the filtrate was analyzed with a Lachat Quick-Chem 8000 automated ion analyzer for NOx, NH4+, PO4−, and dissolved organic nitrogen (DON).

15N stable isotope measures of N2 production

For all 15N tracer analyses, surface sediment (0–3 cm) subsamples were collected from sediment cores, stored immediately on ice, and held for <5 h until sample incubation preparations were performed in the lab. In Trial 1 at St. Jerome Creek and Spencer’s Creek, a sampling transect was conducted in June 2008 with n = 3 replicate sediment cores collected for each of the following treatment types: OY, REF, NOY, FNC, and BIO. In October 2008, both sites were again surveyed including n = 3 cores of the following treatment types: OY, REF, and BIO. In May 2009 at St. Jerome Creek, a methods cross-comparison was performed including n = 3 cores each of OY and REF sediments, and the 15N tracer measures of N2 production were compared to N2 production rates measured from equal numbers of replicate cores using MIMS as part of Trial 2.

The 15N stable isotope enrichment analysis allowed for the direct measure of both DNF and AMX potential production rates in sediments that are well suited for study involving detection of an environmental response to a treatment. The method was performed by incubating sediment samples enriched using 15N-labeled NO3− and or NH4+ substrates (Thamdrup & Dalsgaard 2002). Substrates were supplied in small excess relative to environmental concentrations (as determined by preliminary testing) to measure metabolism of DNF and AMX microbial communities. For each analysis, subsamples (1 g) of wet homogenized sediments or biodeposits were weighed into glass exetainers (Labco International) in 8 replicates per treatment type for time series incubations (t = 0, 60, 150, and 300 min). Four tubes were reserved for 15NO3− / 14NH4+ and 4 tubes for 15NH4+ treatments, allowing us to distinguish between DNF and AMX based on the mass of N2 produced using parallel tracer incubations (Thamdrup & Dalsgaard 2002, Song & Tobias 2011). Exetainers were flushed with ultra-high purity (UHP) He for 10 min (>100 ml min−1), capped immediately, and stored overnight, allowing residual NO3− + NO2− to be consumed. Following the overnight incubation, NO3− drawdown (<1 µM) was confirmed by checking replicate samples for ambient NO3− using a NOx box.

The exetainers were flushed again with UHP He for 15 min prior to introducing an aliquot of injectate to each exetainer. 15NO3− + 15NH4+ or 15NH4+, to a final pore water N concentration of 25 µM. The spiked concentration was chosen as a value consistent with the ranges of measured pore water NO3− + NO2− in site sediments (<1–11 µM) and in biodeposits (<1–36 µM), was squarely within the range of NO3− concentrations reported previously in DNF compilations from other coastal systems (e.g. Steingruber et al. 2001, Seitzinger et al. 2006, Fennel et al. 2008), and served to standardize substrate across all incubations. At each time point, the production of N2 gas was stopped by injecting ZnCl2 (50% wt/vol) into an exetainer and vortexing for 30 s. Isotopically labeled N2 was analyzed from the exetainer headspace using an isotope ratio mass spectrometer (Thermo Electron Delta V). Time series production of 28N2 and 30N2 from the 15NO3− + 14NH4+ incubations were used to calculate AMX and DNF, respectively, according to Thamdrup & Dalsgaard (2002). The 15NH4+ incubations in the absence of NO3− were used to correct AMX rates for any mass 29 production via coupled nitrification–denitrification under the imposed incubation conditions (none was observed). The 15N-based rates originally measured in nmol N g−1 wet sediment h−1 were converted to areal rates by multiplying by the bulk sediment density (1.5 g cm−2) and sediment collection depth (3 cm). These areal rates were scaled to and reported as mmol N m−2 d−1 for ease of comparison to the in situ rates measured by
MIMS and to existing literature values. The bulk density, sampling depth, and all unit conversions can be collapsed into a single value such that mmol N m$^{-2}$ d$^{-1}$ can be back-calculated by multiplying mmol N m$^{-2}$ d$^{-1}$ by 0.926.

**MIMS measures of N$_2$ production**

For Trial 2, sediment core samples were collected at St. Jerome Creek in May 2009 including $n = 3$ cores for each of the following treatment types: OY, REF, and BA. For the purposes of method cross-validation, OY and REF sediment N$_2$ production rates measured using MIMS were compared to $^{15}$N tracer measurements. Sediment cores were collected at both St. Jerome Creek and Spencer’s Creek in August 2009. At St. Jerome Creek, sediment collections included $n = 3$ cores of the following treatment types: OY, REF, FNC, and BA. At Spencer’s Creek, $n = 3$ sediment cores of each of the following treatment types were collected: OY, REF, and BA.

MIMS was used to determine *in situ* N$_2$ production flux rates by measuring dissolved N$_2$ gas in the overlying water entering and exiting sediment cores. MIMS allowed us to measure changes in N$_2$ that result from DNF (including coupled nitrification–denitrification) and AMX, although these 2 processes are not distinguishable separately using this technique. For all MIMS analyses, cores were collected, handled, and transported in a manner designed to maintain the natural redox zones in the sediment (Scott et al. 2008, Whalen et al. 2008, Fulweiler & Nixon 2011). Upon collection at the field sites, cores were immediately submerged uncapped and subsequently transported in ambient water using ice packs and gentle aeration to both reduce metabolism and facilitate gas exchange. Within 5 to 6 h of collection, cores arrived at the University of North Carolina Institute of Marine Sciences, where they were transferred to aerated water baths in an environmental chamber at ambient temperature. The following morning, cores were capped with gas-tight lids equipped with an inflow and outflow sampling port. Lids were plumbed to a multi-channel peristaltic pump that circulated site water through the headspace of the cores at a rate of 1 ml min$^{-1}$ (Lavrentyev et al. 2000, McCarthy & Gardner 2003), facilitating exchange of water overlying the sediment in each core.

Cores were acclimated by incubation in the continuous flow system for at least 18 h to ensure steady-state conditions of dissolved gasses prior to measurements (Eyre et al. 2002). Following the pre-incubation period, 5 ml samples were collected from the outflow of each core at 18, 24, 36, and 48 h increments for dissolved gas analysis. In addition to the outflow from the cores, the concentration of dissolved gasses in the water entering the cores was measured from the ambient water line, which bypassed the cores and flowed directly into the sample vials. These samples also accounted for any changes in water chemistry through tubing and pump effects. Successive results from each core were averaged to core-specific rates. These values were averaged among replicates to calculate mean values.

