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.

KEYWORDS:
Denitrification; DNRA; nitrification; nitrate respiration; clam aquaculture; nitrogen cycling

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48 INTRODUCTION

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

60 As infaunal organisms, cultivated clams both directly and indirectly affect sediment N
61 cycling rates and benthic metabolism through bioturbation, biodeposition, excretion, and
62 respiration, which subsequently influence microbial metabolic pathways (reviewed in Laverock
63 et al. 2011). Clam bioturbation transports particles and water, including solutes (e.g. O_2, NO_3^-),
64 through sediments. Through feeding and biodeposition, clams actively deliver organic carbon to
65 the sediments from the water column, fueling microbial decomposition pathways, enhancing
66 microbial respiration and oxygen demand, and thereby substantially changing redox gradients
67 (Aller 1982, Kristensen et al. 1985) and impacting redox sensitive microbial processes such as
68 nitrification and denitrification (Stief 2013). Benthic infauna, including cultivated clams, also

69 excrete dissolved inorganic and organic N, directly increasing benthic N fluxes to the water
70 column and potentially providing substrate (e.g. NH_4^+) for microbial processes such as
71 nitrification and ANAMMOX (Welsh et al. 2015). Bivalves can thus influence both microbial N
72 removal and recycling pathways in coastal sediments.

73 Bivalves may enhance N removal by promoting denitrification, the microbially mediated
74 pathway that reduces nitrate (NO_3^-) to inert N_2 gas. This bivalve-facilitated, denitrification
75 enhancement results both from biodeposition of organic matter to sediment microbial
76 communities (Newell et al. 2002, Kellogg et al. 2013, Smyth et al. 2013) and by provision of
77 habitats for denitrifying microorganisms (Heisterkamp et al., 2010; Welsh et al., 2015). However,
78 some studies have reported no significant effect of bivalves on denitrification rates (Higgins et al.
79 2013, Erler et al. 2017). Additionally, often overlooked is the effect bivalves have on inorganic
80 N regeneration pathways. High densities of bivalves, found in cultivation settings, may
81 accelerate N recycling processes through bivalve excretion and stimulation of microbial
82 ammonification including dissimilatory nitrate reduction to ammonium (NH_4^+) (DNRA) (Dame
83 2011, Murphy et al. 2016, Erler et al. 2017), which retain bioavailable N in the aquatic
84 ecosystem.

85 The question of whether bivalves promote N removal or retention remains equivocal.
86 The discrepancy among previous studies on how bivalves influence benthic N cycling pathways
87 is in part due to differences in the bivalve species studied (e.g. epifaunal oysters or mussels
88 versus infaunal clams), but also likely due differences in the environmental conditions under
89 which bivalves are farmed. Bivalve aquaculture can occupy expansive regions across estuarine
90 environmental gradients. Few studies that investigate N cycling associated with bivalve
91 aquaculture, and specifically clam aquaculture, have captured the natural environmental

92 variability across which the practice exists. Moreover, few studies have investigated the
93 partitioning of NO_3^- reduction between denitrification and DNRA, which is ecologically
94 important as DNRA retains bioavailable N in the system as NH_4^+ whereas denitrification
95 removes it. Those studies that do provide simulatenouse measurements of denitrification and
96 DNRA are restricted to single study systems. Therefore, we were interested in directly
97 comparing different study systems, which are heavily exploited for infaunal clam cultivation and
98 where previous studies found contrasting results regarding denitrification and DNRA at clam
99 cultivation sites. We chose to sample clam aquaculture in the Sacca di Goro, Italy, where
100 denitrification was reportedly higher than DNRA (Nizzoli et al. 2006) and in coastal Virginia,
101 US, where DNRA generally dominated NO_3^- respiration (Murphy et al. 2016).
102 The objective of this study was to investigate how sediment N cycling associated with
103 clam aquaculture varies across different environmental conditions and between two commonly
104 cultivated infaunal clam species: *Ruditapes philipinarum* (Italy) and *Mercenaria mercenaria*
105 (US). Across the natural environmental gradients in which clam aquaculture exists, we were
106 specifically interested in (1) comparing N cycling and metabolic rates between the two cultivated
107 clam species and determining the direct contribution of these clams to benthic rates and (2)
108 determining the factors that influence the competition between microbial denitrification and
109 DNRA at clam aquaculture sites. By studying two clam species exposed to different
110 environmental conditions and farming practices, we sought to highlight the challenge in
111 generalizing ecological responses across all bivalve aquaculture and, more specifically, across all
112 clam cultivation practices. We hypothesized that both clam species would significantly increase
113 benthic oxygen demand and inorganic N fluxes; however, the contribution of clams to these
114 benthic processes would differ across sites depending on site-specific factors and clam species

115 physiology. We expected that the degree to which N is removed through denitrification relative
116 to N recycled through DNRA would change depending on the availability of labile organic
117 carbon and NO_3^- (Tiedje 1988), factors that would vary broadly across estuarine gradients and
118 clam aquaculture sites.
119
120 METHODS
121 *Study Sites*
122 The Sacca di Goro is a lagoonal system of the Po River Delta, Italy. Approximately 26
123 km^2 with an average depth of 1.5 m, the lagoon hosts a substantial clam aquaculture industry,
124 with about 1/3 of the area occupied by clam cultivation. The system is generally divided into
125 three areas based on hydrologic characteristics: the eastern portion is shallow with low energy
126 and slow water exchange; the central region is tidally influenced, and the western portion is
127 riverine dominated with freshwater flow from the Po di Volano (Figure 1A). The lagoon,
128 particularly the eastern region, typically experiences periodic dystrophic events in the early
129 summer when macroalgae bloom. Drastic changes to the hydrodynamics of the system were
130 made over the past 20 years to improve water flow and alleviate dystrophic events, including
131 channel construction along the southern sand spit and dredging of internal canals to increase flow
132 to the Adriatic Sea (Viaroli et al. 2006). The manila clam, *R. philippinarum*, is farmed in
133 privately leased portions of the lagoon at densities ranging from 100 to >2000 individuals m^{-2}.
134 Growers collect juvenile clams at the mouth and directly outside the lagoon, transport them to
135 individual leases within the lagoon; after approximately 9 months market-sized clams are
136 hydraulically harvested.

