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Rapid recovery of carbon cycle processes after the cessation of chronic nutrient enrichment

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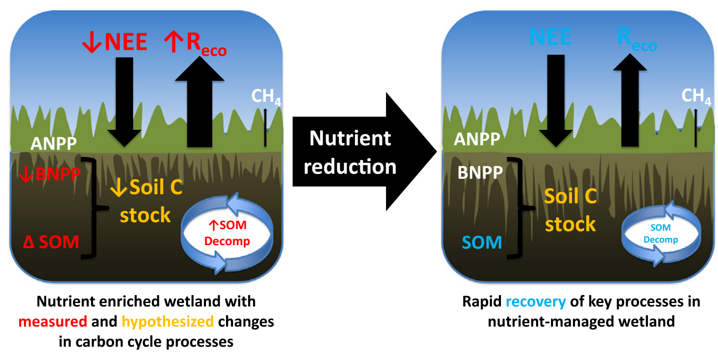
HIGHLIGHTS

- Restoring coastal marshes requires managing nutrient loads but it is not clear how quickly carbon cycling can rebound.
- In a long-term experiment, ceasing enrichment returned rates of ecosystem respiration to reference levels within one year.
- The soil stabilization processes that affect decomposition likewise immediately reverted to reference levels.
- Curbing coastal nutrient enrichment could rapidly improve carbon sequestration in the short-term and increase coastal resilience in the long-term.

GRAPHICAL ABSTRACT



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ABSTRACT

Salt marshes provide critical ecosystem services including some of the highest rates of carbon storage on Earth. However, many salt marshes receive very high nutrient loads and there is a growing body of evidence indicating that this nutrient enrichment alters carbon cycle processes. While many restoration plans prioritize nutrient management in their efforts to conserve salt marsh ecosystems, there has been little empirical investigation of the capacity for carbon cycle processes to recover once nutrient loading is reduced. To address this, we compared rates of greenhouse gas fluxes (i.e., CO_2 and methane) measured using static chambers, and soil organic matter decomposition, using both litter bags and the Tea Bag Index (TBI), during the last two years of a long-term, ecosystem-scale nutrient enrichment experiment (2015–2016) as well as in the first two years of recovery post-enrichment (2017–2018). We found that both ecosystem respiration (R_{eco}) and decomposition processes (i.e., rhizome decomposition and soil organic matter stabilization) were enhanced by nutrient enrichment, but returned to reference ecosystem levels within the first year following the cessation of nutrient enrichment and remained at reference levels in the second year. These results suggest that management practices intended to reduce nutrient loads in coastal systems may, in fact, allow for rapid recovery of carbon cycle processes, potentially restoring the high carbon sequestration rates of these blue carbon ecosystems.

1. Introduction

Salt marsh conservation and restoration has recently garnered strong interest from scientists and policymakers given these systems'

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potential to help mitigate climate change (Emmett-Mattox et al., 2010; Howard et al., 2017; Wylie et al., 2016). The unique combination of high rates of productivity, anaerobic soils, and low rates of methanogenesis (Poffenbarger et al., 2011) result in salt marshes, which are blue carbon ecosystems, supporting some of the highest global rates of carbon sequestration per square meter of habitat (Chmura et al., 2003; Mcleod et al., 2011). Given that nutrient enrichment and the land use practices leading to it are known to negatively influence carbon sequestration in salt marshes (Pendleton et al., 2012), numerous watershed management efforts focus on reducing the nutrient loads that will be carried downstream to coasts. However, very few studies have investigated the rate and extent to which these practices ultimately restore carbon cycle processes. Because salt marshes are among the most nutrient-enriched ecosystems globally (Pardo et al., 2011), it is vital that we understand how nutrient enrichment and watershed management practices that limit nutrient enrichment alter carbon cycle processes.

While certain global change factors, like elevated carbon dioxide concentrations and rising relative sea levels, have been documented to alter carbon cycle processes in salt marsh ecosystems (Kirwan and Megonigal, 2013), the effects of nutrient enrichment are less well understood. In part, this is because the outcomes of nutrient enrichment differ according to the form of the nutrient (e.g., nitrate vs. ammonium) (Johnson et al., 2016; Mozdzer et al., 2020) and the capabilities of dominant plant species to take up these forms through their roots (Cott et al., 2018; Morris, 1977) and leaves (Mozdzer et al., 2011). Salt marshes are considered to be nitrogen limited ecosystems (Valiela and Teal, 1974), but there is a mismatch in the form that scientists typically manipulate (ammonium) vs. the form that typically reaches coastal salt marshes (nitrate). While ammonium addition typically increases net ecosystem exchange (NEE) and gross primary production (GPP) in salt marsh systems (Caplan et al., 2015; Song et al., 2019; Wang et al., 2013), contrasting effects may be observed under nitrate enrichment. Nitrate has the capacity to alter soil microbial processes because it is a strong electron acceptor used by anaerobic bacteria (Bulseco et al., 2019; Giblin et al., 2013); it therefore increases soil (i.e., heterotrophic) respiration and thereby total ecosystem respiration (R_{soil} and R_{eco}, respectively) (Geoghegan et al., 2018; Song et al., 2019; Wigand et al., 2009; Wigand et al., 2018).

It is clear that nutrient enrichment, and specifically nitrate enrichment, fundamentally changes carbon cycle processes in salt marshes. For example, our long-term, ecosystem-level nutrient enrichment experiment in coastal Massachusetts, USA (the TIDE Project) (Deegan et al., 2007; Deegan et al., 2012; Johnson et al., 2016) has found nitrate addition to increase rates of respiration of litter (Deegan et al., 2012), soil (Wigand et al., 2018), and the low-marsh ecosystem (R_{eco}) (Geoghegan et al., 2018). However, nitrate addition has left aboveground plant production unchanged in most years (Johnson et al., 2016), while reducing belowground biomass and the root:shoot ratio of vegetation (Deegan et al., 2012). Cumulatively, these changes have likely reduced the ecosystem's carbon storage capacity. These observations are largely consistent with other N addition studies in wetlands; a meta-analysis reported increases in R_{eco} ($n = 10$ studies), soil respiration ($n = 48$ studies), and gross ecosystem production ($n = 8$ studies) but found no effect of nutrient enrichment on net ecosystem carbon exchange (NEE) (Song et al., 2019). However, other findings of the TIDE study have differed from these other nutrient enrichment studies. While some studies have found increased aboveground net primary production (NPP) ($n = 13$), belowground net primary production (BNPP) ($n = 19$), and aboveground biomass (AGB) ($n = 102$) (Song et al., 2019), we have seen increases in AGB in some years but not every year (Johnson et al., 2016). The differences in NPP, BNPP, and AGB are likely attributable to the experimental addition of nitrate in the flooding water at TIDE rather than the more commonly used ammonium (Mozdzer et al., 2020).

