Eelgrass meadows, *Zostera marina* (L.), facilitate the ecosystem service of nitrogen removal during simulated nutrient pulses in Shinnecock Bay, New York, USA

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Abstract: Seagrass meadows are important sites of nitrogen (N) transformations in estuaries, however, the role of N loading in driving relative rates of N fixation and denitrification in seagrass habitats is unclear. The current study quantified N fluxes in eelgrass meadows (*Zostera marina* (L.)) and nearby unvegetated sand in trials representing *in situ* and N enriched conditions. Net N_2 fluxes were low or negative under *in situ* conditions in both eelgrass and

sand. Under N enriched conditions, denitrification was higher than N-fixation, and denitrification in eelgrass was significantly higher than sand. Denitrification of water column NO_3^- was more significant than coupled nitrification-denitrification in the eelgrass. Denitrification was likely supported by greater organic carbon and N within the eelgrass sediment compared to sand. Eelgrass meadows in Shinnecock Bay may facilitate the ecosystem service of N removal and retention during short-term nutrient pulses that can originate from groundwater discharge and stormwater runoff.

Introduction

Seagrass meadows provide significant ecosystem services including invertebrate and fish habitat (Blandon and zu Ermgassen 2014; Heck et al. 2003; Watson et al. 1993), sediment stabilization (Bos et al. 2007), wave attenuation (Fonseca and Cahalan 1992), carbon (C) sequestration (Macreadie et al. 2014), and nutrient cycling (McGlathery et al. 2007). Recent estimates of their economic value range from \$1,000 ha^{-1} yr^{-1} for nitrogen (N) removal (Piehler and Smyth 2011), \$178,000 ha^{-1} yr^{-1} for enhancing fish biomass (Blandon and zu Ermgassen 2014), and up to \$13.7 billion yr^{-1} in carbon sequestration (Pendleton et al. 2012).

Despite their economic and ecological importance, seagrass coverage has declined an estimated 7% yr^{-1} worldwide since 1990 (Waycott et al. 2009). Currently, 15% of seagrass species are considered threatened (Short et al. 2011). The decline of seagrass habitat has been attributed to a number of anthropogenic activities, especially those that reduce water quality and clarity such as cultural eutrophication (Burkholder et al. 2007) and sediment loadings (Dennison et al. 1993). Thus, it is critical to quantify ecosystem services and ecosystem processes within

existing seagrass habitat to document their ecological and societal value in support of conservation and management efforts.

Seagrasses are typically the dominant primary producer in shallow, nutrient limited coastal ecosystems (McGlathery et al. 2007), and have significant impacts on N transformations. Previous studies suggest that N fixation, or the conversion of inert dinitrogen (N_2) gas into a usable form, can provide a significant portion of total N demand in seagrass ecosystems (Cole and McGlathery 2012). Nitrogen fixation within seagrass meadows may come from autotrophic or heterotrophic epiphytes (Cole and McGlathery 2012; Wetzel and Penhale 1979), although heterotrophic N fixation within the seagrass rhizosphere also occurs (Welsh et al 2000). Heterotrophic N fixers utilize organic C such as foliar photosynthate, root exudates, or sediment organic matter. Rates of N fixation vary widely in seagrass habitats (i.e., 0-22 μmol N m^{-2} h^{-1}; see review in McGlathery 2008) and rates in unvegetated sediment can be similar (Howarth et al. 1988) or higher (Fulweiler et al. 2007; Gardner et al. 2006). A major driver for high N fixation rates appears to be the quantity and quality of organic C available to N-fixing sulfate reducers (Cook et al. 2015; Fulweiler et al. 2013).

Denitrification rates, or the microbial respiration of nitrate (NO_3^-) to N_2, can be highly variable in seagrass habitats and, when exceeding rates of N fixation, can represent a net loss of N from the ecosystem. Environmental factors that influence denitrification rates in shallow coastal ecosystems include organic matter quality and quantity, sediment oxygen, and water column N concentrations (Cornwell et al. 1999; Eyre et al. 2013; Seitzinger et al. 2006). Denitrification is unlikely to be limited by C in seagrass habitats as the below-ground tissues release organic material and their three-dimensional structure promotes settlement and microbial decomposition of seagrass detritus, phytoplankton, and other seston (Eyre et al. 2011; Gacia et

al. 2002). However, the source of N for denitrification is variable and potentially limiting. Denitrifying bacteria acquire NO_3^- from the water column (i.e., direct denitrification) or via nitrification, the aerobic conversion of ammonium (NH_4^+) to NO_3^- by chemolithotrophs (Cornwell et al. 1999). Coupled nitrification-denitrification is considered the main pathway for N removal in ecosystems with low water column NO_3^- (i.e. < 10 µM; Seitzinger et al. 2006), which is typical of most seagrass-dominated ecosystems. Rates of coupled nitrification-denitrification in seagrass meadows can be high (Caffrey and Kemp 1992; Piehler and Smyth 2011), however, direct denitrification is often the dominant form of N removal in seagrass meadows where nitrification is inhibited or water column NO_3^- concentrations are elevated (Bartoli et al. 2008). Inhibition of nitrification in seagrass meadows can occur during periods of sediment anoxia or with competition for sediment NH_4^+ among plants, nitrifying bacteria, and benthic microalgae (Ottosen et al. 1999; Riisgaard-Petersen and Ottosen 2000). Cultural eutrophication affects rates of denitrification and N fixation, but few studies have examined how rates of N cycling in seagrass meadows vary with changing N availability.

The objective of this study was to determine how short-term NO_3^- enrichments affect denitrification and nutrient fluxes in sediment collected within eelgrass meadows (*Zostera marina*) and compare these to fluxes from adjacent, unvegetated sand. The study was conducted in Shinnecock Bay which is part of the Long Island South Shore estuary (Long Island, New York, USA). The estuary has experienced significant losses of eelgrass over the last 85 years (Peterson et al. 2013). Similar to other developed watersheds, water quality in this estuary is affected by NO_3^--enriched submarine groundwater discharge and terrestrial runoff (Bernard et al. 2014; Capone and Bautista 1985; Cole et al. 2006; Gobler and Sanuedo-Wilhelmy 2001). Fluxes of benthic nutrients and N_2 were measured in continuous-flow core incubations from three sites,

and performed in trials with *in situ* water column conditions (control) and simulated N-enriched conditions through an addition of +20 µM $^{15}NO_3$–N to site water (+^{15}N treatment). Anaerobic conditions and available organic C within the eelgrass meadows were predicted to support higher rates of denitrification than the unvegetated sand. In addition, lower rates of N fixation and higher rates of denitrification were expected during the NO_3^--enriched trials (+^{15}N treatment).

Methods

Study sites

Shinnecock Bay is a shallow lagoon and barrier beach estuary located on the south shore of eastern Long Island, New York (Fig 1). The total area of the bay is 39 km^2 and the average depth is 2 m (Green and Chambers 2007). Seagrass coverage in the bay is approximately 2 km^2 (Peterson, unpublished). The bay is connected to the Atlantic Ocean by a narrow inlet with strong tidal velocities (2.5 knots s^{-1}) and it is also connected to the Peconic Bay estuary on its northern shore by a man-made canal (USFWS 2007). The tidal range at the inlet is 0.88 m (Buonaiuto and Bokuniewicz 2008). Salinity in the bay is relatively high (30 PSU) due to the ocean influence and low freshwater inputs (Green and Chambers 2007). The portion of the bay west of the inlet has reduced flushing and higher phytoplankton biomass (Carroll et al. 2008), including annual brown-tide blooms (*Aureococcus anophagefferens*; Gobler et al. 2005). The study sites included one site in the eastern portion (Cormorant Point) and two sites in the western portion of the bay (Tiana Bay and Tiana Beach; Fig 1).

Sediment cores and physiochemical data collection

On 13 July 2013, 12 sediment cores were collected at each site (acrylic, 30 cm or 25 cm length x 7.6 cm i.d.) using a corer designed to minimize disturbance of the sediment-water

interface (Gardner et al. 2006). Six cores were collected within the interior portion of the eelgrass meadows (*Zostera marina*) and 6 were collected from nonvegetated sediments (sand) approximately 10 m removed from the eelgrass. A diver collected the cores from the eelgrass meadows to capture eelgrass within the core and minimize sediment disturbance. Sediment depth in the cores was 12-15 cm which would includes eelgrass rhizosphere. The majority of below-ground biomass is found between 2-5 cm in Shinnecock Bay (Furman, unpublished), which is consistent with other studies of *Z. marina* (McGlathery et al. 1998). Cores were sealed with black rubber caps and placed in a dark cooler for transport to the laboratory (< 3 h). Half of the cores were used to measure sediment microprofiles, and half were used for solute and gas flux measurements. Cores used for microprofiles were 25 cm and flux cores were 30 cm in length. Three 20 l carboys were filled with site water from each sampling site for use in continuous-flow measurements. Temperature and dissolved oxygen (DO) were measured in the middle of the water column at each site with a Hach HQ30d luminescent DO probe, and salinity was measured with a refractometer. These measurements were made from 9am through 12pm. DO stratification is infrequent at the study sites given the shallow water (< 2 m) mixing from wind and tides. At each site, triplicate 20-ml water samples were filtered using 0.2 µm nylon syringe filters (Thermo Scientific, Rockwood, TN, USA) and frozen prior to dissolved nutrient analysis (see below). For water column chlorophyll *a*, triplicate samples of 0.5 l were filtered with a 0.45-µm nitrocellulose membrane and stored in the dark at -20°C until analyzed (N=3 site^{-1}). Membranes were extracted overnight at 4°C in 90% acetone and measured spectrophotometrically (Parsons et al. 1984).

