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Article

Invasive *Phragmites australis* may Protect Long Island Sound Salt Marshes from Sea Level Rise Driven Coastal Erosion

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Simple Summary

This study examined how soil total organic carbon (TOC) stocks vary across space and time beneath invasive *Phragmites australis* and native *Spartina alterniflora* in southern Connecticut tidal marshes. Our findings challenge the common assumption that *P. australis* invasion naturally increases carbon sequestration. While soils colonized by *P. australis* consistently showed significantly higher bulk density and dry weight, this did not result in a consistent carbon advantage. Significant heterogeneity in *P. australis* carbon stocks indicate that its sequestration is less uniform than that of native species. We conclude that while *P. australis* provides a denser soil structure that may assist with physical resilience to sea-level rise, its complex impact on carbon dynamics may reduce the reliability of these marshes as consistent blue carbon sinks.

Abstract

Coastal salt marshes are essential for climate change mitigation due to their high carbon storage capacity, which is influenced by soil type, hydrology, and floristic composition. Over the past century, invasive *Phragmites australis* has displaced native *Spartina alterniflora* across salt marshes on the Long Island Sound, and it is widely hypothesized that its larger biomass and rapid growth enhance soil carbon sequestration. This study tested that hypothesis by comparing TOC stocks and physical soil properties in two southern Connecticut marshes over multiple seasons. Our results show that mean soil bulk density was significantly higher under *P. australis* than *S. alterniflora* at both locations. However, this did not translate to superior carbon storage. Analysis showed significant seasonal effect but no significant overall difference in median TOC between species, indicating that *P. australis* is competitive in total mass only due to its higher soil density. Notably, Levene's test for homogeneity of variance was significant ($P = 0.039$), revealing that *P. australis* creates highly heterogeneous "hot spots" of carbon storage compared to the relatively uniform distribution found in native stands. These findings suggest that while *P. australis* invasion results in a more physically dense and potentially resilient marsh platform—relevant for surviving sea-level rise and filtering nutrient runoff—it may simultaneously compromise the stability and uniformity of regional carbon sinks. Management strategies should consider these tradeoffs when prioritizing the protection of native *S. alterniflora* for consistent carbon sequestration.

Keywords: Blue carbon, bulk density, coastal resilience, Connecticut, *Phragmites australis*, salt marsh, invasive species, sea-level rise, soil heterogeneity, *Spartina alterniflora*, total organic carbon (TOC)

1. Introduction

Coastal salt marshes are among the most productive and valuable ecosystems globally, providing essential services such as carbon sequestration, critical habitat for commercially important fisheries, and physical buffers against storm surges (Duarte et al. 2013). Despite their ecological significance, salt marshes of the northeastern United States are facing escalating threats that challenge

both their functional integrity and physical persistence. Foremost among these are accelerating sea-level rise (SLR), which threatens to drown marshes that cannot accrete vertically (Kirwan et al. 2010); shoreline encroachment (Doody 2008); and excess nutrient loading, which can compromise the root structure necessary to hold marsh soil together (Deegan et al. 2012).

Compounding these global change stressors is the widespread proliferation of the invasive common reed, *Phragmites australis* ssp. *australis* (hereafter *P. australis*). While the native subspecies, *P. australis* ssp. *americanus*, has existed in North American wetlands for millennia (Saltonstall et al. 2004), the non-native invasive lineage—introduced from Europe by the late-1800s (Smithsonian Environmental Research Center-NEMESIS 2024)—is recognized as one of the most problematic invasive wetland plants in eastern North America (Meyerson et al. 2016). While native and invasive *P. australis* can coexist, they exhibit distinct physiological differences; invasive *Phragmites* produce larger inflorescences, leaves, and height (Kettenring et al. 2011, Mozdzer et al. 2013). Crucially, this invasion represents more than a biological displacement of native flora; it drives a fundamental restructuring of marsh geomorphology and soil architecture.

Invasive *P. australis* forms dense, monotypic stands with high aboveground biomass that significantly alter local hydrology by trapping mineral sediment and rapidly elevating the marsh surface (Rooth and Stevenson 2000). While *P. australis* is typically associated with higher elevations to avoid high salinity, it can spread clonally into the low marsh (Amsberry et al. 2000, Bernal et al. 2017), bringing its robust root architecture into zones previously dominated by *Spartina alterniflora*. This structural modification is critical in the context of erosion; while nutrient enrichment has been shown to weaken the peat fabric of *S. alterniflora* marshes leading to creek bank collapse (Deegan et al. 2012), *P. australis* may offer a different geomorphic trajectory. Understanding how the physical density and stability of the soil matrix change during this invasion is paramount to effective marsh management, particularly as physical resilience to wave energy becomes as valuable as biological diversity.

Despite its reputation as a biological threat, invasive *P. australis* has been widely reported to provide superior ecosystem benefits relative to indigenous counterparts like *S. alterniflora* and *S. patens*, specifically regarding sediment stabilization and accretion (Kiviat 2013, Lathrop et al. 2003). A study conducted at a St. Lawrence Estuary marsh found that invasive *P. australis* contributed more to soil volume and carbon stock than native *Spartina* spp. (Gu et al. 2020). Similarly, Windham (2001) found that *P. australis* produced far greater above- and below-ground biomass than *S. patens*. Coupled with slower decomposition rates, it was hypothesized that this robust biomass production would lead to increased carbon sequestration and a physically denser peat layer. However, recent research suggests that anthropogenically driven changes, such as nutrient enrichment, may complicate this picture, potentially resulting in soil organic carbon (SOC) loss (González-Alcaraz et al. 2012, Mozdzer et al. 2023, Zhang et al. 2022).

