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Ribbed mussels *Geukensia demissa* enhance nitrogen-removal services but not plant growth in restored eutrophic salt marshes

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ABSTRACT: Salt marshes are decreasing worldwide. Restoration projects address marsh loss, yet it remains unclear how well restored marshes grow, expand, and function in eutrophic waters. Here, we explored how a facultative mutualism between Atlantic ribbed mussels *Geukensia demissa* and cordgrass *Spartina alterniflora* may enhance marsh growth and nitrogen cycling in a eutrophic setting. We created experimental plots in Jamaica Bay, NY, that contained live mussels, mussel shells, or no mussels (control). After 9 wk, we measured sediment and plant characteristics. We also collected sediment cores for use in continuous flow-through incubations with ambient site water and water enriched with stable isotope-labeled nitrate ($^{15}NO_3^-$). Denitrification in marsh plots with live mussels was significantly higher than the other treatments. Live mussels likely enhanced denitrification as biodeposits increased sediment organic carbon and anaerobic conditions. Mussel treatments did not impact cordgrass growth, possibly due to the eutrophic conditions of our study system or the duration of our trials. Ribbed mussels may be a valuable addition for salt marsh restoration projects in eutrophic estuaries since they increase the ecosystem service of nitrogen removal. Future work should focus on long-term effects of ribbed mussels on nitrogen removal and cordgrass biomass in restored marshes to determine how the mutualism impacts restoration success as sites age.

KEY WORDS: Denitrification · Mutualism · *Spartina* · Coastal restoration

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1. INTRODUCTION

Salt marshes provide many functions to human and ecological communities including protecting coastlines from erosion, providing shelter and food for diverse groups of fauna and flora, and altering nutrient storage and cycling (Costanza et al. 1997, Zedler & Kercher 2005, Deegan et al. 2012). Salt marshes have decreased 50 to 80% worldwide from historical levels due to anthropogenic influences (Grabowski et al. 2012), including the synergistic impacts of nitrogen (N) pollution, sea-level rise, dredging, reduced sediment input, and erosion (Hartig et al. 2002, Peteet et al. 2018). In salt marshes in the Northeastern Atlantic region of the USA, for example, large inputs of anthropogenic N have reduced organic matter storage and sediment stability, which worsens erosion (Deegan et al. 2012, Wigand et al. 2014). Rising sea levels threaten salt marshes when they are unable to grow vertically at the same rate as water levels rise, and vertical growth could be limited by high N levels (Watson et al. 2014).

Given the global decrease of marsh coverage and the resulting loss in ecosystem services they provide, marsh protection and restoration is a goal of many coastal conservation efforts. Salt marsh restoration is

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designed to prevent or remediate environmental damage by optimizing the delivery of critical ecosystem services (Gedan et al. 2009), such as intercepting polluted runoff (Shutes 2001) or mitigating effects of sea level rise (Erwin 2008). Recovery of marshes after anthropogenic disturbance is slow under natural conditions but can be accelerated through management practices that promote marsh growth or moderate negative impacts of environmental stressors (Broome et al. 1988). Various restoration methods have been developed for marsh restoration projects, such as altering hydrology, increasing elevation, and plantings (Broome & Craft 2009). Research is needed to evaluate the success of restoration efforts (Ruiz-Jaen & Mitchell Aide 2005, Staszak & Armitage 2013).

Species interactions can have major consequences for salt marsh growth and ecosystem processes (Silliman & Zieman 2001, Silliman et al. 2004, Silliman 2005, Angelini et al. 2016). For example, Atlantic ribbed mussels *Geukensia demissa* engage in a facultative mutualism with cordgrass *Spartina alterniflora*, the dominant plant in the low marsh throughout the eastern coast of North America (Bertness 1984). Ribbed mussels attach to the stems and roots of cordgrass with proteinaceous byssal threads, which increases the structural stability of the marsh surface. When mussels suspension-feed, they pump water over marsh substrate, oxygenating the sediments and alleviating plant stress from anoxia (Bertness 1984). The production of feces and pseudofeces, collectively known as biodeposits, can enhance cordgrass growth by increasing sediment nutrients. In return, cordgrass provides the mussels with refuge from predators and desiccation (Bertness & Grosholz 1985). Previous studies have shown that the mussel-cordgrass mutualism in salt marshes can enable the ecosystem to recover from or tolerate disturbances (Bertness et al. 2015) and enhance recovery from drought by increasing soil water storage and reducing soil salinity (Angelini et al. 2016).

The presence of ribbed mussels may also increase N removal and recycling in restored salt marshes by providing the substrates and environmental conditions required for denitrification (Kellogg et al. 2013, Bilkovic et al. 2017). Denitrification is the microbial respiratory process of using organic carbon (C) as an energy source and reducing nitrate (NO_3^-) or nitrite (NO_2^-) to nitrogen gas (N_2). Therefore, denitrification provides the important ecosystem service of permanent N removal from ecosystems (Seitzinger 1988). The majority of denitrifying bacteria are heterotrophs that use NO_3^- as an electron acceptor when O_2 becomes depleted. As a result, rates of denitrification are maximized by availability of organic C, NO_3^-, and anaerobic conditions (Davis et al. 2004). Bivalve biodeposition has previously been demonstrated to increase sediment C and N which can increase rates of denitrification (Piehler & Smyth 2011). Ribbed mussels and other bivalves may also affect denitrification via NH_4^+ excretion, diffusion of water column NO_3^- to sediments through burrows, and hosting denitrifying microbes in tissue or shells (Welsh & Castadelli 2004, Hoellein et al. 2015, Turek & Hoellein 2015, Welsh et al. 2015, Humphries et al. 2016, Bilkovic et al. 2017). Biodeposit decomposition along with bivalve respiration can increase local oxygen demand, which may lead to the formation of anoxic microsites that support denitrification (Giles & Pilditch 2006, Smyth et al. 2013). Bivalve suspension feeding may also aid in diffusing water-column O_2 to deeper sediments through burrows and promote coupled nitrification-denitrification (Nizzoli et al. 2006). For example, the combination of ribbed mussels and cordgrass resulted in higher denitrification rates in natural marsh sediments compared to when either were alone (Bilkovic et al. 2017).

