

**IMPACTS OF BIOCHAR ON PHOSPHORUS DISTRIBUTIONS IN SALT
MARSH SURFACE SOILS IN DELAWARE**

by

Pamela Edris

A thesis submitted to the Faculty of the University of Delaware in partial
fulfillment of the requirements for the degree of Master of Science in Marine Studies

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ABSTRACT

Salt marshes store nutrients, including phosphorus (P) in their soils thereby providing the ecosystem service of reduced nutrient export to estuaries. Contrasting other ecosystem services, nutrient storage may be enhanced in stands of the invasive common reed, *Phragmites australis* (hereafter *Phragmites*), compared to native marsh grasses. Restoration efforts to remove *Phragmites* may thus reduce marsh P storage and allow more P into coastal waters where it can contribute to eutrophication. Prescribed burning to control *Phragmites* introduces biochar which, due to its surface chemistry and high surface area, may increase the sorption and storage of P in salt marsh soils and recoup this service. To study this P storage potential, cores were collected from 3 Delaware marshes with different burn histories for analysis of organic and inorganic P contents (OP, IP). These P components were also measured in field incubation plots with and without biochar addition in both native and invasive grass stands to further investigate the impact of biochar and plant species on soil P distributions. Because P form influences its aqueous mobility and storage potential, samples were further differentiated for P speciation on a continuum of mobility/lability (immediately plant available/labile, easily exchangeable, intermediately exchangeable, recalcitrant). P composition and distribution were examined for relationships with depth, tidal cycles, elevation, nutrients, pH, and organic and black carbon concentrations. Total and recalcitrant forms of P were

expected to be highest in previously burned marsh environments and the biochar amended plots demonstrating a P storage ecosystem service provided by the biochar inputs. Further, P concentrations were expected to be higher in the soil of *Phragmites* stands compared to *Spartina*. Neither burn frequency nor biochar application demonstrated a relationship with total organic or inorganic P concentrations suggesting no influence of burned materials on P sorption. Further, the presence of biochar was associated with a reduction in recalcitrant P concentrations. The lack of biochar-induced enhancement in P sorption may be due to the specific characteristics of the biochars produced from the burns and used in the experimental plots. Biomass, grass species, and pH were found to most strongly influence P distribution in the sampled marshes. The data show greater P content in *Phragmites* soils compared to native grass soils, suggesting that burning *Phragmites* and replacing it with native grasses may reduce P retention in marshes. The results of this study will add to the understanding of how prescribed burns and biochar impact P composition and cycling in salt marsh environments. Future work should explore the ubiquity of this finding and whether biochars of differing characteristics (feedstock, production conditions, mixing ratios) may provide a P storage ecosystem service.

Chapter 1

INTRODUCTION

1.1 Salt Marsh Ecosystem Services and the Impact of *Phragmites australis*

Salt and brackish marshes, hereafter referred to as salt marshes, are highly productive coastal habitats that are valued for a variety of ecosystem services. This includes providing habitat, food, shelter, and nursery grounds to a variety of fish and shellfish species, many of which have economic significance like blue crabs (Henton et al., 2013; Jivoff & Able, 2003; Kennish et al., 2014; Weinstein et al., 2019; Wigand et al., 2017). Salt marshes reduce the impact of coastal storms by providing flood abatement and wave attenuation from storm surges thereby limiting erosion (Henton et al., 2013; Kutcher et al., 2018; Wigand et al., 2017; Yao et al., 2018). They also reduce pollution and sediment loads (Neckles et al., 2015) and provide recreational opportunities including bird watching, fishing, hunting, and scenery (Henton et al., 2013; Kutcher et al., 2018).

Beyond these more tangible ecological, coastal protection, and recreational services, marshes also provide biogeochemical benefits by storing carbon (C), nitrogen (N), and phosphorus (P) in soils, sequestering it on decadal to centennial timescales (Giblin et al., 2013; Henton et al., 2013; Yao et al., 2018). It is estimated that salt marshes bury 11-87 Tg C yr⁻¹ globally, 1-36 g N m⁻² yr⁻¹ in marshes along the Atlantic coast of North America, and 0.8-1.3 g P m⁻² yr⁻¹ across the United States (Craft, 2007). Within the mid-Atlantic, specifically, 0.13-2.6 g P m⁻² yr⁻¹ was

accumulated in a New Jersey marsh (Velinsky et al., 2017). Sequestering C can mitigate climate change by keeping CO₂ out of the atmosphere, and the burial of N and P in soils prevents these nutrients from being exported to estuaries and contributing to eutrophication and associated water quality issues (Kutcher et al., 2018; Wigand et al., 2017; Yao et al., 2018).

The ability of tidal marshes to provide these ecosystem services is diminished by a variety of anthropogenic disturbances. Urbanization, deforestation, and sea level rise disrupt marsh sedimentation, which increases their risk of becoming inundated by rising sea level (Wigand et al., 2017). Marsh habitat can be lost when accretion rates are slower than sea level rise due to natural subsidence, which is prevalent in the mid-Atlantic region due to glacial rebound, erosion, and salt inundation, and is often exacerbated by human influences, like road construction and harvesting aquifer and oil reservoirs, that alter hydrology (Cahoon et al., 2010; Henton et al., 2013).

Many of these disturbances promote the invasion of monotypic stands of *Phragmites australis* (hereafter referred to as *Phragmites*), the common reed, to Atlantic and Gulf coast United States marshes. *Phragmites* grows up to 4 m tall in dense stands that outcompete the native grasses, reduce suitable habitat for birds and other wildlife, threaten plant biodiversity, and alter soil conditions (Ruggeri, 2014; Sun et al., 2006). *Phragmites*-dominant wetlands have lower faunal species richness than native *Spartina* wetlands, and fish in *Phragmites* marshes have been found to have less lipid reserves which reduces their fitness to reproduce and overwinter (Ruggeri, 2014). Disturbances that accelerate marsh drainage and reduce sulfides are

associated with *Phragmites* invasions (Ruggeri, 2014), and they are enhanced by high nutrient and low salinity conditions (Kutcher et al., 2018). Marsh restoration efforts are being conducted to promote resilience and allow for marsh migration landward to restore natural marsh functions (Kutcher et al., 2018; Wigand et al., 2017). In mid-Atlantic United States marshes, prescribed burning is employed as a restoration mechanism to remove *Phragmites* and promote the growth of native marsh grasses that provide better habitat and more nutritious food sources (Cahoon et al., 2010; Geatz et al., 2013; Henton et al., 2013; Leonard et al., 2010; Sun et al., 2006; Taillie & Moorman, 2019).

Notably, *Phragmites* does provide some marsh benefits compared to the native grasses including greater sediment trapping to support higher accretion rates (Kutcher et al., 2018; Ruggeri, 2014). *Phragmites* also uptakes more CO₂ and nutrients (N, P) due to their large size, rapid growth, and extensive root systems (Kutcher et al., 2018; Meyerson et al., 2009; Windham & Meyerson, 2003). Such benefits of *Phragmites* expansion should be weighed against its potential harms to inform management decisions.

1.2 Biochar Enhances Phosphorus Storage

The prescribed burns sometimes used to remove *Phragmites* introduce biochar which may enhance C, N, and P storage in marsh soils thereby negating the reduction of sequestration services expected to accompany the replacement of *Phragmites* with native vegetation. Biochar, which is the carbonaceous remains of biomass after

incomplete combustion, has a variable composition but is typically composed of condensed aromatic carbon compounds with high porosity and surface acidic functional groups that give it a high sorption potential (Zhang et al., 2019). Those properties enable biochar to adsorb excess nutrients and pollutants in agricultural and wastewater treatment applications (Bolton et al., 2019; Faridullah et al., 2012; Strømgaard, 1992). Further, its highly condensed aromatic structure makes biochar resistant to degradation and stable over long periods of time (Dittmar et al., 2012; Yao et al., 2018).

Thus, biochar provided by prescribed burns may bestow unexpected N, P, and C sequestration benefits to tidal marshes, but definitive studies have yet to be conducted, particularly on P. However, studies conducted in agricultural soils, wastewater treatment sites, and marine sediments are useful for understanding the potential adsorption of P to biochar in salt marshes. Strømgaard (1992) found higher P stocks in topsoils (upper 40 cm) after controlled burning was conducted using traditional Zambian agricultural practices which also involves adding extra vegetation. The authors concluded that the biomass burning served to mineralize OP thereby providing more plant available P in the burned topsoil and enhancing the microbial community in deeper soil layers. Another agricultural study in Japan using a greenhouse design has found that biochar from poultry litter yielded a significant increase in total P in soils compared to unburned poultry litter and an unmodified control (Faridullah et al., 2012). The majority of the P in the biochar addition was found in the recalcitrant HCl-extracted fraction suggesting it to be less mobile and

susceptible to leaching into runoff. The results from Faridullah et al. (2012) thus suggest biochar to adsorb and immobilize P. Biochar additions enriched with Fe, Ca, and Mg containing minerals have also been shown to remove twice as much PO_4^{3-} from wastewaters relative to a gravel filtration control system with the biochar substrate showing 77% more P content after 7 months compared to the control (Bolton et al., 2019). Scanning electron microscopic analysis of elements and functional groups showed a layer of organic compounds and minerals accumulating on the surface of biochar including the presence of high amounts of cations able to precipitate and adsorb P (Bolton et al., 2019). P precipitation with Ca and Fe in that study and others may have been promoted by an increase in pH brought on by the biochar additions (Bolton et al., 2019; Ojeda et al., 2016). Zhang et al. (2019) added increasing amounts of biochar to marine sediment and found increasing P sorption at each higher concentration of biochar (0, 2, 4, and 7% biochar; Zhang et al., 2019). They also attributed this to biochar's high surface area, acidic surface functional groups, and metal cations. In that work, the exchangeable and Fe-bound P fractions increased in the biochar during sorption, and the organic and Ca-bound P remained constant.

1.3 Phosphorus Distribution and Impact on Marshes and Coastal Waters

Most P in salt marshes is found in soils and above- and below-ground dead plant biomass (Tobias & Neubauer, 2019). 30-65% of total soil P is organic, which is a type of plant-unavailable P that includes dead organisms and microbes (Prasad & Chakraborty, 2019). Inorganic phosphorus (IP) includes the mobile plant-available P

that is dissolved in soil solution, sorbed P (clay-, Fe-, Al-, and Ca oxide- bound) that can be slowly released to the available pool, and mineral P (phosphate minerals) that is released by weathering at a very slow rate (Prasad & Chakraborty, 2019). About 80-90% of P in marsh soils is suggested to be immobile and unavailable to plant uptake from soil since it is incorporated in organic matter, bound to the sediments by microbial immobilization, or associated with Ca, Fe, Al, or Mg (Álvarez-Rogel et al., 2007; Prasad & Chakraborty, 2019; White et al., 2008). In particular, the Ca-bound fraction is the largest and most stable P form (Tobias & Neubauer, 2019; Zhang et al., 2019). Typically, TP is either invariant or shows greatest concentrations in the upper 10 cm and declines with depth, while recalcitrant P concentrations increase with depth due to diagenetic processes (Álvarez-Rogel et al., 2007; Tobias & Neubauer, 2019).

P can be imported to marshes from streams, groundwater, precipitation, tidal exchanges, and weathering processes (Dame et al., 1990; Tobias & Neubauer, 2019; Valiela et al., 1978; Velinsky et al., 2017). Most P exchange is tidal, but groundwater input can be influential in some systems, particularly P-rich areas and non-river dominated systems (Dame et al., 1990; Tobias & Neubauer, 2019; Wilson & Morris, 2012). Studies on relatively closed marsh systems with limited channels for surface water exchange have found marshes to be a small and likely insignificant sink for P from these sources, since the amount of P being exchanged is small compared to the size of the reservoir of P in marshes (Dame et al., 1990; Tobias & Neubauer, 2019; Valiela et al., 1978).

I am unaware of studies that compare the P stocks in soils of mid-Atlantic marshes where *Phragmites* are invasive and *Spartina* are native. However, general trends of *Phragmites* and *Spartina* in locations where their invasion status is reversed (*Phragmites* is native, *Spartina* is invasive, e.g., Jiangsu Province, China; Qing et al., 2015) as well as microbial and decomposition studies in the study region can be used to understand the influence of grass species on soil P. It has been shown that *Phragmites* and invasive wetland grasses in general produce more litter due to greater nutrient uptake (Qing et al., 2015; C. Wang et al., 2020; Windham, 2001), but their decomposition is slower and releases more resources (C. Wang et al., 2020; W. Q. Wang et al., 2015; Windham, 2001). The higher litter content and inefficient decomposition processes suggest that *Phragmites* soils will contain higher P content than those in *Spartina* stands (W. Q. Wang et al., 2015).

Excess P, which is present in watersheds with anthropogenic activities like urbanization, deforestation, and agriculture, can cause eutrophication upon delivery to coastal waterways (Fisher et al., 1992; Volk et al., 2012), especially since P can be a limiting nutrient in salt marshes and adjacent coastal ecosystems (Schafer & Mack, 2018). Nutrient enrichment in marshes has been associated with decreases in belowground production and increases in microbial decomposition yielding destabilized peat and decreased accretion rates (Darby & Turner, 2008; Kutcher et al., 2018), both of which can contribute to marsh loss. Mid-Atlantic coastal plain estuaries are susceptible to eutrophication by residential and agricultural land use exporting nutrients combined with shallow estuaries with long retention times (Volk et al.,

2012), but estuaries can have different responses to nutrient abundances (Sharp et al., 2009). A 25-year study in the Delaware Estuary revealed large seasonal variations in nutrient concentrations as phosphate becomes depleted by the spring bloom to limiting amounts (DON:DOP = 125), then regenerates in early summer in the lower bay. (Sharp et al., 2009). In Rehoboth Bay, improvements in wastewater treatment led to declines in P loads and potential P limitation in lower salinity portions of the bay (Volk et al., 2012). If excess P is introduced to these systems, P may no longer be limiting, and eutrophication could occur. Understanding how biochar could impact P forms transported from marshes to coastal water could elucidate potential impacts to P cycling in these systems since nutrient inputs and eutrophication is variable by estuary.

The current study uses 3 years of field collections in marshes with and without histories of burning to evaluate the influences of biochar on soil P concentrations and distributions. Experimental biochar amended thin layer placement plots are also used to isolate the impacts of biochar, grass species, and environmental conditions on P storage in salt marsh environments. The high porosity, surface cations, and surface acidic functional groups characteristic of biochar were hypothesized to encourage P sorption and retention in soils of the burned and biochar addition samples. Because biochar is expected to promote belowground biomass growth and sorb inorganic and organic P components, the OP and IP concentrations are expected to correlate with black carbon, a proxy for biochar quantity, and total organic carbon and be highest in Roberts Farm relative to the other two marshes.

Analysis of bulk organic and inorganic P concentrations as well as P mobility by sequential extraction will improve our understanding of P cycling between marshes and adjacent coastal waters as well as the biogeochemical impacts of prescribed burns for *Phragmites* removal and native marsh restoration. This information could help to explain and predict the mechanisms that drive vegetative and biogeochemical responses to burning. With this information, salt marsh managers will be able to make more informed decisions on the use of prescribed burns, particularly since post-burn comprehensive nutrient assessments are not frequently conducted.

