

Impacts of invasive *Phragmites australis* on nitrogen cycling in coastal marshes

Project # 2017-R/MG-1711

Project Start Date: 12/1/2017

Project End Date: 6/30/2019

Final Report Submission Date: 8/30/2019

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Abstract

Marshes function as a buffer zone at the land-sea interface and play a key role in global nitrogen (N) cycling. Invasive species pose a threat to these systems globally. One of the most aggressive invasive plant species in marshes across the United States (US) is the European haplotype of *Phragmites australis*, which has spread throughout the US over the past several centuries. We hypothesized that *P. australis* may play an important role in marsh N cycling. In this study, we conducted seasonal gas (N_2 and O_2) fluxes at three sites within the Albemarle-Pamlico Region (Currituck Banks Reserve, Kitty Hawk Woods Reserve, and the Rachel Carson Reserve), focusing on invasive *P. australis*, native *Spartina alterniflora* and/or *Juncus roemerianus*, and unvegetated mudflat. We found net denitrification in sediments associated with *P. australis* was highest across all seasons, though this was not always a significant difference, compared to other marsh species or unvegetated mudflat in the Rachel Carson Reserve. In both the Currituck Banks Reserve and Kitty Hawk Woods Reserve, N_2 fluxes were not distinct between marsh species or unvegetated sediments and were likely driven by other factors such as sediment organic matter and availability of water column nutrients. Addition of nitrate to simulate storm conditions enhanced net denitrification in the Rachel Carson Reserve for all marsh species, indicating nitrate limitation. Furthermore, dissolved nitrogen fluxes measured prior to and following Hurricane Florence indicated this trend was present during actual storm conditions, with *P. australis* having a significant increase in rate of denitrification following the storm. This enhancement across marsh habitats was evident at Currituck Banks during the spring but not during fall and N_2 flux rates at the other brackish site did not respond to nitrate enrichment during either season. These results demonstrate the transition to *P. australis* from native marsh species may alter nitrogen processing to increase removal of excess nitrogen in some areas.

Introduction

The Albemarle-Pamlico estuarine system, comprised of 8 sounds, is second only in size in the United States to the Chesapeake Bay, and supports an array of important habitats for ecologically and economically important fish and shellfish. The system acts as a drainage basin for 36 North Carolina counties and 16 Virginia counties, covering over 77,000 km².

Human activities over the past century have resulted in a major increase in nutrients delivered to coastal ecosystems (Vitousek et al., 1997; Howarth, 1998). Water quality in the Albemarle-Pamlico system is primarily affected by nutrient loads from agricultural runoff and animal waste discharge, with 32% (~1.9 million hectares) of the total management area's land use comprised of farmland and livestock operations (USDA, 2012). Because nitrogen (N) is limiting for primary production in marine ecosystems (Howarth and Marino, 2006), heavy N loads can impact the water quality in the estuarine system by causing eutrophication (Selman, et al., 2008; Seitzinger et al., 2006), anoxia (Diaz and Rosenberg, 2008), and harmful algal blooms (Anderson et al., 2002; Anderson, 2009). This issue is magnified during major

storm events, when large pulses of bioavailable nitrogen are flushed into coastal waters (Paerl et al., 2018).

Marshes are a valuable habitat that can function as a buffer zone at the land-sea interface (Deegan et al., 2012), and play a key role in global biogeochemical cycles of carbon (McLeod et al., 2011) and nitrogen (Valiela et al., 1975, 1976; Chalmers, 1979). This is particularly true in North Carolina, where coastal marshes comprise 17% of the state's total land area, with much of that area in the Albemarle-Pamlico region (Fretwell et al., 1996). Marshes have the capacity to remove and retain excess nutrients (Valiela et al., 1975, 1976) including as much as 20-50% of externally sourced nitrogen (Galloway et al., 2004; Seitzinger et al., 2006; Deegan et al., 2012). Nitrogen removal occurs through biological processes, such as denitrification, during which microbial communities in the sediment transform reactive nitrogen species to unreactive N₂ (Seitzinger et al., 1998, 2006) or physical processes such as the burial of N in the sediments (Merrill and Cornwell, 2002). If excess N is not intercepted by marshes, it flows into adjacent estuaries, eventually making its way to the open ocean. Estuaries provide habitat for over 75% of our commercially important fish (National Safety Council's Environmental Center, 1998) and act as the last line of defense for these important ecosystems. It is vital to understand changes in marsh functions in order to maintain and protect ecosystems on both local and global scales.

Invasive species threaten coastal systems globally (Chambers et al. 1999). One of the most aggressive plant species in marshes across the United States (US) is *Phragmites australis* (Saltonstall, 2012). Though *P. australis* is native to the US, an aggressive European haplotype has spread throughout the US over the past several centuries and has all but completely supplanted its native counterpart, due to its ability to thrive in high nutrient and disturbed conditions (Minchinton and Bertness, 2003). As is the case with other invasive species, invasive *P. australis* is known to negatively impact biodiversity of both flora and fauna (Vitousek et al., 1996; Sala et al., 2000; Silliman and Bertness, 2004).

In North Carolina, *P. australis* is classified as a high-priority aquatic nuisance species, and coastal managers have made a concerted effort to control and eradicate the plant through mowing, burning, and chemical approaches (Hazelton et al., 2014). These eradication efforts have largely been unsuccessful due to the resilient nature of this primary producer, though may result in a temporary decrease in aboveground biomass. In the rare case of complete eradication, there is limited evidence suggesting that marshes return to their previous natural state. Rather than returning native vegetation, the atrophied marsh platform is left susceptible to erosion and rising level.

Though invasive *P. australis* is traditionally considered an undesirable species, recent studies show it performs closely to native counterparts in terms of ecosystem services such as carbon storage and shore stabilization (Theuerkauf et al., 2016). *P. australis* and other invasive species have even been shown to enhance ecosystem function under certain conditions (Ramus et al., 2017, Theuerkauf et al., 2016). In comparison to adjacent marshes dominated by native *Spartina alterniflora*, *P. australis*-dominated marshes also have

significantly higher rates of vertical accretion (Rooth et al., 2003). This is particularly important with regard to sea level rise, as vertical accretion is the predominant means for marshes to keep pace with rising waters, as horizontal migration is typically limited due to human developments (Pontee, 2013).

Nitrogen processing is a critical feature in sustaining ecosystem health and function, and invasive marsh grasses may have a currently unquantified yet significant impact on this ecosystem service. While *P. australis* is largely non-native, understanding its contributions to ecosystem function is critical, particularly due to the complications, uncertainty, and cost of attempting eradication. Quantifying the impact that *P. australis* has on nitrogen cycling in this system has direct application to sustaining water quality, as eutrophication is one of the largest impairments to water quality in these coastal systems.

The objectives of this study were to determine the impact of invasive *P. australis* on the biogeochemical cycling of nitrogen in tidal marshes in the Albemarle-Pamlico estuarine system. Specifically, to quantify the transformations of nitrogen in the sediments associated with non-native *P. australis*, native marsh grasses likely to be displaced (i.e., *Spartina alterniflora* and *Juncus roemarianus*), and unvegetated mudflat sediments during both ambient and storm conditions.

We hypothesized sediments associated with invasive *P. australis* would have distinctive N cycling as compared to native marsh sediments and unvegetated sediments. We also hypothesized that the sediments in different areas with native vegetation would not have significantly different net N₂ fluxes and unvegetated sediments would have significantly lower net N₂ fluxes. In respect to denitrification efficiency, we hypothesized that the removal efficiency in *P. australis* would be lower than in native species, but that it would increase during storm conditions.