Concentrations of O$_2$, N$_2$, and Ar were measured using MIMS (Kana et al. 1998). Ratios of N$_2$:Ar, and O$_2$:Ar in water samples were used to determine the concentration of N$_2$ and O$_2$ in each sample (Kana et al. 1994, Ensign et al. 2008). After 24 h, 50 ml water samples were collected for nutrient analysis from the reservoir water line in each core. Water was filtered through Whatman GF/F filters (25 mm diameter, 0.7 µm nominal pore size), and the filtrate was analyzed for nitrogen and increased oxygen concentrations. Benthic fluxes were reported as mmol N m$^{-2}$ d$^{-1}$ and were calculated using the equation ($C_{\text{out}} - C_{\text{in}}$) × F/A, where C represents the concentration of any analyte, $C_{\text{out}}$ and $C_{\text{in}}$ are the outflow and inflow concentrations (µM), respectively, F is the peristaltic pump flow rate (l h$^{-1}$), and A is the surface area of the core (m$^2$) (Miller-Way & Twilley 1996, Ensign et al. 2008). Although some previous studies have conducted light/dark experiments and analyzed the samples using MIMS (Ferguson & Eyre 2007), we chose to not use light incubations because this approach often results in the formation of gas bubbles, which significantly and selectively affect gas concentrations in water (Reeburgh 1969), an effect reflected by our experience. Our N$_2$ production data were therefore extrapolated based on a 12 h day to reflect both competition with benthic microalgae for nitrogen and increased oxygen concentrations (Tobias 2007, Hochard et al. 2010).

Experiments on the effects of oyster biodeposits were performed by collecting REF sediment cores and amending the cores with increasing quantities of oyster biodeposits and then analyzed the same as all other sediment cores. Biodeposits were collected for the addition experiments at each respective site <24 h prior to core sample collection and stored at 4°C until addition to reference sediment cores. Wet weight biodeposit samples (0.16, 0.64, and 5.00 g) were added to the water overlying reference cores and allowed to settle during the acclimation period.
**Statistical analyses**

All statistical tests were either paired (where OY and REF were compared) or crossed where discerning effects of seasons, sites, or treatments (OY, REF, NOY, FNC, and BA). When data conformed to the expectations of normality and homogeneity of variance, differences among treatment types, sites, and seasons were assessed using 1-way, 2-way, or 3-way ANOVA for total N\textsubscript{2} production, DNF, and AMX (SPSS v.18.0). Post hoc testing was conducted using Tukey’s HSD. Regression and correlation (Pearson product moment correlation) analyses were performed using SigmaPlot (v.11.0) and SPSS (v.18.0). In the one case where data failed to conform to assumptions of normality or constant variance, Mann-Whitney \(U\) non-parametric procedures were used to determine statistical significance (\(p \leq 0.05\)).

**RESULTS**

**Oyster biodeposition rates**

Oyster biodeposition rates at 2 floating raft oyster aquaculture sites followed a seasonal pattern and were significantly higher at St. Jerome Creek than at Spencer’s Creek (\(p < 0.04\)). Biodeposition at St. Jerome Creek, where oysters were larger, ranged from (mean ± SE) 3.8 ± 1.1 to 15.9 ± 1.1 g dry weight (dwt) m\textsuperscript{-2} d\textsuperscript{-1} (both extremes recorded in 2008) produced by trays containing 286 oysters m\textsuperscript{-2}, compared to Spencer’s Creek where aquacultured oyster biodeposition ranged from 1.6 ± 0.2 to 4.8 ± 1.0 g dwt m\textsuperscript{-2} d\textsuperscript{-1} during the same period (Table 1).

Paired tests (seasonal sampling across sites) revealed a seasonal effect for both TN and TC, but site was not a significant factor in determining the TC content of oyster biodeposits (\(p < 0.04\)). A site-season interaction was detected for TN, possibly masking site as a significant factor (\(p < 0.04\)). Biodeposited TN was significantly higher in June and October 2008 than in May 2009, but overall variation was small, ranging from 0.75 to 1.74\% TN across seasons (Table 2; \(p < 0.04\)). The majority of the biodeposit TN consisted of organically bound N, which ranged from 81 to 95\%. Biodeposit NH\textsubscript{4}\textsuperscript{+} (extractable) content ranged from 0.07 to 0.25\%, whereas NO\textsubscript{x}\textsuperscript{-} (extractable) was below the detection limit (<0.0001\%; data not shown). The flux of biodeposit OM to the sediments ranged from 0.36 to 1.21 g m\textsuperscript{-2} d\textsuperscript{-1} at Spencer’s Creek and 0.74 to 2.95 g m\textsuperscript{-2} d\textsuperscript{-1} at St. Jerome Creek, and ranged from 18.6 to 25.5\% OM at both sites across seasons (Table 2). Rates of TN delivery to sediments at both floating raft aquaculture sites ranged from a high of 14.1 ± 2.7 mmol N m\textsuperscript{-2} d\textsuperscript{-1} in June 2008 at St. Jerome Creek to a low of 1.7 ± 0.2 mmol N m\textsuperscript{-2} d\textsuperscript{-1} in October 2008 at Spencer’s Creek (Table 1). On average, one 76 mm oyster deposited 0.06 ± 0.01 mmol N d\textsuperscript{-1} during periods of highest filtration (June 2008, May 2009 at St. Jerome Creek) but as little as 0.01 ± 0.00 mmol N d\textsuperscript{-1} at both sites during October 2008. There was an effect of the floating raft structure based on the results of settled seston collections from control rafts which are a source of habitat heterogeneity as they become substrate for flora, fauna, and fouling organisms. These rates were relatively low, but may be considered as an essential component of the system. Settled seston deposition rates ranged from a low in October 2008 to a high in May 2009 at both sites: 0.34 to 3.29 g m\textsuperscript{-2} d\textsuperscript{-1} at Spencer’s Creek and 0.60 to 3.41 g m\textsuperscript{-2} d\textsuperscript{-1} at St. Jerome Creek.

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<th>Site</th>
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<th>mg oyster\textsuperscript{-1} d\textsuperscript{-1}</th>
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<td>Biodeposition rate (yr\textsuperscript{-1})\textsuperscript{a}</td>
<td></td>
<td></td>
<td>108.01</td>
<td>7.20</td>
<td>1543.50</td>
<td>9.00</td>
<td>1816.20</td>
</tr>
</tbody>
</table>

\(\textsuperscript{a}\)Estimate calculation is sum of mean seasonal flux × 90 d, winter excluded.
Effect of aquacultured oyster biodeposition on sediment N$_2$ production rates

Trial 1 (15N tracer)

Mean OY sediment N$_2$ production rates were ~1 mmol N m$^{-2}$ d$^{-1}$ lower than REF sediments at both study sites; 0.76 mmol N m$^{-2}$ d$^{-1}$ lower at St. Jerome’s Creek and 0.97 mmol N m$^{-2}$ d$^{-1}$ lower at Spencer’s Creek (Table 3). Mean OY and REF sediment N$_2$ production rates did not differ significantly in 4 out of the 5 paired analyses conducted at both sites on 3 separate events between 2008 and 2009 (Table S3 in the supplement). In the one case where a significant difference was detected between OY and REF treatments (June 2008), REF sediments had significantly higher rates than OY sediments at St. Jerome Creek ($df = 5$, $p < 0.04$, Fig. 3A). In separate analyses of seasonal samples within a site, only treatment type was a significant factor associated with DNF, AMX, and N$_2$ production rates when considering treatment, site, and season (3-way ANOVAs, $df$ ranged from 5 to 14, all $p < 0.05$), and no interactions between treatment and site, or among site, treatment, and season were detected. DNF accounted for the vast majority of N$_2$ production, 86.3 to 100%, compared to AMX, which contributed 0.0 to 13.8%; therefore, differences in N$_2$ production rates detected among sediment treatments were due predominantly to differences in DNF (Table 3).