137 Clam aquaculture occupies large subtidal areas on both the Chesapeake Bay-side and
138 Atlantic-side of the Eastern Shore peninsula of Virginia (Emery 2015). Cherrystone Inlet (ES-
139 23), located on the Chesapeake Bay-side of the Eastern Shore, is a small shallow embayment (~6
140 km^2, mean water depth of 1.1 m) that receives little freshwater discharge. Smith Island Bay (ES-
141 33) is the southern-most lagoon, located on the eastern side of the Eastern Shore and is protected
142 from the Atlantic Ocean by a barrier island (Figure 1B). In both locations, the hard clam, *M.*
143 *mercenaria*, is cultivated in privately owned leases in subtidal regions. Clams are sourced from
144 land-based hatcheries and nurseries and planted in the sediments at ~8-15 mm in shell length.
145 Unlike in Italy, growers set plastic mesh nets over the clam beds flush to the sediment surface as
146 protection from natural predation. Macroalgal blooms, supported by nutrients excreted by clams
147 and from microbial mineralization of organic matter in the sediment, occur on the predator-
148 exclusion nets (Murphy et al. 2015). Periodically growers sweep the nets of macroalgae to
149 prevent the algae from suffocating the clams. After about two years the market-sized clams are
150 hydraulically harvested.
151
152 *Site Characterization*
153 Surface water column and sediment samples were collected once in summer 2013 at five
154 sites in the Sacca di Goro, Italy (Figure 1A) and two sites on the Eastern Shore, VA USA (Figure
155 1B). Triplicate water column grab samples were collected at each site at ~50 cm above the
156 sediment, filtered (0.45 μm) and stored frozen in either whirlpak bags or Falcon tubes until
157 analyzed for NH_4^+ and nitrate plus nitrite (NO_X^-). Triplicate sediment cores (polycarbonate core
158 tubes, 30 cm height and 4 cm i.d.) were also collected at each site for determination of sediment
159 organic matter by loss on ignition (450 °C over 3 hours) in the 0-2 cm sediment horizon.

160 Temperature and salinity were measured at each site using a thermometer and refractometer,
161 respectively. Although, both the Sacca di Goro and the Eastern Shore experience seasonal
162 variation in temperature, salinity, and nutrient concentrations (Murphy et al. 2016; Nizzoli et al.
163 2006), capturing this temporal variability was beyond the scope of this study. We focused on the
164 natural spatial variability of environmental parameters across the study sites during the summer
165 season, when biogeochemical rates are typically high. Throughout the study, site identification
166 refers to the location and measured salinity, for example, Goro-13 was collected in the Sacca di
167 Goro and the salinity was 13.
168
169 *Benthic Metabolism and Nutrient Flux Measurements – 'Intact Cores'*
170 Twelve sediment cores (10 cm sediment depth) were collected (Eastern Shore (ES) sites,
171 9.5cm i.d.; Goro sites, 8 cm i.d.) at all sites, except ES-23 where 10 cores were collected, for the
172 determination of benthic metabolism, nutrient fluxes, denitrification and DNRA. From each site,
173 half the cores were incubated in the light and half in the dark. Cores were randomly collected at
174 each site; clam densities varied between sediment cores and some sediment cores contained no
175 clams by chance.
176 Sediment cores collected in the Sacca di Goro were transported to the University of
177 Parma while cores from the Eastern Shore were transported to Virginia Institute of Marine
178 Science, Eastern Shore Laboratory (VIMS ESL) in Wachapreague VA. Cores were placed in
179 water baths at site-specific salinity and temperature and allowed to equilibrate overnight. Oxic
180 conditions in water baths were assured by bubbling with airstones. The water inside the cores
181 was gently stirred avoiding sediment resuspension during the equilibration and incubation
182 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 hours, 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 7M 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} hr^{-1} or μmol N m^{-2} hr^{-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).

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 one hour; 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 & 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 hours, depending on the specific sediment oxygen demand determined in the previous incubation (see above), allowing DO to change by no more than 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 $ZnCl_2$ (7M). 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 uM, 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:

$$D_{15} = p29 + 2p30 \quad (3)$$

$$D_{14} = D_{15} \times (p29/2p30) \quad (4)$$

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):

$$D_w = (^{14}NO_3^- / {}^{15}NO_3^-) * D_{15} \quad (5)$$

$$D_n = D_{14} - D_w \quad (6)$$

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 2M. Samples were shaken for 1 hour, 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 (1989). Water samples were placed in specimen cups; an acidified (25μl of 2.5M sulfuric acid) GFF filter (1cm, 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 & Rysgaard (1995) as:

$$DNRA_t = p^{15}NH_4^+ \times (D_{14}/D_{15}) \quad (7)$$

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:

$$DNRA_w = (^{14}NO_3^- / {}^{15}NO_3^-) * p^{15}NH_4^+ \quad (8)$$

$$DNRA_n = DNRA_t - DNRA_w \quad (9)$$

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

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 hours. 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} hr^{-1} or μmol N m^{-2} hr^{-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.

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 (Steif et al. 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.).

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 2M KCl for an hour; the extractant was filtered and frozen until analysis. The T_f cores were capped and incubated for 24 hours 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 1989, as described above. Rates of gross ammonification were calculated using a model described by Wessel & Tietema 1992 as

$$\text{Ammonification} = \frac{\ln (Tf_{atm\%} - k) / (T_{0atm\%} - k)}{\ln [NH_4^+ T_f] / [NH_4^+ T_0]} * \frac{[NH_4^+ T_0] - [NH_4^+ T_f]}{\text{time}} \quad (10)$$

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 %; $[NH_4^+Tf]$ 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.