The impacts of mussels on denitrification may be especially important for marsh restoration projects. Young salt marshes generally have low denitrification rates due to C and NO_3^- limitation (Tyler et al. 2003, Broome & Craft 2009). N fixation often occurs at greater rates than denitrification in young marshes (Piehler et al. 1998). However, as marshes age, N fixation decreases, and denitrification increases (Tyler et al. 2003). Denitrification rates in natural marshes can be up to 44-fold greater than in restored salt marshes due to oxygen inhibition at low tide and flushing of porewater nutrients at high tide in restored marshes (Thompson et al. 1995). Most ecological functions in restored marshes require 5 to 15 yr to reach the original levels of natural marshes, which is about the time needed to accumulate 1000 g C m^{-2} and 100 g N m^{-2} in the soil (Craft et al. 2003). This suggests that either recovery is slow or that restorations fail to recreate biological and physical factors important to marsh function. The mutualism between ribbed mussels and cordgrass could promote the growth of restored salt marshes and increase N removal.

The impact of ribbed mussels on salt marsh plants may depend on both marsh history and nutrient availability. Whereas past work has shown mussels may increase marsh recovery under oligotrophic and mesotrophic conditions (Bertness et al. 2015, Angelini et al. 2016, Derksen Hooijberg et al. 2018), we have no evidence to determine whether the same relationship would benefit restoration attempts in

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eutrophic ecosystems. Eutrophic conditions could alleviate N and C limitation for both cordgrass and denitrifying bacteria, thus reducing the role of mussel biodeposits and burrows in sustaining denitrification. Moreover, N loading has been found to favor aboveground plant growth over belowground growth (Deegan et al. 2012, Alldred et al. 2017), which would decrease sediment stability. A reduction in belowground plant biomass could affect marsh N cycling because the rhizosphere plays an important role in coupled nitrification-denitrification (McGlathery et al. 2007, Aoki & McGlathery 2018). Therefore, in eutrophic systems, mussel biodeposits may have no effect or a negative effect on plant growth and marsh stability.

To better understand the impact of mussels on marsh growth and denitrification in eutrophic systems, we created field plots with 3 treatments: live mussels, empty mussel shells, and no mussels. Plots were monitored for ~9 wk. We expected that C- and N-rich mussel biodeposits would provide a nutrient subsidy in this young restored marsh, which would lead to increases in aboveground and belowground biomass of marsh plants. We also performed a sediment core incubation study using cores from the field plots to examine how mussels impact denitrification under ambient and nitrate-enriched conditions in this young restored marsh. We hypothesized that the combined effects of mussel presence and biodeposition would increase denitrification compared to the control and mussel shell treatments.

2. MATERIALS AND METHODS

2.1. Study site

We conducted a study in 2017 at a young restored marsh in Jamaica Bay, NY, USA with the goal of determining the impacts of ribbed mussels *Geukensia demissa* on cordgrass *Spartina alterniflora* growth, sediment characteristics, and microbial N removal in eutrophic systems (Wigand et al. 2014). High N loads due to wastewater inputs are considered one of the main threats to the marshes, which are deteriorating at a mean rate of 13 ha yr^{-1} (Wigand et al. 2014). Ongoing restoration efforts seek to recover marsh area, along with the many ecosystem services that these marshes provided (Rafferty et al. 2011, Wigand et al. 2014, Campbell et al. 2017).

Yellow Bar Hassock (Fig. 1) is a large salt marsh island in Jamaica Bay (NY, USA) that declined 13% in area between 1959 and 1998 (Hartig et al. 2002) and

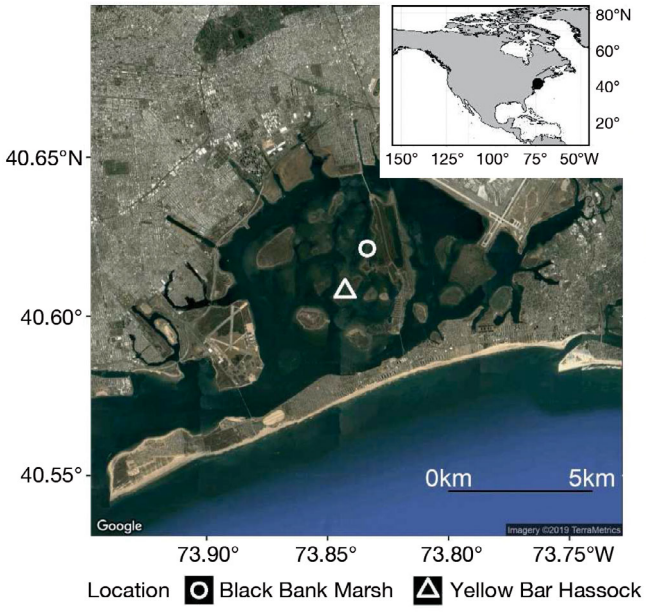

Fig. 1. Study area. Inset shows location in North America

then lost 0.9 ha yr^{-1} from 2003 to 2012 (Campbell et al. 2017). In 2012, ~286 700 m^3 of dredged sand from Ambrose Channel and Rockaway Inlet (NY Harbor) was transferred to the northern half (17 ha) of the island and graded to the desired elevation (Ravit et al. 2015). Cordgrass was then seeded in middle-elevation areas, while high-elevation areas were planted with *Spartina patens* and *Distichlis spicata* plugs (P. Rafferty, National Park Service, pers. comm.). Subsequent monitoring has shown that restoration at Yellow Bar successfully increased elevation and coverage of cordgrass (Campbell et al. 2017).

2.2. Experimental field plots

We established fifteen 0.25 m^2 experimental plots in the southwest corner of the restored portion of Yellow Bar (40.61° N, 73.83° W) in the summer of 2017. The plots were located near the marsh edge in 2 transects parallel to the edge of the waterline. Plots were spaced ~1 to 1.5 m from each other, and all were at a similar elevation. Plots were established so that they contained 18 to 23 naturally occurring cordgrass stems (mean ± SE: 79.47 ± 1.70 stems m^{-2}).

Plots were randomly assigned one of the following treatments: (1) cordgrass alone (control), (2) cordgrass with empty ribbed mussel shells embedded in the sediment, and (3) cordgrass with live ribbed mussels embedded in the sediment. Empty ribbed mussel shells used for the mussel shell treatment were

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sealed using marine epoxy. We applied the mussel shell plot treatment so that we could compare the physical impacts of mussels (shell and burrow) on sediment characteristics and processes (i.e. changes in friction velocity and organic matter accumulation) (Sanford & Chang 1997) to the effects of suspension-feeding and biodeposition of live mussels. The mussel shells and live mussels were collected from Black Bank Marsh, which is ~1 km north of Yellow Bar. Black Bank is a degraded marsh that has become fragmented due to loss of elevation and vegetation (Wigand et al. 2014, Campbell et al. 2017). Ribbed mussels used in the plots had a mean shell length of 70.85 mm (SE = 0.11 mm; n = 70). Live mussels and shells were pushed into the sediment so that ~50% of the shell was buried (Jost & Helmuth 2007, Bertness et al. 2015). Each of the live mussel and mussel shell plots had a density of 400 mussels (~1600 mussels m^{-2}). Although densities of 10 000 m^{-2} have been observed in the eutrophic Jamaica Bay (Franz 2001), salt marshes in the mid-Atlantic and northeastern USA commonly have ribbed mussel populations of 600 to 3000 m^{-2} (Bertness & Grosholz 1985, Bilkovic et al. 2017). Therefore, the experimental density used in this study, while similar to densities observed in other ecosystems, likely represents both the lower limits of natural population sizes in Jamaica Bay and an achievable restoration goal.

