FLORIDA INTERNATIONAL UNIVERSITY

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CARBON PROCESSING BY BENTHIC AND PELAGIC MICROBIAL COMMUNITIES UNDER CHANGING HYDROLOGIC PULSES AND PRESSES IN COASTAL WETLANDS

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To: Dean Michael R. Heithaus
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This dissertation, written by Kenneth J. Anderson, and entitled Carbon Processing by Benthic and Pelagic Microbial Communities Under Changing Hydrologic Pulses and Presses in Coastal Wetlands, having been approved in respect to style and intellectual content, is referred to you for judgment.

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DEDICATION

I dedicate this dissertation to Robin, Howl, and Josie. I wouldn’t have been able to do this without their unwavering support.

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ABSTRACT OF THE DISSERTATION

CARBON PROCESSING BY BENTHIC AND PELAGIC MICROBIAL COMMUNITIES UNDER CHANGING HYDROLOGIC PULSES AND PRESSES IN COASTAL WETLANDS

by

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Wetland ecosystems are rapidly changing as both climate change and human modifications alter their hydrology and biogeochemistry. Coastal wetlands are critical stores of carbon, and how hydrologic presses (e.g., sea-level rise, restoration) and pulses (storms, seasonality) interact to influence carbon cycling in coastal wetlands is uncertain. I investigated how changing nutrient concentrations, carbon sources, and water depths influence carbon cycling in wetlands of the Florida Coastal Everglades, which are both undergoing rapid freshwater restoration and saltwater intrusion from sea-level rise. First, I performed a reciprocal transplant of four dominant species of leaf litter across freshwater-to-marine gradients and showed that marine water is the most consistent driver of increasing litter breakdown across species. I then investigated how gene expression of microbial communities responds to dissolved and particulate chemistries to better understand the effects of seawater on litter breakdown and found a positive correlation between sulfate acquisition and seawater, especially in more anoxic environments. To understand the fate of carbon after litter breakdown, I collected data on the concentration and composition of dissolved organic matter from surface waters along wetland transects with higher and lower phosphorus enrichment receiving restorative fresh water. I found that dissolved organic matter in higher-phosphorus wetlands is becoming “greener” (decreasing in concentration and increasing in algal influence), while DOM in lower-phosphorus wetlands is becoming “browner” (increasing in concentration and in detrital influence). To better understand long-term drivers, I used twenty years of long-term data on the concentration and composition of dissolved organic matter across six sites spanning marsh, ecotone, and mangrove habitats in a lower- and a higher-productivity. I found that the higher-productivity Everglades marsh and mangroves are tightly controlled by changing water depths, but the lower-productivity marsh was not. This suggests that higher water depths suppress the production and dilute the fluxes of carbon in higher-productivity marshes; however, they import carbon to lower-productivity marshes and mobilize carbon within mangrove ecosystems. Overall, my research highlights how changes in hydrology and nutrients drivers carbon processing in coastal wetlands and provides a framework for predicting how these ecosystems will be altered by ongoing sea-level rise and restoration.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BIX	Biological Index
BP	Bacterial Productivity
CERP	Comprehensive Everglades Restoration Plan
COP	Combined Operations Plan
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
ENP	Everglades National Park
FCE	Florida Coastal Everglades
FI	Fluorescence Index
HIX	Humic Index
LTER	Long-term Ecological Research
MWD	Modified Water Deliveries
NESRS	North East Shark River Slough
S_R	Slope Ratio
SRS	Shark River Slough
$SUVA_{254}$	Specific ultraviolet Absorbance at 254nm wavelength
TN	Total Nitrogen
TP	Total Phosphorus
TS/Ph	Taylor Slough/Panhandle

CHAPTER I

INTRODUCTION

Coastal ecosystems are at the forefront of multiple effects of climate change, including sea-level rise, and increased storm frequency, while also facing anthropogenic pressures from coastal communities (Morris et al. 2002, Craft et al. 2016). Coastal wetlands are major stores of carbon, and the regulation of that carbon plays a role in their value both to people using the wetlands, and as an ecosystem (Barbier et al. 2011, Gedan et al. 2011, Fourqurean et al. 2012, Jones et al. 2018). Maintaining carbon storage is especially important for coastal wetlands because it builds soil elevation, which is only becoming more important as wetlands are stressed by sea-level rise, in some places causing the collapse of wetland soils (Chambers et al. 2015). The multiple threats facing coastal wetlands are driving changes to the biogeochemistry of coastal wetlands, which in turn is altering the storage of carbon in these ecosystems.

Climate change and restoration driven presses and pulses of marine and fresh water have led to significant changes to coastal wetlands, in some cases leading to rapid shifts to new environments (Harris et al. 2018). One constant hydrologic press is saltwater intrusion from sea-level rise, that brings novel stressors and subsidies to coastal wetland communities (Kirwan et al. 2013, Smoak et al. 2013, Chambers et al. 2015). Hydrologic pulses include major storm events that are becoming more common, which can lead to the transport and/or transformation of large quantities of carbon (Osburn et al. 2019, Breithaupt et al. 2020, Feher et al. 2020). At the same time restoration efforts are producing competing pulses and presses, commonly originating from fresh water, where many hydrologic disruptions are being restored to their former state at varying levels of success (Hobbs et al. 2007, Lee et al. 2021). This combination of disturbances is producing rapidly changing conditions for coastal wetlands that have led to state shifts in terms of macrophyte communities along with soil collapse, massively changing the function of these ecosystems (Chambers et al. 2013, Charles et al. 2020, Kominoski et al. 2020, 2021).