Gas and nutrient fluxes measured via continuous-flow core incubations

Continuous-flow incubations of sediment cores were performed to measure nutrient and gas fluxes (Gardner and McCarthy 2009; Hoellein et al. 2015). After transporting the cores to the laboratory we removed ~90% of the overlying water in each core and then carefully added new site water using a 60 ml syringe and tubing to adjust the total volume to ~230ml. Cores were fitted with a rubber cap on the bottom and a plunger with a rubber O-ring on the top. The plunger maintained a tight seal and its surface had two holes which were plumbed with polyetheretherketone inlet and outlet tubing (PEEK; Zeus. Inc., Branchburg, NJ, USA). All cores were incubated at *in situ* temperature (water bath of 26°C) and kept dark to avoid photosynthetic activity. There are limitations to studying a photic system under dark conditions, and these are carefully noted when interpreting the results.

Two sequential trials were performed by manipulating the inflow water in the continuous-flow cores. In the first trial, no nutrients were added to the site water (i.e. control). The second trial followed 24 h later and +20 µM $15NO_3$-N (referred to as the $+^{15}N$ treatment) was added to the site water (N=3 cores $habitat^{-1}$ $site^{-1}$) to simulate N-enriched conditions. The isotopic form of NO_3^- was used so that denitrification of added $^{15}NO_3^-$ could be differentiated from natural sources as well as to estimate total denitrification and N fixation (Gardner et al. 2006; Gardner and McCarthy 2009). For each trial, aerated site water was gently passed over the intact cores for 24 h at a rate of 1.1 ml min^{-1} (turnover time = 4.2 h). Water was collected directly from each inflow carboy and from each of the core outflows and filtered into 3, 20 ml scintillation vials and frozen until measurement of water chemistry including ammonium (NH_4^+), nitrate + nitrite (NO_x^-), nitrite (NO_2^-), and soluble reactive phosphorus (SRP). Additional samples from the inflows and outflows were collected into triplicate 12 ml exetainer sample vials (Labco Ltd., Lampeter, United Kingdom) to measure dissolved gases. The inflow samples were

collected directly from the carboy with a 60 ml syringe with attached tubing. Vials were gently filled from the bottom and allowed to overflow for several volumes. The outflow samples were collected by placing the outflow tubing directly in the bottom of the exetainer vials and allowed to overflow for several volumes. The samples were then treated with 200 µl of 50% zinc chloride as a preservative (McCarthy et al. 2007). The vials were carefully capped to avoid air bubbles and stored submerged and below sampling temperature until measurement of dissolved gases. We did not use a bypass line for collecting inflow samples as tubing effects were likely minimal since the experiment was performed near room temperature and the tubing used was chemically inert.

Dissolved gases were measured with membrane inlet mass spectrometry (MIMS; Bay Instruments, Easton, MD, USA; Kana et al. 1994). The mass spectrometer measured abundance of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, $^{32}O_2$, and ^{40}Ar. Artificial seawater (30 PSU) maintained at a constant temperature (24.5^{o}C; Circulating Bath, VWR International, Radnor, PA, USA) and equilibrated to atmospheric gases by stirring (Lab Egg RW11 Basic, IKA Works, Inc., Wilmington, NC, USA) at low speed was used for standards. Instrument drift was corrected throughout sample runs with standard water. We recognize that the quadrupole mass spectrometer in the MIMS can produce O+ ions that form nitric oxide (NO) in the presence of N_2, affecting $^{28}N_2$ and $^{30}N_2$ measurements (Eyre et al. 2002; Kana and Weiss 2004). Ionization rates are machine-specific (McCarthy and Gardner 2003), but have not been quantified for the MIMS used in this study. There was likely minimal effects since no mass 30, representing NO, was detected in control cores (McCarthy et al. 2008). Dissolved inorganic nutrients were measured on an Autoanalyzer III (Seal Analytical, Inc., Mequon, WI) using the phenol hypochlorite technique for NH_4^+ (Solorzano 1969) and the antimonyl tartrate method for SRP (Murphy and Riley 1962). Nitrate +

nitrite (NO_x^-) and nitrite (NO_2^-) were measured with or without cadmium reduction, respectively (APHA 1998).

Gas and nutrient fluxes were calculated from the concentration in the outflow minus the concentration in the inflow, and adjusted for surface area of the core and pump flow rate (flux units = µmol element m^{-2} h^{-1}). Net retention was indicated by a negative value and flux out of the sediment (i.e., efflux) was indicated by a positive value. Simultaneous N fixation and potential denitrification (sum of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$ and N fixation) were calculated using equations from An et al. (2001) with the data collected from the $+^{15}N$ treatment. Comparisons of denitrification between the control and $+^{15}N$ treatment were made using the total denitrification rate (sum of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$; An and Gardner 2002). The data is presented as total denitrification rather than potential denitrification to facilitate comparisons between treatments. The $^{30}N_2 + (^{29}N_2 * 0.5)$ production in the $+^{15}N$ treatment was considered an index of direct denitrification of NO_3^- (Hoellein et al. 2015). Sulfide can inhibit the last step in denitrification, and the N_2 values measured via MIMS do not account for incomplete denitrification to nitrous oxide. Fluxes in each core were calculated after 24 h and averaged using 3 replicate cores for each treatment (Bruesewitz et al. 2013; Gardner and McCarthy 2009). An observed flux where the standard error (SE) of the data points did not overlap with zero was considered above the detection limit (after Gardner and McCarthy 2009).

Sediment microprofiles

Cores collected for sediment microprofiles were submerged in aerated site water without caps and maintained at 26°C in the laboratory under dark conditions for 24-72 h before measurements were performed. The O_2 and H_2S microsensors (50µm tip diameter; Unisense, Aarhus N, Denmark) were equilibrated for 24 h and then calibrated according to the

manufacturer's instructions (Bernard et al. 2014; Osborne et al. 2015). Microsensors were mounted on a computer-controlled micromanipulator and signals were recorded by an A/D converter (Unisense). All components were controlled by a computer using SensorTrace PRO software (Unisense). The O_2 profile was measured in 500-µm intervals and H_2S as [HS^-] in 2000-µm intervals. Replicate profiles (n = 2-4) were performed within each core. The O_2 penetration depth for each profile was estimated from a regression using the measurements starting at the sediment water interface and ending at the first measurement where O_2 was below detection. We note that holding the cores in the laboratory and exposing them to dark conditions can alter oxygen profiles, however, all cores were treated identically so effects were equal across groups.

Sediment and eelgrass characteristics

After completing the continuous-flow measurements, three replicate samples (5 cm^3) of sediment were removed from the top 2.5 cm of each core to measure organic matter (%), sediment C, N , and P relative content, chlorophyll *a*, and porosity. Sediment organic content was determined by drying the sediment at 60°C and then combusting at 500°C for 4 h (Benfield 2006). Sediment porosity was calculated from these data using the methodology of Carroll et al. (2008), and the equations and parameter values of Berner (1971). Sediment organic C and total N content was determined on dried samples that were acidified and re-dried (Nieuwenhuize et al. 1994) and then measured on a Perkin Elmer 2400 Series II CHN analyzer (Perkin Elmer Life and Analytical Sciences, Shelton, CT) . Sediment P was determined by dry oxidation-acid hydrolysis (Aspila 1976) followed by colorimetric analysis (Murphy and Riley 1962) using a Seal AQ2+ discrete analyzer (Seal Analytical, Inc., Mequon, WI). Elemental content was calculated on a dry weight basis and used to calculate molar C:N and N:P ratios. Sediment chlorophyll *a* was extracted overnight at 4°C in 90% acetone and measured spectrophotometrically (Parsons et al.

1984). Extracted sediment was dried at 60ºC and re-weighed for dry mass to calculate chlorophyll *a* as µg per gram dry weight of sediment.

Eelgrass roots and leaves were separated from the core sediments, scraped free of epiphytes, washed, and dried at 60ºC. Samples of the dried eelgrass leaves collected from each site were used to determine C, N, and P content. The C and N content were measured with a Perkin Elmer 2400 Series II CHN analyzer, while P was determined following the dry oxidation-acid hydrolysis procedure as modified by Fourqurean et al. (1992) and colorimetric analysis (Murphy and Riley 1962) using a Seal AQ2+ discrete analyzer. Eelgrass nutrient limitation was assessed using the limitation index (LI = |30-N:P|) (Allgeier et al. 2011; Campbell and Fourqurean 2009) and compared to the ideal seagrass Redfield ratio (30:1, Duarte 1990).

Stoichiometry of nitrogen and phosphorous fluxes

The nutrient and gas fluxes were examined within a stoichiometric framework to determine if Shinnecock Bay sediment (eelgrass and unvegetated sand) would shift from N-limitation to a source of N when exposed to nutrient pulses (+^{15}N treatment). For this analysis, it was assumed that organic matter mineralized in the sediment was similar in C, N, P composition to the Redfield ratio, that the microbial processes produce N and P in expected ratios, and that O_2 uptake was an adequate proxy for benthic metabolism (i.e. CO_2:O_2 = 1:1; Cornwell et al. 2014). In addition, the sum of DIN and total N_2 fluxes ($^{28,29,30}N_2$) was used to estimate N remineralization rates, and SRP fluxes were used to estimate P remineralization (Kellogg et al. 2013).