Methods

Site Description

The Albemarle-Pamlico System is a large, temperate estuary that extends from southeastern Virginia and continues south along the North Carolina coast. It has a total surface area of over 8000 km², and an average depth of 4.9 m. There is a total of six rivers, the Pasquotank, Chowan, Roanoke, Tar-Pamlico, Neuse, and White Oak, that drain into the system, draining over 77,000 km². The system is split into eight different sounds, the Albemarle, Pamlico, Back, Bogue, Core, Croatan, Currituck, and Roanoke. We chose to sample at three sites, all on state or federally protected lands: the Currituck Banks National Estuarine Research Reserve (CBNERR), the Kitty Hawk Woods Coastal Reserve (KHWCR), and the Rachel Carson National Estuarine Research Reserve (RCNERR) (Figure 1) to roughly represent the northern, middle, and southern portions of the system and respectively representing the Currituck Sound, Albemarle Sound, and Back Sound. Both CBNERR and KHWCR are primarily influenced by wind driven tides, while RCNERR experiences a semi-diurnal tide. Sites were sampled during spring, summer, and fall of 2018; however, due to

Hurricane Florence, we were unable to complete flux experiments on samples collected from CBNERR and KHWCR. Following Hurricane Florence, an additional flux experiment was completed at RCNERR, capturing a genuine storm event to compliment simulated ones completed during regular flux experiments. These sites slightly differed from our original proposed sites, as we replaced the Buxton Woods Coastal Reserve with the Rachel Carson National Estuary Research Reserve.

Sample Collection

Sediment cores were collected in triplicate at each site in clear polyvinyl chloride (PVC) tubes measuring 6.4 cm in diameter and 31 cm in height. Sediment height in each core was approximately 17 cm. Cores were collected by hand or with a PVC pole-corer fitted with a check valve. Cores were collected within patches of non-native *P. australis*, *Juncus roemarianus* and/or *Spartina alterniflora* and unvegetated mudflat. Efforts were made to exclude live plant material or living organisms (i.e. snails, crabs, etc) from the cores. Sixty liters of water was collected adjacent to marsh habitats. Samples were transported to the Institute of Marine Sciences in Morehead City, NC. Cores were immediately submerged in site water placed in an environmental chamber (Bally Inc.) set to *in situ* water temperature. The cores and waters were left overnight to ensure temperature equilibration.

Continuous flow-through flux experiments were performed according to the methods detailed in Piehler and Smyth, 2011. Gas-tight caps with inflow and outflow ports were placed on each submerged core excluding air bubbles and subsequently connected to the flow through system. This system used a multichannel peristaltic pump to pump unfiltered, aerated site water into the top of core while drawing water from just above the sediment surface from the bottom of the core at a rate of 1 mL min⁻¹ for an approximate turnover time of 5 hours. Following capping, cores were left in the unlit environmental chamber for approximately 3 complete turnovers over water within the cores.

Water samples flowing from the cores and bypass lines (considered inflow waters) were sampled periodically over the next 2-3 days. Additional water samples were collected, filtered and frozen for later nutrient analysis of NO_x⁻, NH₄⁺, PO₄³⁻, and total nitrogen (TN) (μM) with a Lachat Quick-chem 8000 (Lachat Instruments, Milwaukee, WI, USA) for.

Additional sediment samples were collected directly adjacent to core sampling locations to determine the sediment chlorophyll concentration and the molar concentration of carbon and nitrogen using a CHN elemental analyzer (Dalsgaard et al., 2000). Upon completion of core incubations, we collected a 3 cm subsample from each core, for analysis of sediment organic matter concentration via loss on ignition. Efforts were made to minimize the number of roots and rhizomes present in the subsamples used for these analyses.

Nutrient Enrichment

Following collection of the first three sample timepoints on day 1 of the flux experiment, NaNO_3 was added to the inflow waters, raising the nitrate concentration by $20 \mu\text{M}$ NO_3 , to simulate a storm pulse of nitrate. Cores were then allowed to incubate for a minimum of 15 hours, again allowing for approximately 3 turnover times of water in the cores.

Flux Calculations

Dissolved gas samples were analyzed using a membrane inlet mass spectrometer (MIMS) for $\text{N}_2:\text{Ar}$ and $\text{O}_2:\text{Ar}$ in order to measure the net N_2 flux and O_2 flux within the sediment which were calculated with the following equation (Kana et al., 1998; Fulweiler and Nixon, 2009; Piehler and Smyth, 2011):

$$\text{Flux} = \left(\frac{(\text{Outflow concentration } (\mu\text{m}) - \text{Inflow concentration } (\mu\text{m})) * \text{pump rate } (\text{L hr}^{-1})}{\text{core surface area } (\text{m}^2)} \right)$$

Using equation 1, a positive number is indicative of a net denitrification, and a negative number is indicative of net nitrogen fixation. To convert the O_2 flux to sediment oxygen demand (SOD), results were multiplied by -1.

Denitrification efficiency (DNE) is the percentage of inorganic nitrogen that is released from the sediments as N_2 gas during the flux experiment. This is used as a measure of how much of the proportion of the total inorganic nitrogen flux observed in the water column is accounted for by denitrification in the sediments. DNE was calculated by the methods in Eyre & Ferguson 2002 using the following equation:

$$\text{Denitrification Efficiency } (\%) = \left(\frac{\text{N}_2 \text{ Flux}}{\text{N}_2 \text{ Flux} + (\text{NO}_2 \text{ Flux} + \text{NH}_4^+ \text{ Flux})} \right) * 100$$

Statistical Analysis

All data from this study were analyzed using JMP Pro 13.0 and R software packages. Data were assessed for normality using the Shapiro-Wilk test in JMP and R. It was found that these results were not normally distributed (Shapiro-Wilk ≤ 0.0001). Due to the non-normality of the data, Kruskal-Wallis non-parametric analyses were used for all data analysis.

Results

Sediment Gas Fluxes

Rachel Carson Reserve

Spring flux experiments in RCNERR showed *P. australis* ($65.86 \pm 10.43 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) had the highest rate of denitrification during ambient conditions compared to unvegetated mudflat ($15.55 \pm 12.23 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) and *S. alterniflora* ($-1.26 \pm 5.06 \mu\text{mol N m}^{-2} \text{hr}^{-1}$); however, this was not a significant difference (Fig 1). Following nitrate enrichment, a significant increase in denitrification ($p < 0.05$) was observed in *P. australis* ($226.76 \pm 18.38 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) as compared to the rate for the habitat prior to enrichment.

Summer flux experiments showed *P. australis* ($182.32 \pm 5.60 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) had significantly higher rates of denitrification (< 0.05) than *S. alterniflora* ($38.26 \pm 4.16 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), *J. roemarianus* ($34.47 \pm 6.44 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), and unvegetated mudflat ($40.32 \pm 3.56 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) (Fig 1). *P. australis* also exhibited significantly higher rates than those observed during the spring. An increase in this rate is observed following nitrate enrichment; however, this was not a significant increase. Following nitrate enrichment, *P. australis* ($206.43 \pm 25.03 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) had a significantly higher rate of denitrification than *J. roemarianus* ($82.38 \pm 49.04 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) and unvegetated mudflat ($97.06 \pm 18.26 \mu\text{mol N m}^{-2} \text{hr}^{-1}$).