To understand how changing pulses of fresh and marine water are driving carbon cycling and to predict how they will into the future, we need a better understanding of the carbon cycle and what drives both the organisms involved in it, and the different types and qualities of carbon within wetlands. One of the major reasons that coastal wetlands are so successful at sequestering carbon is that they typically have high rates of production of new vegetation (Kristensen et al. 2008, Duarte et al. 2010). They also tend to have largely anaerobic soils leading to slow breakdown of organic matter when in their soils (Bouillon et al. 2008, Donato et al. 2011). That breakdown also creates much of the dissolved organic carbon in the water column that is utilized by pelagic microbes driving the microbial loop, so changing benthic processing will likely also affect organic matter processing in the water column (Jaffé et al. 2008). However, novel terminal electron acceptors found in seawater act as subsidies to anaerobic microbial communities by releasing redox conditions as a limitation (Helton et al. 2015). Carbon quality is also changing as macrophyte communities shift and increasing pulses and presses of water from restoration, sea level rise and disturbance are altering the importance of both assimilatory and dissimilatory limitations on microbial processing of carbon.

The Florida Coastal Everglades is the perfect environment to investigate drivers of climate change on coastal wetland carbon processing, given the presses of sea-level rise and saltwater intrusions, and hydrologic pulses from freshwater restoration and tropical storms. Florida is vulnerable to sea-level rise with 0.6 m of rise is predicted by

2060 (Zhongming et al. 2021). The Everglades is currently seeing the effect of sea-level rise, and changes in carbon storage where peat soils are collapsing and vegetation are changing as saltwater intrudes (Wilson et al. 2018, Charles et al. 2019, Servais et al. 2019). At the same time as the press of seawater is increasing throughout coastal wetlands, restoration is bringing new pulses of fresh water into systems, which is in turn mobilizing legacy phosphorus from years of past phosphorus loading (Sarker et al. 2020). This environment with pulses of fresh and salt water, containing subsidies of nutrients, and terminal electron acceptors across a landscape that ranges from fresh to marine water is ideal for studying the effects of ecosystem change on carbon processing.

In each chapter of my dissertation, I investigated different questions related to the processing and storage of carbon across coastal wetland ecosystems. In Chapter II, I asked what are the intrinsic and extrinsic drivers of litter breakdown in wetlands with changing environmental chemistries? In Chapter III, I asked how do microbial energetic pathways change, both along freshwater to marine gradients, and between litter of variable quality? In Chapter IV, I asked what are the drivers controlling the sources and concentrations of dissolved organic carbon in restored wetlands that differ in nutrient enrichment? In Chapter V, I asked how are sources and fates of carbon shifting in response to changing hydrology? Finally, in Chapter VI I summarize the results from the previous chapters and give suggestions for future work based on my findings.

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CHAPTER II

INTRINSIC AND EXTRINSIC DRIVERS OF ORGANIC MATTER PROCESSING ALONG PHOSPHORUS AND SALINITY GRADIENTS IN COASTAL WETLANDS

ABSTRACT

1. Sea-level rise and saltwater intrusion are driving large-scale changes in macrophyte species composition, and productivity, as well as the deposition of litter in coastal wetlands. Quantifying macrophyte litter breakdown along freshwater-to-marine coastal gradients is needed to predict how carbon stores will respond to shifts in both macrophyte communities and water chemistry under changing environmental conditions.
2. To test the interactive drivers of changing species identity and water chemistry, we performed a reciprocal transplant of four macrophyte litter species in seven sites along freshwater-to-marine gradients in the Florida Coastal Everglades. We measured surface water chemistry, litter chemistry (nutrients, cellulose and lignin as proxies for recalcitrance) and litter breakdown rates (*k /* degree day) during a 10-month incubation period.
3. Direct effects of salinity, surface water nutrients, and litter species identity were the strongest drivers of *k*, but unexpectedly litter chemistry did not correlate with litter *k*. However, salinity strongly correlated with changes in litter chemistry, whereby litter incubated in brackish and marine wetlands was more recalcitrant and gained more phosphorus compared to litter in freshwater marshes. Our results suggest that litter *k* in coastal wetlands is explained by species-specific interactions among water and litter chemistries. We found that the breakdown of less recalcitrant litter (*Eleocharis cellulosa*, *Rhizophora mangle*) was directly driven by marine subsidies (not nutrients, per se), whereas for more recalcitrant

litter (*Cladium jamaicense*) the direct effects of marine nutrients and indirect effects of changing litter chemistry explained breakdown rates.

4. *Synthesis.* Oligotrophic ecosystems are extremely nutrient limited, and nutrients are often the primary driver of breakdown. However, we found that variation in macrophyte breakdown rates were explained by seawater influence, emphasizing how anaerobic conditions principally constrain organic matter processing in wetlands. Our results expand our understanding of how wetland carbon storage responds to saltwater intrusion as sea-level rise increases and macrophyte composition shifts across coastal ecosystems.

