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Variation in benthic metabolism and nitrogen cycling across clam aquaculture sites

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ABSTRACT

As bivalve aquaculture expands globally, an understanding of how it alters nitrogen is important to minimize impacts. This study investigated nitrogen cycling associated with clam aquaculture in the Sacca di Goro, Italy (*Ruditapes philipinarum*) and the Eastern Shore, USA (*Mercenaria mercenaria*). Ammonium and dissolved oxygen fluxes were positively correlated with clam biomass; *R. philippinarum* consumed ~6 times more oxygen and excreted ~5 times more NH_4^+ than *M. mercenaria*. There was no direct effect of clams on denitrification or dissimilatory nitrate reduction to ammonium (DNRA); rather, nitrate availability controlled the competition between these microbial pathways. Highest denitrification rates were measured at sites where both water column nitrate and nitrification were elevated due to high densities of a burrowing amphipod (*Corophium* sp.). DNRA exceeded denitrification where water column nitrate was low and nitrification was suppressed in highly reduced sediment, potentially due to low hydrologic flow and high clam densities.

1. Introduction

The presence of bivalve aquaculture in coastal ecosystems has large implications for coastal nitrogen (N) dynamics. As nutrient pollution continues to be problematic in coastal waters worldwide concurrent with the rapid expansion of the bivalve industry (FAO, 2014), the influence of bivalve aquaculture on N removal pathways is of increasing interest. Implementing bivalve aquaculture as a means to promote N removal and mitigate coastal eutrophication is a current topic of debate (e.g. Stadmark and Conley, 2011; Rose et al., 2012). Effective resource management requires an understanding of the net effect of bivalve cultivation on N cycling, both recycling and removal pathways, and particularly how this changes with different environmental conditions. This study investigates the mechanistic drivers that influence the effects of clam cultivation on benthic N cycling pathways by sampling two clam species that are farmed across a range of environmental conditions.

As infaunal organisms, cultivated clams both directly and indirectly affect sediment N cycling rates and benthic metabolism through bioturbation, biodeposition, excretion, and respiration, which subsequently influence microbial metabolic pathways (reviewed in Laverock et al., 2011). Clam bioturbation transports particles and water, including solutes (e.g. O_2, NO_3^-), through sediments. Through feeding and biodeposition, clams actively deliver organic carbon to the sediments from the water column, fueling microbial decomposition pathways, enhancing microbial respiration and oxygen demand, and thereby substantially changing redox gradients (Aller, 1982; Kristensen et al., 1985) and impacting redox sensitive microbial processes such as nitrification and denitrification (Stief, 2013). Benthic infauna, including cultivated clams, also excrete dissolved inorganic and organic N, directly increasing benthic N fluxes to the water column and potentially providing substrate (e.g. NH_4^+) for microbial processes such as nitrification and ANAMMOX (Welsh et al., 2015). Bivalves can thus influence both microbial N removal and recycling pathways in coastal sediments.

Bivalves may enhance N removal by promoting denitrification, the microbially mediated pathway that reduces nitrate (NO_3^-) to inert N_2 gas. This bivalve-facilitated, denitrification enhancement results both from biodeposition of organic matter to sediment microbial communities (Newell et al., 2002; Kellogg et al., 2013; Smyth et al., 2013) and

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by provision of habitats for denitrifying microorganisms (Heisterkamp et al., 2010; Welsh et al., 2015). However, some studies have reported no significant effect of bivalves on denitrification rates (Higgins et al., 2013; Erler et al., 2017). Additionally, often overlooked is the effect bivalves have on inorganic N regeneration pathways. High densities of bivalves, found in cultivation settings, may accelerate N recycling processes through bivalve excretion and stimulation of microbial ammonification including dissimilatory nitrate reduction to ammonium (NH_4^+) (DNRA) (Dame, 2011; Murphy et al., 2016; Erler et al., 2017), which retain bioavailable N in the aquatic ecosystem.

The question of whether bivalves promote N removal or retention remains equivocal. The discrepancy among previous studies on how bivalves influence benthic N cycling pathways is in part due to differences in the bivalve species studied (e.g. epifaunal oysters or mussels versus infaunal clams), but also likely due differences in the environmental conditions under which bivalves are farmed. Bivalve aquaculture can occupy expansive regions across estuarine environmental gradients. Few studies that investigate N cycling associated with bivalve aquaculture, and specifically clam aquaculture, have captured the natural environmental variability across which the practice exists. Moreover, few studies have investigated the partitioning of NO_3^- reduction between denitrification and DNRA, which is ecologically important as DNRA retains bioavailable N in the system as NH_4^+ whereas denitrification removes it. Those studies that do provide simulatenouse measurements of denitrification and DNRA are restricted to single study systems. Therefore, we were interested in directly comparing different study systems, which are heavily exploited for infaunal clam cultivation and where previous studies found contrasting results regarding denitrification and DNRA at clam cultivation sites. We chose to sample clam aquaculture in the Sacca di Goro, Italy, where denitrification was reportedly higher than DNRA (Nizzoli et al., 2006) and in coastal Virginia, US, where DNRA generally dominated NO_3^- respiration (Murphy et al., 2016).

The objective of this study was to investigate how sediment N cycling associated with clam aquaculture varies across different environmental conditions and between two commonly cultivated infaunal clam species: *Ruditapes philipinarum* (Italy) and *Mercenaria mercenaria* (US). Across the natural environmental gradients in which clam aquaculture exists, we were specifically interested in (1) comparing N cycling and metabolic rates between the two cultivated clam species and determining the direct contribution of these clams to benthic rates and (2) determining the factors that influence the competition between microbial denitrification and DNRA at clam aquaculture sites. By studying two clam species exposed to different environmental conditions and farming practices, we sought to highlight the challenge in generalizing ecological responses across all bivalve aquaculture and, more specifically, across all clam cultivation practices. We hypothesized that both clam species would significantly increase benthic oxygen demand and inorganic N fluxes; however, the contribution of clams to these benthic processes would differ across sites depending on site-specific factors and clam species physiology. We expected that the degree to which N is removed through denitrification relative to N recycled through DNRA would change depending on the availability of labile organic carbon and NO_3^- (Tiedje, 1988), factors that would vary broadly across estuarine gradients and clam aquaculture sites.

2. Methods

2.1. Study sites

The Sacca di Goro is a lagoonal system of the Po River Delta, Italy. Approximately 26 km^2 with an average depth of 1.5 m, the lagoon hosts a substantial clam aquaculture industry, with about 1/3 of the area occupied by clam cultivation. The system is generally divided into three areas based on hydrologic characteristics: the eastern portion is shallow with low energy and slow water exchange; the central region is tidally

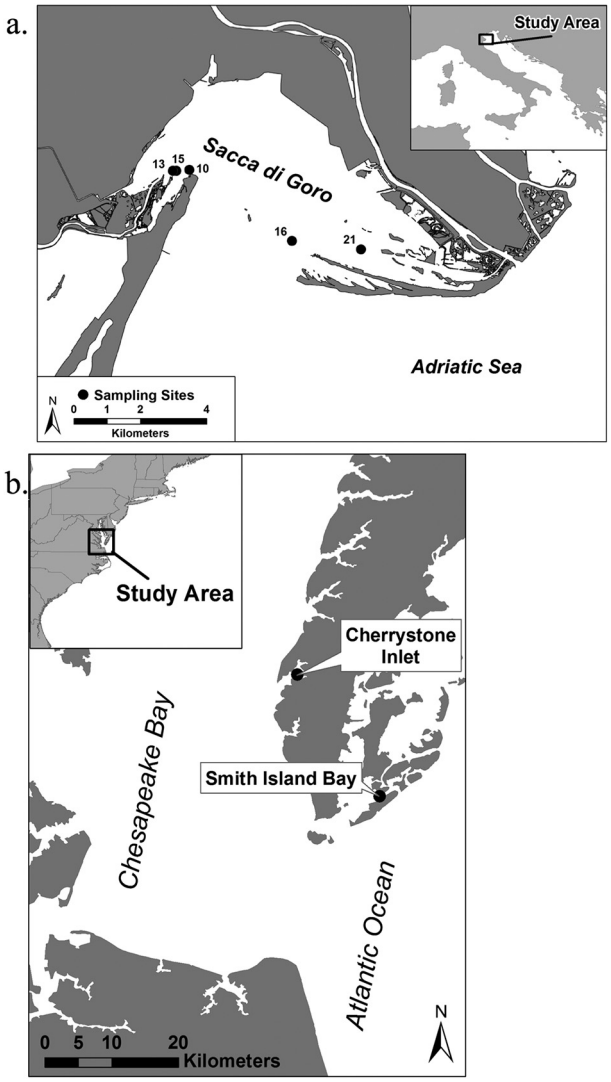

Fig. 1. Study sites in the Sacca di Goro, Italy (a) and the Eastern Shore, VA, USA (b).

influenced, and the western portion is riverine dominated with freshwater flow from the Po di Volano (Fig. 1A). The lagoon, particularly the eastern region, typically experiences periodic dystrophic events in the early summer when macroalgae bloom. Drastic changes to the hydrodynamics of the system were made over the past 20 years to improve water flow and alleviate dystrophic events, including channel construction along the southern sand spit and dredging of internal canals to increase flow to the Adriatic Sea (Viaroli et al., 2006). The manila clam, *R. philippinarum*, is farmed in privately leased portions of the lagoon at densities ranging from 100 to > 2000 individuals m^{-2}. Growers collect juvenile clams at the mouth and directly outside the lagoon, transport them to individual leases within the lagoon; after approximately 9 months market-sized clams are hydraulically harvested.

