# Invasive Phragmites australis Reshapes Soil Biogeochemistry and Microbial Community Structure in a Northeastern U.S. Salt Marsh

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# Abstract

**Background:** Coastal salt marshes of the northeastern United States have experienced major compositional shifts due to invasive species. While previous research has shown that these invasions impact microbial composition and nutrient dynamics, a significant knowledge gap remains regarding taxonomic and biochemical changes. This study investigated the effects of invasive *Phragmites australis* on soil chemistry and microbial community structure in the Quinnipiac River salt marsh in southern Connecticut, comparing invaded zones to areas dominated by the native foundation species, *Sporobolus alterniflorus* (formerly *Spartina alterniflora*). We employed 16S rRNA sequencing to characterize microbial taxa and measured soil organic carbon (SOC), total nitrogen (TN), carbon-to-nitrogen (C:N) ratio, as well as environmental variables.

**Results:** Our findings reveal that although mean TN and SOC stocks did not differ significantly between native and non-native dominated areas, there were spatial and temporal distinctions in soil chemistry and microbial assemblages. Sporobolus soils had a significantly greater C:N ratio, which may favor slower organic matter decomposition leading to greater capacity for carbon sequestration. In contrast, Phragmites soils, with higher pH and bulk density, could support more copiotrophic microbial taxa. Microbial community structure showed a significant separation between the two vegetation types at all taxonomic levels, with plant type explaining between 9.1 and 13.5% of the community variation. While Sporobolus soils had greater diversity at broader taxonomic levels, Phragmites soils supported more diversity from Family to Genus levels.

**Conclusions:** These results underscore the role of vegetation in shaping microbial ecology and soil function, providing crucial insights for the management and restoration of salt marsh ecosystems.


# Introduction

The stability and function of coastal ecosystems are fundamentally governed by the soil microbiome, a diverse community of microorganisms that regulates the global cycling of carbon and nitrogen (Schimel & Schaeffer, 2012). In coastal salt marshes, these microbes underpin blue carbon sequestration and nutrient filtration, services that contribute directly to climate change mitigation and water quality (Chmura et al., 2003; Duarte et al., 2013). Prior salt marsh and coastal wetland work has established that microbially mediated pathways such as denitrification and extracellular enzyme activity are highly responsive to environmental gradients (e.g., inundation, salinity, redox), but much of this literature has emphasized process rates or bulk functional assays rather than attempting to link specific microbial taxa to observed changes (Seitzinger, 1988; Sinsabaugh et al., 2008). Where taxonomic resolution has been achieved, studies demonstrate why it matters mechanistically: along estuarine/salt-marsh salinity gradients, ammonia oxidizers shift among Nitrosomonas- and Nitrosospira-like bacteria (Bernhard et al., 2007), and other coastal sediment studies show contrasting dominance patterns of ammonia-oxidizing archaea (often Nitrosopumilus-like Thaumarchaeota) versus bacterial nitrifiers (Mosier & Francis, 2008). These examples illustrate that similar “net” nitrogen transformation rates can arise from different microbial assemblages, underscoring why genus-level (or finer) community information is increasingly necessary for predicting marsh biogeochemical responses under changing conditions.

These essential microbial processes are increasingly disrupted by biological invasions that alter the plant–soil interface. Invasive plants often possess superior physiological mechanisms, including enhanced nutrient

utilization efficiencies and rapid growth rates, which allow them to displace native assemblages and reshape the belowground environment (Callaway et al., 2004; Mack et al., 2000). Along the Long Island Sound, salt marsh plant communities were historically dominated by the native foundation species *Sporobolus alterniflorus* (formerly *Spartina alterniflora*), which structures sediment conditions and organic matter inputs that, in turn, help shape resident microbial communities and biogeochemical function. When invaders replace such foundation species, these shifts can reorganize microbial community structure and potentially compromise long-term marsh resilience (Loreau et al., 2001). In the northeastern United States, the invasive genotype (haplotype) of *Phragmites australis* (hereafter Phragmites) has become a dominant driver of such ecosystem shifts, in part because cryptic spread of the non-native lineage enabled rapid expansion across coastal regions (Saltonstall, 2002). By modifying soil oxygen dynamics, litter inputs, and nutrient availability at the plant–soil interface, invasions can alter the microbial loop; for example, invasive plants have been shown to restructure soil microbial community composition and associated functioning, with downstream implications for nutrient availability and carbon storage (Kourtev et al., 2002; Wolfe & Klironomos, 2005). Importantly, invasion-driven changes in organic matter quality can change soil C:N, which theory and global empirical syntheses link to microbial biomass stoichiometry and extracellular enzyme allocation—mechanisms that can shift decomposition and nutrient mineralization even without large changes in total microbial biomass (Cleveland & Liptzin, 2007; Sinsabaugh et al., 2008).

Despite recognition of these broad mechanisms, a key knowledge gap persists in linking invasion-driven shifts in soil chemistry (including SOC, TN, and C:N) with microbial community composition at fine taxonomic resolution and at local (within-marsh) spatial scales. High throughput 16S rRNA sequencing now enables inference at much finer taxonomic resolution than earlier approaches, making it possible to associate specific bacterial and archaeal lineages with measured edaphic gradients (Caporaso et al., 2012). In this study, we investigated the link between plant species, soil microbial taxonomy, and chemical properties at a local scale in a southern Connecticut salt marsh. Specifically, we sought (1) to characterize the physical and chemical properties of brackish marsh soils near the mouth of the Quinnipiac River; (2) to quantify and compare microbial diversity and community structure at multiple taxonomic levels between Phragmites and Sporobolus soils using 16S rRNA sequencing; and (3) to evaluate differences in soil organic carbon (SOC), total nitrogen (TN), and C:N ratios. By explicitly pairing fine-resolution microbial community data with co-located soil chemistry across invaded and native vegetation zones, this research is timely for improving mechanistic forecasts of marsh carbon and nitrogen cycling and for informing restoration strategies under accelerating invasion pressure and climate-driven change.

## MATERIALS AND METHODS

# Site description

Our study site was located in the Eugene B. Fargeorge Quinnipiac Meadows a 35-acre nature preserve located in the lower section of the Quinnipiac River in New Haven, Connecticut (41° 19 '00"N 72° 52' 50"W; Fig. 1). The climate is humid subtropical climate with four distinct seasons. Winters (-7 to 4° C) are cold 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).

The preserve is comprised of tidal salt and brackish wetlands, coastal forest, and coastal grasslands. Invasive Phragmites dominates high marsh with native species like Sporobolus closer to the shoreline in the mid to low marsh. Quinnipiac Meadows serves as a vital habitat for ospreys and a diverse array of more than 90 bird species, with occasional sightings of deer and raccoons. (Ewbank, 2017).

