Living shorelines enhance nitrogen removal capacity over time

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A B S T R A C T

Living shorelines are nature-based solutions to coastal erosion that can be constructed as salt marshes with fringing oyster reefs. Each of these habitats can decrease the potential for eutrophication through increased nitrogen (N) removal via denitrification. However, the development of N cycling over time has not been studied in living shorelines. This research measured denitrification rates in a chronosequence of living shorelines spanning 0–20 years in age in Bogue Sound, NC. Analyses were conducted seasonally from summer 2014 to spring 2015 along an elevation transect through the salt marsh, oyster reef, and adjacent sandflat at all sites. Gas fluxes (N_2 and O_2) from sediment core incubations were measured with a membrane inlet mass spectrometer (MIMS) to assess denitrification and sediment oxygen demand. Fluxes of dissolved nutrients and the greenhouse gas N_2O were measured. Sediment properties, inundation frequency, oyster filtration rates, and marsh grass stem density were also quantified. There was no significant difference in denitrification rates among habitats. N removal consistently increased from the 0- to 7-year-old sites. Denitrification efficiency was always greater than 50% and positive N_2O fluxes were negligible. Our results suggest that living shorelines increase net N removal within a relatively short time period following construction, without introducing deleterious greenhouse gas emissions. This demonstrates that living shorelines can play an important role in estuarine N cycling and management.

1. Introduction

Coastal environments provide many ecosystem functions that contribute to human well-being (Costanza et al., 1997; Millennium Ecosystem Assessment, 2005). As a result, estuarine habitat loss or degradation has social and ecological consequences. Estuaries provide functions such as nursery habitat for juvenile fishes and invertebrates (Beck et al., 2001), resilience to rising sea level (Morris et al., 2002), and nutrient cycling (Jordan et al., 2011). Structured estuarine habitats also limit coastal erosion (Koch et al., 2009), which is particularly valuable given high human population densities in coastal areas. Restoration is one way to mitigate for habitat loss and reintroduce ecosystem functions, and the construction of living shorelines can be considered a specific example of oyster reef and salt marsh restoration. Living shorelines are a nature-based solution to coastal erosion. They consist of marsh vegetation with an optional fringing hard substrate: rocks, cement, or natural hard structures like oyster reefs (NOAA Living Shorelines Workgroup, 2015). Living shorelines have been shown to be more effective than bulkheads at attenuating storm impacts (Smith et al., 2017), and they incorporate natural habitats that can provide additional ecosystem services, increasing their overall value (Currin et al., 2010). Since public approval can encourage restoration (Cairns, 2000, Hackney, 2000), increased awareness of the effectiveness of living shorelines could increase their implementation. Given estimates that 14% of the US coast was hardened as of 2005 (Gittman et al., 2015), it is crucial that the environmental benefits of living shorelines are well articulated to contextualize their appropriateness as a management option.

The habitats included in living shorelines have been shown to contribute to a common goal of estuarine restoration: mitigation of nutrient loading. In nutrient-rich systems, eutrophication can lead to a host of deleterious impacts, including harmful algal blooms (Paerl and Otten, 2013), hypoxia (Hagy et al., 2004), and fish kills (Paerl et al., 1998). Nitrogen (N) is a limiting nutrient in coastal ecosystems, but NO_3^- concentration is often high due to N loading from developed watersheds (McClelland and Valiela, 1998; Bowen and Valiela, 2001), creating an imperative to manage N loads both by controlling inputs and maximizing potential for removal. The latter is imperative (Brush, 2009; Passeport et al., 2013) and can be facilitated by ecosystem engineers that promote denitrification (Gutiérrez and Jones, 2006). Denitrification is a globally important microbial pathway that removes bioavailable N from terrestrial and aquatic environments (Herbert, 1999; Sutherland et al., 2010). In shallow coastal waters, NO_3^- reduced by denitrifiers can be derived from land-based sources or

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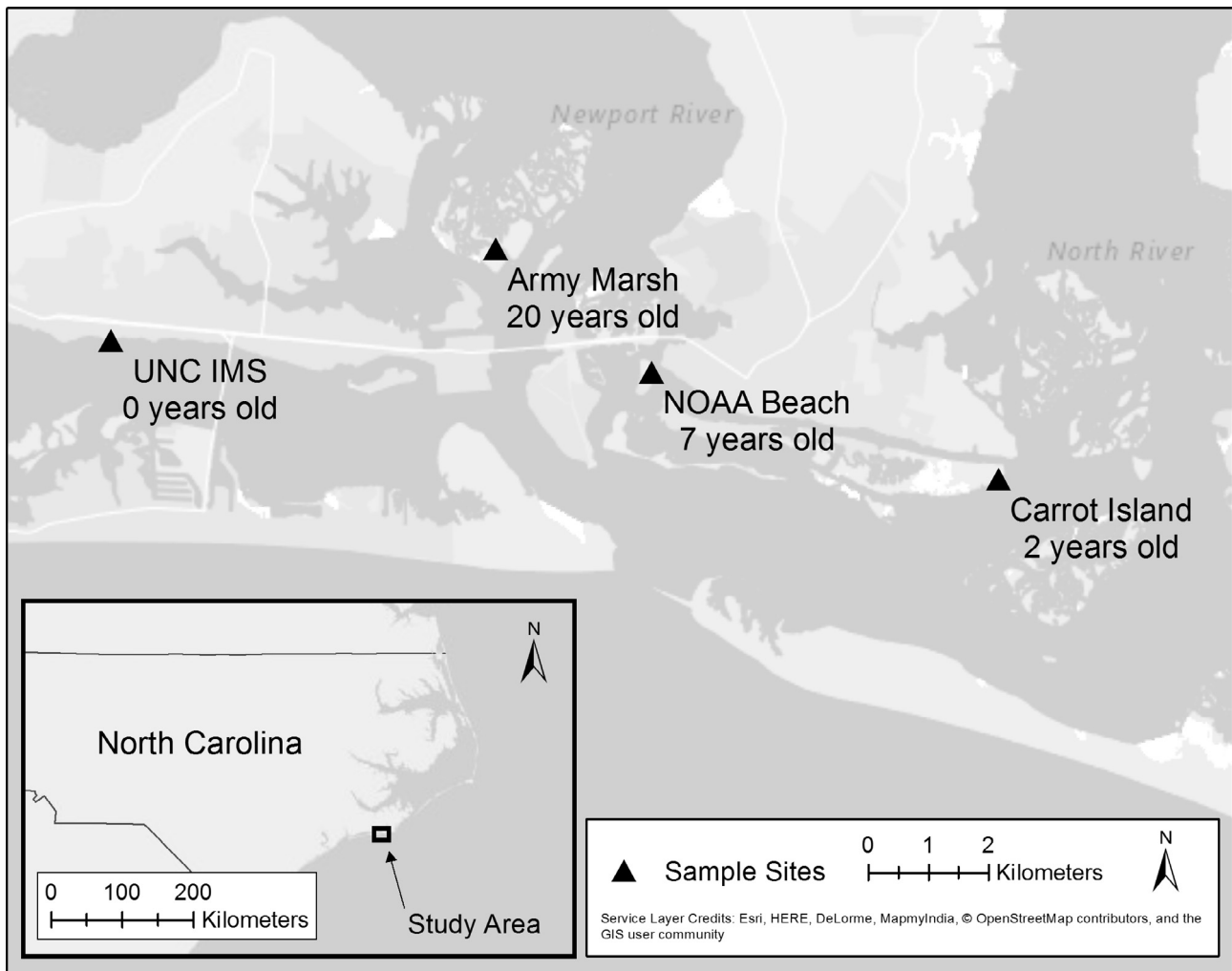
Fig. 1. Locations of the 4 sampling sites included in this study. Sites are identified by name and age, defined as years since living shorelines were constructed. Sites are located within a 13 km radius in Bogue Sound. The inset map identifies the study area within the state of North Carolina, USA.

produced by nitrification, whereby ammonium is oxidized to NO_3^-. The term "coupled nitrification-denitrification" (coupled NF-DNF) is used when the two processes are connected. Salt marshes have long been identified as important habitats for denitrification (George and Antoine, 1982; DeLaune et al., 1983), and recent work has shown that oyster reefs also facilitate high rates of denitrification (Kellogg et al., 2013; Piehler and Smyth, 2011; Seitzinger et al., 2006; Sousa et al., 2012; Pollack et al., 2013). Since living shorelines can include oyster reefs and salt marshes, they are likely to increase denitrification and consequently lower NO_3^- concentrations. However, to our knowledge, no study has directly measured N removal in living shorelines. Understanding the role of living shorelines in N cycling could lead to a more comprehensive valuation of this management option.

When designing and assessing nature-based solutions that modify N dynamics, it is also important to account for other N fluxes that are considered potential ecosystem disservices (Burgin et al., 2013; Lyytimäki and Sipilä, 2009). N_2O is a powerful greenhouse gas that is produced as a byproduct of nitrification and as an intermediate in denitrification. In estuarine environments, higher N_2O emissions are typically associated with denitrification (Dong et al., 2002), although some studies have shown that nitrification can be the main source of N_2O (de Wilde and de Bie, 2000; Ji et al., 2015). N_2O is produced via incomplete denitrification, during which it is released to the atmosphere instead of being reduced to N_2 gas. Coupled NF-DNF can reduce N_2O emissions in estuarine environments dominated by this process

(Cartaxana and Lloyd, 1999; LaMontagne et al., 2003). This may have been observed because coupled NF-DNF relies on adjacent and distinct oxic and anoxic conditions, and persistent anoxia increases the likelihood of complete denitrification. Increased production of NH_4^+ can also be considered a disservice, since it provides a bioavailable N source that can promote algal growth. By measuring fluxes of N_2, N_2O, and NH_4^+, this study aimed to provide a comprehensive assessment of both beneficial and deleterious aspects of nitrogen cycling in living shorelines.

This study employed a chronosequence space-for-time replacement design to analyze living shorelines. Monitoring is time- and resource-intensive, and well-designed chronosequence studies can provide the benefits of long-term monitoring without the expense (Hutto and Belote, 2013). Data from chronosequences can be used to construct restoration trajectories, which are theoretical frameworks that visualize change in an environmental parameter over time (Kentula et al., 1992). Although the concept of restoration success remains a contentious one (Kentula, 2000; Ruiz-Jaen and Mitchell Aide, 2005; Zhao et al., 2016), restoration practitioners have been advised to identify specific habitat functions as goals (Hackney, 2000, Simenstad and Cordell, 2000). Restoration trajectories can illustrate a range of development pathways for a chosen function based on management decisions and environmental stressors (Testa et al., 2017), which can provide a useful framework for evaluating projects.