There is some evidence that, under nutrient enrichment, changes in salt marsh carbon cycling are driven by increased rates of organic matter decomposition and associated decreases in soil organic matter stabilization (Mueller et al., 2018). Stabilization refers to the combined, inhibiting effects of the environment on the fraction of soil organic matter that is labile and hydrolysable; greater values indicate that conditions favor stability (Keuskamp et al., 2013). Reductions in soil organic matter stabilization under nitrate enrichment would be consistent with the diminished soil shear strength (Wigand et al., 2018) and lower root:shoot ratios observed at TIDE, which have led to the collapse of creek banks and therefore marsh loss (Deegan et al., 2012). These changes in the quantity and quality of soil carbon are likely due to nitrate stimulating the microbial decomposition of soil organic matter (Bulseco et al., 2019).

Little is known about the capacity for recovery of carbon cycle processes after the cessation of chronic nutrient enrichment, despite the fact that many conservation efforts are intended to limit the entry of nutrients into coastal systems. This is likely because evaluating such effects requires long-term studies that are beyond the typical lifespan of an ecological experiment. Understanding if and how rapidly marsh communities can recover from chronic nutrient enrichment could have important implications for management decisions related to both marsh restoration and carbon sequestration. Moreover, given the evidence that nutrient-enriched marshes in many areas are not able to keep pace with relative sea level rise (Langley and Megonigal, 2010; Wigand et al., 2014) and may store less carbon (Geoghegan et al., 2018), there is a pressing need to understand how land management actions that limit nutrient enrichment can influence carbon fluxes and decomposition processes.

We investigated whether, and how rapidly, salt marsh carbon cycle processes recover from chronic N-enrichment by measuring carbon fluxes (CO_2 and CH_4) and organic matter decomposition in a salt marsh system that had been experimentally enriched with nitrate for over a decade. We hypothesized that, with the cessation of chronic nutrient enrichment, NEE and R_{eco} rates in the formerly nitrogen enriched (N-legacy) system would eventually return to the range of rates observed in our reference ecosystem. In addition, we hypothesized that decomposition rates and organic matter stabilization would return to reference levels at rates comparable to those of NEE and R_{eco}. To test our hypotheses, we measured greenhouse gas fluxes using static chambers through two growing seasons during the experiment's enrichment phase and through two seasons post-enrichment. We measured soil organic matter decomposition using litter bags containing root and rhizome material from the marsh, as well as via the Tea Bag Index method (Keuskamp et al., 2013).

2. Materials and methods

2.1. The TIDE Project

Our research was conducted using a pair of primary tidal creeks at the Plum Island Ecosystem Long Term Ecological Research site in northeastern Massachusetts (42° 47′ 55″ N, 70° 48′ 40″ W). Salt marshes in this region experience a tidal range of 3 m and a water salinity range of 8–28 ppt (Pascal and Fleeger, 2013). The high-marsh platform is dominated by *Spartina patens* and *Distichlis spicata*, is inundated by ~30% of high tides, and is submerged fully ~4% of the time. The low-marsh is dominated by tall-form *Spartina alterniflora*, inundated twice per day at high tide, and submerged fully ~31–38% of the time. From 2003 through 2016, nitrate was added to one of the primary creeks on each incoming tide during the growing season (May–October), resulting in a load of 171 g N m^{-2} $year^{-1}$ in the low marsh and 19 g N m^{-2} $year^{-1}$ in the high marsh (Deegan et al., 2007; Johnson et al., 2016). These levels of N enrichment are 10-15× the ambient N concentration, representing moderate-to-severe eutrophication conditions (Deegan et al., 2012). Nutrient enrichment was applied at our target concentration, except in 2013, when nutrient enrichment levels were half typical values from May through July, and resumed full enrichment levels in August. In 2014, an earthquake in Chile resulted in a global disruption of nitrate fertilizers, delaying the shipment of nitrate until July, which prevented N enrichment in May and June; nutrient enrichment resumed during the second week of July 2014. Neither disruption directly overlapped with our study (2015–2018). We discontinued nutrient enrichment after the 2016 growing season and tracked the recovery of key ecosystem processes beginning in 2017.

2.2. Greenhouse gas fluxes

To determine the effects of nutrient enrichment on greenhouse gas (GHG) fluxes, and the marsh's ability to recover after the cessation of enrichment, we measured carbon dioxide (CO_2) and methane (CH_4) fluxes at least monthly during the 2015–2018 growing seasons in both the reference creek and the N-enriched/legacy creek, using the static chamber method. GHG flux rates during nutrient enrichment were reported previously (Geoghegan et al., 2018) but are presented again here to facilitate comparison.

We measured fluxes of greenhouse gases using the static chamber approach. This entails temporarily placing a transparent chamber over a vegetated plot and measuring the accumulation or removal of gases in the chamber's airspace. Each year, fluxes were measured at six plots in the low marsh zone dominated by tall *Spartina alterniflora* (2015–2018) and six plots in the high marsh zone dominated by *S. patens* (2017–2018). There were some differences in plot placement spanning the four years (Fig. 1). In 2015, sampling plots had fixed locations for the duration of the season that were distributed throughout the low marsh. In 2016, new locations were selected at each time point to allow for greater spatial coverage. In 2017 and 2018, flux plots were located near previously established, long-term plant productivity transects, which span both the high and low marsh zones. In 2015, 2017, and 2018, we installed aluminum collars (30 × 30 cm) at least three days before any measurement to allow for recovery from disturbance. Flux collars in the high marsh (enclosing *S. patens*) were set in 2017 and not removed between field seasons. Aluminum-framed chambers with clear Lexan® walls (either 30 × 30 × 93 cm or 30 × 30 × 126 cm, depending upon the height of the plant canopy) were set onto the aluminum collars at the time of measurement. Closed-cell neoprene foam was used to create an airtight seal with the chamber and the base, and a small battery-powered fan within the chambers ensured a well-mixed environment. In 2016, we used circular PVC bases (20 cm diameter) and 20 cm diameter, 150 cm-long polycarbonate cylinders. Closed-cell neoprene foam was likewise used at the junction of bases and chambers to prevent air entry or escape.