INTRODUCTION

Coastal wetlands make up less than 0.2% of the planet's ocean surface, but they account for 50% of total carbon burial in ocean sediments (Duarte et al. 2013). With climate change and development reducing wetland area worldwide (Taillardat et al. 2020, Xi et al. 2021, Salimi et al. 2021), understanding the drivers of organic carbon storage and dynamics within all wetlands, particularly in coastal wetlands, is essential (Schmidt et al. 2011). Sea-level rise is one of the major factors affecting carbon in coastal wetlands, as it changes organic carbon production, decomposition, and movement (Chambers et al. 2015). For instance, mangrove expansion, driven by sea-level rise into and within coastal wetlands, is changing macrophyte communities and the storage of organic carbon in coastal ecosystems (Cavanaugh et al. 2014, Charles et al. 2020). Understanding patterns and magnitudes of changes in carbon storage requires quantifying direct (e.g., salinity increasing breakdown of organic matter) and indirect pathways (e.g., salinity changing plant communities, thus changing the quality of litter) through which different drivers alter organic matter breakdown in coastal wetlands (Stagg et al. 2018). In general, the breakdown of organic matter is mediated by microbes, invertebrates, and leaching, each of which are in turn affected by the chemistry of their environments and the chemistry of the litter itself (Rejmánková and Houdková 2006). Although much is known about specific drivers of organic matter breakdown in aerobic conditions, the relative importance of nutrient limitation and redox conditions on organic matter processing in wetlands is uncertain and likely variable (Helton et al. 2015).

Coastal wetlands worldwide are undergoing climate-driven changes in species composition (Chen et al. 2011, Guo et al. 2017). As macrophyte communities change in coastal wetlands, the quality of litter being deposited is changing (Smith et al. 2019, Charles et al. 2020). Deposition of leaf litter plays an important role in the accretion of soil, especially in mangrove basins where thick layers of litter can contribute up to 3.4 mm of sediment accretion each year, and as a source of nutrients to the water column from leachates produced by the litter (Davis et al. 2006, McKee 2011). At the same time, hydrologic pulses, due to increased water flow, or storm events can shift the distribution of litter, either accelerating downstream transport or pushing litter upstream with storm surge or tidal changes (Zhao et al. 2021). Shifting litter species and environmental drivers often alter the structure and function of microbial communities colonizing litter, which drive rates of litter breakdown. Initial litter chemistry can determine the extent of microbial colonisation, which in turn drives further breakdown (Bärlocher and Kendrick 1975, Cleveland et al. 2014). Lignin concentrations and stoichiometry (elemental ratios) play major roles in determining the initial quality of litter (Melillo et al. 1982, Bradford et al. 2016, Hall et al. 2020). Increases in lignin concentrations, even within different tissues of the same plant species, can decrease the quality of litter, making it more difficult to break down (McKee and Seneca 1982, Gallagher et al. 1984).

Organic matter processing and nutrient cycling in coastal wetlands are changing with saltwater intrusion and sea-level rise (Tully et al. 2019). Intrusion of seawater acts as both a stressor and subsidy for microbial communities in coastal wetlands, commonly enhancing microbial processing of organic carbon (Weston et al. 2011, Chambers et al. 2016). Subsidies include alternate terminal electron acceptors, such as sulfur or iron, which can play an important role in enhancing anaerobic communities (Helton et al. 2015). In oligotrophic wetlands, phosphorus from seawater also subsidises nutrient-limited microbial communities (Servais et al. 2019, Kominoski et al. 2020). Terminal electron acceptors in seawater can release microbes from energetic limitations in anaerobic systems where litter is commonly deposited, subsidising breakdown (Helton et al. 2015). However, salinity from seawater can also act as a stressor to microbial communities, decreasing the breakdown rate of cellulose, as well as decreasing rates of denitrification (Mendelssohn et al. 1999, Neubauer et al. 2019).

At the same time, seawater intrusion is causing shifts in vegetation, changing the type and quality of litter being deposited (Charles et al. 2020). That change in quality plays a role in determining the limitations of wetland microbial communities (Stagg et al. 2018). Changing vegetation and mobilization of stored carbon in the soil also change the composition of carbon in the water column (Chen et al. 2013, Bhattacharya and Osburn 2020). Increases in bio-reactive water column DOC (dissolved organic carbon) could create a priming effect, leading to an increase in the breakdown of recalcitrant DOC where there is available labile DOC (Guenet et al. 2010, Howard-Parker et al. 2020). Understanding both the direct and indirect effects of seawater intrusion is important for understanding what constrains microbial processing in different habitats, how litter of different qualities can ease those constraints, and how microbial communities under different limitations respond to changing litter quality.