Therefore, evaluating the trade-offs of this invasion requires looking beyond simple carbon quantities to the stability of the soil itself. While carbon sequestration rates are often reported as offsetting greenhouse gas emissions, carbon stocks provide a clearer indication of the ecosystem's potential to mitigate climate change (Don et al. 2023). Furthermore, the physical properties of these stocks—specifically bulk density—determine the marsh's ability to resist erosion. In this study, we compared total organic carbon (TOC) stocks and physical soil properties in locations dominated by *P. australis* and *S. alterniflora* in salt marshes in southern Connecticut, aiming to determine if the shift in vegetation alters the soil architecture required to withstand rising seas.

2. Materials and Methods

2.1. Study Area

Soil samples were collected at two tidal salt marshes (Figure 1) located in southern Connecticut namely; Branford Trolley Trail (41°16'9.28"N, 72°45'18.60"W) and Quinnipiac Meadows (Eugene B. Fargeorge) Preserve located in the lower section of the Quinnipiac River (41° 19 '00"N 72° 52' 50"W).

Both marshes exchange tides with the Long Island Sound and share a similar temperate climate with an average annual temperature of 11.3 °C, and average annual rainfall of 1273 mm. Temperatures peak in July, with highs of 23.2 °C, while the lowest average temperatures occur in January, at around -0.5 °C. The lowest amount of rainfall occurs in July with an average of 81 mm, while the greatest amount of precipitation occurs in December, with an average of 130 mm (Climate Data 2023). The region experiences four distinct seasons with cold winters (-7 to 4° C) with occasional snow and ice, though tidal flow prevents full soil freezing. Spring (4 to 18 °C) brings increased rainfall and promotes plant growth. Summers (18 to 29 °C) are warm and humid, with peak marsh productivity and frequent thunderstorms. Fall (10 to 21° C) introduces potential for early frost and coastal storm surges, affecting both vegetation and soil chemistry (Nomad Season, 2024; WeatherSpark 2024). At the time of this study, Branford had an average tidal range of about 1.5 m (Branford Land Trust 2023), whereas Quinnipiac Meadows experiences a much greater tidal range of about 2.8 m (NOAA 2025). Marsh vegetation at Branford is dominated by stands of *P. australis*, *S. alterniflora* and *S. patens* interspersed with smaller patches of *Distichlis spicata*. *P. australis* stands occupy higher elevations of the marsh and distinctively along recently disturbed areas such as gravel pathways, roads and railroad tracks. The gravel path along the northern perimeter of the study site is frequented by human traffic and often littered with animal feces. The elevation of the gravel path is approximately 0.7 m above sea level descending to sea level at the lowest southern boundary. The Quinnipiac Meadows site vegetation is comprised of *S. alterniflora* and *P. australis* backed by coastal forest (Ewbank, 2017). Branford marsh soils were dominated by Westbrook mucky peat and Quiambog silt loam, intertidal while Quinnipiac Meadows soils were mainly Westbrook mucky peat in the high and mid-marsh areas and Pishagqua silt loam in the low marsh (USDA-NRCS 2025). Invasive *P. australis* was identified in the field by examining the height of the plant and size of inflorescence which is longer and denser than native species.

2.2. Sample Collection

We employed a stratified random sampling approach to collect soil cores at both sites. Soil cores were stratified and equally distributed by plant type in zones dominated by *P. australis* and *S. alterniflora* respectively. Vegetation at each location was characterized by height (cm) and dry above ground biomass (g). In some instances, *P. australis* or *S. alterniflora* soils were collected under plants growing in transition zones where both species were present, but we were careful to core immediately adjacent or under plants of one species. At Branford, samples also captured a range of elevations with equal distribution across each of three zones (Figure 1). Determination of marsh zone boundaries was based on a perpendicular transect originating from the highest elevation (areas that remain dry even at peak high tide) to the water's edge. Vegetation composition was a secondary determinant of marsh zones based on plant inundation and salinity tolerances (Chapman 1976). High marsh zones lay adjacent to a walking path (approximately 0.7 m above sea level), to the lowest areas as close as possible to the water's edge (0 masl) to assess the influence of inundation depth, flood frequency and human disturbance on carbon stocks. Sample locations at the lowest elevations by the water's edge experienced deeper flooding, more frequent flooding and less disturbance than the sites closer to the walking path at the northern border. A 5.5 cm diameter stainless steel regular soil auger was used to collect soil samples with minimum compaction to a depth of 30 cm. Surface dead vegetative matter such as leaves, rhizomes and roots were removed from the sample in the field. Soil cores were stored in polyethylene Ziploc® bags and transported in a cooler at about 4 °C to the University of New Haven where they were immediately stored at -20 °C until processing.

In 2024, samples were collected from Quinnipiac Meadows on three occasions over three seasons (Spring, Summer and Fall) in 2024, with 20 samples collected at each visit with equal number of samples collected under each species. Soil samples were taken from the rhizosphere to depths between 20-30cm using a 2.5 cm diameter stainless-steel soil corer (Hunnings et al. 2019).