All marsh habitats measured in the RCNERR study showed net denitrification in sediments prior to and following Hurricane Florence (Fig 4). In *P. australis* and *J. roemarianus*, the rates of denitrification following Hurricane Florence were significantly higher (322.83 ± 20.50 and $73.29 \pm 8.61 \mu\text{mol N m}^{-2} \text{hr}^{-1}$, respectively) than the pre storm rates (182.32 ± 5.60 and $34.47 \pm 6.44 \mu\text{mol N m}^{-2} \text{hr}^{-1}$, respectively). *P. australis* exhibited significantly higher rates of denitrification ($p < 0.05$) compared with other marsh and unvegetated sediments measured during both time periods

Similar to the spring and summer, *P. australis* exhibited the highest rate of denitrification ($179.29 \pm 12.57 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) across the habitats sampled in the fall; however, it was not significantly higher than ($p < 0.05$) than *S. alterniflora* ($32.27 \pm 17.21 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), unvegetated mudflat ($40.50 \pm 4.88 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), and *J. roemarianus* ($86.39 \pm 15.04 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) (Fig 1). Though an increase in denitrification was observed in *J. roemarianus* ($90.48 \pm 9.59 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) and *P. australis* following nitrate enrichment, neither were significant changes.

Kitty Hawk Woods

During both the spring and fall in KHWCR, N_2 fluxes were generally positive but low compared to the other sites sampled. No significant differences in sediment N_2 fluxes were present between *S. alterniflora* ($59.43 \pm 1.31 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), unvegetated mudflat ($23.21 \pm 3.93 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), or *P. australis* ($45.04 \pm 8.24 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) during the spring at KHWCR (Fig 3). No significant change in rate of denitrification was observed across any of the three marsh/unvegetated habitats following nitrate enrichment.

There were no significant differences between spring and fall in any of the three habitats, though the average rate of all three habitats is higher in the fall than the spring. No significant differences were observed between *S. alterniflora* ($68.40 \pm 3.88 \mu\text{mol N m}^{-2} \text{hr}^{-1}$),

unvegetated mudflat ($97.82 \pm 41.61 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), and *P. australis* ($68.08 \pm 12.89 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) (Fig 3). No change in rate of denitrification was observed following nitrate enrichment.

Currituck Banks

Rates of denitrification during the spring in CBNERR were low across all habitats, with neither unvegetated mudflat ($5.74 \pm 9.40 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) nor *P. australis* ($-7.58 \pm 4.36 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) being significantly different than 0 (Fig 2). *J. roemarianus* ($23.04 \pm 3.39 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) was significantly different than 0; however, not statistically different than either other habitats. Following nitrate enrichment, a significant increase was observed in *J. roemarianus* ($127.40 \pm 10.84 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), unvegetated mudflat ($75.45 \pm 19.47 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), and *P. australis* ($81.79 \pm 10.35 \mu\text{mol N m}^{-2} \text{hr}^{-1}$), though there was no significant differences between habitats.

Rates of denitrification were significantly higher ($p < 0.05$) in both unvegetated mudflat ($37.74 \pm 6.32 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) and *P. australis* ($75.96 \pm 30.33 \mu\text{mol N m}^{-2} \text{hr}^{-1}$) sediments in the fall compared with those in the spring (Fig 2). *P. australis* also exhibited a significantly higher ($p < 0.05$) rate of denitrification than both unvegetated mudflat and *J. roemarianus* ($39.37 \pm 29.07 \mu\text{mol N m}^{-2} \text{hr}^{-1}$). Following nitrate enrichment there was a significant increase ($p < 0.05$) in rate of denitrification in *J. roemarinaus* ($57.62 \pm 12.61 \mu\text{mol N m}^{-2} \text{hr}^{-1}$).

Denitrification Efficiency (DNE)

Rachel Carson Reserve

Denitrification efficiency in RCNERR was greater than 50% across all environment types except during the spring in *S. alterniflora* and *P. australis* (Fig. 6). An increase in DNE was observed across all habitats and seasons following nitrate enrichment. Between environment types, DNE was relatively stable though *P. australis* had the lowest in the summer and fall. DNE during the spring was slightly lower across all habitats sampled (*S. alterniflora*, *P. australis*, and unvegetated mud flat), than the summer and fall.

Kitty Hawk Woods Reserve

Denitrification efficiency in KHWCR were extremely high across all seasons and environment types and all were greater than 85% (Fig 7). DNE remained stable both seasonally and between marsh species/mid flat habitats. Following nitrate enrichment, a decrease in DNE occurred across all samples.

Currituck Banks Reserve

During the spring, an increase in efficiency was observed in mudflat and *P. australis* samples following nitrate enrichment; however, there was no effect on DNE in *J. roemarianus* following enrichment. Denitrification efficiency in CBNERR appeared to somewhat vary seasonally, with decreases in DNE being present in *J. roemarianus* and unvegetated mudflat

samples between the spring and fall (Fig 8). The opposite was observed in *P. australis*, with an increase in DNE between the spring and fall samples. Unvegetated mudflat sediments shows the same increase in DNE following nitrate enrichment during the fall as in the spring. *P. australis* also exhibits an increase in DNE following nitrate enrichment during the fall, and no change is observed in *J. roemarianus*.

Sediment Organic Matter (SOM)

Rachel Carson Reserve

During the spring at RCNERR, sediments associated with *P. australis* had a significantly greater ($p < 0.05$) sediment organic matter ($39 \pm 11\%$) than both *S. alterniflora* and mudflat sediments. Similarly, during the summer, *P. australis* sediments once again had a significantly greater ($p < 0.05$) SOM percentages than *S. alterniflora*, *J. roemarianus*, and mudflat sediments. This same trend is observed in the fall samples as well.

Kitty Hawk Woods

Sediment organic matter was low across all habitats sampled in KHWCR during the spring, and the average SOM (%) was below 1% for all three habitats. *S. alterniflora* sediments are observed having a significantly greater ($p < 0.05$) percentage OM than *P. australis* sediments. A significant decrease in SOM was observed across all three habitats between the spring and fall, with all three habitats falling under 0.10% OM. During the fall, *P. australis* sediments had a significantly greater ($p < 0.05$) percentage OM than mudflat sediments.

Currituck Banks Reserve

During the spring in CBNERR, organic matter (OM) is particularly high in sediments associated with *J. roemarianus* ($69 \pm 1\%$) and *P. australis* ($54 \pm 3\%$), and *J. roemarianus* is significantly greater than *P. australis* ($p < 0.05$). Both *J. roemarianus* and *P. australis* sediments had significantly greater ($p < 0.05$) OM than mudflat sediments ($11.0 \pm 3.0\%$). A significant decrease ($p < 0.05$) in OM is observed in the fall across all three habitats sampled; however, sediment organic matter in *P. australis* sediments is significantly greater ($p < 0.05$) than both mudflat and *J. roemarianus* sediments.

Discussion

Understanding how the presence of *P. australis* in coastal systems impacts rate and efficiency of denitrification in the underlying sediments is critical for evaluating how the invasive species may be overall impacting the function of coastal marshes, as these systems are heavily relied upon for this important ecosystem service.

Differences between P. australis and native grasses

Across the three sites included in this study, differences are observed between *P. australis* and its native counterparts at different points in the growing season during both ambient and nitrate-enriched conditions in the RCNERR and CBNERR.