Statistical analyses

Water column nutrients and chlorophyll *a* were compared among sites using 1-way ANOVA followed by a Tukey post-hoc test. Sediment characteristics, N fixation, direct

denitrification, the proportion of N_2 attributed to direct denitrification, and O_2 penetration depth were compared using 2-way ANOVA, with site and habitat types (eelgrass and unvegetated sand) as fixed factors. A 2-way ANOVA was performed separately for each study site (Cormorant Point, Tiana Bay and Tiana Beach) to compare dissolved nutrient and gas fluxes. The fixed factors were treatment (control or $+^{15}N$) and habitat (eelgrass or unvegetated sand). Site was not included in the ANOVA since there were no *a priori* hypotheses regarding site differences for flux rates, but sites were not replicates (i.e., variation in ambient water column and sediment characteristics among sites). Comparisons among sites were made qualitatively by aligning the results of the ANOVA for each site in a table (Hoellein and Zarnoch 2014; Lindemann et al. 2016). Relationships between total N_2 fluxes, sediment oxygen demand, sediment C and N abundance, and nutrient fluxes were assessed by regression analysis. Data were transformed as needed to meet the assumptions of equal variance and normal distribution. A p-value of ≤ 0.05 was used as the threshold for significance. All statistical analyses were completed using SigmaPlot 11 (Systat, Inc., Chicago, IL) and IBM SPSS 22 (Armonk, NY).

Results

Site Characteristics

Cores collected within the eelgrass meadows had similar total biomass of eelgrass (ANOVA, $F = 3.5$, df = 2, $p = 0.10$; Table 1), shoot biomass (ANOVA, $F = 2.9$, df = 2, $p = 0.13$; Table 1), and root/rhizome biomass (ANOVA, $F = 4.0$, df = 2, $p = 0.08$; Table 1) among study sites. Physicochemical conditions showed some variation among study sites. Water column NH_4^+ was significantly higher at Cormorant Point relative to the other sites (ANOVA, $F = 75.3$, df = 2, $p < 0.01$; Table 1). Cormorant Point had the highest SRP concentration (ANOVA, $F = 23.2$, df =

2, p <0.01) while Tiana Beach and Tiana Bay were similar (Table 1). Water column NO_x^- concentration was similar among sites (ANOVA, $F = 0.35$, df = 2, p = 0.72; Table 1), and NO_2^- was below detection at each site. Although Tiana Beach had the lowest nutrient concentrations, it had the highest water column chlorophyll *a* concentration (ANOVA, $F = 150.1$, df = 2, $p < 0.01$; Table 1). Dissolved oxygen, temperature, and salinity were similar across the three sites. Dissolved oxygen ranged from 7.7 mg l^{-1} at Tiana Beach to 10.5 mg l^{-1} at Tiana Bay. Water temperatures were 26°C at both Cormorant Point and Tiana Bay, and 28°C at Tiana Beach. Salinity was 30 at all three sites.

Sediment characteristics followed the expected pattern, where the eelgrass meadows had significantly higher organic material and chlorophyll-*a* (µg g^{-1}) than sand (Tables 2 and 3; ANOVA $p < 0.01$). Sediment organic C and total N in the eelgrass meadows was higher than sand (ANOVA, $p < 0.01$), however, the C:N and N:P ratios were the same across sites and habitat types (Tables 2 and 3). Relative abundance of organic matter as well as C and N concentrations were significantly higher at Tiana Bay than Cormorant Point or Tiana Beach, which were similar to each other (ANOVA, $p \leq 0.01$; Tables 2 and 3). Sediment porosity within the eelgrass meadows was greater than sediments from sand (ANOVA, $p < 0.01$), and varied among sites (ANOVA, p = 0.01; Tables 2 and 3).

Eelgrass stoichiometry

The C, N, and P content in eelgrass leaves were not significantly different among sites (ANOVA, $F < 0.66$, df =2, $p > 0.05$) and the mean (±SE) values for all sites were 37.3 (±0.6) %C, 1.35 (±0.1) %N, and 0.81 (±0.1) %P. Molar C:N and N:P ratios were also similar across sites with mean (±SE) values of 32.5 (±1.2) and 3.61 (±0.2), respectively. Finally, since the N:P

ratios were similar across sites, the seagrass limitation index was also similar with a total mean (±SE) of 26.4 (±0.2).

Nutrient fluxes

Fluxes of dissolved nutrients in the control and $+^{15}N$ treatments show variable results, including significant effects of habitat, treatment, and habitat x treatment interaction (Fig 2; Table 4). All sediment at Tiana Beach and Tiana Bay in both control and $+^{15}N$ treatments yielded net SRP efflux, while Cormorant Point sediment had net SRP uptake (Fig 2a). There was no effect of habitat or treatment on SRP flux at Cormorant Point and Tiana Beach. Tiana Bay showed a significant interaction between habitat and treatment, attributable to very high SRP efflux in the sand during the $+^{15}N$ treatment (Fig 2a; Table 4).

Flux of NH_4^+ was variable between habitats and treatments (Fig 2b), while NO_x^- fluxes were largely directed into the sediment (Fig 2c). At Tiana Bay and Tiana Beach, NH_4^+ efflux was higher in the sand compared to eelgrass (ANOVA, $p < 0.05$ and 0.02, respectively), but there was no difference between habitats at Cormorant Point (Table 4; Fig 2b). The NO_x^- fluxes were similar at Tiana Bay and Tiana Beach, while fluxes at Cormorant Point differed. Cormorant Point showed a significant habitat effect (ANOVA, $p = 0.04$; Table 4) due to high NOx^- efflux in the control trial (150 µmol N m^{-2} h^{-1}), but net uptake for sand in the $+^{15}N$ treatment and in both trials for eelgrass (Fig 2c). In contrast, a significant interaction was observed between habitat and treatment at Tiana Bay and Tiana Beach (ANOVA, $p < 0.01$), which occurred because of greater NO_x^- uptake in the eelgrass compared to the sand habitat in the $+^{15}N$ treatment (Fig 2c; Table 4). There were no significant relationships between nutrient fluxes and sediment characteristics or eelgrass biomass.

Gas fluxes

Patterns for sediment oxygen demand (i.e. O_2 flux) were consistent among sites and treatments. At all sites, O_2 flux was higher in eelgrass meadows than in sand (Table 4). In addition, O_2 flux was greater in the $+^{15}N$ treatment compared to the control at all sites (Table 4). No significant interactions were observed between habitat and treatment for O_2 flux (Table 4).

Rates of N_2 flux (µmol N m^{-2} h^{-1}) under control and enriched conditions ($+^{15}N$ treatment) were variable by habitat and treatment (Fig 3; Table 4). At Tiana Bay, total N_2 flux was greater in the $+^{15}N$ treatment (ANOVA, $p < 0.01$) compared to control, and greater in eelgrass meadows compared to sand (ANOVA, $p = 0.03$; Table 4, Fig 3). Tiana Beach and Cormorant Point showed significant, orderly interactions between habitat and treatment for total denitrification (Table 4). At Cormorant Point, N_2 flux in sand was similar between control and $+^{15}N$ treatments (Fig 3b), but in eelgrass, rates were higher during the $+^{15}N$ treatment relative to the control conditions (Fig 3b). The same pattern was found for eelgrass cores at Tiana Bay relative to sand cores.

The relative amount of ^{15}N converted into $^{29,30}N_2$ was greater in eelgrass than in sand among all 3 sites. At Cormorant Point, 10.6% of the total N_2 was $^{29,30}N_2$ in sand, while 20.4% of the N_2 was $^{29,30}N_2$ in the eelgrass. At Tiana Bay, the values were 34.6% in sand and 36.0% in eelgrass, while in Tiana Beach 38.5% of the N_2 pool was isotopically labeled in sand, and 48.9% of the N_2 was $^{29,30}N$ in the eelgrass.

For all three study sites, denitrification exceeded N fixation in the $+^{15}N$ treatment, suggesting N limitation of denitrification (Fig 3b). N fixation was calculated in the $+^{15}N$ treatment using An et al. (2001), and it ranged from 0-15.6 µmol N m^{-2} h^{-1}. N fixation was not recorded in either habitat at Cormorant Point, or in the sand at Tiana Beach. The highest rates of N fixation were observed in the eelgrass at Tiana Beach while rates were similar in the sand

(8.0±8 µmol N m^{-2} h^{-1}) and eelgrass (11.4±6 µmol N m^{-2} h^{-1}) at Tiana Bay. Gas fluxes were not related to eelgrass biomass in cores, however, total denitrification in the $+^{15}N$ treatment was positively related to organic carbon and nitrogen content in the sediment (Fig 4).

Flux stoichiometry

In both the control and $+^{15}N$ treatments, the remineralization of N (Fig 5a, b) and P (Fig 5c, d) resulted in fluxes below what was predicted from the Redfield ratio, indicating net retention of elements. The relationship of total N to SRP flux, however, indicates that SRP flux is similar to or greater than predicted by the Redfield ratio (Fig 5e, f) suggesting that P may be in excess.

Sediment microprofiles

The sediment O_2 profiles for both habitats were similar at Tiana Beach (Fig S1b) and Tiana Bay (Fig S1c). At Cormorant Point, however, the sand habitat had higher O_2 concentrations than the eelgrass meadow (Fig S1a). While the mean O_2 penetration depth across sites was generally deeper for sand habitats (-5.8 ± 2.5 mm) than eelgrass (-2.0 ± 0.2 mm), no significant effects of habitat type or site (ANOVA, $F = 0.8$, $p > 0.05$) were found. The H_2S concentrations in the profiles were similar in both habitat types at Cormorant Point (Fig S1d) and Tiana Bay (Fig S1f), although Cormorant Point had higher H_2S concentrations. At Tiana Beach, sand showed negligible H_2S concentrations to 20 mm depth, but the eelgrass had the highest H_2S concentrations observed (Fig S1e).