2.3. Plant and sediment data collection

Cordgrass and sediment characteristics were measured on 22 June 2017 to ensure initial similarity among plots. We recorded stem density and the height of 5 randomly selected stems (distance from the sediment surface to the tip of the cordgrass stem) for each plot. A sediment sample was collected from each plot with a modified 25 mm diameter syringe to a depth of 3 cm and brought back to our laboratory at Baruch College for analysis. Each sample was homogenized and then subsampled to measure sediment characteristics. Subsamples were dried at 60°C until a constant weight, and then re-weighed to determine bulk density, percent moisture, and porosity. Sediment organic matter (OM) was determined following loss on ignition at 500°C (Benfield 2007). Sediment total organic C and total N was determined by treating samples with 25% HCl and redrying at 60°C (Nieuwenhuize et al. 1994) before analysis using a Series II 2400 CHN Analyzer (Perkin Elmer Life and Analytical Sciences, Shelton, CT) with acetanilide as a standard.

Treatments were then established by adding live mussels and mussel shells on 19 to 21 July 2017. Established plots were monitored for ~9 wk. We measured stem heights and stem densities and analyzed sediment data again on 14 August 2017 using the procedures noted above. At the conclusion of the experiment (29 September 2017), we recorded stem density and heights for all cordgrass stems from each plot, then cut all stems at the sediment surface. Aboveground biomass was determined after drying cordgrass at 60°C for at least 48 h. We reserved 2 leaf fragments from each plot to measure the C:N ratio. Leaf tissue was cut to 5 cm long sections, dried at 60°C until a constant weight, ground into homogenous samples using a mortar and pestle, and analyzed for C and N content as described above. We sampled belowground biomass (i.e. roots and rhizomes) by inserting 7.6 cm diameter acrylic cores into marsh sediments to a depth of 15 cm. Three replicate cores were taken from each plot (n = 45). Belowground material was wet-sieved through a 1.0 mm mesh sieve to remove sediment, dried at 60°C until a constant weight, and weighed to determine biomass. Dried belowground material was ground into homogenous samples to measure C and N content. These samples were also used to analyze sediment characteristics using the procedures noted above.

2.4. Benthic nutrient and gas fluxes from continuous-flow core incubations

We measured nutrient and gas fluxes using intact sediment cores from each plot in continuous-flow incubation studies (Hoellein et al. 2015, Bilkovic et al. 2017, Zarnoch et al. 2017). Intact sediment cores (30 cm long × 7.6 cm diameter) were collected from each of the plots on 19 September 2017 (2 mo after plot establishment) using a PVC coring device (Gardner et al. 2006) and brought back to the laboratory. Each core contained ~15 cm of sediment along with 3 mussels (applicable to only mussel shell and live mussel treatment cores; equivalent to 661.3 mussels m^{-2}) and cordgrass. Live mussels had a mean (±1 SE) shell length of 70.2 ± 1.72 mm and tissue dry mass of 0.8 ± 0.05 g. The mean (±1 SE) shell length of shells used in the mussel shell treatment was 71.5 ± 2.10 mm. Stems in the cores were cut and plugged with silicone gel to reduce oxygen and organic C leakage (Caffrey et al. 2007). All cores were capped with gas-tight lids and maintained at *in situ* water temperatures (22°C) for 48 h. We first introduced

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ambient site water (NO_x^- = 8 µmol l^{-1}) to cores at a flow rate of 1.1 ml min^{-1} for 24 h (ambient incubation hereafter) to determine net nutrient uptake and net denitrification. After the initial incubation, we enriched the site water with $^{15}NO_3^-$ for 24 h to determine total denitrification and N fixation (final NO_x^- concentration = 24 µmol l^{-1}). After the incubations were complete, 10 ml sediment samples were collected from each core and analyzed for OM and C:N content as previously described.

We determined concentrations of soluble reactive phosphorus (SRP), NH_4^+, and NO_x^- for water samples collected from inflow carboys and from outflow samples of each core. Samples were filtered through a 0.2 µm nylon filter (Thermo Scientific) into three 20 ml scintillation vials and frozen until analysis. Samples were analyzed following established protocols (SRP: antimonyl tartrate method following Murphy & Riley 1962; NH_4^+: phenol, hypochlorite method following Solorzano 1969; NO_x^-: cadmium reduction method following APHA 1998) with a Seal AQ2+ discrete nutrient analyzer (Seal Analytical).

Samples for dissolved gases ($^{28}N_2$, $^{29}N_2$, $^{30}N_2$, $^{32}O_2$, and ^{40}Ar) were collected directly from carboys for inflow measurements, and outflows dripped directly into triplicate 12 ml Labco Exetainer® vials (Lampeter) so that vials overflowed 3-fold the volume. Samples were preserved with 200 µl of 50% zinc chloride, then capped and stored underwater at 4°C. Samples were analyzed using membrane inlet mass spectrometry (MIMS; Bay Instruments; Kana et al. 1994) at Loyola University, Chicago, IL. The standard used for the MIMS was artificial seawater held at 22°C and a salinity of 27.2 (circulating bath, VWR International), stirred at a low speed to equilibrate to atmospheric gases (Lab Egg RW11 Basic, IKA Works). O_2 and N_2 concentrations were determined by using the ratio with Ar following standard protocols (Kana et al. 1994, Hoellein et al. 2015). MIMS measurements were corrected for instrument drift by interspersing standards throughout the run.