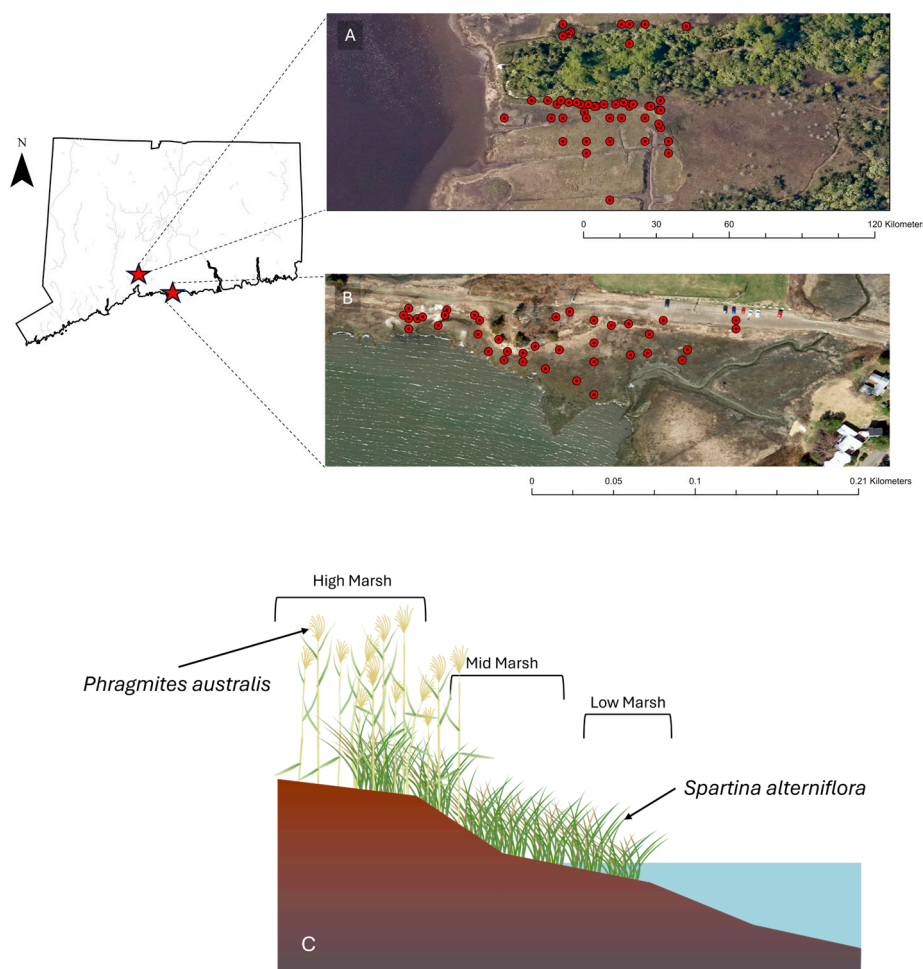


Figure 1. Soil sampling locations at (A) Quinnipiac Meadows and (B) Branford marsh. Distribution of dominant vegetation in a typical tidal salt marsh profile in southern Connecticut (C).

2.3. Sample Processing and Analysis

In both Branford and Quinnipiac Meadows, dead vegetative matter including leaves and rhizomes were removed from the surface of the soils prior to coring. Samples were transported in a cooler containing dry ice to maintain a temperature of approximately 4 °C in the field upon return to the University of New Haven where they were immediately stored at -20 °C until processing. During processing, entire soil samples from each site were thawed, homogenized and weighed in grams to obtain wet weight. Samples were then dried at 60 °C for 24 h and re-weighed to obtain dry weight. For all cores, dry bulk density was calculated by dividing the dry weight by the initial volume. Dried samples were then ground using an electric spice grinder. Soil samples were then milled to fine powder in a mortar and pestle, sieved through a 2mm mesh, and packed into tin capsules (Carter and Gregorich 2008). All samples were acidified with 2% HCl and analyzed for total organic carbon. Branford samples were analyzed using an Eltra® CS580 elemental analyzer at the Yale Analytical and Stable Isotope Center (YASIC) while Quinnipiac Meadows samples were analyzed at the University of California, Merced Stable Isotope Ecosystems Laboratory using a Costech 4010 Elemental Analyzer coupled with a Delta V Plus Continuous Flow Isotope Ratio Mass Spectrometer. These yielded Carbon percent in each sample which was then used to estimate TOC. Data analysis was conducted in Microsoft Excel® and program R (R Core Team 2023, Version 4.1.2).