Gas flux measurements took place from 900 to 1500 h, with low marsh sites measured within 3 h of low tide to avoid flooding the collars. During flux measurements (each lasting 3–5 min), CO_2 and CH_4 concentrations in the airspace enclosed by the chamber and base were measured every second with an Ultraportable Greenhouse Gas Analyzer (UGGA 915-0011; Los Gatos Research, San Jose, USA). GHG fluxes were measured at or near saturating solar radiation levels (>1000 μmol photons m^{-2} s^{-1}) to determine daytime net ecosystem exchange (NEE). During flux measurements in 2015, temperature sensors were located both inside and outside the chamber; we observed a mean (±SD) warming effect of 2.5 ± 2.9 °C. After each NEE measurement (determined from the rate of decline in CO_2 concentration), a reflective tarp was placed over the chamber to prevent light from entering and thus facilitating a measurement of ecosystem respiration (R_{eco}; determined from the resulting accumulation of CO_2). R_{eco} measurements began no more than 5 min after preceding NEE measurements were complete. GPP was calculated as the sum of NEE and R_{eco} from paired flux measurements. CO_2 and CH_4 fluxes were measured from the linear portion of the concentration time series collected when chambers were closed; regressions had $R^2 > 0.98$ for CO_2 and > 0.8 for CH_4. Rates were converted from fractional units (ppm) to molar units using the ideal gas law in combination with the volume of the enclosed space.

Air temperature and soil temperature were recorded simultaneously with fluxes; see Geoghegan et al. (2018) for details on 2015–16. In 2017, we used a HOBO Micro Station Data Logger (Onset H21-002; Onset Corporation, Bourne, USA) with air and soil temperature probes (Onset S-

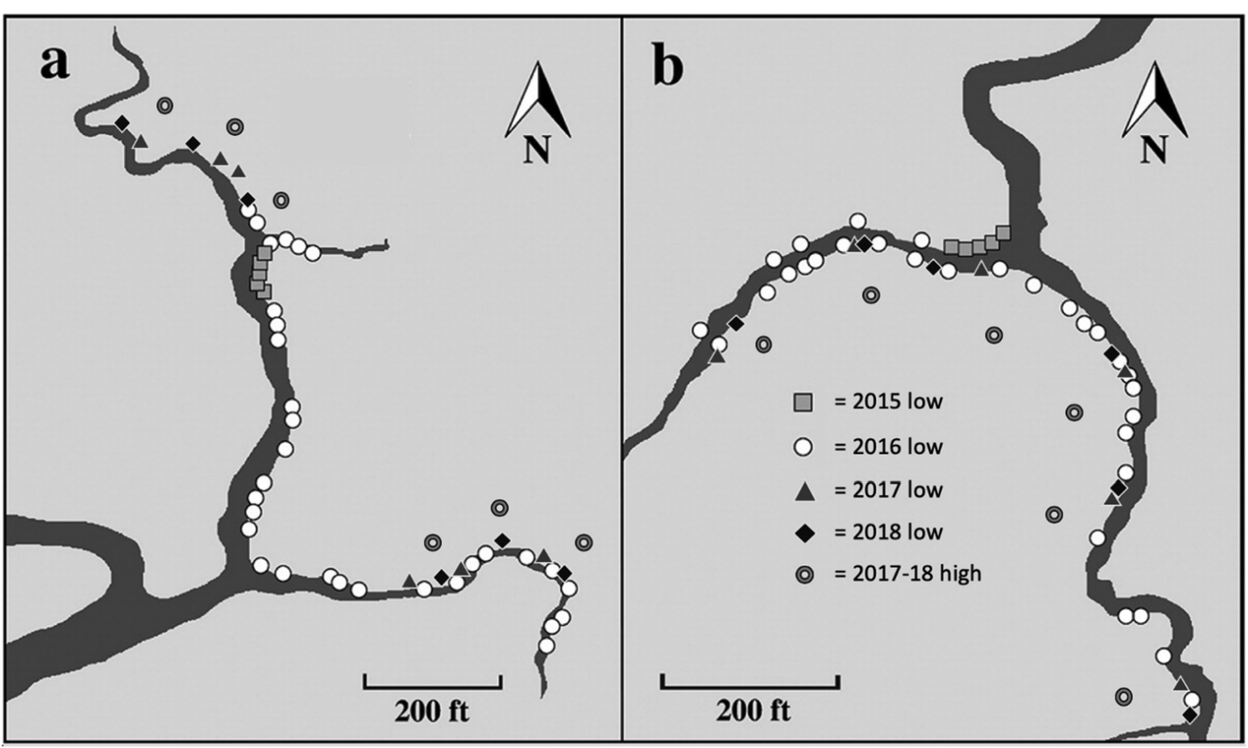

Fig. 1. Location of greenhouse gas flux plots for (a) long-term reference creeks and (b) N-legacy creeks. Sites in the high marsh (open circles) were the same for 2017 and 2018, while the low marsh sites were replaced each year. Tea Bag Index (TBI) decomposition assays in the high and low marsh took place near the flux collar locations from 2017, and low marsh root and rhizome decomposition bags were deployed within 5 m of these same locations in 2014.

TMB-M006; Onset Corporation, Bourne, USA). In 2018, we used a CR1000X datalogger (Campbell Scientific, Logan, USA) with temperature probes (Campbell 109-17-PW) Campbell Scientific, Logan, USA) to record soil and air temperature.

2.3. Soil organic matter decomposition

We used two methods to investigate soil decomposition processes during nutrient enrichment (2015 and 2016) and in the first year of recovery (2017). First, we used high-resolution litter bags containing root or rhizome material from plants growing at the site, following Kirwan et al. (2012). To obtain plant material, we collected five soil cores (10 cm diameter) from nutrient-enriched and reference creek banks on 5 June 2014. We removed sediment with tap water and separated the live belowground biomass into root and rhizome material. After being oven dried (60 °C) to constant mass, roots and rhizomes were cut into 2-cm long segments, manually homogenized within groups (i.e., combinations of litter type and source creek), and redried (60 °C). Litter bags were created by heat sealing root or rhizome material between a pair of 5 μm membranes (Versapore, Pall Life Sciences, Ann Arbor, USA) cut to approximately 5 × 7 cm. Bags contained approximately 670 mg of material (mean ± SD: 668 ± 68 mg) prior to deployment.