The goal of this study was to evaluate the development of N removal

capacity in living shorelines composed of salt marshes and oyster reefs over time. We aimed to contextualize N cycling by calculating denitrification efficiency and measuring N_2O production, and we assessed other biotic and abiotic parameters to identify influences on denitrification rates. It was hypothesized that denitrification rates would increase with age and eventually reach a plateau. Based on comparable prior studies (Piehler and Smyth, 2011; Smyth et al., 2013), it was expected that structured habitats would exhibit higher denitrification rates than unstructured adjacent sandflats, and that seasonal variability in denitrification would be observed among habitats (Thompson et al., 1995; Nowicki et al., 1997; Cabrita and Brotas, 2000). Overall, this study aimed to provide some of the first measurements of N cycling in living shorelines and thereby inform expectations regarding the development of biogeochemical processes.

2. Methods

2.1. Study sites

Sampling was conducted in 4 sites located in Bogue Sound in the southern Outer Banks of North Carolina, USA (Fig. 1). Bogue Sound has relatively good water quality: monitoring over the past decade shows a mean chl a concentration of $3.97 \pm 2.00 \, \mu g/L$ (S.P. Thompson, unpublished data). The system is highly N-limited, with maxima of 5 and $30 \, \mu M$ measured for NO_x and NH_4^+, respectively, during the study. Sites were restored 0–20 years prior to this study as living shorelines constructed for ecosystem restoration or mitigation purposes (Sutton-Grier et al., 2015). All sites consisted of salt marshes dominated by *Spartina alterniflora* with fringing *Crassostrea virginica* oyster reefs. The sites chosen for this study are located within a 13 km radius and are exposed to similar environmental conditions, such as rainfall and temperature. Wave energy and inundation vary between sites. The 2-year-old site is exposed to wind-driven waves in Bogue Sound, the 0-year-old site experiences frequent boat wakes, and the 7-year-old site is in a more protected no-wake zone. The 20-year-old site is in a semi-enclosed marsh complex that reduces wave energy and may influence tidal exchange. Average annual tidal inundation frequency varied across sites and sampling zones (Table 1). The 7-year-old site typically had the highest average inundation frequency. The chosen sites compare to other chronosequence studies (Salmo et al., 2013) and are within a tighter radius than other published studies (e.g. Ballantine and Schneider, 2009).

Sampling was conducted each season from summer 2014 through spring 2015 along transects of 5 elevations at each site: seaward and landward sides of the oyster reef and 3 elevations in the salt marsh (Fig. 2). Fieldwork was conducted at approximate low tide to maximize access to lower elevations. Adjacent tidal sandflats within 15 m were sampled at an elevation matching the reef/marsh border to evaluate the impact of the living shoreline on surrounding sediment.

Table 1

Average annual inundation frequency (%) ($\pm$ SE) for the 5 sampling zones at each site. Methods used to calculate inundation frequency are explained later in this section.

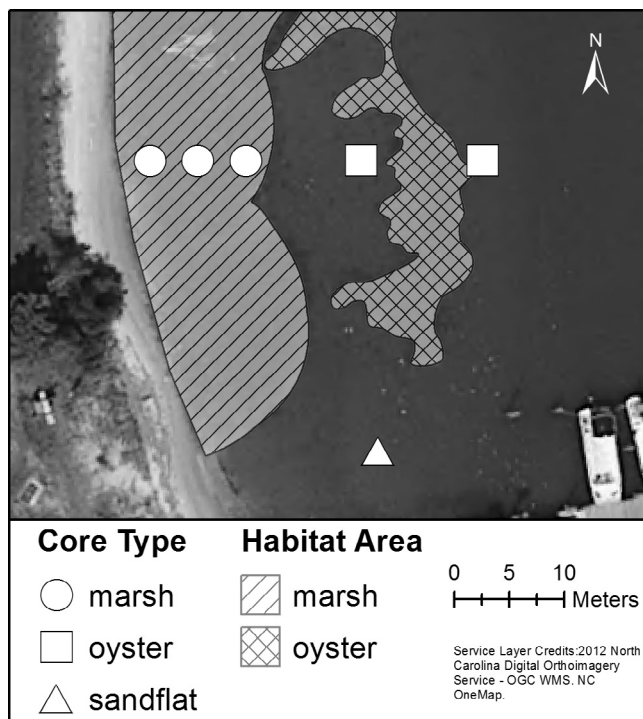| Sampling Zone	Inundation (%)			
	0 years old	2 years old	7 years old	20 years old
Low Oyster	100 ± 0	100 ± 0	100 ± 0	89.6 ± 6.7
High Oyster	96.5 ± 3.2	70.7 ± 8.3	96.6 ± 3.0	78.6 ± 8.6
Low Marsh	N/A	71.8 ± 8.3	98.1 ± 1.8	64.1 ± 7.5
Mid Marsh	78.3 ± 8.5	63.2 ± 7.4	95.4 ± 4.1	52.0 ± 7.3
High Marsh	N/A	43.9 ± 6.8	65.7 ± 7.8	44.9 ± 7.0

Fig. 2. Diagram of the sampling scheme, illustrated for the 7-year-old site. Marsh and oyster habitat areas are shaded. Oyster sediment cores were collected from the landward and seaward sides of the oyster reef; marsh cores were collected from low, mid, and high marsh elevations; and sandflat cores were collected in adjacent sandflats. Sampling was conducted in triplicate.

2.2. Sediment core collection

Sediment cores were collected in triplicate using plastic polycarbonate tubes (6.4 cm diameter x 30 cm). Cores were inserted by hand into the sediment to 17 cm, topped with site water, and capped with rubber stoppers. Care was taken to exclude vegetation and megafauna. Oyster habitat cores were collected from sediment adjacent to the reef. Site water was also collected for flow-through incubation. All cores were immediately stored in a cooler and transported to an environmental chamber at UNC Institute of Marine Sciences (IMS), where they were immersed in site water in the dark at average seasonal *in situ* temperature. During the study period, temperatures ranged from 9 to 27 °C and salinity ranged from 27 to 35.

Following overnight equilibration, cores were capped underwater to exclude bubbles. They were connected to a flow-through core incubation system, whereby site water was pumped through an inflow tube into the top and out from the bottom of the water column of each core at a rate of $1 \, mL \, min^{-1}$ with an approximate turnover time of 5 h. The continuous flow experiments were conducted in an environmental chamber (Bally Inc.) at *in situ* temperatures as described above. Each core was capped with a Plexiglas top milled to accommodate to O-rings to assure an air- and watertight seal. Each cap contained two ports plumbed with Tygon tubing, one for inflow and one for outflow. Water column volume was maintained at approximately 400 mL. Unfiltered inflow water was held in the reservoir and aerated until it was passed over cores using a multichannel peristaltic pump.

2.3. N_2 and O_2 measurements

Water samples (5 mL) were collected from the outflow of sediment cores at least 16 h after capping. Samples were also collected from an inflow line to assess background concentrations of dissolved gas and to control for influence of the plastic tubing. Sampling was repeated

several times at 5 h intervals following approximate turnover of overlying water to assess the duration of steady state. Net dissolved N_2 and O_2 concentrations were measured using a Balzers Prima QME 200 quadropole mass spectrometer (MIMS; Pfeiffer Vacuum, Nashua, NH, USA; Kana et al., 1994). Without the use of stable isotopic tracers, the MIMS cannot distinguish between N_2 produced through denitrification or anammox, although the latter is often negligible in estuarine habitats (Koop-Jakobsen and Giblin, 2009). The MIMS also cannot distinguish between denitrification and sediment N_2 uptake through nitrogen fixation, which has been shown to occur in restored salt marshes (Piehler et al., 1998). Cores experience a gradual draw-down of oxygen over the course of the incubation that was quantified as sediment oxygen demand (SOD).

2.4. Flux calculations

All fluxes (μmol m^{-2} h^{-1}) were calculated per the following equation (Piehler and Smyth, 2011):

$$flux = ([x]_{outflow} - average\,[x]_{inflow}) * \frac{pump\ rate}{core\ area} \qquad (1)$$

where $[x]_{outflow}$ is concentration in the sediment core outflow tube (μM), average $[x]_{inflow}$ is average concentration in the inflow tubes (μM), pump rate is the incubation flow-through rate (L h^{-1}), and core area is the surface area of the sediment sample in the core (m^2). Positive and negative dissolved gas fluxes were interpreted as net flux out of and into the sediment, respectively. Denitrification was calculated as net positive N_2 flux (μmol N m^{-2} h^{-1}) and SOD was calculated as net negative O_2 flux (μmol O_2 m^{-2} h^{-1}).

Denitrification efficiency (DNE) was calculated per the following equation (Eyre and Ferguson, 2002):

$$DNE\,(\%) = \frac{N_2\,flux}{N_2\,flux + DIN\,flux} * 100 \qquad (2)$$

where DIN flux included NH_4^+ and NO_x (μmol m^{-2} h^{-1}).

2.5. N_2O flux measurements

Water samples (100 mL) were collected in vented N_2-sparged glass serum bottles (260 mL) to eliminate contamination with ambient N_2O. Bottles were shaken vigorously to equilibrate gases, and headspace gas was transferred to an evacuated glass vial (13 mL). A syringe was used to inject an air sample into a 1 mL sample loop of a Shimadzu GC-2014 (Shimadzu Corporation, Kyoto, Japan) for detection of N_2O gas. $[N_2O]$ was calculated based on the assumptions of Henry's Law. $[N_2O]$ (μM) in each water sample was calculated using the Bunsen solubility coefficient (β), which was in turn was calculated from the Henry's Law solubility constant (K_o). Equations from Weiss and Price (1980) were used to calculate K_o based on published constants and in situ temperature and salinity. Flux (μmol N_2O m^{-2} h^{-1}) was calculated per Eq. (1).

2.6. Nutrient concentration and flux measurements

Water samples (40 mL) were collected from inflow and outflow tubing midway through the incubation. Samples were analyzed on a Lachat Quick-Chem 8000 (Lachat Instruments, Milwaukee, WI, USA) to measure concentrations of NO_x, NH_4^+, PO_4^{3-}, total nitrogen (TN), and organic nitrogen by difference (ON) (μM). Detection limits were 0.05 μM N-NO_x, 0.24 μM N-NH_4^+, 0.02 μM P-PO_4^{3-}, and 0.75 μM N-TN. Nutrient flux was calculated per Eq. (1).

2.7. Sediment characteristics

Surface sediment (0–3 cm) was collected from each core at the end of the incubation and analyzed for sediment organic matter (SOM) via loss on ignition (LOI) (Byers et al., 1978). Known volumes of surface sediment from spring 2015 samples were dried and combusted to determine bulk density (g cm^{-3}). Sediment samples from spring 2015 were pulverized and analyzed with a Costech ECS 4010 CHNS-O Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) to determine bulk %N, %C, and C:N ratios. Bulk density and CHN data were assumed to represent sediment conditions during the study period.

2.8. Inundation calculations

Inundation for the 0-, 2-, and 7-year-old sites was calculated by deploying HOBO water level loggers (Model: U20-001-01, Onset Corporation, Bourne, MA, USA) on the seaward side of the oyster reef. Water level data were logged at 15-min intervals for at least 1 month. Data were corrected for local barometric pressure and brackish conditions, and standardized to local tide records from the NOAA monitoring station in Beaufort, NC. The relationship was used to hindcast seasonal tides at each study site (Smyth et al., 2013). NOAA tide records were not standardized for the 20-year-old site, where water level data were not successfully recorded. Elevation data and field records indicated that this substitution was appropriate. Inundation frequency was calculated for each season by comparing hindcasted water levels to sampling elevations at each site. Elevations were obtained in the field using an automatic laser level (Model SAL24N, CST/Berger, Watseka, IL, USA).