Total organic carbon (kg ha^{-1}) stock of each sample was estimated using the equation proposed by Carter & Gregorich (2008) (Equation 1).

$$\text{TOC}_{\text{stock}} = \%C \times \rho_b \times D \times 100 \quad (1)$$

where:

%C = Percent carbon in sample

ρ_b = Soil dry bulk density (g cm^{-3})

D = Soil depth (cm)

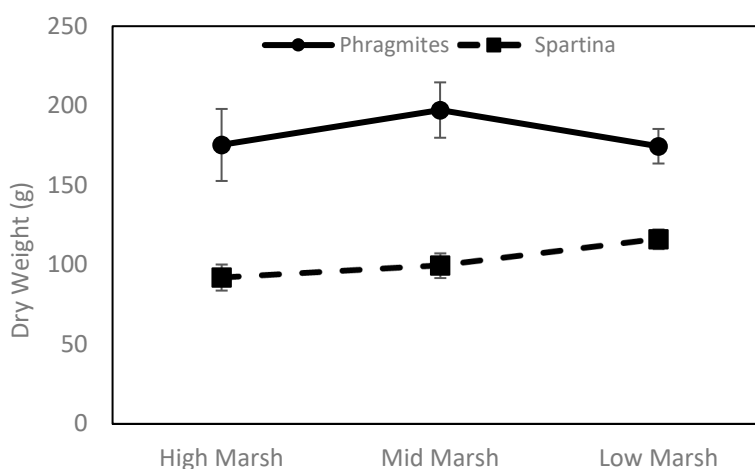
Above ground vegetation was dried and weighed to stable mass. A sub-sample of remaining dry soil samples underwent additional sorting to extract all traces of below-ground live and dead rhizomes and fine roots to determine the vegetation fraction within the soils. Below ground vegetative material was rinsed, patted dry and dried at 60 °C for 24 h. The proportion of vegetation was then estimated as a fraction of the dry sub-sample weight. All response variables were tested for normality using a Shapiro-Wilk test.

3. Results

A total of 48 soil samples were collected over a 5-month period between October 2022 and February 2023 at Branford and 60 samples from Quinnipiac Meadows from April to November 2024. Below ground vegetative matter including dead and live roots, rhizomes constituted 10-11% of soil samples under both species. A Shapiro-Wilk test run on soil data from both sites revealed that bulk density data was significantly non-normal (Branford $W = 0.95$, $p < 0.01$; Quinnipiac Meadows $W = 0.86$, $p < 0.001$), as was dry weight (Branford $W = 0.97$, $p < 0.01$; Quinnipiac Meadows $W = 0.88$, $p < 0.001$). Consequently, a non-parametric Wilcoxon rank sum test was used to compare between species. Results indicated a significant difference in bulk density and dry weight between species at both locations, with *P. australis* generally exhibiting higher variability and higher mean and median bulk density values compared to *S. alterniflora* (Tables 1 and 2). Soil moisture content was greater under *S. alterniflora* at Branford but greater in *P. australis* at Quinnipiac Meadows (Figures 2 and 3).

Total organic carbon (TOC; kg C ha^{-1}) varied spatially and temporally but was greater in *S. alterniflora* than *P. australis* at Branford and vice versa for Quinnipiac Meadows (Figures 4-6). A Scheirer-Ray Hare test revealed no interaction between species and zone at Branford ($P = 0.89$) and a Welch's ANOVA detected a significant effect of season ($F = 12.52$, $P < 0.001$) but no effect by species at Quinnipiac Meadows. The post-hoc Dunn's Test found significantly greater TOC in *S. alterniflora* across all zones ($P = 0.04$).

Above-ground vegetation measured in 2023 and 2024 found that *P. australis* grew taller ($\bar{x} = 126.2 \pm 15.1$ cm) than *S. alterniflora* ($\bar{x} = 64.5 \pm 8.6$ cm). Above ground biomass was greater in Phragmites in 2023 but lower in 2024 (Figure 7)