Chemical differences among different species of litter influence the microbial communities that colonise it, and mixing litter types can lead to emergent communities based on the mixture (Kominoski et al. 2009, Chapman et al. 2013). Specific formation of litter microbial communities, and their specialization within their own environment, can additionally lead to a home-field advantage for litter breaking down where it is normally produced (Kominoski et al. 2012, Yeung et al. 2019). Environmental chemistry is especially important where it can reduce stoichiometric mismatch between litter chemistry and decomposing microbes, making up for nutrients missing in the litter (Kominoski et al. 2015, Manning et al. 2015). That mismatch can change the nutrient quality of litter, and changing nutrient availability often has the most significant effects on the worst quality litter, relative to environmental limitations (Cheesman et al. 2010, Suberkropp et al. 2010). Microbial communities can additionally be “primed” to increase breakdown of more recalcitrant material when more labile carbon is available in their environment (Guenet et al. 2010). Over time, microbial colonisation changes the quality of litter, making it more palatable to invertebrate consumers (Bärlocher and Kendrick 1975), which further facilitates invertebrate breakdown of litter (Graça 2001, Motomori et al. 2001).

Our goal was to determine the influence of interacting drivers of changing species composition and environmental chemistry on organic matter processing and nutrient cycling. To achieve this, we performed a reciprocal transplant of different qualities of wetland macrophyte litter into novel environments across a gradient of salinity and phosphorus in coastal ecosystems. Here, we tested the following questions: 1. How does variation in litter chemistry across species interact with surface water physicochemistry to drive litter breakdown in coastal wetland ecosystems? 2. How does litter carbon recalcitrance (cellulose:lignin) and nutrient availability affect litter breakdown along freshwater to marine gradients in different ways? Our reciprocal transplant experiment explicitly evaluated the interaction between litter quality and the environment in freshwater, ecotone, and mangrove wetlands in long- and short-hydroperiod wetlands, and in marine seagrass meadows of Florida Bay within the Florida Coastal Everglades. We anticipated that litter chemistry (in terms of both phosphorus and carbon recalcitrance) would be a major driver of litter breakdown rates and that marine influences that alleviate anaerobic constraints would release both stoichiometric (with phosphorus subsidies) and redox constraint (with terminal electron acceptor subsidies) to increase breakdown rates in brackish and marine sites (Figure 1).

METHODS

Site Description and Experimental Design

Our study took place within Everglades National Park (Florida, USA) (Figure 2), an International Biosphere Reserve, a World Heritage Site, and a Ramsar Wetland of International Importance. The Everglades begins at Lake Okeechobee in central Florida and flows from there to Florida Bay at the southern tip of the state. Everglades wetlands are highly oligotrophic and heterogeneous with wide variation in hydrology, productivity, and relative nutrient limitation (Noe et al. 2001, Childers et al. 2003, Castañeda-Moya et al. 2013). The hydrology of the Everglades was radically altered starting in the early 1900s with the construction of drainage canals to create agricultural areas and to develop inundated areas (Light and Dineen 1994). Under the Comprehensive Everglades Restoration Plan (CERP) and associated projects, one of the world's largest restoration projects, efforts are in place to restore sheet flow across the Everglades, which, among other changes, is shifting vegetation communities as legacy phosphorus is mobilised by restorative freshwater entering the system (Sarker et al. 2020). Legacy phosphorus has built up in different portions of the central and southern Everglades as a consequence of upstream farming around Lake Okeechobee. As a component of restoration efforts, a series of treatment wetlands have been created to prevent the movement of nutrients from the farming areas to the Everglades wetlands further downstream, the legacy of phosphorus remains stored within wetland soils at hotspots in previously degraded wetlands (Sarker et al. 2020). Phosphorus is also entering the Everglades from the coast, because despite extremely low concentrations of phosphorus in the Gulf of Mexico (0.25-0.65 µmol/L), they are typically higher than that of the extremely oligotrophic wetlands further inland (Fourqurean and Zieman 2002, Boyer 2006). With this dynamic, sea-level rise is pushing more phosphorus into coastal wetlands, leading to shifts in vegetation communities (Childers et al. 2006, Charles et al. 2019, 2020). Both fresh and marine water contribute to changing phosphorus concentrations and vegetation communities. The combination of these shifting conditions and the robust data availability, due to monitoring by the Florida Coastal Everglades Long Term Ecological Research (FCE-LTER) program, make the Everglades an ideal location to study how spatio-temporal variation in biogeochemistry affects the processing of changing communities of organic matter.

Research was conducted at seven long-term sampling sites of the FCE-LTER program: three along each of the two major drainages, Shark River Slough (SRS) and Taylor Slough/Panhandle (TS/Ph), and one in Florida Bay (Figure 2). For the Everglades, SRS is a high productivity, long-hydroperiod wetland that transitions from sawgrass dominated ridge and slough peat marshes to tidal riverine mangroves (Childers et al. 2006, Ewe et al. 2006, Castañeda-Moya et al. 2013). TS/Ph is a lower productivity (compared to SRS), short-hydroperiod wetland that transitions from sawgrass and periphyton dominated marl prairies to microtidal mangrove scrub forests. We deployed litterbags at three sites (see sub-section: Litter breakdown rates and chemistry for details) in each drainage corresponding to marsh (SRS-2, TS/Ph-2), ecotone (SRS-4, TS/Ph-3), and mangrove (SRS-6, TS/Ph-7). We also deployed bags at a single site in the seagrass meadows in Florida Bay (TS/Ph-10).

