Title: Plant biomass and rates of carbon dioxide uptake are enhanced by successful restoration of tidal connectivity in salt marshes

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Abstract:

Salt marshes, due to their capability to bury soil carbon (C), are potentially important regional C sinks. Efforts to restore tidal flow to former salt marshes have increased in recent decades in New England (USA), as well as in some other parts of the world. In this study, we investigated plant biomass and carbon dioxide (CO_2) fluxes at four sites where restoration of tidal flow occurred five to ten years prior to the study. Site elevation, aboveground biomass, CO_2 flux, and porewater chemistry were measured in 2015 and 2016 in both restored marshes and adjacent marshes where tidal flow had never been restricted. We found that the elevation in restored marsh sites was 2-16 cm lower than their natural references. Restored marshes where porewater chemistry was similar to the natural reference had greater plant aboveground biomass, gross ecosystem production, ecosystem respiration, as well as net ecosystem CO_2 exchange (NEE) than the natural reference, even though they have the same plant species . We also compared respiration rates in aboveground biomass (AR) and soil (BR) during July 2016, and found that restored marshes had higher AR and BR fluxes than natural references. Our findings indicated that well-restored salt marshes can result in greater plant biomass and NEP, which has the potential to enhance rates of C sequestration at 10-yrs post restoration. Those differences were likely due to lower elevation and greater flooding frequency in the recently restored marshes than the natural marsh. The inverse relationship between elevation and productivity further suggests that, where sea-level rise rate does not surpass the threshold of plant survival, the restoration of these salt marshes may lead to enhanced organic and mineral sedimentation, extending marsh survival under increased sea level, and recouping carbon stocks that were lost during decades of tidal restriction.

Key words: Salt marsh, greenhouse gas, restoration, carbon dioxide

Introduction

Coastal wetlands are considered a significant sink for carbon (C), since the rates of C burial are generally greater and, as importantly, the rates of C mineralization are generally lower in coastal wetlands than in terrestrial ecosystems (McLeod et al., 2011). Salt marshes play an important role in the global C cycle, and the large C reservoirs in salt marsh sediments are important pools for conservation (Holmquist et al., 2018; Wang et al., 2019b). Globally, there is increasing interest in coastal wetlands as targets for greenhouse gas emission offset projects through preservation and restoration of these ecosystems to increase future C sequestration and reduce anthropogenic greenhouse gas emissions (Crooks et al., 2018; Kroeger et al., 2017; Pendleton et al., 2012).

Despite their importance, salt marshes have been under pressure from human activities including conversion to farms and developed land, dam construction, among others (Emery and Fulweiler, 2017; Gedan et al., 2011). In New England, over half the salt marshes present before the pre-industrial era have been lost (Portnoy and Giblin, 1997a; Portnoy and Giblin, 1997b). Some of these salt marshes were converted to other ecosystems after undersized culverts, dikes, and similar structures were put in place for the construction of roads, insect control, wildfowl habitat, or agriculture (Portnoy and Giblin, 1997a; Portnoy and Giblin, 1997b; Roman and Burdick, 2012). These dikes and undersized culverts not only reduce the geographic extent of these wetlands, but also impact the biogeochemical processes by restricting tidal exchange between estuaries and upstream wetlands, and alter the processes controlling soil C accumulation and greenhouse gas emissions (Drexler et al., 2013; Emery and Fulweiler, 2017; Warren et al., 2002).

Restriction of tidal exchange commonly causes retention of freshwater and lowers the salinity in wetlands landward of the restriction (Emery and Fulweiler, 2017; Roman and Burdick, 2012). In turn, large ecological shifts occur that alter biogeochemical processes. For example, plant community composition often transitions from salt-tolerant cordgrasses (*Spartina alterniflora* and/or *S. patens*) to freshwater or brackish wetland species such as cattail (*Typha latifolia*) and common reed (*Phragmites australis*) in restricted marshes

(Warren et al., 2002). If the tidal restriction results in drainage of the wetland, C decomposition may increase as buried soil C is exposed to oxygen (Portnoy, 2012; Portnoy and Giblin, 1997b). In a 90-year old diked and drained salt marsh in New England, Portnoy and Giblin (1997b) observed 90 cm soil surface subsidence relative to the nearby unrestricted reference marsh. If tidal restrictions promote decomposition of buried organic matter or enhanced methane emissions, the salt marshes may shift from a CO_2 sink to a source of greenhouse gases (Kroeger et al., 2017).

Restoration of tidal flow has the potential to reverse these effects by extending flooding depth and duration, raising the water table, and increasing salinity (Portnoy and Giblin, 1997b). A longer flooding duration, or higher flooding frequency, can enhance salt marsh plant biomass (Cadol et al., 2014; Kirwan and Guntenspergen, 2012; Morris et al., 2002), potentially resulting in greater CO_2 assimilation in salt marshes after tidal restoration. In a recent laboratory experiment, we found reduced C decomposition rates in the higher inundation levels that occur with tidal restoration (Wang et al., 2019a). While there have been a number of investigations of biogeochemical processes and plant community composition in response to salt marsh restriction and restoration (Roman et al., 2002; Smith and Medeiros, 2013; Warren et al., 2002), there is relatively limited knowledge of the C storage and greenhouse gas (GHG) flux responses associated with these management actions (Emery and Fulweiler, 2017; Kroeger et al., 2017; Negandhi et al., 2019; Pendleton et al., 2012). Considering the large area of tidally restricted wetlands globally (Kroeger et al., 2017), quantifying the effect of tidal restriction and restoration on C biogeochemical cycles and subsequent storage will support future restoration scenarios (Drexler et al., 2013).

In New England, there is increasing occurrence of tidal restoration in salt marshes in the last few decades (Warren et al., 2002). While post-restoration studies are critical for determining whether restoration goals were achieved through returning tidal exchange to salt marshes, data collection is inconsistent and mostly limited to evaluating animal and plant community composition (Raposa, 2008; Roman et al., 2002; Warren et al., 2002). Greenhouse gas (GHG) exchange is a critical component of evaluating the effectiveness of restored wetlands as efficient C sinks. To fill this knowledge gap, we aim to evaluate soil porewater chemistry and CO_2 fluxes in restored and natural salt marshes. Here, we compare two types of coastal wetlands: 1) 'natural' marshes at seaward of tidal restrictions where water exchange has never been restricted, and 2) landward 'restored' marshes that were reestablished from previously tidally restricted marshes.

Methods:

Study Sites

Four marsh sites along Cape Cod Bay, MA, USA, were chosen for this study (Fig 1). According to Massachusetts Division of Ecological Restoration (MA-DER), restrictions at these sites were constructed over 100 years ago. Between 2005 and 2010, tidal exchange was restored when a restriction, such as an undersized culvert in the tidal creek, was enlarged, thereby increasing connectivity between the marsh and Cape Cod Bay (Fig. 1). Measurements in these four sites were conducted in 2015 and 2016.

Quivett Creek (41.7470, -70.1434)**:** The site was restored in 2005 with construction of a new bridge that allowed full tidal flow in the landward direction. *Spartina alterniflora* is the dominant vegetation at the natural site, while at the restored site, *S. alterniflora* and *Spartina patens* are the dominant species.

State Game Farm (41.7314, -70.4272): In 2006, a small culvert was removed, and replaced with a bridge which increased tidal flow to former restricted marsh. At the natural site, *S. patens* is the dominant species. In the restored marsh, *S. alterniflora* is dominant, with *P. australis* distributed along the marsh edge.

Bass Creek (41.7162, -70. 2376): The site was restored in 2008 with a new bridge, which fully restored the tidal flow in landward salt marsh. At the seaward of the former restriction, *Distichlis spicata* is the main species in the natural marsh site. In the restored marsh, *S. alterniflora* and *S. patens* dominate.