2.9. Oyster filtration and marsh grass density

Oyster density was measured in summer 2015 to assess conditions during the study period. A total of four 1/16 m^2 quadrats were randomly tossed onto the oyster reef: half on the reef crest and half on the landward side. Live oysters were excavated and transported to IMS for processing. The number of mature oysters and their shell heights (SH) were recorded. Spat $<$ 25 mm SH were excluded (zu Ermgassen et al., 2013).

Oyster filtration provides a functional representation of oyster populations and was calculated for each season per the following equation (zu Ermgassen et al., 2013):

$$filtration\ rate\,(L\ m^{-2}h^{-1}) = N\,(8.02W^{0.58}e^{[-0.015*((T-27)^2)]}) \qquad (3)$$

where N is oyster density (number of mature oyster m^{-2}), W is dry tissue weight (g), and T is temperature ($^\circ$C). Dry tissue weight was estimated from SH using an equation developed for South Carolina (Grizzle et al.2008; zu Ermgassen et al., 2016). Seasonal filtration rates do not include an error term because oyster density and dry tissue weight were assessed once.

S. alterniflora density was measured in fall 2015 prior to senescence to assess density during the growing season. Three marsh elevation zones (low, mid, and high) were sampled at each site. Three ¼ m^2 quadrats were tossed in each zone in a manner favoring vegetated areas. The number of live S. alterniflora culms in each quadrat was recorded and adjusted by estimating percent cover to calculate overall density for each zone.

2.10. Statistical analysis

N_2 flux data were analyzed for normality and heteroscedasticity. A constant was added to convert all data to positive values before applying a Box-Cox transformation (Box and Cox, 1964). Transformation achieved homogeneity of variances and improved normality. A three-way ANOVA was used to identify significant differences (α = 0.05) in denitrification rates, with site, season, and habitat as interactive fixed factors. The effect of habitat was not significant (p = 0.19), so a two-way ANOVA was conducted with site and season as interactive fixed factors. A Tukey post-hoc test was also conducted.

Restoration trajectories were created for seasonal and annual data using regressions fitted with second-order polynomial equations. This provided the best fit for the observed data; it was not necessarily expected that denitrification would decrease with age. All data from the entire study year were included in the annual restoration trajectory. Analysis of the seasonal trajectories focused on summer, when N removal is an important potential regulator of an annual peak in biotic activity. Correlations were conducted to identify collinearity among site parameters and between LOI and CHN data. Regression and correlation results are reported with the p-value and Pearson correlation coefficient.

A regression tree was constructed to explore the relative impact of site parameters (SOD, O_2 concentration, SOM, NH_4^+ flux, and NO_x flux) and factors (site age, season, and habitat) on annual N_2 fluxes. Seasonal regression trees were also constructed to eliminate the potentially confounding influence of temperature (Seitzinger, 1988; Bachand and Horne, 2000). Regression trees were constructed using the ANOVA version of recursive partitioning, and pruned with a complexity parameter corresponding to the smallest tree with a cross-validation error within one standard deviation of the minimum (De'Ath and Fabricius, 2000). If this method did not sufficiently prune the tree, the next smallest complexity parameter was applied.

All analyses were conducted using R Version 3.3.1 (R Core Team 2016). Regression trees were constructed using the rpart package (Therneau et al., 2015).

3. Results

3.1. Nitrogen cycling

N_2 flux rates (μmol N m^{-2} h^{-1}) were generally positive, indicating net denitrification (Fig. 3). N_2 fluxes were not significantly different across habitats (p = 0.19). Site and season each had a significant impact on N_2 fluxes (p = 2×10^{-16} & 2.3×10^{-11}, respectively). The interaction of site and season was also significant (p = 9.7×10^{-11}), indicating that N_2 flux did not respond to seasonal variation in the same way across sites. Post-hoc testing indicated that all combinations of seasons were significantly different from one another except winter and fall (p = 0.99) and spring and summer (p = 0.16), and that the 7-year-old site was significantly different from all other sites (p = 0.00). N_2 flux was highest in the summer for most sites and habitats, and was consistently highest at the 7-year-old site.

N_2 flux for all sites combined was positively correlated with SOD (μmol O_2 m^{-2} h^{-1}) (p < 0.01, R^2 = 0.54; Fig. 4). The highest values of N_2 flux and SOD were observed in the summer and spring; the lowest were observed in the winter. N_2 flux was significantly correlated with SOM only during the summer (p < 0.01, R^2 = 0.49).

Regression of all data collected during the study year indicated that N_2 flux and SOD were significantly associated with age, although neither relationship explained a large amount of variability (p < 0.01; Fig. 5, Table 2). Both parameters increased from the 0- to 7-year-old sites and were lower at the 20-year-old site. During the summer, N_2 flux plateaued at the 7-year-old site (p < 0.01, R^2 = 0.54).

The best fit for the regression tree of all annual data included 4 splits (R^2 = 0.72; Fig. 6). Samples were initially split by SOD (530.7 μmol O_2 m^{-2} h^{-1}). Splits in the second row were based on SOD (232.1 μmol O_2 m^{-2} h^{-1}) and age (4.5 years). There was one split in the third row: data from sites older than 4.5 years were split by SOD (1393 μmol O_2 m^{-2} h^{-1}). None of the seasonal regression trees was notably different than the annual tree, indicating that controlling for temperature did not alter regression tree results.

3.2. N removal efficiency

Positive fluxes of NO_x and NH_4^+ were variable and low, with maxima of 33 and 305 μmol m^{-2} h^{-1}, respectively. Denitrification
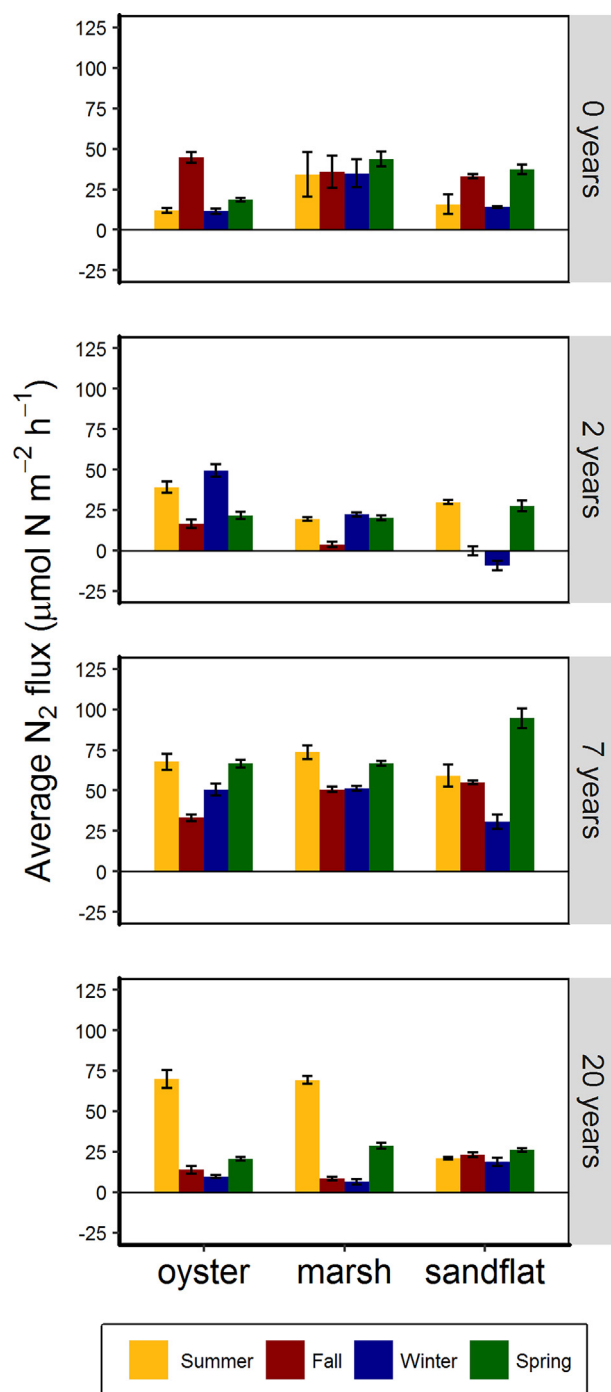
Fig. 3. Seasonal average net N_2 flux (μmol N m^{-2} h^{-1}) ($\pm$ SE) for each habitat at each site.

efficiency was always greater than 50%, indicating net N removal, and typically exceeded 75% (Fig. 7). DNE did not follow a discernable seasonal pattern, although the lowest rates were generally recorded in the winter. DNE remained relatively stable across restored age and habitats. There was no clear relationship between DNE and SOD. DNE generally increased with SOD to 1000 μmol O_2 m^{-2} h^{-1}, then plateaued. NH_4^+ flux was not positively correlated with SOD or SOM.

Average annual N_2O fluxes were < 0.5 μmol N_2O m^{-2} h^{-1} (Fig. 8). There were 2 positive average fluxes: the oyster habitat at the 2-year-old site and the marsh habitat at the 20-year-old site. All other fluxes were negative or had a range of error that included 0. The 0-year-old site exhibited the largest negative fluxes.
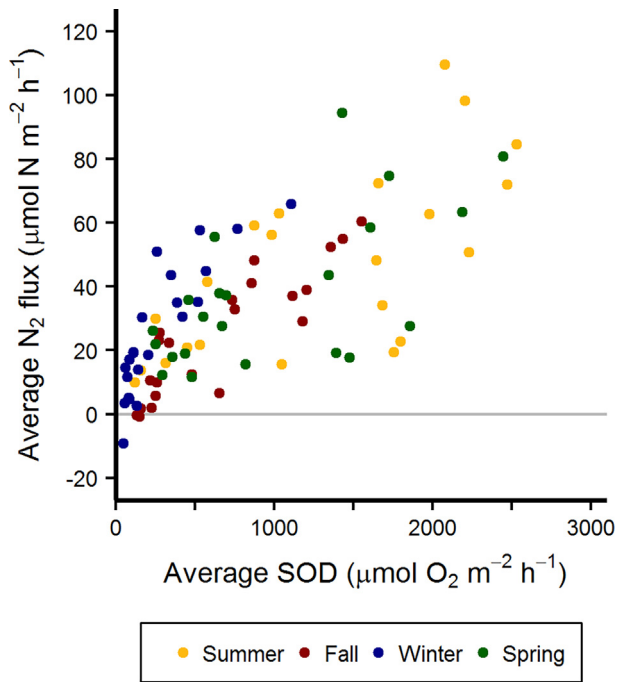
Fig. 4. N_2 flux (μmol N m^{-2} h^{-1}) is positively and significantly correlated with sediment oxygen demand (SOD) (μmol O$_2$ m^{-2} h^{-1}) ($p < 0.01$, $R^2 = 0.54$).