Surface Water Physicochemistry

We used long-term data from monthly surface water grab samples for the period that litter was deployed at all sites to calculate the average concentrations of DOC, TN, TP, and salinity across the periods that litterbags were deployed (Briceño 2020, Gaiser and Childers 2021, Troxler 2021, Troxler and Childers 2021). Total phosphorus (TP) was measured following the method of Solórzano and Sharp (1980). Total nitrogen (TN) and total carbon (TC) were measured using an Antek TN analyser (Antek Instruments, Houston, Texas, USA). DOC concentrations were measured using filtered water samples (0.7-µm GF/F filters; Whatman, Maidstone, UK) with a Shimadzu TOC Analyser (Shimadzu Corporation, Columbia, Maryland, USA). All water chemistry analyses were conducted at the CREST CAChE Nutrient Core Facility that is NELAC Certified for non-potable water-General Chemistry under State Lab ID E76930. Surface water temperature was collected at the locations of gas flux towers for SRS-2, SRS-6, TS/Ph-1, TS/Ph-7, and TS/Ph-10. Missing data were estimated using water temperatures at the closest tower: water temperature data at TS/Ph-1, SRS-6 and TS/Ph-3 were used as an estimate for TS/Ph-2, SRS-4, and TS/Ph-7, respectively.

Litter Breakdown Rates and Chemistry

Cladium jamaicense (sawgrass) and *Eleocharis cellulosa (*spikerush) are the dominant species in Everglades freshwater marshes, *Rhizophora mangle* (red mangroves) dominates at mangrove sites and coincides with higher salinity in estuarine habitats, and *Thalassia testudinum* (seagrass) is the dominant species in the seagrass meadows of Florida Bay, which are fully marine. We collected litter to be deployed as live stems from SRS-2 (*C. jamaicense* and *E. cellulosa*), SRS-6 (*R. mangle*), and TS/Ph-10 (*T. testudinum*). Except for the seagrass, all litter was air dried for at least one week and weighed prior to being sealed into litterbags. The seagrass litter was not dried, as drying can alter its chemical makeup. The wet mass of seagrass was measured for each litterbag, and a subset of seagrass was oven dried to calculate the ratio of dry to wet mass of litter deployed in each litterbag. All deployed seagrass was collected within 48 hours of being deployed and was stored at 4°C until being deployed.

We deployed six litterbags at each of two sub-sites for each site for a total of n = 12 litterbags of each species deployed to each site. We deployed litterbags with 3 grams of air-dried litter of a single species inside both fine (1 mm) and coarse mesh (5 mm) bags with *C. jamaicense, R. mangle,* or *E. cellulosa* litter at each of the seven sites, or *T. testudinum* at each mangrove site (TS/Ph-7, SRS-6) and in Florida Bay (TS/Ph-10). We collected two litterbags from each subsite at 1, 4, and 10 months after deployment. Litter was rinsed with deionised water to remove sediment and oven-dried at 45°C for at least 3 d. After drying, the dry mass remaining was weighed and then ground using a ball mill. We calculated the ash free dry mass (AFDM) for each sample by combusting a subsample at 550°C for 4 h and subtracting the ash mass remaining from the sample dry mass. We calculated the breakdown rate (k) for each sample as: $k = \ln(\text{AFDM}_t/\text{AFDM}_0)$ / days incubated. We calculated degree-days as: degree-days = summed daily mean temperature °C / number of days deployed. We then scaled breakdown rate (k) by temperature as: k / degree-day.

We measured percent total nitrogen (TN) and total carbon (TC) for each litter sample using a CE Flash 1112 Elemental Analyser (City, State, Country). We measured percent total phosphorus (TP) using a UV-2101 Shimadzu Spectrophotometer using a modified colorimetric method (Solórzano and Sharp 1980). We analysed the recalcitrance of each litter sample with ramped pyrolysis. To do this, we combusted each sample at a series of thermal intervals associated with the loss of different qualities of carbon using thermal intervals validated by Trevathan-Tackett et al. (2017): T1: 180°C (hemicellulose), T2: 300°C (cellulose) T3: 400°C (lignin), 550°C (inorganic carbon). After each combustion, we measured the mass lost and the percentage of the remaining material that was carbon, using a CE Flash 1112 Elemental Analyser. We calculated the percent total carbon lost at each thermal interval as: mass carbon post-combustion / mass of carbon pre-combustion.

Data Analyses

Principal component analysis was used to reduce the dimensionality of litter chemistry for initial litter. We used both one and two-way analysis of variance (ANOVA) to test the effects of site and species on metrics of litter chemistry and breakdown rates, differences between groups were determined by a post-hoc Tukey HSD test. Linear regressions were used to test significant correlations between metrics of litter chemistry, site chemistry, and breakdown rates. All analyses were performed using R version 4.2.0 (R Core Team 2022). All plots were constructed with the ‘ggplot2’ package (Wickham 2009).

We use path analysis to examine (or assess) the hypothesised direct and indirect effects of environmental variables and litter chemistry on litter breakdown rates, using the ‘lavaan’ package in R (Rosseel 2012). We constructed a hypothesised model using previous literature on how the interplay between site and litter chemistry determines the breakdown of litter (Manning et al. 2015, Bradford et al. 2016, Stagg et al. 2018, Hall et al. 2020). We used six predictor variables for litter *k* / degree day, measured from both litter and site: litter N:P ratio, litter cellulose:lignin ratio, surface water salinity, surface water TN:TP ratio, site as a factor, species as a factor.