At RCNERR *P. australis* exhibits the highest rate of denitrification across all habitats sampled during ambient conditions in all three seasons, though this difference is only significant during the summer (Fig. 2). During nitrate enriched conditions, which would be expected following a major storm event, *P. australis* once again has the highest rate of denitrification in the spring and summer, significantly greater than that of *S. alterniflora* and unvegetated mudflat in the spring and significantly greater than all three habitats in the summer (Fig. 2). This difference is observed following a major storm event, Hurricane Florence, with *P. australis* exhibiting significantly higher rates of denitrification than rates measured in the same site previous to the storm and to all other habitats following the storm (Fig. 3). Denitrification efficiency is high across all the habitats sampled and consistently increases following nitrate enrichment; however, efficiency is lowest in *P. australis* both prior to and following nitrate enrichment (Fig. 6).

The KHWCR had the lowest overall rates of denitrification of the three sites included in this study, and there was both no differences between species and no change in denitrification rate in any of the habitats sampled following nitrate enrichment or between season (Fig. 4). Given the extremely low sediment organic matter percentages in this location (Fig. 10), it is likely the lack of carbon that is predominately driving the consistently low rates of denitrification.

Though CBNERR experienced extremely low rates of denitrification during spring sampling, with only *J. roemarianus* performing at a rate that significantly differed from 0, a significant increase was observed across all three habitats following nitrate enrichment, which is indicative of low water column nutrient concentrations limiting denitrification in the system (Fig 5, Table 1). This is also supported by the extremely high sediment organic matter percentages, making it unlikely that carbon in the sediments would act as a limiting factor (Fig. 11).

During the fall, a significant increase was observed across all three habitats, potentially indicative of higher ambient nutrient concentrations in the water column (Fig. 5, Table 1). It was also observed during the fall that *P. australis* had significantly higher rates of denitrification than both the native *J. roemarianus* and unvegetated mudflat, which may indicate that during time periods of higher water column nutrient concentrations, *P. australis* will outperform native counterparts in this respect (Fig. 5). Unlike during the spring, there was no change in denitrification rate following nitrate enrichment, which could potentially be due to significantly lower amounts of available carbon, indicated by lower sediment organic matter percentages (Fig. 11).

These data suggest that, during some points in the growing season in RCNERR, sediments associated with *P. australis* have the ability to remove greater loads of bioavailable N as it enters the system than native counterparts, though they may do so less efficiently than *S. alterniflora*, *J. roemarianus*, or unvegetated mudflat. This is observed during both simulated (nitrate enriched) and actual (Hurricane Florence) storm conditions.

At CBNERR and KHWCR, other factors may inhibit denitrification from occurring, such as lack of available nutrients in the water column or lack of available organic matter in the sediments.

Management Implications

Traditional ecosystem management has focused on attempting to control and/or eradicate *P. australis* from habitats, primarily due to the plant being attributed to decreased biodiversity of fauna (Dibble et al., 2013). In the face of a rapidly changing natural world, management decisions need to be based on ecosystem health as a complete unit. As a high nutrient specialist, *P. australis* can thrive in coastal marshes that are impacted by nutrient pollution (Minchinton & Bertness, 2003), and is capable of outcompeting native counterparts in these conditions. Additionally, marshes dominated by *P. australis* have higher rates of vertical accretion as compared to adjacent marshes dominated by native plants (Rooth et al., 2013), making them more resilient to sea level rise. It has also been observed to often be more effective in stabilizing marsh platforms than its native counterparts (Theuerkauf et al., 2017), and its growth is promoted in areas of the United States, such as the Gulf Coast, which already are heavily impacted by sea level rise and coastal erosion.

This study has highlighted *P. australis*' ability to foster higher rates of denitrification in underlying sediments than native species such as *S. alterniflora* and *J. roemarianus* during certain ambient and storm conditions, suggesting that it enhances the capacity of saltmarshes to act as a sink for nutrients. However, despite higher rates of denitrification, the efficiency of the process may be lower in many cases than in the sediments of the native counterparts.

As coastal managers are faced with this highly invasive species, the first course of action is typically to attempt eradication; however, it is well documented that eradication is both costly and largely ineffective. Considering this study and other recent works regarding the role that *P. australis* plays in providing ecosystem services such as nitrogen removal, shoreline stabilization, and increased vertical accretion, coastal managers should consider whether eradication efforts are justified.

Outreach

The results of this project have been communicated to both the general public and scientific communities in several formats. Graduate student, Mollie Yacano, has presented these data in both private and public events, including the 2018 Fall meeting of UNC-CH's IE Board of Visitors, NCSG Federal Review Poster Session, NCSG Advisory Board, APNEP Science & Technical Advisory Committee, American Geophysical Union (AGU)'s 2018 Fall Meeting, and Research Applied to Managing the Coast Symposium (RAMCS). An Outstanding

Student Presentation Award (OSPA) was awarded for the AGU presentation. An abstract has also been submitted and accepted for these data to be presented at the Coastal & Estuarine Research Federation (CERF) biennial meeting in November 2019. A lesson plan targeting elementary and middle school students was developed for the Science and Research Education Network (SciREN), distributed to area teachers during the 2019 Coast exchange event, and is currently available through the SciREN lesson plan portal.

Students Supported

This minigrant supported Mollie Yacano's graduate research. In addition, it supported an undergraduate student, Peggy Mullin, assisting in lab and field components of the project during Summer 2018.

NOAA Data Management Plan

The Albemarle-Pamlico Nitrogen Processing Project, implemented by Mollie Yacano (graduate student) generated environmental data and information, including: the rates of denitrification and/or nitrogen fixation in sediments underlying invasive *P. australis*, dominant marsh grasses (*Spartina*, *Juncus*, etc), and unvegetated sediments, pore water and water column concentrations of nitrogen and phosphorus, ambient water quality data (temperature, salinity, dissolved oxygen, and turbidity), percent organic matter, sediment chlorophyll concentrations, and carbon to nitrogen ratios. Nitrogen flux and nutrient data was collected by the Piehler Lab according to the procedures described Piehler and Smyth, 2011. Data was stored in excel documents. Water quality data was collected and stored using a YSI automatic sampler and was downloaded and transferred to excel files upon return from the field. Percent organic matter, sediment chlorophyll, and carbon to nitrogen ratio analysis was performed using standard operating procedures, (Dalsgaard et al., 2000) and all results were recorded in a laboratory notebook and transferred into an excel document upon completion. Field data collected was recorded in waterproof field notebooks and transferred into excel spreadsheets for storage and analysis. Field data included sampling locations (latitude, longitude), dates of sample collection, and any other pertinent information. A master excel document was kept that includes all field and laboratory results and includes a metadata tab with descriptions of the project, all of the data types included in the file, abbreviations used, contact information, and any other pertinent information. All data is saved locally on the laboratory computer, on an external hard drive, and backed up on both a Dropbox and One Drive server. The data will be available to our PIs and collaborators on the project until the results are published in peer-reviewed journals, or January 1, 2021, whichever comes first. Contact Mollie Yacano (moyacano@live.unc.edu) or Dr. Michael Piehler (mpiehler@email.unc.edu) for more information or to make a data request. In the past, we have shared similar data by publishing peer-reviewed manuscripts, conference presentations, and through our Institution's website. All future sub-awardees not identified in this plan will have as a condition of their contract acceptance of this data-sharing plan. Any additional data sharing stipulations for future sub-awardees may be outlined at that time and described in their contract.