Discussion

Eelgrass habitat responds to short-term N-enrichment with increased denitrification

There is conflicting evidence within the literature as to whether eelgrass meadows support greater N fixation or denitrification compared to unvegetated reference sediment

(McGlathery et al. 2007; Piehler and Smyth 2011; Smyth et al. 2013). Our results during typical summer conditions in Shinnecock Bay (i.e., control treatment), indicate that co-occurrence of denitrification and N fixation in eelgrass sediments can result in low or negative values for net N_2 flux, which supports previous findings (Fulweiler et al. 2013; McGlathery et al. 2007). The observed rates of N fixation in the present study were similar to values reported for sediment N fixation in eelgrass meadows (Herbert 1999). The highest N fixation was observed in eelgrass at Tiana Beach which may be due to sulfate reducing bacteria as this site also had the highest observed H_2S in the sediment microprofiles (Fig S1e). It is also possible that measured N fixation could be attributed to N_2 diffusion into the lacunae, however, it is unlikely that this was significant since N fixation was similar between eelgrass and unvegetated sediment. More significantly, our study reveals that short-term nutrient enrichment (+20 µM $^{15}NO_3^-$-N) of eelgrass meadows generates higher rates of total denitrification than bare sand (109-232 µmol N m^{-2} h^{-1} and 4-86 µmol N m^{-2} h^{-1}, respectively). The N_2 fluxes from the control cores in this study were similar to those reported elsewhere (Piehler and Smyth 2011; Riisgaard-Peterson and Ottosen 2000; Smyth et al. 2013). The total N_2 fluxes from the $+^{15}N$ treatment were generally higher than other reported N_2 fluxes (Piehler and Smyth 2011), but were similar to those reported by Riisgaard-Peterson and Ottosen (2000) from a site with high water column N. Previous research has demonstrated eelgrass responds to NO_3^--enriched groundwater discharge by increasing direct assimilation (via nitrate reductase activity; Maier and Pregnall 1990). Our data add to the literature by showing eelgrass meadows in Shinnecock Bay support N-limited denitrifying communities which have the capacity to provide the ecosystem service of N removal during short-term nutrient pulses, as simulated in $+^{15}N$ treatment. We note that this result was

obtained in summer under dark conditions and may vary spatially and temporally. Future studies will need to examine this effect across diel and seasonal time-scales and in other ecosystems.

Mechanisms of eelgrass nitrogen cycling

Denitrification in eelgrass meadows may be supported by the anaerobic conditions, labile carbon generated from root exudates (Cole and McGlathery 2012; Moriarty et al. 1986), and the retention of organic material (i.e., drift macroalgae and seagrass detritus; Gacia et al. 2002). Comparison of our results in the control and $+^{15}N$ treatments suggest that denitrification was primarily limited by NO_3^-. Sources of NO_3^- for denitrification include the water column ($\leq$ 2.1 µM across study sites) or nitrification. Previous studies suggest denitrification rates are limited by low nitrification in eelgrass meadows (Ottosen et al. 1999; Riisgaard-Petersen and Ottosen 2000). We did not measure nitrification directly, but infer a similar pattern based on eelgrass cores response to $+^{15}N$ treatment. At all sites, the total N_2 flux increased significantly in the eelgrass cores during the $+^{15}N$ treatment suggesting that denitrifiers were NO_3^- limited. The eelgrass leaves were also low in %N as compared to the global average (Duarte 1990) which may be indicative of competition for sediment NH_4^+.

The source of N for denitrification among sites and habitats could be inferred by tracing the $^{15}NO_3^-$ into $^{29,30}N_2$ gas, and comparing that value among sites and habitats. For both Cormorant Point and Tiana Beach, the relative isotope enrichment in N_2 was higher in eelgrass than sand. At all 3 sites, $^{28}N_2$ was 51-89% of the N_2 produced in the $+^{15}N$ treatment. Potential sources of ^{14}N for $^{28}N_2$-denitrification are water column $^{14}NO_3^-$ or nitrification-derived $^{14}NO_3^-$. Because water column NO_3^- was similar among sites and low compared to the $^{15}NO_3^-$ enrichment, the sites with lowest $^{29,30}N_2$ production (e.g. 11% in sand at Cormorant Point), were most likely to have nitrifier-derived $^{14}NO_3^-$ supporting denitrification. Other factors also suggest

conditions at Cormorant Point could support greater nitrification than Tiana Bay and Tiana Beach. Water column NH_4^+ was elevated at Cormorant Point compared to the other sites, and the sand at this site showed a positive flux of both NO_x^- and N_2 in the control trial (Figs 2c, 3a). It also had higher sediment oxygen than the other sites and had the lowest benthic microalgae concentration (Table 2). The composite factors suggest oxygen conditions and substrate availability favored conditions for nitrification at Cormorant Point. Studies using $^{15}NH_4^+$ enrichments with light and dark treatments are needed to clarify the role of coupled nitrification-denitrification across sites and seasons in eelgrass meadows.

Denitrification in Shinnecock Bay eelgrass meadows was clearly influenced by NO_3^- availability, but the sediment microprofiles also enabled exploration of the potential for sediment oxygen and C to affect denitrification. We predicted that anaerobic conditions and C availability would generate greater denitrification in eelgrass compared to sand habitats, however, we found little evidence for differences in sediment oxygen conditions between habitats. With the exception of Cormorant Point, O_2 microprofiles were similar between the eelgrass and the unvegetated sand (Fig S1b, c). Sulfide microprofiles were also similar between habitat types (except Tiana Beach sand, where no significant H_2S was measured). This site and habitat also had the lowest total denitrification rates in the $+^{15}N$ treatment (19 µmol N m^{-2} h^{-1}). Sediment oxygen conditions may have been homogenized by high reoxidation of sediment from overlying water due to strong tidal flow and wind effects across shallow Shinnecock Bay. Higher sulfide concentrations in *Thalassia testudinum* beds as compared to bare sediments in Texas bays was attributed to higher current velocities which better exchange anoxic porewater with overlying water. (Lee and Dunton 2000). Oxygen conditions within our cores could have also been homogenized by being held in the dark. Unfortunately, our study design could not include a light

treatment due to interference from bubble formation, however, oxygen release from roots and rhizomes during photosynthesis can alter sediment oxygen and sulfide concentrations (Caffrey and Kemp 1991; Lee and Dunton 2000). Future studies that use continuous-flow core incubations with eelgrass may be able to avoid the challenges of photosynthetic bubble formation by employing a light pretreatment, which can enhance oxygen root diffusion in dark conditions (Thursby 1984; Pederson et al 1998). Overall, differences in sediment oxygen conditions were not likely to be the primary driver of higher denitrification rates in the eelgrass cores compared to sand. Instead, organic matter was the more probable explanatory factor. For example, sediment organic C (Fig 4a) and total N (Fig 4b) were strongly related to total denitrification rates, and both were higher in eelgrass meadows. Carroll et al. (2008) found that organic and inorganic N largely influenced productivity of eelgrass meadows in Shinnecock Bay. Our results show that sediment organic C and total N in eelgrass meadows also enhance denitrification rates.

Denitrification rates were greatest in the eelgrass cores when water column NO_3^- was enriched ($+^{15}NO_3^-$ treatment), suggesting that eelgrass may have the capacity to provide an ecosystem service of N removal for a short period of time (i.e., hours to days). Pulsed delivery of water column nutrients to Long Island estuaries can be expected in spring when submarine groundwater discharge is maximal (Gobler and Sanuedo-Wilhelmy 2001), after severe storms (via runoff or sediment resuspension), and from nutrient regeneration after phytoplankton bloom demise. Previous studies of long-term nutrient enrichment, however, suggest direct negative influences on seagrass health and indirect effects via shading as N stimulates both water-column and epiphytic growth (Cabaco et al. 2013). Laboratory studies show that long-term exposures of eelgrass, *Zostera marina,* to daily pulses of enriched concentrations are lethal (3.5 – 35 μM;

Burkholder et al. 1992). A 14-week NO_3^- enrichment (8 µM) significantly lowered shoot production and density (Burkholder et al. 1994; Touchette et al. 2003). Eelgrass lack the capacity to inhibit nitrate reductase, which is the enzyme responsible for NO_3^- assimilation and for both dark and light N uptake (Burkholder et al. 2007). This appears to be an adaption resulting from evolution within nutrient-limited ecosystems (Burkholder et al. 1992).