Chapter 2

METHODS

2.1 Sample Collection and Processing

2.1.1 Field Collection from Marshes of Different Burn Histories

To compare marshes with different burn histories, 10 cores were collected from 3 marshes of different burn histories in 2 sampling seasons (Fig. 1; January 2022 and November 2022). The marsh at St. Jones National Estuarine Research Reserve (SJ; Dover, DE) has never been burned due to potential effects on power lines that run over the marsh. Roberts Farm (RF; Townsend, DE) has a known recent burn history with several locations within the marsh having been burned between 2017 and 2021. Rocks Tract (RT; Townsend, DE) has not been burned in at least 20 years (Eric Ludwig, personal communication). It is, however, located adjacent to Roberts Farm and may have experienced hydrological or atmospheric transport of biochar from prescribed burns in that marsh. SJ has an average salinity of 10.8 ± 7.0 ppt while the average salinity in RF and RT is 2.0 ± 2.1 ppt (Kelly, 2023 using <https://cdmo.baruch.sc.edu/>). Each of the three marshes has 10 sample sites that were characterized by their vegetation type and elevation above mean sea level. Nine of the core locations at each marsh were dominated by native *Spartina* vegetation, and a tenth core was taken from a *Phragmites*-dominated stand. An abridged third sampling collected 8 cores to enhance comparisons of P distribution in soils of *Phragmites* versus *Spartina* grasses (Fig. A1; November 2023). These 8 cores consisted of four

Phragmites stand locations and four *Spartina* dominated areas within Roberts Farm and Rocks Tract. All coring sites were selected to be relatively dry at the surface and within vegetative stands. Cores were taken from areas in between vegetation to facilitate isolation of the soil from belowground biomass. Core collection involved 10.5 cm diameter and 30 cm long push cores being hammered into the soil. The top was sealed, the core was dug out and capped at the bottom. Compaction measurements and vegetation type were recorded.

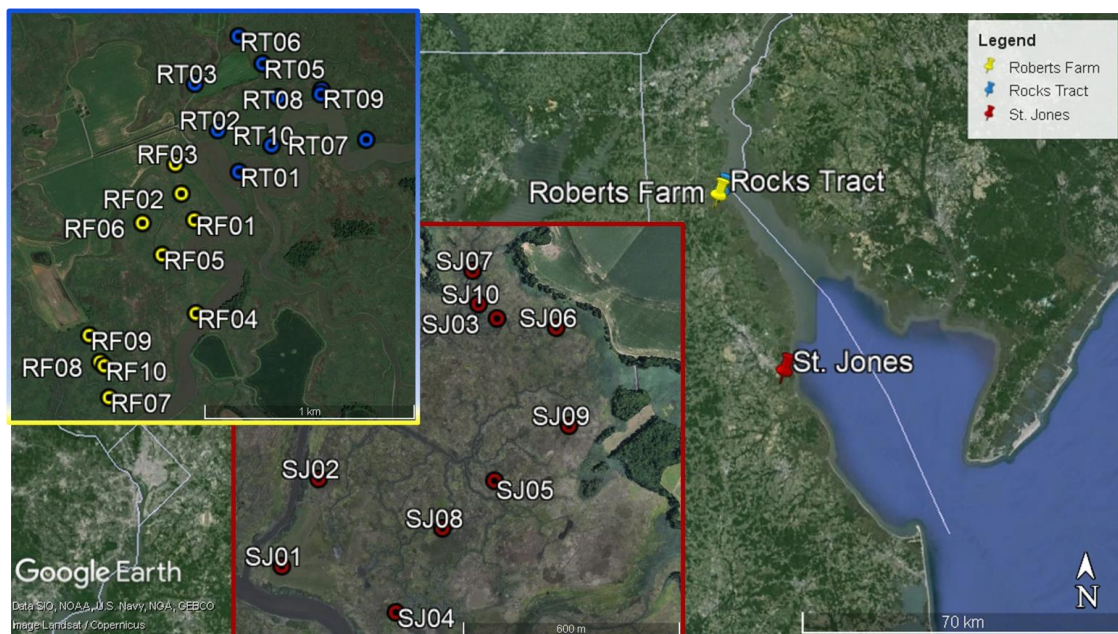


Figure 1. Locations of core collection sites along Delaware Bay. Thumbtacks note marsh sites. The inset at top left shows the locations of core collections at the Roberts Farm and Rocks Tract sites, and the bottom left shows the core collections at the St. Jones site.

In an effort to account for effects due to a core's position in the marsh, core sites were assessed for tidal cycles and elevation relative to sea level. Other influences on P cycling include physical (salinity, temperature), biogeochemical (pH, Fe, S), and biological factors (seasonal variations in microbial and plant uptake and output) that lead to spatial and temporal variations in P distributions (Paludan & Morris, 1999; Rozan et al., 2002; Sundareswar & Morris, 1999; Tobias & Neubauer, 2019; Upreti et al., 2015). These confounding factors were attempted to be controlled for in the experimental design, but they could be important to determining P availability and are considered when contextualizing the data.

Core processing was conducted in an anoxic environment to preserve redox-sensitive analytes for analyses not reported in this thesis. Whole sealed cores were frozen upon return to the lab the same day as collection. Cores were later thawed and sectioned to 5 cm increments to assess a depth profile of P concentration in a glove bag of N₂ gas by pounding up from the bottom or gently sliding the sediment out of the core. These sections were then frozen, freeze dried, and sieved at 500 µm. Sieved, homogenized samples were placed in jars and topped with N₂ gas before being frozen until analysis.

2.1.2 Biochar Thin Layer Placement Experiments

Thin layer placement (TLP) experiment plots were conducted in St. Jones Reserve to simulate the effects of burning as well as investigate the effects of amending TLP sediments with biochar which is thought to enhance C and nutrient

storage services in marshes (Raposa et al., 2023). The potential of biochar amended TLP to retain P will be examined using the following experimental setup.

Five 1 m x 1 m experimental plots were constructed using untreated wood and installed in June 2022. Vegetation was clipped to the soil base (<5 cm height) to enable even distribution of sediment- biochar amendments. Three plots were located in a *Spartina alterniflora* vegetated stand: one of these plots was unamended and serves as a control, one was amended with estuarine sediment (~8 cm thick layer), and one was amended with a sediment and biochar mixture (9 sediment: 1 biochar by volume, ~8 cm thick; Raposa et al., 2023). The other 2 plots (one sediment amended, one sediment/ biochar mixture amended) were in a neighboring *Phragmites* stand. The applied sediment was collected from a nearby tidal creek and processed in a cement mixer before being added to the plots. The biochar used for these experiments is a custom char made for this project from *Phragmites* biomass by Sustainable Material Solutions using a custom-built pyrolyzer. The detailed char properties are listed in Table A9. The original creek sediment, 10% biochar amended mixture, and the biochar itself were analyzed for bulk and sequential P concentrations. This allows for comparison between the initial and final P concentrations in the biochar to determine if P sorption occurred. Three cores were collected from each of these plots in July 2023 in the same way as described above.

Core processing and sample preservation were done similarly in the biochar TLP plots except for the sectioning increments. Core sections were separated into the top of the core (~0-8 cm depth), which visually consisted of the applied sediment, and

the bottom of the core (~8-15 cm depth), which represented the original marsh surface and now are in the root zone. Separating the applied and original sediment allows for characterization of the creek sediment applications 1 year after deployment and also allows us to distinguish if the biochar is in the root zone where its interaction with P can directly impact plant P uptake. Initial analyses of the 3 marsh locations showed no trends of P with depth allowing for this simple sectioning.

2.2 Phosphorus Analysis

2.2.1 Bulk Phosphorus Analysis

Bulk OP and IP were extracted using hydrochloric acid and analyzed using the spectrophotometric molybdate blue (ascorbic acid) method (Murphy & Riley, 1962). For the extraction, homogenized soils from each depth increment were dried at 105 °C for 24 hours to a constant mass. Then, 0.25 g aliquots of the samples were kept dry for inorganic P extraction. The remaining sample aliquots were ignited at 550 °C for 2 hours to oxidize organic material for total P analysis (Ann et al., 2000; Aspila et al., 1976). All the 0.25 g samples were then extracted overnight with 25 mL HCl in 50 mL Falcon tubes on a shaker table at 200 RPM (14-18 hours). Samples were vortexed for 30 seconds before extraction to break up any lumps. Finally, samples were centrifuged for 20 min at ~ 2,500 RPM. This technique was conducted on all collected samples from the 3 marshes and the biochar TLP plots.

To analyze phosphorus from each of the experimental isolates, the molybdate blue method was adapted (Aspila et al., 1976; Murphy & Riley, 1962). Calibration standards of 0-20 μM P were created using a 10 mM potassium dihydrogen phosphate with sulfuric acid stock that was diluted to 100 μM daily. Bulk P samples were diluted by a factor of 100 for measurement to ensure that concentrations were in the range that would produce a linear trend between absorbance values and P concentration (Aspila et al., 1976). A mixed reagent of sulfuric acid, ammonium heptamolybdate tetrahydrate, and potassium antimony tartrate was made daily. Then, 0.5 mL of the mixed reagent and 0.5 mL of an ascorbic acid solution were added to the standards and the samples. The color was developed and ready for analysis on a Horiba Aqualog at a wavelength of 880 nm after 10 minutes. The absorbance data were converted to mg P kg^{-1} soil. Inorganic P data were derived from measurements made on the unignited samples with organic P calculated as P from the unignited samples subtracted from that of the ignited samples.

2.2.2 Sequential Phosphorus Analysis

The sequential extractions were conducted following Hedley et al. (1982) as adapted by Faridullah et al. (2012) and Gu et al. (2020). Sequential extraction protocols operationally define P pools based on their mobility. Recalcitrant P is the least mobile P form extracted by these procedures (Anton et al., 2021). 0.5 g of sieved and dried soil samples were first extracted by 30 mL MQ water mixed to match the salinity of the sampled salt marsh using artificial seawater mix. Roberts Farm and

Rocks Tract samples were extracted in 5 ppt seawater, and 10 ppt seawater was used for St. Jones samples. The water extraction removed immediately plant available P during shaking for 16 hours at room temperature. After separation by centrifugation, the soil residue was rinsed with the previous extract solution (MQ water for step 1) by shaking for 1 hour to reverse any secondary adsorption (Song & Liu, 2015). After centrifuging the rinse, the residue was dried as described above. Next, the soil residue was similarly extracted in sequence using 0.5 M sodium bicarbonate (to isolate the easily exchangeable P), followed by 0.1 M sodium hydroxide (to isolate the intermediately available/Fe-P), then by 1 M hydrochloric acid (to isolate the recalcitrant P). The extract to soil ratio was increased from 60 (used for the subset of field collected samples) to 100 for the biochar TLP plot samples to improve the yield of the sum of all OP species compared to the bulk OP concentrations measured by the ignition method. Organic P was measured for each P species using wet chemical oxidation by adding 0.18 M potassium persulfate to the extracts and autoclaving at 121 °C for 2 hours, which was found to be the duration needed to maximize P release (Ridal & Moore, 1990). This procedure was conducted for the subset of 21 samples from the 3 marshes as well as all biochar TLP plot samples.

The molybdate blue method was also used to quantify P concentrations in the sequentially extracted samples, but the dilution factors were adjusted since these concentrations are lower than bulk P. The subset of samples from the 3 marsh field collections were diluted by a factor of 30 for the easily exchangeable to recalcitrant P forms and 1 or 2 for the immediately available P to avoid concentrations below the

limit of detection of the instrument. The inorganic P was derived from measurements made on the unautoclaved samples, and the organic P was calculated as P from the unautoclaved samples subtracted from the autoclaved samples.

2.3 Supporting Data

Black C, OC, and TN data for the field collected samples in January 2022 (year 1) was provided by Kelly (2023). These parameters were evaluated with the corresponding P values from year 1. The benzene polycarboxylic acid marker (BPCA) method following Wagner et al. (2017) adapted for sediment analysis (Barton & Wagner, 2022) were used to quantify black C concentration and used to assess biochar inputs at each sampling site. TOC and TN were measured by an adaptation of Menzel & Vaccaro (1964) and Ehrhardt & Koeve (1999) by CHNS elemental analysis using a COSTECH ECS4010. TN was used along with TP from bulk P measurements to calculate molar N:P ratios.

Elevation data were provided by Daniel Warner (Delaware Geological Survey). LiDAR data as a 3x3 m average were plotted in QGIS with the sites from the three marshes.

The position of sampling time within tide cycles was assessed as the number of days between sampling and the last spring tide (lunar tidal cycles) as well as the number of hours between sampling and the last high tide (diurnal tides).

Additional biochar properties were measured by Control Laboratories in Watsonville, CA according to the International BioChar Initiative Laboratory Tests for

Certification Program. The measurements listed in this thesis and their associated methods are as follows: H:C (Dry Combustion-ASTM D 4373), pH (4.11USCC:dil. Rajkovich), % ash (ASTM D-1762-84), surface area correlation (Butane Activity Surface Area Correlation Based on McLaughlin, Shields, Jagiello, and Thiele's paper: Analytical Options for Biochar Adsorption and Surface Area), iron and total P (EPA 3050B/EPA 6010).

An oxidation reduction potential (ORP) meter was used to collect *in situ* pH and redox potential (Eh) measurements in the biochar TLP plot experiments. After cores were collected, porewater was allowed to fill in the hole, and an HI I991003 probe was used to measure the Eh, pH, and temperature at each core location as an average of 3 measurements.

2.4 Statistical Analysis

Pearson's correlation coefficients with t-Tests give p-values that indicate if linear relationships between studied variables exist. When p values are significant, R^2 values define the strength of these relationships as follows: 0-0.25 = very weak, 0.25-0.50 = weak, 0.50-0.75 moderate, and 0.75-1 = strong. T-tests were used to determine if 2 groups were significantly different including paired 2-sample t-Tests and 2-sample t-Tests of unequal variance. 1-way ANOVA indicated if a group was significantly different and 2-way ANOVA determined if grass type and/or biochar application significantly influenced P concentration in the biochar TLP plot experiments. Multiple regression analyses were conducted to determine the influence of environmental

factors on P concentrations. A model describing the influence of the measured environmental parameters on IP, OP, and TP was created and variables that independently contribute to variation in P concentrations are highlighted. Environmental variables examined for the year 1 field collected sites were P, black C, organic C, and N concentrations as well as time since high and spring tides, core depth, elevation, and N:P. Variables available for biochar plot samples were P concentrations, pH, and redox potential (Eh). Statistically significant differences were determined at $p < 0.05$.

2.5 Replication and Accuracy

Replication precision was determined for various aspects of P analysis. Full process replication was assessed on subsamples from the same sample extracted and analyzed in the same way. Subsamples were taken directly before colorimetric analysis from the same tube of diluted sample with the colorimetric reagents added to assess instrumental replication and to assess any errors due to the dilution process. All replicates were found to be statistically similar (Table A1).

An external sediment P standard was also used to verify the P concentrations (CRM BCR-684). While IP concentrations were accurate for both techniques, OP measured by the ignition method over-reported P concentration while the autoclave oxidation used for the sequential extraction technique under-reported P concentration (Fig. 2).