On 7 August 2014, we deployed a total of 96 litter bags (48 containing roots and 48 containing rhizomes), evenly distributed in six locations near the low marsh flux collars at both the reference and nutrient enriched ecosystems (Fig. 1). This allowed us to retrieve two root and two rhizome bags per location per year. We deployed material sourced from a creek at that creek to ensure any differences in tissue composition or structure associated with N fertilization would be reflected in the results. Approximately half of the bags ($n = 46$) were retrieved after one year (6 August 2015) while the remainder that could be found ($n = 41$) were retrieved after two years (11 August 2016). Of those recovered ($n = 87$), only 58 could be used for decomposition measurements due to live roots growing into bags or seams failing. After retrieval, bags were cleaned of sediment, dried (60 °C), and reweighed to determine the fractional mass lost during deployment (M_L).

In addition, we investigated decomposition using the Tea Bag Index (TBI) (Keuskamp et al., 2013); this method has been used in >570 sites across multiple biomes (Djukic et al., 2018), including in salt marshes (Mueller et al., 2018, Djukic et al., 2018; Tang et al., 2020), to understand short-term (~90 day) soil organic matter dynamics. In this method, pairs of Lipton® green (EAN: 8722700 055525; Lipton, Unilever, UK) and red (i.e., rooibos) (EAN: 8722700188438, Lipton, Unilever, UK) tea bags are buried (~10 cm soil depth), then recovered after a specified incubation time. Although the rates it provides are not specifically the rates at which material originating onsite would decompose, the use of a standardized material allows for more reliable comparisons to be made across sites and treatments and is minimally influenced by bag-to-bag variation in the source material.

The mass lost from tea bags over time was used to calculate the rate of decomposition (k) and the stabilization factor (S); S represents the inhibitory effect of environmental conditions on decomposition (Keuskamp et al., 2013). The TBI parameters k and S were determined using the protocols adapted for tidal wetlands by Mueller et al. (2018):

$$W_r(t) = a_r e^{-kt} + (1 - a_r) \tag{1}$$

$$S = 1 - a_g / H_g \tag{2}$$

$$a_r = H_r(1 - S) \tag{3}$$

where $W_r(t)$ is the weight of the rooibos tea remaining after the incubation time (t in days); a_r is the labile portion of the rooibos substrate and 1-a_r is the recalcitrant part; k is the decomposition rate; S is the stabilization factor; a_g is the decomposable fraction of the green tea; and H is the hydrolysable fraction of the green tea. Eq. (3) calculates the decomposable part of the rooibos tea based on the hydrolysable fraction (H_r) and S. We used values of H_g and H_r from the tidal wetland protocols described by Mueller et al. (2018).

Two sets of tea bags were deployed during the enrichment phase. In 2015, 10 pairs of red and 10 pairs of green tea were buried on 10 June and recovered 14 September; these data previously contributed to a global study investigating decomposition in wetlands (Mueller et al., 2018). In 2016, four pairs of red and green tea bags were deployed within two meters of each of the high-marsh flux plot locations ($n = 6$) (Fig. 1) and recovered after three months (6–7 July to 6–7 October); these data contributed to the TeaComposition initiative (Djukic et al., 2018). During the first year of recovery (2017), we deployed an additional array in both the high and low marsh zones, roughly paired with each of the gas flux collar sites (Fig. 1). Tea bags were deployed on 8 June and recovered on 16–17 October. All recovered tea bags were processed according to the TeaCompostion protocols (Djukic et al., 2018), and oven dried to constant mass at 60 °C. Decomposition rate (k) and organic matter stabilization factor (S) were then calculated following Keuskamp et al. (2013) using updated coefficients (see Mueller et al., 2018). These data allowed us to compare estimates of decomposition (k) and stabilization (S) of soil organic matter between the enrichment and post-enrichment experimental phases in order to better understand the processes driving differences in gas fluxes between the two phases.

2.4. Statistical analysis

Data were analyzed using linear modeling in R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). Separate models were fit for each response variable ($R_{eco,}$ NEE, GPP, methane emissions, M_L for root litter, M_L for rhizome litter, k, and S) and, where applicable, ecosystem zone (low marsh and high marsh). Mixed-effects linear models were used for the flux variables and high-marsh decomposition parameters (via the *lme4* code library; Bates et al. 2015); random effects terms were used to account for repeated measurements within sampling locations and years. All flux models included fixed effects for Creek (i.e., reference vs. N-manipulation), day of year (DOY), and air temperature (Temp). Low marsh flux models further included a term for experimental phase (Phase; during vs. after N-enrichment). Models for M_L, both of rhizomes and roots, included fixed effects for Creek and Year. Models for S and k in the high marsh included fixed effects for Creek and Phase; Year was entered as a random effect. Models for k and S in the low marsh included only fixed effects, namely those for Creek and Phase. All possible two-way and three-way interaction terms were evaluated as well.

To facilitate the interpretation of coefficients, response variables and all continuous explanatory variables were standardized (by dividing by two standard deviations) while binary variables were coded as ±0.5; this method enables coefficients (hereafter, β) to be interpreted on a single scale that is approximately proportional to the size of their effect (Gelman, 2008). Models were fit using maximum likelihood estimation. The statistical significance of coefficients ($\alpha = 0.05$) was evaluated using Wald χ^2 tests for mixed-effects models and F-tests for fixed-effects models; these were computed from Type III sums of squares (R function *Anova* in the library *car*). Residual normality and variance inflation were checked and deemed acceptable in all cases.

3. Results

3.1. Greenhouse gas fluxes in the low marsh

There was no difference in R_{eco} observed between the N-legacy and reference creeks post-enrichment (Fig. 2A), whereas, during N enrichment, ecosystem respiration was significantly higher in the enriched creek than the reference. Statistically, this manifested as a significant interaction between creek and experimental phase (Creek × Phase effect, $P = 0.03$, $\beta = -0.39$, Table S1). Further, R_{eco} generally increased through the growing season (DOY effect, $P = 0.03$, $\beta = 0.20$) and may have been modestly enhanced by higher air temperatures, though statistical evidence of this was weak (Temp effect, $P = 0.06$, $\beta = 0.16$).