Clam aquaculture occupies large subtidal areas on both the Chesapeake Bay-side and Atlantic-side of the Eastern Shore peninsula of Virginia (Emery, 2015). Cherrystone Inlet (ES-23), located on the Chesapeake Bay-side of the Eastern Shore, is a small shallow embayment (~6 km^2, mean water depth of 1.1 m) that receives little freshwater discharge. Smith Island Bay (ES-33) is the southern-most lagoon, located on the eastern side of the Eastern Shore and is protected from the Atlantic Ocean by a barrier island (Fig. 1B). In both locations, the hard clam, *M. mercenaria*, is cultivated in privately owned leases in subtidal regions. Clams are sourced from land-based hatcheries and nurseries and planted in the sediments at ~8–15 mm in shell length.

Unlike in Italy, growers set plastic mesh nets over the clam beds flush to the sediment surface as protection from natural predation. Macroalgal blooms, supported by nutrients excreted by clams and from microbial mineralization of organic matter in the sediment, occur on the predator-exclusion nets (Murphy et al., 2015). Periodically growers sweep the nets of macroalgae to prevent the algae from suffocating the clams. After about two years the market-sized clams are hydraulically harvested.

2.2. Site characterization

Surface water column and sediment samples were collected once in summer 2013 at five sites in the Sacca di Goro, Italy (Fig. 1A) and two sites on the Eastern Shore, VA USA (Fig. 1B). Triplicate water column grab samples were collected at each site at ~50 cm above the sediment, filtered (0.45 μm) and stored frozen in either whirlpak bags or Falcon tubes until analyzed for NH_4^+ and nitrate plus nitrite (NO_x^-). Triplicate sediment cores (polycarbonate core tubes, 30 cm height and 4 cm i.d.) were also collected at each site for determination of sediment organic matter by loss on ignition (450 °C over 3 h) in the 0–2 cm sediment horizon. Temperature and salinity were measured at each site using a thermometer and refractometer, respectively. Although, both the Sacca di Goro and the Eastern Shore experience seasonal variation in temperature, salinity, and nutrient concentrations (Murphy et al., 2016; Nizzoli et al., 2006), capturing this temporal variability was beyond the scope of this study. We focused on the natural spatial variability of environmental parameters across the study sites during the summer season, when biogeochemical rates are typically high. Throughout the study, site identification refers to the location and measured salinity, for example, Goro-13 was collected in the Sacca di Goro and the salinity was 13.

2.3. Benthic metabolism and nutrient flux measurements – 'intact cores'

Twelve sediment cores (10 cm sediment depth) were collected (Eastern Shore (ES) sites, 9.5 cm i.d.; Goro sites, 8 cm i.d.) at all sites, except ES-23 where 10 cores were collected, for the determination of benthic metabolism, nutrient fluxes, denitrification and DNRA. From each site, half the cores were incubated in the light and half in the dark. Cores were randomly collected at each site; clam densities varied between sediment cores and some sediment cores contained no clams by chance.

Sediment cores collected in the Sacca di Goro were transported to the University of Parma while cores from the Eastern Shore were transported to Virginia Institute of Marine Science, Eastern Shore Laboratory (VIMS ESL) in Wachapreague VA. Cores were placed in water baths at site-specific salinity and temperature and allowed to equilibrate overnight. Oxic conditions in water baths were assured by bubbling with airstones. The water inside the cores was gently stirred avoiding sediment resuspension during the equilibration and incubation periods with magnetic stirrers driven by a large magnet rotated by an external motor at 40 rpm. The following day, half the cores were illuminated while the other half remained dark. The water inside the tanks was replaced with new water prior to initiating the incubation. To initiate incubations, the water level in the tank was lowered to below the core tops and all cores were sealed with clear lids. Short term batch incubations were conducted over 4–5 h, ensuring cores never became hypoxic or anoxic. At each time point, DO was measured and samples of overlying water were collected for determinations of NH_4^+ and NO_x^-. Water column nutrient samples were immediately filtered (0.45 μm) and stored frozen until analysis. For the Sacca di Goro incubations, a polargraphic microsensor (50 μm; Unisense, DK) connected to an amperometer (PA2000, Unisense, DK) was used to measure DO concentrations in water samples collected during the incubation, stored in 12 ml exetainers (Labco Inc.) and preserved with $ZnCl_2$ (100 μl of 7 M solution). For the Eastern Shore sites, DO was measured using Hach LDO101 Luminescent dissolved oxygen (DO) sensors (Hach Co., Loveland, CO, USA) secured in the lids of the cores. Hourly fluxes for each analyte (mmol O_2 m^{-2} h^{-1} or μmol N m^{-2} h^{-1}) were calculated as the change in concentration over time multiplied by the core water volume and divided by the core surface area. Fluxes from the sediment to the water column are represented by positive values (production), while fluxes to the sediment from the water column are negative (consumption).

2.4. Denitrification and DNRA rate measurements – 'intact cores'

After the initial flux incubations for NH_4^+, NO_x^-, and DO, all cores were uncapped and the overlying water was replaced. Cores were allowed to equilibrate in the water bath for at least 1 h; the light cores remained illuminated and the dark cores remained dark. The isotope pairing technique (IPT) was used to measure denitrification (Nielsen, 1992) and DNRA (Risgaard-Petersen and Rysgaard, 1995). The water bath level was dropped to just below the core tops; $^{15}NO_3^-$ (98.9 atom %, targeting a final concentration of ~100 μM) was added to the overlying water of each core. A water sample was collected from each core immediately before and after $^{15}NO_3^-$ addition to determine the ^{15}N-enrichment of the NO_3^- pool. Then the cores were capped and sealed. Incubations typically lasted 3–4 h, depending on the specific sediment oxygen demand determined in the previous incubation (see above), allowing DO to change by no > 30% of the initial concentration (Dalsgaard et al., 2000). After the incubation, each core was gently homogenized and slurries were sampled for $^{29}N_2$, $^{30}N_2$, and extractable $^{15}NH_4^+$.

Dissolved $^{29}N_2$ and $^{30}N_2$ gas samples were collected by siphoning the homogenized core slurry into 12 ml exetainer vials (Labco, Inc) without headspace and preserving them with 100 μl of $ZnCl_2$ (7 M). The abundances of $^{29}N_2$ and $^{30}N_2$ in the dissolved N_2 pool were determined within a month on a membrane inlet mass spectrometer (MIMS, detection limits for $^{29}N_2$ and $^{30}N_2$ are 0.011 and 0.0004 μM, respectively) (Kana et al., 1994) using a PrismaPlus mass spectrometer with an inline furnace operated at 600 °C to allow for O_2 removal (limits of detection for $^{29}N_2$ and $^{30}N_2$ are 10 nM and 0.4 nM, respectively). Denitrification rates were calculated based on the production of $^{29}N_2$ (p29) and $^{30}N_2$ (p30), assuming a binomial distribution of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ (Nielsen, 1992) as follows:

$$D_{15} = p29 + 2p30 \quad (1)$$

$$D_{14} = D_{15} \times (p29/2p30) \quad (2)$$

where D_{15} represents denitrification of the added $^{15}NO_3^-$ and D_{14} is the ambient denitrification rate of $^{14}NO_3^-$. Direct denitrification of NO_3^- from the water column (D_w) and coupled denitrification (D_n) were calculated as described by Nielsen (1992):

$$D_w = (^{14}NO_3^-/^{15}NO_3^-) * D_{15} \quad (3)$$

$$D_n = D_{14} - D_w \quad (4)$$

where $^{14}NO_3^-$ is equal to the ambient unlabeled NO_3^- concentration (μM) and $^{15}NO_3^-$ is equal to the isotopically-labeled NO_3^- concentration at the start of the incubation. Previous manipulation experiments in which denitrification rates were measured with various concentrations of added $^{15}NO_3^-$, demonstrated that at all sites ANAMMOX contributed a negligible amount of N_2 relative to denitrification (Murphy, unpublished). Thus, the assumptions upon which the isotope pairing technique is based were met and the equations are valid for these systems (Nielsen, 1992).