Stony Brook (41.45275, -70. 6762.): A small culvert was removed in 2010 by local government and replaced with a large opening which restored the full tidal flow, aiming to restore the natural marsh in the restricted region. In both the natural and restored sites, *S. alterniflora* dominated plots were selected in 2015. In 2016, additional plots where *S.patens* dominated were included to evaluate any species effect.

Experimental Design

In this study, we conducted two experiments to detect the effect of salt marsh restoration on CO_2 fluxes, plant biomass and pore water chemistry. The first experiment was conducted at the four sites mentioned above. At each site, plots were placed approximately 10 m downstream and upstream from the former restriction edge, and 4-5 m from the creek edge, except at Stony Brook, where we utilized a boardwalk to access plots over 100 m away from the former restriction, and 40-50 m away from creek edge (Fig. 1). Four 1m x 1m plots were randomly distributed within the selected area (10 x 10 m), within 1-2 meters of each other. Plots were kept in similar geomorphic settings (e.g. elevation, plant types) to minimize the influence of other factors when comparing upstream restored and downstream natural salt marshes. In each plot, a 30-cm-diameter circular collar was installed for static gas chamber measurements. The collar was 5 cm high, and inserted into sediment to 2 cm and left in place for the entire two years of the study. Within each study site, a 1-m-deep well was installed within 2 m of the collars in 2015. In each well, an *In-Situ* Aquatroll 200 (In-Situ Inc., Fort Collins, CO, USA) CTD sensor collected continuous water table, water temperature, and salinity data. Further details on sensor deployment can be found in an accompanying data release (O'Keefe Suttles et al., 2019).

While the first experiment was designed to evaluate the difference between natural and restored marsh sites, the dominant plant species usually differed between these sites. To resolve this issue, we conducted the second experiment at one site - Stony Brook, where *S. alterniflora* and *S. patens* dominated communites were observed in both restored and natural marsh sites. In 2016, we established four *S.patens* plots (Elevation (NAVD88) : 1.48 m) not far from the orginal four *S. alterniflora* plots upstream and down stream of the former restriction, respectively. At this site, two species (*S. alterniflora* and *S.patens*) and two restoration types (restored and natural marsh), with each replicated by four plots, were used to identify the species effect and restoration effect on plant biomass and gas flux.

CO_2 flux

CO_2 flux was measured in situ with a Picarro G2301 gas Analyzer (Picarro Inc, Santa Clara, CA, USA). Gas flux was measured monthly during the growing season (April to September) in 2015 and 2016. A 60-cm tall x 30-cm diameter transparent chamber, with a recirculation fan to mix the chamber headspace, was used for gas measurements. Air temperature and solar radiation were monitered during each gas flux measurement. Fluxes were measured under both light and dark conditions. To exclude photosynthetic CO_2 uptake during dark sampling, we covered chambers with an opaque curtain. Pressure equilibration occurred through a 10 cm length of 0.6 mm inner diameter steel tubing on the chamber that is open to the atmosphere. All CO2 flux measurements lasted 4-5 minutes per plot (with approximately 1 second sampling intervals), based on observed periods for linear rates of gas concentration change and to avoid excessive chamber warming (Martin & Moseman-Valtierra, 2015; Brannon *et al.*, 2016). The gas flux was determined by the following formula:

1) $F=(dC/dT)\times(1/V_0)\times(P/P_0)\times(T_0/T)\times(V/S)$

Where F is the flux rate, dC/dT is the slope of the CO_2 concentration *vs.* time, V_0 is the CO_2 molar volume under standard conditions (*i.e.*, 22.4 L mol^{-1}), P is the air pressure, P_0 is the standard air pressure, T is the air temperature during each measurement, T_0 is the standard temperature, V is the effective head space volume, including the tubing volume, and S is the soil surface area of the soil core. CO_2 fluxes were calculated with code developed by Eckhardt and Kutzbach (2016) using Matlab 2016a Mathworks. Here, the net ecosystem CO_2 exchange was measured in light, and expressed as NEE, and ecosystem respiration (ER) was measured in dark after 1 minute equilibration after the light measurement. We calculated gross ecosystem production (GEP) as the balance of NEE and

ER. Soil temperature, soil water-filled pore space (WFPS, ProCheck soil moisture meter, Decagon Devices, Inc. Pullman WA, USA), pH (Spectrum FieldScout SoilStik pH meter, Spectrum Inc. Aurora IL, USA), and oxidation/reduction potential (Spectrum FieldScout SoilStik electrode meter, Spectrum Inc. Aurora, IL) were also measured for surface soils (0-5 cm) during the gas measurement in the field.

Water samples

During the gas flux measurements in 2016, pore-water samples were taken from each well (0.3 m depth) for pH, redox, salinity, dissolved organic C (DOC), Cl^-, SO_4^{2-} and S^{2-} measurements. Analyses were targeted to provide information on redox conditions, porewater chemical properties, and likely metabolic pathways for C cycling. Water pH (Spectrum FieldScout SoilStik pH meter, Spectrum Inc. Aurora, IL), redox (Spectrum FieldScout SoilStik electrode meter, Spectrum Inc. Aurora, IL), and salinity (refractometer) were measured in the field. The DOC samples were filtered *in-situ* with a 0.45 µm syringe filter (Millipore) into a 40 mL amber glass vial and acidified with 10 µL of 50% hydrochloric acid. Sulfate and Cl^- samples were also filtered but not acidified. Sulfide samples were collected into a 10 mL vial preloaded with 0.2 mL of 2.0 N zinc acetate and 0.5 mL of 15 M sodium hydroxide, and flushed with a nitrogen atmosphere, and then vacuumized. During field sampling, 8 mL porewater was injected into the vial with a needle to avoid exposure to air. All porewater samples were stored on ice immediately after collection, and then stored at 4°C at the USGS Woods Hole laboratory prior to analysis. DOC samples were run on a total organic carbon analyzer (OI Analytical, Aurora, IL) by high-temperature catalytic oxidation/non-dispersive infrared detection (HTCO-NDIR). DOC concentrations are reported relative to potassium hydrogen phthalate (KHP) standard. Hansell deep seawater (University of Miami Hansell Laboratory, Lot# 01-14), and Suwannee River NOM (IHSS, Lot# 2R101N) reference materials were analyzed daily as additional checks on precision and accuracy. Coefficient of variation of DOC concentration of KHP standards, reference materials and samples is typically less than or equal to 5%. Porewater sulfate concentrations were measured using a Dionex ion chromatograph (ThermoFisher, Waltham, MA). Sulfide concentrations were measured upon formation of a methylene blue-sulfide complex and measurement of its concentration with a spectrophotometer (Cline, 1969; Reese et al., 2011).

Plant samples

In 2015, we sampled total aboveground biomass in each plot in mid-August by clipping one 25 cm x 25 cm quadrat near each gas chamber collar (n=4). The clipped plants from each plot were initially stored on ice, then at 4°C until they were dried at 65 °C for 48 hours and weighed to calculate above ground biomass.

In June 2016, we installed four additional collars at each site. After the July gas flux measurement, we clipped the plants to harvest the aboveground biomass, and then repeated the gas flux measurement. The goal was to separate aboveground respiration (AR) from soil respiration (BR, including root and soil microbial respiration).

Surface elevation and water table

Gas collar and well elevation were measured with a Trimble Real-Time Kinematic Geographic Positioning System (RTK GPS). All data were projected to NAD 1983 Massachusetts State Plane FIPS 2001 and elevations are given relative to NAVD88 with an elevation accuracy of 2-3 cm. The depth to the water table was calculated from the continuous (15 minute) CTD sensor data. Flooding frequency was determined as the percent of time water elevation was above the soil elevation during the sensor deployment.