Net ecosystem exchange in the low marsh was not elevated by N enrichment. However, there were occasionally periods when NEE was greater in the reference than in the fertilized creek. The frequency of these effects was more common during vs. after enrichment, with the effect size also being greater on warmer days (Creek × Temp × Phase effect, $P = 0.01$, $\beta = 1.02$, Table S2). This interaction manifested most strongly during early June and late July 2016 (DOY ~160 to 180) but also occurred in August 2018 (DOY ~215 to 230) (Fig. 2B). In addition, NEE typically increased over the course of the growing season, though this was not observed in August 2016 (DOY effect, $P = 0.002$, $\beta = -0.53$). There was also weak evidence of a difference in NEE between the first two years of measurement and the second two years (Phase effect, $P = 0.054$, $\beta = 0.25$); this did not appear to be driven by nutrient enrichment as the two creeks did not respond differently (Creek × Phase effect, $P = 0.26$, $\beta = 0.2$).

Like R_{eco}, GPP was greater, on average, in the enriched systems during enrichment, but this difference was not observed after nutrient enrichment ceased (Creek × Phase effect, $P = 0.04$, $\beta = 0.36$, Fig. 2C). As with NEE, differences between the creeks were more pronounced at higher temperatures during enrichment (Creek × Temp × Phase effect, $P = 0.009$, $\beta = 0.89$, Table S3). GPP also tended to increase over the course of the growing season (DOY effect, $P < 0.001$, $\beta = -0.40$).

There was no effect of nutrient enrichment on methane efflux in the low marsh and fluxes were very low. Methane flux increased over the

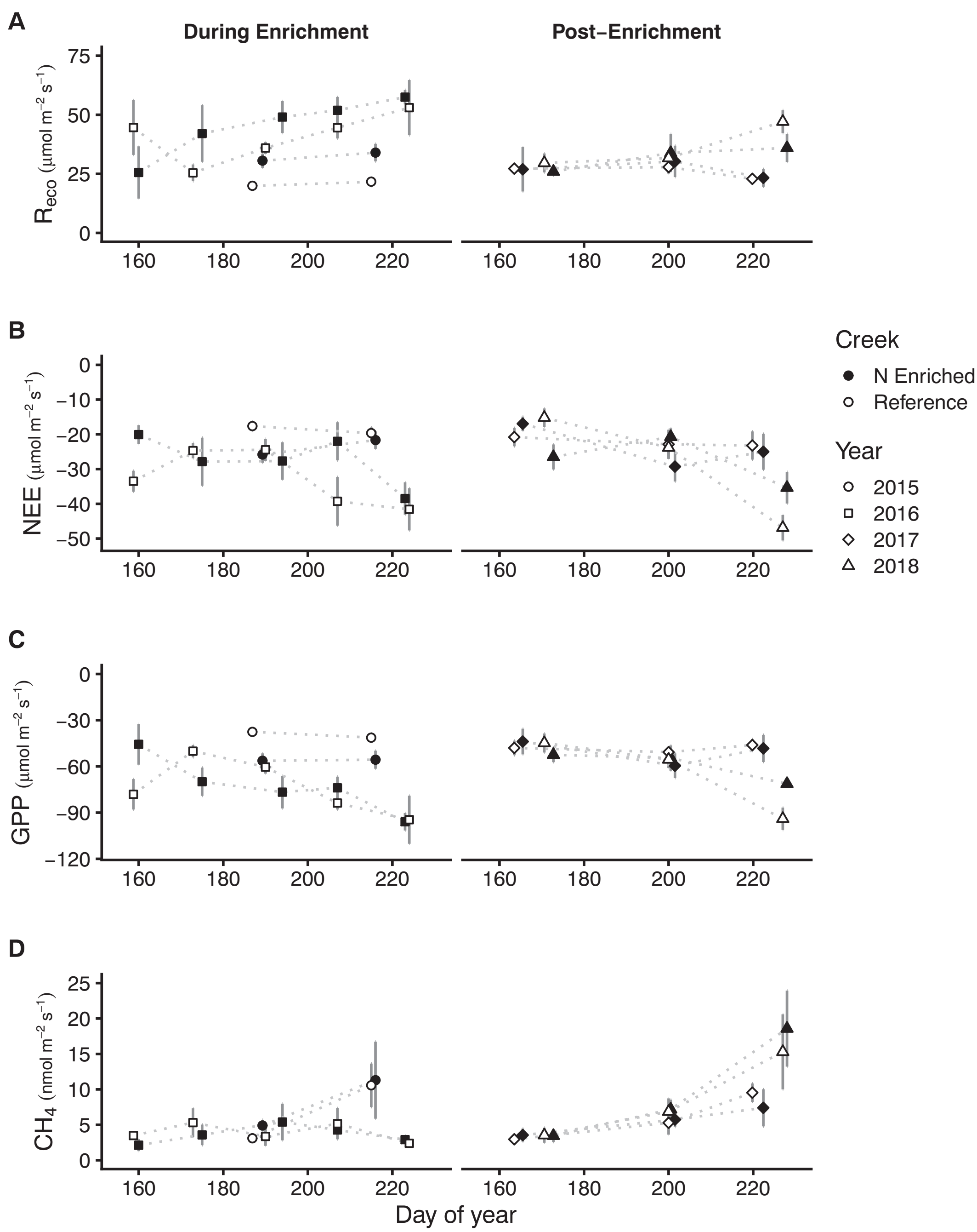

Fig. 2. Mean (±SE) carbon flux rates in the low marsh. (a) Ecosystem respiration (R_{eco}); (b) net ecosystem exchange (NEE); (c) gross primary productivity (GPP); (d) methane efflux (CH_4).

growing season in three out of four years of the study (DOY effect, $P < 0.001$, $\beta = 0.48$, Table S4, Fig. 2D). Methane emissions were higher on average for both creeks in the years after cessation of nutrient enrichment (Creek, $P = 0.009$, $\beta = -0.28$) but there were no differences in response between the two creeks (Creek × Phase, $P = 0.904$, $\beta = -0.03$).

3.2. Greenhouse gas fluxes in the high marsh

There was no difference in R_{eco} in the high marsh between the N-legacy and reference creeks (Creek effect, $P = 0.932$, $\beta = -0.01$, Fig. 3A, Table S5). We note that flux measurements were not made in the high marsh in 2015 or 2016 so we cannot confirm that the increase in R_{eco} we observed in the low marsh also occurred in the high marsh (but see decomposition results below). R_{eco} also increased with air temperature in the high marsh (Temp effect, $P = 0.00$, $\beta = 0.39$).

On average, NEE and methane efflux in the high marsh were both greater in the reference creek than the nutrient enriched creek (NEE: Creek effect, $P = 0.02$, $\beta = -0.24$, Fig. 3B, Table S6; Methane: Creek effect, $P = 0.02$, $\beta = 0.22$, Fig. 3D, Table S7). GPP may have also been modestly greater in the reference system, though the difference was not significant (Creek effect, $P = 0.08$, $\beta = -0.18$, Fig. 3C, Table S8). NEE, GPP, and methane all appeared to increase substantially in 2018. However, variation between years was accounted for as a random effect within our statistical models, so differences could not be evaluated with Wald χ^2 tests.