References:

- Anderson, D. M., Glibert, P. M., and Burkholder, J. M. (2002). Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25, 704–726. doi: 10.1007/BF02804901
- Anderson, D. M. (2009). Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean Coast. Manag.* 52, 342–347. doi: 10.1016/j.ocecoaman.2009.04.006
- Bridgham SD, Megonigal JP, Keller JK, Bliss NB, Trettin C. The carbon balance of North American wetlands. *Wetlands*. 2006; 26: 889–916.
- Chalmers AG (1979) The effects of fertilization on nitrogen distribution in a *Spartina alterniflora* salt marsh. *Estuar Coast Mar Sci* 8:327–337
- Chambers, R. M., Meyerson, L. A., & Saltonstall, K. (1999). Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic botany*, 64(3), 261-273.
- Choi, W. J., Ro H.M., Chang S.X. 2005. Carbon isotope composition of *Phragmites australis* in a constructed saline wetland. *Aquatic Botany* 82: 27–38.
- "Comprehensive Conservation and Management Plan, 2012-2022." *Albemarle-Pamlico National Estuary Partnership*. 14 Mar. 2012.
- Dalsgaard, Tage. Protocol Handbook for NICE - Nitrogen Cycling in Estuaries: A Project under the EU Research Programme: Marine Science and Technology (MAST III). Silkeborg: National Environmental Research Institute, Department of Lake and Estuarine Ecology, 2000. Print.
- Deegan, L. A., Johnson, D. S., Warren, R. S., Peterson, B. J., Fleeger, J. W., Fagherazzi, S., & Wollheim, W. M. (2012). Coastal eutrophication as a driver of salt marsh loss. *Nature*, 490(7420), 388-392.
- Diaz, R. J., and Rosenberg, R. (2008). Spreading dead zones and consequences for marine ecosystems. *Science* 321, 926–929. doi: 10.1126/science. 1156401
- Dibble, K. L., Pooler, P. S., & Meyerson, L. A. (2013). Impacts of plant invasions can be reversed through restoration: a regional meta-analysis of faunal communities. *Biological Invasions*, 15(8), 1725-1737.
- Emery, H. E., & Fulweiler, R. W. (2014). *Spartina alterniflora* and invasive *Phragmites*

- australis stands have similar greenhouse gas emissions in a New England marsh. *Aquatic botany*, 116, 83-92.
- Fretwell, J. D., Williams, J. S., & Redman, P. J. (1996). *National water summary on wetland resources* (No. 2425). US Government Printing Office.
- Fulweiler, R. W., & Nixon, S. W. (2009). Responses of benthic–pelagic coupling to climate change in a temperate estuary. *Hydrobiologia*, 629(1), 147-156.
- Hazelton, E. L., Mozdzer, T. J., Burdick, D. M., Kettenring, K. M., & Whigham, D. F. (2014). Phragmites australis management in the United States: 40 years of methods and outcomes. *AoB plants*, 6.
- Hesslein, R. H. 1976. An in-situ sampler for close interval porewater studies. *Limnology & Oceanography*. 21:912-914.
- Howarth R.W. 1998. An assessment of human influences on fluxes of nitrogen from the terrestrial landscape to the estuaries and continental shelves of the North Atlantic Ocean. *Nutr. Cycl. Agroecosyst.* 52: 213–223.
- Howarth R.W., Marino Roxanne, (2006), Nitrogen as the limiting nutrient for eutrophication in coastal marine ecosystems: Evolving views over three decades, *Limnology and Oceanography*, 51(1, part 2), doi: 10.4319/lo.2006.51.1_part_2.0364.
- Kana, T. M., M. B. Sullivan, J. C. Cornwell, and K. Groszkowski. 1998. Denitrification in estuarine sediments determined by membrane inlet mass spectrometry. *Limnol. Oceanogr.* 43:334–339.
- Merrill, J. Z., & Cornwell, J. C. (2002). The role of oligohaline marshes in estuarine nutrient cycling. *Concepts and controversies in tidal marsh ecology*, 425-441.
- Minchinton, T., Bertness, M., 2003. Disturbance-mediated competition and the spread of Phragmites australis in a coastal marsh. *Ecol. Appl.* 13, 1400–1416.
- National Safety Council's Environmental Center. 1998. Coastal Challenges: A Guide to Coastal and Marine Issues. Prepared in conjunction with Coastal America. <http://www.nsc.org/ehc/guidebks/coasttoc.htm>
- "North Carolina Sea Grant Strategic Plan 2018-2021." *North Carolina Sea Grant*.

- Paerl, H. W., Crosswell, J. R., Van Dam, B., Hall, N. S., Rossignol, K. L., Osburn, C. L., & Harding, L. W. (2018). Two decades of tropical cyclone impacts on North Carolina's estuarine carbon, nutrient and phytoplankton dynamics: implications for biogeochemical cycling and water quality in a stormier world. *Biogeochemistry*, *141*(3), 307-332.
- Piehl, M. F., and A. R. Smyth. 2011. Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services. *Ecosphere* 2(1): art12. doi:10.1890/ES10-00082.1
- Pontee, N. (2013). Defining coastal squeeze: A discussion. *Ocean and Coastal Management*, *84* (November), 204–207. <https://doi.org/10.1016/j.ocecoaman.2013.07.010>
- Ramus, A. P., Silliman, B. R., Thomsen, M. S., & Long, Z. T. (2017). An invasive foundation species enhances multifunctionality in a coastal ecosystem. *Proceedings of the National Academy of Sciences*, *114*(32), 8580-8585.
- Ravit, B., Ehenfeld J.G., Haggblom, M.M. 2003. A comparison of sediment microbial communities associated with *Phragmites australis* and *Spartina alterniflora* in two brackish wetlands of New Jersey. *Estuaries* 26: 465–474.
- Rooth, J. E., Court Stevenson, J., & Cornwell, J. C. (2003). *Increased Sediment Accretion Rates Following Invasion by Phragmites australis: The Role of Litter* (Vol. 26).
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., & Leemans, R. (2000). Global biodiversity scenarios for the year 2100. *science*, *287*(5459), 1770-1774.
- Seitzinger, S., Harrison, J. A., Bohlke, J. K., Bouwman, A. F., Lowrance, R., Peterson, B., et al. (2006). Denitrification across landscapes and waterscapes: a synthesis. *Ecol. Appl.* 16, 2064–2090. doi: 10.1890/1051-0761(2006)016[2064:DALAWA]2.0.CO;2
- Seitzinger, S. P. (1988). Denitrification in freshwater and coastal marine ecosystems: ecological and geochemical significance. *Limnol. Oceanogr.* 33, 702–724. doi: 10.4319/lo.1988.33.4_part_2.0702
- Selman, M., Greenhalgh, S., Diaz, R., and Sugg, Z. (2008). *Eutrophication and Hypoxia in Coastal Areas: A Global Assessment of the State of Knowledge*. Washington, DC: World Resources Institute Policy Note.
- Silliman, B. R., & Bertness, M. D. (2004). Shoreline development drives invasion of *Phragmites australis* and the loss of plant diversity on New England salt marshes. *Conservation Biology*, *18*(5), 1424-1434.

- Teasdale, P. R., Batley, G. E., Apte, S. C., & Webster, I. T. (1995). Pore water sampling
- Theuerkauf, S. J., Puckett, B. J., Theuerkauf, K. W., Theuerkauf, E. J., & Eggleston, D. B. (2017). Density-dependent role of an invasive marsh grass, *Phragmites australis*, on ecosystem service provision. *PloS one*, *12*(2), e0173007.
- USDA 2012 North Carolina census publications. Retrieved from USDA Census of Agriculture Website.
- Valiela I, Teal JM, Sass WJ (1975) Production and dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge. Biomass, production, and species composition. *J Appl Ecol* 12:973–981
- Valiela I, Teal JM, Persson NY (1976) Production and dynamics of experimentally enriched salt marsh vegetation: belowground biomass. *Limnol Oceanogr* 21:245–252
- Vitousek, P. M., Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American scientist*, *84*(5), 468.
- Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D.W., & Tilman, D. G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological applications*, *7*(3), 737-750.