<table>
<thead>
<tr>
<th>Site, date</th>
<th>TN</th>
<th>TON</th>
<th>NH$_4^+$</th>
<th>TC</th>
<th>OM</th>
<th>C:N</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spencer’s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 2008</td>
<td>1.74 ± 0.09</td>
<td>1.66 ± 0.09</td>
<td>0.08 ± 0.00</td>
<td>12.92 ± 1.20</td>
<td>25.51 ± 1.61</td>
<td>8.79 ± 1.08</td>
</tr>
<tr>
<td>October 2008</td>
<td>1.49 ± 0.00</td>
<td>1.37 ± 0.00</td>
<td>0.12 ± 0.00</td>
<td>9.92 ± 0.31</td>
<td>23.07 ± 0.00</td>
<td>7.76 ± 0.24</td>
</tr>
<tr>
<td><strong>St. Jerome</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 2008</td>
<td>1.26 ± 0.07</td>
<td>1.12 ± 0.04</td>
<td>0.14 ± 0.03</td>
<td>9.99 ± 0.72</td>
<td>18.61 ± 0.21</td>
<td>9.38 ± 1.01</td>
</tr>
<tr>
<td>October 2008</td>
<td>1.78 ± 0.05</td>
<td>1.63 ± 0.09</td>
<td>0.15 ± 0.04</td>
<td>8.47 ± 0.21</td>
<td>19.13 ± 0.05</td>
<td>5.57 ± 0.11</td>
</tr>
<tr>
<td>May 2009</td>
<td>0.75 ± 0.06</td>
<td>0.68 ± 0.05</td>
<td>0.07 ± 0.01</td>
<td>5.56 ± 0.16</td>
<td>19.60 ± 0.27</td>
<td>8.81 ± 0.57</td>
</tr>
<tr>
<td>August 2009</td>
<td>1.40 ± 0.08</td>
<td>1.14 ± 0.08</td>
<td>0.25 ± 0.04</td>
<td>8.43 ± 0.96</td>
<td>19.63 ± 0.81</td>
<td>7.16 ± 0.94</td>
</tr>
</tbody>
</table>

Table 2. Seasonal nutrient content of oyster biodeposits (mean ± SE) measured at 2 aquaculture sites in Chesapeake Bay. Total nitrogen (TN), total organic nitrogen (TON), ammonia (NH$_4^+$), total carbon (TC), organic matter (OM), and carbon to nitrogen ratio (C:N). Nitrogen oxides (data not shown) were below detection limit (<0.0001 mg l$^{-1}$). Sample sizes for each test during each sampling event were $n = 3$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date</th>
<th>DNF</th>
<th>AMX</th>
<th>Total N$2$</th>
<th>DNF</th>
<th>AMX</th>
<th>Total N$2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OY</strong></td>
<td>June 2008</td>
<td>0.93 ± 0.55</td>
<td>0.07 ± 0.02</td>
<td>0.99 ± 0.58</td>
<td>0.73 ± 0.37</td>
<td>0.07 ± 0.04</td>
<td>0.81 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>October 2008</td>
<td>1.31 ± 0.45</td>
<td>0.14 ± 0.02</td>
<td>1.46 ± 0.46</td>
<td>0.65 ± 0.31</td>
<td>0.04 ± 0.01</td>
<td>0.69 ± 0.32</td>
</tr>
<tr>
<td></td>
<td>May 2009</td>
<td>0.63 ± 0.27</td>
<td>0.10 ± 0.04</td>
<td>0.73 ± 0.31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>0.96 ± 0.24</td>
<td>0.10 ± 0.02</td>
<td>1.06 ± 0.25</td>
<td>0.69 ± 0.22</td>
<td>0.05 ± 0.02</td>
<td>0.75 ± 0.24</td>
</tr>
<tr>
<td><strong>REF</strong></td>
<td>June 2008</td>
<td>2.49 ± 0.25</td>
<td>0.15 ± 0.02</td>
<td>2.64 ± 0.26</td>
<td>1.58 ± 0.20</td>
<td>0.08 ± 0.01</td>
<td>1.66 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>October 2008</td>
<td>1.93 ± 0.18</td>
<td>0.10 ± 0.02</td>
<td>2.02 ± 0.20</td>
<td>1.69 ± 0.35</td>
<td>0.09 ± 0.03</td>
<td>1.78 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>May 2009</td>
<td>0.69 ± 0.10</td>
<td>0.11 ± 0.01</td>
<td>0.80 ± 0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td>1.70 ± 0.28</td>
<td>0.12 ± 0.01</td>
<td>1.82 ± 0.30</td>
<td>1.64 ± 0.18</td>
<td>0.08 ± 0.01</td>
<td>1.72 ± 0.19</td>
</tr>
<tr>
<td><strong>NOY</strong></td>
<td>June 2008</td>
<td>2.29 ± 0.25</td>
<td>0.11 ± 0.02</td>
<td>2.40 ± 0.25</td>
<td>1.61 ± 0.28</td>
<td>0.06 ± 0.02</td>
<td>1.67 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>June 2008</td>
<td>0.98 ± 0.19</td>
<td>0.04 ± 0.02</td>
<td>1.02 ± 0.21</td>
<td>1.21 ± 0.40</td>
<td>0.04 ± 0.01</td>
<td>1.26 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>June 2008</td>
<td>0.04 ± 0.12</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.12</td>
<td>0.26 ± 0.07</td>
<td>0.02 ± 0.01</td>
<td>0.28 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>October 2008</td>
<td>0.09 ± 0.04</td>
<td>0.01 ± 0.00</td>
<td>0.10 ± 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BIO</strong></td>
<td>June 2008</td>
<td>0.06 ± 0.07</td>
<td>0.00 ± 0.00</td>
<td>0.07 ± 0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Trial 1 denitrification (DNF), anammox (AMX), and total N$_2$ production rates (all in mmol N m$^{-2}$ d$^{-1}$; mean ± SE) measured using the 15N tracer method in sediments at 2 Chesapeake Bay sites with various oyster biodeposition treatments: directly underneath oyster aquaculture rafts (OY), no oyster aquaculture (REF), 5 to 10 m outside of the oyster aquaculture array (NOY), forced accumulation of oyster biodeposits in the field (FNC), and collected oyster biodeposits (BIO). Original rates measured in nmoles N g$^{-1}$ wet sediment h$^{-1}$ can be back-calculated from mmol N m$^{-2}$ d$^{-1}$ by multiplying by 0.926. For all treatments, $n = 3$. For treatments measured over several periods, the overall treatment mean ± SE is shown (bold).
With respect to paired seasonal N$_2$ production data (each test df = 5), no significant differences were detected between oyster aquaculture sites with respect to sample type; therefore, we did not find that site selection significantly impacted N$_2$ production rates (1-way ANOVA, p > 0.05). Sediment N$_2$ production rates of OY sediments at St. Jerome Creek and Spencer’s Creek were not significantly different from one another, whether combining seasons or considering seasons separately, and were 1.22 ± 0.32 and 0.75 ± 0.32 mmol N m$^{-2}$ d$^{-1}$, respectively. Significant differences in REF sediments between the 2 sites were detected only in June 2008, when N$_2$ production rates were 0.91 mmol N m$^{-2}$ d$^{-1}$ higher at St. Jerome Creek than Spencer’s Creek (1-way ANOVA, df = 5, p < 0.05, Table 3).