The homogenized cores were also sampled for extractable $^{15}NH_4^+$ to calculate ambient DNRA rates from the production of $^{15}NH_4$. Potassium chloride (KCl) was added to approximately 200 ml of sediment slurry for a final concentration of 2 M. Samples were shaken for 1 h, filtered (0.45 μm Whatman PES), and frozen until they were diffused and trapped for analyses of $^{15}NH_4^+$ enrichment and

concentration using a method modified from Brooks et al. (1989). Water samples were placed in specimen cups; an acidified (25 µl of 2.5 M sulfuric acid) GFF filter (1 cm, i.d.), threaded onto a stainless steel wire, was suspended on the lip of the cup; magnesium oxide was added and the samples were allowed to diffuse for 2 weeks, after which samples were placed in tin capsules and analyzed on an isotope ratio mass spectrometer (IRMS) at the University of California Davis Stable Isotope Facility for $^{15}NH_4^+$.

DNRA rates of the ambient $^{14}NO_3^-$ ($DNRA_t$) were calculated according to Risgaard-Petersen and Rysgaard (1995) as:

$$DNRA_t = p^{15}NH_4^+ \times (D_{14}/D_{15}) \quad (5)$$

where $p^{15}NH_4^+$ is equal to the production of $^{15}NH_4^+$. This assumes that DNRA occurs in the same sediment horizon as denitrification, resulting in the same proportional use of $^{14}NO_3^-$ and $^{15}NO_3^-$ as denitrification (Rysgaard et al., 1993). Direct DNRA of NO_3^- from the water column ($DNRA_w$) and coupled DNRA ($DNRA_n$) were calculated as:

$$DNRA_w = (^{14}NO_3^-/^{15}NO_3^-) * p^{15}NH_4^+ \quad (6)$$

$$DNRA_n = DNRA_t - DNRA_w \quad (7)$$

Nitrification rates were estimated as the sum of denitrification, DNRA, and NO_x^- effluxes.

2.5. Clam respiration and excretion rate measurements – 'clam-only incubations'

After the 'intact sediment core' incubations, all sediment cores were sieved and the clams from each core were collected and rinsed to remove any sediment; these clams were placed back into the same polycarbonate tubes they were sieved from for a 'clam-only' (i.e. no sediment) incubation. Therefore, the number of clams in each tube varied across samples and reflected the ambient clam density at each study site. 'Clam-only' static flux incubations were then conducted as described for the 'intact sediment core' incubations. Chambers with the clams were placed back in the water bath, filled with unfiltered water, allowed to equilibrate for at least an hour, and capped for 2–3 h. Over the incubation, samples were collected for DO, NH_4^+ and NO_3^-. As described above, hourly fluxes for each analyte (mmol O_2 m^{-2} h^{-1} or µmol N m^{-2} h^{-1}) were calculated as the change in concentration over time multiplied by the core water volume and divided by the core surface area. All these incubations were conducted under dark conditions. After the incubations, all clams were measured (shell length) and tissue dry weight (DW) and ash-free DW (loss on ignition) were obtained.

2.6. Infauna sampling

After initial observations during field sampling, it was determined that a burrowing amphipod, *Corophium* sp., was present at Goro-10, Goro-13, and Goro-15. As these organisms likely strongly influence N cycling rates (Stief, 2013), we collected, counted, and determined biomass (g DW m^{-2}) of the amphipods. As this decision was made after sampling Goro-10 and Goro-16, amphipod data were not collected at these sites, although it was clear that amphipods were also abundant at Goro-10. Amphipods were not abundant at the Eastern Shore sites and were not quantified (pers. obs.).

2.7. Gross microbial ammonification rates

Additional core samples were collected at each site for gross ammonification rate measurements using the isotope pool dilution technique (Anderson et al., 1997). Cores (5.7 cm i.d, with approximately 5 cm overlying water and 5 cm sediment depth) were collected in pairs at each sampling site, carefully avoiding inclusion of clams, however other infauna were retained. It is important to note that this method cannot decipher between microbial and infaunal NH_4^+ production; it is not possible to remove infaunal organisms without disturbing the natural sediment gradients important to microbial metabolic pathways. Cores were transported to the laboratory, placed in site water, and held overnight uncapped with gentle mixing and aeration. The following day the sediments were uniformly spiked with $^{15}N\text{-}NH_4^+$ (3.6 ml of $[NH_4]_2SO_4$, 30 at.%, 10 mM). One paired core, T_0, was immediately sacrificed after spiking by shaking in 2 M KCl for an hour; the extractant was filtered and frozen until analysis. The T_f cores were capped and incubated for 24 h in the dark at in situ temperatures, after which the cores were processed the same as the T_0 cores above. NH_4^+ was processed and analyzed using the diffusion method modified by Brooks et al. (1989), as described above. Rates of gross ammonification were calculated using a model described by Wessel and Tietema, 1992 as

$$\text{Ammonification} = \frac{\ln(Tf_{atm\%} - k)/(T0_{atm\%} - k)}{\ln[NH_4^+T_f]/[NH_4^+T_0]} * \frac{[NH_4^+T_0] - [NH_4^+T_f]}{\text{time}} \quad (8)$$

where $Tf_{atm\%}$ and $T0_{atm\%}$ refer to the $^{15}NH_4^+$ enrichment of the T_f and T_0 cores; k is equal to natural abundance of $^{15}NH_4^+$ expressed as atom %; $[NH_4^+T_f]$ and $[NH_4^+T_0]$ are the concentrations of NH_4^+ in the T_f and T_0 cores, respectively, and time is the incubation time.

2.8. Denitrification efficiency calculation

Denitrification efficiency, the percent of organic N that is mineralized via denitrification, was calculated as:

$$\text{Denitrification Efficiency (\%)} = \frac{D_{14}}{NO_X^- + NH_4^+ + D_{14}} \times 100 \quad (9)$$

where D_{14} is denitrification and NO_x^- and NH_4^+ represent the positive fluxes of these nutrients (effluxes).

2.9. Statistical analyses

Data from the 'clam-only' incubations were analyzed using analysis of covariance (ANCOVA) to test the effect of and interaction between clam biomass and species on rate measurements (NH_4^+, NO_x^-, and DO fluxes). Clam physiological rates (respiration and excretion), were calculated using the slope estimates of the linear models within each species (mmol O_2 g DW^{-1} h^{-1} or µmol NH_4^+ g DW^{-1} h^{-1}). To determine the clam contribution to total benthic fluxes, clam physiological rates were scaled to per m^2 by multiplying by the clam biomass present within each core and dividing by the surface area of the core and compared to the 'intact core'.

A two-way analysis of variance (ANOVA) was used to examine the interactive effects of light condition and site, which refers to all 7 study sites, on 'intact sediment' nutrient fluxes, DO fluxes, denitrification, and DNRA. The Tukey HSD post hoc analysis was used to compare means when an effect was significant. For further analysis, all fluxes, regardless of whether they were made in the light or dark were included and the effect of light was ignored because (1) the ANOVAs revealed light condition had minimal effects on the response variables and (2) the effect of light on benthic biogeochemical rates was not a priority of our study, however we included paired light and dark cores to capture the variability associated with light in our measurements.

Linear models were used to assess the relationship between clam biomass and 'intact core' rate measurements (nutrient and DO fluxes, denitrification, and DNRA) within each site individually. Across all sites, the overall effects of clam biomass and species on 'intact sediment' nutrient fluxes, DO fluxes, denitrification, and DNRA, were assessed using mixed effects models, which accounted for the variance due to site. The mixed effects models (*lme* function from the 'nlme' package (Pinheiro et al., 2017)) were constructed with clam biomass and species as fixed effects while site was included as a random effect. Both the intercept and slope were allowed to vary by site to account for intrinsic

Table 1
Environmental characteristics at each site. Mean values and (standard error).

Site	Salinity	Temp. (°C)	NO_x^- (μM)	NH_4^+ (μM)	Sediment Organic Matter (0–2 cm)
Goro-10	10	20	53.98 (3.43)	19.11 (1.45)	1.36 (0.06)
Goro-13	13	21	33.96 (1.13)	8.50 (0.41)	1.74 (0.05)
Goro-15	15	21	40.04 (0.66)	9.51 (0.36)	2.38 (0.35)
Goro-16	16	20	34.84 (0.59)	38.4 (2.32)	0.92 (0.08)
Goro-21	21	20	1.07 (0.03)	18.43 (1.06)	1.62 (0.09)
ES-23	23	25	0.20 (0.02)	2.10 (0.55)	1.21 (0.11)
ES-33	33	27	0.25 (0.03)	0.88 (0.27)	1.50 (0.15)

site differences that may affect baseline benthic rates as well as differences in clam behavior or metabolisms across the sites.