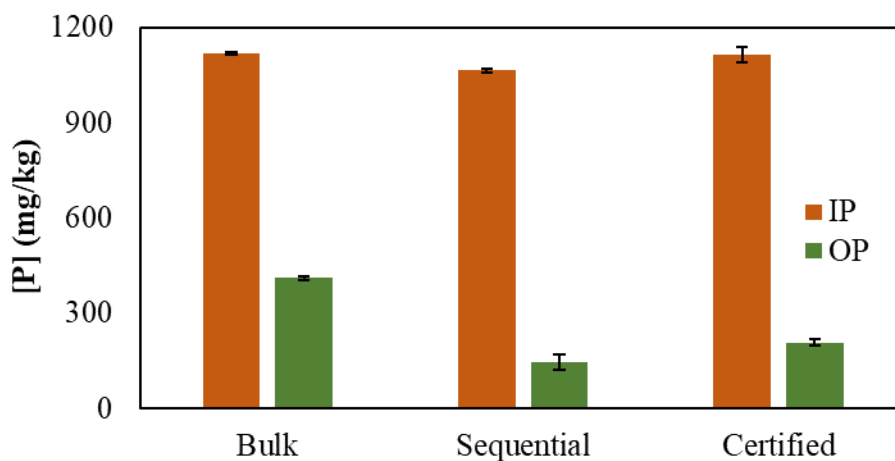


Figure 2. Average IP (orange) and OP (green) concentrations of the certified soil standard during bulk and sequential P analysis as well as the reported certified values. Error bars represent uncertainty as the half-width of the 95% confidence interval.

Additionally, 18 of the field collected samples and all biochar TLP plot samples were oven dried instead of freeze dried. There was no significant difference between the oven or freeze dried bulk IP and OP concentrations (IP: $t(14) = -1.12$, $p = 0.282$; OP: $t(14) = -0.188$, $p = 0.854$).

Chapter 3

RESULTS AND DISCUSSION

3.1 Field Collection from Marshes of Different Burn Histories

There were no significant differences in OP and IP concentrations between collection years 1 and 2 (January vs November 2022 collections) for each sampled marsh and all marshes grouped together (Table A2). Thus, the year 1 and 2 data are pooled together when applicable and as specified in the text.

Comparison of P concentrations between the marshes binned by depth shows that the only significant difference in P concentration between the marshes and their burn frequencies is significantly higher [OP] in the 2.5 cm depth in SJ relative to those in the 2.5 cm depth range at the other two marshes (Fig. 3, $p < 0.009$, Table A3). These results contradict the hypothesis that RF would have the greatest P concentrations due to its recent frequent exposure to burning and presumably biochar inputs that would facilitate P sorption and/or char P inputs. Comparing OP:IP in each marsh at the separated depth increments shows that SJ has the highest OP:IP ratio between the marshes, with SJ having a significantly greater OP:IP at the surface depth of 2.5 cm compared to the other marshes (Fig. A2). The higher OP concentration and OP:IP at the 2.5 cm depth at St. Jones suggest higher surface organic inputs at this marsh and are consistent with results for organic C concentration measured for a parallel study which showed SJ to have higher organic C concentration relative to the other study marshes (Fig. 5; Kelly, 2023). There were no other significant

relationships between OP:IP and marsh burn history or depth (Tables A5 & A6). Figure 3 also shows [P] trends with depth at each marsh, and there is a significant decrease in [IP] with depth in RF and [OP] with depth in SJ ($p = 0.007$ & $p = 0.0007$ respectively, Table A4). P concentrations have been found to be steady or declining with depth in salt marsh soils (Álvarez-Rogel, 2007; Tobias and Neubauer, 2019, Velinsky et al., 2017), so this observed trend is consistent with that expectation.

The soil P concentrations and compositions in the study marshes shown in Figure 3 follow similar trends to those observed in the literature along the Mid to Southeast Atlantic coast. TP concentrations averaged 588-635 mg P kg⁻¹ soil_{dry weight} in 3 brackish marshes in Georgia (Loomis & Craft, 2010), 921 mg TP kg⁻¹ soil_{dry weight} in the top 10 cm and 697 mg TP kg⁻¹ soil_{dry weight} in the 10-20 cm section of a brackish soil in a South Carolina marsh (Sundareshwar & Morris, 1999), and 710 mg TP kg⁻¹ soil_{dry weight} (up to 3,900 mg TP kg⁻¹ soil_{dry weight}) in a tidal New Jersey marsh affected by urban and agricultural runoff (Velinsky et al., 2017). OP represented about 42-55% of TP in the upper 15 cm of marsh soil in the 3 marshes in the current study, which is within the wide expected range of 30-90 % OP for wetland soils (DeLaune et al., 2013).

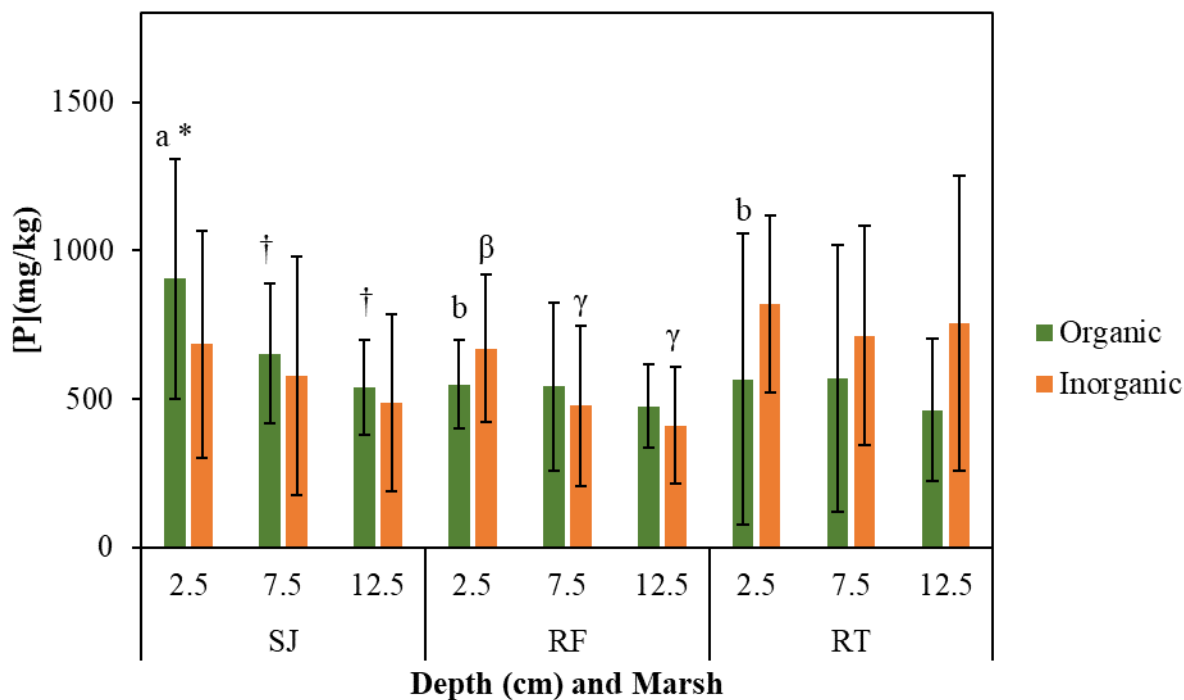


Figure 3. Organic (green) and inorganic (orange) P concentrations at the 3 marshes binned by 5 cm depth increments. Concentrations represent the average of all cores from years 1 and 2 (n=18-20) with error bars showing standard deviation. ‘a’ and ‘b’ notations indicate significant differences in P concentrations between marshes at a given depth, ‘*’ and ‘†’ notations indicate depths that have significantly different OP concentrations within a site, and ‘β’ and ‘γ’ notations indicate depths that have significantly different IP concentrations within a site by 1-way ANOVA (Tables A3 & A4).

In an effort to quantitatively assess the relationship between biochar inputs and P concentration, black C concentrations were quantified for year 1 samples. While black C concentrations were highest at RF followed by RT then SJ confirming the

expected trend based on burning exposure (Fig. A5), there are only very weak correlations between bulk IP or OP with black C concentration ($R^2 = 0.09$ and 0.0005 , respectively, Fig. A3). Exposure to recent burning and black C thus does not appear to be a dominant factor governing P concentrations in the study marshes, and any additional P retained due to sorption to biochar (or other means) is unobservable due to factors unrelated to burning.

Soil black C concentrations were also compared to sequentially extracted IP and OP pools to evaluate its influence (and, therefore, the influence of burning) on P mobility (Fig. 4). The less mobile IP pools (Intermediately Available, Recalcitrant) show significant negative correlations with black C concentration ($R^2 = 0.37$ and 0.30 , $p = 0.010$ and 0.033 , respectively). However, this negative trend runs counter to the hypothesis that greater amounts of black C, and thus biochar, would decrease P mobility and result in greater concentrations of the less mobile P pools. The observed decline in [IP] with [black C] could be attributed to plant uptake since porewater nutrient concentrations were found to decline in annual burn regimes likely due to increases in belowground biomass (Bickford et al., 2012; Cahoon et al., 2010; Gaetz et al., 2013). However, most of the sites in these companion studies are sedge dominated, and the grass dominated sites were not associated with significant differences in biomass by burn treatments (Bickford et al., 2012). A positive relationship between OP:IP and [black C] could be interpreted as a reflection of IP being converted to OP in biomass and its subsequent incorporation into the soil OP pool with increased burning. However, no statistically significant trends were observed when comparing total or

sequentially extracted P pool OP:IP to [black C], except for a very weak significant correlation ($R^2=0.15$, $p = 0.047$) in the intermediately available P pool (Figs. A3 & A4).

Sequential P pool quantities can be difficult to compare to other marsh studies since they are defined operationally by extraction techniques that vary according to the needs of the study goal. Wetlands studies with similar definitions of P pools to ours can be used to verify our reported values. Our study found 91-98% of TP to not be immediately plant available, which is greater than other findings that 80-90% of wetland P is not available and instead bound to sediment, Fe/Al, or OM (Álvarez-Rogel et al., 2007; Prasad & Chakraborty, 2019; White et al., 2008), but closer to Fox et al. (2014) reporting that plant available P is <1% of TP in agricultural soils. 21% of soil P in the sampled marshes is easily exchangeable, which is less than the reported value of 40% suggested to be important to maintain porewater P equilibrium for salt marsh plant use, but similar to the amount of this P pool in South Carolina marshes (up to 24%) (Paludan & Morris, 1999; Tobias & Neubauer, 2019). Low N:P ratios in the studied marshes indicate there is likely sufficient P for the marsh plants (Fig. A6). Recalcitrant P is 13-38% of TP in brackish marshes, which is similar to what was observed in our marshes (35% recalcitrant P in SJ, 39% recalcitrant P in RF and RT; Paludan & Morris, 1999).

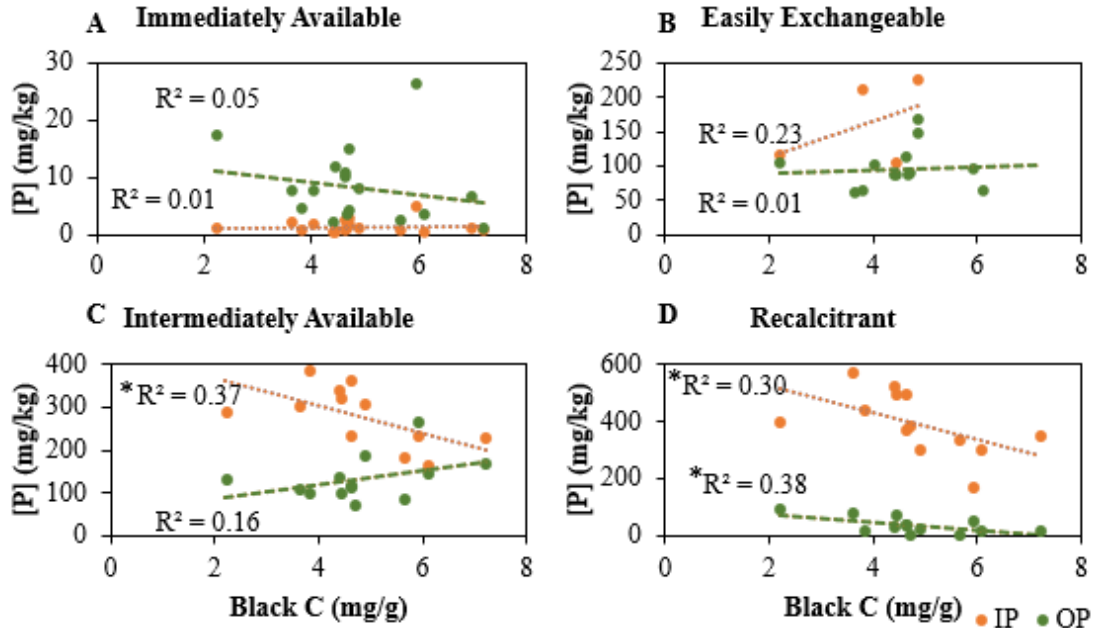


Figure 4. Concentrations (mg P kg^{-1} soil_{dry weight}) of OP (green) and IP (orange) in each sequentially extracted P pool versus black C concentration (mg C g^{-1} soil dry weight) for a subset of the year 1 core samples. P mobility decreases from A to D. * R^2 indicates a significant correlation between black C concentration and the specified P pool ($p < 0.05$).

3.1.1 Environmental Factors that Could Influence P Distribution

Since field collection results do not indicate that burning or biochar are influencing P distribution, a suite of additional environmental variables was examined including tidal inundation and N and C influence. Vegetation is expected to influence soil [TP] due to litter and root production cycles that take up IP from porewaters and release OP into the soil (Zhang et al., 2015). Burning has been found to increase above and belowground biomass (Bickford et al., 2012; Cahoon et al., 2010; Wang et al.,

2019); the effects of vegetation enhancement may mask impacts of biochar on P sorption. Variations in vegetation type and density could also influence P distributions since plants with more biomass can assimilate and return more P to soils, particularly OP concentrations which are associated with belowground biomass (Zhang et al., 2015). The impact of differences in vegetation type and overall biomass are important to this study since *Phragmites* generally has greater biomass than native grasses and could be influencing P distributions after their removal (Wang et al., 2015; Windham, 2001).

Organic C (OC) and total N (TN) concentrations were compared to P as a comparison of biomass proxies on soil P content since OC concentrations are positively correlated with plant biomass (Zhang et al., 2015). Only the year-1 [P] data are used in this analysis since [N] and [OC] values are only available for year-1. [OP] and [OC] are significantly moderately correlated, which suggests that the OP and OC are derived from a similar OM source (Fig. 5). The relationship between [OC] and [IP] is significant but very weak. Similar to [OC], [TN] shows a significant but weak positive relationship with [OP] and a very weak significant relationship with [IP] (Fig. 6). The weaker relationship between [OP] and [TN] relative to that between [OP] and [OC] may be explained by TN containing both inorganic and organic forms. These direct comparisons demonstrate that the TN and OC biomass proxies influence the OP fractions but have little to no influence on inorganic forms of P.

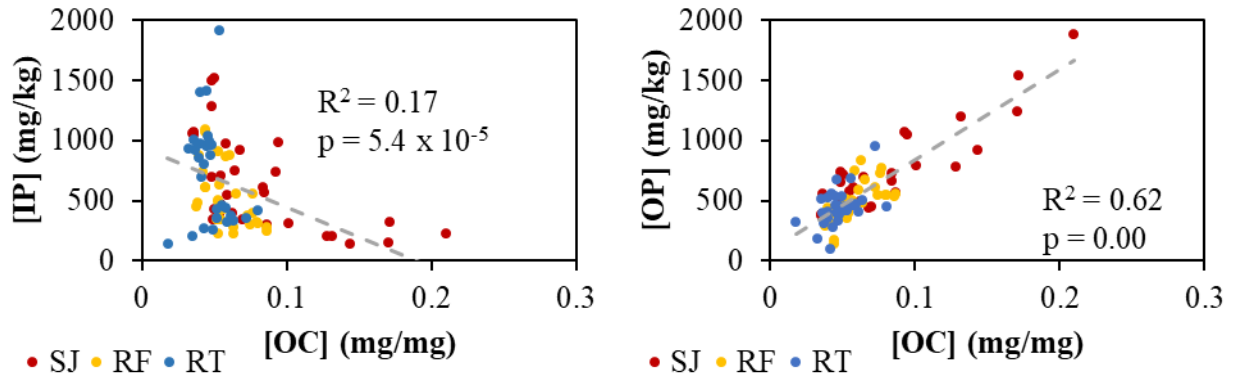


Figure 5. Plots of organic C concentration versus IP (left) and OP (right) concentrations from all year 1 core samples. Data points are color-coded by marsh of collection.