319 *Denitrification Efficiency Calculation*

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

322

323 ———————— (11)

324

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

327

328 *Statistical Analyses*

329 Data from the 'clam-only' incubations were analyzed using analysis of covariance
330 (ANCOVA) to test the effect of and interaction between clam biomass and species on rate
331 measurements (NH_4^+, NO_X^-, and DO fluxes). Clam physiological rates (respiration and excretion),
332 were calculated using the slope estimates of the linear models within each species (mmol O_2 g
333 DW^{-1} hr^{-1} or μmol NH_4^+ g DW^{-1} hr^{-1}). To determine the clam contribution to total benthic fluxes,
334 clam physiological rates were scaled to per m^2 by multiplying by the clam biomass present
335 within each core and dividing by the surface area of the core and compared to the 'intact core'.
336 A two-way analysis of variance (ANOVA) was used to examine the interactive effects of
337 light condition and site, which refers to all 7 study sites, on 'intact sediment' nutrient fluxes, DO
338 fluxes, denitrification, and DNRA. Tukey HSD post hoc analysis was used to compare means
339 when an effect was significant. For further analysis, all fluxes, regardless of whether they were
340 made in the light or dark were included and the effect of light was ignored because (1) the
341 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 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.

RESULTS

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.

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} hr^{-1}) compared to *M. mercenaria*, which averaged

387 0.006 ± 0.001 mmol O_2 g DW^{-1} hr^{-1} (Table 3). Clam respiration accounted for between 18 and
388 176% of the 'intact sediment' dark DO fluxes across sites.

389 The 'intact sediment' incubations revealed all sites to be net heterotrophic (DO
390 consuming) and ranged from a mean of -3.0 ± 0.6 mmol m^{-2} hr^{-1} in the light at ES-23 to a mean
391 of -21.8 ± 3.2 mmol m^{-2} hr^{-1} in the light at Goro-15 (Figure 2A). There was no significant effect
392 of light on DO fluxes; a significant site effect was observed, with highest consumption at Goro-
393 13 and Goro-15 (Supplemental Table 1, Figure 2A). Within each site individually, 'intact
394 sediment' DO fluxes were significantly correlated with clam biomass, except at Goro-10 and ES-
395 23 (Supplemental Table 2). Across all sites, there was a significant effect of clam biomass on
396 'intact sediment' DO fluxes, while the effect of clam species was not significant (Figure 3A,
397 Table 4).

398

399 *NH_4^+ Fluxes*

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

405 There was no significant effect of light or site on the 'intact sediment' NH_4^+ fluxes
406 (Supplemental Table 1). All sites had a net efflux of NH_4^+ in the light and dark, ranging from an
407 average of 101.6 ± 42.7 to 1258.7 ± 173.5 μmol m^{-2} hr^{-1} at Goro-10 and Goro15, respectively.
408 (Figure 2B). Within each site individually, 'intact sediment' NH_4^+ fluxes were significantly
409 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 (Figure 3B, Table 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} hr^{-1}, and shifted to a net source of NO_X^- to the water column at the low salinity sites (Goro-10 and Goro-13), which averaged 1349.2 ± 238.3 μmol m^{-2} hr^{-1} and 606.2 ± 120.0 μmol m^{-2} hr^{-1}, respectively (Figure 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 (Figure 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 ($R^2 = 0.23$, $p < 0.001$).

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).

Denitrification, DNRA, and Nitrification

Average denitrification rates ranged from 1.6 ± 0.2 μmol m^{-2} hr^{-1} at ES-23 to 259.1 ± 54.1 μmol m^{-2} hr^{-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 (Figure 4A). Overall nitrification was the main 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% (Figure 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 2). 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} hr^{-1} at Goro-13 to 87.7 ± 22.5 μmol m^{-2} hr^{-1} at Goro-16 (Figure 4B). There was no significant effect of light on DNRA rates (Supplemental Table 1). DNRA was significantly higher at Goro-16 compared to all other sites (Figure 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

455 increased with clam biomass at Goro-10 and Goro-13, while clam biomass had no significant
456 effect on DNRA at any of the other sites (Supplemental Table 2).
457 Across sites in which *Corophium* sp. abundances were quantified (i.e. Goro-13, Goro-15,
458 and Goro-21), DNRA rates were significantly negatively correlated with *Corophium* sp.
459 abundances (Figure 5A), while rates of denitrification and calculated nitrification were
460 significantly positively correlated with *Corophium* sp. abundances (Figure 5B and 5C).
461 However, these relationships should be considered with caution as the environmental variability
462 across the three sites may be confounding and could not be fully assessed statistically with the
463 limited number of sites in which *Corophium* sp. were quantified (e.g. using a mixed effects
464 model).
465 The ratio of DNRA relative to denitrification (DNRA : DNF) was highest at ES-33,
466 averaging 14.9, and lowest at Goro-13, averaging 0.06 (Table 5). Denitrification exceeded
467 DNRA at Goro-10, Goro-15, Goro-13, while DNRA exceeded denitrification at Goro-21, ES-23,
468 and ES-33; at Goro-16 DNRA : DNF was close to 1. The means of DNRA : DNF across sites
469 were positively correlated with the ratio of ammonification (a proxy for labile carbon
470 availability) relative to water column NO_x^- concentration ($p < 0.001$) (Figure 6).
471
472 DISCUSSION
473 This study demonstrates the importance of considering environmental factors,
474 specifically those influencing NO_3^- supply, when determining the effects of clam cultivation on
475 N removal and recycling processes. By sampling across clam aquaculture sites that spanned two
476 countries and a range of environmental conditions, this study captured some of the natural
477 environmental variability under which clam aquaculture is practiced. However, as this study was