3.3. Decomposition

Rhizome litter bags buried in the nutrient enriched marsh lost a significantly greater percentage of their mass regardless of the incubation period (1 or 2 years) than did litter bags buried in the reference marsh (Creek effect, $P = 0.003$, $\beta = -0.40$, Fig. 4B, Table S9). However, there was no difference in mass loss for root litter (Creek effect, $P = 0.50$, $\beta = 0.09$, Fig. 4A, Table S10). Both substrates lost more mass with a longer burial time (Year effect, $P < 0.001$, $\beta > 0.60$, Tables S9 and S10).

Although we previously observed marginally greater rates of TBI-derived decomposition (k) in the low marsh during enrichment (Mueller et al., 2018), this pattern was not identifiable in the larger dataset presented here (Creek × Year effect, $P = 0.56$, $\beta = 0.23$, Table S11). That said, sample means for the low marsh were consistent with enrichment raising k over those of the reference creek in 2015, with this margin decreasing by the first year of recovery (Fig. 5A). Regardless, a similar pattern was not apparent in the high marsh; we found no statistically distinguishable difference in k due to enrichment status (Fig. 5B; Creek × Phase effect, $P = 0.83$, $\beta = 0.07$, Table S12).

Soil organic matter stabilization rates (S) increased between 2015 and 2017 in the low marsh (Year effect, $P = 0.001$, $\beta = 0.60$, Table S13) and sample means were lower in the N-legacy creeks during both years (Fig. 5C). However, the interaction effect was not statistically significant (Creek × Year effect, $P = 0.69$, $\beta = 0.13$). In contrast, S in the high marsh was significantly, and appreciably, lower in the enriched creek during the final two years of enrichment (Creek effect, $P < 0.01$, $\beta = 0.21$, Fig. 5D, Table S14) than in the first year of recovery (Creek × Phase effect, $P = 0.05$, $\beta = 0.27$). This occurred despite large interannual variability in S; means were approximately 4× greater in 2016 than 2015.

4. Discussion

This study suggests that land and water management practices that limit excess N loading can be effective in restoring nutrient-altered carbon sequestration processes in at least some, but possibly many, salt marshes globally. Previously, we found that nutrient enrichment stimulated R_{eco} by 8–65% in the salt marsh investigated through the TIDE Project, intermittently reducing NEE (Geoghegan et al., 2018, Fig. 2) and

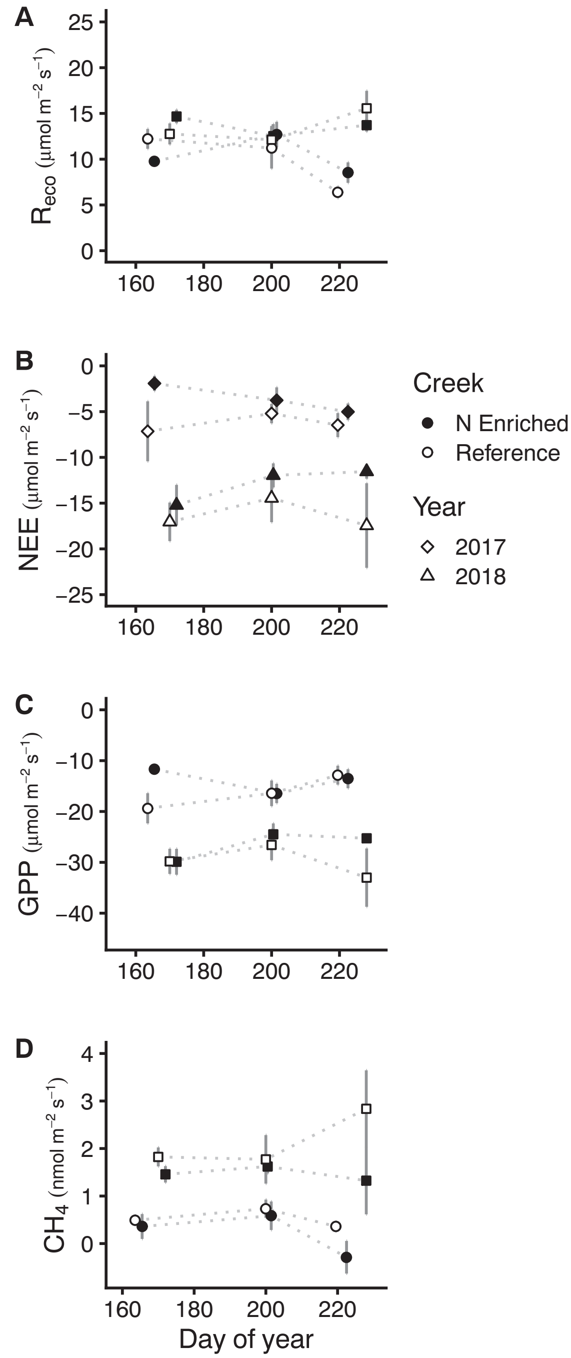

Fig. 3. Mean (±SE) carbon flux rates in the high marsh. (a) Ecosystem respiration (R_{eco}); (b) net ecosystem exchange (NEE); (c) gross primary productivity (GPP); (d) methane efflux (CH_4). Data are presented only for 2017 and 2018 because fluxes were not measured in the high marsh during the N enrichment phase of the experiment.

likely diminishing the carbon sequestration capacity of the marsh. Our findings are consistent with others that reported stimulations or R_{eco} in the high and low marsh platforms (Martin et al., 2018; Song et al., 2019; Wigand et al., 2009; Wigand et al., 2018). We now show that rates of R_{eco} returned to reference levels in the first two years after nutrient enrichment ceased, with no significant differences in CO_2 fluxes (R_{eco}, NEE, or GPP) between the long-term reference and N-legacy ecosystems in the low marsh (Figs. 2 & S1–3). This pattern suggests that reductions in nitrate loading to coasts can rapidly restore the carbon storage function of nutrient-enriched salt marshes to reference levels. Since there are no other ecosystem-scale longitudinal studies on salt marsh carbon cycle processes that include reductions in nitrate