Nutrient (SRP, NH_4^+, NO_x^-) and gas (O_2, N_2) fluxes were calculated by subtracting the concentration in the outflow from the concentration in the inflow, multiplying by the pump flow rate, and dividing by the surface area of the core (flux units = µmol element m^{-2} h^{-1}). The $^{28}N_2$ data were used to determine the net N_2 flux of the control incubation, whereas the sum of dissolved gases of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ were used to calculate total denitrification in the $^{15}NO_3^-$ enriched incubation. The production of $^{29}N_2$ and $^{30}N_2$ in the enriched incubation was considered as an index of direct denitrification of the $^{15}NO_3^-$. We also calculated nitrification, percentage of coupled nitrification-denitrification, and denitrification efficiency for each core, focusing on $^{28}N_2$ flux for ambient cores and the sum of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ fluxes for enriched cores. Nitrification was calculated by summing the NO_x^- and N_2 fluxes (Kellogg et al. 2013). The percentage of coupled nitrification-denitrification was calculated by dividing the calculated nitrification by the N_2 fluxes and multiplying by 100%. If NO_x^- fluxes were positive, we assumed all denitrification was coupled (Gonzalez et al. 2013). Lastly, denitrification efficiency was calculated by dividing N_2 efflux by the sum of the N_2, NH_4^+, and NO_x^- effluxes (only positive values used) and multiplying by 100 (Eyre & Ferguson 2009).

2.5. Statistical analysis

One-way ANOVAs with treatment as a factor were used to assess data collected at one point per sampling period for each plot or core (plot stem density and aboveground biomass, June and July sediment data, core sediment data). Mixed-effect models with treatment as a fixed factor and sampling unit as a random effect were used to assess data collected at multiple points per plot per sampling period (stem heights, belowground biomass and plant C:N, September sediment data) or multiple times from a core (core incubation flux data) to account for within sampling unit variation and pseudoreplication (Zuur 2009, Bates 2010). Residuals were visually checked for normality and homogeneity among groups for all models (Zuur 2009, Zuur et al. 2010); data were transformed when needed to meet these assumptions. The p-values were found by analyzing Type III sums of squares for models (Fox & Weisberg 2011). We considered p-values ≤ 0.05 to be statistically significant. When significant differences were found among treatments, we ran post hoc tests using the Tukey method (Lenth 2016) to compare treatment means. Relationships among OM, sediment oxygen demand, and denitrification in ambient cores were also analyzed using linear regression. Residuals were checked as noted above.

Data were analyzed using R v.3.6.1 (R Core Team 2019). Graphs and figures were produced in R using the 'ggplot2'and 'ggmap' packages, and the 'plyr' and 'reshape' package were used for data manipulation (Wickham 2007, 2009, 2011, Bivand et al. 2016). Map data were provided by Google Earth and Natural Earth.

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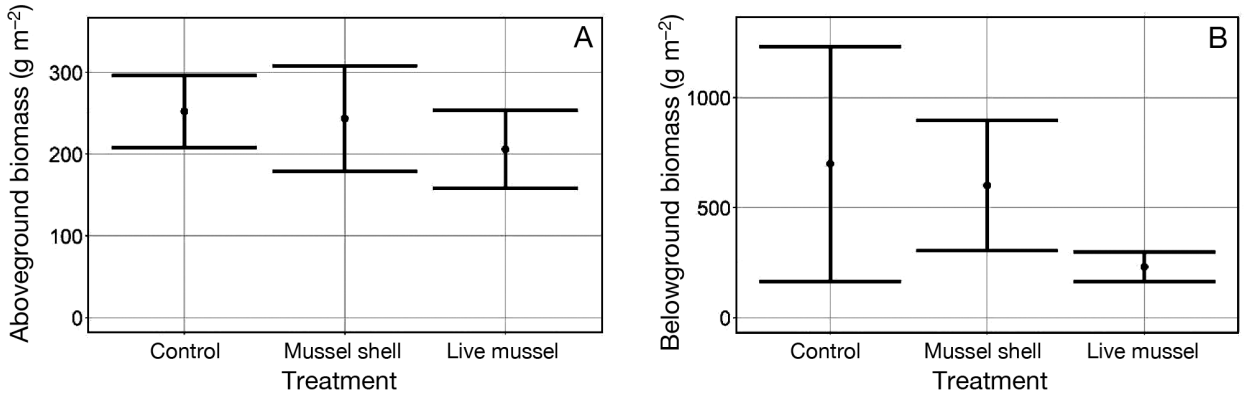

Fig. 2. (A) Aboveground biomass collected from plots at conclusion of experiment (n = 5 per treatment), and (B) belowground biomass collected from plots at conclusion of experiment (n = 15 per treatment). Dots represent mean, and error bars represent standard error

3. RESULTS

3.1. Initial plot measurements

No significant differences were observed in stem density ($F_{2,12}$ = 0.659, p = 0.535, total mean = 79.5 stems m^{-2}) or stem height (χ^2_2 = 0.459, p = 0.795, total mean = 23.7 cm) for salt marsh plants among the study plots prior to the start of the mussel *Geukensia demissa* manipulation. Sediment conditions also showed no significant differences among study plots (bulk density: $F_{2,12}$ = 0.860, p = 0.448, total mean = 1.12 g cm^{-3}; % moisture: $F_{2,12}$ = 0.389, p = 0.686, total mean = 0.12%; porosity: $F_{2,12}$ = 0.425, p = 0.663, total mean = 26%; OM: $F_{2,12}$ = 0.036, p = 0.964, total mean = 0.64%; C:N: $F_{2,12}$ = 0.885, p = 0.438, total mean = 7.97).

3.2. Impact of treatments on cordgrass *Spartina alterniflora* and sediment characteristics

We focused our methods and analysis on data collected in September, which was ~9 wk after treatments were established, due to the short time period between the July and August sampling dates. Throughout the experiment, overall mean stem density across all plots increased from 79.5 to 319.2 stems m^{-2}, and mean stem height increased from 23.7 to 39.0 cm. However, we found no treatment impact on stem density ($F_{2,12}$ = 0.276, p = 0.763), stem height (χ^2_2 = 1.719, p = 0.423), or aboveground biomass ($F_{2,12}$ = 0.216, p = 0.808; Fig. 2A). Although not significant ($F_{2,12}$ = 3.222, p = 0.076), we noted the C:N molar ratio of leaf tissue appeared to differ strongly among treatments, with the largest difference existing between control and mussel shell plots. Treatments did not impact belowground biomass (χ^2_2 = 0.820, p = 0.663; Fig. 2B); log-transformed data were also analyzed due to differences in variance among treatments but yielded the same result (χ^2_2 = 0.509, p = 0.775). We also noted a trend for treatment impact on belowground C:N molar ratio (χ^2_2 = 4.683, p = 0.096), with a slightly higher C:N ratio observed in the control plots (measures for each treatment type provided in Table A1 in the Appendix).