Statistical analysis

We used linear mixed effects models (LME) to evaluate differences in CO_2 fluxes, elevation, salinity, and soil pH between restored plots and natural plots. In the LME analysis, study sites and restoration treatment (natural marsh vs restored marsh), and their interactions as fixed factors, and sampling time and plots within each site and treatment as random factors. Since there were significant effects of inter-site, and the interaction of site and restoration treatment on gas flux, we further investigated the restoration effect at each site using a linear mixed effects model with restoration type as a fixed effect, and plots and sampling time as random effects.

For pore-water data in 2016, since there was only one well at each restored or natural marsh site, we thus treated sampling time as a random effect, and site, restoration type and their interaction as fixed effects. Plant aboveground biomass data in each year was analyzed by linear mixed model with site, restoration type, and their interaction as fixed effects and plots as a random effect.

At the Stony Brook site, we have two species (*S. alterniflora* and *S. patens*) and two restoration types (natural and restored marshes) in 2016. To detect the species-specific effect on gas flux during salt marsh restoration, a linear mixed model was used to analyze the species and restoration types effect, with species, restoration type and their interaction the fixed effects, and plots and sampling date random effects. To obtain p-values to assess significance of each fixed effect on the above variables, we compared the Akaike information criterion (AIC) of full models against models with each of the fixed effects removed.

Principal component analysis (PCA) was conducted for the July 2016 aboveground biomass data, porewater data, site parameters (elevation, salinity), and CO_2 gas flux (including GEP, NEP, ER, aboveground respiration, soil respiration).

All statistics were performed in R 3.6.0 (R Core Team, 2016) and interpreted significance at $p \leqslant 0.05$.

Results

Surface elevation, water table, and flooding frequency

The elevation of the restored marsh plots were generally 2-16 cm lower than plots in the natural reference sites (Table 1). Specifically, there was generally lower elevation in three of four restored sites, excluding Bass Creek site (BC). The flooding duration was greater in three of the restored marshes due to the lower surface elevation (Table 1).

Pore-water properties

The pore water results indicate that inter-site variability dominated over restored versus natural treatments. There was no difference in porewater salinity between restored and natural marshes at three sites (Fig. 2), with the only exception of State Game Farm (SGF), which was restored in 2006, but still had much lower salinity in restored side (10 SI) than downstream in the natural marsh (21 SI). While natural marshes generally had higher soil pH than restored marshes, there was no statistically significant difference (Fig. 2). Disolved organic carbon (DOC) concentrations were significantly different among sites ($p<0.01$), and there was a significant interaction effect between sites and restoration treatments ($p<0.01$), but no significant differences between natural and restored sites. The highest DOC (5,526 uM L^{-1}) was observed in the natural marsh at the QC site, where DOC concentrations were over four times higher than its restored counterpart (Fig. 2a). At SB and SGF sites, there were generally 10% to 20% higher DOC concentrations in restored marshes than natural reference (Fig. 2a). BC had the lowest DOC in both natural and restored marshes, and there was no difference between them. Porewater sulfate concentration was greatly affected by sites and the interaction of site and restoration types ($p<0.01$ for both).

The highest sulfate concentration (28.1 mM) was observed in BC sites (Fig 2b), which was similar to full-salinity seawater sulfate concentration but was 2-3 times higher than the values at other sites. At QC site, sulfate concentration was significantly higher in the restored marsh than the natural marsh. However the opposite pattern was observed at the SGF site ($p<0.01$). There was no difference in sulfate concentration between restored and natural marshes in SB and BC sites (Fig. 2b). The sulfide concentration was very low in comparison to sulfate, as expected. Due to the large variability observed between each sampling events, there were no significant differences among sites or restoration treatments. Porewater chloride concentration was 1-3 times higher at BC sites (over 500 mM) compared to other sites. The interaction between sites and restoration treatments also significantly affected chloride concentrations ($p<0.01$, Fig. 2d). At QC and SGF sites, there were higher chloride concentrations in natural marshes than restored marshes. However, at BC site, restored marsh had higher chloride concentrations than natural marsh ($p<0.05$, Fig. 2d).

Plant biomass

In both 2015 and 2016, restored marshes had higher biomass than their natural references in three out of four sites, with the SGF site serving as an exception with the opposite pattern observed (Fig. 3). At SB site in 2016, we investigated the biomass of two dominant salt marsh communities in restored and natural marsh: *S. alterniflora* and *S. patens*. *S. patens* generally had higher aboveground biomass than *S. alterniflora* at both restored and natural marsh sites. Moreover, at the SB site, the restored marsh had higher plant biomass than the natural marsh for both *S. patens* and *S. alterniflora* plots.

Gross Ecosystem Production，Ecosystem Respiration and Net Ecosystem Exchange

Gross ecosystem production (GEP) varied significantly among sites and restoration treatments, as well as their interactions ($P<0.01$ for all, Table 2). The full factor LME model showed that restored marshes had on average 2.47 μmol CO_2 m^{-2} s^{-1} greater (negative values means uptake of atmosphere CO_2) GEP than natural marshes, but this varied across sites ($p<0.01$). We further conducted a separate analysis for each site (Fig. 4). At three out of four sites, the restored marsh had significantly higher GEP than the natural reference ($p<0.01$), while at SGF there was no difference between restored and natural marsh sites (Fig. 4).

Ecosystem respiration (ER) was also typically greater at the restored sites than the natural marsh sites (Fig. 5, $p<0.01$, Table 2). In QC, BC and SB marshes, the restored site generally had higher ER than natural reference ($p<0.01$ for QC and BC, $p<0.05$ for SB, Fig. 5). At SGF, the natural marsh had higher ER than the restored marsh ($p<0.01$).

Net ecosystem CO_2 exchange (NEE) was generally higher (more negative values means higher capability to uptake CO_2) in the restored marshes (Fig. 6 &Table 2). This pattern was clear in QC ($p<0.05$), BC ($p<0.01$) and SGF sites ($p<0.01$, Fig. 6). At SB sites, the restored marsh had lower NEE during the Aug. and Sep. 2016 samplings mainly due to the cloudy weather or flooded plots by tides. As a result, we did not observe similar pattern as other three sites. However, in spring and early summer samplings of 2016, the NEE in restored site was higher than natural marsh (Fig. S4 & S5).

To examine the species effect during restoration, we conducted gas flux measurements in two dominated plant communities (*S. alterniflora* and *S. patens*) in Stony Brook. There was significantly higher (more negative) GEP in restored marshes for the two *Spartina* species ($p<0.01$, Fig. 7a&b). It was harder to resolve patterns in the ER data, which vaired across species and restoration treatment ($p<0.01$, Fig. 7c, d), mainly due to the Aug. and Sep. samplings, which experienced cloudy weather (lower PAR) and floodings during the measurement in restored marshes. There was significantly higher ER in restored marsh *S. alterniflora* communites, but greater ER in natural marsh *S. patens* community than natural marsh *S. alterniflora* ($p<0.05$, Fig. 7d). The NEE data indicate that restored marshes had higher NEE ($p<0.01$, Fig. 7e), and this pattern varied between species (interaction effect: $p<0.01$). As we mentioned above, this was mainly due to cloudy weather and flooding conditions in the restored marsh measurements. The reduced data set, derived by filtering out data collected during cloudy weather, indicated that restored marshes had significantly higher NEP than natural marsh at both *S. patens* and *S. alterniflora* communities (Fig. S5). Moreover, *S. alterniflora* had higher NEP than *S. patens* communities in either natural or restored marshes (Fig S5. $P<0.01$).