To better evaluate species-specific dynamics of litter breakdown, we constructed three sub-models using the same predictor variables for individual litter species *C. jamaicense, E. cellulosa,* and *R. mangle*. We did not construct a sub-model for *T. testudinum* because we did not have adequate data points. For all models, we similarly evaluated model fit using the chi square test statistic and compared best-fit models based on Akaike's Information Criterion (AICc). We removed model links which were not significant when it improved the model fit.

RESULTS

Surface Water Physicochemistry

Total phosphorus concentrations in surface water were higher in Shark River Slough (SRS), with averages (± SD) ranging from 0.75 (± 0.15) µmol/L to 0.90 (± 0.28) µmol/L, than in Taylor Slough/Panhandle (TS/Ph), with averages ranging from 0.54 (±0.09) µmol/L to 0.72 (±0.15) µmol/L. Florida Bay had the lowest surface water TP with an average of 0.27 (±0.05) µmol/L (Table S1). Nitrogen concentrations in surface water were higher in the marsh of SRS with an average of 80.10 (± 19.14) µmol/L, compared to TS/Ph with an average of 41.62 (± 5.46) µmol/L. Nitrogen concentrations in the ecotone of TS/Ph (86.98± 30.18 µmol/L) were higher than SRS (64.21± 9.48 µmol/L). Total nitrogen in the mangroves of TS/Ph (62.13± 8.99 µmol/L) were also higher than in SRS (36.70± 8.12 µmol/L). Dissolved organic carbon was higher in SRS (1,077 ±926.50 µmol/L to 2,003 ±1304.13 µmol/L) compared to TS/Ph (604 ±58.19 µmol/L to 1,006 ±320.51 µmol/L) and Florida Bay (906 ±940.51 mol/L), but it was highly variable across sites (Table S1). Salinity significantly increased from freshwater to more marine sites and was significantly higher in SRS ecotone and mangrove sites than TS/Ph (ANOVA, $F_{(5,54)} = 38.86$, $P < 0.001$; Table S1). Temperature was not significantly different among sites (ANOVA, $F_{(5,54)} = 1.41$, $P = 0.24$; Table S1).

Litter Chemistry

We decomposed the metrics of initial litter chemistry using principal component analysis of seven measured parameters: total carbon (TC), total phosphorus (TP), total nitrogen (TN), and estimates of carbon quality measured by ramped pyrolysis: % hemicellulose, % cellulose, % lignin, and % inorganic carbon (Figure 3). The first principal component explained 68% of the variation, primarily driven by % cellulose (20%), TP (18%), and TN (18%). The second principal component explained 19% of the variation and was primarily driven by % inorganic carbon (40%), and TC (24%). *R. mangle* and *T. testudinum* litter had higher average TP (both with 0.11%) than *E. cellulosa* (0.03%), and *C. jamaicense* (0.003%) had the lowest.

Litter species remained chemically distinct after 6 months of incubation; *Rhizophora* and *Thalassia* had lower N:P ratios compared to *E. cellulosa* and *C. jamaicense* (ANOVA, $F_{(3,178)} = 5.79$, $P < 0.001$; Table 1). Litter N:P ratios were lower at marine sites compared to freshwater sites (ANOVA, $F_{(6,178)} = 26.87$, $P < 0.001$; Table 1). Litter carbon lability (measured as the ratio of cellulose:lignin) was lower in *R. mangle* and *T. testudinum* as compared to *E. cellulosa* and *C. jamaicense* (ANOVA, $F_{(3,175)} = 20.88$, $P < 0.001$; Table 1). Carbon lability was also lower at marine sites as compared to freshwater sites (ANOVA, $F_{(6,175)} = 12.81$, $P < 0.001$; Table 1). In comparison to initial values, total phosphorus of *R. mangle* and *E. cellulosa* were unchanged, while phosphorus in *C. jamaicense* increased after 10 months of incubation at all sites, and *T. testudinum* lost phosphorus at all sites in those same 10 months (Table 1). In comparison to initial values, hemicellulose increases in all species, and, at brackish and marine sites (SRS-6, TS/Ph-7, TS/Ph-10), the cellulose:lignin ratio decreased, indicating decreasing lability (Table 1).

There was a significant negative correlation between litter N:P and salinity in all species: *C. jamaicense* ($R^2 = 0.43$, $P < 0.001$), *E. cellulosa* ($R^2 = 0.59$, $P < 0.001$), *R. mangle* ($R^2 = 0.44$, $P < 0.001$), and *T. testudinum* ($R^2 = 0.77$, $P < 0.001$) (Figure 4). There was a significant negative correlation between litter lability (cellulose:lignin ratio) and salinity in *C. jamaicense* ($R^2 = 0.19$, $P < 0.001$), *E. cellulosa* ($R^2 = 0.13$, $P < 0.01$), and *R. mangle* ($R^2 = 0.38$, $P < 0.001$), but there was no significant correlation in *T. testudinum* ($R^2 = 0.03$, $P = 0.46$; Figure 4).