## Soil Sampling and Chemical analysis

Soil samples were collected using a stratified random sampling approach to capture variability across vegetation types, following standardized protocols for ecological soil studies (Carter and Gregorich 2008). Two dominant vegetation zones were targeted: (1) native sites primarily populated by *Sporobolus alterniflorus* and (2) invaded sites dominated by non-native *Phragmites australis*. Thirty soil samples were collected under each species for a total of 60 samples spread across three key seasons - early growing season (May), peaking growing season (August), and dormant season (December) with 10 replicate samples per vegetation type in each period.

## Field sampling

Sixty soil samples were collected over three seasons (Spring, Summer and Fall) in 2024, with 20 samples collected at each visit. Soil samples were taken from the rhizosphere to a depth of approximately 20 to 30 cm using a stainless-steel soil corer (Hunnings et al. 2019). Site locations were marked using a handheld GPS unit (Garmin eTrex 10, model 010-00970-00; Garmin Ltd., Olathe, KS, USA). On-site measurements included soil temperature, pH (Extech PH100 Waterproof ExStik pH Meter) and wet weight. To prevent cross-contamination, equipment was cleaned with a 20% bleach solution between samples. 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.

About 20–30 grams of soil were extracted from each original sample and all visible roots removed prior to air drying for 24 hours followed by then oven-drying at 55°C for another 24 hours to remove excess moisture. Once dried, the soil samples were ground with a mortar and pestle, sieved through a 2mm mesh, and the dust packed into tin capsules (Carter and Gregorich 2008). The δ13C and δ15N values and elemental carbon and nitrogen contents of all 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 Nitrogen and Carbon percent in each sample which was then used to estimate TN and SOC.

## Microbial activity and analysis

Microbial DNA was extracted from 0.250 g of wet-weight soil from each of the 60 samples using the Qiagen Dneasy PowerSoil® DNA Isolation Kit following the standardized protocol described by Lin et al. (2022). Post-

extraction, DNA concentration and purity were assessed using a Nanodrop spectrophotometer. All samples yielded DNA concentration within the desired range, and A260/A280 absorbance ratios, were between the optimal range of 1.8–2.0, indicating high purity suitable for downstream sequencing applications. Samples were prepared for high throughput sequencing at Novogene Corporation (Novogene 2024). According to their 16S amplicon sequencing guidelines, each DNA sample was required to meet a minimum concentration of 5ng/μL, a volume of at least 40 μL, and a total DNA yield of at least 200 ng. Although Qubit quantification is recommended by Novogene for more accurate DNA measurements, Nanodrop readings were used for preliminary screening prior to shipping.

Each sample was allotted into a 1.5 mL flip-cap microcentrifuge tube, labeled with freezer-safe ink, sealed with parafilm, and packed on dry ice for shipping.

Amplicon sequencing was conducted using the Illumina NovaSeq 6000 platform Sequencing targeted the V4 region of 16S rRNA was conducted using primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) generating paired end reads (2 x 250 bp) with an average of approximately 30,000 raw reads per sample.

Novogene completed internal quality control checks, including removal of adapter sequences, trimming of low-quality reads, and demultiplexing of raw sequences. The demultiplexed, high-quality FASTQ files were delivered through secure FTP access and used for downstream microbial diversity and taxonomic analysis.

# Statistical Analysis

# Environmental variables

All statistical analyses were conducted in RStudio (version 2023.03.0 + 386) using R version 4.3.4 using the dplyr, ggplot2, and ggpubr packages. To assess variation in environmental variables (soil temperature, pH, and bulk density) between Phragmites- and Sporobolus- dominated soils, mean values and standard errors (SE) were calculated for each vegetation type. Normality was tested using the Shapiro-Wilk test. For variables that followed a normal distribution, Welch's two-sample t-tests were used to compare group means. For non-normally distributed variables (e.g., temperature and bulk density), Wilcoxon rank sum tests were performed to compare between plant species and Scheirer–Ray–Hare tests to assess interactions with season.

## TN and SOC

Total nitrogen (TN) and soil organic carbon (SOC) contents were converted from weight percent to kilograms per hectare (kg/ha) by multiplying by bulk density and sample volume ($cm^3$). For this we used an equation proposed by Carter & Gregorich (2008):

$$Nutrient\ stock\ \left(\frac{kg}{ha}\right) = Concentration\ (\%) \times\ Bulk\ density\ \left(\frac{g}{cm^3}\right)\ \times\ Soil\ depth\ (cm) \times\ 100$$

0

The C:N ratio, a metric is widely used to assess the nutrient balance, was calculated by dividing carbon weight percent by nitrogen weight percent. The C:N ratio can affect microbial metabolism, decomposition, and nitrogen cycling (Bui and Henderson 2013).

## Microbial Community Analysis

Microbial diversity and composition were assessed at five taxonomic levels: phylum, class, order, family, and genus. Alpha Diversity (Shannon Index): Shannon diversity indices were calculated from operational taxonomic unit (OTU) abundance tables. Bray-Curtis dissimilarity matrices were computed to assess beta diversity which assesses differences in whole community composition. These matrices were visualized using a non-metric multidimensional scaling (NMDS) ordination. Stress values were used to assess ordination quality, and 95% confidence ellipses were plotted to illustrate vegetation-based clustering. A Permutational Multivariate Analysis of Variance (PERMANOVA) was used to asses differences in microbial structure, The PERMANOVA was conducted using the adonis2() function in R with 999 permutations of the Bray-Curtis dissimilarity indices for each sample. Presences/absence matrices were generated at each taxonomic level to identify shared and unique taxa between vegetation types. Taxa were categorized as shared (present in both Phragmites and Sporobolus soils) or unique to one vegetation type, helping to identify vegetation-specific microbial assemblages.

# Seasonal variation

Differences in environmental conditions, soil nutrients, and microbial alpha diversity were compared across three seasons (Spring, Summer and Fall) using a one-way ANOVA or a Kruskal-Wallis Rank Sum Tests depending on the data distribution normality. When significant differences were found ($p < 0.05$), a post hoc pairwise Dunn's test with Bonferroni correction was applied to account for multiple variables (temperature, pH, and bulk density) and lab-derived metrics such as TN, SOC, and C:N ratios.

## RESULTS

# Environmental variables

Soil temperature did not differ significantly between the two vegetation types. suggesting broadly similar thermal conditions across vegetation zones (Table 1). Conversely, soil pH was significantly greater in Phragmites-dominated regions indicating possible variations in microbial buffering ability or root exudation. Bulk density was also significantly greater in Phragmites soils.