At St. Jerome Creek in June 2008, significant differences in sediment N$_2$ production rates among the 5 treatments were detected (1-way ANOVA, df = 14, p < 0.01). Post hoc testing revealed that REF and NOY sediment N$_2$ production rates were both significantly higher than OY sediments by 1.65 and 1.41 mmol N m$^{-2}$ d$^{-1}$, respectively, and did not differ significantly from one another (Tukey’s HSD, p < 0.04, Fig. 3A). FNC sediment N$_2$ production rates were 1.02 ± 0.21 mmol N m$^{-2}$ d$^{-1}$ and were not significantly different from OY, REF, or NOY treatments (Tukey’s HSD, p > 0.05, Fig. 3A). BIO samples had very low activity, 0.04 ± 0.12 mmol N m$^{-2}$ d$^{-1}$, and were significantly lower than REF and NOY sediments (Tukey’s HSD, p < 0.01, Fig. 3A). In October 2008 at St. Jerome Creek, OY and REF sediment N$_2$ production rates did not differ significantly from one another (1-way ANOVA, df = 5, p > 0.05). In May 2009 at St. Jerome Creek, no significant difference was detected between the total N$_2$ production rates of OY and REF sediments (1-way ANOVA, df = 5, p > 0.05, Fig. 3A).

For June and October 2008, Spencer’s Creek OY sediment N$_2$ production rates were ~1 mmol N m$^{-2}$ d$^{-1}$ lower than REF sediments (range 0.85–1.09 mmol N m$^{-2}$ d$^{-1}$); however, due to the large variance, these differences were not statistically significant (1-way ANOVA, df = 11, p > 0.05, Table 3). In June 2008, REF and NOY sediment N$_2$ production rates were ~0.85 mmol N m$^{-2}$ d$^{-1}$ higher than OY sediments, but not significantly different (1-way ANOVA, df = 14, p > 0.05, Fig. 3A). FNC sediments did not differ from any other treatments and had N$_2$ production rates 0.40 mmol N m$^{-2}$ d$^{-1}$ lower than REF sediments (Tukey’s HSD, p > 0.05). As expected, biodeposits exhibited little DNF and AMX
activity, 0.28 ± 0.07 mmol N m⁻² d⁻¹, and were significantly different from all treatments except FNC sediments (Tukey’s HSD, p < 0.04). In October 2008 at Spencer’s Creek, there was no significant difference between OY and REF sediment N₂ production rates, although REF rates were 1.09 mmol N m⁻² d⁻¹ higher than OY sediment rates (1-way ANOVA, df = 5, p > 0.05).

**Trial 2 (MIMS)**

In May 2009, comparative methods analyses of OY and REF sediments at St. Jerome Creek detected no significant difference between sediment N₂ production rates measured by the ¹⁵N tracer and MIMS methods, nor was an interaction between treatment type and method detected (Table S3, 2-way ANOVA, df = 11, p > 0.05). With regard to REF sediment rates, MIMS data were more highly variable than ¹⁵N tracer data, with MIMS ranging from 0.00 to 2.08 mmol N m⁻² d⁻¹ versus ¹⁵N tracer REF rates which ranged from 0.61 to 0.95 mmol N m⁻² d⁻¹, yet mean rates were not different, 0.71 ± 0.69 and 0.80 ± 0.10 mmol N m⁻² d⁻¹, respectively (1-way ANOVA, df = 5, p > 0.05, Fig. 3A). The ¹⁵N tracer method detected activity in OY sediments which had rates of 0.73 ± 0.31 mmol N m⁻² d⁻¹, compared to MIMS, which detected no N₂ production activity in OY sediments (1-way ANOVA, df = 5, p > 0.05, Fig. 3A).

Trial 2 OY sediment N₂ production increased by 1.56 mmol N m⁻² d⁻¹ in August 2009 at St. Jerome Creek, compared to 3 months earlier, May 2009, when no N₂ production activity was detected (Table 4). In the August 2009 trial, OY sediments exhibited significantly higher N₂ production rates than REF sediments at both sites, a trend also observed in the BA and FNC experiments. At St. Jerome Creek, mean OY sediment N₂ production rates were 1.14 mmol N m⁻² d⁻¹ higher than REF rates (Mann-Whitney U, df = 5, p = 0.05). In contrast to June 2008 Trial 1 data when OY sediments exhibited lower N₂ production, the significant differences in August 2009 paired summer tests were explained by a decline in August 2009 REF rates at St. Jerome Creek and Spencer’s Creek, whereas OY sediment rates remained relatively unchanged (Fig. 4). At St. Jerome Creek in August 2009, FNC sediments exhibited significantly increased rates that were 1.6 times higher than OY sediments and 6.0 times higher than REF sediments (Fig. 3A; df = 8, p < 0.03).

Addition of oyster biodeposits to REF sediment cores yielded no significant difference in N₂ production rates among the 3 levels of biodeposit addition (df = 8, p > 0.05); however, a seasonal difference was observed between Trial 2 May and August 2009 tests. In May 2009 at St. Jerome Creek, sediment N₂ production rates decreased with increasing additions