3.3. Sediment & site physical characteristics

Oyster reef sediment exhibited the highest SOM at all sites except the 20-year-old site (Table 3). SOM and bulk density were significantly associated with age ($p < 0.01$; Fig. 9, Table 2). SOM plateaued at the 7-year-old site, whereas bulk density exhibited the opposite trajectory. Correlations of sediment parameters quantified using LOI and CHN methods indicated that SOM was significantly correlated with %C and %N ($p < 0.01$).

Oyster filtration rates (L m^{-2} h^{-1}) were highest at the 0-year-old site, followed by the 7-year-old site (Table 4). The 20-year-old site exhibited the lowest filtration rates. Filtration rates are proportional to

Table 2
Results of regressions conducted using annual or seasonal data for various site parameters regressed against restored age. Data were fitted with a second-order polynomial equation. Only significant relationships ($p < 0.05$) are included in the table. Pearson's correlation coefficients (R^2) and p-values are reported.

Timespan	Parameter	Pearson's correlation coefficient (R^2)	p-value
Annual	SOD	0.13	< 0.01
Annual	N$_2$ flux	0.30	< 0.01
Summer	N$_2$ flux	0.54	< 0.01
Annual	SOM	0.39	< 0.01
Annual	Bulk density	0.44	< 0.01

temperature, so summer values representing the maximum rates are reported. Adjusted *S. alterniflora* stem density (number of stems m^{-2}) roughly corresponded to restored age (Table 4). There was no clear relationship between N_2 flux and either oyster filtration or stem density. Oyster filtration was not correlated with other site parameters such as SOM, NH$_4^+$ flux, or SOD. Stem density was positively correlated with annual average SOM ($p = 0.0502$, $R^2 = 0.9$).

4. Discussion

Annual denitrification rates were not significantly different between the oyster reef and salt marsh habitats restored as part of a living shoreline. The habitats comprising the living shorelines effectively functioned as a unit in terms of N removal within 20 years of construction. During the first 7 years following living shoreline construction, restoration trajectories for every season demonstrated an increase in denitrification rates. This suggests that nitrogen removal increases in marshes, oyster reefs, and adjacent sandflats during at least the first 7 years post-construction. The regression tree results also demonstrated that early development is important for denitrification in living shorelines, since some variation was explained by splitting the data at 4.5 years. Taken together, these results suggest that living shorelines are an effective way to bolster N removal within a relatively short time.

The similarity among habitats (salt marsh, oyster reef, and sandflat) was surprising, because sediment cores were collected along transects encompassing a range of elevations and inundation frequencies. Differences in sediment characteristics, redox conditions, and
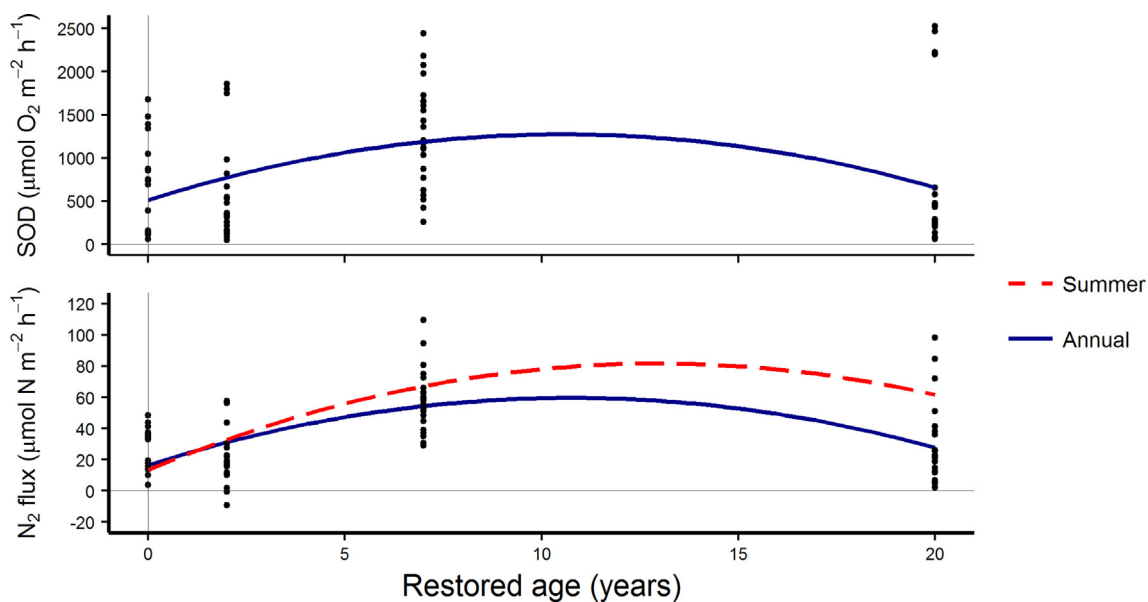

Fig. 5. Regression for sediment oxygen demand (SOD) (μmol O$_2$ m^{-2} h^{-1}) and N_2 flux (μmol N m^{-2} h^{-1}) as a function of restored age (years). Annual data include all data collected during the study year. The red dotted line illustrates the regression for data collected in summer. Regressions are fitted with a second-order polynomial equation. Pearson's correlation coefficient (R^2) and p-values are reported in Table 2.
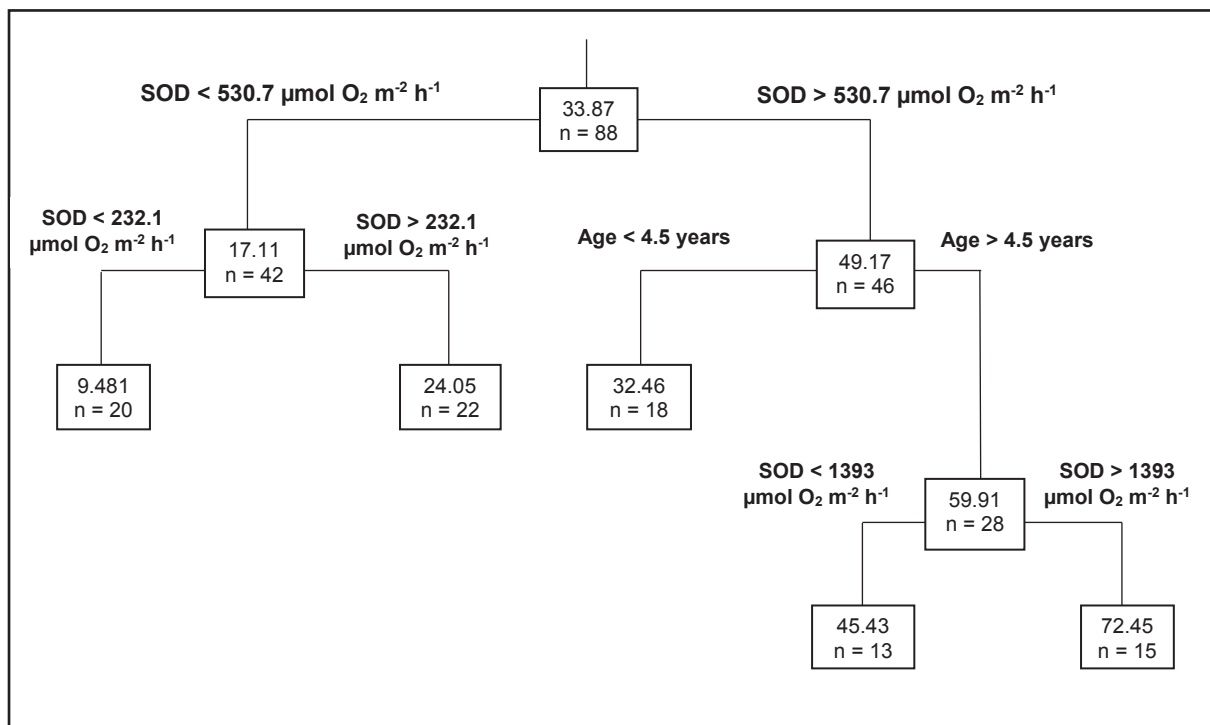
Fig. 6. Pruned regression tree for N_2 flux ($\mu mol\ N\ m^{-2}\ h^{-1}$). N_2 flux rates and the number of samples are reported in the boxes at each split. The regression tree was constructed using annual site parameters and factors: season, age, habitat, sediment organic matter (SOM) (%), sediment oxygen demand (SOD) ($\mu mol\ O_2\ m^{-2}\ h^{-1}$), O_2 concentration ($mg\ O_2\ L^{-1}$), NH_4^+ flux ($\mu mol\ NH_4^+\ m^{-2}\ h^{-1}$), and NO_x flux ($\mu mol\ NO_x\ m^{-2}\ h^{-1}$).
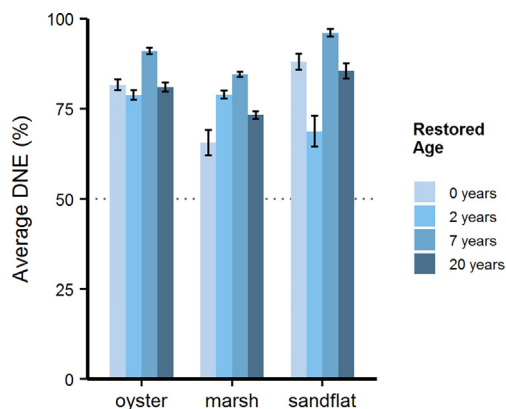
Fig. 7. Average annual denitrification efficiency (DNE) (± SE) for each habitat across living shoreline age. DNE greater than 50% (dotted line) indicates net N removal.

inundation were predicted to lead to distinctions in denitrification (Fernandes et al., 2016, Sousa et al., 2012). Conceptual models have even been developed to predict the timing and duration of sediment redox conditions favorable for denitrification in estuaries (Ensign et al., 2008). It is possible that because core incubation conditions mimicked high tide, the impact of inundation, and therefore elevation, was obscured. Wave energy, shoreline morphology, and shoreline orientation relative to prevailing wind direction could have affected the spatial distribution of denitrifiers at each site. Sediment characteristics such as bioturbation or $[S^{2-}]$ could have also constrained denitrification rates (Banks et al., 2013, Gilbert et al., 1995, Seitzinger, 1988). However, the range of elevations surveyed should have accounted for site-specific differences. In practical terms, our results suggest that the habitats in living shorelines enhance denitrification in ways that might not be predicted by elevation and inundation patterns.