Sediment OM differed significantly among treatments (χ^2_2 = 15.842, p < 0.001; Fig. 3) and was significantly higher in live mussel plots compared to control (t = –3.133, p = 0.022) and mussel shell plots (t = –3.693, p = 0.008). We found no effect of the treatments on bulk density (χ^2_2 = 1.005, p = 0.605, total mean = 1.07 g cm^{-3}), moisture (χ^2_2 = 1.316, p = 0.518, total mean = 0.12%), porosity (χ^2_2 = 1.281, p = 0.527, total mean = 25%), or C:N (χ^2_2 = 1.652, p = 0.438, total mean = 7.34).

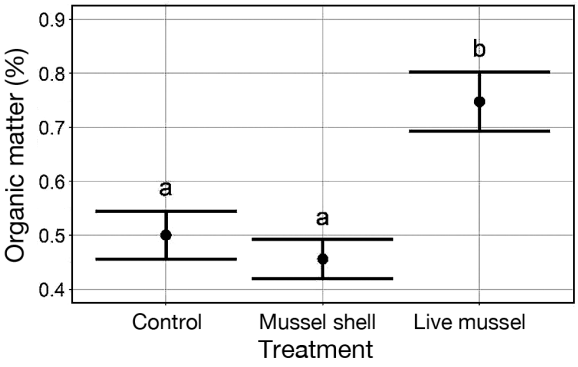

Fig. 3. Percent sediment organic matter at the end of the experiment. Sediment organic matter was higher in live mussel plots than control or mussel shell treatments ~9 wk following mussel introductions. Dots represent the mean, and error bars represent standard error (n = 15 per treatment). Different letters indicate treatments which are significantly different

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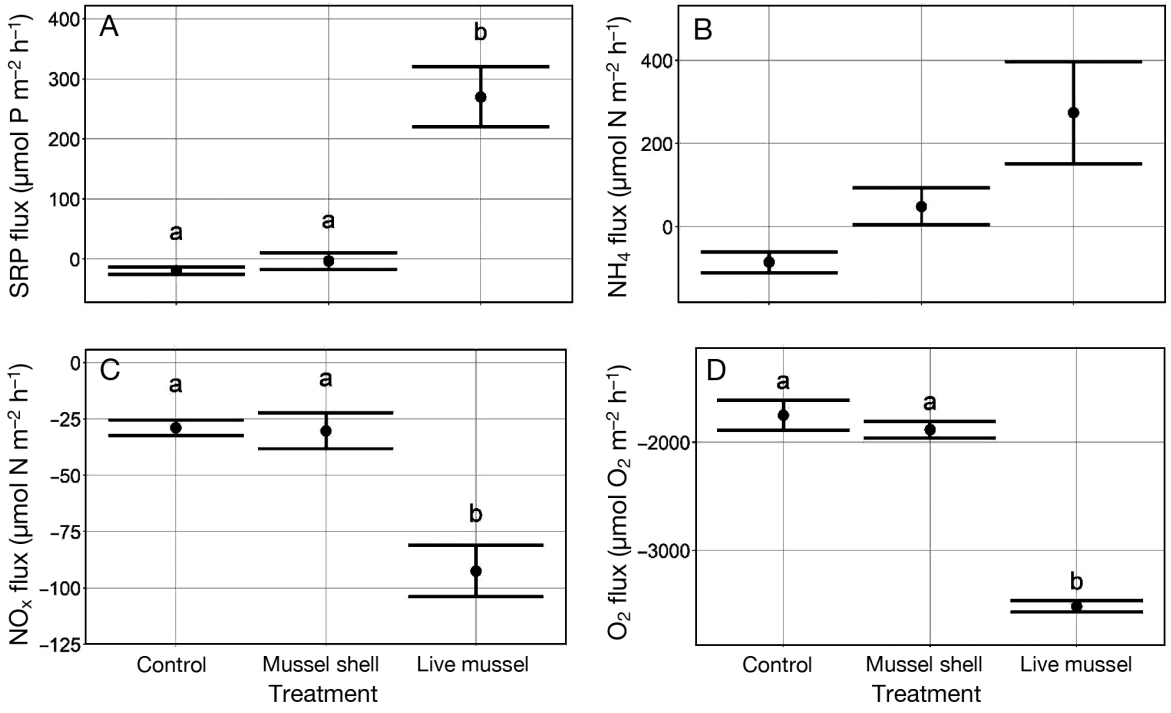

Fig. 4. Mean (±1 SE) flux of (A) soluble reactive phosphorus (SRP), (B) NH_4^+, (C) NO_x^-, and (D) O_2 from continuous-flow core incubations under ambient conditions. Dots represent mean, and error bars represent standard error (n = 15 per treatment). Different letters indicate treatments which are significantly different

3.3. Continuous-flow core incubations

Live mussels had a significant effect on the fluxes for 2 of the 3 solutes measured. We found significant differences among treatments for fluxes of SRP (χ^2_2 = 18.287, p < 0.001; Fig. 4A) and NO_x^- (χ^2_2 = 17.596, p < 0.001; Fig. 4C) in the ambient core incubations. Post-hoc analysis indicated that live mussel plots had significantly greater SRP efflux and NO_x^- uptake than control and mussel shell plots (post-hoc tests, all p < 0.01). In contrast, NH_4^+ fluxes did not differ statistically among plot treatments but were highest in the live mussel plots (χ^2_2 = 3.391, p = 0.184; Fig. 4B).

Gas fluxes also showed significant effects of the mussel treatments. Oxygen fluxes were significantly different among treatments (χ^2_2 = 19.249, p < 0.001; Fig. 4D), with live mussel plots showing greater net O_2 consumption than the control and mussel shell treatments (all p < 0.009). For N_2 flux, there was a significant interaction between incubation (ambient vs. enriched) and plot (control, mussel shell, or live mussel) treatments (χ^2_2 = 36.038, p < 0.001; Fig. 5) that was driven by differences in N_2 flux from live mussel and mussel shell plots between incubation treatments. Examining the ambient and enriched results separately showed higher denitrification rates in live