478 field-based with randomly selected sites, there was little control over environmental conditions.
479 Strong negative covariance between water column NO_3^- concentrations and salinity made it
480 difficult to determine the mechanistic controls on the observed differences in rates across
481 sampling sites. Despite this, the data provide insight into the influence of bivalve aquaculture on
482 sediment biogeochemistry and specifically N processing. The study shows the effects of bivalves
483 depends on the local environment and the specific bivalve species cultivated. As such, the
484 ecosystem impact of clam aquaculture should be assessed accordingly.
485
486 *Clam bioenergetics directly affect NH_4^+ and DO fluxes*
487 Our results highlight the difference in metabolic rates between the two infaunal clam
488 species. *R. philippinarum* consumed approximately 6 times more oxygen and regenerated
489 approximately 5 times more NH_4^+ than *M. mercenaria*. These differences could be due to
490 intrinsic species-specific physiological and/or behavioral differences, size/age differences, and/or
491 variation in food sources between the regions. The fact that *R. philippinarum* has higher
492 metabolism may suggest that this species also has higher filtration rates than *M. mercenaria*.
493 Depending on food availability, which varies by location, *R. philippinarum* may deliver more
494 organic carbon to the sediments than *M. mercenaria*. The methods used to estimate clam
495 respiration and excretion in this study assume that clams behave similarly when removed from
496 the sediment as they do *in situ*. However, our rates reflect reasonable approximations, as they
497 are similar to previously reported rates for *M. mercenaria* (Srna & Baggaley 1976, Hofmann et
498 al. 2006) and *R. philippinarum* (Magni & Montani 2005, Han et al. 2008) measured at similar
499 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, Figure 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, 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 (Steif 2013; Figure 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.

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.

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 (Figure 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

568 more energetically favorable pathway than DNRA (Tiedje 1988; Hardison et al. 2015). This
569 occurred in the western portion of the Sacca di Goro (Goro-10, Goro-13, and Goro-15) where not
570 only was water column NO_3^- high (~60 μM) but nitrification rates and NO_3^- effluxes were also
571 high (Table 5; Figure 2C; Figure 4). Approximately 50-65% of denitrification was coupled to
572 nitrification at these sites despite the ample NO_3^- in the water column, indicating high sediment
573 nitrification rates. Elevated nitrification may be associated with the high abundances of the
574 amphipod *Corophium* sp. found at these sites (~4,800-35,600 individual m^{-2}). These amphipods
575 can stimulate nitrification (Figure 5C) by creating extensive oxic niches associated with their
576 shallow 'U'-shaped burrows and increasing exchanges of porewater through the sediment profile
577 and overlying water (Henriksen et al. 1983, Middelburg et al. 1996, Kristensen 2000).
578 Additionally, as this study and previous studies have shown, denitrification is enhanced in
579 sediments with high densities of *Corophium* sp., likely due to a tight coupling between
580 nitrification and denitrification within the burrow walls (Pelegri et al. 1994; Figure 5B).
581 At sites where NO_3^- was limiting due to a combination of low ambient water column
582 NO_3^- concentrations, low nitrification rates, and possibly competition with benthic microalgae for
583 NO_3^- (although not directly measured), DNRA dominated NO_3^- respiration (i.e ES-23, ES-33,
584 and Goro-21). Since water column NO_3^- concentrations were low at these sites both
585 denitrification and DNRA were tightly coupled to nitrification (~78-98%) (Table 5). However,
586 low oxygen availability likely suppressed nitrification at these sites. The generally reduced state
587 of the sediments at the US sites was evidenced by a net release of NH_4^+ and high sediment
588 oxygen consumption with clam metabolism only accounting for approximately 25% of these
589 rates. Additionally, the US sites and the eastern region of the Sacca di Goro were reported as
590 having high sulfide concentrations (Murphy et al. 2016, Giordani et al. 1997), which may

591 directly inhibit nitrification (Joye & Hollibaugh 1995). The use of predator exclusion nets at the
592 US sites, which become fouled by macroalgae (Murphy et al. 2015), likely leads to reduced
593 conditions limiting water flow and exchange between the sediments and water column (Secrist
594 2013). Similarly, in the shallow, sheltered, eastern region of the Sacca di Goro, where the
595 hydrological residence time is long, significant macroalgal blooms occur seasonally and have
596 been associated with large dystrophic events (as reviewed in Viaroli et al. 2006).
597 Highest rates of DNRA occurred in the central portion of the Sacca di Goro (Goro-16),
598 where denitrification rates were also relatively high and the ratio between the two pathways was
599 close to one. Strong competition for NO_3^- between these two NO_3^- respiring pathways was likely
600 due to high water column NO_3^- concurrent with high densities of clams that continuously deliver
601 labile carbon to the sediments. This results in rapid utilization of NO_3^-, as demonstrated by the
602 net influx of NO_3^- (Figure 2C), and a balance between denitrification and DNRA.
603
604 *Denitrification efficiency*
605 Denitrification efficiency is a metric often used to assess the percent of organic N that is
606 microbially mineralized via denitrification and related to organic carbon load to the benthos
607 (Eyre and Ferguson 2009). However, it also includes any N 'mineralized' by infauna (i.e.
608 excretion). In this study, the sediments associated with clam cultivation had low denitrification
609 efficiency (<30%; Table 5). This was not necessarily because denitrification was an unimportant
610 mineralization pathway, in fact it was important in the up-estuary Sacca di Goro sites, but rather
611 because of the high NH_4^+ production by the clams and other infauna. Additionally, bioturbating
612 infauna such as *Corophium* sp., which stimulate denitrification also promote nitrification (Figure
613 5). As observed at the low salinity sites in the Sacca di Goro (Goro-10, Goro-13, and Goro-15),