To better understand the effect of the $+^{15}N$ treatment on the mass balance of N in the core incubations, we divided total denitrification by the negative NO_x^- flux (Table 5). This provides an index of N_2 produced relative to NO_x^- consumed. As we expected, NO_x^- uptake in the $+^{15}N$ treatment was significantly greater in the eelgrass compared to the sand cores (312-408 µmol N m^{-2} h^{-1} vs 82-216 µmol N m^{-2} h^{-1}, respectively), but there was no difference in NO_x^- uptake between habitats in control conditions (Fig. 2c; Table 4). This suggests eelgrass habitats have the capacity to 'absorb' a pulse of added NO_x^-. Other data in this study gives some suggestion as to the fate of the NO_x^- taken up in each habitat. Measured N_2 production rates accounted for 2-53% of the total NO_x^- uptake in the sand and 31-77% in the eelgrass (Table 5). The remainder of the unexplained NO_x^- uptake could be assimilation by eelgrass, benthic microalgae (Anderson et al. 2003), epiphytes, or microbes. Alternatively, some NO_x^- uptake could be attributed to dissimilatory nitrate reduction to ammonia (DNRA; Gardner et al. 2006). In that case, net efflux of NH_4^+ could be expected in the cores, however, most of our results showed net NH_4^+ uptake (Fig 2b). This does not preclude DNRA altogether, as the relatively high turnover of NH_4^+ in seagrass sediment (Lee and Dunton 1999) and eelgrass uptake (Sandoval-Gil et al. 2015), could have reduced NH_4^+ efflux thus limiting our ability to detect potential DNRA. In particular, the sand cores at Tiana Bay and Tiana Beach had high NH_4^+ efflux which could be due to DNRA and the lack of NH_4^+ assimilation by eelgrass. The observed NH_4^+ efflux at these sites was higher

in the $+^{15}N$ treatment but there was no significant treatment effect (Table 4) which suggests that the enrichment did not promote N release to the water column.

Measurements using both $^{15}NO_3^-$ and $^{15}NH_4^+$ enrichments are needed to clarify the relative roles of denitrification, N assimilation, and DNRA. For example, Sandoval-Gil et al. (2016) showed isotopic enrichment of eelgrass shoots when in close proximity to oyster aquaculture presumable due to enriched N conditions from mineralization of biodeposits. Additional studies should also measure response to episodic nutrient pulses across seasons to develop annual budgets of N removal and to better understand the drivers for N cycling in eelgrass meadows. We expect there may also be seasonal effects on sediment C availability which were strongly related to total denitrification rates, particularly within the eelgrass meadows. Eelgrass N demand as well as available N in water column and porewater are also likely to change across seasons (Lee and Dunton 1999).

The flux stoichiometry shows mineralization and retention of N and P in Shinnecock Bay sediments are similar to a properly functioning "coastal filter" (McGlathery et al. 2007). The unvegetated and eelgrass sediments appear to be a biological buffer for short-term N loading through N assimilation and denitrification. These may be mechanisms that contribute to the resilience of Shinnecock Bay to N loading. The N:P fluxes however, show greater release of P than would be predicted by the Redfield ratio. Availability of P is largely dependent upon binding with iron oxides where release can be caused by low redox and sulfate reduction (Cornwell et al. 2014). The higher P availability may due to changes in redox within submarine groundwater which results in desorption from iron oxides (Spiteri et al. 2008). This mechanism has been suggested for another NY embayment within Long Island Sound (Young et al. 2015). Shinnecock Bay eelgrass also has lower N and greater P content than average seagrass (Duarte

1990), supporting our conclusion of N-limitation and presence of excess P. It should be noted, however, that applying the Redfield ratio to eelgrass meadows may not be ideal due to the potential contribution of eelgrass detritus, benthic microalgae, and organic exudates. Our measurements of sediment C:N and N:P across all study sites, however, show values (total means = ~10.2 and ~1.6, respectively) that are more consistent with seston organic matter rather than eelgrass (Duarte 1990). There was also no statistical difference in sediment C:N and N:P among the eelgrass and unvegetated sites. Lastly, eelgrass generally has greater N and phosphorous limitation than seston organic material Duarte 1990) which would give C:N:P ratio lines that result in the same interpretation (e.g. net N retention or net P release) as the Redfield ratio.

Societal value of eelgrass N removal

The ecosystem service of N removal through enhanced denitrification is of particular importance in Long Island (New York, USA) and other highly developed coastal systems where submarine groundwater discharge is a major source of N (Giblin and Gaines 1990; Moran et al. 2014). The potential monetary value of N removal was estimated for current and historic estimates of eelgrass coverage (New York State Seagrass Taskforce 2009) in New York with intent to demonstrate the potential ecological and economic significance of denitrification in eelgrass meadows. The mean denitrification rate in the three eelgrass habitats in the current study ($+^{15}N$ treatment = 173 μmol m^{-2} h^{-1}) was extrapolated to a daily rate by multiplying by 12 (i.e. hours in dark per day as per the incubations) then multiplied by the estimated areal coverage of the eelgrass meadows. This product was then multiplied by the 2013 trading price of N removal in the Connecticut (USA) N trading program ($12.34 kg^{-1} or 5.61 lb^{-1}; New York does not have a N trading program).

Calculations were completed for the Long Island South Shore Estuary (2009 measurements of eelgrass area), Peconic Estuary (1930 and 2009 eelgrass area), and Long Island Sound (1930 and 2009 eelgrass area; Table 6). The Long Island South Shore estuary currently has the greatest areal coverage of eelgrass meadows of the 3 sites, and has the potential to remove 4,695 kg N day^{-1} (>10,000 lbs N day^{-1}), valued at $57,396 day^{-1}. Historical eelgrass in the Peconic Estuary and Long Island Sound (New York coastline in 1930) provided N removal rates comparable to modern estimates for the South Shore Estuary ($25,248 and $68,672, respectively; Table 6) but current eelgrass meadows are only a fraction of their former area. Thus, eelgrass meadows might play a critical role for Long Island estuaries exposed to periodic N-enriched submarine groundwater discharge (Gobler and Sanudo-Wilhemly 2001; Young et al. 2015). In addition, as the number and intensity of storms increase with changing climate (Groisman et al. 2005), eelgrass meadows could play an increased role in mediating periodic nutrient inputs from storm-water runoff. Eelgrass meadows should not, however, be used as a tool to manage N loadings via groundwater or runoff. The monetary estimates generated from these calculations (Table 6) may be conservative since the cost of removed N is low compared to other published values (Rose et al. 2015). In addition, denitrification under light conditions was not included in this estimate but was found to be significant in *Z. capricorni* meadows (Eyre et al. 2011). Future studies that measure denitrification in eelgrass meadows under both light and dark conditions, across larger spatial scales, and across seasons are needed to refine these preliminary estimates of ecosystem services.

Summary

This study demonstrates that eelgrass meadows in Shinnecock Bay may facilitate denitrification of water column NO_3^- during enrichment events (i.e., submarine groundwater

discharge and stormwater) thereby providing an important ecosystem service. Future studies should examine this phenomena over longer (i.e., annual) and shorter (i.e., diel) time scales to develop N budgets for modeling efforts. The NO_3^- uptake through eelgrass leaves during these short-term events may provide an important subsidy supporting eelgrass growth in what is otherwise an N limited system (*this study*; Murray et al. 1992). The ecosystem service of N removal during enrichment events can be of monetary value where eelgrass coverage is significant. Lastly, the consistent indications of N limitation within both control and $+^{15}N$ treatments among the stoichiometric relationships demonstrates the system's capacity to process N during short-term pulses of N. These findings on eelgrass N dynamics in Shinnecock Bay as well as other studies on ecosystem services (i.e., blue carbon; Macreadie et al. 2014) provide additional support for seagrass management, conservation and, where applicable, active restoration.

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Table 1. Mean (±SE) eelgrass biomass (g DW m^{-2}; n = 3 samples $site^{-1}$) and water column nutrients (µM; n = 3 samples $site^{-1}$) including ammonium (NH_4^+-N), nitrite + nitrate (NO_x^--N), soluble reactive phosphorous (SRP), and chlorophyll *a* (µg l^{-1}) for each study site. Study sites were compared using ANOVA. A Tukey post-hoc test was performed when a significant difference was found ($p < 0.05$) and superscript letters are used to indicate significant differences. There were no statistical differences in biomass among sites. Study sites include Cormorant Point (Cor. Pt.), Tiana Bay (T. Bay), and Tiana Beach (T. Beach) within Shinnecock Bay (New York, USA).

Eelgrass	**Cor. Pt.**	**T. Bay**	**T. Beach**
Total	180.6 (39.2)	326.9 (64.4)	186 (13.1)
Shoot	96.2 (43)	187.9 (23.2)	172.35 (9)
Root/Rhizome	85.5 (32.3)	142.5 (42.5)	17.52 (9)
Water Column	**Cor. Pt.**	**T. Bay**	**T. Beach**
NH_4^+-N	17.4^a (0.6)	10.9^b (0.9)	5.1^c (0.6)
NO_x^- -N	1.5 (0.2)	2.1 (1.5)	1.1 (0.4)
SRP	1.3^a (0.1)	0.7^b (0.1)	0.7^b (0.0)
Chl *a*	4.4^a (0.0)	4.2^a (0.2)	8.9^b (0.3)

Table 2. Mean (±SE) sediment organic matter (% dry weight), sediment organic carbon and total nitrogen (µmol g^{-1}), C:N (molar), N:P (molar), chlorophyll *a* (µg g^{-1}), and porosity for sand and eelgrass habitats at each of the study sites. Study sites include Cormorant Point (Cor. Pt.), Tiana Bay (T. Bay), and Tiana Beach (T. Beach) within Shinnecock Bay (New York, USA).

	Site		**Habitat**		**Interaction**	
	F	***P***	**F**	***P***	**F**	***P***
% Organics	4.9	**0.03**	111.5	**<0.01**	6.3	**0.01**
Sediment C	9.9	**<0.01**	15.2	**<0.01**	2.0	0.18
Sediment N	11.4	**<0.01**	8.6	**0.01**	1.0	0.39
C:N	0.7	0.53	1.2	0.29	0.7	0.73
N:P	3.1	0.08	0.5	0.49	2.4	0.13
Chl *a*	3.5	0.07	11.0	**<0.01**	0.1	0.9
Porosity	7.0	**0.01**	35.7	**<0.01**	2.2	0.15

Table 3. Results of two-way ANOVA of sediment characteristics with study site and habitat (unvegetated sand and eelgrass meadow) as fixed factors and the dependent variables of sediment organic matter (% organics), sediment organic carbon, sediment total nitrogen, C:N, N:P , chlorophyll *a* (Chl *a*), and porosity.