Figures



Fig. 1 Study sites

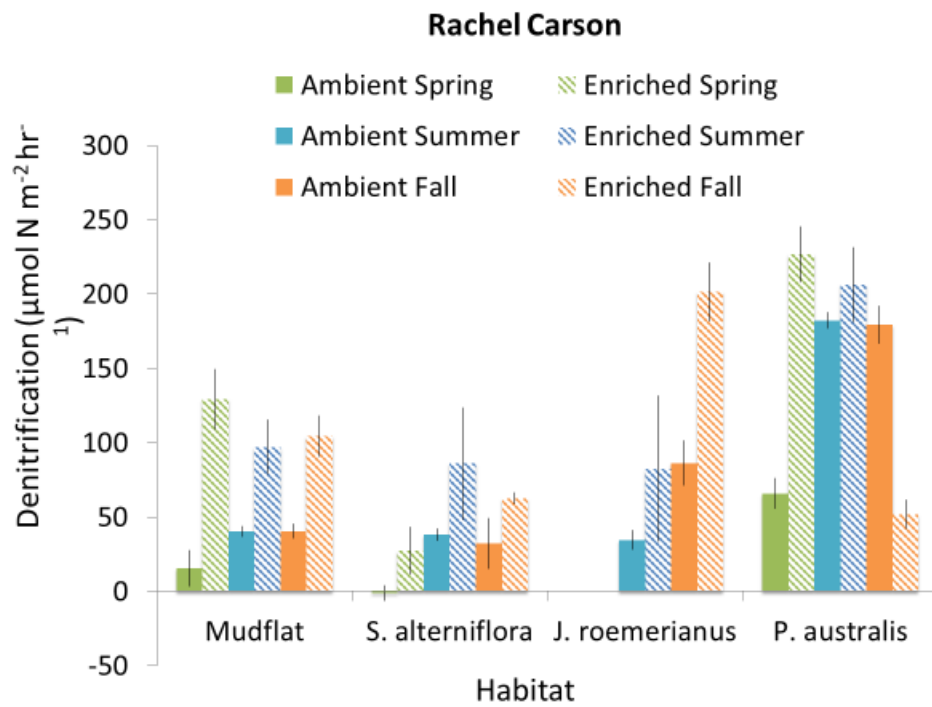


Fig. 2 Denitrification rates in the Rachel Carson Reserve

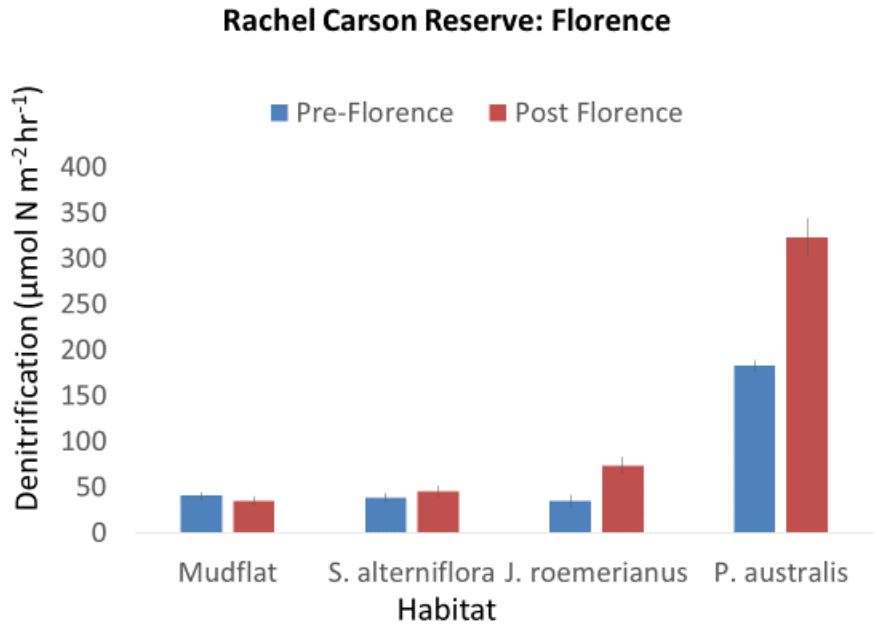


Fig 3. Denitrification rates in the Rachel Carson Reserve prior to and following Hurricane Florence