Factor analysis

To identify the dominant environmental variables that impact variance in gas flux, two orthogonal latent factors were extracted for the July 2016 data (Fig. 8). The high loadings of GEP, ER, AR, BR, NEE and biomass on Dim 2 suggest strong linkages between these parameters, whereas the relatively small loading of porewater variables (water table, SO_4, Chloride, Salinity, S^{2-}, DOC) and site elevation on this factor indicate relatively small to moderate relationships to CO_2 fluxes components and biomass. Moreover, the similar orientations among biomass and BR indicated aboveground biomass had a closer relation with BR rather than ER and AR. Soil water-filled pore space (WFPS), temperature and pH had relative short length in either Dim 1 or Dim 2, indicted their weak relations with CO_2

Comparing across sites, BC site data were generally different than other sites along Dim 1, which is mainly related with porewater variables (Fig. 8). Moreover, the relative difference between the restored marsh and natural marsh at BC site was mainly loaded on Dim 2, indicating significant biomass and CO_2 flux differences. Whereas at SGF site, the restored and natural marsh difference was mainly loaded onto Dim 1, reflecting differences in porewater chemistry and elevation across the sites. QC restored marsh site had much lower Dim 2 loading, likely resulting from higher biomass and CO_2 fluxes (GEP, ER, AR, BR, NEE) than other sites. SB restored and natural marsh sites grouped near each other, irrespectively of species composition.

Discussion

Salt marsh restoration has been proposed as one potential pathway to mitigate rising atmospheric CO_2 levels (National Academies of Sciences and Medicine, 2018). In this study, we found that biomass and CO_2 gas fluxes in restored marshes, with lower elevation and higher flooding duration, were 50%-100% greater than those in their natural counterpart in three studied sites, where the porewater salinity was comparable between the restored marsh and the natural reference. Previous studies have reported that the duration of flooding controls coastal plant biomass (Cadol et al., 2014; Kirwan and Guntenspergen, 2012; Morris et al., 2002). At intraspecies levels, the productivity of marsh plants had a subsidy/stress response to increasing flood frequency (Morris et al., 2013). Marsh biomass and productivity increase with decreasing elevation and increasing flooding frequency, until frequency exceeds the optimum for plant growth. This can create a positive feedback between increased flooding and plant biomass and CO_2 uptake. This feedback was observed at many of the marsh sites in this study.

Restored marsh sites generally had similar porewater salinity as their natural reference, indicating that tidal flow was substantially restored to the previously restricted marshes, except State Game Farm, which is discussed further below. However, generally lower present-day elevation in the restored sites, relative to their reference sites, suggests that elevation loss and/or diminished accretion rate occurred while the sites were tidally restricted. As a result, flooding duration was greater in the restored marshes. These sites also had higher plant biomass than the natural marsh, indicating that more frequent flooding likely increased plant biomass in both *S. alterniflora* or *S. patens* communities (Fig S1). Thus, while these marshes lost elevation due to previous restriction, upon restoration of tidal flow, they had sufficient elevation capital to respond positively to increased flooding. It is likely that the enhanced CO_2 uptake in restored versus natural reference sites observed here will continue until the restored marshes reach the same elevation and flooding frequency as the natural marsh.

Moreover, species occurrence is largely a function of the flooding regime, as different species are adapted to different degrees of flooding (Morris et al., 2002; Morris et al., 2013). Following tidal restoration, in some cases we observed different salt marsh vegetation species in restored and natural marsh sites. Due to the lower elevation and greater inundation duration in the restored marsh, *S. alterniflora* was generally the dominant species. For example, the restored marsh sites at SGF and QC were dominated by tall form *S. alterniflora*, while their natural references were predominantly *S. patens* and short form *S. alterniflora,* respectively. This difference in dominant species at natural and restored sites could result in a mixed effect of restoration on gas flux and plant biomass. To separate the species effect from restoration treatment, our second experiment at SB site included both *S. patens* and *S. alterniflora* in each of natural and restored treatments*. S. patens* generally had higher biomass than short form *S. alterniflora* in both restored and natural salt marshes. Both species had significantly greater aboveground biomass in the restored marsh than in the natural reference (Fig. 3). Our interpretation is that this was mainly due to the greater inundation frequency at restored marshes.

Other studies have reported the net ecosystem CO_2 exchange (NEE) for New England salt marshes. For example, Moseman-Valtierra et al. (2016) reported that *S. alterniflora* low marsh zones had much higher NEE rate (up to 14 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) than the nearby high marsh zone, with dominant plant species including *S. patens* (less than 2 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$). At the marshes in the present study, NEE was up to 20 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ at *S. alterniflora* sites. We also compared the NEE in both the *S. alterniflora* and *S. patens* dominated communities at Stony Brook (SB) marsh, and found *S. alterniflora* generally had much higher NEP than *S. patens* in both natural and restored marshes, despite similar elevation across restoration treatment. This species-specific effect on CO_2 emissions in salt marshes suggests *S. alterniflora* can assimilate more CO_2 than *S. patens* under similar environmental conditions. Given predicted increases future sea-level rise rates, *S. patens* dominated high marsh will likely retreat and be replaced by *S. alterniflora* dominated low marsh (Gonneea et al., 2019; Kirwan and Mudd, 2012; Raposa et al., 2017). If SLR rates do not surpass thresholds for marsh plant survival, we could expect higher CO_2 uptake during the plant community shift, coincident with increasing vertical increments of accommodation space for soil storage, thus promoting higher rates of soil organic matter accumulation (Gonneea et al., 2019), enhancing salt marsh elevation resilience to sea level rise (Kirwan et al., 2016).

In the present study, the pattern of ecosystem respiration (ER) between restored marsh sites and their natural references was similar to aboveground biomass variability. ER rates at three restored sites (SB, QC and BC) were higher in the restored marsh than the natural marsh. The opposite pattern was observed at SGF, likely due to the high freshwater input resulting in low coverage of *S. alterniflora* at this site. Thus, plant biomass likely exerts a strong control on ER during the growing season. Ecosystem respiration in this study was up to 25 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, much higher than the soil respiration rate alone (including root respiration and soil microbial respiration) measured at the same marshes (up to 15 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, Fig 7). Previous studies of New England salt marshes report soil microbial respiration ranging from 1.7 to 7.8 µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ in peak summer season (Wigand et al., 2009). At Great Sippewisset Marsh, Falmouth, Massachusetts, near the sites in this study, Teal and Howes (1996) reported peak (August) soil microbial respiration in *S. alterniflora* over a seven year period ranged 3.1– 3.7 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$. When we parse respiration into plant aboveground respiration (AR) and soil respiration (BR), we find that AR contributes 26%-50% of total ecosystem respiration in these salt marshes. Since salt marsh root biomass is likely higher than shoot biomass (Moseman-Valtierra et al., 2016; Valiela et al., 1976), belowground roots might contribute more to CO_2 emissions than plant shoots (Moseman-Valtierra et al., 2011; Spivak and Reeve, 2015).

Long-term salt marsh stability requires soil surface vertical elevation to increase at a rate similar to the local rate of relative sea-level rise (SLR). This process relies on net positive sedimentation of mineral sediment and increasing volume of plant-derived soil organic matter (Fagherazzi et al., 2013). Marsh grass abundance can influence the rate of sediment deposition by slowing water velocity over the marsh platform, thereby promoting deposition (Morris et al., 2002). Changes in marsh plant abundance and biomass can therefore affect both organic matter supply and mineral sediment deposition, and ultimately vertical accretion (Fagherazzi et al., 2013). In the present study, restored marshes (SB, QC and BC sites) generally had greater NEP than natural saltmarsh, indicating a greater rate of supply of plant organic matter. Moreover, the higher biomass can enhance trapping of suspended sediment particles by slowing water velocity, which would further accelerate the sedimentation in restored marsh. In addition, due to the lower elevation in the restored sites, greater flooding frequency, and depth occurred, the ecogeomorphic feedbacks mentioned above tend to increase rates of organic and mineral sediment accumulation as marshes become progressively more flooded. Anisfeld et al. (1999 has observed significantly greater accretion rate (~10 mm yr^{-1}) in restored marsh than in natural marsh (3.6 mm yr^{-1}).