614 NO_3^- production can exceed consumption, likely due to the *Corophium* sp. flushing their burrows,
615 actively transporting NO_3^- to the water column. This results in high NO_3^- effluxes and
616 subsequently low denitrification efficiencies.
617
618 *Conclusions*
619 This study demonstrates the variability in N cycling processes in sediments dominated by
620 clam aquaculture. The growth of the clam aquaculture industry in coastal systems worldwide
621 has increased interest in the influence of these operations on coastal N dynamics and specifically
622 the question of whether N removal is promoted through bivalve-facilitated denitrification. This
623 study shows that numerous factors affecting sources of labile carbon, NO_3^-, and O_2 including
624 clam biomass, the presence of other dominant infauna, cultivation practices, and the
625 environmental context determine whether bivalve cultivation favors N loss (i.e. denitrification)
626 or N recycling (i.e. DNRA). Our study further highlights the challenge in generalizing about the
627 influence of clam aquaculture on denitrification and the importance of considering
628 environmental factors and competing pathways (i.e. DNRA). A commonality that was apparent
629 across all study sites was that clams promoted high N recycling and NH_4^+ release to the water
630 column, due to high excretion rates; thus, determination of whether clam aquaculture promotes
631 denitrification or not should be considered within the context of its influence on N regeneration.
632

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Figure Captions

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

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

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.

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

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)

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.

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 (0-2cm)
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)

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.*	n.d.*
Goro-13	365 (117)	87.1 (26.0)	28.0 (1.03)	20,783 (2,307)	5.46 (0.60)
Goro-15	1161 (268)	188.9 (40.6)	25.8 (0.44)	19,550 (2,581)	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.

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

*

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 gDW^{-1} hr^{-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 x 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 gDW^{-1} hr^{-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 x 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 x Species	1.71	1.14	1.50	0.14					

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. Figure 3 depicts DO and NH_4^+ mixed models graphically.

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		

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.

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.

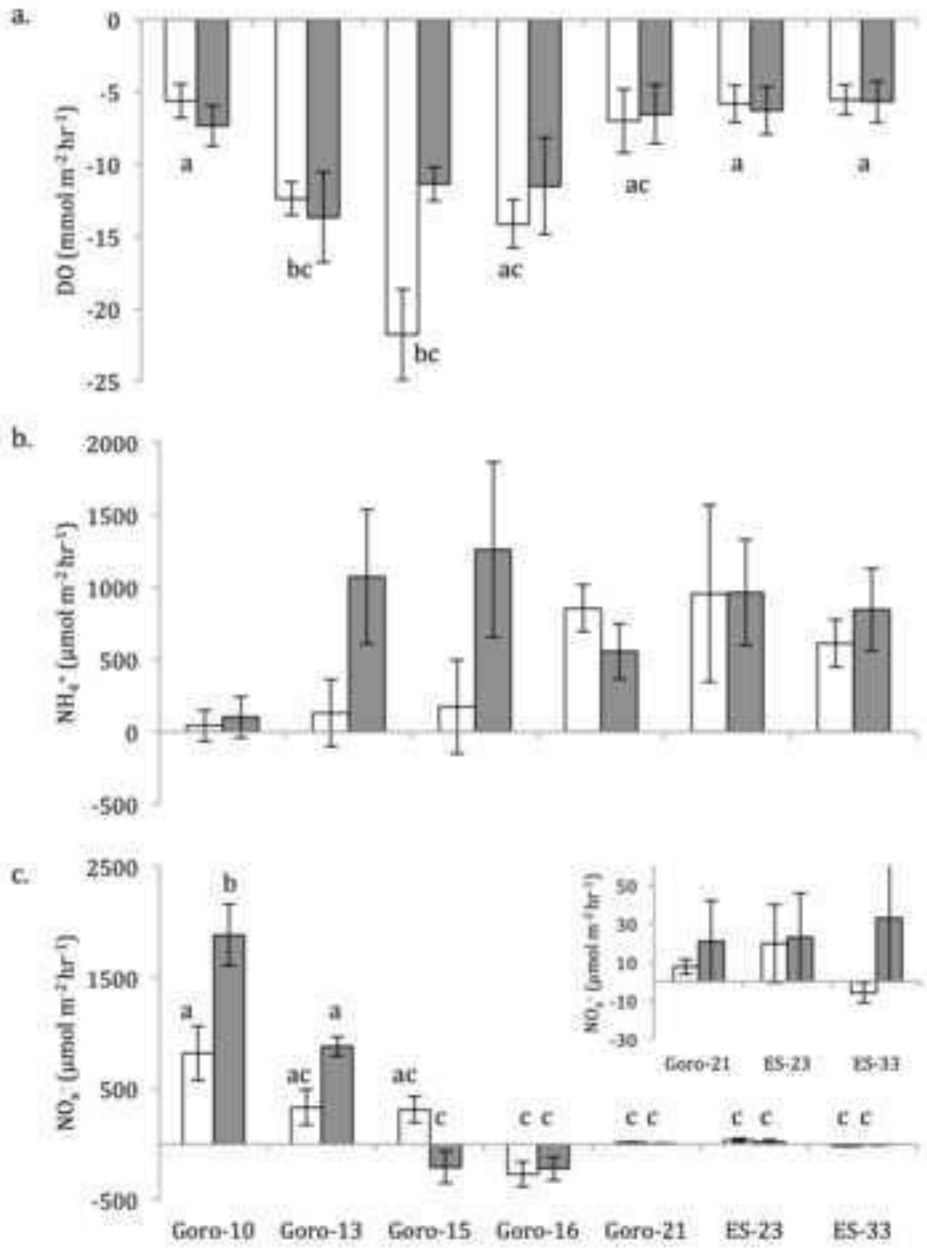
Site	Ammonification (mmol $m^{-2} d^{-1}$)	Calculated Nitrification (μmol m^{-2} hr^{-1})	Percent coupled DNF (%)	DNF Efficiency (%)	DNRA : DNF	AMN : NO_X^-
Goro-10	8.06 (0.98)	1656.9 (249.2)	51.4 (7.1)	18.0 (4.3)	0.11 (0.02)	0.14 (0.01)
Goro-13	11.47 (1.6)	762.5 (112.4)	64.5 (1.5)	25.3 (10.3)	0.06 (0.01)	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.