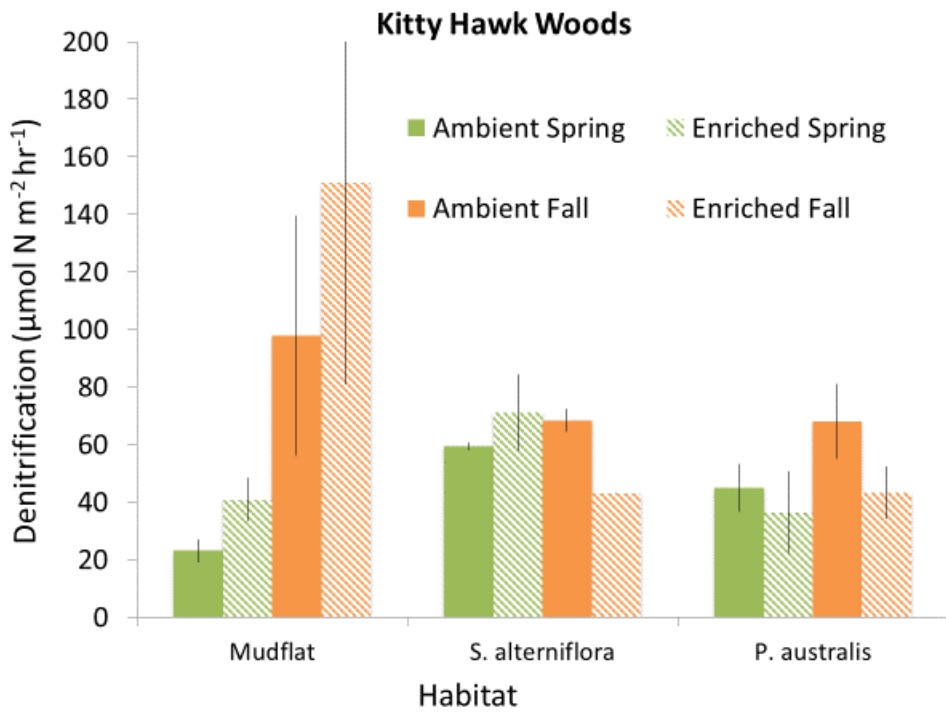


Fig. 4 Denitrification rates in the Kitty Hawk Woods Reserve

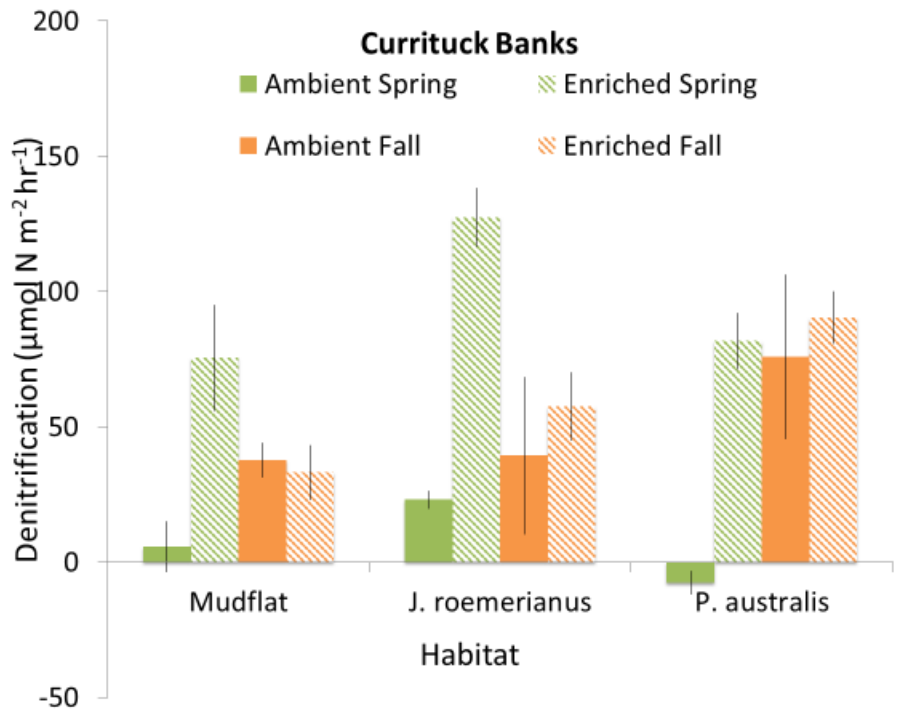


Fig 5. Denitrification rates in the Currituck Banks Reserve

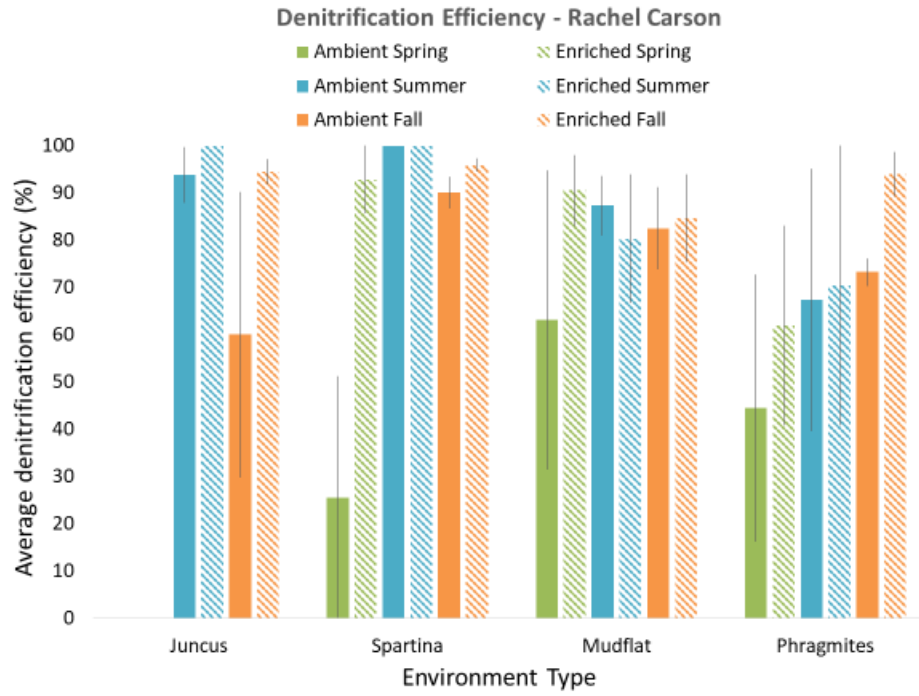


Fig. 6 Denitrification Efficiency in the Rachel Carson Reserve

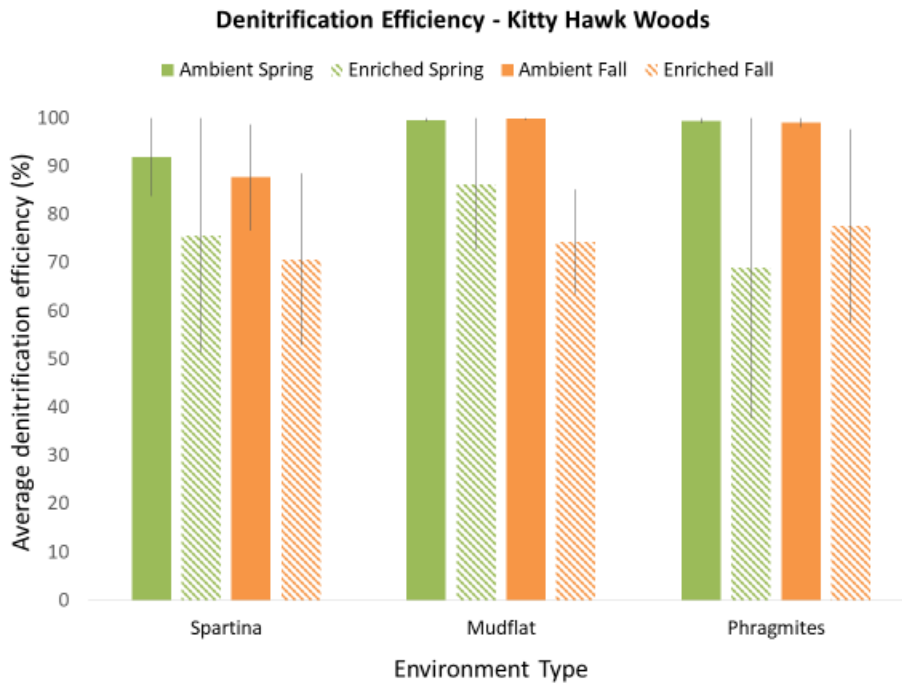


Fig. 7 Denitrification Efficiency in the Kitty Hawk Woods Reserve

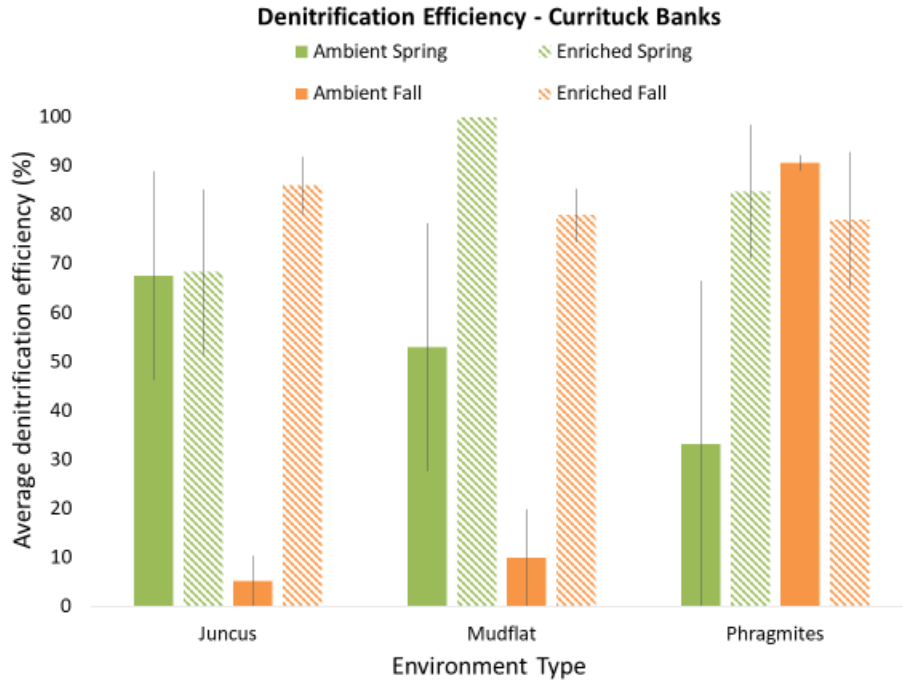


Fig. 8 Denitrification Efficiency in the Currituck Banks Reserve

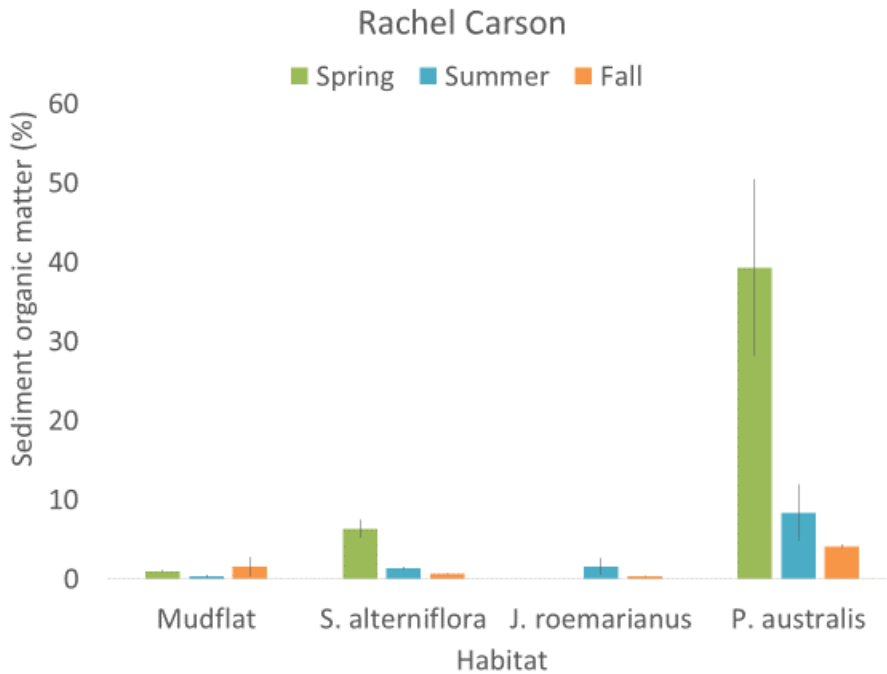


Fig. 9 Sediment organic matter percentages in the Rachel Carson Reserve