Our comparison between restored and natural marshes indicates that recently restored (5 to 10 years) marshes, with similar porewater chemistry and salinity as natural marshes, had greater biomass and NEP than reference sites. Thus, these systems may be poised to have greater C storage and vertical accretion rates after restoration than either prior to restoration or compared to marshes that were never tidally restricted. This capacity to store carbon and build elevation is critical for coastal wetlands restoration as these previously restricted and/or diked marshes experienced substantial loss of elevation and carbon stocks while tidally restricted. The results presented here, in context of well-documented ecogeomorphic feedbacks, suggest that enhanced carbon storage rates are likely to continue into the future, until elevation has been regained to a level similar to that in reference marshes. Therefore, the carbon stock deficit that has occurred as a result of decades of tidal restriction may be recouped, given sufficient time, through enhanced net uptake and storage of atmospheric carbon dioxide. However, we should be aware that some restricted tidal wetlands may subside too much to recover vegetation and associated carbon storage without the restoration of elevation in addition to tidal flow. In these cases, engineering practice, like the control of tidal flow or sediment addition may be needed to successfully restore tidal wetlands.

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Tables:

Table 1: Dominant species, elevation （NAVD88）, porewater chemistry and flooding duration for each salt marsh site.

Sites	Treatment	Dominant Species	Elevation (m)	Salinity (‰)	pH	Flooding Duration (%)
Quivett Creek	Natural	*S. alterniflora*	1.39±0.01	21.0±0.9	6.97±0.13	13.2±0.3
	Restored	*S. alterniflora*	1.31±0.01	19.0±0.4	6.72±0.20	17.8±0.6
State Game Farm	Natural	*S. patens*	1.35±0.01	21.2±1	7.04±0.31	7.2±0.3
	Restored	*S. alterniflora*	1.25±0.00	10.3±0.5	6.72±0.24	12.0±0.2
Bass Creek	Natural	*D. spicata*	1.60±0.01	32.8±2.8	6.85±0.10	4.2±0.3
	Restored	*S. alterniflora & S. patens*	1.58±0.00	33.5±2.4	6.51±0.11	4.9±0.1
Stony Brook	Natural	*S. alterniflora*	1.46±0.01	18.8±0.5	6.38±0.11	6.2±0.2
	Natural	*S. patens*	1.46±0.01			5.94±0.1
	Restored	*S. alterniflora*	1.33±0.01	19.4±0.4	6.16±0.15	14.7±0.1
	Restored	*S. patens*	1.31±0.01			16.3±0.1

Note: SGF: State Game Farm, QC: Quivett Creek, BC: Bass Creek, SB: Stony Brook.

Table 2. The p-values of LME model results for gross ecosystem production (GEP), net ecosystem CO_2 exchange (NEE) and ecosystem respiration (ER).

Variables	Restoration Type	Site	Site* Restoration interaction
GEP	<0.01	<0.01	<0.01
NEE	<0.01	<0.01	<0.01
ER	<0.01	<0.01	<0.01

Figures:

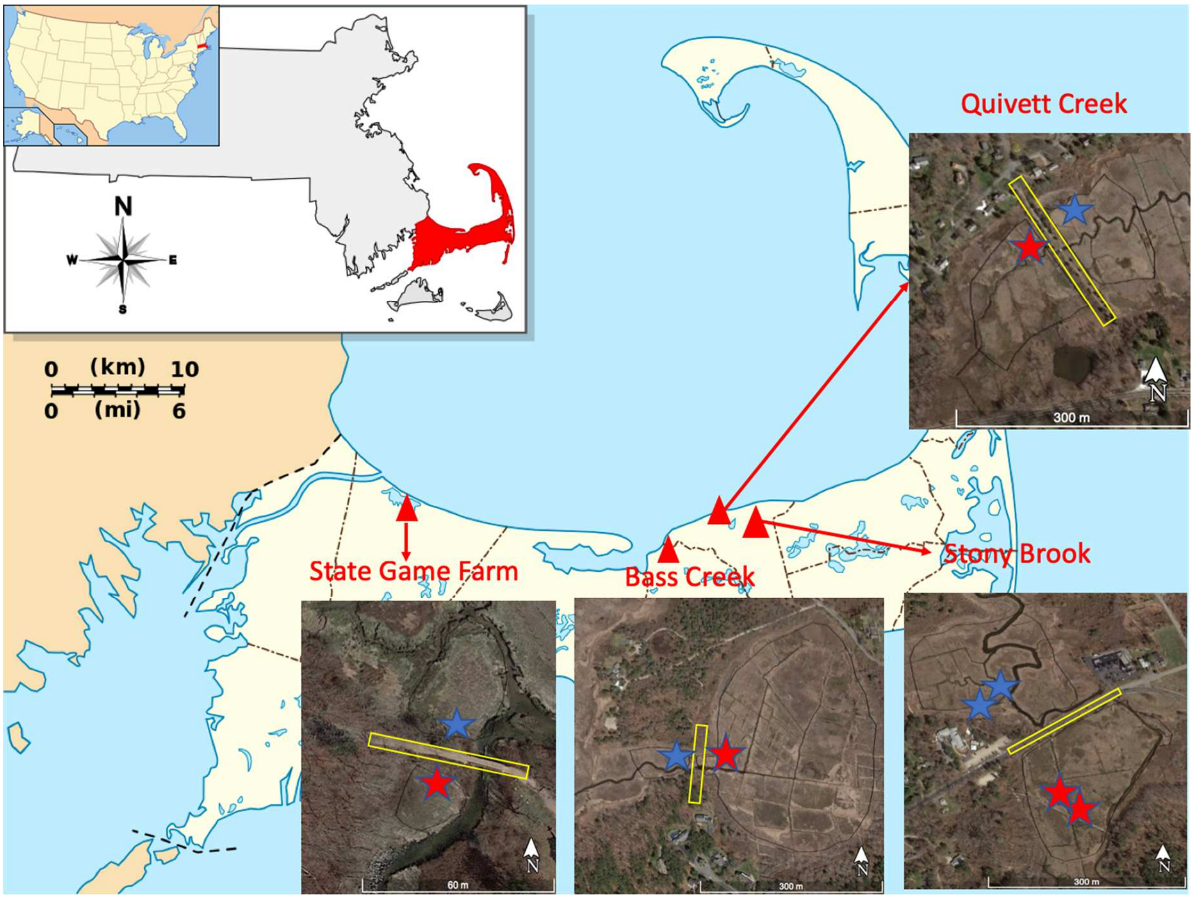

Fig 1. The location of research sites in Cape Cod, MA, USA. Red stars indicate restored marsh GHG and biomass sampling sites; Blue stars indicate natural reference marsh GHG and biomass sampling sites; Yellow rectangles indicated the locations at each site where tidal restriction was removed.