Table 1
Mean (± Standard Error) of environmental variables measured in Phragmites- and Sporobolus- dominated soils, including soil temperature (°C), pH, and bulk density ($g/cm^3$).

| Vegetation | Temperature (°C) | pH | Bulk Density ($g/cm^3$) |
|---|---|---|---|
| *Phragmites australis* | 13.14 ± 1.79 | 6.71 ± 0.07 | 0.24 ± 0.03 |
| *Sporobolus alterniflorus* | 14.56 ± 1.74 | 6.47 ± 0.08 | 0.11 ± 0.02 |

# Soil Chemistry results

Total nitrogen (TN) and soil organic carbon (SOC) did not differ significantly between the two plant species (Table 2). However, the C:N ratio was significantly greater in Sporobolus soils (Table 2).

Table 2
Mean ± Standard error (SE) of soil nitrogen (kgN/ha), soil organic carbon (kgC/ha), and C:N ratio in soils dominated by Phragmites and Sporobolus.

| Species | Nitrogen (kg/ha) | Carbon kg/ha) | C:N ratio |
|---|---|---|---|
| *Phragmites australis* | 0.13 ± 0.02 | 2.16 ± 0.25 | 17.32 ± 0.37 |
| *Sporobolus alterniflorus* | 0.12 ± 0.01 | 2.29 ± 0.19 | 18.92 ± 0.36 |

# Microbial Taxonomic Distribution

Microbial community composition revealed that a core microbiome is shared across vegetation types, with increased taxonomic exclusivity at finer resolutions (Table 3).

# Alpha Diversity

Shannon diversity indices revealed contrasting patterns across taxonomic levels. Microbial alpha diversity peaked at the Order level for both species (Fig. 2). Although Phragmites tended to support greater alpha diversity from class to genus levels, no statistically significant difference was detected between the two plant species. The plants shared more microbial taxa than there were unique microbial species, but Sporobolus held more unique species across most taxonomic levels (Table 3).

Table 3
Summary of microbial taxa shared and uniquely distributed between *Phragmites australis* and *Sporobolus alterniflorus*– dominated soils across six taxonomic levels.

| Taxonomic Level | Shared Taxa | Phragmites Only | Sporobolus Only |
|---|---|---|---|
| Phylum | 81 | 2 | 3 |
| Class | 163 | 9 | 15 |
| Order | 396 | 23 | 40 |
| Family | 493 | 35 | 62 |
| Genus | 1049 | 271 | 256 |

# Beta Diversity and Community Composition

Beta diversity analysis revealed strong and consistent vegetation effects on microbial community structure with significant compositional separation between Phragmites and Sporobolus soils across all taxonomic levels (Table 4). PERMANOVA analysis yielded $R^2$ values ranging from 0.091 at the Genus level to 0.135 at the Class level (all $p < 0.001$), Indicating that vegetation type explained approximately 9.1–13.5% of variation in microbial community composition. NMDS stress values were all < 0.1, indicating reliable two-dimensional

ordination (Fig. 3). Visual clustering by vegetation type was observed in ordination plots from Phylum through Genus levels, supporting distinct microbial community structure associated with the dominant plant species.

Table 4
Summary of beta diversity metrics across taxonomic levels in soils dominated by Phragmites and Sporobolus. Community composition differences were assessed using PERMANOVA based on Bray-Curtis dissimilarity values to assess the influence of vegetation type on microbial community composition.

| Taxonomic Level | $R^2$ | F-value | p-value | NMDS Stress |
|---|---|---|---|---|
| Phylum | 0.128 | 8.490 | 0.001 | 0.066 |
| Class | 0.135 | 9.070 | 0.001 | 0.076 |
| Order | 0.129 | 8.580 | 0.001 | 0.080 |
| Family | 0.107 | 6.970 | 0.001 | 0.065 |
| Genus | 0.091 | 5.816 | 0.001 | 0.040 |

The NMDS analysis consistently demonstrated a strong and reliable fit across all taxonomic levels, from phylum to genus, as indicated by low stress values (all below 0.080). The clear and distinct clustering of samples based on vegetation type across all levels confirms that dominant plant species are a primary driver shaping the composition and structure of soil bacterial communities.

# Seasonal Variation

# Environmental Conditions

Soil temperature varied significantly with season in both vegetation types (Fig. 4). Temperatures followed expected seasonal patterns, peaking in summer. In Phragmites soils, seasonal variation was statistically significant (Kruskal-Wallis: $\chi^2 = 19.483$, $P < 0.001$), with post hoc analysis showing significant differences between spring and fall ($P = 0.015$) and between summer and fall ($P < 0.001$). A similar pattern was observed for Sporobolus ($\chi^2 = 24.714$, $P < 0.001$), with a significant difference between spring and fall ($P = 0.023$) and summer and fall ($P < 0.001$). Mean summer temperature under Sporobolus was ~ 24.7°C and slightly lower under Phragmites at ~ 23.5°C.

In both vegetation types, pH was highest in fall, possibly due to reduced microbial respiration and root activity. The effect of season was significant in Phragmites ($\chi^2 = 13.03$, $P = 0.0015$), with post hoc comparisons showing that fall pH was significantly higher than both spring ($P = 0.037$) and summer ($p = 0.003$). In Sporobolus, the seasonal effect was also significant ($\chi^2 = 14.568$, $P = 0.0007$), with higher pH in fall compared to spring ($p = 0.002$) and summer ($P = 0.024$).

Sporobolus soils had significantly lower bulk density in fall, likely due to freeze-thaw cycles and moisture retention. For Phragmites, seasonal differences were also significant ($\chi^2 = 9.844$, $P = 0.0073$), with post hoc analysis indicating lower density in fall compared to spring ($P = 0.018$). In Sporobolus soils, the pattern was more pronounced ($\chi^2 = 19.904$, $P = 0.001$), with both spring-fall ($P = 0.005$) and summer-fall ($P = 0.004$). The highest recorded bulk density was observed in Phragmites spring soils (~ 0.58 g/cm³).

# Nutrient Analysis

There was no statistically significant difference in the TN and SOC stocks between species (H = 0.42, df = 1, *P* = 0.52), but differences were detected among seasons (H = 10.28, df = 2, *P* = 0.006). Mean SOC in Phragmites soils increased steadily throughout the year and was greatest in the fall, In comparison, SOC under Sporobolus peaked in the summer and dropped in the fall (Fig. 5). Nitrogen followed a similar pattern as SOC (Fig. 5). A Scheirer-Hare test was used to compare C:N ratios between species, seasons and interactions. Results found significantly greater C:N ratios in Sporobolus relative to Phragmites (H = 12.59, df = 2, *P* = 0.0004). Though no seasonal effect was detected and both species exhibited relatively stable C:N ratios throughout the year, a post-hoc Dunn's Test revealed the primary difference between the species occurred in summer and fall (Fig. 5; *P* = 0.04), suggesting species- and season-dependent difference in organic matter quality.