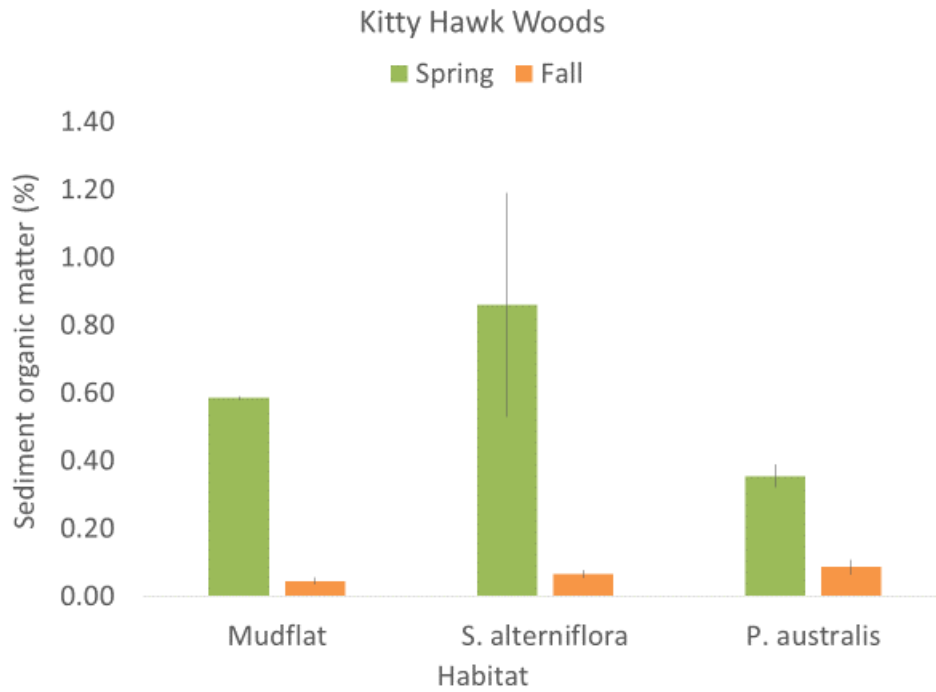


Fig. 10 Sediment organic matter percentages in the Kitty Hawk Woods Reserve

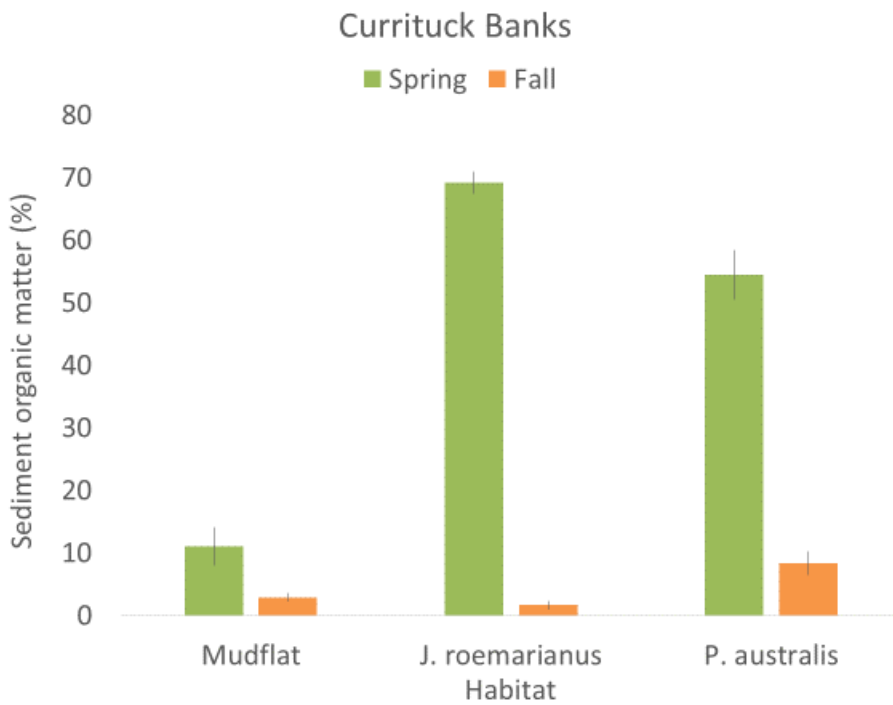


Fig. 11 Sediment organic matter percentages in the Currituck Banks Reserve

Site	Season	NOX (uM)	NH4 (uM)	PO4 (uM)	TN (uM)	ON (uM)
RCNERR	Spring	0.00	0.21	0.22	14.96	14.76
	Summer	0.0	0.8	0.2	10.6	9.8
	Fall	0.15	1.27	0.23	13.29	11.86
KHWCR	Spring	0.00	1.97	0.24	30.00	28.03
	Fall	0.00	0.33	0.08	34.39	34.06
CBNERR	Spring	0.00	0.56	0.19	49.86	49.30
	Fall	0.94	1.12	0.10	55.61	53.55

Table 1 Water column nutrient concentrations