<table>
<thead>
<tr>
<th>Site</th>
<th>Treatment</th>
<th>Date</th>
<th>Total N₂ (mmol m⁻² d⁻¹)</th>
<th>OM (%)</th>
<th>SOD (mmol m⁻² d⁻¹)</th>
<th>NO₃ (mmol m⁻² d⁻¹)</th>
<th>NH₄⁺ (mmol m⁻² d⁻¹)</th>
<th>DON (mmol m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Jerome</td>
<td>OY</td>
<td>May 2009</td>
<td>0.00 ± 0.00</td>
<td>2.46 ± 0.06</td>
<td>8.72 ± 0.75</td>
<td>-0.04 ± 0.07</td>
<td>2.01 ± 0.60</td>
<td>0.09 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 2009</td>
<td>1.56 ± 0.37</td>
<td>2.00 ± 0.47</td>
<td>34.57 ± 1.40</td>
<td>0.12 ± 0.00</td>
<td>3.58 ± 1.31</td>
<td>0.54 ± 0.58</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.78 ± 0.39</td>
<td>2.23 ± 0.40</td>
<td>21.64 ± 5.82</td>
<td>0.09 ± 0.03</td>
<td>2.95 ± 0.84</td>
<td>0.29 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>REF</td>
<td>May 2009</td>
<td>0.71 ± 0.69</td>
<td>2.10 ± 0.36</td>
<td>7.16 ± 2.99</td>
<td>0.01 ± 0.01</td>
<td>1.04 ± 0.26</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 2009</td>
<td>0.42 ± 0.05</td>
<td>2.27 ± 0.38</td>
<td>20.97 ± 3.56</td>
<td>0.08 ± 0.05</td>
<td>2.78 ± 0.61</td>
<td>0.79 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.56 ± 0.31</td>
<td>2.19 ± 0.34</td>
<td>14.07 ± 3.72</td>
<td>0.05 ± 0.03</td>
<td>1.91 ± 0.49</td>
<td>0.21 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>FNC</td>
<td>August 2009</td>
<td>2.50 ± 0.11</td>
<td>7.48 ± 1.55</td>
<td>40.56 ± 0.73</td>
<td>0.13 ± 0.00</td>
<td>11.91 ± 0.30</td>
<td>1.81 ± 1.03</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>May 2009</td>
<td>0.39 ± 0.17</td>
<td>1.62 ± 0.82</td>
<td>9.50 ± 0.87</td>
<td>0.04 ± 0.03</td>
<td>1.21 ± 0.23</td>
<td>0.26 ± 0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August 2009</td>
<td>1.09 ± 0.66</td>
<td>2.30 ± 0.13</td>
<td>27.58 ± 5.76</td>
<td>0.00 ± 0.06</td>
<td>6.17 ± 3.06</td>
<td>1.81 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.65 ± 0.27</td>
<td>1.88 ± 0.72</td>
<td>16.28 ± 3.84</td>
<td>0.03 ± 0.03</td>
<td>3.07 ± 1.36</td>
<td>0.51 ± 0.43</td>
</tr>
<tr>
<td>Spencer’s</td>
<td>OY</td>
<td>August 2009</td>
<td>0.93 ± 0.34</td>
<td>11.05 ± 0.50</td>
<td>34.55 ± 3.90</td>
<td>0.17 ± 0.05</td>
<td>3.90 ± 1.18</td>
<td>0.11 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>REF</td>
<td>August 2009</td>
<td>0.08 ± 0.04</td>
<td>7.97 ± 2.27</td>
<td>7.99 ± 0.80</td>
<td>0.03 ± 0.02</td>
<td>1.07 ± 0.04</td>
<td>0.16 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>BA</td>
<td>August 2009</td>
<td>0.73 ± 0.49</td>
<td>7.69 ± 2.25</td>
<td>17.88 ± 4.96</td>
<td>0.03 ± 0.01</td>
<td>6.49 ± 4.15</td>
<td>1.72 ± 1.42</td>
</tr>
</tbody>
</table>
of biodeposits when the highest N₂ production rate was observed in the 0.16 g biodeposit addition (0.97 mmol N m⁻² d⁻¹), and the rate decreased with increasing amounts of biodeposits to 0.29 mmol N m⁻² d⁻¹ for the 5.0 g addition (Fig. 4A). Conversely, in the August 2009 experiments, the opposite trend was observed at both sites when rates of N₂ production increased from a low of 0.38 mmol N m⁻² d⁻¹ (0.16 g biodeposit addition) to 1.69 mmol N m⁻² d⁻¹ (5.0 g biodeposit addition; Fig. 4C).

Sediment N₂ production across trials: site and seasonal comparisons

We found no evidence that the method of measuring sediment N₂ production had a significant effect on the conclusion of OY and REF comparisons. Season and site were significant factors in determining N₂ production, although these differences were marginal (p < 0.03). Overall, for paired sediment N₂ production data across seasons measured by both methods, OY and REF sediments were not significantly different, although the mean (± SE) REF rate was slightly higher than OY sediments, 1.26 ± 0.20 and 0.89 ± 0.15 mmol N m⁻² d⁻¹, respectively (df = 47, p > 0.05).

The N₂ production rates measured in REF sediments were not significantly different between St. Jerome Creek and Spencer’s Creek, which were 1.32 ± 0.25 and 1.17 ± 0.33 mmol N m⁻² d⁻¹, respectively, indicating that the condition of the sediments did not play an important role in determining the impact of oyster aquaculture at the sites (df = 20, p > 0.05). For paired data across seasons and methods, no significant differences in OY sediment N₂ production rates were detected between St. Jerome Creek and Spencer’s Creek which were 0.95 ± 0.19 and 0.81 ± 0.25 mmol N m⁻² d⁻¹, respectively; therefore, we did not find that aquaculture site selection significantly impacted N₂ production rates (df = 20, p > 0.05).

Considering REF sediments only, a significant effect of season was detected (df = 20, p < 0.003) due to the August 2009 reduction in DNF. Considering all seasonal data for all treatment comparisons, OY sediments generally exhibited N₂ production rates lower or no different than the comparative REF sites, with the exception of the August 2009 tests when OY sediment N₂ production was 1.6 times higher due to the decline in N₂ production in the REF sediments (Fig. 3B). In August 2009, OY sediment N₂ production rates were ~1 mmol N m⁻² d⁻¹ higher at both sites (St. Jerome Creek, df = 5, p < 0.005 and Spencer’s Creek, df = 5, p = 0.05) than comparative REF sediments, which had very little activity at either site (Table 4).

Relationships among sediment N₂ production and biogeochemical parameters

Trial 2 nutrient and oxygen flux

SOD and NH₄⁺ flux from sediment cores were strongly correlated with N₂ production rates and tended to increase as N₂ production increased from
sediment cores (Pearson, df = 29, p < 0.000, R > 0.75), whereas NO\textsubscript{x} flux had a weak negative correlation with N\textsubscript{2} production rates, decreasing as N\textsubscript{2} production rates increased (Pearson, df = 29, p < 0.02, R = 0.452). Linear regression analysis revealed a significant relationship between N\textsubscript{2} production rates and both SOD and NH\textsubscript{4}\textsuperscript{+}, explaining 57 and 56% of variation in N\textsubscript{2} production rates, respectively (Fig. 5A, df = 29, p < 0.01). An interaction between SOD and NH\textsubscript{4}\textsuperscript{+} also was detected (df = 29, p < 0.05), as well as a strong positive correlation between SOD and NH\textsubscript{4}\textsuperscript{+}, which was exponential to a maximum (R\textsuperscript{2} = 0.67; Fig. 5B). The relationship between SOD and NH\textsubscript{4}\textsuperscript{+} initially followed a linear pattern and then plateaued at higher loads. Thereafter, regardless of input, SOD did not increase above ~40 mmol O\textsubscript{2} m\textsuperscript{-2} d\textsuperscript{-1}, whereas NH\textsubscript{4}\textsuperscript{+} continued to increase. Sediment treatments with the highest OM loads (BA and FNC) had SOD rates similar to those found at the OY sites, ~40 mmol O\textsubscript{2} m\textsuperscript{-2} d\textsuperscript{-1}, but these sediments continued to release NH\textsubscript{4}\textsuperscript{+}, more than doubling NH\textsubscript{4}\textsuperscript{+} efflux rates to ~12 to 15 mmol N m\textsuperscript{-2} d\textsuperscript{-1} (Fig. 5B).