# Seasonal microbial diversity

The analysis of seasonal microbial alpha diversity using the Shannon index reveals that seasonal variation is not a consistent pattern across all taxonomic levels or plant species. The most notable seasonal trend was observed at the phylum level, specifically in soils dominated by Phragmites. At the phylum level, Phragmites soils exhibited a significant seasonal pattern, with diversity being significantly higher in fall (2.55 ± 0.09) compared to both spring (2.14 ± 0.08) and summer (2.13 ± 0.07). Statistical significance of this difference was confirmed by the Kruskal-Wallis test ($\chi^2 = 9.76$, *P* = 0.007) and subsequent post-hoc tests. In contrast, Sporobolus soils showed a modest, but not statistically significant, seasonal increase in diversity from spring (2.08 ± 0.12) to summer (2.38 ± 0.11) and fall (2.23 ± 0.09).

At all other taxonomic levels examined, microbial diversity remained relatively stable throughout the seasons for both Phragmites and Sporobolus soils. No significant seasonal differences were detected at the class, order, family or genus levels for either vegetation type. Although slight, non-significant increases in diversity were often observed in fall, seasonal changes did not have a strong impact on diversity at these finer taxonomic scales.

Table 5: Microbial alpha diversity (Shannon) across all taxonomic levels by species and season.

| Species | Taxonomic Level | Spring $\bar{x} \pm SE$ | Summer $\bar{x} \pm SE$ | Fall $\bar{x} \pm SE$ | Kruskal-Wallis $\chi^2$ (P-value) |
|---|---|---|---|---|---|
| Phragmites | **Phylum** | **2.14 ± 0.08** | **2.13 ± 0.07** | **2.55 ± 0.09** | **9.76 (0.007)** |
| | Class | 2.84 ± 0.06 | 2.81 ± 0.07 | 2.98 ± 0.06 | 3.63 (0.162) |
| | Order | 2.87 ± 0.07 | 2.91 ± 0.08 | 2.99 ± 0.05 | 2.11 (0.348) |
| | Family | 2.85 ± 0.08 | 2.87 ± 0.07 | 2.97 ± 0.06 | 3.36 (0.186) |
| | Genus | 2.94 ± 0.06 | 2.96 ± 0.07 | 3.08 ± 0.06 | 4.33 (0.115) |
| Sporobolus | Phylum | 2.08 ± 0.12 | 2.38 ± 0.11 | 2.23 ± 0.09 | 5.19 (0.075) |
| | Class | 2.71 ± 0.06 | 2.78 ± 0.07 | 2.92 ± 0.06 | 2.26 (0.322) |
| | Order | 2.73 ± 0.06 | 2.78 ± 0.07 | 2.95 ± 0.07 | 4.68 (0.096) |
| | Family | 2.74 ± 0.06 | 2.79 ± 0.07 | 2.92 ± 0.07 | 3.92 (0.141) |
| | Genus | 2.86 ± 0.07 | 2.90 ± 0.08 | 3.04 ± 0.07 | 3.41 (0.181) |

# DISCUSSION

The invasion of tidal salt and brackish wetlands of the eastern U.S. by the non-native genotype of *Phragmites australis* has long been a concern, with extensive research focusing on its impacts on aboveground biodiversity and carbon sequestration. Results of this study of soil microbes provide compelling evidence that plant invasions trigger a microbial restructuring impacting key ecosystem processes like nutrient cycling and wetland resilience (Kourtev et al., 2002; Negesse et al., 2025). Our findings illuminate a complex interplay between plant identity, soil conditions, and microbial community structure, with significant implications for wetland biogeochemistry and management. Results show that abiotic characteristics of Phragmites soils differed from those of native Sporobolus stands. For instance, soil temperature was slightly higher in Sporobolus soils, while Phragmites-dominated soils showed significantly greater bulk density and higher pH.

The elevated pH in Phragmites soils, particularly during fall, could be attributed to decreased microbial and root activity, both of which lower pH during the growing season. This contrasts with the lower bulk density observed in Sporobolus soils during fall, likely a result of freeze-thaw cycles and greater soil moisture (Schindler Wildhaber et al., 2012). The increased bulk density in Phragmites areas likely reflects the extensive, dense, belowground biomass of this species, a characteristic that alters the physical architecture of the soil and has been observed in other invaded marsh systems (Silliman and Bertness, 2004). These physical and chemical changes are critical, as they can act as powerful environmental filters selecting for specific microbial taxa (Lauber et al., 2009; Cheng et al., 2013). While some earlier research suggested that broad environmental factors like salinity or site history could outweigh vegetation as the primary driver of microbial communities in some contexts (Ravit et al., 2003), our results demonstrate that even within the same marsh system, vegetation type is a primary determinant of soil chemistry and, consequently the microbiome.

The influence of dominant vegetation on microbial communities was a central finding of this study, revealing a scale-dependent restructuring of the salt marsh microbiome. The use of high-resolution 16S rRNA amplicon sequencing allowed us to move beyond the broad "fingerprinting" characterizations of earlier methodologies (e.g., FAME analysis) to precisely identify taxonomic shifts. Sporobolus-dominated soils exhibited more unique taxa than Phragmites at most taxonomic levels suggesting that some taxa may have been lost when Phragmites invaded the area. Conversely, at finer taxonomic resolutions (Order, Family, and Genus), Phragmites-dominated soils showed greater alpha diversity. This suggests that invasive vegetation generates more heterogeneous microhabitats through unique root exudates and soil aeration, promoting a broader range of specialized microbial taxa (Bernard et al., 2007; Windham and Ehrenfeld, 2003). The presence of hundreds of genera exclusive to each vegetation type further supports the hypothesis that plant-microbe interactions foster specialized niches (Weinstein et al., 2019).

Beyond alpha diversity, our findings consistently demonstrated a significant compositional restructuring of microbial communities from the phylum through the genus levels. This is consistent with global meta-analyses showing that invasive plants often restructure microbiomes, even when overall richness remains unchanged (Malacrinò et al., 2020; Torres et al., 2021). The distinct partitioning of over 500 genera between the two vegetation types underscores that Phragmites fundamentally alters the microbial landscape of salt marshes. This work modernizes the foundational findings of studies like Ravit et al. (2003), which first proved that microbial communities were different in invaded marshes. Our manuscript now explains who these microbes are taxonomically and how specific changes in soil chemistry create the environment that selects for them, with potential downstream effects on critical processes such as nutrient cycling and ecosystem function (Holdredge et al., 2010; Jackson et al., 2020).