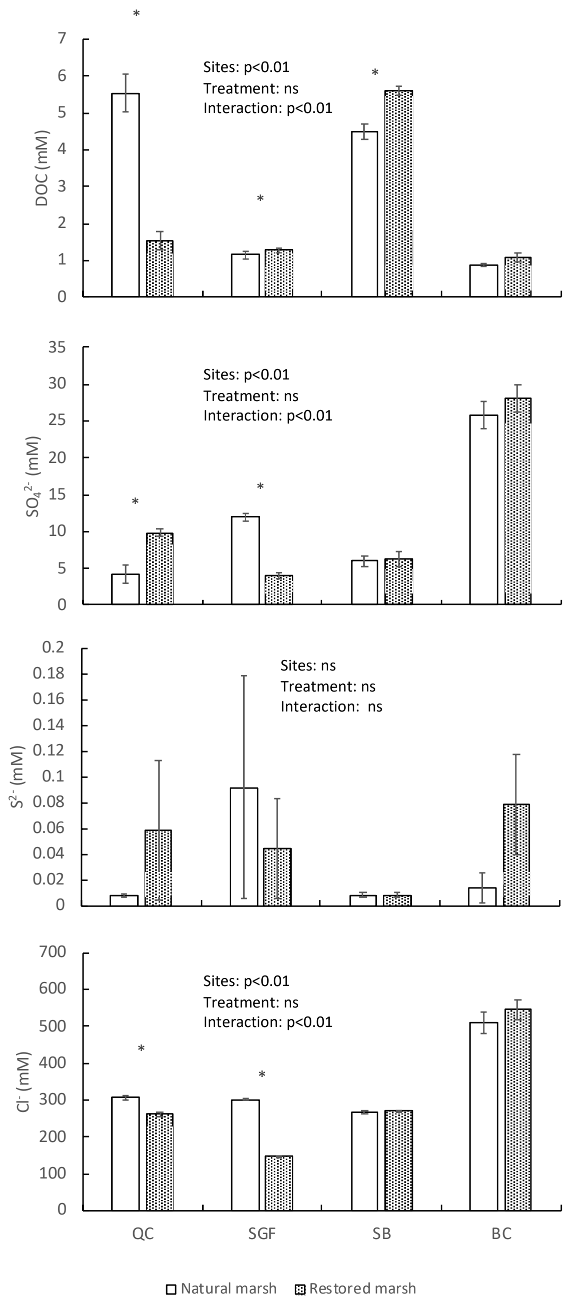

Fig. 2 Porewater chemistries in Cape Cod restored and natural salt marshes. DOC: Dissolved organic carbon, SGF: State Game Farm, QC: Quivett Creek, BC: Bass Creek, SB: Stony Brook. Treatment effect is the difference between restored marsh and natural marsh. Error bars indicate one SE.

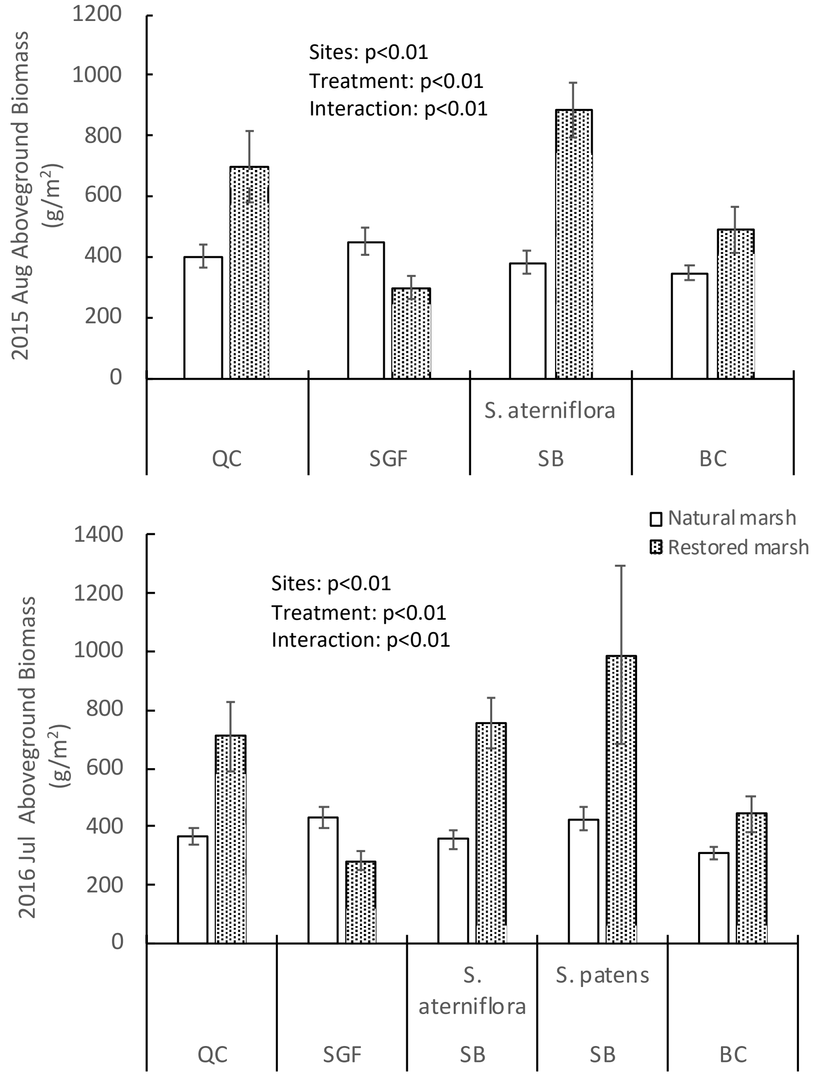

Fig. 3. Aboveground dry biomass in 2015 and 2016 at restored and natural salt marsh sites in Cape Cod, MA, USA. SGF: State Game Farm, QC: Quivett Creek, BC: Bass Creek, SB: Stony Brook. Error bars indicate one SE.

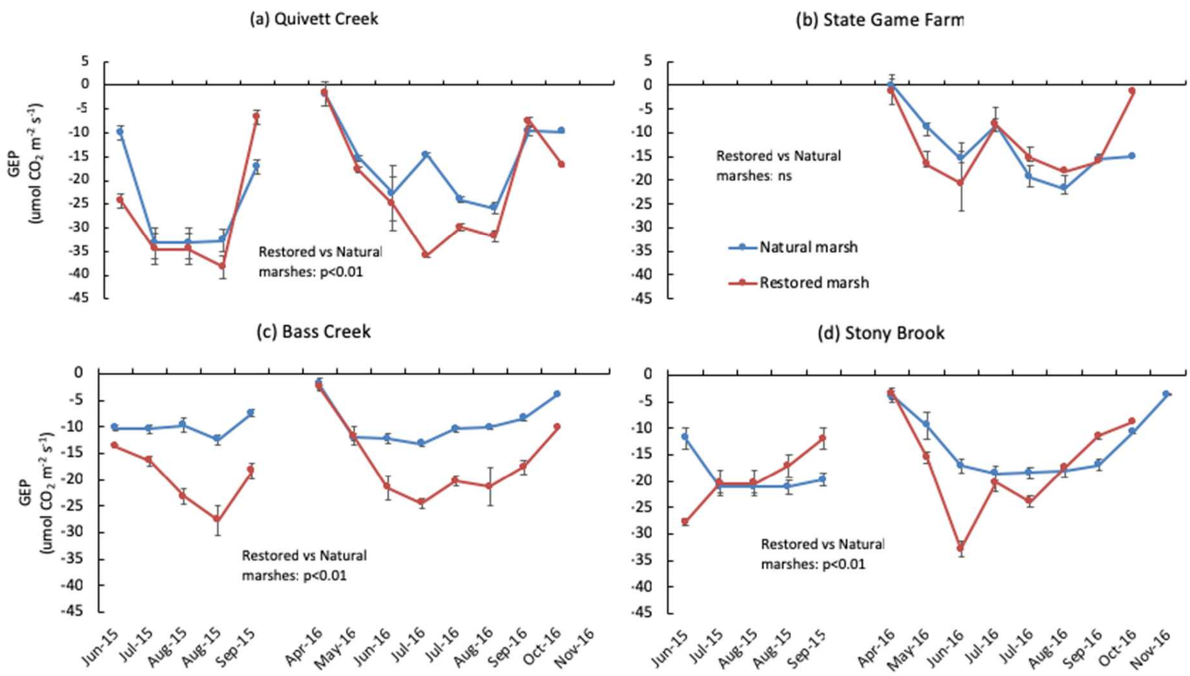

Fig 4. Gross ecosystem production (GEP) at restored (red) and natural (blue) marshes in four sites at Cape Cod, MA. The negative GEP values means uptake of atmosphere CO_2. Error bars indicate one SE.