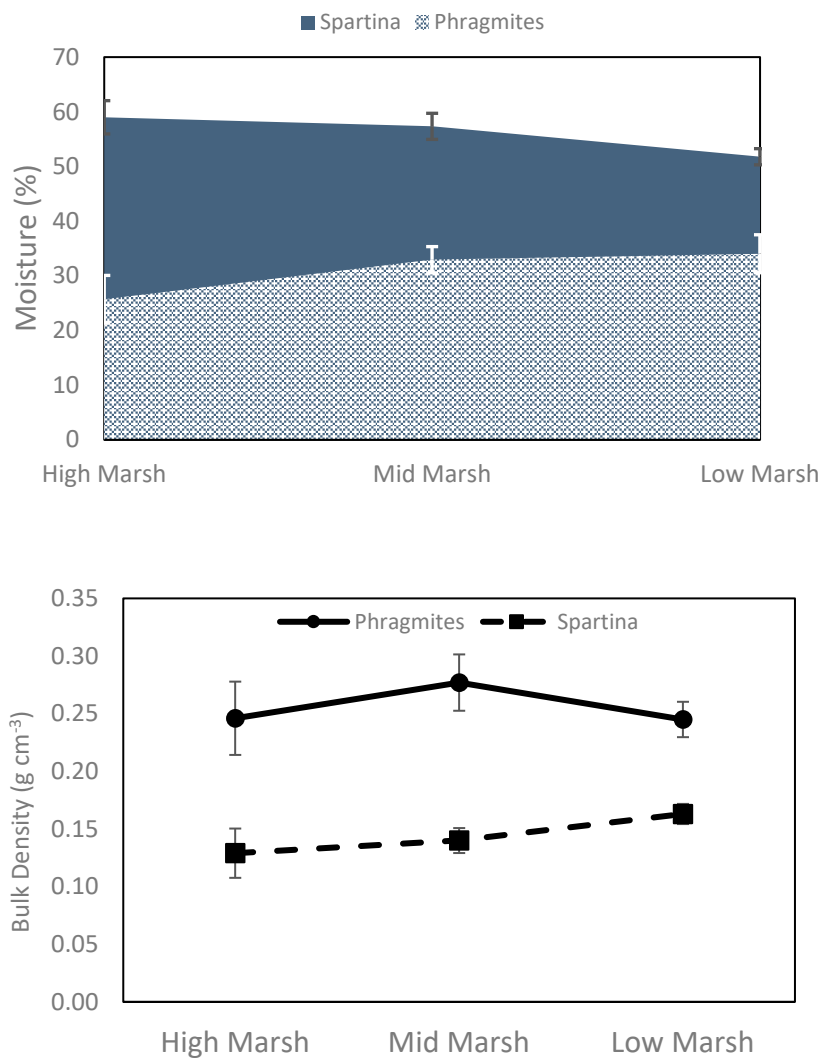
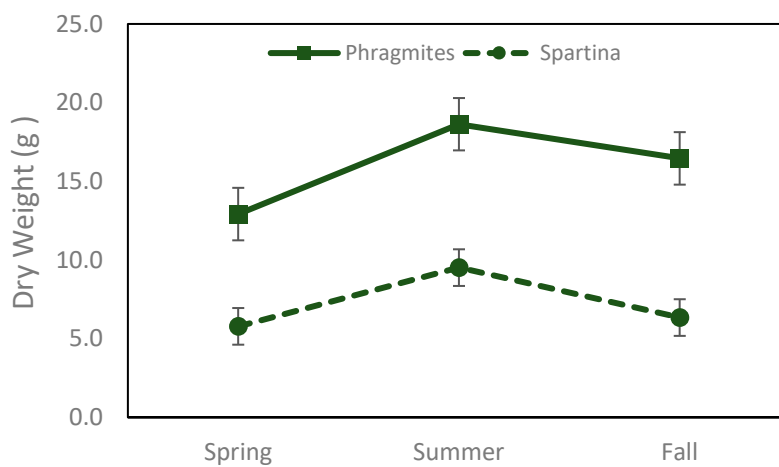


Figure 2. Dry weight, percent moisture and bulk density of soils collected over the fall and winter of 2022-2023 under two dominant species in a tidal salt marsh located in Branford, CT.



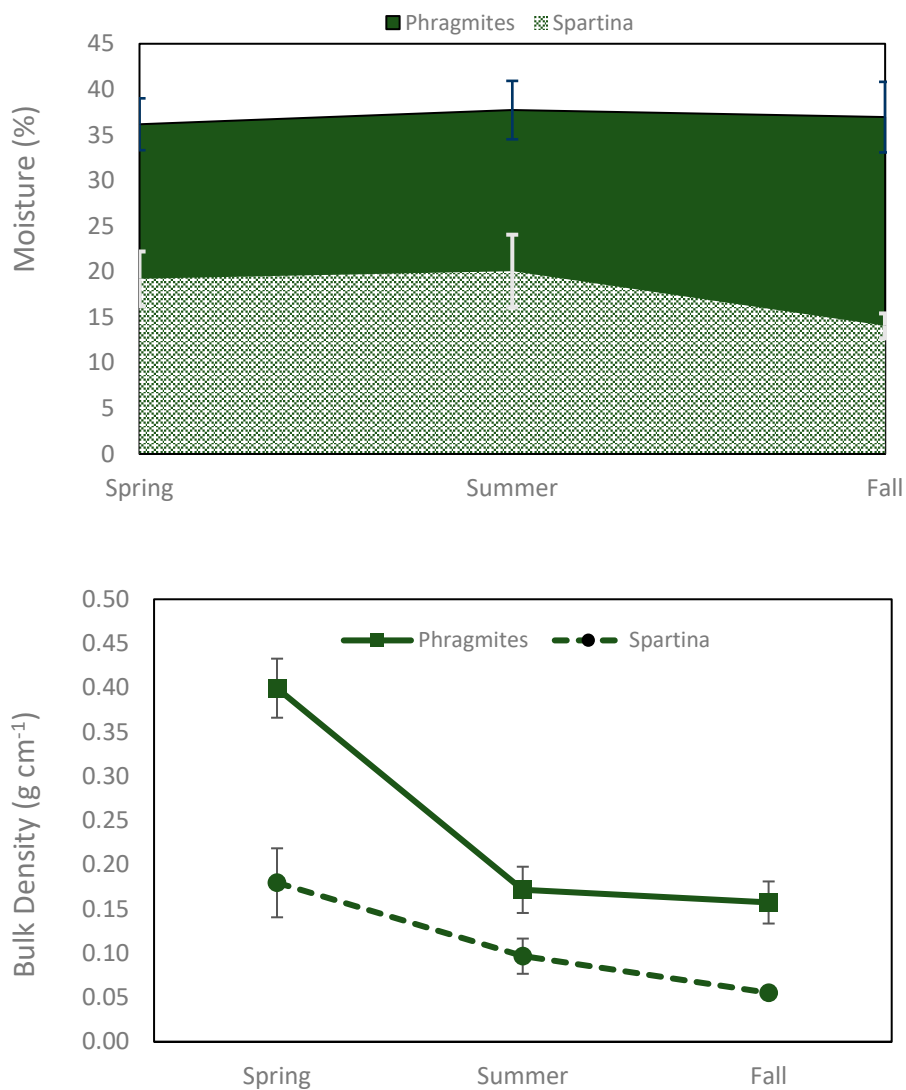


Figure 3. Seasonal variation in dry weight, percent moisture and bulk density of soils collected over the spring, summer and fall of 2024 under two dominant species in a tidal salt marsh located in Quinnipiac Meadows, CT.