Despite these profound shifts in microbial composition, total soil nitrogen (TN) and soil organic carbon (SOC) stocks did not differ significantly between the vegetation types. This outcome is consistent with prior studies that report minimal changes in bulk nutrient pools following plant invasion (Windham, 2001; Mozdzer et al., 2010). However, the C:N ratio presented a more nuanced picture. Sporobolus soils exhibited a significantly higher C:N ratio, which likely reflects the presence of more recalcitrant organic matter that decomposes slowly and may support oligotrophic microbial taxa (Chapin et al., 2002). In contrast, the lower C:N ratio in Phragmites soils indicates that its litter is more labile, decomposing faster and facilitating accelerated nutrient turnover. This finding provides a high-resolution, mechanistic explanation for earlier hypotheses regarding nitrogen dynamics in invaded marshes. For example, Ravit et al. (2003), using broader biochemical indicators, found that Phragmites stands had higher potential for denitrification. Our results align with this, suggesting that the lower C:N ratio in Phragmites soils creates conditions favorable for faster nutrient cycling, likely driven by the distinct nitrifying and denitrifying communities we identified. Thus, it is not simply the quantity of nutrients but the quality and form of organic matter input that appears to be a key driver of microbial community assembly.

Seasonal dynamics also played a crucial role in shaping both soil conditions and microbial communities. Soil temperature, pH, and bulk density all exhibited clear seasonal shifts, which in turn influenced microbial diversity and nutrient availability. For instance, the pH of the soil was consistently higher in both vegetation types during the fall, attributable to decreased microbial and root activity (Ravit et al., 2007). At the phylum level, Phragmites-dominated soils showed a significant seasonal pattern, with diversity noticeably higher in

fall. This suggests that colder conditions may select for a different range of microbial taxa adapted to lower temperatures or changes in resource availability (Yeh et al., 2022). Although the overall community structure demonstrated resilience to short-term seasonal variability, the more pronounced seasonal response in Phragmites soils likely reflects the species' aggressive growth strategy, which creates a more dynamic environment for microbial activity (Chambers et al., 2012; Bernal and Mitsch, 2013).

Collectively, our findings highlight that dominant vegetation and seasonal rhythms interact to shape the belowground microbial world in salt marsh soils. The invasion of Phragmites fosters a more diverse and compositionally distinct microbiome at finer taxonomic levels, driven by qualitative differences in organic matter and microhabitat heterogeneity. Understanding these dynamics is critical, as soil microbial communities are central to biogeochemical processes in coastal ecosystems (Mitsch and Gosselink, 2015). For wetland managers, our research underscores that while bulk nutrient stocks may remain unchanged, the shifts in microbial communities could have profound implications for ecosystem function. Future research should integrate microbial gene analysis to clarify how these vegetation-driven taxonomic changes translate into functional, ecosystem-level changes, thereby developing more effective management strategies for maintaining the health and resilience of these vital coastal ecosystems.

We thank Gather New Haven for granting access to the Quinnipiac Meadows Eugene B. Fargeorge Preserve. We also thank N. Stasulli and J-P. Simiouw for their extensive edits of a previous version of this manuscript and P. Khanal for the map.

# Declarations

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Availability of data and material

The 16S rRNA gene sequence datasets generated and analyzed during the current study are available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) repository under BioProject accession number PRJNA1426752.

### Competing interests

The authors declare that they have no competing interests

### Funding

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### Authors' contributions

R.N. conceptualized the project, collected and analyzed all samples, wrote the initial manuscript and assisted in securing funding. J.S. assisted with all field data collection and laboratory analysis as well as contributed to the initial manuscript. S. K. conceptualized the project, was the PI on the grant, administered the grant, supervised field work, reviewed the manuscript and made final edits.

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### Funding Declaration

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### Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work the authors used Google Gemini 2.5 Flash and Chat GPT 5.2 in order to generate efficient diagram and analysis codes in RStudio as well as identify grammatical errors and redundancies. After using this tool/service, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the published article.

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# Figures

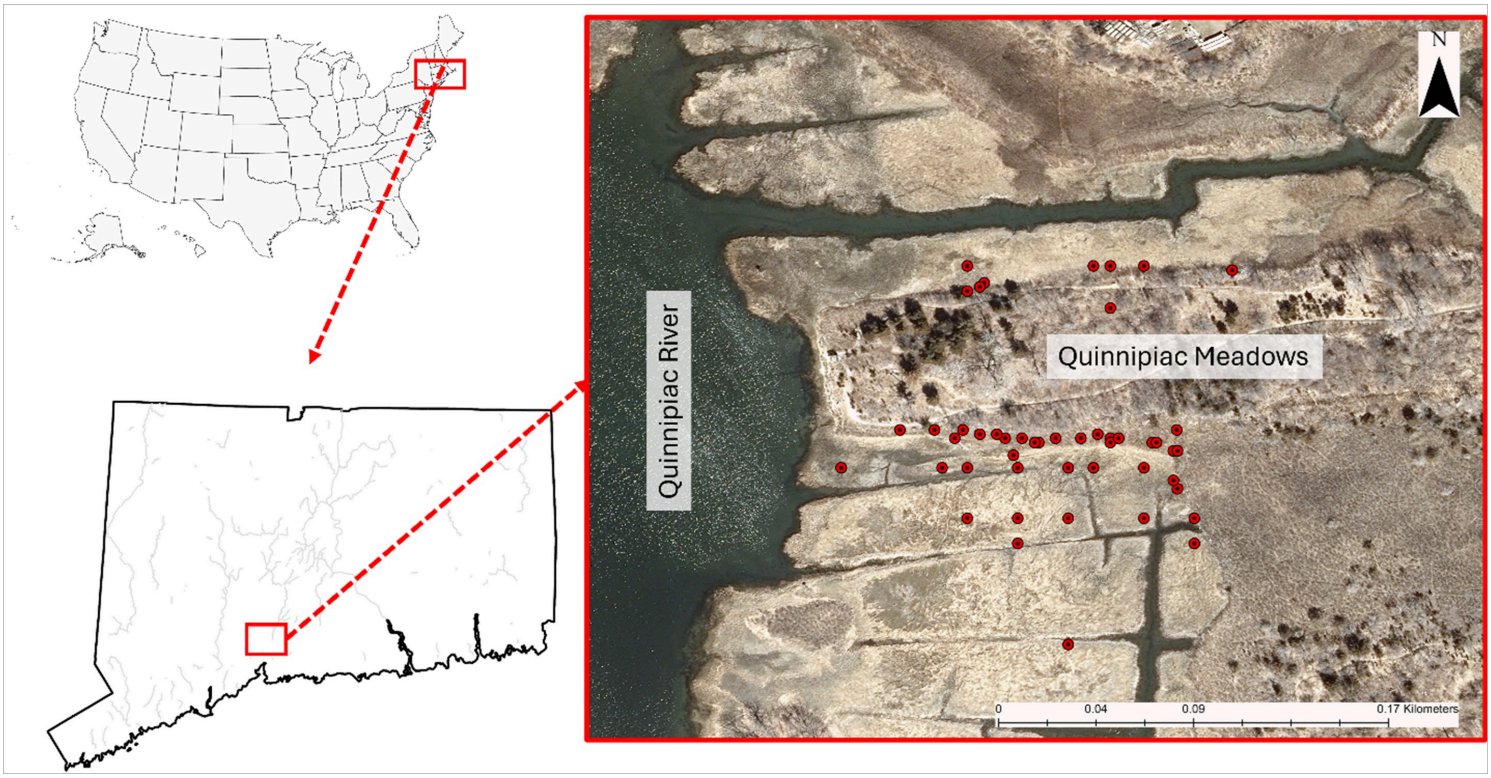



**Figure 1**

Location of the study area and distribution of soil samples collected at Quinnipiac Meadows Preserve, New Haven Connecticut, USA.