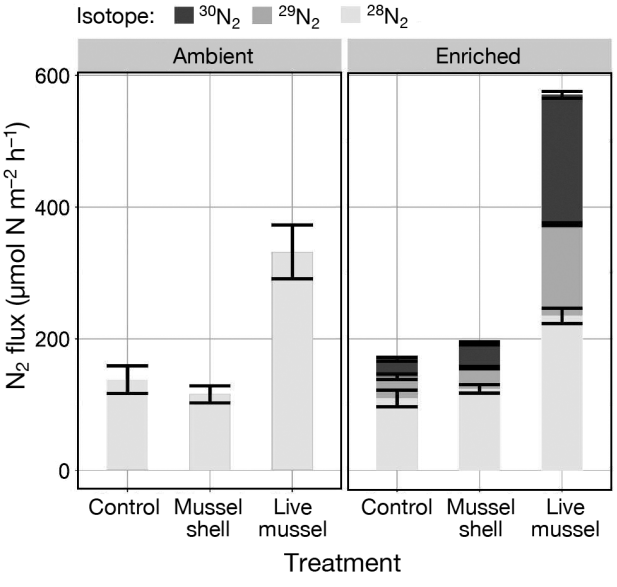

Fig. 5. N_2 flux including contributions of $^{28}N_2$, $^{29}N_2$, and $^{30}N_2$ fluxes from ambient and enriched trials. Bars represent mean and error bars represent SE (n = 45 per isotope per treatment)

mussel plots within both incubations (ambient: χ^2_2 = 9.711, p = 0.008; enriched: χ^2_2 = 47.582, p < 0.001). In the ambient core incubation, live mussel plots had significantly higher $^{28}N_2$ efflux than the mussel shell

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treatment (t = −2.832, p = 0.037). We also noted the live mussel plots tended to have a higher $^{28}N_2$ efflux than the control treatment, though the results were not significant (t = −2.543, p = 0.062). In the enriched core incubation, live mussel plots had significantly greater total N_2 efflux than both the control (t = −6.148, p = 0.001) and mussel shell plots (t = −5.783, p = 0.002). We measured N fixation (11 µmol N m^{-2} h^{-1}) in only 1 core from the control treatment during the enriched trial. The live mussel plots had the highest nitrification rate, lowest DN efficiency, and lower coupled nitrification-denitrification than control plots (Table 1).

We detected a significant relationship between sediment oxygen demand and sediment OM under ambient conditions ($F_{1,13}$ = 6.993, R^2 = 0.350, p = 0.020; Fig. 6A). Regression results also showed a significant positive relationship between N_2 flux and sediment oxygen demand from the ambient trial ($F_{1,13}$ = 13.695, R^2 = 0.513, p = 0.003; Fig. 6B).

Table 1. Mean (standard error) of calculated nitrification rate, percent of denitrification coupled to nitrification, and denitrification efficiency from ambient trial. Treatments include the control plots, empty mussel shell plots, and live mussel plots. n = 5 per treatment

Treatment	Nitrification rate (µmol N m^{-2} h^{-1})	Coupled nitrification-denitrification (%)	Denitrification efficiency (%)
Control	109.09 (43.49)	84.35 (5.32)	83.31 (16.69)
Mussel shell	85.59 (22.85)	62.52 (15.27)	72.37 (13.75)
Live mussel	239.65 (80.64)	65.09 (8.34)	0.96 (16.84)

4. DISCUSSION

Our results demonstrate the importance of ribbed mussels *Geukensia demissa* for enhancing the ecosystem service of N removal via denitrification at a recently restored salt marsh in eutrophic Jamaica Bay, NY. However, contrary to previous studies, we found no evidence that mussels enhance plant growth, and thus sediment stability, in this system. Our findings with respect to mussel effects on N removal are consistent with an experiment conducted in a natural marsh, which also detected significant positive effects of ribbed mussels on microbial denitrification rates (Bilkovic et al. 2017). Rates from both studies were similar (~411 µmol N m^{-2} h^{-1} in Bilkovic et al. 2017 and ~350 µmol N m^{-2} h^{-1} in this study), which was unexpected as this study was performed at a young restored marsh (5 yr) while the other measurements were from mature, natural marshes. Studies at restored marshes have typically found that N fixation is predominant over denitrification during early development (Currin & Paerl 1998) and that young marshes could take >15 yr before N-removal services matched those of natural reference marshes (Broome & Craft 2009). We attribute the high rates of denitrification in our study to the highly eutrophic conditions of Jamaica Bay. The abundance of mineralized N and labile C inputs could be sufficient to promote denitrification, even in young, primarily inorganic marsh sediments.

Contrary to previous studies, and despite compelling reasons to believe that plants would benefit from mussels in newly constructed marshes, we found no evidence that mussels enhanced cordgrass *Spartina alterniflora* biomass. It is possible that our results were simply due to the short duration of our study,

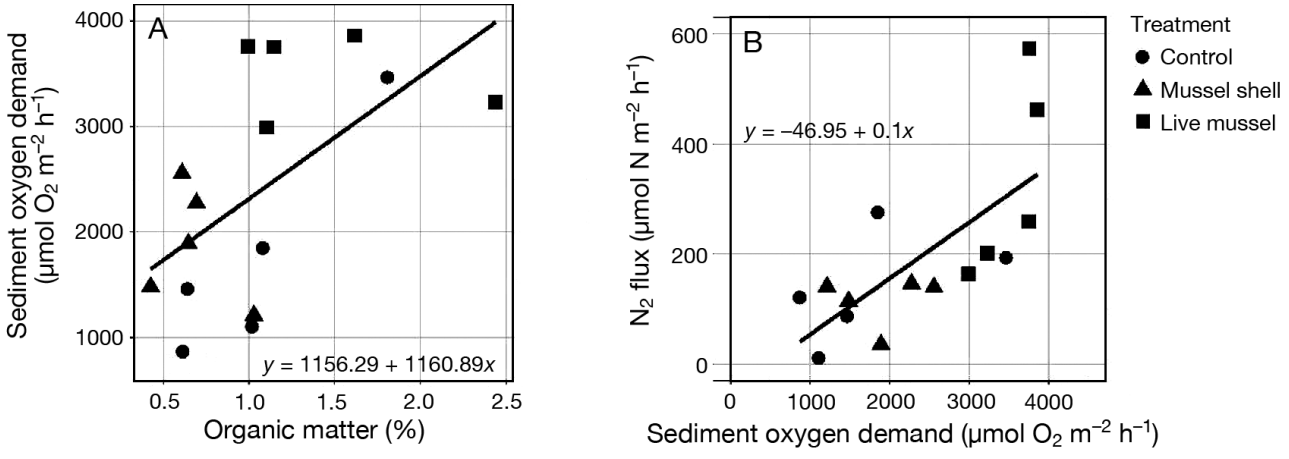

Fig. 6. Linear regression of (A) sediment oxygen demand and sediment OM and (B) net N_2 flux and sediment oxygen demand from the ambient incubation