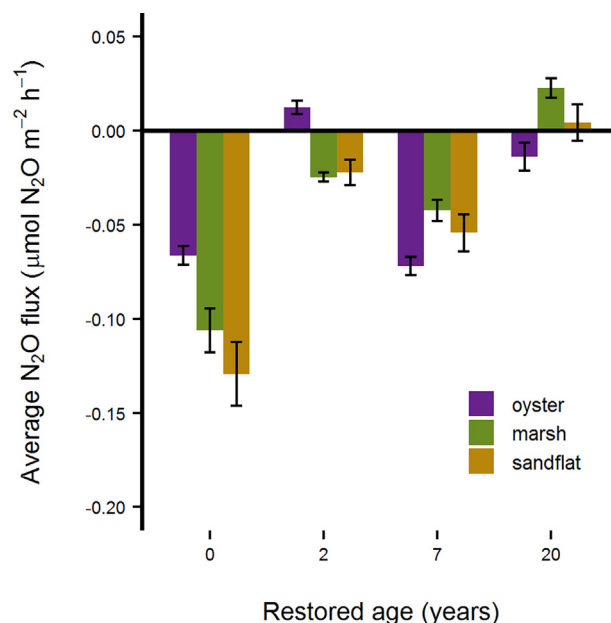It is useful to review denitrification rates in natural habitats for

Fig. 8. Annual average N_2O flux ($\mu mol\ N_2O\ m^{-2}\ h^{-1}$) (± SE) by restored age, divided by habitat.

context. Previous research in Bogue Sound found that among habitats, oyster reefs exhibited the highest denitrification rates, with average annual values of approximately $410 \pm 200\ \mu mol\ N\ m^{-2}\ h^{-1}$, whereas salt marshes exhibited average annual rates of approximately $170 \pm 20\ \mu mol\ N\ m^{-2}\ h^{-1}$ (Piehler and Smyth, 2011). Humphries et al. observed average annual denitrification rates of $582 \pm 164\ \mu mol\ N\ m^{-2}\ h^{-1}$ in oyster reefs in Rhode Island (2016), and Smyth et al. reported summer denitrification rates of approximately $210 \pm 10\ \mu mol\ N\ m^{-2}\ h^{-1}$ in restored oyster reefs adjacent to natural

Table 3

Sediment characteristics for habitats at each study site. Average annual sediment organic matter (SOM) (%) (± SE) and spring bulk density (g cm^{-3}) values are reported.

Site	Habitat	SOM (%)	Bulk density (g cm^{-3})
0 years old	Oyster	1.60 ± 0.05	1.31
	Marsh	1.01 ± 0.04	1.42
	Sandflat	1.16 ± 0.05	1.51
2 years old	Oyster	1.17 ± 0.02	1.42
	Marsh	0.78 ± 0.01	1.34
	Sandflat	0.75 ± 0.02	1.32
7 years old	Oyster	4.22 ± 0.10	0.90
	Marsh	3.34 ± 0.05	0.91
	Sandflat	1.35 ± 0.04	1.53
20 years old	Oyster	3.08 ± 0.04	0.92
	Marsh	4.41 ± 0.03	0.75
	Sandflat	2.99 ± 0.06	1.09

Table 4

Physical characteristics at each study site. SE is included for *S. alterniflora* stem density to reflect varying conditions across 3 marsh elevations.

Site	Summer oyster filtration rate (L m^{-2} h^{-1})	Oyster density (mature oysters m^{-2})	Average adjusted *S. alterniflora* stem density (stems m^{-2})
0 years old	95,040	972	26 ± 14
2 years old	10,254	411	11 ± 10
7 years old	49,259	361	71 ± 41
20 years old	2441	57	287 ± 142

salt marshes in Bogue Sound (2015). A range of denitrification rates in salt marshes have been recorded, including 25 μmol N m^{-2} h^{-1} in the summer in the St. Lawrence Estuary (Poulin et al., 2007) and up to 420 μmol N m^{-2} h^{-1} in Narragansett Bay (Davis et al., 2004). Although the denitrification rates documented by this study are lower than those in natural habitats, they are notable and contribute to the overall functional value of living shorelines, which includes shoreline stabilization and habitat provision.

Environmental context could also contribute to the comparatively lower rates observed in living shorelines. Oyster reefs and salt marshes naturally co-occur in NC estuaries and elsewhere on the East Coast, but living shorelines are often constructed in areas that have not previously fostered these habitats. As a result, some environmental parameters may have distinct influence on denitrification rates in living shorelines compared to oyster/marsh habitats that naturally recruited over time. For example, SOM may have less of an impact on denitrification in older natural marshes than in constructed marshes, especially considering delays in SOM accretion following restoration (Davis et al., 2015, Craft et al., 2002).

The impact of a living shoreline may extend beyond the complex itself, as suggested by the fact that there were no significant differences between denitrification rates in the living shoreline habitats and the adjacent sandflat. It has been suggested that biotic structured habitats provide organic matter subsidies to adjacent unstructured habitats,

increasing denitrification rates in the latter (Piehler and Smyth, 2011). The living shorelines studied here may also be exporting organic matter to adjacent sandflats. Since living shorelines are part of a broader ecological context, further exploration of whether organic matter outwelling is occurring would be beneficial. Ecosystem functions provided by living shorelines are typically described for the entire complex, rather than being delineated by habitat. The homogeneity of biogeochemical processes we observed underscores this paradigm and increases the attractiveness of living shorelines as an ecologically sound coastal management strategy.

Restoration practitioners are often limited by time and financial resources, making it useful to employ constructs such as restoration trajectories to anticipate changes in ecosystem functions over time. This study asked whether denitrification could be reliably predicted by restored site age, and the differences between annual and seasonal trajectories suggest that it would be misleading to identify a single trajectory. This is primarily due to seasonal differences in denitrification rates between the 7- and 20-year-old sites. The annual trajectory indicated a decrease in denitrification rates between the 7- and 20-year-old sites, reflecting the pattern during most seasons. However, during the summer, denitrification remained elevated beyond the 7-year-old site, recalling La Peyre et al.'s proposed trajectory for biogeochemical factors (2014). Older restored sites may be able to sustain high rates of denitrification during periods of elevated microbial activity in the summer, providing a critical ecosystem service by helping to control algae growth. Additional summer sampling of living shorelines between 7 and 20 years old is recommended to explore this pattern. The latter two-thirds of the trajectory was driven by the 20-year-old site, and although sites are treated as snapshots in time, they are discrete sites subject to local influences. Inundation frequency was particularly high
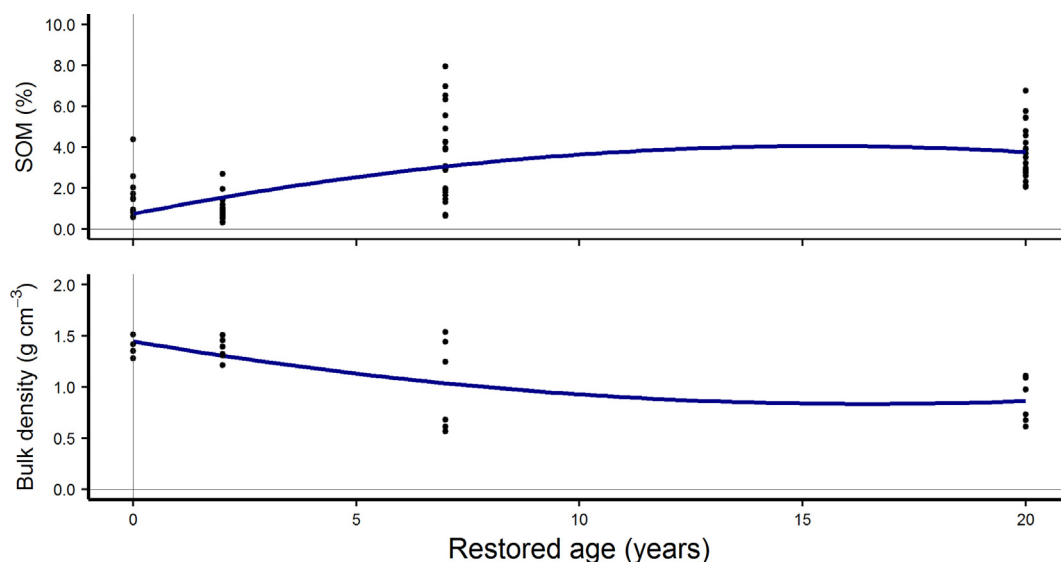

Fig. 9. Regression for sediment organic matter (SOM) (%) and bulk density (g cm^{-3}) as a function of restored age (years). SOM data include all data collected during the study year; bulk density data were collected during spring 2015 to describe conditions during the study period. Regressions are fitted with a second-order polynomial equation. Pearson's correlation coefficient (R^2) and p-values are reported in Table 2.

at the 7-year-old site, for example, which could have fostered a balance of oxic/anoxic sediment conditions optimal for coupled NF-DNF. Results were interpreted with the awareness that site features, rather than age, could have shaped the observed trendline, but the oldest site does have a disproportionate influence as the endpoint of the trajectory.

The disparity among seasonal trajectories means that a single trajectory cannot capture the variability in denitrification rates during a typical year. Results from other studies suggest that seasonal variability among trajectories is not unusual. Seasonal peaks in denitrification were observed from summer to fall in Japanese estuaries (Senga et al., 2010), during the fall in North Carolina salt marshes (Thompson et al., 1995), and from winter to spring in intertidal environments in the Netherlands (Kieskamp et al., 1991). Many studies have recorded a positive relationship between denitrification and temperature (Nowicki et al., 1997; Barnes and Owens, 1999; Cabrita and Brotas, 2000; Kellogg et al., 2013; Kuschk et al., 2003), which illustrates the importance of seasonal sampling and suggests that it may be more informative to construct restoration trajectories for individual seasons.

Other studies have also found it challenging to identify a consistent relationship between restored age and denitrification (Veldkamp et al., 1999; Ahn and Peralta, 2012), and there is some speculation as to whether restoration trajectories are a practical construct given that many factors, including random events, can influence site development (Zedler and Callaway, 1999). Evaluation of genes related to denitrification along a glacial retreat chronosequence suggested that denitrifying communities develop at different rates (Kandeler et al., 2006). Analysis of genetic diversity was beyond the scope of this research, but it could have contributed to some differences observed among sites. It has also been suggested that chronosequence studies may be best suited for studying soil development (Walker et al., 2010), implying that it might be easier to identify a restoration trajectory for soil properties than biogeochemical processes. For example, Peyre et al. (2009) and Osland et al. (2012) identified restoration trajectories for the development of sediment characteristics in chronosequences of brackish marshes and mangroves, respectively. In their salt marsh chronosequence study, Craft et al. suggested that sediment characteristics could be predicted by a chronosequence spanning < 30 years, but that nutrient dynamics might require more time to develop (1988). Our study found that, when all annual parameters were regressed against restored age, SOM and bulk density explained the highest amount of variability, possibly reinforcing the idea that restoration trajectories may be more suitable for tracking soil parameters.

It is beneficial to calculate denitrification efficiency (DNE) to compare N removal rates to positive NH_4^+ and NO_x fluxes (Eyre and Ferguson, 2009; Piehler and Smyth, 2011), because net N input could be considered an ecosystem disservice (Burgin et al., 2013; Lyytimäki and Sipilä, 2009). DNE results indicate that average annual denitrification in our study sites was consistently efficient and that positive fluxes of NH_4^+ and NO_x were low. Although DNE did not follow a clear pattern, it was always greater than 50%, indicating that these systems result in net N removal and augmented ecosystem function. There was no correlation between NH_4^+ fluxes and oyster filtration, challenging suggestions that oyster reefs are a source of NH_4^+ (Dame et al., 1984; Dame and Libes, 1993). It has been suggested that denitrification could become less efficient as SOD increases, reducing O_2 concentrations and increasing the likelihood of incomplete denitrification (Gardner and McCarthy, 2009). However, our results demonstrated sustained DNE with increasing SOD, which agreed with similar work in the Chesapeake Bay (Kellogg et al., 2013).