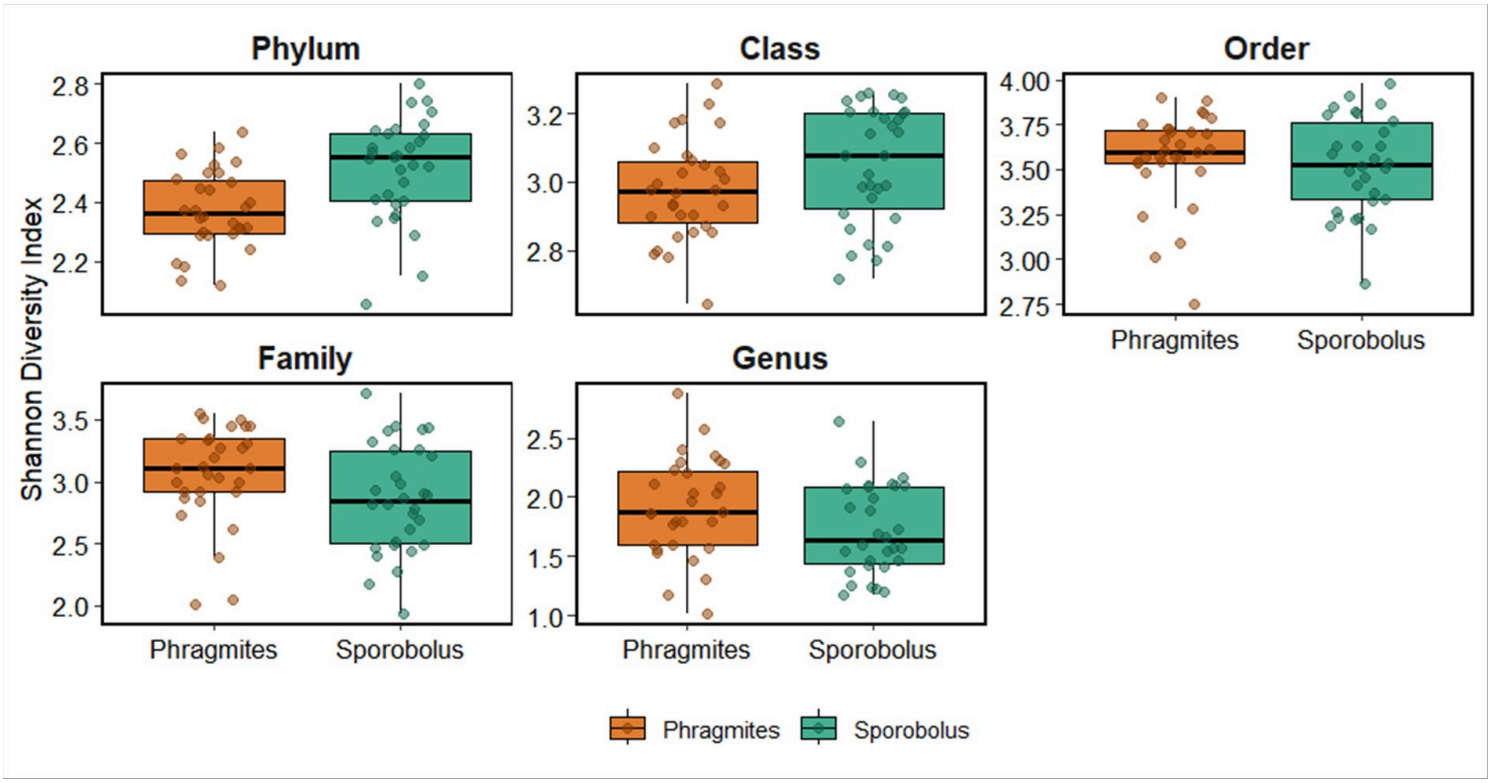
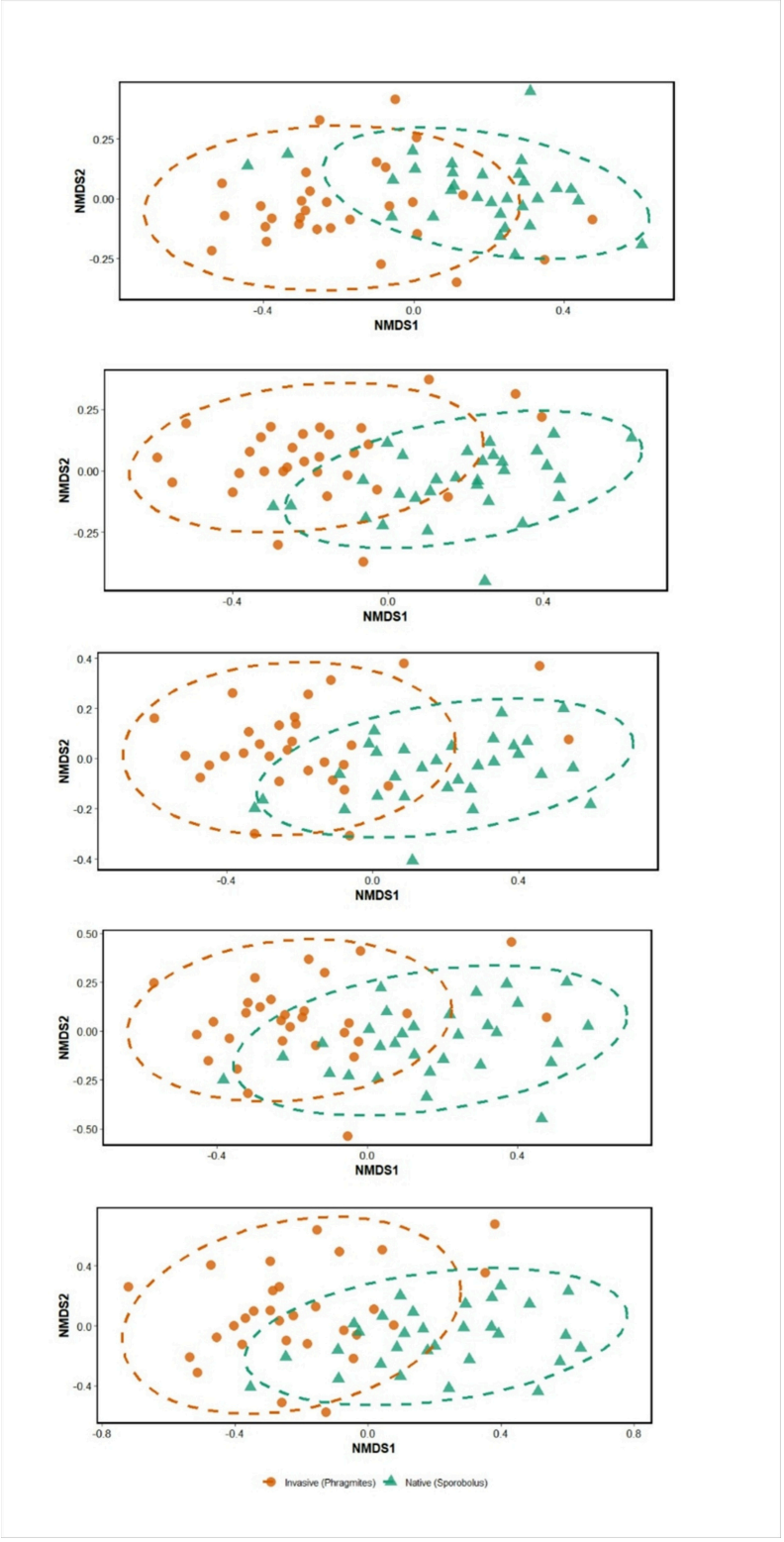