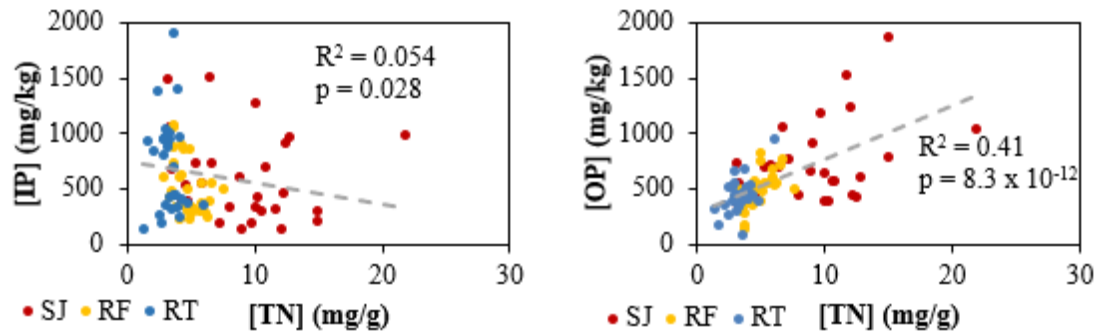


Figure 6. Total N concentration compared to IP (left) and OP (right) concentrations from year 1 collection. Data points are color-coded according to the marsh of collection.

The influence of tides was examined since they are known to import and export P as well as black C (Axelrad et al., 1976; Ditmar et al., 2012; Reimold, 1972;

Tobias & Neubauer, 2019). Elevation of the sample site above sea level showed a weak correlation with P concentrations (Fig. A9). No correlation was observed between time since high tide (diurnal tidal cycle) and time since spring tide (lunar tidal cycle) on OP or IP concentration except for a significant but very weak relationship between OP concentration and diurnal tides (Figs. A7 & A8).

Because no one parameter was observed to have a major impact on any P concentration parameters, multiple regression analysis was used to determine how the measured environmental parameters may combine to impact the variability in year 1 IP, OP, and TP soil concentrations. Results from multiple regression analysis demonstrate that [black C], [OC], elevation, and molar N:P ratio uniquely contribute to explaining the variation in soil [IP] and together explain 33% of [IP] variability. Depth, [OC], lunar tide, and N:P ratio uniquely contribute to variation in OP concentration and together explain 78% of the variation in soil [OP], and depth, [black C], elevation, [TN], and N:P ratio were unique contributors to [TP] variability and explained 34% of the variation in soil [TP]. All p-values of the models were significant, and variables that significantly contributed to unique variation in [P] are listed below (Table 1). Diurnal tidal cycles were the only examined parameter that did not uniquely contribute to any [P] variability. In sum, the multiple regression models show that the measured environmental variables explain soil [OP] variability very well, but they only weakly explain relationships between these environmental factors and soils [IP] and [TP]. Further, [black C] has significant, independent contribution to variations in IP and TP but not OP concentrations. These results support the moderate

correlation between intermediately available and recalcitrant [black C] and [IP] (Figure 4).

Table 1. Summary of multiple regression analysis of the contribution of the measured environmental factors on IP, OP, and TP. See Table A8 for the coefficients and p-values of each independently significant variable.

| | Model Significance | Adjusted R ² of All Variables | Independently Significant Variables | Adjusted R ² of Significant Variables |
|----|------------------------|--|--|--|
| IP | F(8,78)=8.02, p<0.000 | 0.40 | [Black C], [OC], Elevation, N:P | 0.33 |
| OP | F(8,78)=41.56, p<0.000 | 0.79 | Depth, [OC], Lunar Tide, N:P | 0.78 |
| TP | F(8,78)=6.31, p<0.000 | 0.33 | Depth, [Black C], Elevation, [TN], N:P | 0.34 |

Notably, the SJ soils show higher year-1 TN, OC, and OP concentrations (Figs. 5,6) as well as higher N:P ratios compared to RF and RT (Fig A6). Marsh location and associated variables (i.e., salinity, allochthonous P sources, pH, and vegetation type and density) may combine to explain P distribution differences between the sites.

Land use and water quality data demonstrate the St. Jones Watershed to have greater urban land use and deliver higher amounts of nutrient pollution due to more impervious land (Piorko, 2012). The St. Jones River watershed has significant development upstream from Dover, and the downstream portion is primarily agricultural (DNREC, 2022). The land use in this watershed is 37% agriculture, 33% urban, 18% wetlands, and 8% forest (Piorko, 2012). The Blackbird Creek watershed

that influences the RT and RF sites is dominated by forests and wetlands (50%), agriculture (44%), and urban use is 3% (Kennedy et al., 2017). Water quality stations near our sites measured average P concentrations of 0.19 mg/L in St. Jones River that are >25% higher than those at Blackbird Creek (0.15 mg/L) in 2022 (<https://cema.udel.edu/applications/waterquality/>). Additionally, earlier land use reports define 53% of the St. Jones watershed as agricultural, so historical fertilization application could be enhancing soil P and N (DNREC, 2005; Dunne et al., 2010; Kennedy et al., 2017).

The northern RF and RT sites are lower in salinity than SJ. At higher salinities, P adsorption to soil is less favored and instead released to porewater (Sundareshwar & Morris, 1999; Tobias & Neubauer, 2019; Upreti et al., 2015; Zhang & Huang, 2011) and presumably exported from the system due to the decoupling of Fe-P associations in favor of Fe-S associations (Upreti et al., 2015). If this were an important process, there would be more TP in the fresher marshes, as observed by Loomis and Craft (2010); however, RF and RT do not have greater P concentrations than SJ (Fig. 3), and salinity does not explain the higher P concentrations at SJ.

Differences in pH may explain the P concentration differences between SJ and RF and RT. There is likely a pH increase from the northern RT and RF sites towards the more marine-influenced SJ sites due to a positive correlation between salinity and pH that could influence P cycling (Paludan & Morris, 1999; Sundareshwar & Morris, 1999). P tends to be more readily available at higher pH (Fox et al., 2014; Gao & DeLuca, 2020). P and S compete for binding sites on Fe, so at low pH, more hydrogen

sulfide is produced and binds to soluble Fe(II) to form pyrite, leaving P to be released from precipitation in soils to its available dissolved form (Kim et al., 2016; Reimold & Daiber, 1970; Rozan et al., 2002; Sundareshwar & Morris, 1999; Upreti et al., 2015). P is more soluble at a higher pH, so precipitation with Al, Ca, and Fe is favored, and soil P concentration is greater (Bolton et al., 2019; Rozan et al., 2002; Shen et al., 2016).

Finally, the differences in vegetation could be influencing P differences between the marsh sites since OC, TN, and N:P were important factors contributing to P variability, and OC and TN concentrations are related to OP concentration (Figs. 5 & 6, Table 1). Field observations of the marshes found SJ to be strongly dominated by dense *S. alterniflora* and *Phragmites* stands. RF and RT had more varied grass species that include *S. alterniflora*, *S. cynosuroides*, *S. patens*, and *Phragmites*. *S. alterniflora* has greater stem density than *Phragmites* (Coleman et al., 2023; Leonard et al., 2002), so *S. alterniflora* growing in higher density in SJ could be contributing to the larger concentrations of OM at that site and the associated influence on P concentrations.

3.2 Biochar Thin Layer Placement Experiments

The biochar itself had 1091 mg IP kg⁻¹ soil_{dry weight} and 4,100 mg OP kg⁻¹ soil_{dry weight}, which is in the observed range of TP concentrations in biochar (130-42,790 mg P kg⁻¹ soil_{dry weight} depending on feedstock and pyrolysis conditions; Ghodszad et al., 2021) and higher than that of the creek sediments (Fig. 7). Analysis of OP in the 10% biochar-creek sediment mixture and creek sediment alone reveal the biochar

amendments to have higher OP concentrations as measured for bulk OP but similar quantities of the sequentially extracted fractions. Higher IP concentrations were also observed in the biochar amended mixture than the creek sediment by both analysis techniques. These samples collected during experimental setup indicate that the biochar is a P source within the amended plots.

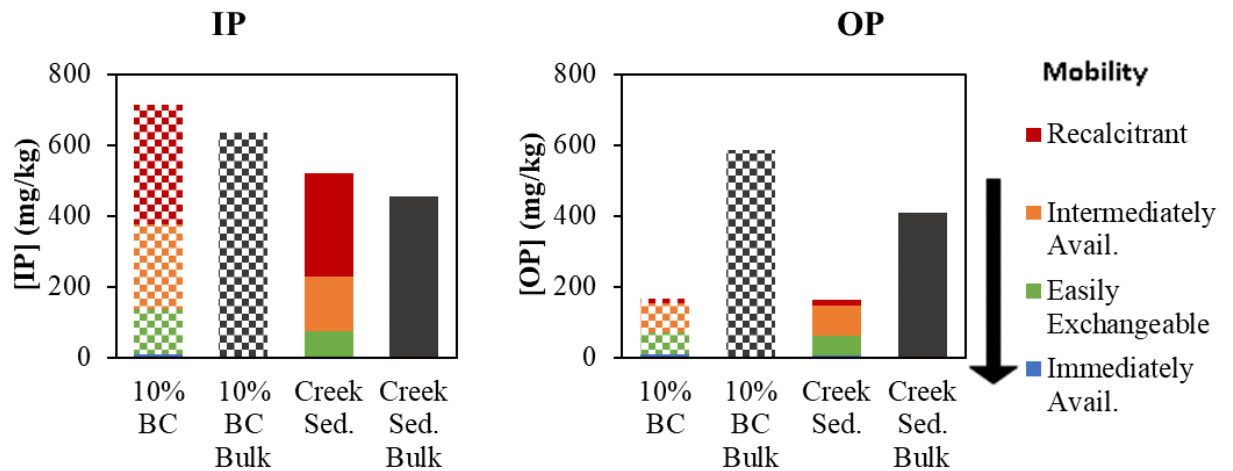


Figure 7. Bulk (black) and sequentially extracted (colored by mobility fraction) IP and OP concentrations in the creek sediment (solid) and 10% biochar (BC) mixtures (textured) added to the biochar TLP plots.

It was expected that final P concentrations in the biochar amended plots will be greater than initial concentrations since biochar will increase P concentrations in soils by sorption, in particular, there will be greater proportions of intermediately exchangeable and recalcitrant P after the experiment since P mobility will be reduced by sorption to biochar. No such changes were observed. Similar to the results from the

analysis of the field-collected cores, no statistically significant positive correlations are observed between biochar and P in the thin layer placement experimental plots at St. Jones. 2-way ANOVA ($p = 0.25-0.92$) shows there to be no difference in [IP] or [OP] in the biochar amended versus unamended plots for either grass species within the top (applied sediment) and bottom (original sediment/ root zone) of the core sections (Fig. 8, Table A10).

The results of the sequential analysis of P in the biochar plots agree with the bulk P analysis; there are no substantial differences in the abundance of any of the P fractions in the biochar amended versus unamended plots within each grass species. A 2-way ANOVA found that the immediately available OP, a very small portion of TP, was the only P pool to show a significant difference in the biochar amended and unamended plots (Fig. 9, Table A11). P concentrations overall did not increase in the soils, and there was no reduction in P mobility in the biochar treatment plots as hypothesized. Thus, there is no evidence within the experimental plots for enhanced P storage in biochar amended TLPs.

The top sections of the cores can be compared to the P concentrations in the applied sediment at the start of the experiment to evaluate if P was sorbed to biochar during the experiment. From experimental setup to sampling 1 year later, [IP] in the biochar amended sediment decreased by about $250 \text{ mg IP kg}^{-1} \text{ soil}_{\text{dry weight}}$. In contrast, [OP] increased by about $220 \text{ mg OP kg}^{-1} \text{ soil}_{\text{dry weight}}$. Unamended sediment slightly decreased in IP (about $30 \text{ mg IP kg}^{-1} \text{ soil}_{\text{dry weight}}$) and increased in OP (about $300 \text{ mg IP kg}^{-1} \text{ soil}_{\text{dry weight}}$). The amended and unamended soil P concentrations changed

similarly during the experiment, which further shows no evidence for biochar sorbing P. Rather, it seems to show that IP may have been taken up and incorporated into OM as OP. Additionally, the greater concentrations of P in the biochar amended sediment that were observed at the start of the experiment was not seen 1 year later after sampling, which suggests that the P from the biochar was exported from the plots in that amount of time. Dittmar et al. (2012) found dissolved black C to be tidally exported from a salt marsh tidal creek system, and recent work shows biochar to be more mobile than previously expected (Bostick et al., 2018; Wagner et al., 2018; Wozniak et al., 2020; Wozniak et al., 2023), so the biochar itself may have been partially exported from the plots.

While the TLP experiments did not show a difference in P distribution with the application of biochar, they did show that *Phragmites* stored more soil P than *S. alterniflora*. There are significantly greater bulk [P] in the bottom section of the *Phragmites* plots of both TLP treatments than the *S. alterniflora* stands (Fig 8, Table A10). This difference in [P] by grass species is also evident in the sequentially analyzed P pools with grass type significantly impacting Easily Exchangeable and Intermediately Available P pools (Fig. 9, Table A11). The results of this experiment indicate that removing *Phragmites* would reduce P storage in soils and increase its mobility in the marsh without any sorption benefits from biochar.

It is worth noting that there is also no evidence for an effect of TLP alone on [P] or distribution since the bottom of the core shows the control plot to have similar [P] to the other *Spartina* plots (Figs. 8 & 9). The comparison of the control to the

treatments in the top section of the cores is not relevant since having had no sediment added, the control core doesn't have an equivalent top section for comparison.