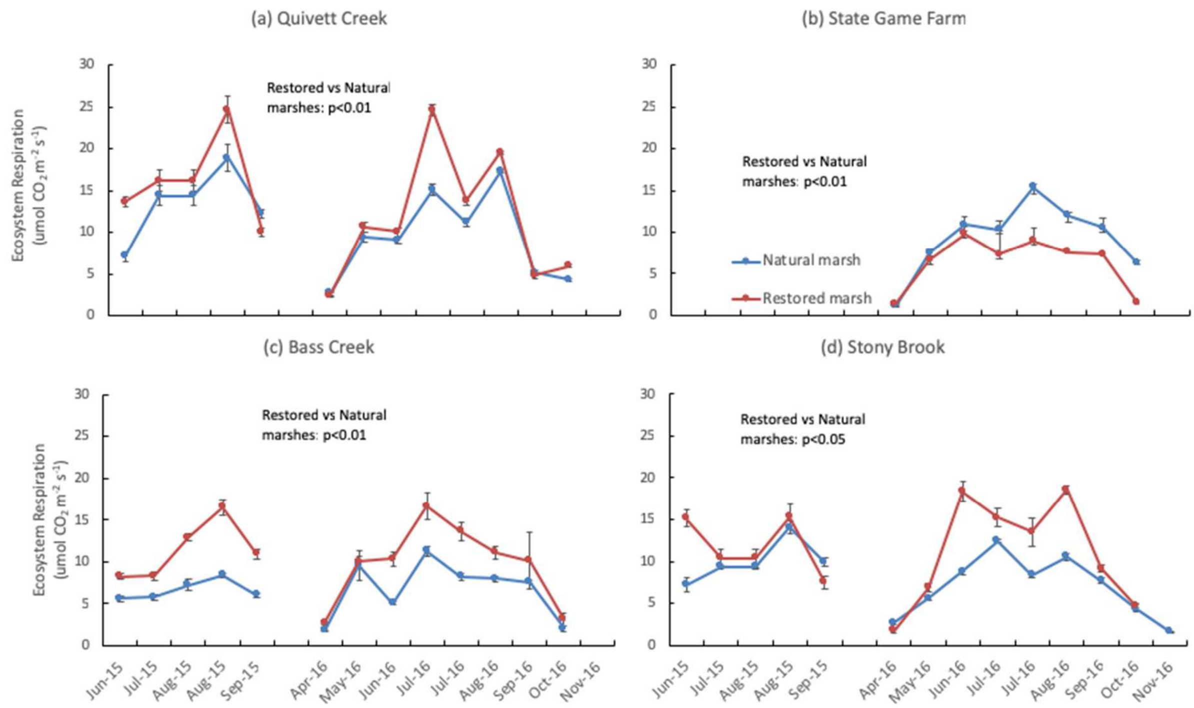

Fig 5. Ecosystem respiration (ER) in restored (red) and natural (blue) marshes at four sites on Cape Cod, MA, USA. Three marshes have greater ER in the restored site, while the opposite is observed at State Game Farm. Error bars indicate one SE.

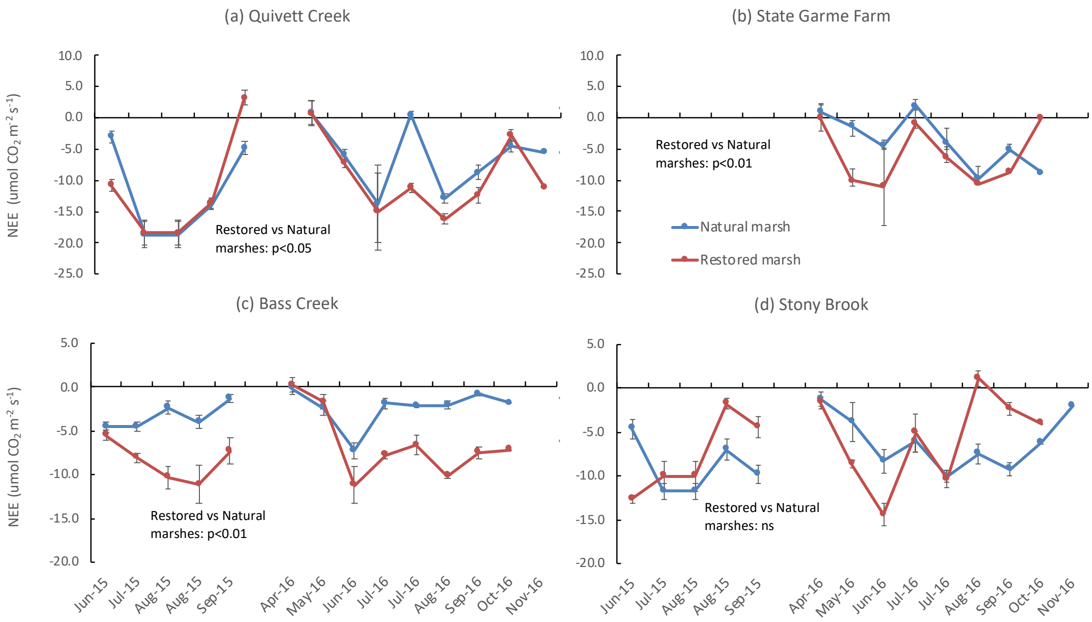

Fig. 6. Net ecosystem CO_2 exchange (NEE) in restored and natural marshes in four sites at Cape Cod, MA, USA. The statistical results between restored and natural marshes in each site were shown in each panel. The negative GEP values means uptake of atmosphere CO_2. Error bars indicate one SE.