**Figure 2**

Alpha diversity (Shannon) of microbial communities in non-native *Phragmites australis* and native *Sporobolus alterniflorus*soils at Quinnipiac Meadows salt marsh in Connecticut.




## Figure 3

Microbial community dissimilarity at each taxonomic level (From top to bottom; Phylum, Class, Order, Family Genus)

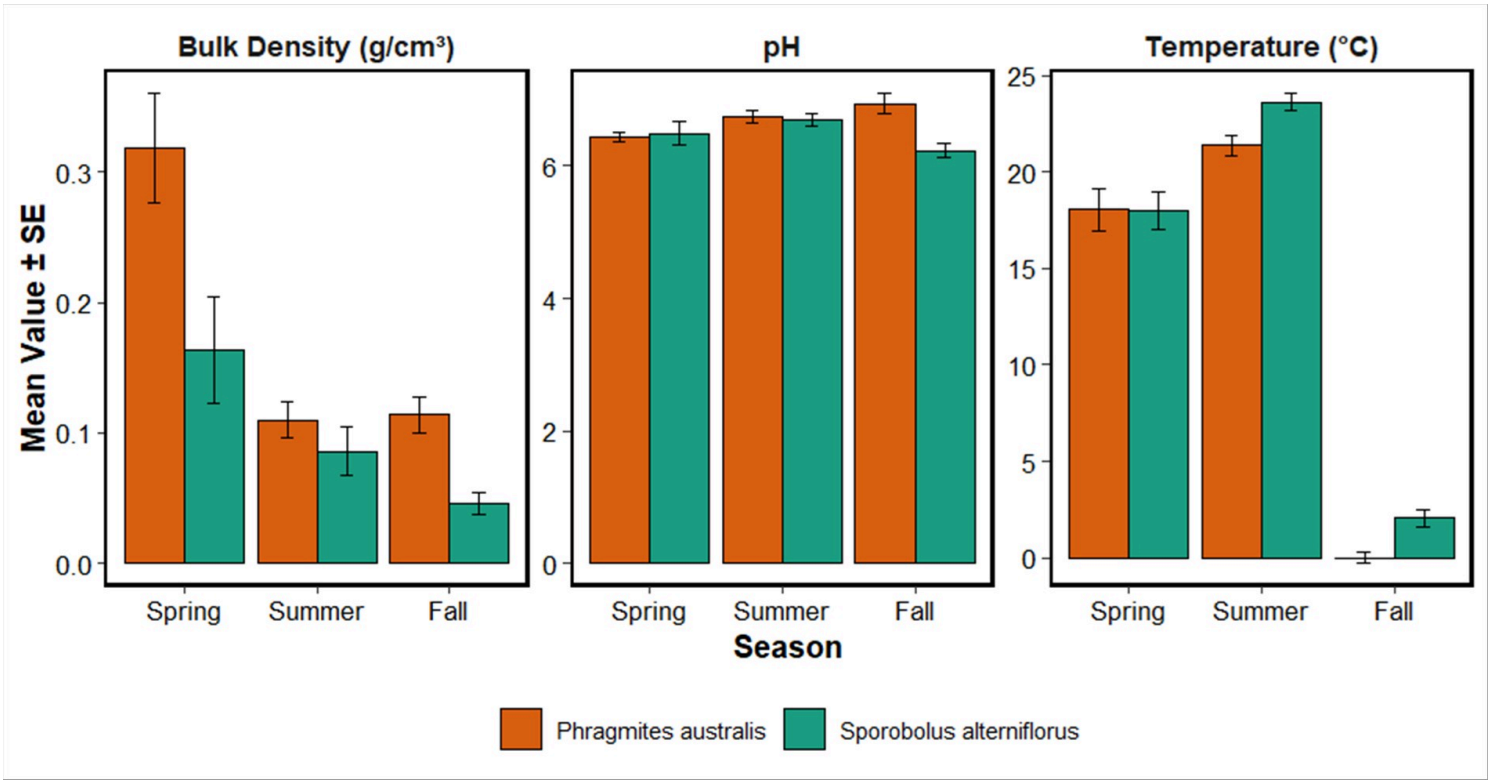



Figure 4

Physical characteristics of salt marsh soils under two species (*Phragmites australis*and *Sporobolus alterniflorus*) over three seasons (Spring, Summer, and Fall) in southern Connecticut.