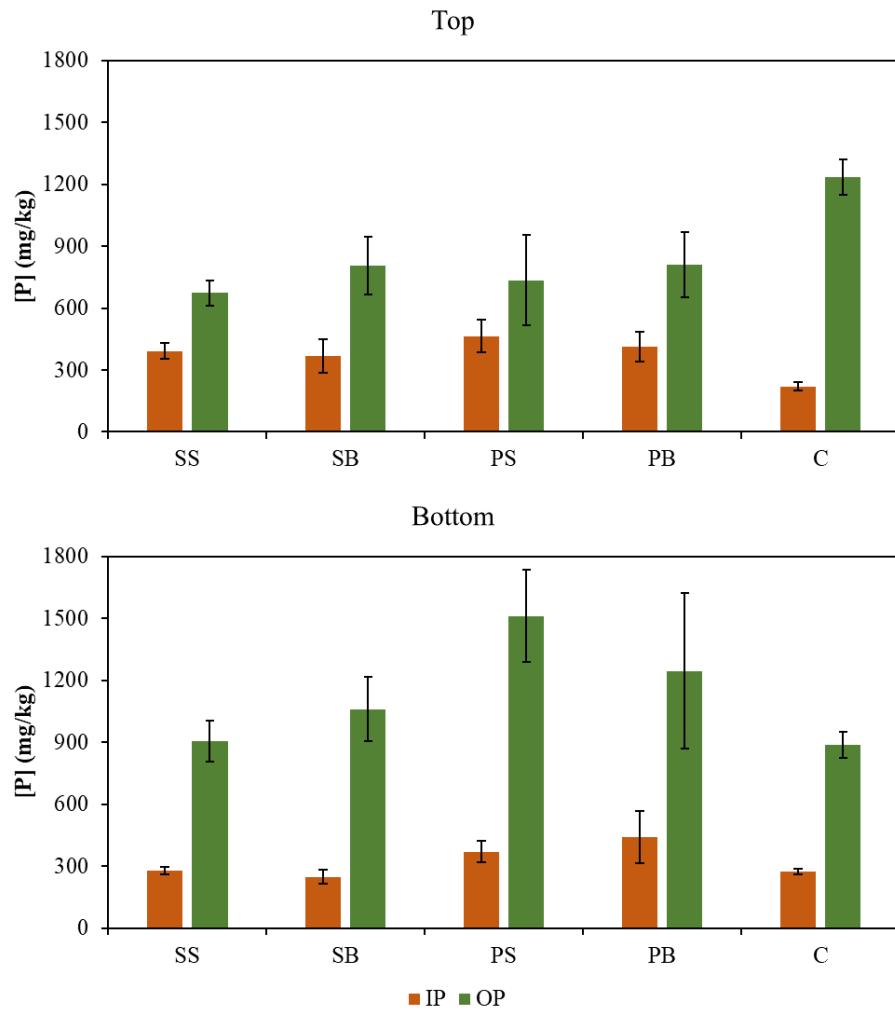


Figure 8. Mean inorganic (orange) and organic (green) P concentrations in the top (applied sediment) and bottom (original sediment) of triplicate cores taken from the biochar TLP experiment plots. Sample IDs are as follows: SS = sediment applied to *Spartina* grass, SB = biochar applied to *Spartina* grass, PS = sediment applied to *Phragmites* grass, PB = biochar added to *Phragmites* grass, and C = control plot (no additions to *Spartina* grass).

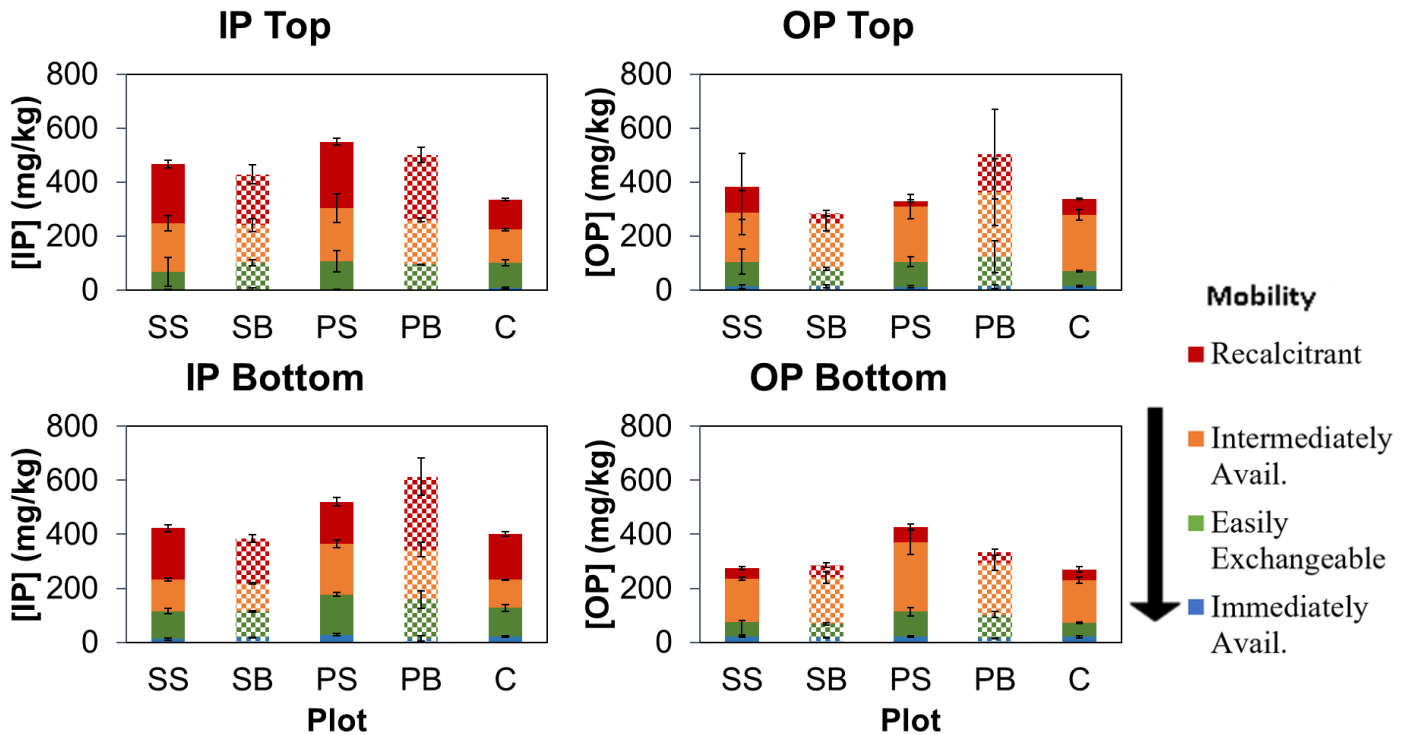


Figure 9. Concentrations of IP (left) and OP (right) in each sequentially extracted P pool (indicated by color) in the top and bottom core sections from the biochar TLP plots. SS, SB, and C are in the *Spartina* stand while PS and PB are located in the *Phragmites*. Solid bars have creek sediment applied and textured bars have sediment with biochar applied. The control (C) has no sediment applied. Error bars represent standard deviation.

Examining OP:IP of each P pool does not reveal any trends in P distribution with biochar application or grass species, but it does highlight the importance of quantifying the OP in each P pool, which is not typically performed in sequential

extraction protocols (Fig. A10). OP is typically measured at the end of the extraction procedure since it is assumed to be immobile, but this study has found OP in every pool of the field-collected and biochar TLP experiment samples (Figs. 4 & 9). Notably, OP:IP is higher in the immediately available pool than the recalcitrant pool (Fig. A10). Therefore, it is recommended that OP and IP be measured at every extraction step.

3.2.1 Environmental Factors that Could Influence P Composition

The biochar amended TLP plots represent a more controlled experiment than the field collection approach since the plots are expected to have experienced similar inundation regimes and environmental conditions. However, since biochar has been used in some applications for its liming effects (Bolton et al., 2019; Fox et al., 2014; Gao & DeLuca, 2020; Shen et al., 2016; Tesfaye et al., 2021), the difference in redox conditions was examined between the plots. P is influenced by redox reactions due to its competition with S for binding sites on Fe. With greater pH, P is more soluble and more likely to precipitate with Fe or Ca, which moves P from water to soil (Rozaan et al., 2002). Increasing pH by biochar addition should add to the expected increase in soil P from sorption by encouraging P precipitation.

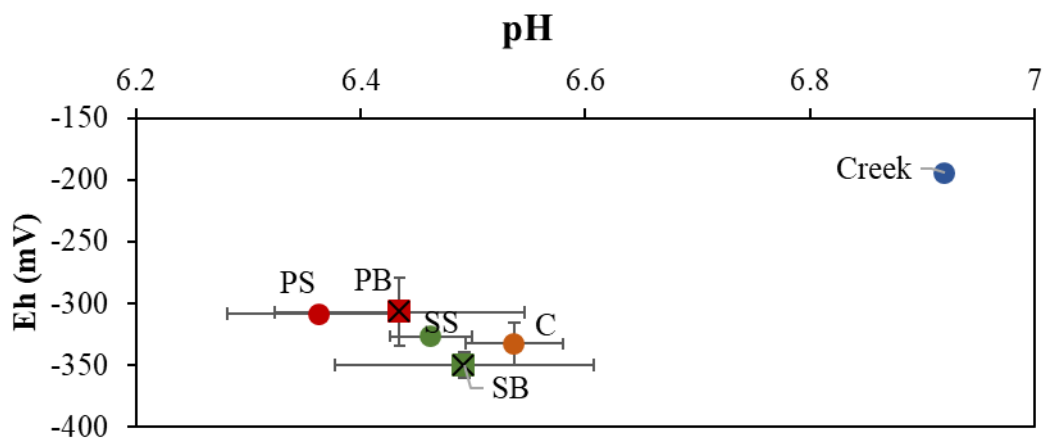


Figure 10. Mean (n=3) redox potential (Eh) and pH values of the biochar TLP plot experiment porewaters and a nearby tidal creek. Plot ID explanations are as follows: SS = sediment applied to *Spartina* grass, SB = biochar applied to *Spartina* grass, PS = sediment applied to *Phragmites* grass, PB = biochar added to *Phragmites* grass, C = control plot of no additions to *Spartina* grass, and Creek = a nearby tidal creek. Error bars show standard deviation.

A liming effect of biochar did occur in this experiment as observed by the higher pH values in the biochar amended TLP plots relative to the sediment only TLP plots of the same grass species (Fig. 10). Interestingly, the TLP additions decreased the pH relative to the control plot by 0.1-0.2 pH units. When comparing pH to P concentrations, the impact of the increase in pH by biochar addition is apparent. The two moderately mobile P pools (Easily Exchangeable & Intermediately Available) have a strong, negative correlation with porewater pH (Fig 11). The moderately mobile P can be Fe- bound, so there is less in the porewater at high pH and likely more

in the soils since the observed pH values are in the range that favors P sorption to marsh sediments (6-6.8; Upreti et al., 2015). Biochar is impacting pH as expected, but this liming effect should add to soil P concentrations which was not observed. Precipitation of P at higher pH due to biochar addition is also not driving P distribution overall.

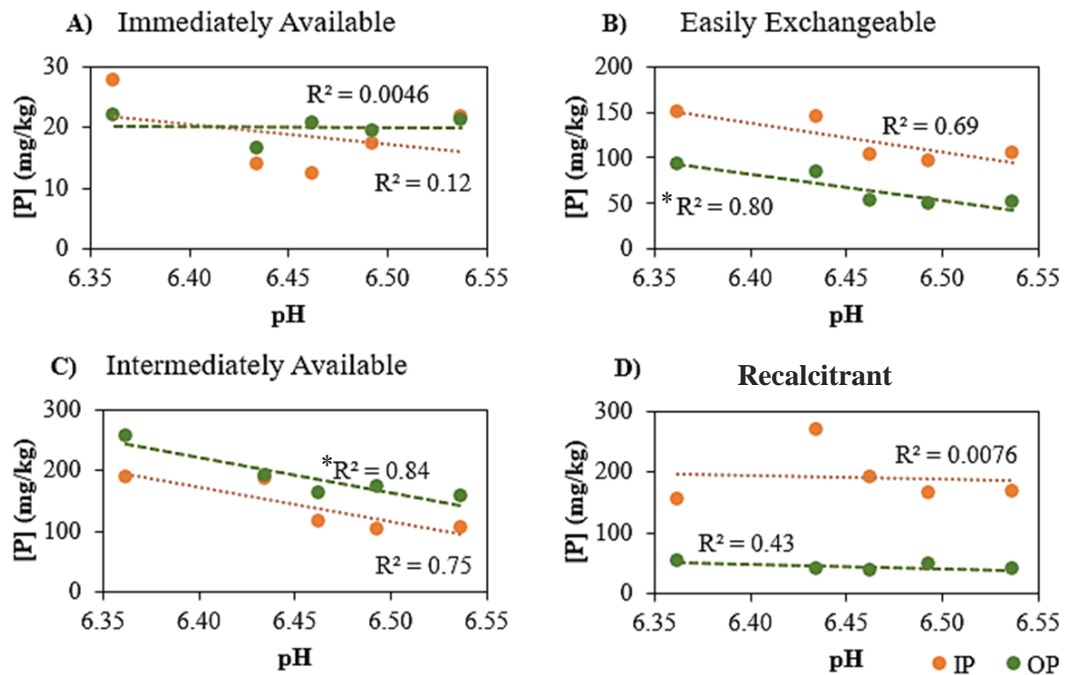


Figure 11. Inorganic (orange) and organic (green) P mobility fractions (mobility decreasing from A to D) compared to *in situ* porewater pH. * R^2 indicates a significant correlation between pH and the specified P pool.

3.3 Phosphorus Storage in Native Vegetation vs. *Phragmites*

Eight additional cores were collected from Roberts Farm and Rocks Tract in November 2023 (year 3) to further assess the differences in *Phragmites* and *Spartina* soil P storage that was observed in the biochar TLP experiments. Four cores were collected in *Spartina* stands in Roberts Farm. Two cores were collected from *Phragmites* stands in both Roberts Farm and Rocks Tract. Concentrations of OP and IP in the *Phragmites* stands were significantly different between RF and RT (Fig. A11), so only the *Phragmites* sites from RF were compared to *Spartina* sites from RF (Fig. 12).

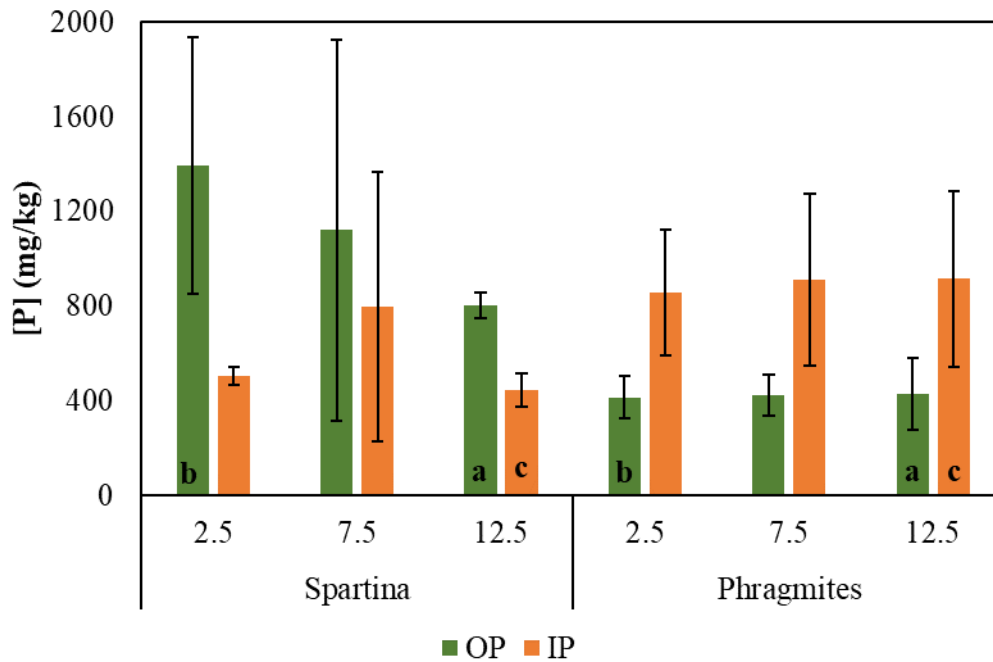


Figure 12. Organic (green) and inorganic (orange) P concentrations in the soil 5 cm depth increments of *Spartina* and *Phragmites* stands in Roberts Farm. Error bars represent standard deviations. Letters indicate pairs of significantly different P concentrations between *Spartina* and *Phragmites* stands at that depth (Table A12).