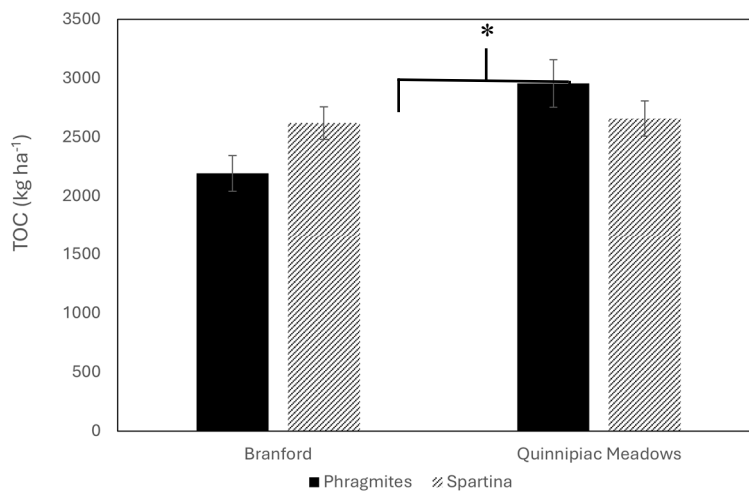


Figure 4. Total organic carbon (TOC) under the dominant species at two tidal salt marshes in southern Connecticut. Asterisk denotes a significant difference ($P < 0.05$).

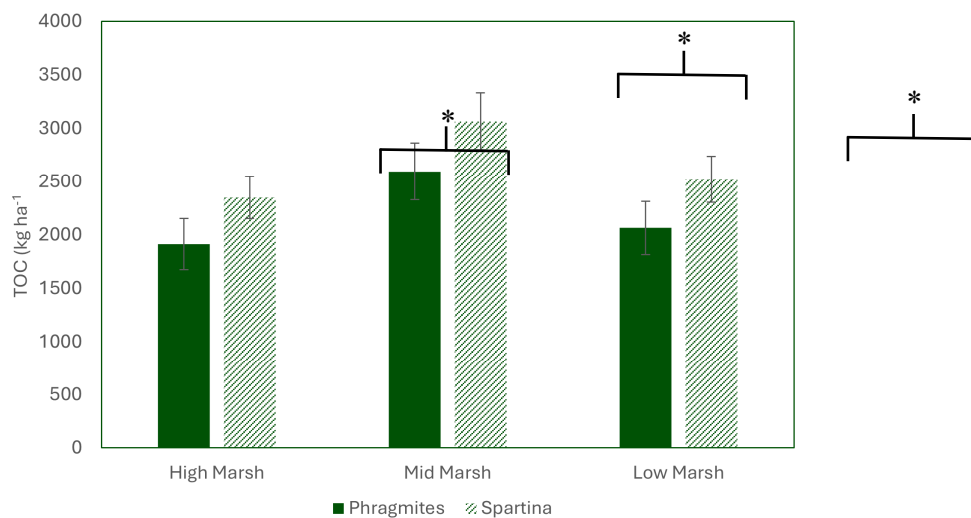


Figure 5. Spatial variation in total organic carbon (TOC) at Branford, Connecticut. Total Organic Carbon was significantly greater in soils under *Spartina alterniflora* than *Phragmites australis* across all zones. Asterisk denotes a significant difference ($P < 0.05$).

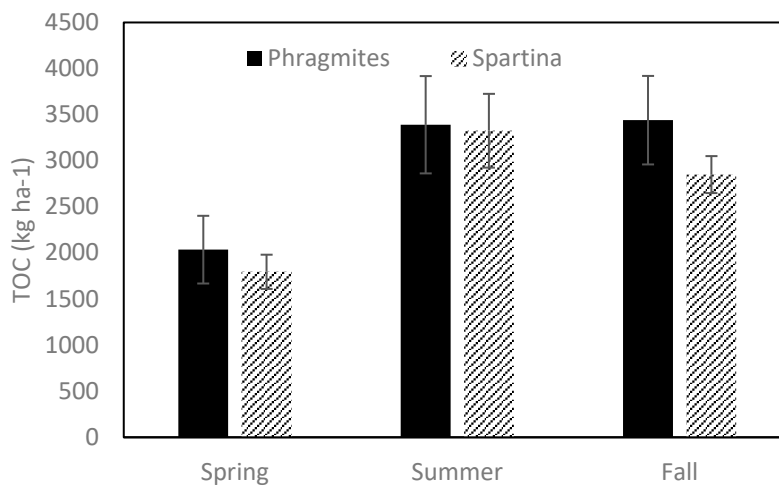


Figure 6. Seasonal variation in total organic carbon (TOC) at Quinnipiac Meadows, Connecticut.