This study identified only 2 instances of positive average annual N_2O fluxes by habitat, suggesting that living shoreline construction is not associated with an increase in N_2O production within 20 years. The average N_2O fluxes recorded compare favorably with published values for estuarine environments. Foster and Fulweiler (2016) reported negative fluxes of -0.5 to -1 μmol N_2O m^{-2} h^{-1} in sediments from a Massachusetts estuary, and a review by Murray et al. (2015) cited numerous instances of negative N_2O fluxes smaller than -5 μmol N_2O m^{-2} h^{-1} in salt marshes.

Living shorelines can provide N removal, but denitrification is a complicated and expensive parameter to measure, making it useful to identify a proxy. SOD consistently explained the most variation in denitrification rates, and other studies have also identified a strong correlation between denitrification and SOD (Fennel et al., 2009; Piehler and Smyth, 2011; Seitzinger and Giblin, 1996). SOD reflects the cumulative influence of all oxygen-utilizing processes in sediment, and coupled NF-DNF has been shown to constitute a large percentage of SOD in N-limited environments (Seitzinger, 1994). We also observed an overall increase in microbial activity as C availability increased over time. Denitrification was significantly correlated with SOM only during the summer, when denitrifiers likely experience limitation by C availability (Groffman and Tiedje, 1989, Eyre et al., 2013).

Our results suggest that maximizing habitat structure and function does not necessarily result in higher levels of biogeochemical function. There was no connection between oyster filtration or marsh grass density and denitrification. This result addresses the "field of dreams" restoration model: that restoring physical features of a site will ultimately translate to ecosystem function (Hilderbrand et al., 2005). It also points to the fact that other site parameters can complicate the development of denitrification (Theriot et al., 2013). Since biotic growth may not be linear, the cascading impact on denitrification over time may not be straight-forward. For example, marsh grass density was positively correlated with SOM (also observed by Craft et al., 2003), but that did not translate to a corresponding increase in annual denitrification, possibly because SOM was only correlated with denitrification in the summer. Greater structural complexity is expected to increase SOM in oyster reefs (Carlsson et al., 2012), but this study found no correlation between oyster filtration and SOM.

State and federal agencies are increasingly advocating for naturebased solutions like living shorelines to sustain ecosystems and their functions into the future. Living shorelines enable coastal communities to protect shorelines while building resiliency in the face of uncertain future conditions (Harris et al., 2006; Seastedt et al., 2008). As living shorelines continue to be constructed, there is ample potential for future research on their impact on nutrient cycling. Our chronosequence study demonstrated that living shorelines can help reduce bioavailable N by augmenting efficient N removal without introducing ecosystem disservices such as increased N_2O or NH_4^+ fluxes. Denitrification was not significantly different between habitats, illustrating that living shorelines express habitat homogeneity in terms of biogeochemical function during at least the first 7 years following construction. Overall, this research indicates that living shorelines can play an important role in estuarine N cycling and management.

Acknowledgements

References

Ahn, Changwoo, Peralta, Rita M., 2012. Soil properties are useful to examine denitrification function development in created mitigation wetlands. Ecol. Eng. 49, 130–136. http://dx.doi.org/10.1016/j.ecoleng.2012.08.039.

Bachand, Philip A.M., Horne, Alexander J., 2000. Denitrification in constructed freewater surface wetlands: II. Effects of vegetation and temperature. Ecol. Eng. 14, 17–32. http://dx.doi.org/10.1016/S0925-8574(99)00017-8.

Ballantine, Katherine, Schneider, Rebecca, 2009. Fifty-five years of soil development in restored freshwater depressional wetlands. Ecol. Appl. 19 (6), 1467–1480. http://dx.doi.org/10.1890/07-0588.1.

Banks, Joanne L., Jeff Ross, D., Keough, Michael J., Macleod, Catriona K., Keane, John,

Eyre, Bradley D., 2013. Influence of a burrowing, metal-tolerant polychaete on benthic metabolism, denitrification and nitrogen regeneration in contaminated estuarine sediments. Mar. Pollut. Bull. 68, 30–37. http://dx.doi.org/10.1016/j.marpolbul.2013.01.002.

Barnes, J., Owens, N.J.P., 1999. Denitrification and nitrous oxide concentrations in the Humber Estuary, UK, and adjacent coastal zones. Mar. Pollut. Bull. 37 (3–7), 247–260. http://dx.doi.org/10.1016/S0025-326X(99)00079-X.

Beck, Michael W., Heck, Kenneth L., Able, Kenneth W., Childers, Daniel L., Eggleston, David B., Gillanders, Bronwyn M., Halpern, Benjamin, et al., 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51 (8), 633. http://dx.doi.org/10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2.

Beseres Pollack, Jennifer, Yoskowitz, David, Kim, Hae-Cheol, Montagna, Paul A., 2013. Role and value of nitrogen regulation provided by oysters (Crassostrea virginica) in the Mission-Aransas Estuary, Texas, USA. PLoS One 8 (6), e65314. http://dx.doi.org/10.1371/journal.pone.0065314.

Bowen, Jennifer L., Valiela, Ivan, 2001. The ecological effects of urbanization of coastal watersheds: historical increases in nitrogen loads and eutrophication of Waquoit Bay estuaries. Can. J. Fish. Aquat. Sci. 58, 1489–1500. http://dx.doi.org/10.1139/f01-094.

Box, G.E.P., Cox, D.R., 1964. An analysis of transformations. J. R. Stat. Soc., Ser. B (Methodolog.) 26 (2), 211–252 URL: http://www.jstor.org/stable/2984418.

Brush, Grace S., 2009. Historical land use, nitrogen, and coastal eutrophication: a paleoecological perspective. Estuaries Coasts 32, 18–28. http://dx.doi.org/10.1007/s12237-008-9106-z.

Burgin, Amy J., Lazar, Julia G., Groffman, Peter M., Gold, Arthur J., Kellogg, D.Q., 2013. Balancing nitrogen retention ecosystem services and greenhouse gas disservices at the landscape scale. Ecol. Eng. 56, 26–35. http://dx.doi.org/10.1016/j.ecoleng.2012.05.003.

Byers, Shelia C., Mills, Eric L., Stewart, Patrick L., 1978. A comparison of methods of determining organic carbon in marine sediments, with suggestions for a standard method. Hydrobiologia 58 (1), 43–47. http://dx.doi.org/10.1016/0022-0981(77)90042-9.

Cabrita, Maria Teresa, Brotas, Vanda, 2000. Seasonal variation in denitrification and dissolved nitrogen fluxes in intertidal sediments of the Tagus Estuary, Portugal. Mar. Ecol. Prog. Ser. 202, 51–65. http://dx.doi.org/10.3354/meps202051.

Cairns, John Jr, 2000. Setting ecological restoration goals for technical feasibility and scientific validity. Ecol. Eng. 15, 171–180. http://dx.doi.org/10.1016/S0925-8574(00)00068-9.

Carlson, Marita Sundstein, Engström, Pia, Lindahl, Odd., Ljungqvist, Lars, Petersen, Jens Kjerulf, Svanberg, Linda, Holmer, Marianne, 2012. Effects of Mussel Farms on the Benthic Nitrogen Cycle on the Swedish West Coast. Aquaculture Environment Interactions 2, 177–191. http://dx.doi.org/10.3354/aei00039.

Cartaxana, P., Lloyd, D., 1999. N2, N2O and O2 profiles in a Tagus Estuary salt marsh. Estuar. Coast. Shelf Sci. 48, 751–756. http://dx.doi.org/10.1006/ecss.1998.0475.

Costanza, Robert, D'Arge, Ralph, de Groot, Rudolf, Farber, Stephen, Grasso, Monica, Hannon, Bruce, Limburg, Karin, et al., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.

Craft, C.B., Broome, S.W., Seneca, E.D., 1988. Nitrogen, phosphorus and organic carbon pools in natural and transplanted marsh soils. Estuaries 11 (4), 272–280 http://www.jstor.org/stable/1352014.

Craft, Christopher, Broome, Stephen, Campbell, Carlton, 2002. Fifteen years of vegetation and soil development after brackish-water marsh creation. Restor. Ecol. 10 (2), 248–258. http://dx.doi.org/10.1046/j.1526-100X.2002.01020.

Craft, Christopher, Megonigal, Patrick, Broome, Stephen, Stevenson, Jan, Freese, Robert, Cornell, Jeff, Zheng, Lei, Sacco, John, 2003. The pace of ecosystem development of constructed Spartina alterniflora marshes. Ecol. Appl. 13 (5), 1417–1432. http://dx.doi.org/10.1890/02-5086.

Currin, C.A., Chappell, W.S., Deaton, A., 2010. Developing alternative shoreline armoring strategies: the living shoreline approach in North Carolina. In: Shipman, H., Dethier, M.N., Gelfenbaum, G., Fresh, K.L., Dinicola, R.S. (Eds.), Puget Sound Shorelines and the Impacts of Armoring: Proceedings of a State of the Science Workshop, pp. 91–102 USGS Scientific Investigations Report 2010-5254.

Dame, Richard F., Zingmark, Richard G., Haskin, Elizabeth, 1984. Oyster reefs as processors of estuarine materials. J. Exp. Mar. Biol. Ecol. 83, 239–247. http://dx.doi.org/10.1016/S0022-0981(84)80003-9.

Dame, R., Libes, S., 1993. Oyster reefs and nutrient retention in tidal creeks. J. Exp. Mar. Biol. Ecol. 171, 251–258. http://dx.doi.org/10.1016/0022-0981(93)90007-B.

Davis, Jenny L., Currin, Carolyn A., O'Brien, Colleen, Raffenburg, Craig, Davis, Amanda, 2015. Living shorelines: coastal resilience with a blue carbon benefit. PLoS One 10 (11), e0142595. http://dx.doi.org/10.1371/journal.pone.0142595.

Davis, Jenny L., Nowicki, Barbara, Wigand, Cathleen, 2004. Denitrification in fringing salt marshes of Narragansett Bay, Rhode Island, USA. Wetlands 24 (4), 870–878. http://dx.doi.org/10.1672/0277-5212(2004) 024[0870:DIFSMO]2.0.CO;2.

de Wilde, Hein P.J., de Bie, Monique J.M., 2000. Nitrous oxide in the Schelde Estuary: production by nitrification and emission to the atmosphere. Mar. Chem. 69, 203–216. http://dx.doi.org/10.1016/S0304-4203(99)00106-1.

De'Ath, Glenn, Fabricius, Katharina E., 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. Ecology 81 (11), 3178–3192 10.1890/0012-9658(2000) 081[3178:CARTAP]2.0.CO;2.