Linear models were used to examine the effect of *Corophium* abundances on rates of denitrification, DNRA, and estimated nitrification across the three sites in which *Corophium* were quantified. Finally, the ratio of DNRA to denitrification (DNRA:DNF) as a function of labile organic carbon (ammonification rates were considered a proxy) relative to NO_3^- availability (ammonification rate: water column NO_3^-) was explored with a linear model.

Data were checked for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests and transformed using Box-Cox to meet assumptions. All statistical analyses were considered significant at the $p < 0.05$ level and were conducted in R Studio, version 3.4.1.

3. Results

3.1. Environmental characteristics

Salinity ranged from 10 to 33, while temperature was relatively consistent with lower temperatures at the Sacca di Goro sites (20–21 °C) than the Eastern Shore sites (25–27 °C) (Table 1). Water column NO_x^- was inversely correlated with salinity ($R^2 = 0.74$, $p = 0.01$), with the highest concentration at Goro-10 (54 μM) and lowest concentration at ES-23 (0.2 μM). Water column NH_4^+ ranged from 0.88 μM at ES-33 to 38.4 μM at Goro-16, with no significant relationship with salinity. Sediment organic matter (0–2 cm sediment horizon) was highest at Goro-15 (2.38) and lowest at Goro-16 (0.92), but was generally similar across sites.

Average clam densities in the Sacca di Goro ranged from 365 to 2089 individuals m^{-2}, and increased with salinity in this system ($R^2 = 0.88$, $p = 0.01$), while average densities on the Eastern Shore ranged from 258 to 630 individuals m^{-2} and did not follow the salinity trend (Table 2). Average clam biomass ranged from 82.9 to 553 g DW m^{-2} and was not significantly related to salinity (Table 2). *M. mercenaria* were generally larger, averaging 39.7 mm in shell length, compared to the *R. philippinarum*, which ranged from 24.5 to 32.5 mm.

Corophium densities ranged from an average of 534 ind m^{-2} at Goro-21 to 20,783 ind m^{-2} at Goro-13 (Table 2). Based on visual estimation during sampling the densities at Goro-10 were similar to densities measured at the nearby sites (Goro-13 and Goro-15); however, densities were not directly quantified.

3.2. Dissolved oxygen fluxes

The 'clam only' incubations revealed significantly different respiration rates between the two species (ANCOVA, $p < 0.001$); *R. philippinarum* had significantly higher rates of respiration (0.024 ± 0.002 mmol O_2 g DW^{-1} h^{-1}) compared to *M. mercenaria*, which averaged 0.006 ± 0.001 mmol O_2 g DW^{-1} h^{-1} (Table 3). Clam respiration accounted for between 18 and 176% of the 'intact sediment' dark DO fluxes across sites.

The 'intact sediment' incubations revealed all sites to be net heterotrophic (DO consuming) and ranged from a mean of -3.0 ± 0.6 mmol m^{-2} h^{-1} in the light at ES-23 to a mean of -21.8 ± 3.2 mmol m^{-2} h^{-1} in the light at Goro-15 (Fig. 2A). There was no significant effect of light on DO fluxes; a significant site effect was observed, with highest consumption at Goro-13 and Goro-15 (Supplemental Table 1, Fig. 2A). Within each site individually, 'intact sediment' DO fluxes were significantly correlated with clam biomass, except at Goro-10 and ES-23 (Supplemental Table 2). Across all sites, there was a significant effect of clam biomass on 'intact sediment' DO fluxes, while the effect of clam species was not significant (Fig. 3A, Table 4).

3.3. NH_4^+ fluxes

Similar to clam respiration, the clam excretion rates, measured in the 'clam only' incubations, were significantly higher for *R. philippinarum*, averaging 2.73 ± 0.27 μmol N g DW^{-1} h^{-1}, compared to *M. mercenaria*, which averaged 0.75 ± 0.10 μmol N g DW^{-1} h^{-1} (ANCOVA, $p < 0.001$, Table 3). Clam excretion accounted for between 28 and 575% of the total benthic NH_4^+ fluxes.

There was no significant effect of light or site on the 'intact sediment' NH_4^+ fluxes (Supplemental Table 1). All sites had a net efflux of NH_4^+ in the light and dark, ranging from an average of 101.6 ± 42.7 to 1258.7 ± 173.5 μmol m^{-2} h^{-1} at Goro-10 and Goro15, respectively. (Fig. 2B). Within each site individually, 'intact sediment' NH_4^+ fluxes were significantly positively correlated with clam biomass, except at Goro-15, ES-23, and ES-33 (Supplemental Table 2). Across all sites, net NH_4^+ fluxes were significantly positively correlated with clam biomass, while the effect of clam species was not significant (Fig. 3B, Table 4).

Table 2
Clam and *Corophium* sp. data. Mean values and (standard error). n.d., no data collected.

Site	Clam density (ind m^{-2})	Clam biomass (g DW m^{-2})	Clam shell length (mm)	*Corophium* sp. density (ind m^{-2})	*Corophium* sp. biomass (g DW m^{-2})
Goro-10	398 (139)	82.9 (31.7)	28.0 (0.79)	n.d.[a]	n.d.[a]
Goro-13	365 (117)	87.1 (26.0)	28.0 (1.03)	20,783 (2307)	5.46 (0.60)
Goro-15	1161 (268)	188.9 (40.6)	25.8 (0.44)	19,550 (2581)	7.10 (1.20)
Goro-16	1127 (193)	553.0 (103.4)	32.5 (0.64)	n.d.	n.d.
Goro-21	2089 (478)	316.9 (64.4)	24.5 (0.35)	533 (154)	0.36 (0.10)
ES-23	630 (102)	192.4 (27.8)	35.5 (1.81)	n.d.	n.d.
ES-33	258 (95)	192.4 (84.9)	43.9 (2.02)	n.d.	n.d.

[a] High abundances of *Corophium* sp. were observed at Goro-10, comparable to the nearby Goro-13 and Goro-15 (pers. obs.).

Table 3
ANCOVA results of the 'clam only' incubation data. A significant interaction term suggests significant differences in metabolic rates between the two clam species. Fig. 3 depicts DO and NH_4^+ mixed models graphically.

Response	Source of Variation	Estimate	Standard Error	t value	*p* value	R^2	F Stat	*p* value	Residual SE	Metabolic Rate
NH_4^+	Intercept	− 198.3	148.1	− 1.34	0.18	0.69	$F_{(3,88)} = 68.9$	< 0.001	455.4	Excretion (μmol g DW^{-1} h^{-1})
	Clam biomass	0.75	0.35	2.14	0.04					*M. mercenaria*: 0.75
	Species	279.89	168.3	1.66	0.10					*R. philippinarum*: 2.73
	Clam × Species	1.98	0.41	4.8	< 0.001					
DO	Intercept	− 1.65	1.01	− 1.64	0.11	0.80	$F_{(3,85)} = 115.1$	< 0.001	2.92	Respiration (mmol g DW^{-1} h^{-1})
	Clam biomass	− 0.006	0.002	− 2.62	0.02					*M. mercenaria*: 0.006
	Species	− 0.77	1.13	− 0.68	0.50					*R. philippinarum*: 0.026
	Clam × Species	− 0.02	0.003	− 6.36	< 0.001					
NO_x^-	Intercept	4.35	398.6	0.011	0.99	0.11	$F_{(3,87)} = 4.81$	0.003	1205	
	Clam biomass	− 0.028	0.98	− 0.029	0.98					
	Species	− 1082	451.3	− 2.30	0.02					
	Clam × Species	1.71	1.14	1.50	0.14					