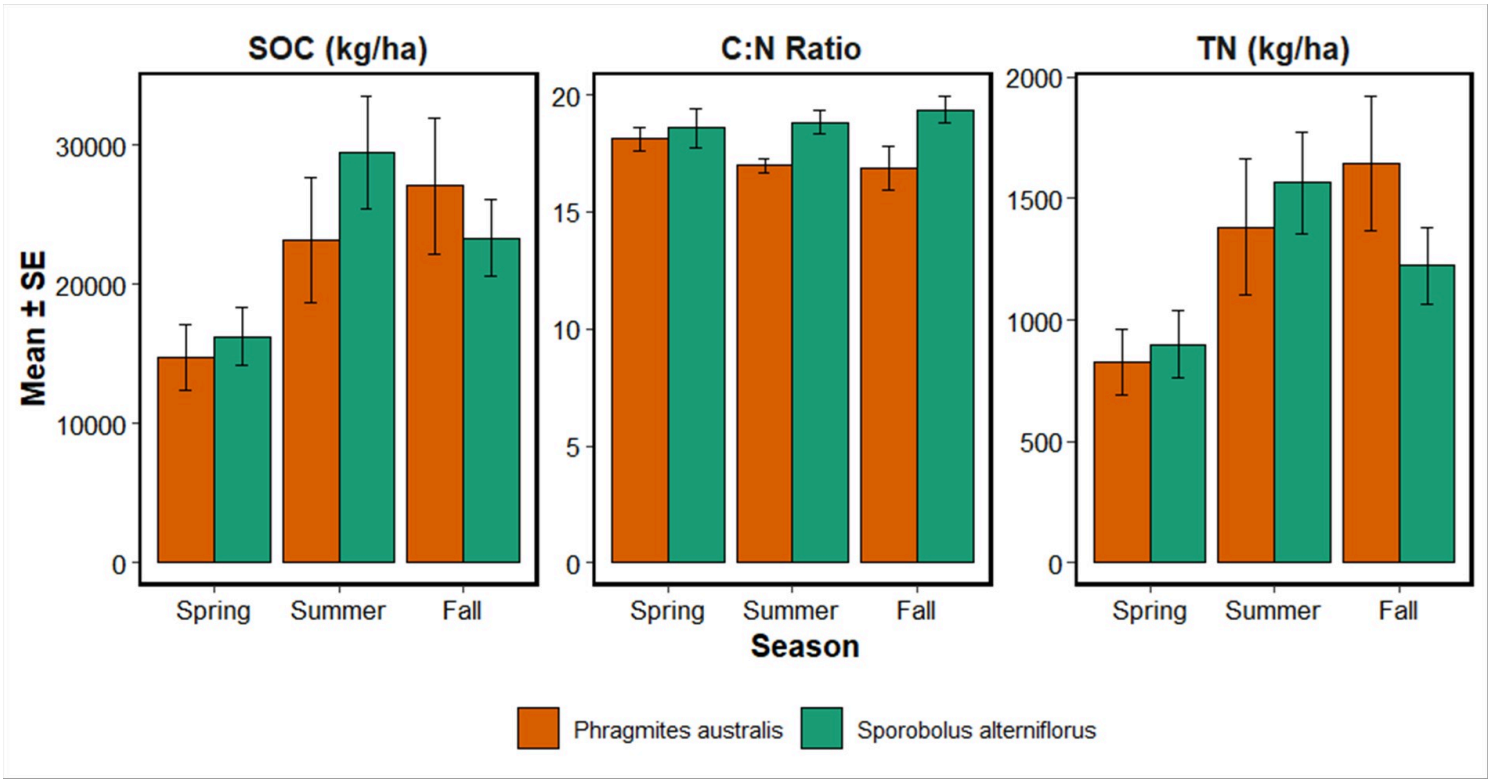



Figure 5

Mean (+- SE) seasonal soil nutrients under Phragmites australis and *Sporobolus alterniflorus* at Quinnipiac Meadows, CT.

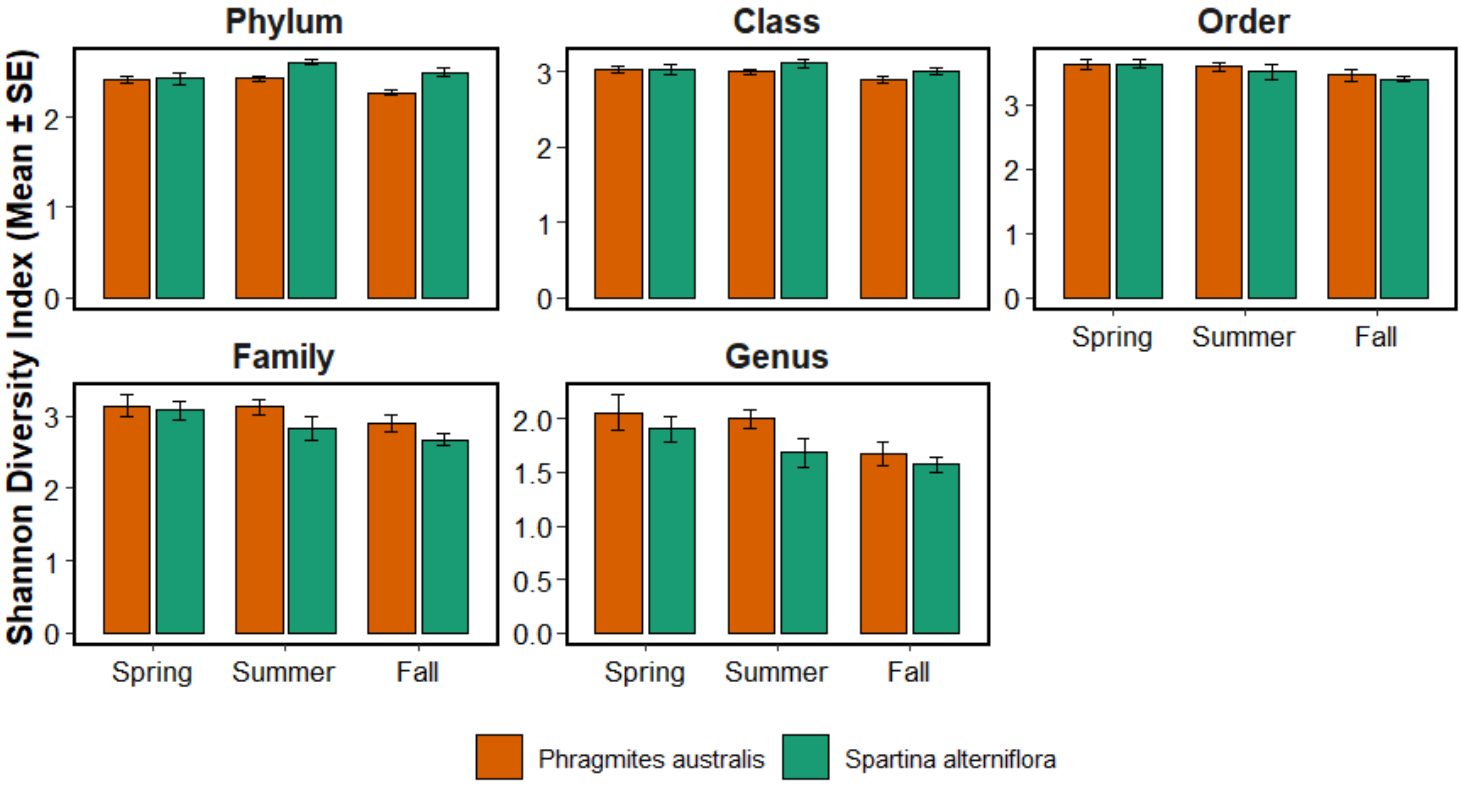



**Figure 6**

Seasonal comparison of Shannon diversity index (alpha diversity) at the Genus level between Phragmites and Sporobolus soils.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- oturitwiktable.csv
- RitwikNutrients.csv