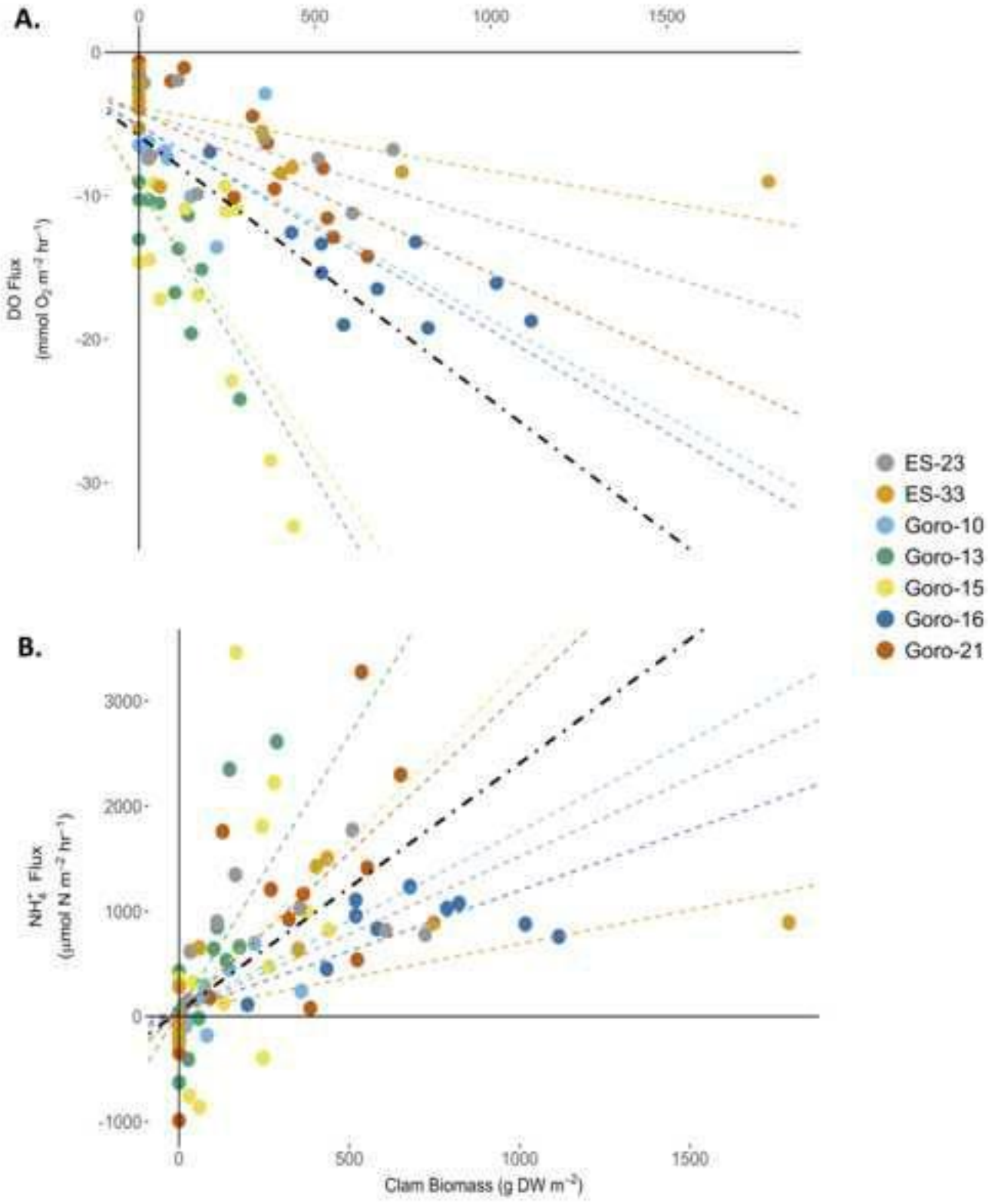
a.