Sediment	**Cor. Pt. Sand**	**Cor. Pt. SAV**	**T. Bay Sand**	**T. Bay SAV**	**T. Beach Sand**	**T. Beach SAV**
Organic (%)	0.4 (0.0)	1.2 (0.1)	0.6 (0.0)	1.6 (0.0)	0.7 (0.2)	1.1 (0.0)
Organic C	3.9 (1.5)	4.6 (1.0)	6.8 (1.3)	12.3 (2.0)	3.4 (0.6)	8.7 (0.7)
Total N	0.3 (0.1)	0.4 (0.1)	0.7 (0.2)	1.2 (0.2)	0.4 (0.1)	0.8 (0.0)
C:N	10.8 (1.3)	10.8 (1.2)	8.9 (0.2)	10.2 (0.3)	9.5 (1.7)	11.2 (1.2)
N:P	3.0 (1.1)	1.5 (0.3)	1.0 (0.1)	1.3 (0.2)	1.1 (0.3)	1.5 (0.3)
Chl *a*	1.8 (0.3)	3.3 (0.6)	3.3 (0.9)	4.3 (0.5)	2.6 (1.1)	3.8 (1.1)
Porosity	0.4 (0.0)	0.5 (0.0)	0.4 (0.0)	0.5 (0.0)	0.5 (0.0)	0.5 (0.0)

Table 4. Results of two-way ANOVA of nutrient and gas fluxes with habitat (unvegetated sand and eelgrass) and treatment (control and $+^{15}N$) as fixed factors. Study sites include Cormorant Point (Cor. Pt.), Tiana Bay (T. Bay), and Tiana Beach (T. Beach) within Shinnecock Bay (New York, USA). Results are aligned for qualitative comparisons among sites.

	SRP flux		**NH_4^+ flux**		**NO_3^- flux**		**N_2 flux**		**O_2 flux**	
	F	***P***	**F**	***P***	**F**	***P***	**F**	***P***	**F**	***P***
Cor. Pt.										
Habitat	1.7	0.24	0.4	0.56	3.6	0.1	0.4	0.56	5.9	**0.04**
Treatment	2.6	0.14	0.0	0.88	6.4	**0.04**	6.8	**0.03**	9.6	**0.02**
Interaction	0.1	0.73	0.0	0.94	0.257	0.63	6.9	**0.03**	1.3	0.29
T. Bay										
Habitat	6.3	**0.04**	5.5	**0.04**	133.7	**<0.01**	7.2	**0.03**	21.4	**<0.01**
Treatment	3.1	0.12	2.6	0.14	1635.5	**<0.01**	22.0	**<0.01**	5.2	0.05
Interaction	6.0	**0.04**	4.8	0.06	150.8	**<0.01**	2.6	0.15	1.6	0.23
T. Beach										
Habitat	0.5	0.49	9.5	**0.02**	22.7	**<0.01**	35.7	**<0.01**	13.6	**<0.01**
Treatment	0.3	0.60	0.1	0.75	231.5	**<0.01**	28.9	**<0.01**	5.4	**0.04**
Interaction	1.2	0.31	1.4	0.28	20.2	**<0.01**	7.5	**0.03**	0.5	0.5

Table 5. The mean ratio (±SE) of total denitrification to NO_X^- fluxes from the $+^{15}N$ treatment using sediment collected at three sites and two subtidal habitat types (eelgrass and unvegetated sand) located in Shinnecock Bay (Long Island, NY). Study sites include Cormorant Point (Cor. Pt.), Tiana Bay (T. Bay), and Tiana Beach (T. Beach). Values are negative due to NOx- uptake.

Site	**Habitat**	**Total N_2:NO_x^-**
Cor. Pt.	Sand	-0.02 (0.14)
Cor. Pt.	Eelgrass	-0.31 (0.07)
T. Bay	Sand	-0.53 (0.21)
T. Bay	Eelgrass	-0.77 (0.14)
T. Beach	Sand	-0.09 (0.03)
T. Beach	Eelgrass	-0.43 (0.08)

Table 6. The daily mass and monetary value of nitrogen removed from New York estuaries (South Shore Estuary, Peconic Estuary, and Long Island Sound) through denitrification in eelgrass meadows when exposed to enriched NO_3^- conditions. Denitrification is assumed to occur in dark hours only (12h day^{-1}). Value of nitrogen removed (\$12.34 kg^{-1}) is based on the 2013 price (USD) for the Connecticut (USA) nitrogen trading program. Estimates of current and historic eelgrass areal coverage are from the New York State Seagrass Taskforce 2009.

Site	**Year**	**Eelgrass area (km^2)**	**N removal day^{-1} (kg)**	**Value of N removal day^{-1} (USD)**
South Shore Est.	2009	81	4,695	\$57,936
Peconic Est.	1930	35.29	2,046	\$25,248
Peconic Est.	2009	6.28	364	\$4,492
Long Island Sd.	1930	96	5,565	\$68,672
Long Island Sd.	2009	0.96	56	\$691

Figure Legends

Fig 1 Map of the three study sites in Shinnecock Bay, which is located on the south shore of eastern Long Island, New York, USA.

Fig 2 Sediment fluxes of soluble reactive phosphorous (**a**), ammonium (**b**), and nitrate +nitrite (**c**) at the three study sites in Shinnecock Bay. Sediment cores were collected from unvegetated sand (unshaded bars) and eelgrass meadows (shaded bars) at each site. Measurements were performed in continuous-flow cores with site water (Control; Ctl) and with site water amended with $^{15}NO_3^-$ ($+^{15}N$ treatment).

Fig 3 Net $^{28}N_2$ flux (**a**) and total denitrification ($^{28,29,30}N_2$ flux; **b**) at the three study sites in Shinnecock Bay. Sediment cores were collected from unvegetated sand (unshaded bars) and eelgrass meadows (shaded bars) at each site. Measurements were performed in continuous-flow cores with site water (Control; Ctl) and with site water amended with $^{15}NO_3^-$ ($+^{15}N$ treatment).

Fig 4 The relationship of sediment organic carbon (**a**) and sediment total nitrogen (**b**) to total denitrification ($^{28,29,30}N_2$ flux) when exposed to site water enriched with $^{15}NO_3^-$ at the three study sites in Shinnecock Bay. Sediment cores were collected from unvegetated sand (unshaded) and eelgrass meadows (shaded) at each site.

Fig 5 a-b The relationship of sediment oxygen demand and the total N flux (DIN + $^{28,29,30}N_2$) at the three study sites in Shinnecock Bay in control cores (**a**; unamended) and $^{15}NO_3^-$ enriched cores (**b**). The lines represent the molar ratio between O_2 flux and $\sum$N flux for each treatment. **c-**

d The relationship of sediment oxygen demand and the soluble reactive phosphorus flux (SRP) at the three study sites in Shinnecock Bay in control cores (**c**; unamended) and $^{15}NO_3^-$ enriched cores (**d**). The lines represent the molar ratio between P flux and ∑N flux for each treatment. **e-f** The relationship of total N flux (DIN + $^{28,29,30}N_2$) and SRP flux at the three study sites in Shinnecock Bay in control cores (**e**; unamended) and $^{15}NO_3^-$ enriched cores (**f**). The lines represent the molar ratio between ∑N flux and P flux for each treatment.

Supplementary Data 1. Sediment microprofiles of oxygen (**a-c**) and hydrogen sulfide (measured as $[HS]^-$; **d-f**) measured at the three study sites in Shinnecock Bay. Measurements were performed in sediment cores collected from unvegetated sand (unshaded circles) and eelgrass meadows (shaded circles) at each site.