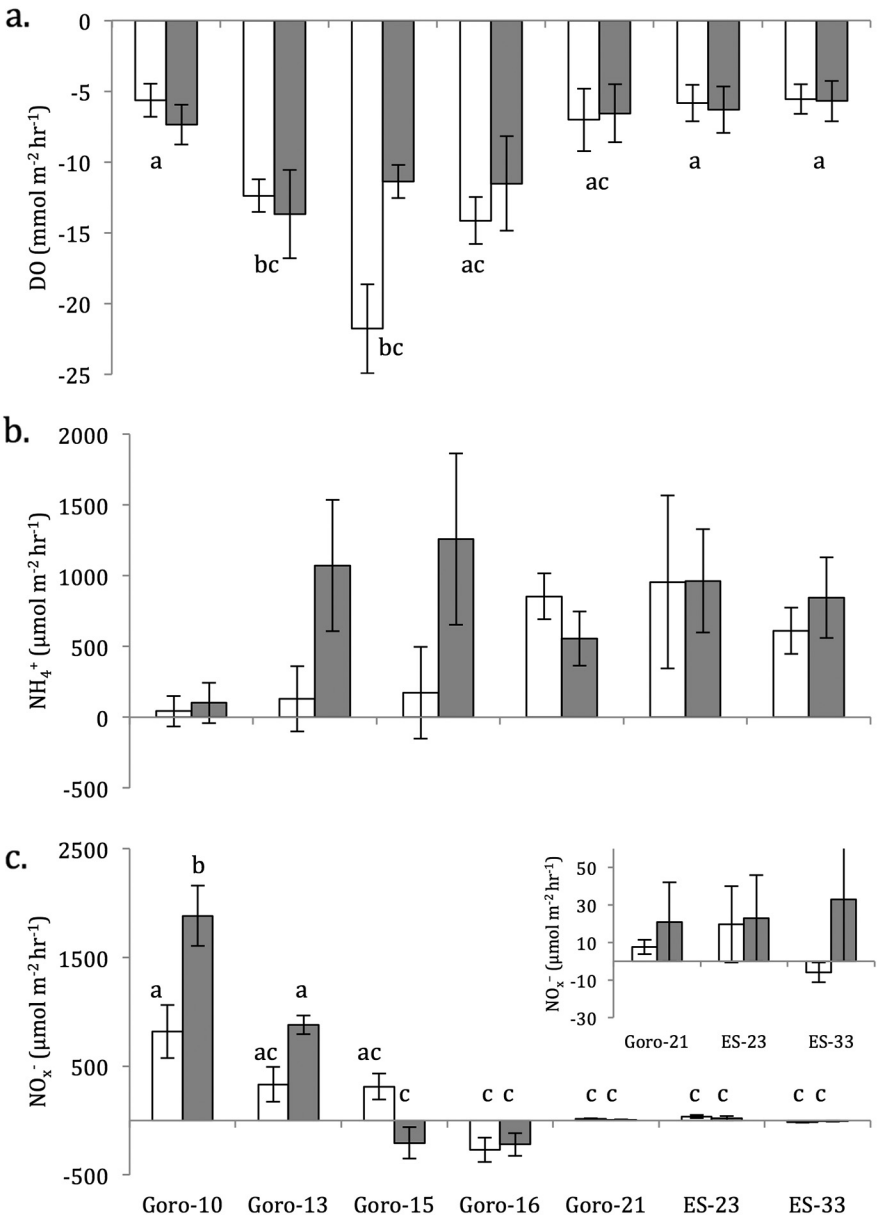

Fig. 2. Intact sediment fluxes of dissolved oxygen (a), NH_4^+ (b), and NO_x^- (c), in the light (white) and dark (gray). Letters designate significant differences due to site (DO fluxes; panel a) or the significant interaction of site and light condition (NO_x^- fluxes; panel c). No significant difference due to site or light condition was observed for the NH_4^+ fluxes (b). Sites are organized by salinity. Error bars are standard errors. Inset in (c) shows Goro-21, Cherrystone Inlet (ES-23) and Smith Island (ES-33) on a smaller scale.

3.4. NO_x^- fluxes

In the 'clam only' incubations NO_x^- fluxes were not significantly related to clam biomass for either species (ANCOVA, $p = 0.97$). In the 'intact sediment' incubations NO_x^- fluxes were negligible at the high salinity sites (ES-33, ES-23, and Goro-21). Sediments were a net sink of NO_x^- at the mid-salinity site (Goro-16), averaging -250.0 ± 73.6 μmol m^{-2} h^{-1}, and shifted to a net source of NO_x^- to the water column at the low salinity sites

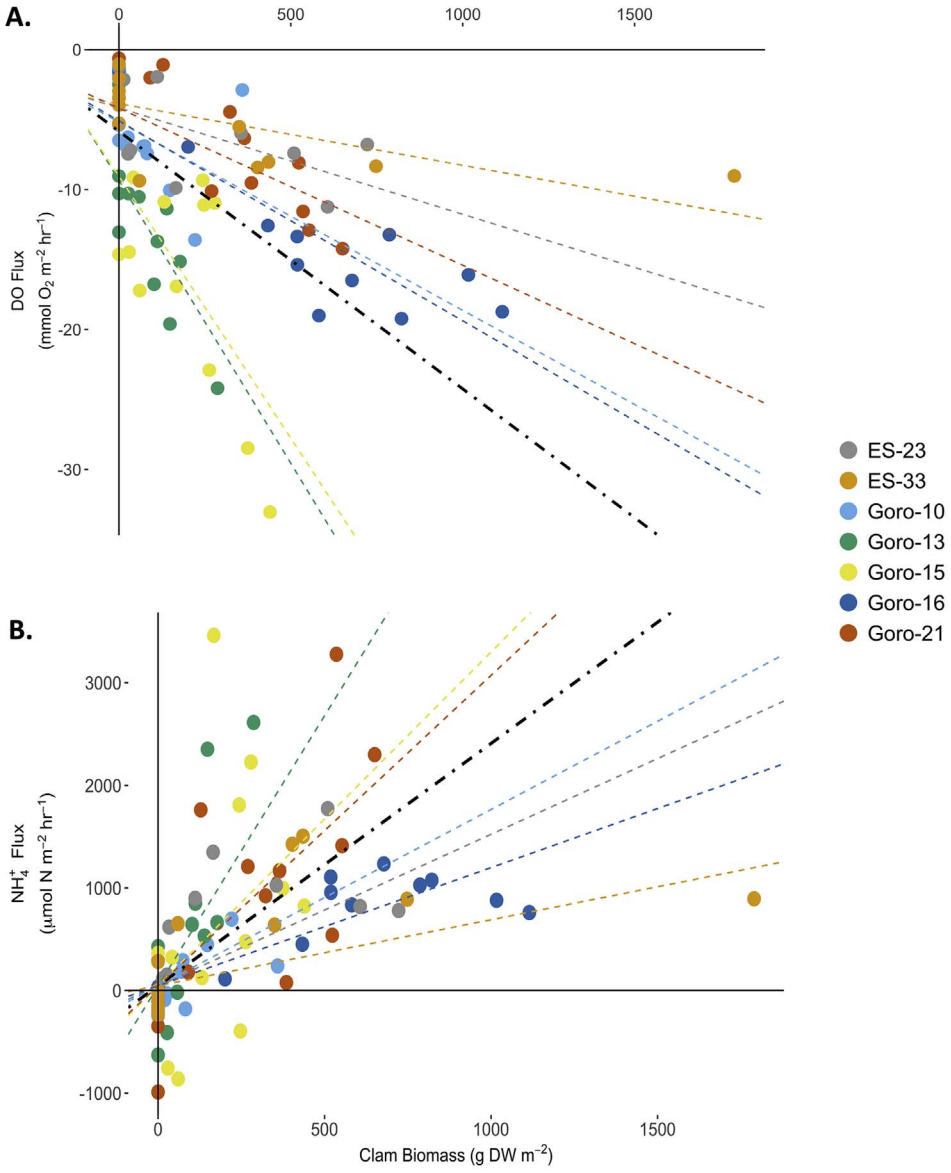

Fig. 3. 'Intact sediment' fluxes of dissolved oxygen (a) and NH_4^+ (b) as a function of clam biomass (g DW m^{-2}) at each site from the intact sediment incubations. Data were analyzed using mixed effects models with site as a random effect. The black dashed line represents the fixed effects (clam biomass) while the colored lines show the random effect coefficients for each site. Statistical results are provided in Table 4.

Table 4
Statistical results of the mixed effects models that accounted for the variance associated with site as random, allowing both the intercept and slope to vary: lme(response ~ clam biomass + Species, random = ~Clam Biomass | Site). Interactive effects between clam biomass and species were not significant for any response variable and thus were removed from the models.