Litter Breakdown Rates

There were significant effects of both site (ANOVA, $F_{(6,166)} = 4.263$, $P < 0.001$) and species (ANOVA, $F_{(3,166)} = 9.99$, $P < 0.001$) on the breakdown rate (k / degree day) of litter over the course of 10 months (Figure 5). We found no significant effect of mesh size on litter k (ANOVA, $F_{(1,166)} = 0.18$, $P = 0.67$). There was a significant but weak effect of salinity on *R. mangle* k / degree day ($R^2 = 0.09$, $P < 0.05$), but there was no effect of salinity on k / degree for *C. jamaicense* ($R^2 = 0.04$, $P = 0.12$), *E. cellulosa* ($R^2 = 0.00$, $P = 0.78$), or *T. testudinum* ($R^2 = 0.15$, $P = 0.10$). To better understand both direct and indirect effects of litter and site chemistry, we constructed a path analysis model of long-term rates of litter k over the course of 10 months (Figure 6). The best-supported model predicted 37% variation in leaf litter *k*. Litter species (-0.48), salinity (0.40), and site N:P (-0.25) ratio were all directly correlated with leaf litter *k* (Figure 6). Salinity had indirect effects on litter *k* through site N:P (0.36), and litter N:P (-0.63), where site N:P additionally had an indirect effect on litter *k* through litter N:P (-0.18; Table 2). However, the direct effect of litter N:P on leaf litter *k* was marginally insignificant ($P = 0.051$). Salinity (-0.33) and litter N:P (0.21) were both correlated with the litter recalcitrance (cellulose:lignin ratio), which was not a significant driver of leaf litter *k.*

To better explain species specific dynamics of litter breakdown, we constructed sub-models for *C. jamaicense, E. cellulosa,* and *R. mangle*. The best-supported model for *C. jamaicense* explained 28% of variation in leaf litter *k* (Figure S1). In the *C. jamaicense* model, there were direct effects of litter recalcitrance (cellulose:lignin ratio; 0.29), litter N:P (-0.51), and site N:P (-0.29). Salinity had an indirect effect on leaf litter *k* through all three of those direct effects (Table S2A). The best-supported model for *E. cellulosa* explained 40% of the variation in leaf litter *k* (Figure S2); salinity was the only explanatory factor of leaf litter *k* (0.53). Salinity was also correlated with changing litter N:P (-0.68), and litter recalcitrance (cellulose:lignin ratio; -0.40), but neither had effects on leaf litter *k* (Table S2B). The best-supported model for *R. mangle* explained 47% of variation in leaf litter *k* (Figure S3); salinity (0.90) and litter N:P (0.46) were both correlated with leaf litter *k*. There were also indirect effects of salinity through its effects on litter N:P (-0.56) and site N:P (0.44; Table S2C). Site N:P indirectly affected leaf litter *k* through an effect on litter N:P (-0.24).

DISCUSSION

Our goal was to understand how saltwater intrusion will affect the breakdown of litter as it changes available total phosphorus, litter quality, and redox conditions (as a subsidy associated with changing salinity) across coastal wetland habitats. Our results support redox conditions and environmental chemistry as the major drivers of litter breakdown. There have been studies of how litter quality and decomposition vary along freshwater-to-marine gradients (Scarton et al. 2002, Lopes et al. 2011, Trevathan-Tackett et al. 2017, Stagg et al. 2018, Batistel et al. 2021). However, to our knowledge this study is the first to use reciprocal transplants of litter species along such gradients to investigate interactions among litter quality, breakdown, and marine influence in highly oligotrophic coastal wetlands. We found that salinity and litter species played the biggest roles in determining breakdown of litter, followed by site phosphorus availability, but direct (litter) variation in nutrient availability did not explain litter breakdown rates.

Litter quality is often the most important factor influencing litter breakdown, with species specific differences playing a bigger role than environmental variation (Windham 2001, Scarton et al. 2002, Lopes et al. 2011, Stagg et al. 2018, Guo et al. 2023). In our study, we found differences in litter chemistry and breakdown among litter species, however variation in litter chemistry only had a weak explanatory effect on litter breakdown rates. Our results suggest that chemical analysis of litter alone may not be sufficient to understand the recalcitrance of litter from different species and support studies showing that structural traits such as leaf toughness, and cuticle thickness may be more important to litter breakdown (Simões et al. 2021). Our sub-models showed that although salinity increased breakdown among species, all three litter species responded to changing surface water phosphorus availability differently. We found higher phosphorus in water and litter led to lower rates of breakdown for *C. jamaicense*, but higher rates of breakdown for *R. mangle*, while *E. cellulosa*, did not have a significant interaction with salinity. This pattern may reflect microbial priming, where the breakdown of highly recalcitrant *C. jamaicense* is only energetically efficient when phosphorus is very low and *C. jamaicense* is an available source of phosphorus (Horvath 1972, Guenet et al. 2010). This could indicate that understanding microbial responses to litter mixtures (Kominoski et al. 2009, Chapman et al. 2013) will become increasingly important as vegetation communities change in response to sea-level rise and saltwater intrusion (Smith et al. 2019, Charles et al. 2020). Additionally, as rates of litter breakdown change the importance of litter as a source of nutrients through leachates to wetland environments is also likely to change (Davis et al. 2003, 2006).