These additional field collection results generally show *Spartina alterniflora* to have greater OP concentrations and *Phragmites* have greater IP concentrations which contradicts the biochar TLP plot results that show a consistently significantly greater [IP] and [OP] in the *Phragmites* stands relative to the *Spartina alterniflora*. These contrary results could be due to differing dominant vegetation in the marshes or differing environmental conditions in RF and RT compared to the southern and more saline SJ site where the biochar TLP plots were located. SJ does not seem to be a comparable reference marsh to RF and RT given that SJ has greater concentrations of OC, TN, and higher N:P, all of which were determined to significantly contribute to variations in P concentrations by multiple regression analysis (Table 1, Figs. 5, 6, A6). SJ is more saline and likely has more alkaline soils than RF and RT since its more southern location has more marine influence. SJ is dominated by *S. alterniflora* and *Phragmites* while RF and RT have *S. alterniflora*, *S. patens*, *S. cynosuroides*, *Phragmites*, and some *Typha*. The differences in salinity, alkalinity, and vegetation could be reasons that the hypothesis that *Phragmites* may have more soil P retention than *Spartina* was supported by the biochar TLP plots but not the additional field collection results (Figs. 8, 12).

To further examine if marsh site impacts differences in P concentration by grass species and to increase the number of samples, the average P concentration of all *Spartina* collection sites versus all *Phragmites* sites (years 1, 2, 3) are compared (Fig. A12). There were no significant differences between grass type or marshes by 2-way ANOVA without replication. However, there is a general trend of SJ having greater [IP] than RF and RT, which explains why this difference in [P] with grass species was observed in biochar TLP plot experiments. The effect of *Phragmites* on soil [P] relative to native soils may thus be dependent on the marsh system.

There also is generally more OP in the *Spartina* stands relative to *Phragmites*. OP concentrations are associated with OM and biomass, (Table 1) and SJ has greater [OC] and [N] (Figs. 5 & 6), so greater biomass in SJ could be contributing to the different concentrations of P in the different grass species soils.

3.4 Implications and Recommendations

Grass species and biomass influence soil P concentrations in this study. Removing *Phragmites* may reduce the P retention capability of marshes, even if native *Spartina* grasses recolonize after burns as seen by the greater P concentrations in the *Phragmites* plots in the biochar TLP experiments. Therefore, in the context of a P storage ecosystem service, it may not be advisable to remove *Phragmites* in marshes similar to SJ with high OM and biomass and areas where marshes and adjacent coastal waters are at risk of nutrient enrichment impacts. Salt marsh nutrient ecosystem services, whereby nutrient loads are reduced via their retention in salt marsh soils and

biomass (Neckles et al., 2015; Wigand et al., 2017; Yao et al., 2018) would be diminished, potentially causing nutrient pollution in coastal systems, decreasing water quality, and contributing to blooms during seasonal P limitation (Fisher et al., 1992; Pennock & Sharp, 1994; Sharp et al., 2009; Wigand et al., 2017; Yao et al., 2018). Additionally, excess nutrients in marshes have been associated with decreases in belowground production, increased microbial decomposition rates, peat collapse, decreased accretion rates, and greater emission of the greenhouse gas, nitrous oxide (Darby & Turner, 2008; Kutcher et al., 2018; Roman, 2017).

Biochar was shown to have only weak associations with P concentration in the field-collection experiments, and no clear evidence for an added P ecosystem service was observed in the field collections of the biochar TLP experiments. This absence of biochar-induced recouping of P storage contradicts previous work in agricultural and wastewater systems (Bolton et al., 2019; Faridullah et al., 2012; Strømgaard, 1992) and may result from the properties of the *Phragmites* biochar in the TLP plots and produced from the prescribed burns. Biochar properties that influence P sorption capacity like surface area and porosity, ion exchange capacity, surface acidic functional groups, and pH are largely determined by the feedstock material and pyrolysis conditions like temperature (Dugdug et al., 2018; Ghodszad et al., 2021; Jassal et al., 2015; Oliveira et al., 2017; Tesfaye et al., 2021; Zhang et al., 2017). The exact burn temperature of our applied biochar is unknown but was designed to be relatively low (<350°C) to simulate a prescribed fire. Using trends for herbaceous biochars described by a synthesis of 154 published studies, we can estimate that our

char was pyrolyzed at around 400°C based on its pH, ash content, and H:C ratio (Li et al., 2019; Table A8). Higher pyrolysis temperatures are associated with increased surface area, porosity, pH, TP, and ion abundances, with some studies finding declines in these properties above 600 °C (Eduah et al., 2019; Ghodszad et al., 2021; Zhang et al., 2017). Zhang et al. (2017) suggests that biochar from Mg- and Ca-rich feedstocks that are pyrolyzed above 400°C with potential activation measures to increase surface area would be the most efficient biochar for P sorption purposes. The biochar used in this study may thus not have efficiently sorbed P due to its low temperature of formation and lack of activation measures.

Feedstocks can also influence biochar properties like surface area, P-binding ion concentrations, pH, surface functional groups, and TP concentration. *Phragmites* biochar is not well documented in the literature, but comparisons of other feedstocks inform the potential influence of biochar feedstock on P sorption. Wood and crop-based biochars have greater surface area than biochars from solid waste and animal litter due to variations in cellulose, lignin, and moisture content (Ghodszad et al., 2021). Surface area was found to be elevated in pine chars relative to maize straw (Zhao et al., 2017). However, corn stover and switchgrass derived biochars sorbed more P than pine chars, which was attributed to their higher pH (11.4 and 10.4 vs 5.82) and anion exchange with surface functional groups (Chintala et al., 2014). *Phragmites*, like the maize straw, is likely to have less surface area than woody chars resulting in poor sorption performance, but this may be overcome by its relatively high pH (8.43) and presumably surface ion exchange. Ca and Mg concentration in the

feedstock (and corresponding biochar) increases P sorption capacity by binding with P on biochar surfaces and precipitating it out of solution (Dugdug et al., 2018; Ghodszad et al., 2021). Specifically, higher Ca and Mg concentrations in a willow wood biochar (19.6 mg Ca g⁻¹, 2.14 mg Mg g⁻¹) were attributed to greater P sorption than by a wheat straw biochar (1.4 mg Ca g⁻¹, 0.18 mg Mg g⁻¹; Dugdug et al., 2018). Greater Fe concentrations in biochar could also provide more sorption sites for P, and our *Phragmites* char had much higher [Fe] than a corn husk char reported by Eduah et al. (3,442 vs 620 mg Fe kg⁻¹; 2019). Higher P concentration in the applied biochar can slow or even reverse P sorption to biochar due to saturation of sorptive sites, which was seen by pine biochar (11,070 mg P kg⁻¹) sorbing more P than maize-straw biochar (16,200 mg P kg⁻¹; Eduah et al., 2019; Zhao et al, 2017). The *Phragmites* biochar in this study did not have extreme P concentration relative to the range of TP concentration in the previously mentioned studies (~5,200 mg P kg⁻¹ soil_{dry weight} in our study; 1,320-1,940 mg P kg⁻¹ soil_{dry weight} in Eduah et al., 2019; 11,070-16,200 mg P kg⁻¹ soil_{dry weight} in Zhao et al., 2017), and this site saturation is unlikely to have occurred.

The soil environment where biochar is applied also impacts P sorption. Biochar application to low pH soils can release P, while soils with a pH near 6.5, similar to the current study, were found to have increased P sorption with biochar due to precipitation with Mg and Ca at higher pH (Chintala et al., 2014; Eduah et al., 2019). Dugdug et al. (2018) found that there is an optimal pH range for P sorption that is determined by biochar and environmental properties. Overall, the most P sorption

will occur on biochar formed at high temperatures ($>400^{\circ}\text{C}$) from Mg- and Ca-abundant feedstocks of high pH (>8) and low TP ($<12,000\text{ mg P kg}^{-1}$) concentration since P will precipitate with Ca and Mg, P anions will exchange with biochar surface groups, and there are abundant binding sites with high surface area.

This study can also add to the conversation of P quantification methodology. Total OP from the bulk ignition method and the sequential wet chemical oxidation method were not equal. Further, OP measured by sequential extraction is typically only measured at the end of the sequence after igniting or digesting the sample residue (Álvarez-Rogel et al., 2007; Ruttenberg, 1992; Zhang et al., 2019). This study has found OP in all sequentially separated P pools, which suggests that the traditional quantification of OP at the end of the extraction sequence is likely an underestimate.

Future studies monitoring salt marsh P distributions should measure *in situ* pH, Fe, Ca, and Mg concentrations to determine how pH dependent P precipitation interactions are influencing P compositions. Ratios of Al, Fe, and P can indicate P mobility (Joshi et al., 2015; Kleeberg et al., 2010). Quantifying vegetation density and/or above and belowground biomass to compare to P concentrations would also further elucidate the impacts of vegetation and grass species on marsh P retention. There is an interest in amending thin layer placement sediments used to promote marsh resilience with biochar to add C and nutrient storage benefits to marshes (Raposa et al., 2023). Experiments that vary the biochar feedstock and production conditions (temperature, activation) and resulting sorption characteristics may

differentially impact P sorption in marshes and inform biochar design specifically for that service.

Chapter 4

CONCLUSIONS

Current marsh management practices remove *Phragmites* to promote marsh habitat. However, their removal, even by burning, may negatively impact P retention in marshes. The vulnerability of the specific marsh and estuary system to nutrient loading impacts should be considered when determining how best to manage *Phragmites*. The prescribed burns in our study site did not produce biochar that showed a clear P storage benefit; neither did the low temperature *Phragmites*-derived biochar in our TLP plots. However, biochars have heterogeneous compositions, and a more carefully designed biochar may yield the desired P storage ecosystem service. While temperatures in prescribed burns are likely to be difficult to optimize, creating biochar at high pyrolysis temperature from feedstock of high surface area that is enriched with ions that interact with P (e.g. Ca, Mg, Fe) could enhance P sorption within TLP projects. Future work should evaluate the effects of char feedstock and temperature on this P storage ecosystem service. Biomass and grass species, pH, and tidal inundation have the greatest impact on P distributions in the studied sites. Therefore, efforts to understand salt marsh P distributions should include vegetation metrics (e.g. above and belowground biomass, stem density), as well as pH, Fe, Ca, and Mg concentration measurements.

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Appendix A

ADDITIONAL FIGURES



Figure A1. Collection sites of the year 3 field collection samples. There were 4 samples collected in *Spartina* stands in Roberts Farm and 4 samples from *Phragmites* stands split between Roberts Farm and Rocks Tract.

Table A1. p-values from the results of paired 2 sample t-Tests with one tail of replication types on both total IP and OP measured by the ignition method (bulk P) and sequentially extracted samples (sequential P). Replication was assessed on soil subsamples subjected to the full extraction and analysis process (process) and through multiple analyses of diluted extracts with the colorimetric reagents added (instrument). No p-values are significant.

| Analysis | Replication Type | p | df |
|--------------|------------------|-------|----|
| Bulk P | Instrument | 0.421 | 35 |
| | Process | 0.381 | 24 |
| Sequential P | Instrument | 0.340 | 38 |
| | Process | 0.292 | 36 |

Table A2. p-values from the results of paired 2 sample, 1-tail t-Tests showing no significant differences in OP or IP concentrations between sample years at each marsh.

| | Marsh | p |
|----|-------|-------|
| OP | SJ | 0.121 |
| | RF | 0.159 |
| | RT | 0.050 |
| IP | SJ | 0.063 |
| | RF | 0.248 |
| | RT | 0.382 |

Table A3. p-values from the results of a 1-way ANOVA comparing OP and IP concentrations between the three marshes separated by the 3 sampled depth increments. Bolded p-values indicate that at least one marsh had significantly different P concentrations at the specified depth interval.

| | Depth (cm) | p |
|----|------------|---------------------------|
| OP | 2.5 | 0.0082^a |
| | 7.5 | 0.5172 |
| | 12.5 | 0.3853 |
| IP | 2.5 | 0.4557 |
| | 7.5 | 0.3388 |
| | 12.5 | 0.0553 |

^a Tukey's *post hoc* test found SJ to have significantly more OP at the 2.5 cm increment than RF ($F(54) = 4.01$, $p = 0.0174$) and RT ($F(54) = 3.88$, $p = 0.0222$).

Table A4. p-values from the results of 1-way ANOVA comparing OP and IP concentrations for each of the depth increments separated by the three marshes. Bolded p-values indicate that the average P concentrations are not equal at all depths at the specified marsh location.

| | Marsh | p |
|----|-------|---------------------------|
| OP | SJ | 0.0007^a |
| | RF | 0.5127 |
| | RT | 0.6799 |
| IP | SJ | 0.2416 |
| | RF | 0.0063^b |
| | RT | 0.7014 |

^a Tukey's *post hoc* test found the 2.5 cm increment in SJ to have significantly more OP than the 7.5 cm increment ($F(57) = 3.88$, $p = 0.0219$) and the 12.5 cm increment ($F(57) = 5.63$, $p = 0.00057$).

^b Tukey's *post hoc* test found the 2.5 cm increment in RF to have significantly more IP than the 12.5 cm increment ($F(51) = 4.56$, $p = 0.00611$).

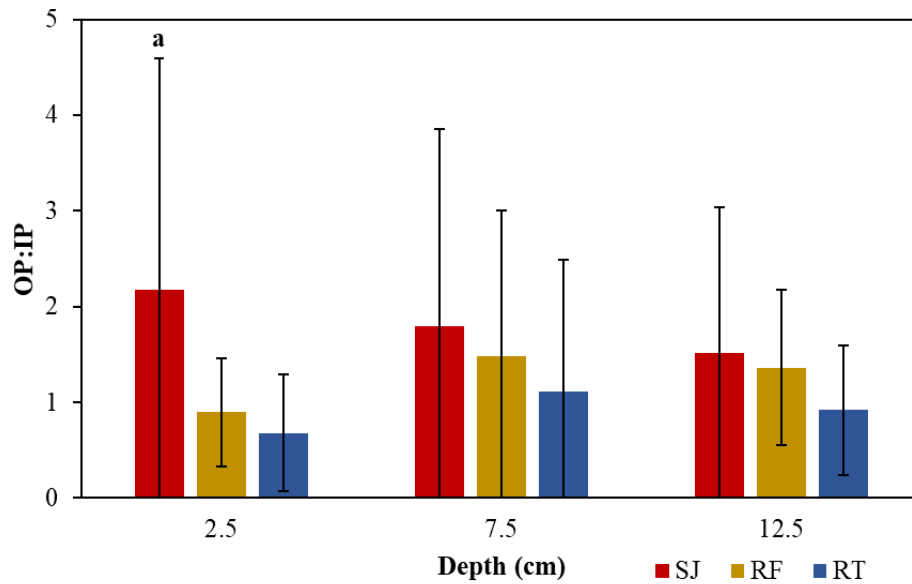


Figure A2. The OP:IP ratio at each marsh (indicated by color) and depth increment. Ratios are an average of all cores from year 1 and 2 with error bars showing standard deviation. ‘a’ indicates there is a significantly greater OP:IP at SJ than the other marshes at 2.5 cm deep.

Table A5. Results of 1-way ANOVA comparing OP:IP ratios between marsh sites at each depth increment. Bolded p-values indicate a significant difference in OP:IP between marshes at the specified depth.

| Depth (cm) | p |
|------------|---------------------------|
| 2.5 | 0.0044^a |
| 7.5 | 0.3594 |
| 12.5 | 0.1788 |

^a Tukey’s *post hoc* test found there to be significantly higher OP:IP in SJ than RF ($F(54) = 3.83, p = 0.0243$) and RF ($F(54) = 4.53, p = 0.0064$).