DeLaune, R.D., Smith, C.J., Patrick, W.H., 1983. Nitrogen losses from a Louisiana Gulf Coast salt marsh. Estuar. Coast. Shelf Sci. 17, 133–141. http://dx.doi.org/10.1016/0272-7714(83)90058-6.

Dong, Liang F., Nedwell, David B., Underwood, Graham J.C., Thornton, Daniel C.O., Rusmana, Iman, 2002. Nitrous oxide formation in the Colne Estuary, England: the central role of nitrite. Appl. Environ. Microbiol. 68 (3), 1240–1249. http://dx.doi.org/10.1128/AEM.68.3.1240.

Ensign, Scott H., Piehler, Michael F., Doyle, Martin W., 2008. Riparian zone denitrification affects nitrogen flux through a tidal freshwater river. Biogeochemistry 91, 133–150. http://dx.doi.org/10.1007/s10533-008-9265-9.

Eyre, Bradley D., Ferguson, Angus J.P., 2002. Comparison of carbon production and decomposition, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperate Australian lagoons. Mar. Ecol. Prog. Ser. 229, 43–59. http://dx.doi.org/10.3354/meps229043.

Eyre, Bradley D., Ferguson, Angus J.P., 2009. Denitrification efficiency for defining critical loads of carbon in shallow coastal ecosystems. Hydrobiologia 629 (1), 137–146. http://dx.doi.org/10.1007/s10750-009-9765-1.

Eyre, Bradley D., Maher, Damien T., Squire, Peter, 2013. Quantity and quality of organic matter (detritus) drives N2 effluxes (net denitrification) across seasons, benthic habitats, and estuaries. Global Biogeochem. Cycles 27, 1083–1095. http://dx.doi.org/10.1002/2013GB004631.

Fennel, Katja, Brady, Damian, DiToro, Dominic, Fulweiler, Robinson W., Gardner, Wayne S., Giblin, Anne, McCarthy, Mark J., et al., 2009. Modeling denitrification in aquatic sediments. Biogeochemistry 93, 159–178. http://dx.doi.org/10.1007/s10533-008-9270-z.

Fernandes, Sheryl Oliveira, Dutta, Pinky, Gonsalves, Maria-Judith, Bonin, Patricia C., LokaBharathi, P.A., 2016. Denitrification activity in mangrove sediments varies with associated vegetation. Ecol. Eng. 95, 671–681. http://dx.doi.org/10.1016/j.ecoleng.2016.06.102.

Foster, Sarah Q., Fulweiler, Robinson W., 2016. Sediment nitrous oxide fluxes are dominated by uptake in a temperate estuary. Front. Marine Sci. 3. http://dx.doi.org/10.3389/fmars.2016.00040.

Gardner, Wayne S., McCarthy, Mark J., 2009. Nitrogen dynamics at the sediment-water interface in shallow, sub-tropical Florida Bay: why denitrification efficiency may decrease with increased eutrophication. Biogeochemistry 95, 185–198. http://dx.doi.org/10.1007/s10533-009-9329-5.

George, Usha S., Antoine, Alan D., 1982. Denitrification potential of a salt marsh soil: effect of temperature, pH and substrate concentration. Soil Biol. Biochem. 14, 117–125. http://dx.doi.org/10.1016/0038-0717(82)90054-2.

Gilbert, Frank, Bonin, Patricia, Stora, Georges, 1995. Effect of bioturbation on denitrification in a marine sediment from the west Mediterranean littoral. Hydrobiologia 304, 49–58. http://dx.doi.org/10.1007/BF02530703.

Gittman, Rachel K., Joel Fodrie, F., Popowich, Alyssa M., Keller, Danielle A., Bruno, John F., Currin, Carolyn A., Peterson, Charles H., Piehler, Michael F., 2015. Engineering away our natural defenses: an analysis of shoreline hardening in the US. Front. Ecol. Environ. 13 (6), 301–307. http://dx.doi.org/10.1890/150065.

Grizzle, Raymond E., Greene, Jennifer K., Coen, Loren D., 2008. Seston removal by natural and constructed intertidal eastern oyster (Crassostrea virginica) reefs: a comparison with previous laboratory studies, and the value of in Situ methods. Estuaries Coasts 31, 1208–1220. http://dx.doi.org/10.1007/s12237-008-9098-8.

Groffman, Peter M., Tiedje, James M., 1989. Denitrification in north temperate forest soils: relationships between denitrification and environmental factors at the landscape scale. Soil Biol. Biochem. 21 (5), 621–626. http://dx.doi.org/10.1016/0038-0717(89)90054-0.

Gutiérrez, Jorge J., Jones, Clive G., 2006. Physical ecosystem engineers as agents of biogeochemical heterogeneity. Bioscience 56 (3), 227–236. http://dx.doi.org/10.1641/0006-3568(2006) 056[0227:PEEAAO]2.0.CO;2.

Hackney, Courtney T., 2000. Restoration of coastal habitats: expectation and reality. Ecol. Eng. 15, 165–170. http://dx.doi.org/10.1016/S0925-8574(00)00067-7.

Hagy, James D., Bonton, Walter R., Keefe, Carolyn W., Wood, Kathryn V., 2004. Hypoxia in Chesapeake Bay, 1950–2001: long-term change in relation to nutrient loading and river flow. Estuaries 27 (4), 634–658. http://dx.doi.org/10.1007/BF02907650.

Harris, James A., Hobbs, Richard J., Higgs, Eric, Aronson, James, 2006. Ecological restoration and global climate change. Restor. Ecol. 14 (2), 170–176. http://dx.doi.org/10.1111/j.1526-100X.2006.00136.x.

Herbert, R.A., 1999. Nitrogen cycling in coastal marine ecosystems. FEMS Microbiol. Rev. 23, 563–590 http://www.ncbi.nlm.nih.gov/pubmed/10525167.

Hilderbrand, Robert H., Watts, Adam C., Randle, April M., 2005. The Myths of Restoration Ecology. Ecology and Society 10 (1). http://dx.doi.org/10.5751/ES-01277-100119.

Humphries, Austin T., Ayvazian, Suzanne G., Carey, Joanna C., Hancock, Boze T., Grabbert, Sinead, Cobb, Donald, Strobel, Charles J., Fulweiler, Robinson W., 2016. Directly measured denitrification reveals oyster aquaculture and restored oyster reefs remove nitrogen at comparable high rates. Front. Marine Sci. 3. http://dx.doi.org/10.3389/fmars.2016.00074.

Hutto, Richard L., Belote, R.T., 2013. Distinguishing four types of monitoring based on the questions they address. For. Ecol. Manage. 289, 183–189. http://dx.doi.org/10.1016/j.foreco.2012.10.005.

Ji, Qixing, Babbin, Andrew R., Peng, Xuefeng, Bowen, Jennifer L., Ward, Bess B., 2015. Nitrogen substrate-dependent nitrous oxide cycling in salt marsh sediments. J. Mar. Res. 73, 71–92. http://dx.doi.org/10.1357/002224015815848820.

Jordan, Stephen J., Stoffer, Jonathan, Nestlerode, Janet A., 2011. Wetlands as sinks for reactive nitrogen at continental and global scales: a meta-analysis. Ecosystems 14, 144–155. http://dx.doi.org/10.1007/s10021-010-9400-z.

Kana, Todd M., Christina Darkangelo, M., Hunt, Duane, Oldham, James B., Bennett, George E., Cornwell, Jeffrey C., 1994. Membrane inlet mass spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples. Anal. Chem. 66, 4166–4170. http://dx.doi.org/10.1021/ac00095a009.

Kandeler, Ellen, Deiglmayr, Kathrin, Tscherko, Dagmar, Bru, David, Philippot, Laurent, 2006. Abundance of narG, nirS, nirK, and nosZ Genes of denitrifying bacteria during primary successions of a glacier foreland. Appl. Environ. Microbiol. 72 (9), 5957–5962. http://dx.doi.org/10.1128/AEM.00439-06.

Kellogg, M. Lisa, Cornwell, Jeffrey C., Owens, Michael S., Paynter, Kennedy T., 2013. Denitrification and nutrient assimilation on a restored oyster reef. Mar. Ecol. Prog. Ser. 480, 1–19. http://dx.doi.org/10.3354/meps10331.

Kentula, Mary E., Brooks, Robert P., Gwin, Stephanie E., Holland, Cindy C., Sherman, Arthur D., Sifneos, Jean C., 1992. An approach to improving decision making in wetland restoration and creation. In: Ann, J. (Ed.), Hairston. Island Press, Washington, DC.

Kentula, Mary E., 2000. Perspectives on setting success criteria for wetland restoration. Ecol. Eng. 15, 199–209. http://dx.doi.org/10.1016/S0925-8574(00)00076-8.

Kieskamp, Willem Michel, Lohse, Lutz, Epping, Eric, Helder, Willem, 1991. Seasonal variation in denitrification rates and nitrous oxide fluxes in intertidal sediments of the Western Wadden Sea. Mar. Ecol. Prog. Ser. 72, 145–151. http://dx.doi.org/10.3354/meps072145.

Koch, Evamaria W., Barbier, Edward B., Silliman, Brian R., Reed, Denise J., Perillo, Gerardo M E, Hacker, Sally D., Granek, Elise F., et al., 2009. Non-linearity in ecosystem services: temporal and spatial variability in coastal protection. Front. Ecol. Environ. 7 (1), 29–37. http://dx.doi.org/10.1890/080126.

Koop-Jakobsen, Ketil, Giblin, Anne E., 2009. Anammox in tidal marsh sediments: the role of salinity, nitrogen loading, and marsh vegetation. Estuaries Coasts 32, 238–245. http://dx.doi.org/10.1007/s12237-008-9131-y.

Kuschk, P., Wießner, A., Kappelmeyer, U., Weißbrodt, E., Kästner, M., Stottmeister, U., 2003. Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. Water Res. 37, 4236–4242. http://dx.doi.org/10.1016/S0043-1354(03)00163-5.

La Peyre, Megan, K., Gossman, Bryan, Piazza, Bryan P., 2009. Short- and long-term response of deteriorating brackish marshes and open-water ponds to sediment enhancement by thin-layer dredge disposal. Estuaries Coasts 32, 390–402. http://dx.doi.org/10.1007/s12237-008-9126-8.

La Peyre, Megan, K., Humphries, Austin T., Casas, Sandra M., La Peyre, Jerome F., 2014. Temporal variation in development of ecosystem services from oyster reef restoration. Ecol. Eng. 63, 34–44. http://dx.doi.org/10.1016/j.ecoleng.2013.12.001.

LaMontagne, Michael G., Duran, Robert, Valiela, Ivan, 2003. Nitrous oxide sources and sinks in coastal aquifers and coupled estuarine receiving waters. Sci. Total Environ. 309, 139–149. http://dx.doi.org/10.1016/S0048-9697(02)00614-9.

Lyytimäki, Jari, Sipilä, Maija, 2009. Hopping on one leg – the challenge of ecosystem disservices for urban green management. Urban For. Urban Greening 8, 309–315. http://dx.doi.org/10.1016/j.ufug.2009.09.003.