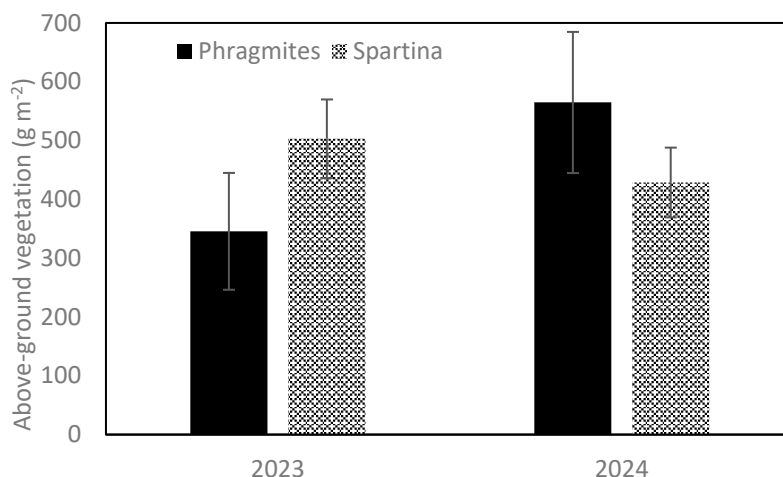


Figure 7. Above-ground vegetation biomass by species at Branford, Connecticut.

Table 1. Mean soil parameters along transects surveyed at a tidal salt marsh in Branford, CT from October 2022 to February 2023. Mean elevation at the high marsh was approximately 0.7 m above sea level.

	Species	n	High marsh ($\bar{x} \pm SE$)	Mid marsh ($\bar{x} \pm SE$)	Low marsh ($\bar{x} \pm SE$)
Dry Weight (g)	Phragmites	9	175.33 \pm 22.67	197.25 \pm 17.39	174.49 \pm 10.87
	Spartina	7	91.98 \pm 9.31	102.70 \pm 8.12	116.02 \pm 6.21
Bulk density (g cm ⁻³)	Phragmites	9	0.26 \pm 0.03	0.28 \pm 0.02	0.24 \pm 0.02
	Spartina	7	0.14 \pm 0.03	0.14 \pm 0.01	0.16 \pm 0.01
Carbon weight percent (%)	Phragmites	9	30.00 \pm 4.13	31.91 \pm 2.89	29.67 \pm 5.77
	Spartina	7	56.04 \pm 8.24	74.90 \pm 9.68	52.00 \pm 4.21
Total Organic Carbon (kg ha ⁻¹)	Phragmites	9	1915.40 \pm 239.23	2596.41 \pm 267.58	2065.79 \pm 249.58
	Spartina	7	2351.09 \pm 197.21	3065.70 \pm 266.47	2522.15 \pm 217.15

Table 2. Temporal variations in soil parameters along transects surveyed Quinnipiac Meadows over the Spring, Summer and Fall of 2024.

	Species	n	Spring ($\bar{x} \pm SE$)	Summer ($\bar{x} \pm SE$)	Fall ($\bar{x} \pm SE$)
Dry Weight (g)	Phragmites	10	12.92 \pm 8.37	18.63 \pm 2.82	16.46 \pm 2.61
	Spartina	10	5.78 \pm 1.74	9.52 \pm 2.33	6.34 \pm 0.88
Bulk density (g cm ⁻³)	Phragmites	10	0.40 \pm 0.03	0.17 \pm 0.03	0.16 \pm 0.02
	Spartina	10	0.18 \pm 0.04	0.10 \pm 0.02	0.06 \pm 0.01
Carbon weight percent (%)	Phragmites	10	8.13 \pm 1.34	9.45 \pm 1.67	11.06 \pm 2.09
	Spartina	10	19.03 \pm 2.66	20.84 \pm 2.68	22.30 \pm 1.87
Total Organic Carbon (kg ha ⁻¹)	Phragmites	10	2035.10 \pm 367.45	3389.83 \pm 528.19	3438.59 \pm 480.02
	Spartina	10	1794.80 \pm 185.53	3324.65 \pm 400.73	2849.43 \pm 200.84

4. Discussion

4.1. Alterations in Soil Physical Architecture and Geomorphology

The results of this study highlight a distinct and functional divergence in the physical soil properties between the invasive *Phragmites australis* and the native *Spartina alterniflora*, even in the absence of significant disparities in overall carbon storage capacities. While general plant characteristics—such as aboveground biomass, stand density, and height—followed expected phenological patterns, the subsurface interactions reveal a more complex narrative. Our bulk density

estimates align with regional observations from St. Lawrence Bay (Gu et al., 2020), though they fall below values recorded in southern ranges (Unger et al., 2016). However, the critical finding lies in the significantly higher and more variable bulk density associated with *P. australis*.

This variance suggests that *P. australis* acts as a distinct geomorphic engineer compared to its native counterpart. The elevated bulk density indicates that *P. australis* likely facilitates higher rates of mineral sediment deposition or possesses a root architecture robust enough to physically compact the surrounding substrate to a greater degree than *S. alterniflora*. This aligns with established research indicating that *P. australis* increases surface elevation in tidal marshes more rapidly than native species, utilizing a dual mechanism of organic matter accumulation and the efficient trapping of inorganic sediments (Rooth et al., 2003). The pronounced variance within the *P. australis* data further supports the “plasticity” hypothesis; this species is renowned for its ability to colonize a wider spectrum of micro-topographies and salinity gradients. Consequently, *P. australis* creates a more heterogeneous soil environment, contrasting sharply with the relatively uniform, monocultural stands typical of *S. alterniflora* (Meyerson et al., 2000).