The FNC treatment showed significantly higher SOD and NH\textsubscript{4}\textsuperscript{+} flux rates than all other sample types, emphasizing that oyster biodeposition elicits higher rates of OM decomposition and promotes recycling of N in the form of NH\textsubscript{4}\textsuperscript{+} (p < 0.01). The SOD rates for FNC sediments were 2 to 3 times higher than rates observed in sediments underneath oyster rafts (OY), in REF sediments, or BA sediments, consuming 40.56 mmol O\textsubscript{2} m\textsuperscript{-2} d\textsuperscript{-1}. Similarly OY sediment SOD rates were 1.5 times REF sediment rates (21.64 ± 5.82 versus 14.07 ± 3.72) and were significantly higher in August than in May 2009, 4 times in OY sediments and 3 times in REF sediments (p < 0.000, Table 4). We observed a stepwise increase in NH\textsubscript{4}\textsuperscript{+} flux rates in sediments treated with oyster biodeposits. Compared to REF sediments, NH\textsubscript{4}\textsuperscript{+} flux rates in OY sediments were 2 to 4 times higher, 2 to 6 times higher in BA sediments, and 2 times higher in FNC sediments, indicating that much of the N in oyster biodeposits is recycled and released back to the water column as dissolved inorganic N (DIN; Table 4). Sediments consumed NO\textsubscript{x} at the aquaculture sites, whereas NO\textsubscript{x} was either released or was consumed at a lower rate in REF sediments (p < 0.04). No difference between treatments was detected in TON sediment fluxes (p > 0.05).

Sediment pore water

Trial 1 and 2 sediment pore water NH\textsubscript{4}\textsuperscript{+} concentrations in OY and FNC sediments were 3 to 6 times higher than REF and NOY sediments, 24.73 ± 3.45 and 38.90 ± 6.10 mg l\textsuperscript{-1}, respectively (df = 41; p < 0.001; Fig. 6A), with OY, FNC, and NOY concentrations significantly higher at Spencer’s Creek than at St. Jerome Creek (df = 41, p < 0.03; Table S4 in the supplement). A weak negative correlation was detected between NO\textsubscript{x} flux and N\textsubscript{2} production (Pearson, df = 41, p < 0.02, R = 0.452), i.e. substrate uptake.

Fig. 5. Regression analyses of (A) sediment oxygen demand (SOD) and ammonia (NH\textsubscript{4}\textsuperscript{+}) efflux rates versus sediment N\textsubscript{2} production from membrane inlet mass spectrometry (MIMS) sediment core samples, and (B) SOD and NH\textsubscript{4}\textsuperscript{+} efflux from MIMS sediment cores for 3 oyster biodeposit treatments and reference sediments (sample designations are as described in Fig. 3). Solid line is the model. Dashed lines are 95% prediction bands.
was positively correlated with N₂ production. A significant negative correlation was detected between sediment pore water NH₄⁺ concentrations and N₂ production values for all sample types (R = −0.47, df = 41, p < 0.01). Non-linear regression analysis revealed that pore water NH₄⁺ concentrations increased in sediments as N₂ production rates decreased (Fig. 6B).

Oxygen demand, chl a, and extractable nutrients

Measures of BIO chl a, BOD, COD, NH₄⁺ (extractable), and organic N were not significantly different between the 2 aquaculture sites, and all BIO parameters, except COD, were significantly higher than the sediment treatment types (i.e. OY, REF, NOY, and FNC) at both sites (df = 76, p < 0.001). Overall, at Spencer’s Creek versus St. Jerome Creek, BOD was 1 to 4 times higher and COD was 4 to 7 times higher for OY, REF, and NOY sediment treatments (each test df = 23, p < 0.05). Sediment chl a measures at both sites were 3 times higher in St. Jerome Creek REF sediments than at Spencer’s Creek (df = 24, p < 0.05), and no significant differences were detected between the 2 sites for OY, NOY, or FNC treatments. In REF sediments, total extractable NH₄⁺ was not significantly different between the 2 sites, but was 4 to 9 times higher at Spencer’s Creek than St. Jerome Creek (each test df = 23, p < 0.05). No difference in sediment organic N was detected between the sites for OY or REF sediments, whereas NOY and FNC sediments were 3 to 11 times higher at Spencer’s Creek than at St. Jerome Creek (each test df = 23, p > 0.05). Comparing sediment treatments within each site, the impact of oyster aquaculture was detectable only at Spencer’s Creek (Table S4), where OY sediments had significantly higher chl a (Fig. S1 in the supplement), BOD, NH₄⁺, and TON than REF sediments (df = 41, all p < 0.05). No significant correlations were detected between sediment N₂ production rates and sediment chl a, BOD, COD, extractable NH₄⁺, or TON at either site (df = 31 St. Jerome Creek, df = 23 Spencer’s Creek, all p > 0.05). Even though OY sediment chl a (a proxy for benthic microalgal biomass as well as an indicator of pseudofeces production) was significantly higher than REF sediments at Spencer’s Creek during each sampling event (df = 17, p < 0.05), OY sediment N₂ production rates were not significantly different from St. Jerome Creek rates (df = 17, p < 0.05), where OY sediment chl a was not significantly higher than REF sediments (df = 23, p > 0.05).

Relationship of sediment N₂ production and oyster biodeposition

The rate of oyster biodeposition (g dwt m⁻² d⁻¹) explained 87% of variation in the rate of TN delivery to sediment via biodeposition (N influx, Fig. 7A). However, the rate of oyster biodeposition explained only 10% of variation in N₂ production (N efflux,
Fig. 7A), and no statistically significant relationship was detected between N₂ production and oyster biodeposition rates of OM (df = 23, p > 0.05). A slight but significant positive correlation (Pearson R = 0.459, df = 23, p < 0.02) between the rate of oyster biodeposition of TN to sediments (N influx) and sediment N₂ production (N efflux) was detected, and linear regression analysis showed that biodeposition of TN explained slightly more of the variation in sediment N removal than oyster biodeposition rates alone, 21 versus 10% (Fig. 7B, R² = 0.21).