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though other studies have detected significant effects within a single growing season (Bertness et al. 2015, Crotty & Bertness 2015). However, it is possible that the nature of the mussel-cordgrass mutualism is contingent on nutrient limitation, which may be absent in an urban, eutrophic system like Jamaica Bay. Eutrophic conditions at our study sites may alleviate N-limitation of plant growth and remove the benefit of mussel biodeposition for the plants. As the sediments in the restored marsh were dominated by sand, it is also possible that over a longer period (i.e. >5 yr) mussel biodeposits may enhance organic C accumulation and result in greater retention of nutrients in sediments and enhanced plant growth. Overall, it is unclear if the high N loads into Jamaica Bay decouple the benefit of mussels for cordgrass just as other forms of disturbances have impacted mutualistic interactions (Palmer et al. 2008, Hoek et al. 2016, de Fouw et al. 2018). Measuring these effects will likely take follow up studies that persist over longer time periods to better capture the potential mutualistic relationship in restored marsh ecosystems. For example, low oxygen and/or acidic conditions may reduce mussel suspension feeding (Clements & Darrow 2018), which can alter their interaction with cordgrass. Though not significant, our data also revealed the potential for a negative trend in belowground plant biomass with mussel addition (Fig. 2B), which is worthy of investigation in future studies. From these results, we can conclude that the addition of mussels to marsh restoration programs in eutrophic ecosystems may enhance N removal via denitrification but may not enhance cordgrass biomass in the short term.

4.1. No effect of mussels on cordgrass biomass

We expected that vegetation within a newly constructed marsh would be N-limited due to its young age, low sediment organic matter, and coarse sediments with low nutrient retention. This would then result in the mussel addition positively affecting cordgrass biomass. Instead, our results indicate that the addition of live ribbed mussels and mussel shells had no influence on cordgrass biomass. Positive feedbacks between ribbed mussels and cordgrass have been shown to enhance salt marsh resilience and marsh recovery (Bertness 1984, Crotty & Bertness 2015, Angelini et al. 2016, Derksen Hooijberg et al. 2018). For example, mussels reduced soil salinity stress, increased cordgrass aboveground growth, and promoted survival during drought (Angelini et al. 2016). In addition, previous transplant experiments in a southeastern US salt marsh found cordgrass growth and clonal expansion increased by 50% due to mussel presence because co-transplanted mussels increased nutrients in the porewater and reduced sulfide stress (Derksen Hooijberg et al. 2018) and that mussels led to a 94% increase in belowground biomass (Derksen Hooijberg et al. 2019). It is possible we did not see an increase in cordgrass biomass in response to the mussel addition due to the short time frame of our experiment. The previous study was conducted over a period of >16 mo (Derksen Hooijberg et al. 2018), while our study was performed within a single plant growing season (3 mo). However, other studies have shown responses to positive interactions between mussels and cordgrass over a period of 2 (Crotty & Bertness 2015) or 4 mo (Bertness 1984). The more likely explanation is that the eutrophic conditions of our study system may have reduced positive feedbacks between mussels and cordgrass. If true, then the impacts of mussels on cordgrass biomass may be context-dependent.

Fertilization experiments simulating high N loadings (Valiela et al. 1976, Turner et al. 2009, Deegan et al. 2012) and field measurements across N loading gradients (Darby & Turner 2008, Alldred et al. 2017) demonstrated that N addition increases aboveground biomass and reduces belowground biomass in cordgrass. This effect may occur because fertilized cordgrass requires fewer roots to obtain N to support growth of aboveground biomass. For example, Alldred et al. (2017) found 60 to 70% less belowground biomass in marshes with high dissolved inorganic N across a land-use gradient in Long Island, NY. If fertilization does reduce belowground plant growth, we would expect mussel additions to enhance marsh growth and stability in oligotrophic or mesotrophic estuaries, while their addition may have no benefit for plant growth in eutrophic estuaries and may in fact reduce sediment stability by reducing root growth (Fig. 2). Experiments at a greater number of eutrophic marshes, conducted over longer time frames, will be required to distinguish among these possible outcomes.

In this study, above- and belowground biomass measurements were low compared to natural marshes, consistent with a relatively young restored marsh (Craft et al. 1999, 2003). A degrading natural marsh located ~1.3 km from our study site had reduced belowground biomass and a high above: belowground biomass ratio compared to a stable marsh in Jamaica Bay (Wigand et al. 2014), which is consistent with the previous studies. Jamaica Bay is a sediment-limited system, and marsh elevation is sus-

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tained primarily through organic matter accumulation (Peteet et al. 2018). Future studies should document above- and belowground biomass at restored marshes in Jamaica Bay over a longer time period to understand the combined effect of N loading and organic matter accumulation.

Sediment characteristics that change over long time scales with marsh maturity may affect the role of mussels in providing plants with nutrients in restored marshes of Jamaica Bay and other eutrophic locations. Our analyses showed live mussels did not increase above- and belowground biomass and only led to slight changes in C:N ratios, suggesting the cordgrass may not have been N-limited. The cordgrass likely received adequate N through water column N, organic matter deposition, and sediment N recycling. As the marsh ages, we may observe a shift towards greater reliance upon biogenic processes that could alter cordgrass allocations to growth, sediment N recycling, and competition for N with sediment microbes. These changes with marsh maturity may also affect the role of mussels within the mutualistic interaction.

4.2. Mussel impact on denitrification

Our results suggest that mussel biodeposition was likely responsible for increases in denitrification in plots containing live mussels. Sediment OM concentrations and denitrification rates in live mussel plots were double those in control and mussel shell plots (Figs. 3 & 5, respectively). This result supports our hypothesis that mussel biodeposition enhances conditions for denitrifying microbes by increasing sediment C and creating a more anaerobic environment. Denitrification is likely to be strongly limited by organic C in young restored marshes like the one examined in this study, as sediments are typically lower in organic content than those of mature marshes (Craft et al. 2003). Decomposition of biodeposits likely altered sediment redox conditions to promote denitrification (Poulin et al. 2007). The positive relationship between organic matter and sediment oxygen as well as the positive relationship between sediment oxygen demand and net N_2 flux support this argument. Sediment oxygen demand and SRP efflux were highest in the live mussel plots, which suggests that these plots had the most reduced sediment conditions (Fig. 4). The SRP fluxes observed in the control and mussel shell treatments were consistent with previous measurements in coastal sediments (−25 to 100 µmol P m^{-2} h^{-1}; Boynton et al. 2018), while the higher rates of SRP efflux (270 µmol P m^{-2} h^{-1}) from the live mussel plots suggest that anoxic conditions promoted SRP desorption from iron oxides and/or transformation to iron sulfate (Kemp et al. 2005).