4.2. Carbon Storage Dynamics: Quantity vs. Density

Our results indicate that the transition from native to invasive dominance does not equate to a collapse in carbon sequestration potential, challenging the assumption that native marshes are invariably superior carbon sinks. The lack of a significant difference in total carbon stock between the two species contradicts literature suggesting that *P. australis* significantly outperforms native marshes (Templer et al., 2016), while also complicating views that native species are superior due to litter quality (Yu et al., 2013).

Instead, our data suggests a compensatory mechanism: while *P. australis* plots may exhibit lower carbon percentages per unit of soil, the total mass of carbon stored remains competitively high because the soil is more densely packed. This density-dependent storage suggests that *P. australis* creates a physically heavier carbon platform. The discrepancy between our findings and studies favoring native storage (Yu et al., 2013) or invasive storage (González-Alcaraz et al., 2012) highlights that factors beyond species identity—such as site hydrology, sediment supply, and microbial activity—are significant drivers of carbon stocks. For instance, while *S. alterniflora* may produce recalcitrant litter that stabilizes soil carbon, *P. australis* may encourage higher mineralization rates by promoting microbial decomposition (Bernal et al., 2023). Additionally, the variability in our *P. australis* carbon data may reflect its flexibility in belowground biomass allocation, a trait responsive to nutrient availability and flooding stress (Windham, 2001). The inconsistency among various studies strengthens the argument that local environmental drivers often override species-specific carbon traits.

4.3. Nutrient Synergy and Structural Integrity

When viewed from the perspective of nutrient enrichment, high nutrient runoff along coastal habitats of the Long Island Sound may stimulate aboveground growth at the expense of belowground root biomass. In *S. alterniflora* marshes, this allocational shift can be catastrophic, leading to “marsh collapse” where the root mat weakens, causing the soil to disintegrate (Deegan et al., 2012). On the other hand, *P. australis*, a documented “nitriphile” is capable of rapidly absorbing excess nutrients to fuel massive biomass production without suffering the same level of belowground structural degradation (Mozdzer & Megonigal, 2013). Its high nitrogen demand drives it to extract deep nitrogen stores, a trait that fuels its invasiveness (Mozdzer et al., 2023) but also results in a denser soil matrix. Therefore, the higher bulk density and variable carbon distribution in *P. australis* stands suggest this species may be more effective at “locking away” nutrients into a dense soil matrix. In eutrophic coastal waters, *P. australis* may function as a superior bio-filter compared to *S. alterniflora*, stabilizing the physical platform even as it alters the biological community.

4.4. Resilience to Sea-Level Rise: The “Islands of Resilience”

The observed heterogeneity in *P. australis* soil properties at these sites has profound implications for resilience against sea-level rise (SLR). The high variance in carbon stocks and bulk density suggests a trade-off: while *P. australis* builds a denser physical platform, its sequestration is less spatially uniform. However, in the context of rapid SLR, this heterogeneity may be advantageous. The “hot spots” of high carbon and high bulk density within *P. australis* stands may function as “islands of resilience.” These dense patches resist drowning and erosion more effectively than the uniform, but potentially slower-accreting, *S. alterniflora* stands (Cahoon et al., 2021). Since higher bulk density typically correlates with increased soil shear strength (Turner et al., 2000), the invasion of *P. australis* may represent a shift toward a physically “hardened” coastline, capable of withstanding higher wave energy and deeper inundation, albeit at the cost of the micro-topographical uniformity preferred by some native fauna.

5. Conclusions

In summary, the transition from *S. alterniflora* to *P. australis* in southern Connecticut salt marshes does not represent a net loss of ecosystem function regarding carbon storage, but rather a fundamental transformation in the physical and spatial organization of the marsh platform. Our findings suggest that while total carbon stocks remain comparable, the mechanism of storage shifts from a system driven by native biomass traits to one driven by the high-density, sediment-trapping, and nutrient-responsive architecture of *P. australis*.

The implications of this study extend into the management of coastal wetlands in the Anthropocene. As sea levels continue to rise and anthropogenic nutrient loading persists, the restoration preference for *S. alterniflora* must be weighed against the geomorphic stability provided by *P. australis*. While traditionally managed as an aggressive invasive threat to biodiversity, *P. australis* demonstrates a capacity to maintain high bulk density and substantial carbon stocks under conditions that might destabilize native marshes.

Consequently, in areas subject to rapid subsidence, high wave energy, or extreme eutrophication, *P. australis* may provide a critical “geomorphic service.” The species’ ability to create a dense, heterogeneous, and nutrient-absorbent soil matrix suggests it could act as a buffer against marsh collapse. Future management strategies should consider this functional trade-off: the preservation of native biodiversity versus the maintenance of a physically resilient marsh platform capable of keeping pace with rising seas. The “islands of resilience” created by *P. australis* may well prove to be essential components of coastal defense in a rapidly changing climate.

Data Availability Statement: We are willing to submit our raw data and codes to MPDI but have not placed them in any repository.

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Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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