DISCUSSION

Effect of oyster biodeposition on sediment N₂ production rates

Given the accelerated rate of TN delivery to sediments via oyster biodeposition, it is reasonable to expect that increasing TN inputs may stimulate sediment N₂ production, thereby enhancing the permanent removal of excess N from the system. A synthesis of data across a range of lake, coastal marine, estuarine, and continental shelf ecosystems indicates that sediment DNF increases linearly with TN inputs, explaining as much as 77% of variation in DNF (Seitzinger 1988, 2000, Seitzinger et al. 2006). However, at the relatively smaller scale of shallow oyster aquaculture sites in mid-Chesapeake Bay, we found that the mass fluxes of TN and organic N via oyster biodeposition exhibited weak positive correlations with N₂ production, explaining 21 and 23% of the variation, respectively. Our dual methods approach permitted constraining the rate estimates, which yielded the same conclusion regardless of methodology (¹⁵N tracer or MIMS), i.e. no stimulation or inhibition of rates of N₂ production due to oyster biodeposition at either study site. Therefore, we did not find evidence that mass load rates of either oyster biodeposits or oyster biodeposition of TN were effective predictors of N loss from the system via sediment N₂ production. Rather, the combined measures of SOD and sediment core NH₄⁺ explained more variation in sediment N₂ production, accounting for 67% of variation observed. At the lower range of biodeposit loading (Fig. 5B), the relationship between the 2 variables appears to be linear, but as sediments became anoxic, SOD reached a maximum with the higher OM loads experienced at the oyster aquaculture sites, indicating that oyster biodeposition enhanced reducing conditions in the sediments underneath oysters. This effect has been observed in sediments beneath cultivated mussels (Christensen et al. 2003, Nizzoli et al. 2006, Minjeaud et al. 2009).

At 2 oyster aquaculture sites representing different hydrological conditions and sediment characteristics under which oysters are typically cultivated in the mid- to lower Chesapeake Bay, we did not find evidence that the capacity of the sediment microbial community to remove N was significantly affected by delivery of oyster biodeposits to the system. Overall, we found that the annual N removal capacity of sediments at an oyster aquaculture site was no different than if no oyster cultivation activity were taking place. Thus, oyster biodeposit stimulation of sediment N₂

![Fig. 7. Regression analysis of oyster biodeposit total nitrogen (TN) to sediment N₂ production at 2 aquaculture sites in Chesapeake Bay. (A) Oyster biodeposit TN (influx) and sediment N₂ production rates (efflux) as compared to oyster biodeposit delivery rates to sediments, and (B) linear regression analysis of oyster biodeposit TN and sediment N₂ production](image-url)
production does not appear to be a ubiquitous process or one that occurs throughout periods of active suspension feeding. On only one occasion were OY sediment N$_2$ production rates found to differ from REF sediments (mid-summer at both sites, August 2009). This difference was moderate, yet significant, with OY sediment rates ~1 mmol N m$^{-2}$ d$^{-1}$ higher than REF sediments, due to an 88% decline in REF rates relative to early summer rather than an increase in N$_2$ production from OY sediments. REF sediment N$_2$ production rates were similar to other reported estuarine rates, which tend to cluster around 1 to 3 mmol N m$^{-2}$ d$^{-1}$ (Fennel et al. 2008), and somewhat higher than previously reported for other sites studied in Chesapeake Bay (Kemp et al. 1990, Kana et al. 1998, 2006). Our findings also are similar to other studies that have investigated the effect of bivalve cultivation on sediment DNF rates. Studies of the effects of aquacultured oysters, mussels, and clams in locations as disparate as the Mediterranean Sea and coastal New Zealand (Kaspar et al. 1985), but inhibited DNF in a more recent study (Tasman and Beatrix Bays, New Zealand; Christensen et al. 2003). Other studies of bivalve aquaculture (oysters, clams, and mussels) culture was previously found to stimulate DNF (Sundbäck & Miles 2000, Sundbäck et al. 2000, 2004), dark incubations are not likely to have resulted in underestimates of N$_2$ production rates. To better capture the uncertainty in rate estimates that arise from these and other factors, we compared reference sediments to oyster biodeposit treatments, examined sediment treatments at 2 study sites, and compensated for potential method bias by conducting cross-validation analysis comparing both MIMS and $^{15}$N tracer methods between which no statistically significant disparities were observed.

**Effect of experimentally concentrated oyster biodeposits**

Oyster biodeposits do not appear to be a significant source of denitrifying or anammox microorganisms to sediments, and we found no evidence that biodeposits alone drive significantly increased rates of N$_2$ production (and thus N removal) from the system by transferring microbes from the pelagic to the benthic zone. The August 2009 trend in the biodeposit-addition core experiments for both sites (Fig. 4B,C) was similar to the findings of Newell et al. (2002). However, the opposite trend was observed in spring at St. Jerome Creek, when N$_2$ production tended to decrease with increasing biodeposit additions to sediment cores (Fig. 4A). Furthermore, controlling for the effect of biodeposit transport away from the site and maximizing the concentration of TN in sediments, a similar trend was also observed in the August 2009 FNC experiments when biodeposits did not increase N$_2$ production above sediment REF or OY rates at either site in June 2008, although significantly higher rates above REF sediments were observed in August 2009 at St. Jerome Creek (Table 4).

**Quantification of N loss potential**

Suspension-feeding bivalves play an important role in coastal marine ecosystem N cycling (Cranford et al. 2007, Dame 2012); however, studies on the potential of bivalve aquaculture to combat eutrophication by stimulating sediment N$_2$ production have been inconsistent. Mussel *Perna canaliculus* aquaculture was previously found to stimulate DNF (Kenepuru and Marlborough Sounds, New Zealand; Kaspar et al. 1985), but inhibited DNF in a more recent study (Tasman and Beatrix Bays, New Zealand; Christensen et al. 2003). Other studies of bivalve aquaculture (oysters, clams, and mussels) demonstrated either no stimulation of DNF or inhibition (Gilbert et al. 1997, Minjeaud et al. 2009). It has been argued that where intensive bivalve aquaculture causes sediment anoxia, aquaculture has the potential to exacerbate eutrophication by stimulating...
release of nutrients sequestered in the sediments (Stadmark & Conley 2011). The current study adds to this body of knowledge by providing data on the effect of oyster aquaculture across 2 sites with differing hydrographic conditions in the mesohaline portion of Chesapeake Bay. Across these sites, we found that the impact of oysters on sediment N₂ production was consistent, variability was generally low across seasons, and there was neither a net increase nor significant inhibition of annual sediment N₂ production.

Modeled estimates of N removal via oyster-stimulated sediment N₂ production (coupled nitrification–denitrification) for Chesapeake Bay are 0.5 g N per g dwt oyster yr⁻¹ (Newell et al. 2005). For a commercial-scale farm producing 5 × 10⁵ oysters (covering 1750 m² sediment), this would equate to a potential removal rate of 250 kg N yr⁻¹. However, our data from 2 floating raft aquaculture sites indicate that ~37.8 kg N yr⁻¹ were transferred to sediments through oyster biodeposition and that N removal via sediment N₂ production ranged from 0.49 to 12.60 kg N yr⁻¹, compared to 2.27 to 16.72 kg N yr⁻¹ at a reference site of the same area. Comparatively, permanent N removal through the biomass harvest of 5 × 10⁵ oysters (76.2 mm) is 66 kg N (Higgins et al. 2011), or 26% of the modeled sediment removal estimate. Therefore, whereas sediment N₂ production varies by site and season, is generally no different than background N₂ production, and is impractical to verify, biomass harvest is a reliable nutrient removal mechanism that can be estimated with highly confident models (Higgins et al. 2011), and harvest of aquacultured biomass is a substantial net N removal mechanism available to balance nutrient inputs across many marine ecosystems (Petersen et al. 2012, Rose et al. 2012).