Response	Predictor	Estimate	Standard Error	p value	Marginal R^2	Conditional R^2
NH_4^+	Clam Biomass	2.36	0.81	0.005	0.37	0.7
	Species	293.2	227.1	0.25		
DO	Clam Biomass	− 0.01	0.001	< 0.001	0.3	0.61
	Species	5.65	3.47	0.16		
NO_x^-	Clam Biomass	− 0.14	0.16	0.41	0.06	0.66
	Species	− 349.1	462.8	0.48		
D_{14}	Clam Biomass	2.3E-03	0.03	0.94	0.01	0.44
	Species	− 135.5	70.7	0.12		
DNRA	Clam Biomass	0.01	0.01	0.42	0.02	0.44
	Species	− 9.19	23.3	0.711		

(Goro-10 and Goro-13), which averaged 1349.2 ± 238.3 µmol m^{-2} h^{-1} and 606.2 ± 120.0 µmol m^{-2} h^{-1}, respectively (Fig. 2C). A significant interaction was observed between site and light condition, driven mainly by the significantly higher NO_x^- efflux in the dark at Goro-10 (Fig. 2C, Supplemental Table 1). There was no significant relationship between 'intact sediment' NO_x^- fluxes and clam biomass (mixed effect model, $p = 0.41$, Table 4). Within each site individually, 'intact sediment' NO_x^- fluxes were not related to clam biomass, except at ES-33, where the relationship was significantly negative (Supplemental Table 2). NO_x^- fluxes across the sites were significantly inversely related to salinity ($R^2 = 0.21$, $p < 0.001$) and directly related to water column NO_3^- concentrations ($R^2 = 0.23$, $p < 0.001$).

Table 5
Average measured gross ammonification rates, calculated nitrification (the sum of D_n, $DNRA_n$, and NO_x^- flux), percent of denitrification coupled to nitrification (% D_n), denitrification efficiency (DNF efficiency), relative proportion of DNRA to denitrification (DNRA:DNF), and ammonification rates relative to water column NO_x^- concentrations (AMN: NO_x^-) at each site. n.d. no data collected.

Site	Ammonification (mmol $m^{-2} d^{-1}$)	Calculated Nitrification (μmol $m^{-2} h^{-1}$)	Percent coupled DNF (%)	DNF Efficiency (%)	DNRA:DNF	AMN:NO_x^-
Goro-10	8.06 (0.98)	1656.9 (249.2)	51.4 (7.1)	18.0 (4.3)	0.11 (0.02)	0.14 (0.01)
Goro-13	11.47 (1.6)	762.5 (112.4)	64.5 (1.5)	25.3 (10.3)	0.06 (0.01)	0.25 (0.03)
Goro-15	11.64 (2.6)	405.9 (76.0)	60.2 (1.9)	30.5 (8.2)	0.11 (0.01)	0.24 (0.04)
Goro-16	4.81 (0.79)	185.6 (29.4)	27.4 (8.7)	12.8 (3.3)	1.53 (0.70)	0.15 (0.02)
Goro-21	4.71 (1.17)	58.1 (6.2)	78.2 (2.3)	11.7 (5.8)	2.27 (0.40)	3.18 (0.50)
ES-23	2.38 (0.29)	53.9 (11.8)	93.0 (1.2)	6.6 (3.8)	9.73 (2.30)	11.96 (1.40)
ES-33	n.d.	46.4 (14.0)	97.9 (0.1)	20.5 (11.9)	14.94 (6.10)	n.d.

3.5. Gross ammonification rates

Gross microbial ammonification rates were significantly lower at ES-23, averaging 2.4 ± 0.3 mmol $m^{-2} d^{-1}$, compared to the other sites (Table 5). The high salinity sites in the Sacca di Goro (Goro-16 and Goro-21) had rates similar to ES-23 and were significantly lower than the up-estuary sites (Goro-15 and Goro-13), which averaged 11.5 mmol $m^{-2} d^{-1}$ (Table 5).

3.6. Denitrification, DNRA, and nitrification

Average denitrification rates ranged from 1.6 ± 0.2 μmol $m^{-2} h^{-1}$ at ES-23 to 259.1 ± 54.1 μmol $m^{-2} h^{-1}$ at Goro-10. There was no significant effect of light on denitrification rates, however rates were significantly different across sites (Supplemental Table 1). ES-23, ES-33, and Goro-21 had similar denitrification rates, which were significantly lower than the other sites (Fig. 4A). Overall nitrification was the main

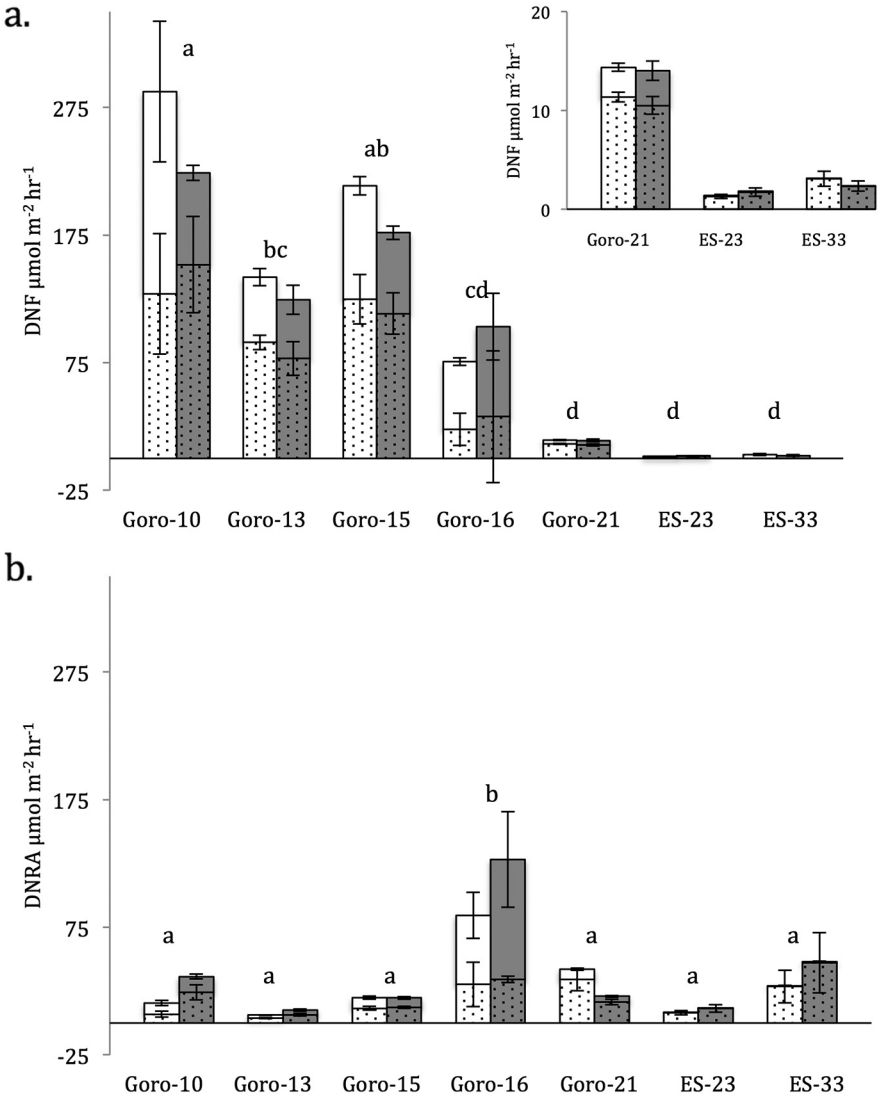

Fig. 4. Denitrification (DNF) (a) and DNRA rates (b), in the light (white) and dark (gray), including the portion coupled to nitrification, D_n and $DNRA_n$ (dotted) and direct (NO_x^- from the water column), D_w and $DNRA_w$ (solid). No significant effect of light condition was observed for either parameter. Letters indicate significant differences across sites. Error bars are standard errors. Inset in (a) shows Goro-21, Cherrystone Inlet (ES-23) and Smith Island (ES-33) on smaller scale.

nitrate source for denitrification at ES-23, ES-33, and Goro-21, where D_n ranged from 78 to 98% of D_{14} (Table 5). Despite the high water column NO_x^- concentrations at the low salinity sites (Goro-10, Goro-13, and Goro-15), the percent of denitrification coupled to nitrification was > 50%, suggesting high nitrification rates (Table 5). At Goro-16, where water column NO_x^- was high (~30 μM), the percent denitrification coupled to nitrification was only 27% (Fig. 4A, Table 5). Within each site individually, there was no effect of clam biomass on denitrification except at Goro-13, where denitrification increased with clam biomass (Supplemental Table 1). Across all sites, there was no significant effect of clam biomass or species on denitrification rates (Table 4). Denitrification efficiency was generally low at all sites, ranging from 6.6% in ES-23 to 30.5% at Goro-15 (Table 5).

DNRA rates ranged from 8.2 ± 1.2 μmol m^{-2} h^{-1} at Goro-13 to 87.7 ± 22.5 μmol m^{-2} h^{-1} at Goro-16 (Fig. 4B). There was no significant effect of light on DNRA rates (Supplemental Table 2). DNRA was significantly higher at Goro-16 compared to all other sites (Fig. 4B; Supplemental Table 1). In general, there was no significant effect of clam biomass or species on total DNRA (Table 4). However when considered within each site, total DNRA significantly increased with clam biomass at Goro-10 and Goro-13, while clam biomass had no significant effect on DNRA at any of the other sites (Supplemental Table 2).