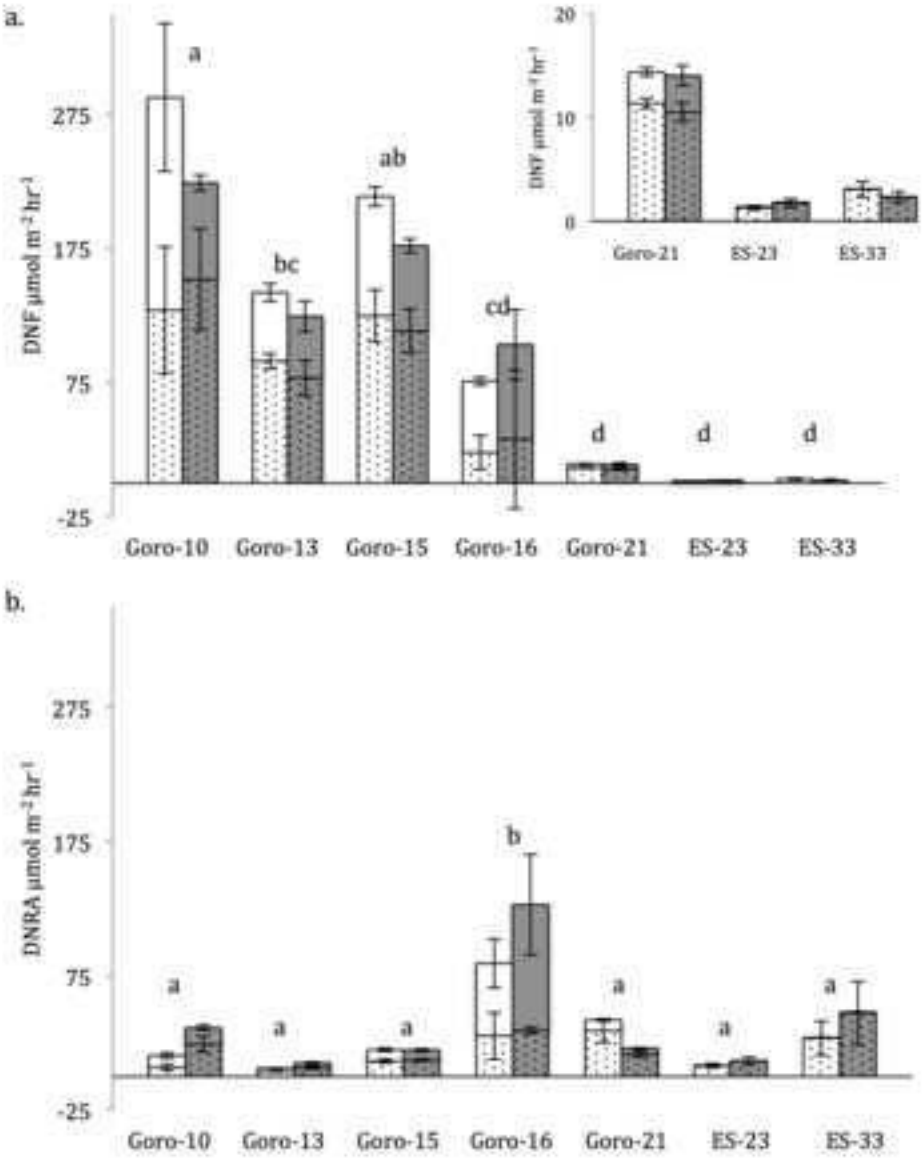

Study Area
Sacca di Goro
Adriatic Sea
Sampling Sites
Kilometers

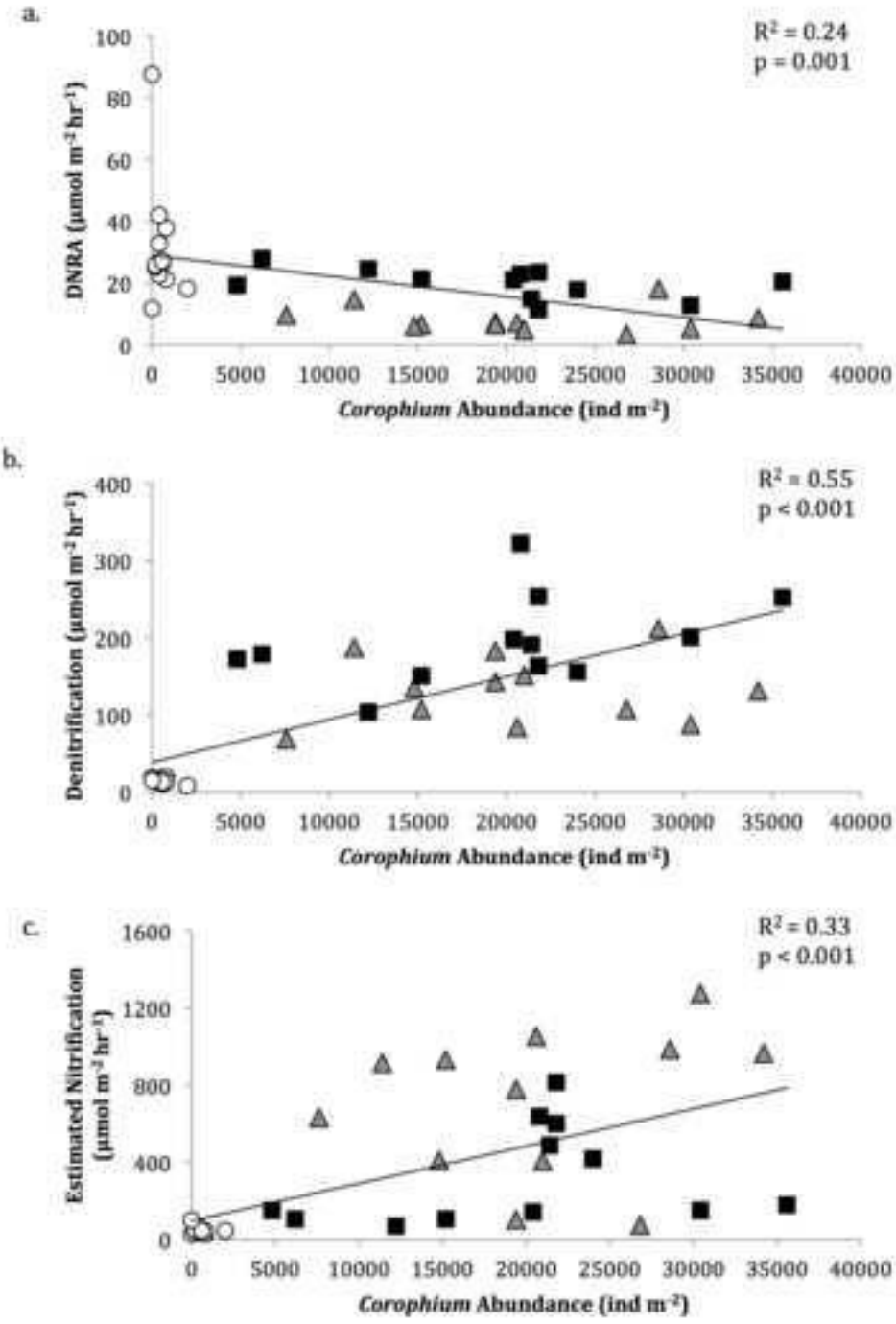
b.