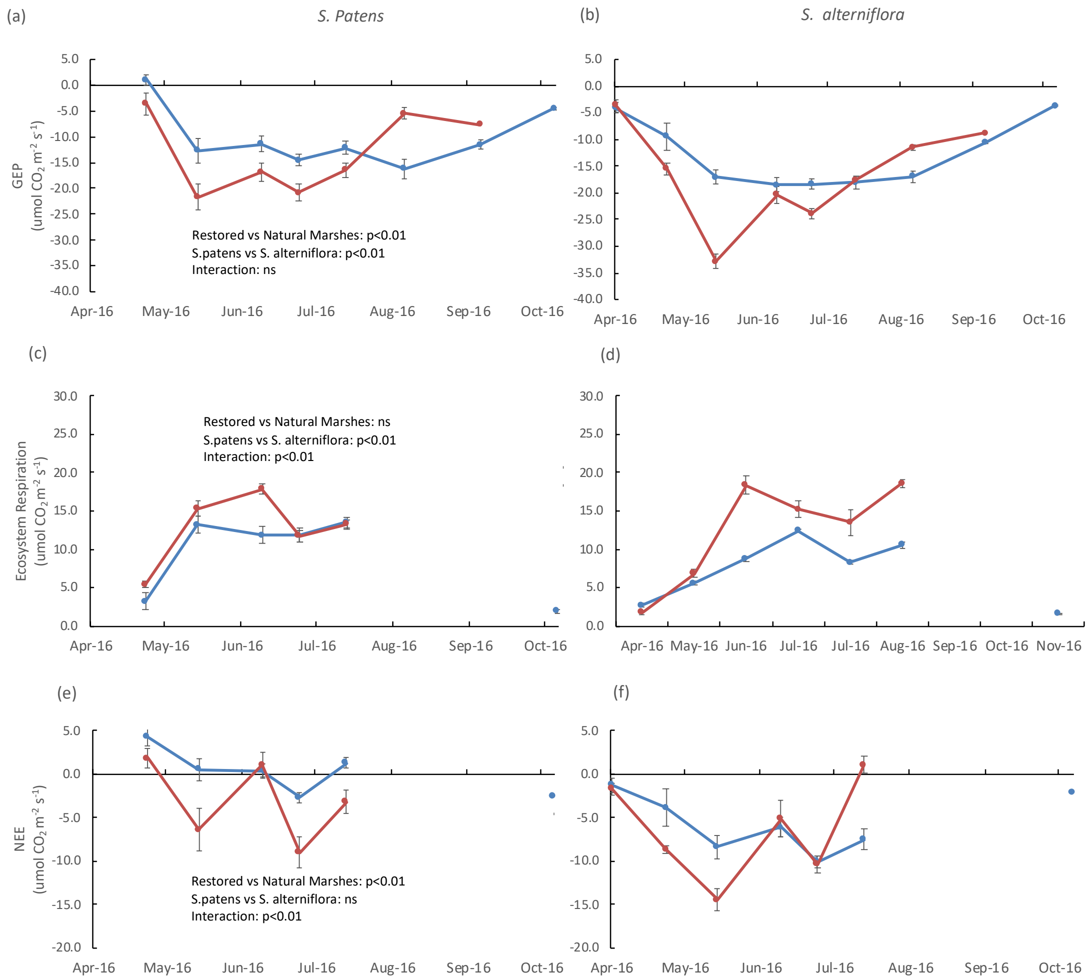

Fig. 7 GEP, ER and NEE of *S. patens* and *S. alterniflora* communities at natural and restored salt marshes in Stony Brook site in 2016. Error bars indicate one SE.

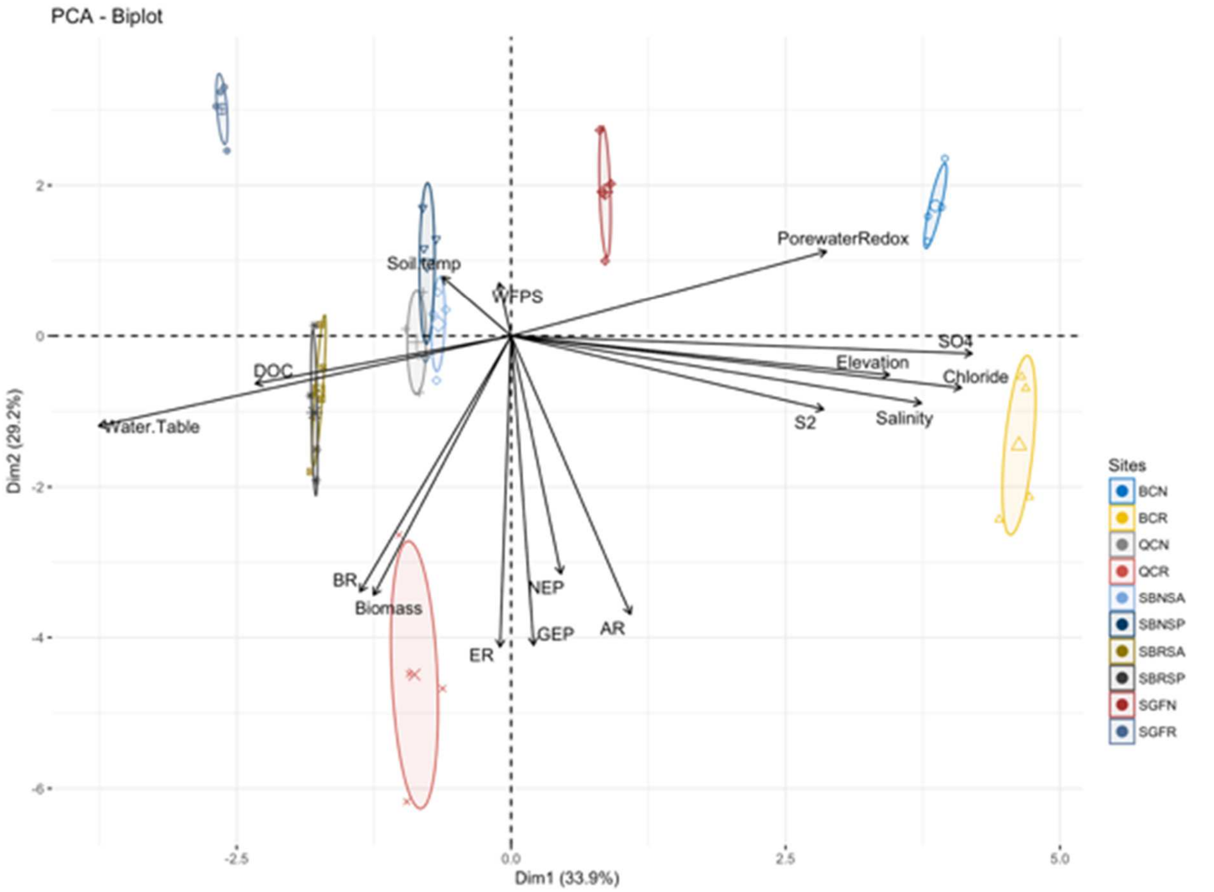

Fig. 8 Biplot showing relative orientations and interrelations of environmental properties and different CO_2 fluxes components in July 2016 sampling for salt marsh data. Percentage explained by each dimension (Dim) is given in parentheses. Dim. 1 and 2 retained about 63.1% (33.9% + 29.2%) of the total information contained in salt marsh data. Positively correlated variables point to the same side of the plot, while negatively correlated variables point to opposite sides of the graph. Individual sites were shown as different points. Each colored ellipse represents one Site, and the size of the ellipse indicated its 95% confidence. GEP=gross ecosystem production, ER=ecosystem respiration, NEP=net ecosystem CO_2 exchange, AR = aboveground plant respiration, BR=soil respiration (including root respiration and microbial respiration), Biomass = aboveground biomass, WFPS=soil water-filled pore space, S2= sulfide concentration in porewater, SO4=sulfate concentration in porewater. SBNSA=Stony brook natural marsh *S. alterniflora* zone, SBNSP=Stony brook natural marsh *S. patens* zone, SBRSA=Stony brook restored marsh *S. alterniflora* zone, SBRSP=Stony brook restored marsh *S. patens* zone, BCN= Bass creek natural marsh, BCR=Bass creek restored marsh, SGFN=State game farm natural marsh, SGFR=State game farm restored marsh, QCN= Quivett creek natural marsh, QCR=Quivett creek restored marsh.

Graphical abstract

The photos showing the tidal channel before (A) and after restoration (B) at Bass creek, Barnstable, MA, USA. Our study indicated that successful restoration of salt marshes leads to greater rates of C sequestration for a decade, at minimum. Moreover, the negative relationship between elevation and plant productivity suggested that sea level rise may lead to enhanced sedimentation, extending marsh survival under the increased sea level, and recouping carbon stocks that were lost during tidal restriction periods.