For the predicted oyster sediment N removal to occur, N₂ production rates would have to be 5 to 30 times those typically measured in estuarine sediments or would have to exhibit increased N₂ production rates over an area of sediment 5 to 20 times more extensive than we found to be impacted by oyster biodeposition. The difference between previously modeled estimates and our empirical results may be explained by our observational interpretation that there is a limited capacity of the study site sediments to remove N via N₂ production and that the area impacted by oyster biodeposition is restricted. Investigation of NOY sediments at both aquaculture sites revealed that the area impacted by oyster biodeposition is limited to within 5 to 10 m of an oyster aquaculture array, regardless of variable flow conditions between the 2 sites. Furthermore, we found no evidence to support the ability to predict N removal based on rates of oyster biodeposition because sediment N₂ production was only weakly correlated with biodeposition of TN (Fig. 5B). Experimentally concentrating biodeposits (in the field and in the lab) yielded at best a modest increase in N₂ production that did not exceed the maximum we observed under any other treatment. Based on our results viewed in the context of the data compilation and models produced by Fennel et al. (2008), we estimate that ~2.0 to 2.5 mmol N m⁻² d⁻¹ is likely the maximum capacity of these Chesapeake Bay sediments to remove N via sediment N₂ production regardless of increasing N inputs, a value that approximates the rates observed in August 2009 with the highest BA (5.0 g wet wt) and FNC experiments. N₂ production rates in August 2009 OY sediments were 0.85 to 1.14 mmol N m⁻² d⁻¹ above REF rates, a comparative increase in the warmest months when REF rates decreased at both sites (Table 4). Our findings parallel a study of natural oyster reef sediments in a North Carolina estuary that reported summer DNF rate increases of ~1 mmol N m⁻² d⁻¹ above spring and fall rates at the same location, and summer rates of an intertidal flat without oysters, indicating that oyster biodeposit-stimulated increase in N removal is likely a natural occurrence in the warmest summer months (Piehler & Smyth 2011). Our observed decrease in REF sediment N₂ production rates during mid-summer also concur with the results of Kemp et al. (1990), who found that DNF rates in mesohaline sediments of Chesapeake Bay were higher in spring and fall and decreased to minimal levels during the summer.

Combining our biodeposition and nutrient content data, we see that oysters deposited 1.7 to 14.1 mmol N m⁻² d⁻¹ to sediments at the aquaculture sites during periods of active suspension feeding, while N removal via sediment N₂ production ranged from 0.63 to 1.56 mmol N m⁻² d⁻¹ in sediments directly underneath the aquaculture rafts. In the absence of a comparison between OY and REF, one might erroneously conclude that N₂ production is 7 to 41% of the oyster biodeposit TN load; however, we did not find that any significant percentage of oyster biodeposit N is removed through N₂ production because N removal was not increased above REF rates in the OY sediments. Rather, given high concentrations of NH₄⁺ found in BIO and ~2-fold increases in NH₄⁺ detected in field and experimental tests of the effects of increasing levels of biodeposit treatments to sediments, our results indicate that biodeposits are rapidly remineralized and released back into the water column as DIN.
There is precedence for this interpretation. Previous studies of bivalve reefs and aquaculture sites have predicted that these systems may act as sinks for PON and sources for DIN, finding that nutrients are regenerated and ambient water NH₄⁺ levels increase in the vicinity (Dame et al. 1984, 1989, 1992, Kaspar et al. 1985, Dame & Dankers 1988, Prins & Smaal 1990, Hatcher et al. 1994, Dame 2012). We found that SOD initially increased linearly with sediment NH₄⁺ efflux rates, but by concentrating OM loading in the fence and biodeposit addition experiments, we observed that SOD ultimately reached a maximum at ~30 to 40 mmol O₂ m⁻² d⁻¹ while NH₄⁺ efflux rates continued to increase (Fig. 5B). OY and FNC sediment NH₄⁺ levels increased 1 to 3 times and 3 to 6 times above REF and NOY sediments, respectively, at the high OM site (Spencer’s Creek), indicating that biodeposits were remineralized in the sediments. Evidence of oyster biodeposit ammonification also was observed in sediment pore water NH₄⁺ concentrations, which were 3 to 6 times higher than REF and NOY sediments, whereas pore water NO₃⁻ concentrations did not differ significantly among biodeposit-treated sediments and did not fluctuate seasonally (Fig. 6).

These observations indicate that DNRA may play an important yet currently undetermined role in recycling biodeposited N as it is understood to be favored over DNF in NO₃⁻ limited but labile-C rich sediments (Tiedje 1988, Tobias et al. 2001, Burgin & Hamilton 2007). Yet to be examined is whether aquacultured oyster biodeposition enhances DNRA due to high organic loading and increases in sulfate reduction as observed by Christensen et al. (2000) under trout Oncorhynchus mykiss aquaculture cages. It also is possible that DNF was incomplete in OY sediments, and that gaseous N was removed in the form of NO or N₂O (not measured in this study). Whatever the mechanism involved, this study illustrates that oyster biodeposition transfers large quantities of nutrients and energy from the water column to the benthos and that the lack of N₂ emission indicates that aquacultured oyster biodeposition serves as a nutrient regeneration rather than removal mechanism, promoting release of DIN that in turn has the potential to further promote phytoplankton growth (Asmus & Asmus 1991, Dame & Libbes 1993, Dame 2012).

CONCLUSIONS

For oyster aquaculture facilitation of enhanced N₂ production to be considered an effective policy initiative for eutrophication mitigation in Chesapeake Bay, this effect would have to be documented consistently and broadly across variable site conditions and seasons. Overall, at 2 mid-Chesapeake Bay mesohaline aquaculture sites, we estimated annual N removal rates via sediment N₂ production at an oyster aquaculture site to be the same as or less than a comparable reference site of the same area. On the sole occasion when increased OY sediment N₂ production was observed at both sites (August 2009), this increase was modest (0.85 to 1.14 mmol N m⁻² d⁻¹ above REF rates) and did not translate into overall enhancement of N removal because the rates observed in OY sediments during other seasons were either lower than or not different from REF sediments. The N removal potential via sediment N₂ production is presumably limited by both sediment microbial capacity and area, making the issue of enhancing a sediment N sink in the Bay a matter of scale relative to system-wide inputs. Intensive oyster biodeposition increased sediment NH₄⁺ remineralization accompanied by a significant decrease in N₂ production, indicating that oyster-biodeposited N is rapidly recycled and returned to the water column as DIN rather than permanently removed from the system. The implication of our findings is that despite increased N delivery to sediments as a result of cultivated oyster biodeposition, no additional N₂ is removed through sediment biogeochemical processes at aquaculture sites with similar environmental conditions. Ultimately, sediment N₂ production is costly and difficult to measure, and to be applicable as a practical policy initiative for Chesapeake Bay eutrophication mitigation, oyster cultivation would likely need to elicit a ubiquitously enhancing effect on N removal, an effect not observed in this study.

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