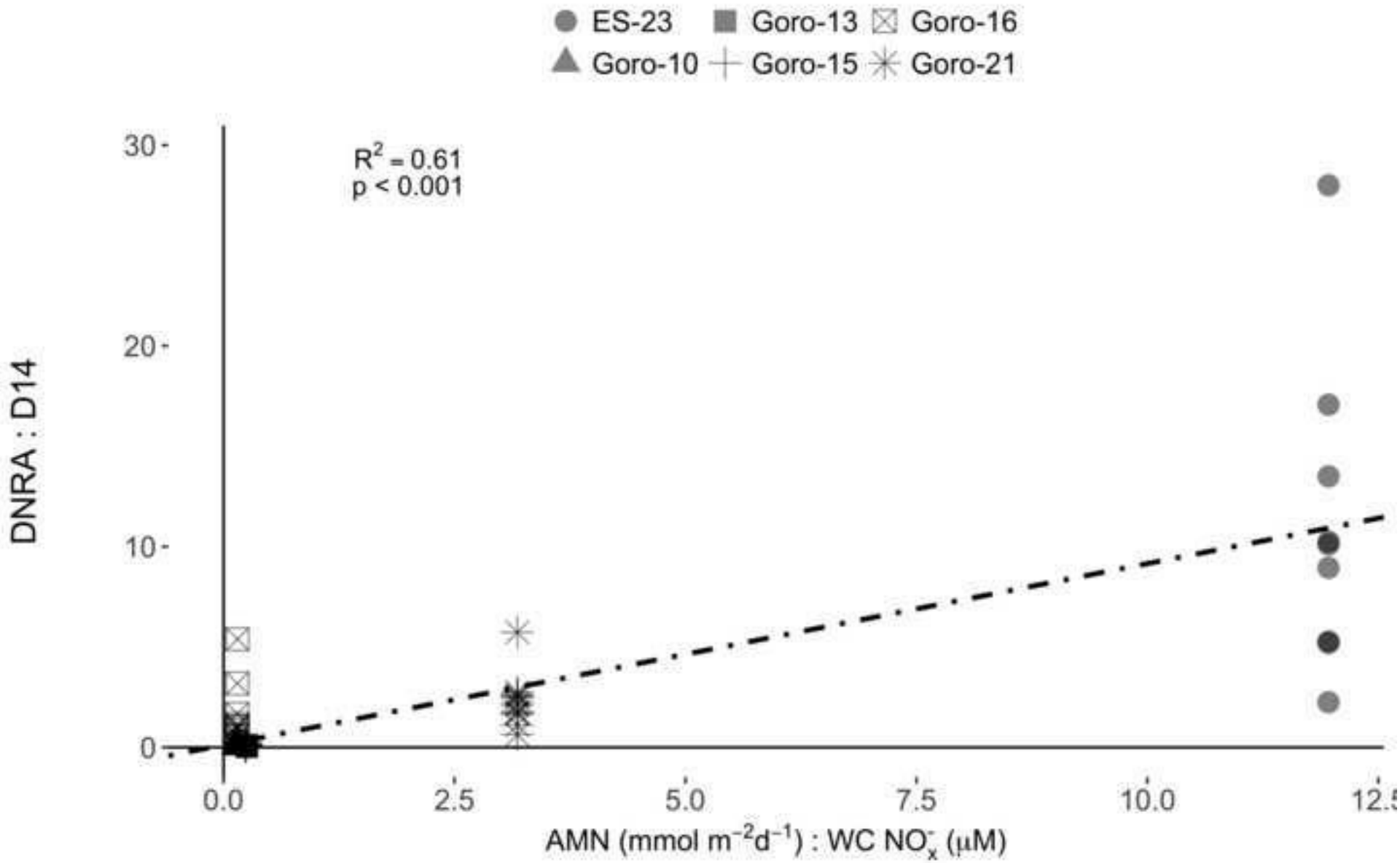

Study Area
Cherrystone Inlet
Smith Island Bay
Chesapeake Bay
Atlantic Ocean
N
0 5 10 20
Kilometers


a.
DO (mmol m-2 hr-1)
0
-5
-10
-15
-20
-25
a
bc
bc
ac
ac
a
a
b.
NH4+ (µmol m-2 hr-1)
2000
1500
1000
500
0
-500
c.
NOx- (µmol m-2 hr-1)
2500
1500
500
-500
a
b
ac
a
ac
c
c c
c c
c c
c c
50
30
10
-10
-30
Goro-21
ES-23
ES-33
Goro-10
Goro-13
Goro-15
Goro-16
Goro-21
ES-23
ES-33


A.
0
500
1000
1500
0
-10
-20
-30
DO Flux
(mmol O_2 m^{-2} hr^{-1})
B.
3000
2000
1000
0
-1000
NH_4^+ Flux
(µmol N m^{-2} hr^{-1})
0
500
1000
1500
Clam Biomass (g DW m^{-2})
ES-23
ES-33
Goro-10
Goro-13
Goro-15
Goro-16
Goro-21


a.
DNF µmol m-2 hr-1
275
175
75
-25
a
bc
ab
cd
d
d
d
Goro-10
Goro-13
Goro-15
Goro-16
Goro-21
ES-23
ES-33
20
10
0
Goro-21
ES-23
ES-33
b.
DNRA µmol m-2 hr-1
275
175
75
-25
a
a
a
b
a
a
a
Goro-10
Goro-13
Goro-15
Goro-16
Goro-21
ES-23
ES-33


a.
R² = 0.24
p = 0.001
DNRA (µmol m⁻³ hr⁻¹)
Corophium Abundance (ind m⁻²)
b.
R² = 0.55
p < 0.001
Denitrification (µmol m⁻³ hr⁻¹)
Corophium Abundance (ind m⁻²)
c.
R² = 0.33
p < 0.001
Estimated Nitrification (µmol m⁻³ hr⁻¹)
Corophium Abundance (ind m⁻²)


ES-23
Goro-13
Goro-16
Goro-10
Goro-15
Goro-21
$R^2 = 0.61$
$p < 0.001$
DNRA : D14
30
20
10
0
0.0
2.5
5.0
7.5
10.0
12.5
AMN (mmol $m^{-2}d^{-1}$) : WC NO_x^- (μM)