McClelland, James W., Valiela, Ivan, 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnol. Oceanogr. 43 (4), 577–585. http://dx.doi.org/10.4319/lo.1998.43.4.0577.

Millennium Ecosystem Assessment. 2005. Ecosystems and Human Well-Being: Wetlands and Water Synthesis. Washington, DC: World Resources Institute. doi:10.1007/BF02987493.

Morris, James T., Sundareshwar, P.V., Nietch, Christopher T., Kjerfve, Bjorn, Cahoon, D.R., 2002. Responses of coastal wetlands to rising sea level. Ecology 83 (10), 2869–2877. http://dx.doi.org/10.1890/0012-9658(2002) 083[2869:ROCWTR]2.0.CO;2.

Murray, Rachel H., Erler, Dirk V., Eyre, Bradley D., 2015. Nitrous oxide fluxes in estuarine environments: response to global change. Glob. Change Biol. 21, 3219–3245. http://dx.doi.org/10.1111/gcb.12923.

NOAA Living Shorelines Workgroup. 2015. "Guidance for Considering the Use of Living Shorelines." Accessed 12 May 2017. http://www.habitat.noaa.gov/pdf/noaa_guidance_for_considering_the_use_of_living_shorelines_2015.pdf.

Nowicki, Barbara L., Requintina, Edwin, Van Keuren, Donna, Kelly, John R., 1997. Nitrogen losses through sediment denitrification in Boston Harbor and Massachusetts Bay. Estuaries 20 (3), 626–639. http://dx.doi.org/10.1007/BF02692175.

Osland, Michael J., Spivak, Amanda C., Nestlerode, Janet A., Lessmann, Jeannine M., Almario, Alejandro E., Heitmuller, Paul T., Russell, Marc J., et al., 2012. Ecosystem development after mangrove wetland creation: plant-soil change across a 20-year chronosequence. Ecosystems 15, 848–866. http://dx.doi.org/10.1007/s10021-012-9551-1.

Paerl, Hans W., Otten, Timothy G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65 (4), 995–1010. http://dx.doi.org/10.1007/s00248-012-0159-y.

Paerl, Hans W, Pinckney, James L, Fear, John M, Peierls, Benjamin L, 1998. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River Estuary, North Carolina, USA. Mar. Ecol. Prog. Ser. 166, 17–25.

Passeport, Elodie, Vidon, Philippe, Forshay, Kenneth J., Harris, Lora, Kaushal, Sujay S., Kellogg, Dorothy Q., Lazar, Julia, Mayer, Paul, Stander, Emilie K., 2013. Ecological engineering practices for the reduction of excess nitrogen in human-influenced landscapes: a guide for watershed managers. Environ. Manage. 51, 392–413. http://dx.doi.org/10.1007/s00267-012-9970-y.

Piehler, M.F., Currin, C.A., Cassanova, R., Paerl, H.W., 1998. Development and N₂-Fixing activity of the benthic microbial community in transplanted *Spartina alterniflora* marshes in North Carolina. Restor. Ecol. 6 (3), 290–296. http://dx.doi.org/10.1046/j.1526-100X.1998.00633.x.

Piehler, M.F., Smyth, A.R., 2011. Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. Ecosphere 2 (1). http://dx.doi.org/10.1890/ES10-00082.1.

Poulin, Patrick, Pelletier, Emilien, Saint-Louis, Richard, 2007. Seasonal variability of denitrification efficiency in northern salt marshes: an example from the St. Lawrence Estuary. Marine Environ. Res. 63, 490–505.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org.

Ruiz-Jaen, Maria C., Mitchell Aide, T., 2005. Restoration success: how is it being measured? Restor. Ecol. 13 (3), 569–577. http://dx.doi.org/10.1111/j.1526-100X.2005.00072.x.

Salmo, I.I.I., Severino, G., Lovelock, Catherine, Duke, Norman C., 2013. Vegetation and soil characteristics as indicators of restoration trajectories in restored mangroves.

Hydrobiologia 720, 1–18. http://dx.doi.org/10.1007/s10750-013-1617-3.

Seastedt, Timothy R., Hobbs, Richard J., Suding, Katharine N., 2008. Management of novel ecosystems: are novel approaches required? Front. Ecol. Environ. 6 (10), 547–553. http://dx.doi.org/10.1890/070046.

Seitzinger, Sybil P., 1988. Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. Limnology Oceanography 33 (4, part 2), 702–724. http://dx.doi.org/10.4319/lo.1988.33.4part2.0702.

Seitzinger, Sybil P., 1994. Linkages between organic matter mineralization and denitrification in eight riparian wetlands. Biogeochemistry 25, 19–39. http://dx.doi.org/10.1007/BF00000510.

Seitzinger, S.P., Giblin, A.E., 1996. Estimating denitrification in North Atlantic continental shelf sediments. Biogeochemistry 35, 235–260. http://dx.doi.org/10.1007/BF02179829.

Seitzinger, S., Harrison, J.A., Böhlke, J.K., Bouwman, A.F., Lowrance, R., Tobias, C., Van Drecht, G., Van Drecht, G., 2006. Denitrification across landscapes and waterscapes: a synthesis. Ecol. Appl. 16 (6), 2064–2090. http://dx.doi.org/10.1890/1051-0761(2006) 016[2064:DALAWA]2.0.CO;2.

Senga, Yukiko, Okumura, Minoru, Seike, Yasushi, 2010. Seasonal and spatial variation in the denitrifying activity in estuarine and lagoonal sediments. J. Oceanogr. 66, 155–160. http://dx.doi.org/10.1007/s10872-010-0013-0.

Simenstad, Charles A., Cordell, Jeffrey R., 2000. Ecological assessment criteria for restoring anadromous salmonid habitat in Pacific Northwest estuaries. Ecol. Eng. 15, 283–302. http://dx.doi.org/10.1016/S0925-8574(00)00082-3.

Smith, Carter S., Gittman, Rachel K., Neylan, Isabelle P., Scyphers, Steven B., Morton, Joseph P., Joel Fodrie, F., Grabowski, Jonathan H., Peterson, Charles H., 2017. Hurricane damage along natural and hardened estuarine shorelines: using homeowner experiences to promote nature-based coastal protection. Marine Policy 81, 350–358. http://dx.doi.org/10.1016/j.marpol.2017.04.013.

Smyth, Ashley R., Thompson, Suzanne P., Siporin, Kaylyn N., Gardner, Wayne S., McCarthy, Mark J., Piehler, Michael F., 2013. Assessing nitrogen dynamics throughout the estuarine landscape. Estuaries Coasts 36, 44–55. http://dx.doi.org/10.1007/s12237-012-9554-3.

Smyth, Ashley R., Piehler, Michael F., Grabowski, Jonathan H., 2015. Habitat context influences nitrogen removal by restored oyster reefs. J. Appl. Ecol. 52, 716–725. http://dx.doi.org/10.1111/1365-2664.12435.

Sousa, Ana I., Lillebø, Ana I., Risgaard-Petersen, Nils, Pardal, Miguel A., Caçador, Isabel, 2012. Denitrification: an ecosystem service provided by salt marshes. Mar. Ecol. Prog. Ser. 448, 79–92. http://dx.doi.org/10.3354/meps09526.

Sutherland, William J, Clout, Mick, Côté, Isabelle M, Daszak, Peter, Depledge, Michael H, Fellman, Liz, Fleishman, Erica, et al., 2010. A horizon scan of global conservation issues for 2010. Trends Ecol. Evol. 25 (1), 1–7. http://dx.doi.org/10.1016/j.tree.2009.10.003.

Sutton-Grier, Ariana E., Wowk, Kateryna, Bamford, Holly, 2015. Future of our coasts: the potential for natural and hybrid infrastructure to enhance the resilience of our coastal communities, economies and ecosystems. Environ. Sci. Policy 51, 137–148. http://dx.doi.org/10.1016/j.envsci.2015.04.006.

Testa, Jeremy M., Michael Kemp, W., Harris, Lora A., Woodland, Ryan J., Boynton, Walter R., 2017. Challenges and directions for the advancement of estuarine ecosystem science. Ecosystems 20, 14–22. http://dx.doi.org/10.1007/s10021-016-0004-0.

Theriot, Jared M., Conkle, Jeremy L., Reza Pezeshki, S., DeLaune, Ronald D., White, John R., 2013. Will hydrologic restoration of Mississippi River riparian wetlands improve their critical biogeochemical functions? Ecol. Eng. 60, 192–198. http://dx.doi.org/10.1016/j.ecoleng.2013.07.021.

Therneau, Terry, Beth Atkinson, and Brian Ripley. 2015. rpart: Recursive Partitioning and Regression Trees. R package version 4.1-10. URL: https://CRAN.R-project.org/package=rpart.

Thompson, Suzanne P., Paerl, Hans W., Malia, C. Go., 1995. Seasonal patterns of nitrification and denitrification in a natural and a restored salt marsh. Estuaries 18 (2), 399–408. http://dx.doi.org/10.2307/1352322.

Veldkamp, Edzo, Davidson, Eric, Erickson, Heather, Keller, Michael, Weitz, Antje, 1999. Soil nitrogen cycling and nitrogen oxide emissions along a pasture chronosequence in the humid tropics of Costa Rica. Soil Biol. Biochem. 31, 387–394. http://dx.doi.org/10.1016/S0038-0717(98)00141-2.

Walker, Lawrence R., Wardle, David A., Bardgett, Richard D., Clarkson, Bruce D., 2010. The use of chronosequences in studies of ecological succession and soil development. J. Ecol. 98, 725–736. http://dx.doi.org/10.1111/j.1365-2745.2010.01664.x.

Weiss, R.F., Price, B.A., 1980. Nitrous oxide solubility in water and seawater. Mar. Chem. 8, 347–359. http://dx.doi.org/10.1016/0304-4203(80)90024-9.

Zedler, Joy B., Callaway, John C., 1999. Tracking wetland restoration: do mitigation sites follow desired trajectories? Restor. Ecol. 7 (1), 69–73. http://dx.doi.org/10.1046/j.1526-100X.1999.07108.x.

Zhao, Qingqing, Bai, Junhong, Huang, Laibin, Binhe, Gu., Qiongqiong, Lu., Gao, Zhaoqin, 2016. A review of methodologies and success indicators for coastal wetland restoration. Ecol. Ind. 60, 442–452. http://dx.doi.org/10.1016/j.ecolind.2015.07.003.

zu Ermgassen, Philine S.E., Spalding, Mark D., Grizzle, Raymond E., Brumbaugh, Robert D., 2013. Quantifying the loss of a marine ecosystem service: filtration by the eastern oyster in US estuaries. Estuaries Coasts 36, 36–43. http://dx.doi.org/10.1007/s12237-012-9559-y.

zu Ermgassen, Philine, Hancock, Boze, DeAngelis, Bryan, Greene, Jennifer, Schuster, Elisabeth, Spalding, Mark, Brumbaugh, Robert, 2016. Setting Objectives for Oyster Habitat Restoration Using Ecosystem Services: A Manager's Guide. The Nature Conservancy, Arlington, VA.