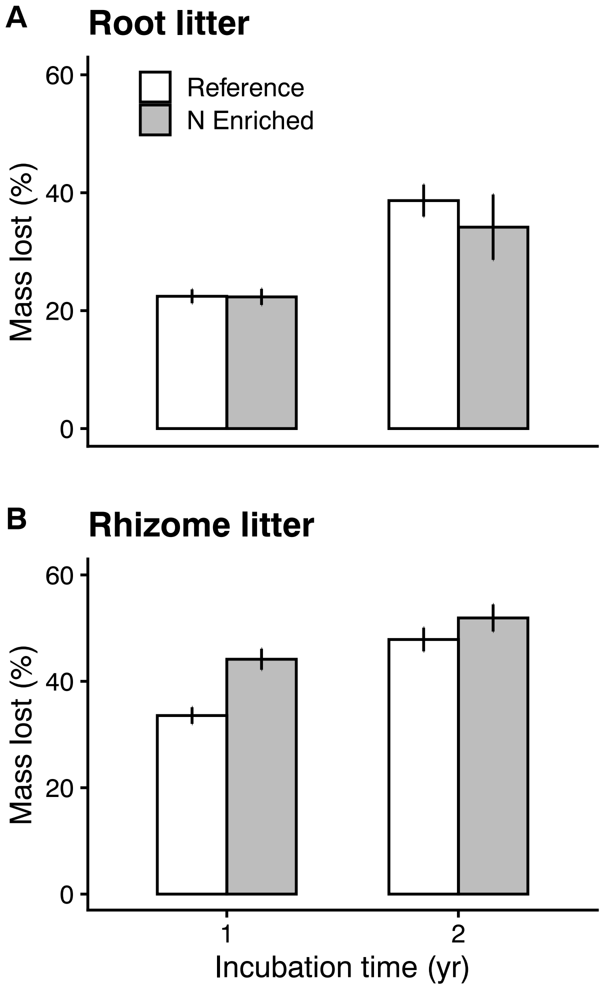

Fig. 4. Mean (±SE) mass loss from litter bags containing root or rhizome material from the field site. Litter bags were buried during the experiment's N-enrichment phase. All litter bags were buried in 2014 and recovered in 2015 or 2016.

enrichment, this is among the strongest evidence currently available that nutrient-stimulated losses of blue carbon may be reversed with management practices targeted at reducing nitrate enrichment.

Changes in microbial activity are likely the dominant reason for the observed changes in ecosystem respiration under nutrient enrichment, as well as in the recovery. Under nutrient enrichment, this and prior studies at TIDE have observed higher rates of rhizome decomposition (Fig. 4), aboveground litter respiration (Deegan et al., 2012), and soil respiration (Wigand et al., 2018), as well as lower soil organic matter stabilization (*S*) (Fig. 5C&D, Mueller et al., 2018). We also note that the decomposition bag assays for rhizome material and the TBI yielded similar results under nutrient enrichment. The mechanism for these changes was described by Bulseco et al. (2019), who showed that nitrate enrichment stimulated microbial decomposition, and by Kearns et al. (2017), who observed a large shift in the active microbial communities in the marsh soil under nutrient enrichment. Nitrate is a powerful terminal electron acceptor in anaerobic soils, and can stimulate certain classes of microbes to enhance soil organic matter decomposition (Bulseco et al., 2019). In addition, the within-season increases in R_{eco} that we observed are consistent with increased microbial activity due to soil warming (Mozdzer et al., 2014). If autotrophic respiration played a role in stimulating R_{eco}, it was likely secondary, as aboveground primary productivity and tissue N content remained unchanged during nutrient enrichment (Johnson et al., 2016). This suggests that the recovery of R_{eco} and decomposition metrics (M_L, *k*, and *S*) to reference levels are most likely attributed to the cessation of nitrate-stimulated microbial processes.

We found no legacy effect of N enrichment on methane emissions in the low marsh, which is not surprising given that effects of nutrient enrichment on methanogenesis are indirect, driven by root exudation in the rhizosphere (Olsson et al., 2015) or increased methane oxidation from stimulated root growth (Mozdzer and Megonigal, 2013). Further, methane fluxes in polyhaline marshes are typically too low to appreciably alter the carbon sink function of coastal wetlands (Poffenbarger et al., 2011). Although we found elevated rates of methane emissions and NEE in the reference high marsh post-enrichment, we lack similar data during the enrichment phase to determine if this is a legacy of enrichment, or if there was a shift following its cessation. Regardless, the difference in mean methane emission rates (< 2 nmol m^{-2} s^{-1}) were small enough to have a negligible effect on the carbon sequestration capacity of the ecosystem. Nonetheless, the observation of higher rates of methane emissions in the reference system after the cessation of nutrient warrants further investigation, as the effect could have important implications if it occurs widely.

Variation in rainfall may be responsible for much of the interannual variation in our results. Most notably, in 2016, our site received 50% less rainfall compared to the long-term average for the growing season (May–September); monthly mean temperatures were relatively consistent across years (Fig. S1). Interannual variation in carbon cycle processes (measured by eddy covariance) have been demonstrated to be driven by precipitation (Forbrich et al., 2018). Specifically, lower precipitation increased salinity in a nearby salt marsh within the PIE LTER (Forbrich et al., 2018); this could explain the elevated rates of R_{eco} in 2016 since higher salinity is associated with greater respiration even in salt-tolerant species (Jacoby et al., 2011). The lack of precipitation in 2016 likely contributed to the observed decreases in *k* and increased *S* as well (Fig. 5), especially in the infrequently flooded high marsh. A previous study in salt marshes has also reported decreased decomposition rates from drought (Charles and Dukes, 2009), though the pattern has not been observed consistently (Emery et al., 2019). While variation in the magnitude of the above-mentioned rates apparently shifted in response to precipitation, differences between reference and N-enriched marshes were apparent nonetheless. In other words, varying environmental conditions between years were highly unlikely to be responsible for the recovery-phase patterns we observed in R_{eco} and decomposition.

While decomposition processes largely, if not wholly, recovered following reductions in nutrient enrichment, the previous stimulation of microbial activity and altered belowground plant production could have long-term consequences if they reduced belowground carbon stocks. Nutrient enrichment can decrease belowground productivity in some systems (Deegan et al., 2012; Kirwan and Megonigal, 2013; Langley et al., 2009), thereby reducing belowground carbon inputs. Although we do not yet have estimates of belowground net primary productivity during the recovery phase, we predict that belowground productivity will return to reference levels in future years as nutrient-limited plants scavenge for nutrients and increase their root:shoot ratios. Theoretically, the combined effects of lower carbon inputs, lower root:shoot ratios, and more rapid organic matter decomposition under nutrient enrichment would have resulted in losses of soil carbon and soil volume, potentially lowering surface elevation and thereby decreasing the resilience of this and similar salt marsh ecosystems to sea level rise (Kirwan and Megonigal, 2013).