Across sites in which *Corophium* sp. abundances were quantified (i.e. Goro-13, Goro-15, and Goro-21), DNRA rates were significantly negatively correlated with *Corophium* sp. abundances (Fig. 5A), while rates of denitrification and calculated nitrification were significantly positively correlated with *Corophium* sp. abundances (Fig. 5B and C). However, these relationships should be considered with caution as the environmental variability across the three sites may be confounding and could not be fully assessed statistically with the limited number of sites in which *Corophium* sp. were quantified (e.g. using a mixed effects model).

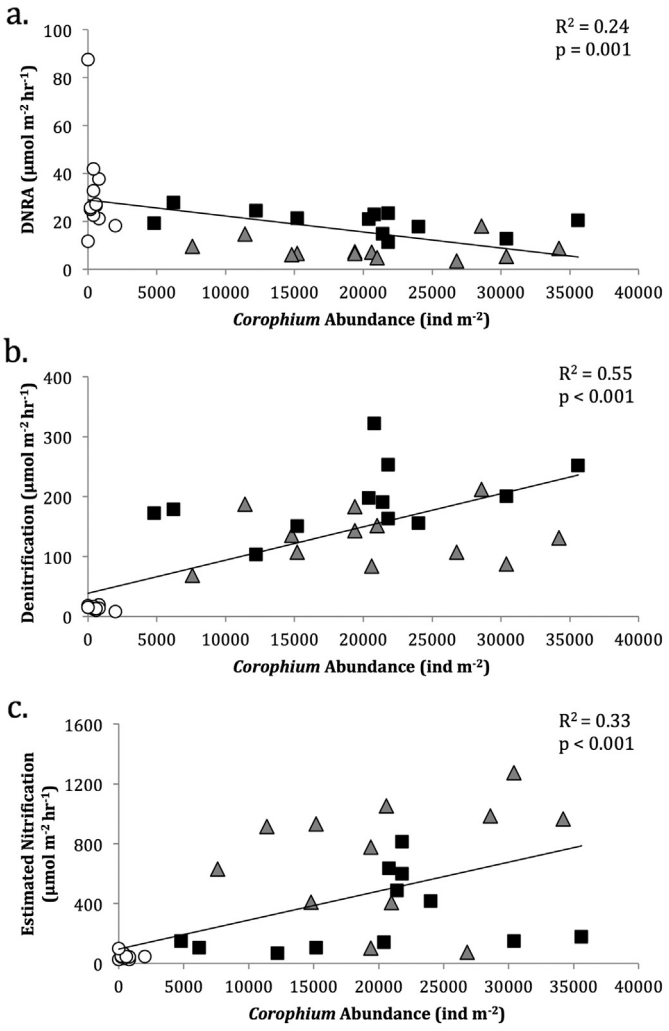

Fig. 5. Relationship between *Corophium* sp. abundance and DNRA (a), denitrification (b), estimated nitrification (calculated as the sum of denitrification, DNRA, and NO_x^- efflux) (c) at Goro-13 (triangles), Goro-15 (squares), and Goro-21 (circles).

The ratio of DNRA relative to denitrification (DNRA:DNF) was highest at ES-33, averaging 14.9, and lowest at Goro-13, averaging 0.06 (Table 5). Denitrification exceeded DNRA at Goro-10, Goro-15, Goro-13, while DNRA exceeded denitrification at Goro-21, ES-23, and ES-33; at Goro-16 DNRA: DNF was close to 1. The means of DNRA: DNF across sites were positively correlated with the ratio of ammonification (a proxy for labile carbon availability) relative to water column NO_x^- concentration ($p < 0.001$) (Fig. 6).

4. Discussion

This study demonstrates the importance of considering environmental factors, specifically those influencing NO_3^- supply, when determining the effects of clam cultivation on N removal and recycling processes. By sampling across clam aquaculture sites that spanned two countries and a range of environmental conditions, this study captured some of the natural environmental variability under which clam aquaculture is practiced. However, as this study was field-based with randomly selected sites, there was little control over environmental conditions. Strong negative covariance between water column NO_3^- concentrations and salinity made it difficult to determine the mechanistic controls on the observed differences in rates across sampling sites. Despite this, the data provide insight into the influence of bivalve aquaculture on sediment biogeochemistry and specifically N processing. The study shows the effects of bivalves depends on the local environment and the specific bivalve species cultivated. As such, the ecosystem impact of clam aquaculture should be assessed accordingly.

4.1. Clam bioenergetics directly affect NH_4^+ and DO fluxes

Our results highlight the difference in metabolic rates between the two infaunal clam species. *R. philippinarum* consumed approximately 6 times more oxygen and regenerated approximately 5 times more NH_4^+ than *M. mercenaria*. These differences could be due to intrinsic species-specific physiological and/or behavioral differences, size/age differences, and/or variation in food sources between the regions. The fact that *R. philippinarum* has higher metabolism may suggest that this species also has higher filtration rates than *M. mercenaria*. Depending on food availability, which varies by location, *R. philippinarum* may deliver more organic carbon to the sediments than *M. mercenaria*. The methods used to estimate clam respiration and excretion in this study assume that clams behave similarly when removed from the sediment as they do in situ. However, our rates reflect reasonable approximations, as they are similar to previously reported rates for *M. mercenaria* (Srna and Baggaley, 1976; Hofmann et al., 2006) and *R. philippinarum* (Magni and Montani, 2005; Han et al., 2008) measured at similar temperatures.

The relative importance of clam metabolism to total benthic community respiration and NH_4^+ production varied across sites depending on clam biomass present. However, clam biomass only explained 30% and 37% of the variation in DO and NH_4^+ fluxes, respectively (marginal R^2 of mixed effect models, Fig. 3). This indicates that other processes are likely important in dictating DO and NH_4^+, such as microbial metabolism and the metabolism of other dominant infauna present. Clam respiration accounted for a high percentage of dark DO consumption at the down-estuary sites in the Sacca di Goro (68–176%) where clam biomass was high, concurrent with low ammonification rates and low sediment organic matter relative to the other sites,

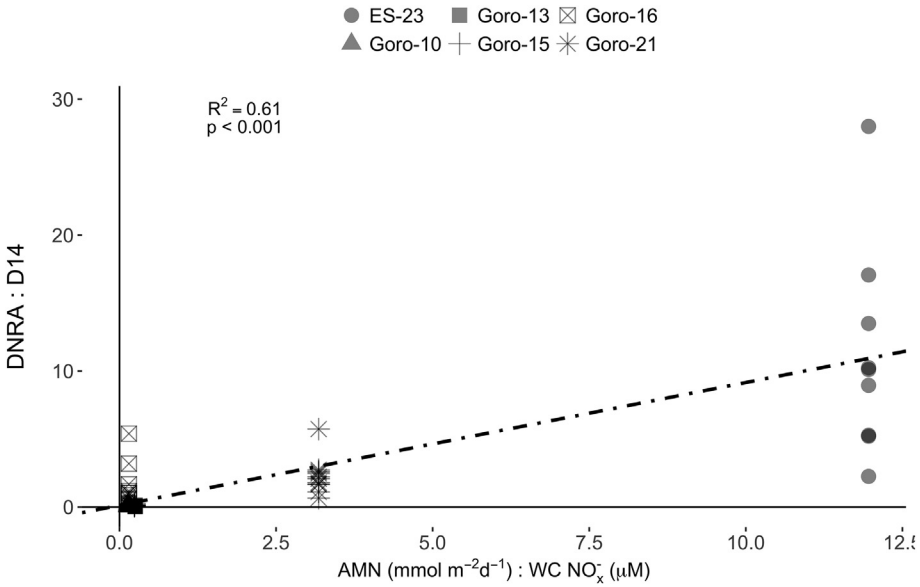

Fig. 6. The competition between DNRA and denitrification (DNRA: D_{14}) as a function of the ratio of labile carbon (estimated as ammonification rate (AMN) to water column NO_x^-). Dashed line represents the linear model.

suggesting lower microbial respiration. By contrast, clam respiration accounted for < 50% of total dark DO consumption where high abundances of the burrowing amphipod *Corophium* sp. were present (~20,000 ind m^{-2}) (Goro-10, Goro-13, and Goro-15). *Corophium* sp. not only contribute directly to benthic community respiration but, through bioirrigation, may stimulate oxygen-consuming microbial pathways such as nitrification and aerobic decomposition (Stief, 2013; Fig. 5). Finally, despite the high clam biomass present at the US sites, clam respiration accounted for < 20% of the benthic DO consumption. These sediments have been reported as being highly reduced with high pore water sulfide concentrations (Murphy et al., 2016; Smyth et al., *in review*); therefore, microbial respiration and the re-oxidation of reduced compounds such as sulfide may consume the majority of oxygen at these sites.