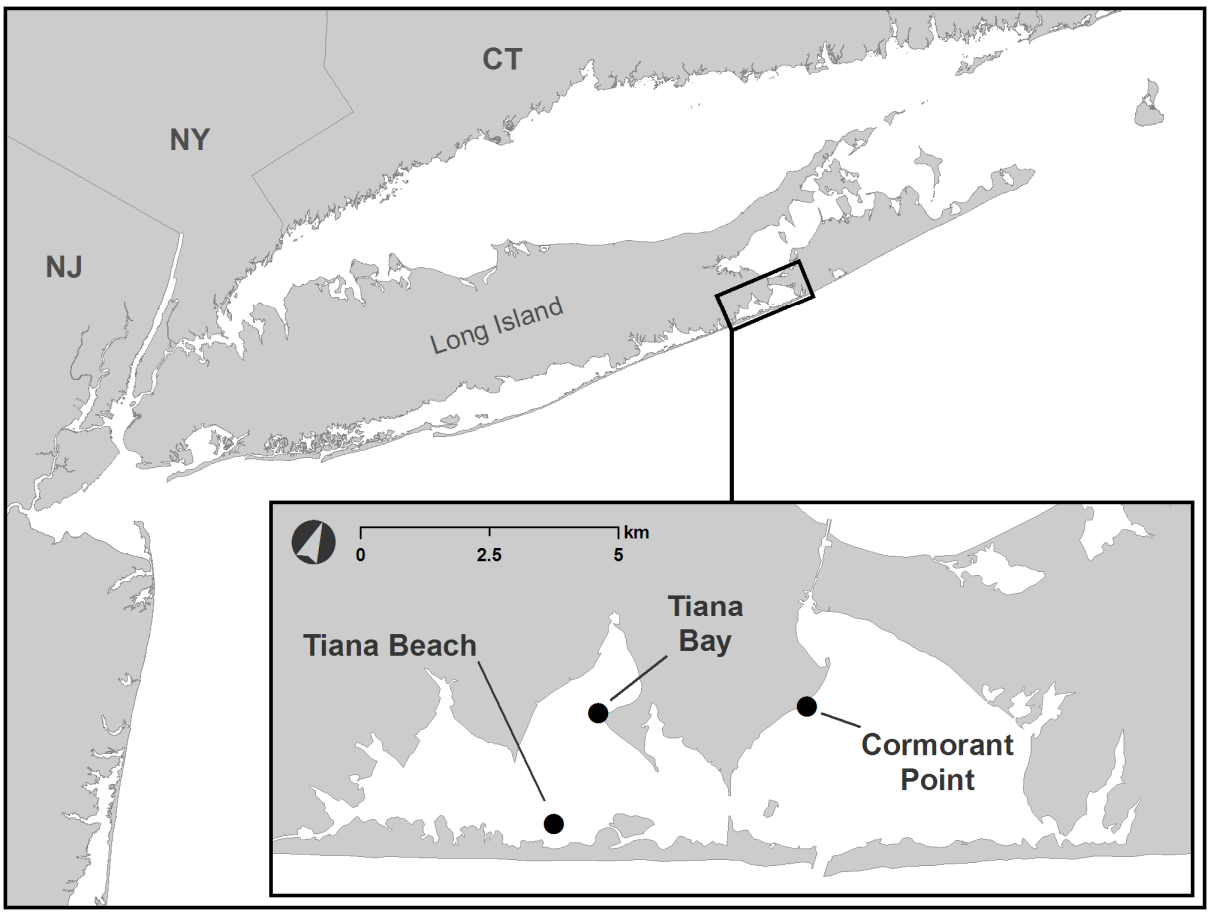

CT
NY
NJ
Long Island
km
0
2.5
5
Tiana Beach
Tiana Bay
Cormorant Point

Figure 1.

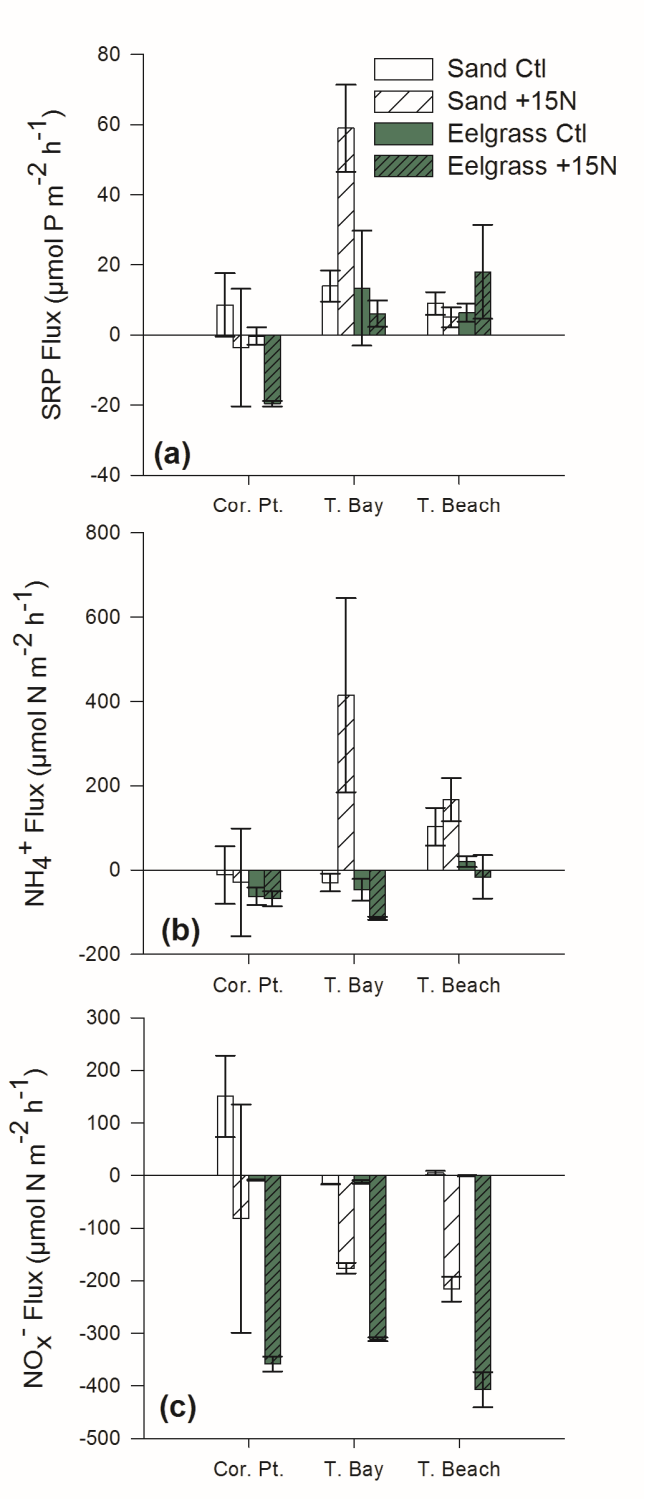

Sand Ctl
Sand +15N
Eelgrass Ctl
Eelgrass +15N
SRP Flux (µmol P m-2 h-1)
80
60
40
20
0
-20
-40
(a)
Cor. Pt.
T. Bay
T. Beach
NH4+ Flux (µmol N m-2 h-1)
800
600
400
200
0
-200
(b)
Cor. Pt.
T. Bay
T. Beach
NOX- Flux (µmol N m-2 h-1)
300
200
100
0
-100
-200
-300
-400
-500
(c)
Cor. Pt.
T. Bay
T. Beach

Figure 2.

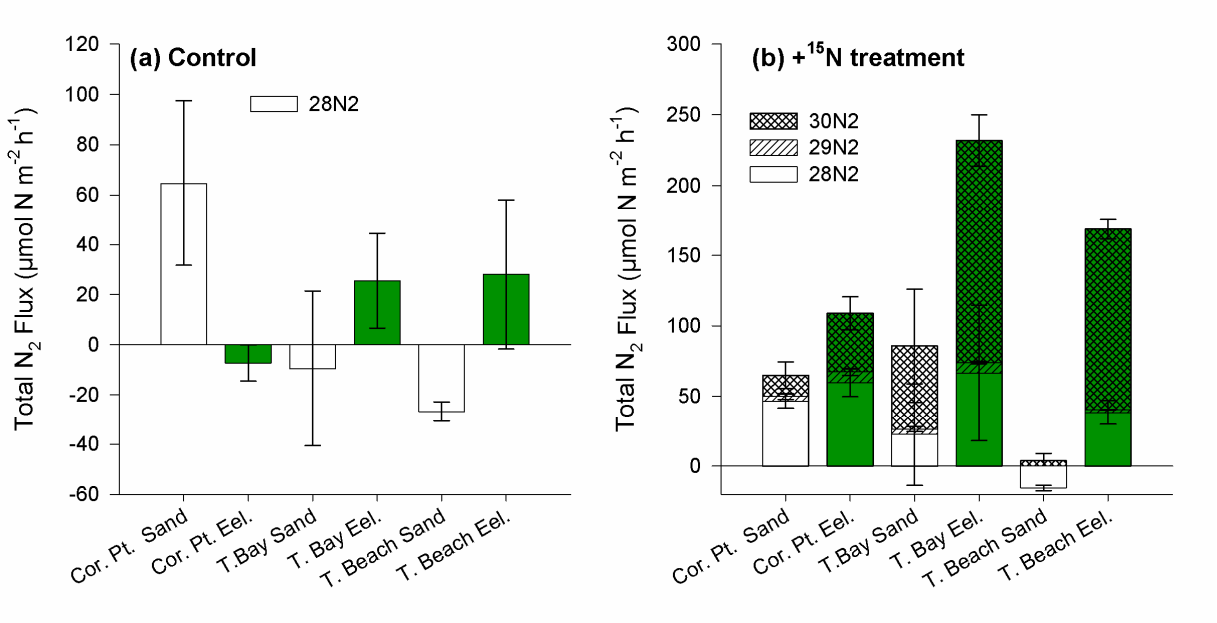


Figure 3.