Future research should investigate how various levels of nutrient enrichment affect the capacity for carbon cycle processes to recover and determine if threshold effects exist. The TIDE experiment simulated moderate levels of enrichment in the tidal flooding water (70 to 150 μM nitrate), resulting in average N loading rates of 171 g N m^{-2} yr^{-1} in the low marsh and 19 g N m^{-2} yr^{-1} in the high marsh (Johnson et al., 2016). These nutrient enrichment levels are at the low end of those experienced by eutrophic New England salt marshes (up to 700 g N m^{-2} yr^{-1}) (Wigand et al., 2009), suggesting that the potential for rapid

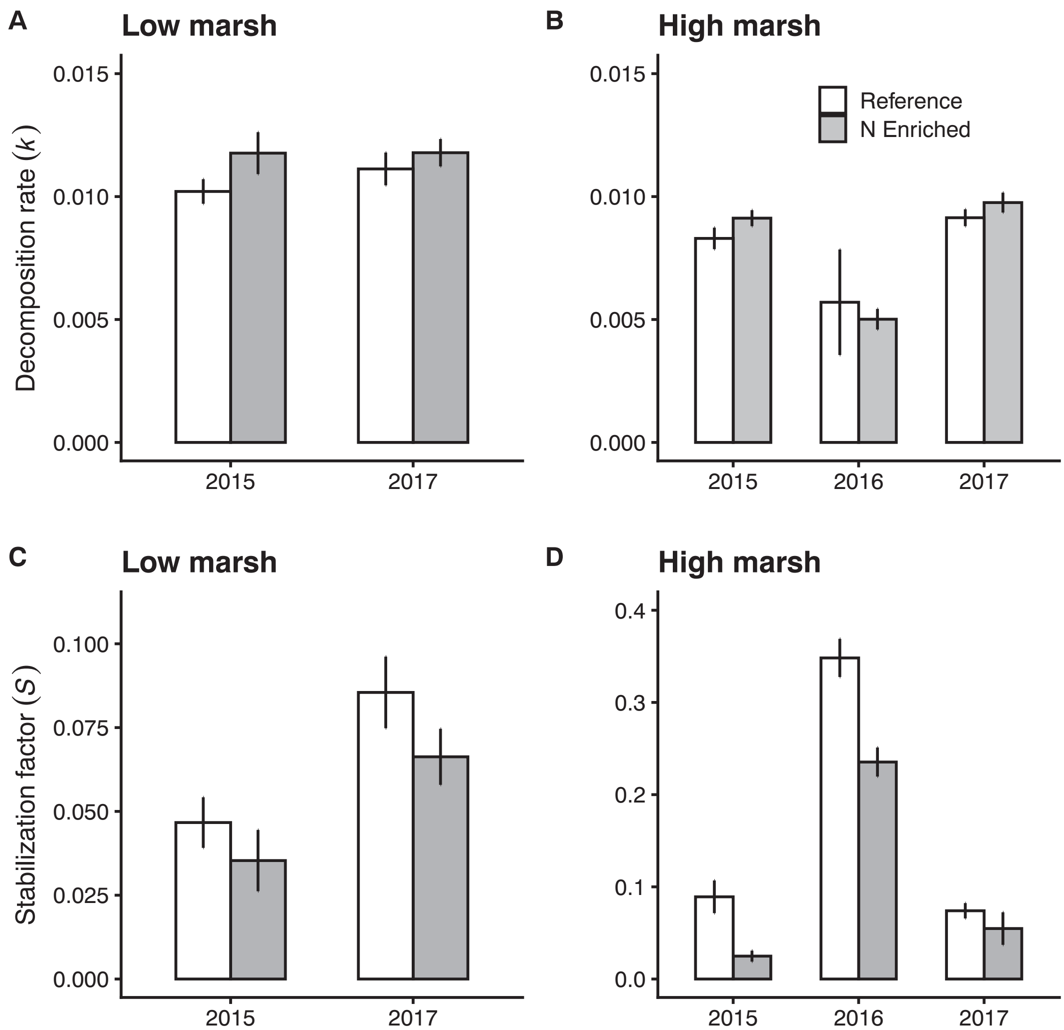

Fig. 5. Mean (±SE) decomposition rate (k) and stabilization factor (S) in the reference and N-enriched creeks both during the enrichment phase (2015 and 2016) and in recovery post-enrichment (2017).

recovery demonstrated here may not apply everywhere. Moreover, factors like the mode of nutrient delivery (i.e. tidal water vs. groundwater delivery), the nutrient form (nitrate, ammonium, or organic N), and soil composition (organic or mineral dominated) almost certainly shape the ecosystem response to nutrient enrichment (Mozdzer et al., 2020). There may be threshold effects or other non-linearities in these relationships that have yet to be identified that may alter the pace and trajectory of recovery from eutrophication in salt marshes.

5. Conclusions

This study suggests that salt marshes similar to the ones studied here have the capacity to return key carbon cycle processes to reference levels when nutrient over-enrichment ceases. These findings have implications for restoration via land and water management; they suggest that curbing coastal nutrient enrichment has the potential to improve carbon sequestration in the short-term and increase coastal resilience in the long-term. Furthermore, because reductions in nutrient enrichment may restore the carbon storage capacity of these blue carbon ecosystems, it may be possible to couple nutrient management to the carbon credit market (Kroeger et al., 2017; Sapkota and White, 2020; Wylie et al., 2016). Finally, our focal system experienced moderate levels of nutrient enrichment and was quick to reverse elevated heterotrophic processes; future studies should evaluate the degree to which higher levels of nutrient enrichment may have enhanced heterotrophic processes and exacerbated salt marsh loss and geomorphic changes, thereby determining the potential of a wider array of salt marsh ecosystems to recover from nutrient loading.

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CRediT authorship contribution statement

Thomas J. Mozdzer: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Sophie E. Drew:** Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization. **Joshua S. Caplan:** Investigation, Data curation, Formal analysis, Writing - review & editing, Visualization. **Paige Weber:** Methodology, Investigation, Writing - review & editing. **Linda A. Deegan:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors have no conflicts of interest to report.

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Appendix A. Supplementary data

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