4.2. Locally, clams have little effect on denitrification, DNRA, and NO_x^- fluxes

Previous studies have shown that by depositing organic matter to the sediment surface and by providing substrate for bacteria to colonize (i.e. clam microbiome), clams increase nitrate respiration rates (e.g. Nizzoli et al., 2006; Kellogg et al., 2013; Welsh et al., 2015). However, in this study, within each of the seven study sites, clam biomass had little to no direct effect on denitrification, DNRA, or net NO_x^- fluxes as demonstrated by the linear model analyses of these rates as a function of clam biomass within each site individually (Supplemental Table 2). When the relationship was significant, the effect was small, generally an order of magnitude lower than the effect of clams on NH_4^+ and DO fluxes. This suggests that on a local scale, other factors aside from labile clam biodeposits (assuming clam biomass is related to biodeposition) are important in regulating NO_3^- reduction pathways. For example, as discussed below, factors that strongly influence NO_3^- supply (e.g. burrowing *Corophium*) may be more important in controlling N-cycling rates.

There was no effect of clam biomass on denitrification or NO_x^- flux, which is in contrast to a previous study conducted in the winter in the central portion of the Sacca di Goro; a positive relationship between denitrification and NO_x^- consumption with *R. philippinarum* biomass was reported (Welsh et al., 2015). Differences in sampling locations within the Sacca di Goro and season (i.e. water column NO_x^- concentrations and temperature) likely contribute to the conflicting findings. Based on incubations of isolated clams with water column NO_3^- approximately 70 μM, Welsh et al. (2015) concluded that nitrifying and denitrifying microorganisms are harbored within the clam tissue and thus, clams directly exert strong controls on benthic N cycling processes. It is possible that our study did not indicate a major control of clams on these processes during the summer because other factors that affect organic carbon and NO_3^- availability (e.g. salinity, bioturbation, and sulfide) are more important than the clams themselves in regulating NO_3^- respiration pathways, as discussed in more detail below. For example, at the sites where water column NO_3^- was high, the presence of *Corophium* sp. and their strong influence on denitrification may have masked the relationship between clams and denitrification.

4.3. Spatial variability of denitrification and DNRA is likely driven by NO_3^- and C supply

The mixed effect models which tested the overall effect of clam biomass on rates of denitrification and DNRA while controlling for the variance across sites, showed no significant effect of clam biomass on denitrification or DNRA (Table 4). We expected clam biodeposition to directly provide organic carbon for heterotrophic denitrification and DNRA. It is possible that clam biomass was not the best predictor to capture clam influences on these microbial pathways. Alternatively or in addition, other environmental factors may be driving organic carbon and nitrate dynamics aside from the clams across these heterogenous sites.

Assuming ammonification is a reasonable proxy for the lability of organic carbon, the ratio of ammonification to water column NO_3^-, was an important predictor for the partitioning of NO_3^- between DNRA and denitrification across study sites (Fig. 6). At sites with a high labile carbon to NO_3^- ratio, DNRA dominated (i.e. the Eastern Shore sites and eastern region of the Sacca di Goro). Denitrification outcompeted DNRA at sites with lower labile carbon to NO_3^- ratios (i.e. low salinity sites in the Sacca di Goro). These trends corroborate previous studies that show strong mechanistic controls of labile carbon relative to NO_3^- on the competition among these two pathways (Hardison et al., 2015; Algar and Vallino, 2014). In this study, NO_3^- supply to the sediments and factors that influence this supply strongly affected the competition between DNRA and denitrification across the study sites.

When NO_3^- was readily available either from the water column or nitrification, denitrification was favored over DNRA. This is likely due to the fact that denitrification is a more energetically favorable pathway than DNRA (Tiedje, 1988; Hardison et al., 2015). This occurred in the

western portion of the Sacca di Goro (Goro-10, Goro-13, and Goro-15) where not only was water column NO_3^- high (~60 μM) but nitrification rates and NO_3^- effluxes were also high (Table 5; Fig. 2C; Fig. 4). Approximately 50–65% of denitrification was coupled to nitrification at these sites despite the ample NO_3^- in the water column, indicating high sediment nitrification rates. Elevated nitrification may be associated with the high abundances of the amphipod *Corophium* sp. found at these sites (~4800–35,600 individual m^{-2}). These amphipods can stimulate nitrification (Fig. 5C) by creating extensive oxic niches associated with their shallow 'U'-shaped burrows and increasing exchanges of porewater through the sediment profile and overlying water (Henriksen et al., 1983; Middelburg et al., 1996; Kristensen, 2000). Additionally, as this study and previous studies have shown, denitrification is enhanced in sediments with high densities of *Corophium* sp., likely due to a tight coupling between nitrification and denitrification within the burrow walls (Pelegri et al., 1994; Fig. 5B).

At sites where NO_3^- was limiting due to a combination of low ambient water column NO_3^- concentrations, low nitrification rates, and possibly competition with benthic microalgae for NO_3^- (although not directly measured), DNRA dominated NO_3^- respiration (i.e ES-23, ES-33, and Goro-21). Since water column NO_3^- concentrations were low at these sites both denitrification and DNRA were tightly coupled to nitrification (~78–98%) (Table 5). However, low oxygen availability likely suppressed nitrification at these sites. The generally reduced state of the sediments at the US sites was evidenced by a net release of NH_4^+ and high sediment oxygen consumption with clam metabolism only accounting for approximately 25% of these rates. Additionally, the US sites and the eastern region of the Sacca di Goro were reported as having high sulfide concentrations (Murphy et al., 2016; Giordani et al., 1997), which may directly inhibit nitrification (Joye and Hollibaugh, 1995). The use of predator exclusion nets at the US sites, which become fouled by macroalgae (Murphy et al., 2015), likely leads to reduced conditions limiting water flow and exchange between the sediments and water column (Secrist, 2013). Similarly, in the shallow, sheltered, eastern region of the Sacca di Goro, where the hydrological residence time is long, significant macroalgal blooms occur seasonally and have been associated with large dystrophic events (as reviewed in Viaroli et al., 2006).

Highest rates of DNRA occurred in the central portion of the Sacca di Goro (Goro-16), where denitrification rates were also relatively high and the ratio between the two pathways was close to one. Strong competition for NO_3^- between these two NO_3^- respiring pathways was likely due to high water column NO_3^- concurrent with high densities of clams that continuously deliver labile carbon to the sediments. This results in rapid utilization of NO_3^-, as demonstrated by the net influx of NO_3^- (Fig. 2C), and a balance between denitrification and DNRA.

4.4. Denitrification efficiency

Denitrification efficiency is a metric often used to assess the percent of organic N that is microbially mineralized via denitrification and related to organic carbon load to the benthos (Eyre and Ferguson, 2009). However, it also includes any N 'mineralized' by infauna (i.e. excretion). In this study, the sediments associated with clam cultivation had low denitrification efficiency (< 30%; Table 5). This was not necessarily because denitrification was an unimportant mineralization pathway, in fact it was important in the up-estuary Sacca di Goro sites, but rather because of the high NH_4^+ production by the clams and other infauna. Additionally, bioturbating infauna such as *Corophium* sp., which stimulate denitrification also promote nitrification (Fig. 5). As observed at the low salinity sites in the Sacca di Goro (Goro-10, Goro-13, and Goro-15), NO_3^- production can exceed consumption, likely due to the *Corophium* sp. flushing their burrows, actively transporting NO_3^- to the water column. This results in high NO_3^- effluxes and subsequently low denitrification efficiencies.

4.5. Conclusions

This study demonstrates the variability in N cycling processes in sediments dominated by clam aquaculture. The growth of the clam aquaculture industry in coastal systems worldwide has increased interest in the influence of these operations on coastal N dynamics and specifically the question of whether N removal is promoted through bivalve-facilitated denitrification. This study shows that numerous factors affecting sources of labile carbon, NO_3^-, and O_2 including clam biomass, the presence of other dominant infauna, cultivation practices, and the environmental context determine whether bivalve cultivation favors N loss (i.e. denitrification) or N recycling (i.e. DNRA). Our study further highlights the challenge in generalizing about the influence of clam aquaculture on denitrification and the importance of considering environmental factors and competing pathways (i.e. DNRA). A commonality that was apparent across all study sites was that clams promoted high N recycling and NH_4^+ release to the water column, due to high excretion rates; thus, determination of whether clam aquaculture promotes denitrification or not should be considered within the context of its influence on N regeneration.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2017.12.003.

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