Table A6. Results of 1-way ANOVA comparing OP:IP ratios from different depth bins within each marsh site. There are no significant differences in OP:IP with depth.

| Marsh | p |
|-------|--------|
| SJ | 0.5423 |
| RF | 0.2172 |
| RT | 0.3573 |

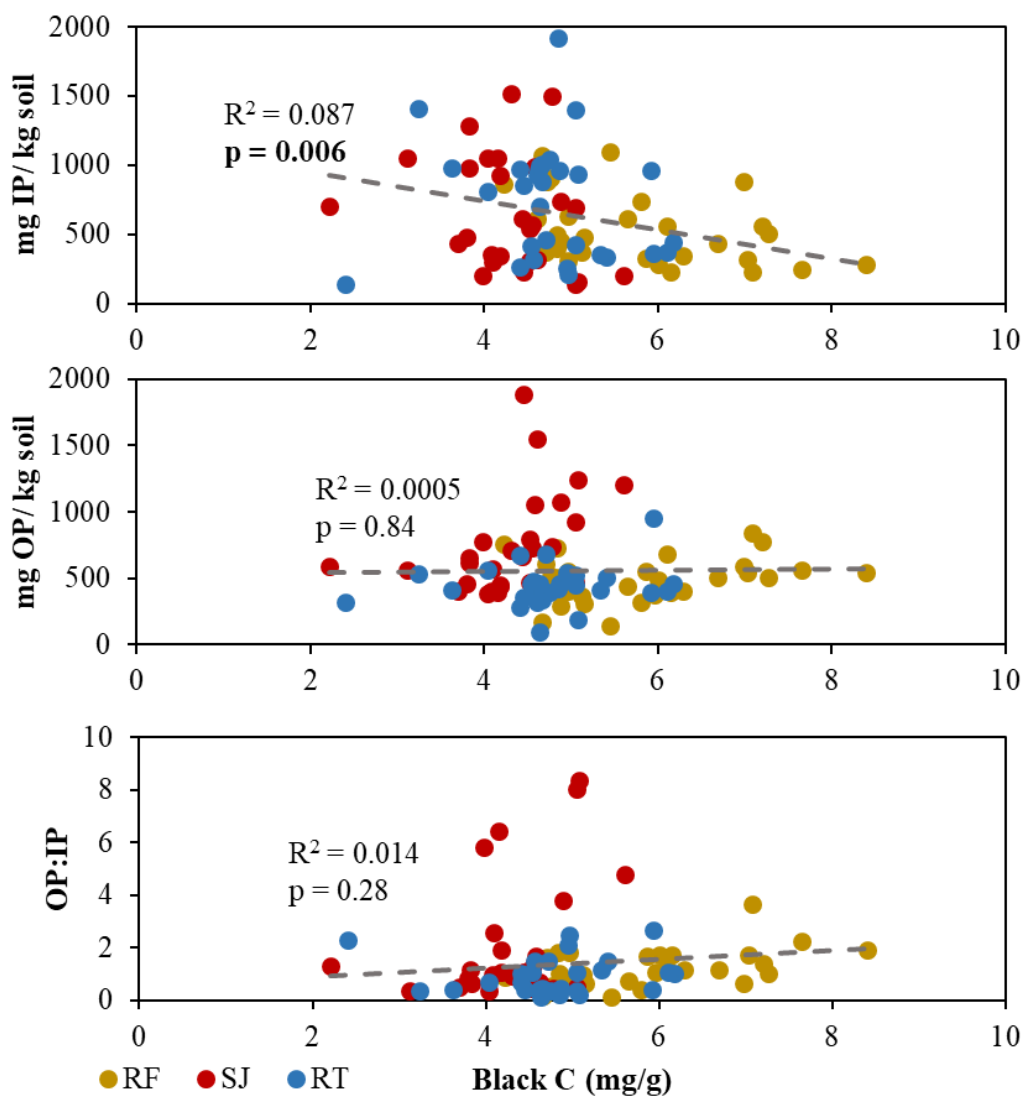


Figure A3. Bulk IP (top) & OP (middle) concentrations and OP:IP (bottom) plotted versus black carbon concentrations. Color indicates the marsh where samples were collected and the trendline represents a linear correlation for samples from all 3 locations.

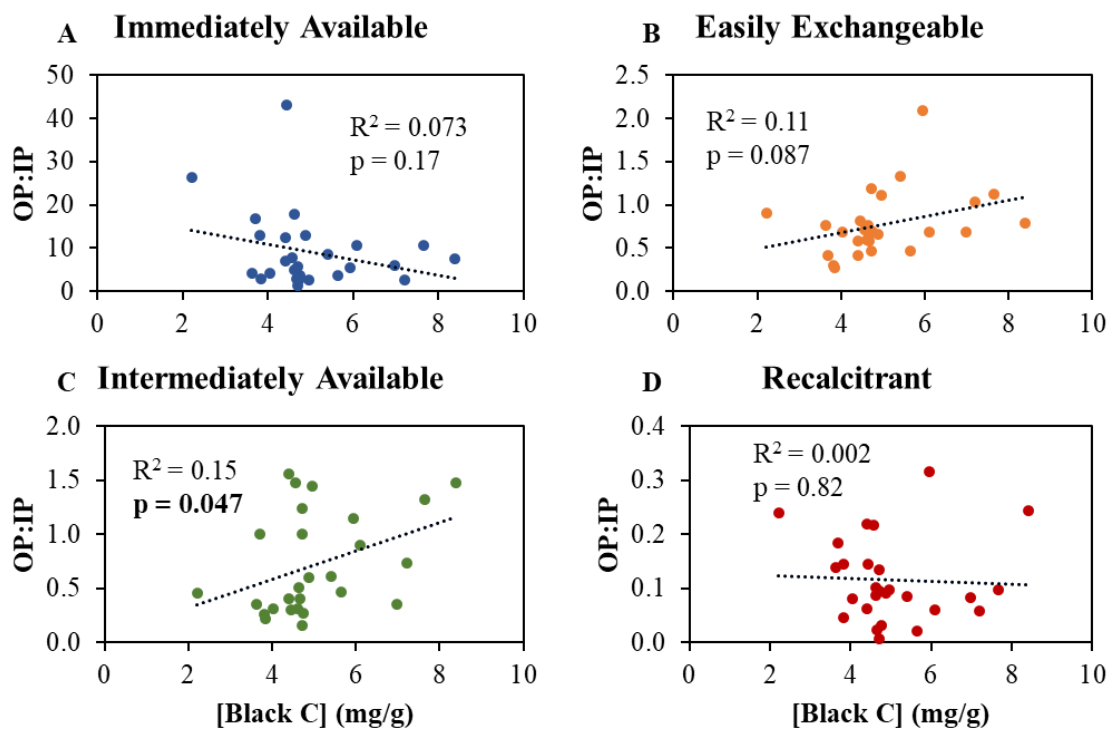


Figure A4. The OP:IP ratios in each of the 4 sequentially extracted P mobility pools plotted against black C concentration. Mobility decreases from A to D.

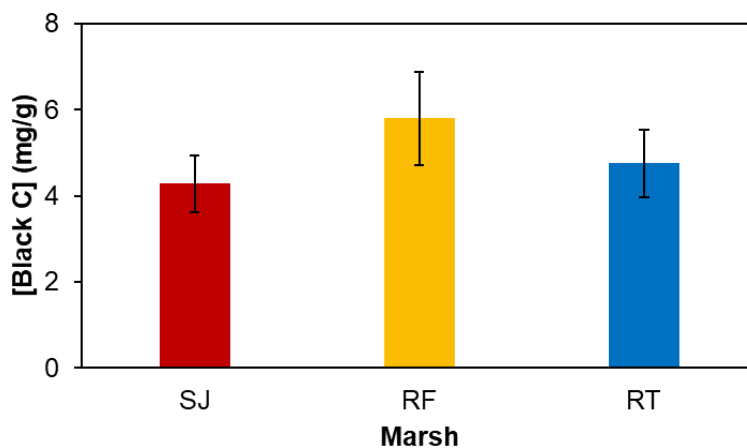


Figure A5. Mean black C concentration measured in core subsamples from the 3 sampled marshes. Error bars represent standard deviations ($n=30$ for each marsh). Results of 1 way ANOVA were significant ($F(2,78)=23.85$, $p < 0.000$). Results of Tukey's *post hoc* test indicate that black C concentration at RF is significantly greater than SJ ($F(78) = 9.43$, $p < 0.000$) and RT ($F(78) = 6.92$, $p < 0.000$).

Molar N:P was also compared at each marsh since it was an explanatory variable for IP, OP, and TP soil concentration variability by multiple regression analysis (Fig. A6). This ratio can also indicate the nutrient limitation status of the studied marshes. Our N:P ratios were found to be within or below the reported N:P range for marsh plants and soils (10-25; Tobias and Neubauer, 2019) at all marshes, which suggests there to be sufficient P in the marshes. Average C:N:P ratios at each marsh are as follows: SJ = 174:16:1, RF = 154:10:1, RT = 107:6:1. These values are lower than reported C:N:P of brackish marsh surface sediments reported in a South Carolina brackish marsh by Sundareshwar and Morris (1297:59:1; 1999) and C:N observed in a New Jersey tidal marsh by Velinsky et al. (14-41; 2017).

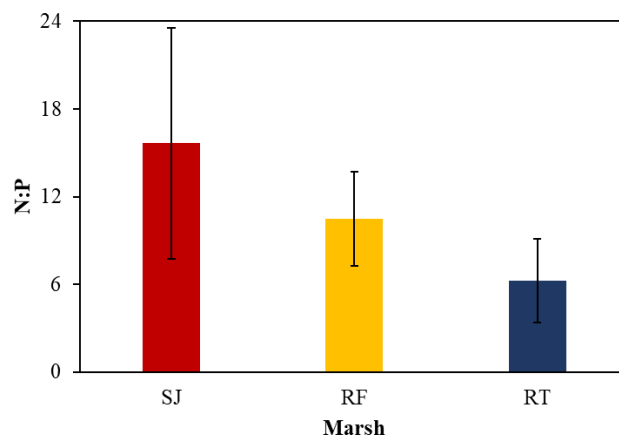


Figure A6 Mean molar N:P ratio in each marsh with [P] as TP from year 1 bulk P measurements and error bars showing standard deviation. 1-way ANOVA found a significant difference in N:P by marsh ($F(2,87) = 24.50$, $p < 0.000$). Tukey's *post hoc* test found all marshes to have significantly different N:P (Table A7).

Table A7. p-values for Tukey's *post hoc* test examining differences in N:P between the marshes. Bolded p-values are statistically significant.

| Marsh Pair | p |
|------------|-----------------------------|
| SJ-RF | 0.0007 |
| SJ-RT | 1.6 x10⁻⁹ |
| RF-RT | 0.006 |

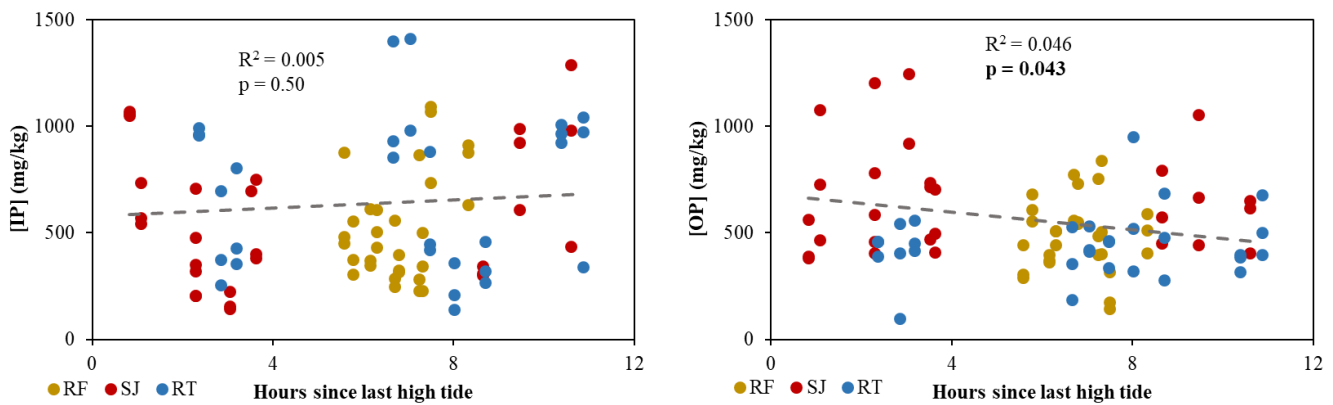


Figure A7. Time between sample collection and last high tide compared to IP (left) and OP (right) concentration. Color indicates sampled marsh.

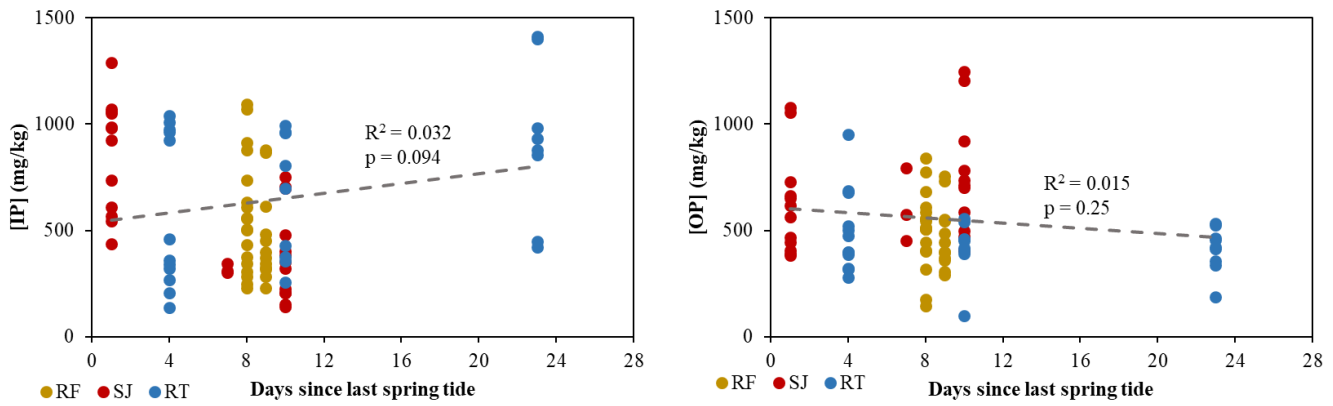


Figure A8. The number of days between sample collection and the last spring tide compared to IP (left) and OP (right). Color indicates sampled marsh.