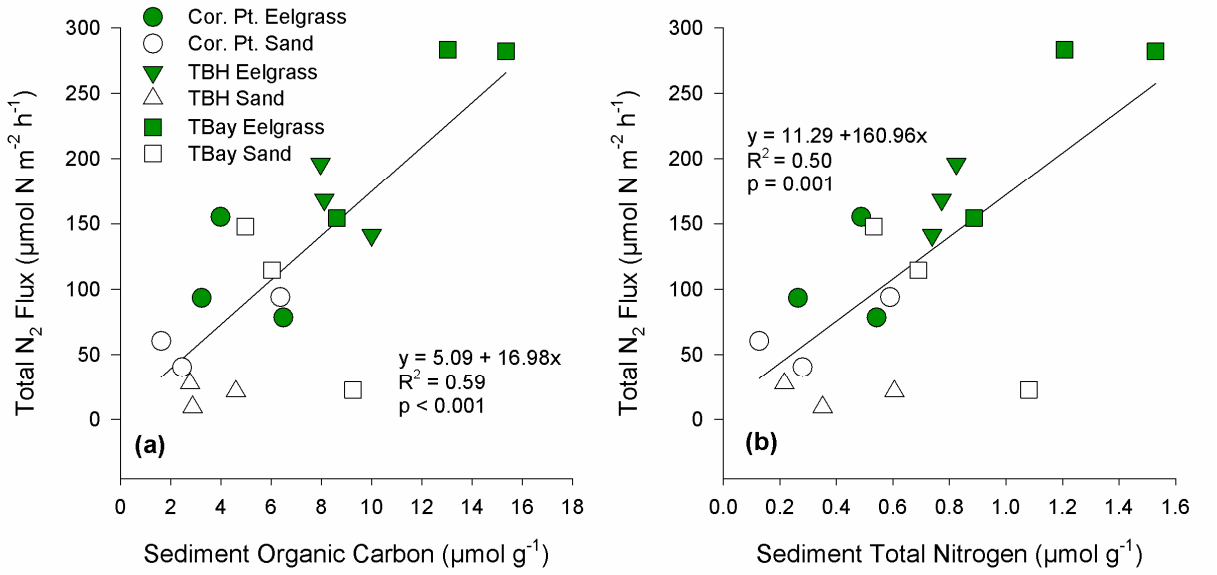


Figure 4.

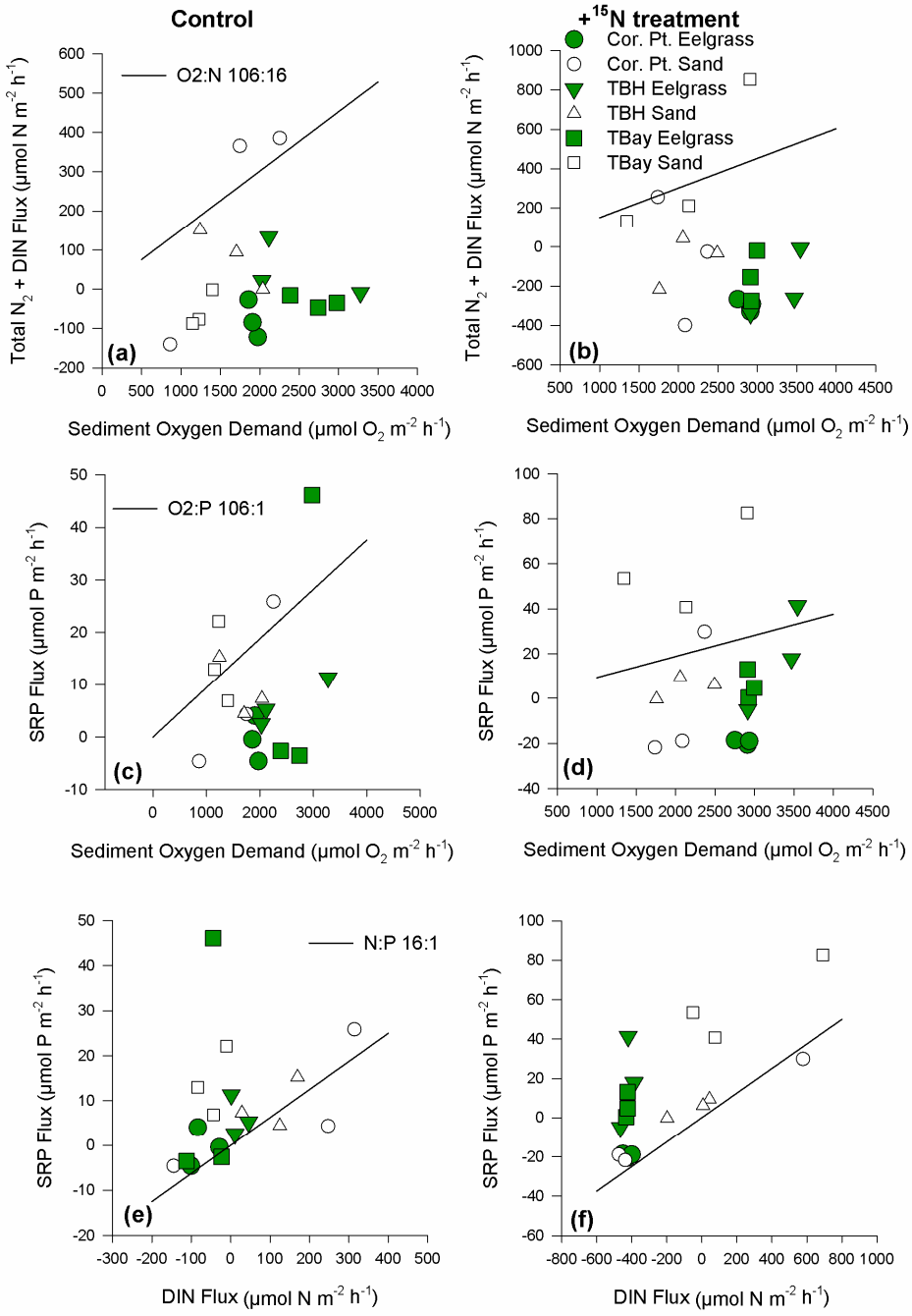
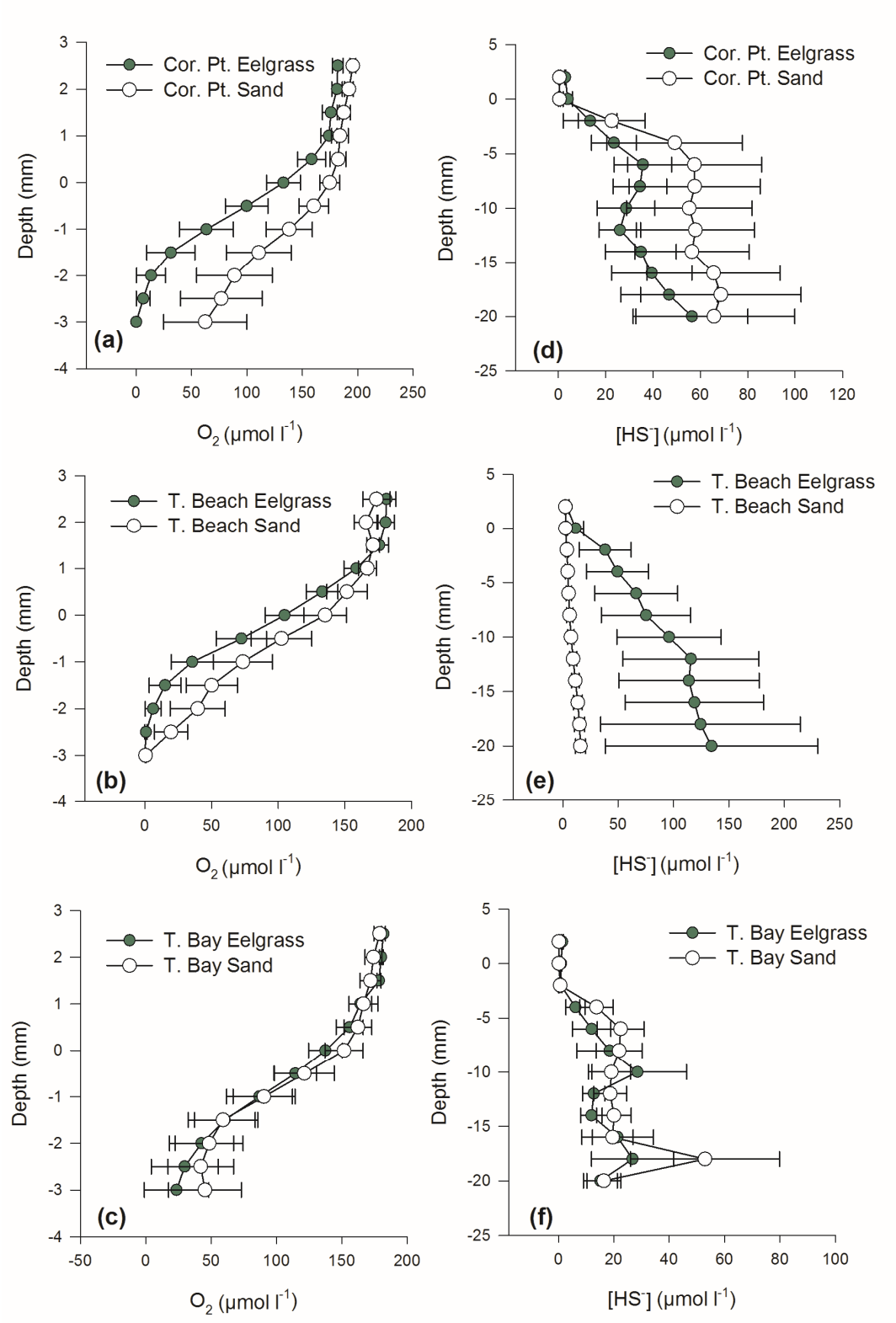

Control
+15N treatment
Cor. Pt. Eelgrass
Cor. Pt. Sand
TBH Eelgrass
TBH Sand
TBay Eelgrass
TBay Sand
O2:N 106:16
O2:P 106:1
N:P 16:1
Total N2 + DIN Flux (µmol N m-2 h-1)
SRP Flux (µmol P m-2 h-1)
Sediment Oxygen Demand (µmol O2 m-2 h-1)
DIN Flux (µmol N m-2 h-1)
(a)
(b)
(c)
(d)
(e)
(f)

Figure 5.



Supplementary Data 1