The increase in $^{29}N_2$ and $^{30}N_2$ flux in the mussel shell and live mussel treatments (Fig. 5) may have been due to the biological effects and physical presence of the mussels altering environmental conditions. We found that the total N_2 flux in the control treatment changed −4 % due to enrichment, 55 % in the mussel shell treatment, and 63 % in the live mussel treatment (Fig. 5). The lack of response in the control treatment indicates that the denitrifying community was not NO_3^--limited in the absence of mussels. Our calculations show that 84 % of the measured denitrification in the control plots was coupled to nitrification, so NO_3^- demand from denitrifying bacteria was primarily met by nitrification. In addition, water column NO_x^- was 8 µmol l^{-1} during the control incubations, which could support direct denitrification (Seitzinger et al. 2006). The presence of mussels on the marsh surface likely reduced horizontal flux of O_2 into the sediment due to lower flow velocity. This would then increase anaerobic microsites and promote denitrification potential. Similarly, altered friction velocity over oyster reefs likely creates microsites that support high rates of denitrification (Sanford & Chang 1997, Kellogg et al. 2013, Humphries et al. 2016). Other studies have found that bivalve burrows or the presence of shell hash could enhance direct denitrification as it increases the sediment surface area (Hoellein et al. 2015, Turek & Hoellein 2015).

The diffusion of NO_3^- through mussel burrows may have also increased $^{29}N_2$ and $^{30}N_2$ fluxes in the mussel shell and live mussel treatments. We note, however, that N_2 and NO_x^- fluxes were similar between the control and mussel shell treatments in the ambient trial. This suggests that NO_3^- diffusion through mussel burrows alone could not enhance denitrification and that enriched conditions coupled with anaerobic sediment would more likely lead to the increase in $^{30}N_2$ production in the mussel shell treatment during the enriched trial. However, if active suspension feeding, as opposed to the existence of burrows, is the main pathway through which NO_3^- diffuses into the sediment, our mussel shell plots would not have captured this effect. Other organisms (e.g. fiddler crabs *Uca pugnax*; Bertness & Grosholz 1985, Laverock et al. 2011) that are more active bioturbators can impact the diffusion of oxygenated water into the sediment and increase the exchange of solutes from the water column to sediment. Similarly, mussel suspension

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feeding may facilitate transport of C-rich biodeposits to sub-surface, anaerobic sediments where denitrifiers commonly occur (Norkko & Shumway 2011). The strength of these effects is likely to be context- and location-dependent. For example, mussels under higher tidal or thermal stress may form larger mounds or burrow deeper into the substrate. In such cases, diffusion may become a more important factor in their effect on salt marsh dynamics.

We also found that in the mussel shell plots, only 63% of denitrification was coupled to nitrification as compared to 84% in the control treatment. This difference is likely due to nitrification being inhibited by O_2 availability in the mussel shell treatment. Calculated nitrification was highest in the live mussel plots, likely due to NH_4^+ excretion and ammonification. Bruesewitz et al. (2008) found that the NH_4^+-rich waste of zebra mussels increased sediment nitrification, which increased NO_3^- availability for denitrification. Collectively, these results suggest that mussel additions to young restored marshes may enhance denitrification by alleviating the limitations of C availability and creating anaerobic conditions.

4.3. Implications for management

Study results indicated that mussel additions to a restored, eutrophic salt marsh in Jamaica Bay, NY did not increase cordgrass biomass at this stage of marsh maturity over a period of a single growing season. However, the mussel-cordgrass interaction may provide other important benefits for marsh restoration projects. For example, mussel addition increased sediment organic matter, which is a critical component of ecosystem structure and growth in the restored salt marshes in Jamaica Bay. Study plots with live mussels had ~200% greater levels of organic matter compared to control plots after only 9 wk of treatment. Since Jamaica Bay marshes rely upon organic matter deposition as compared to mineral sediments to sustain themselves (Peteet et al. 2018), mussel biodeposition may be a subsidy to maintaining marsh elevation (Smith & Frey 1985). Mussel C deposition is likely to be important to the development of restored marshes as well (Craft et al. 2003) and may contribute to C sequestration (i.e. blue carbon).

In addition to benefits for organic matter deposition, mussel addition increased denitrification, which is a valuable ecosystem service (Vaughn & Hoellein 2018). For example, mussel addition increased denitrification 140% compared to the control plots (ambient NO_3^- = 8 µmol l^{-1}), and denitrification in mussel-amended plots increased 235% under enriched conditions (NO_3^- = 24 µmol l^{-1}). Together these results suggest that mussels support higher baseline rates of sediment denitrification and allow sediment microbes to respond quickly to pulses of water-column nutrients, such as we would expect to observe following sewage-overflow events. Water column NO_3^- varies seasonally in Jamaica Bay and often exceeds enriched conditions used in our study (Hoellein & Zarnoch 2014). Including mussels in marsh restoration programs will significantly increase the ecosystem service of N removal in restored marshes even in their early stages of development. Mussels, like other bivalves, also sequester N in tissues and shells, which can be removed from the ecosystem when mussel populations or the predator populations they support are harvested (Kellogg et al. 2013, Clements & Comeau 2019). N removal is an important measurable outcome in restoration projects whose value can be quantified monetarily and used in cost-benefit analyses (Piehler & Smyth 2011, Zarnoch et al. 2017). Future analyses of salt marsh restoration will benefit from careful calculations of the monetary value of marsh-mussel denitrification, which may help evaluate the use of mussels to sustain ecosystem services. We also note that the long-term influence of mussels on plant growth, and thus sediment stability, remains poorly understood within eutrophic estuaries and is deserving of future study. Beyond eutrophic estuaries, the impacts of N availability on marsh plant growth and allocation may need to be considered when decisions are made on whether to fertilize restored or degraded marshes.

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Appendix

Table A1. Mean (standard error) of aboveground (AG) and belowground (BG) cordgrass traits measured at the end of the study. The carbon:nitrogen (C:N) data are expressed as molar ratios. n = 5 per treatment for AG biomass, stem density, stem height (plot average height), and AG C:N; n = 15 per treatment for BG biomass and BG C:N

Treatment	Stem density (stems m^{-2})	Stem height (cm)	AG biomass (g m^{-2})	AG C:N	BG biomass (g m^{-2})	BG C:N
Control	300 (26.32)	40.05 (1.46)	252.25 (43.94)	126.18 (15.14)	698.88 (533.8)	76.41 (6.70)
Mussel shell	319.19 (43.03)	36.88 (1.40)	243.66 (64.53)	72.09 (9.61)	601.02 (296.56)	72.09 (8.56)
Live mussel	338.4 (38.17)	35.69 (1.05)	206.08 (47.58)	87.43 (20.04)	230.45 (67.38)	50.66 (4.55)

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