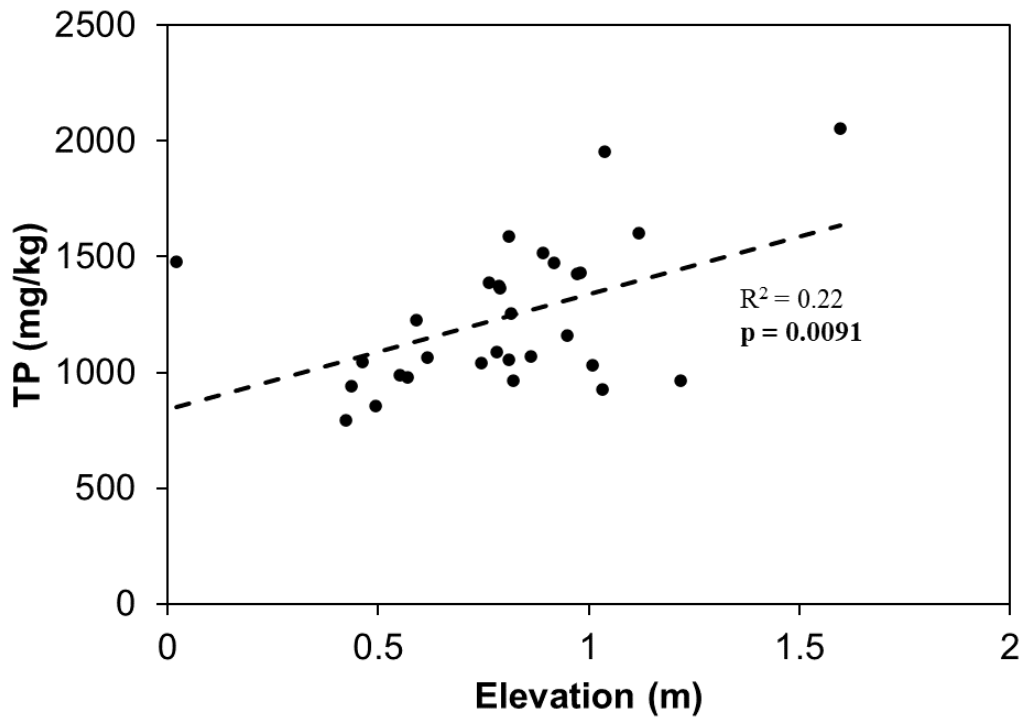


Figure A9. Total P compared to elevation above sea level as obtained from LiDAR measurements (Dan Warner, DGS). IP and OP variability due to elevation were also low ($R^2 = 0.12$, 0.018 respectively)

Table A8. Coefficients and p-values of the independently significant environmental variables on each form of bulk P determined by multiple regression analysis.

| | Variable | Coefficient | p-value |
|----|------------|-------------|---------|
| IP | [Black C] | -90 | 0.008 |
| | [OC] | -4072 | 0.0003 |
| | Elevation | 357 | 0.002 |
| | N:P | -14 | 0.009 |
| OP | Depth | -17 | 0.0001 |
| | [OC] | 7477 | <0.0000 |
| | N:P | -2.8 | 0.24 |
| | Lunar Tide | -5.8 | 0.026 |
| TP | Depth | -24 | 0.011 |
| | [Black C] | -81 | 0.026 |
| | Elevation | 378 | 0.004 |
| | [TN] | 49 | 0.0008 |
| | N:P | -28 | 0.0004 |

Table A9. Physicochemical properties of our *Phragmites* biochar compared to similar* chars from other studies.

| Property | Our Biochar | Literature Value | Reference |
|--|------------------|---|---|
| H:C ^a | 0.46 | 0.86-0.39 | Horel et al., 2019; Jassal et al., 2015; Wei et al., 2019 |
| pH ^a | 8.43 | 7.94-10.4 | Chintala et al., 2014; Dugdug et al., 2018; Eduah et al., 2019; Horel et al., 2019; Zhao et al., 2017 |
| % Ash ^a | 24.6 | 26-42 | Dugdug et al., 2018; Horel et al., 2019; Jassal et al. 2015; Wei et al., 2019; Zhao et al., 2017 |
| Surface Area (mg ² g ⁻¹) ^a | 233 ^c | 4.05-55.2 ^d , 199 ^c | Chintala et al., 2014; Dugdug et al., 2018; Horel et al., 2019; Zhao et al., 2017 |
| [Fe] (mg kg ⁻¹) ^a | 3442 | 620-950 | Eduah et al., 2019 |
| [P] (mg kg ⁻¹) ^a | 857 | 1500-16200 | Chintala et al., 2014; Eduah et al., 2019; Zhao et al., 2017 |
| [P] (mg/kg) ^b | 5,191 | 1500-16200 | Chintala et al., 2014; Eduah, Zhao |

*Comparable biochars have similar feedstocks to *Phragmites* like wheat straw, corn husk, switchgrass, and grain husk.

^a denotes properties that were analyzed by Control Laboratories for our biochar.

^b denotes properties that were analyzed by this study.

^c Surface area correlation and measured by butane activity.

^d Specific surface area by the N₂ BET method.

Table A10. p-value results from 2-way ANOVA comparisons of the impact of grass type and the presence of biochar on IP and OP concentrations in the top and bottom of biochar TLP plot cores as well as *in situ* pH and Eh. Bolded p-values are statistically significant.

| | p Biochar Presence | p Grass Type | p Interaction |
|-----------|--------------------|---------------|---------------|
| Top IP | 0.9210 | 0.8661 | 0.8450 |
| Top OP | 0.8669 | 0.9601 | 0.8612 |
| Bottom IP | 0.5196 | 0.0022 | 0.1325 |
| Bottom OP | 0.2538 | 0.0192 | 0.0408 |
| pH | 0.3620 | 0.1740 | 0.7001 |
| Eh | 0.2265 | 0.0072 | 0.1842 |

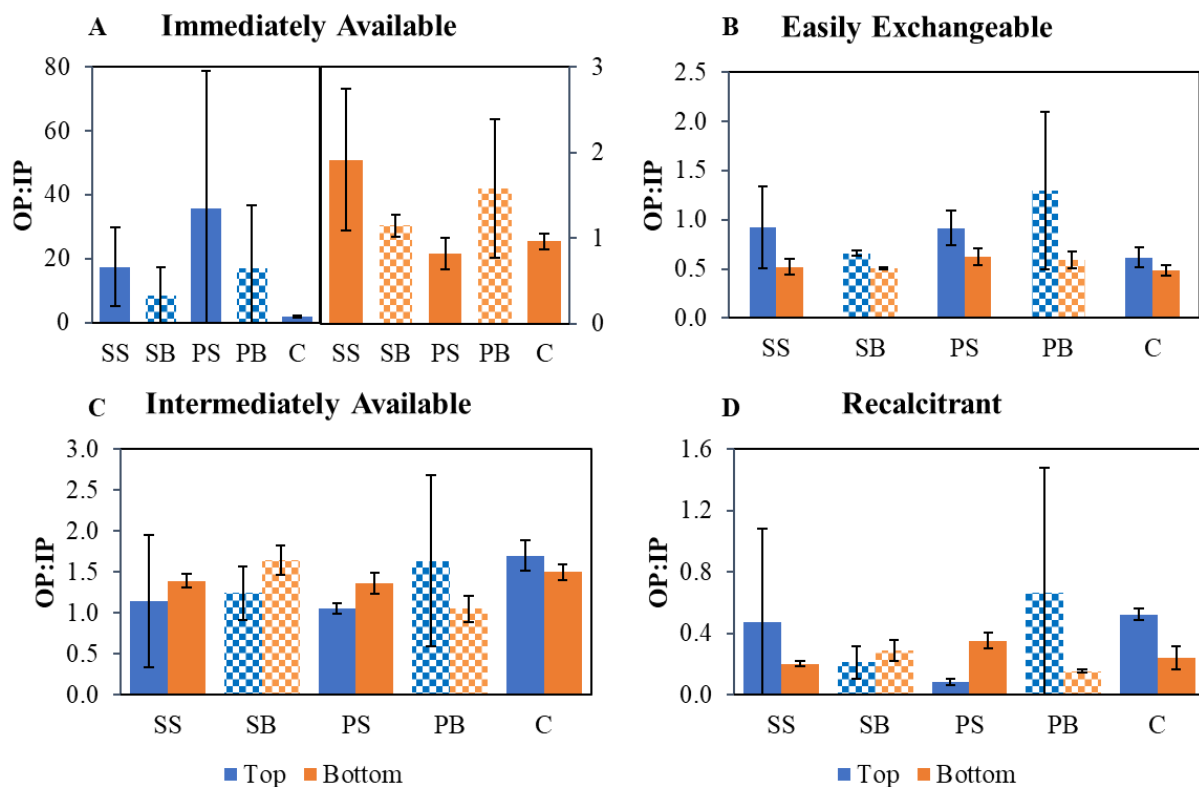


Figure A10. OP:IP ratios of each sequentially extracted P pool (mobility decreasing from A to D) in the top (blue) and bottom (orange) core sections from the biochar TLP plots. SS, SB, and C are in the *Spartina* stand while PS and PB are *Phragmites*. Solid bars have creek sediment applied and dotted bars have sediment with biochar applied.

Table A11. p-value results from 2-way ANOVA comparisons of the impact of grass type and the presence of biochar on concentrations of IP and OP pools in biochar TLP plot cores. Bolded p-values are statistically significant.

| | | p Biochar Presence | p Grass Type | p Interaction |
|--------------------------|----|--------------------|---------------|---------------|
| Immediately Available | IP | 0.2660 | 0.1220 | 0.0300 |
| | OP | 0.0024 | 0.2830 | 0.0230 |
| Easily Exchangeable | IP | 0.5920 | 0.0016 | 0.9510 |
| | OP | 0.2770 | 0.0002 | 0.6700 |
| Intermediately Available | IP | 0.4100 | 0.0000 | 0.6070 |
| | OP | 0.1410 | 0.0084 | 0.0610 |
| Recalcitrant | IP | 0.0694 | 0.1430 | 0.0115 |
| | OP | 0.6700 | 0.3790 | 0.0680 |

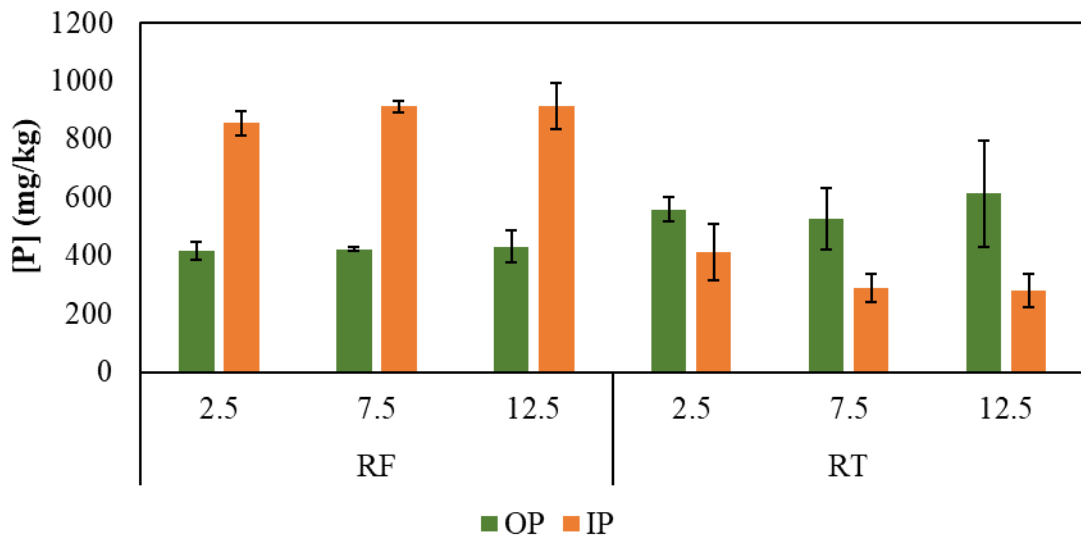


Figure A11. OP and IP concentrations in the *Phragmites* stands in the year 3 collection from Roberts Farm compared to Rocks Tract. There is significantly more IP in RF ($t(10) = 14.06$, $p < 0.000$) and significantly more OP in RT ($t(10) = -3.26$, $p = 0.009$).

Table A12. p-values from 2-sample t-tests of unequal variances comparing P concentrations in the soil of *Spartina* and *Phragmites* stands in Roberts Farm. Bolded p values are statistically significant.

| | Depth (cm) | p |
|----|------------|---------------|
| OP | 2.5 | 0.0588 |
| | 7.5 | 0.2231 |
| | 12.5 | 0.0496 |
| IP | 2.5 | 0.0100 |
| | 7.5 | 0.4391 |
| | 12.5 | 0.0179 |

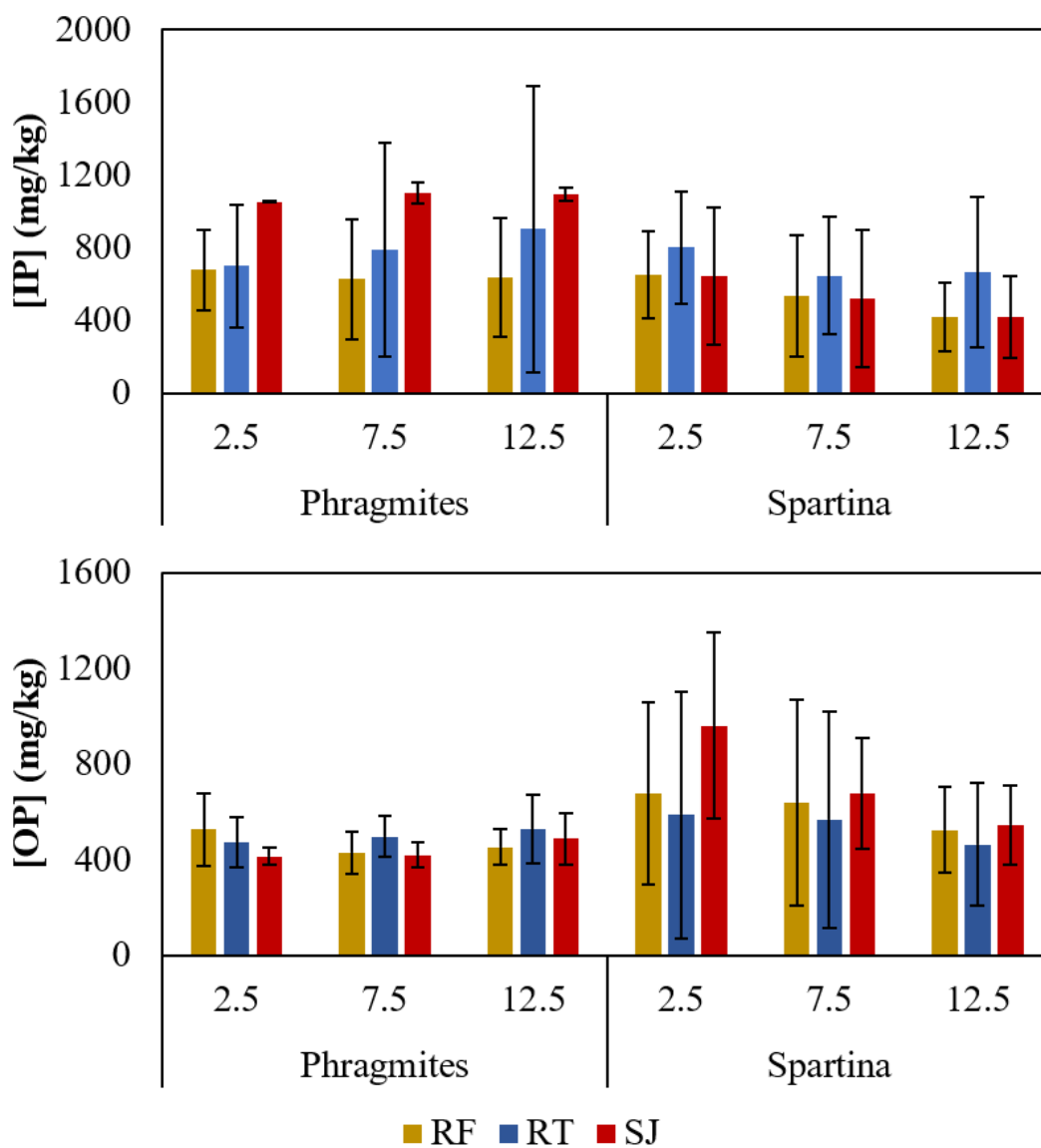


Figure A12. Concentrations of IP (top) and OP (bottom) in *Phragmites* and *Spartina* stands in the three marshes, indicated by color, separated by depth (cm). 2-way ANOVA results found no differences in IP or OP concentrations binned by depth between *Phragmites* and *Spartina* stands.