Understanding litter quality and its effect on breakdown is important for predicting the effects of sea-level rise. Even infrequent pulses of marine water can have significant effects on the composition of litter, as it alters vegetation communities (Birnbaum et al. 2021, Batistel et al. 2022, Guo et al. 2023). We showed that changing vegetation communities can potentially drive significant differences in the breakdown of organic matter. In the Everglades, a shift from *Cladium jamaicense* to *Rhizophora mangle* is likely to lead to higher litter breakdown, but a more comprehensive understanding also requires an analysis of how net primary production and litter deposition rates will change in transition zones. The interaction between litter breakdown and salinity, through both indirect (changes to macrophyte species, or growth rates), and direct (salinity effects on breakdown) pathways can lead to significant changes to the buildup and storage of blue carbon in coastal ecosystems (Cragg et al. 2020, Xia et al. 2021).

We found that increasing salinity enhanced litter breakdown across all species and additionally modified water column total phosphorus. Without taking water column phosphorus into account, we did not see a strong effect of salinity, suggesting an interaction between phosphorus (as indicated by the path analysis approach) and salinity as drivers of breakdown. This finding supports previous studies that have shown salinity increasing breakdown of litter (Stagg et al. 2018, Hu et al. 2019, Trevathan-Tackett et al. 2021, Frainer and Tiegs 2022). Similarly, our data show that in addition to breakdown rates, salinity has a strong effect on the nutrient makeup of litter, even stronger than environmental chemistry, but we found no direct effect of litter chemistry on breakdown rates. Further study is needed to fully understand the mechanism driving these increases as there is little evidence that salinity itself (as opposed to seawater) is driving these changes (Martínez et al. 2020). Seawater can act as both stressor and subsidy depending on the local conditions, where marine subsidies of sulfate likely play an important role, especially where litter is deposited in largely anaerobic environments like the Everglades (Chambers et al. 2011, Weston et al. 2011, Zhang et al. 2023). Our data represent one of the first studies that reciprocally transplants litter across a full range from fresh to fully marine water, showing clear differences between breakdown at ecosystem endmembers, as well as different drivers between species. This is important for understanding the effects of salinity because of the many conflicting factors that affect rates of breakdown including salt-stress interactions on both macro- and micro- decomposers where salinity is variable in the ecotone, as well as changing communities of microbial and invertebrate decomposers (Gómez et al. 2016, Tyree et al. 2016, Canhoto et al. 2017). We found little effect of mesh size on the breakdown of litter suggesting that in Everglades wetlands macro-invertebrates play only a minor role in the breakdown of litter. The lack of change caused by mesh size indicates that salinity is specifically enhancing microbial breakdown of litter, and a limited effect of both litter phosphorus and carbon quality, in this highly phosphorus limited environment suggests that redox constraints may be especially important drivers of litter breakdown.

Marine subsidies associate with changing salinity enhance microbial breakdown of organic matter. Our results support the prediction that microbial limitation of organic carbon breakdown involves both stoichiometric and energetic (redox) constraints in highly reducing environments like wetlands that receive subsidies of both nutrients and terminal electron acceptors (Helton et al. 2015). In anaerobic marine sediments, nearly half of carbon mineralization is catalysed by sulfate reducing microbes that use sulfate as their terminal electron acceptor (Jørgensen 1982). Saltwater intrusion brings subsidies of sulfates to coastal wetlands, where it quickly allows the more energetically efficient sulfate reducing microbes to outcompete methanogens (Muyzer and Stams 2008; Poffenbarger et al. 2011; Bridgham et al. 2013). The increase in breakdown with increasing salinity is likely a response to sulfate subsidies increasing anaerobic breakdown of organic matter in these largely anaerobic wetlands. Studies at the same sites where our litter was deployed in this study have found greater gene abundance of sulfate reducers in the soils of more marine sites, further supporting this interpretation of our data (Zhao et al. 2023). Our data suggest that the release of redox constraints by marine subsidies allows for microbial uptake of nutrients (specifically phosphorus) and increases breakdown rates of litter.

Conclusions

Saltwater intrusion is rapidly changing litter deposition in coastal wetland soils, with changing species composition and rate of litter decomposition. A holistic understanding of the drivers of organic matter processing is necessary to predict how accelerating sea-level rise will affect carbon storage in coastal wetlands (Birnbaum et al. 2021). Our study contributes to that understanding, highlighting the importance of both litter quality and redox conditions as key contributors to rates of litter breakdown, especially in highly oligotrophic wetlands. Our results suggest that direct measurements of litter chemistry may not be sufficient to understand breakdown, and that structural traits are likely important. Increases in breakdown with higher salinity suggests that marine subsidies to anaerobic communities are likely important drivers of litter breakdown where they are available. Additionally, our data suggest that marine subsidies transform litter chemistry, increasing its phosphorus content and making it more recalcitrant (likely as a result of breakdown). Understanding the drivers of organic matter processing in highly dynamic coastal wetland ecosystems is critical to preserving blue carbon stores in the face of sea-level rise (Mcleod et al. 2011, Cragg et al. 2020).

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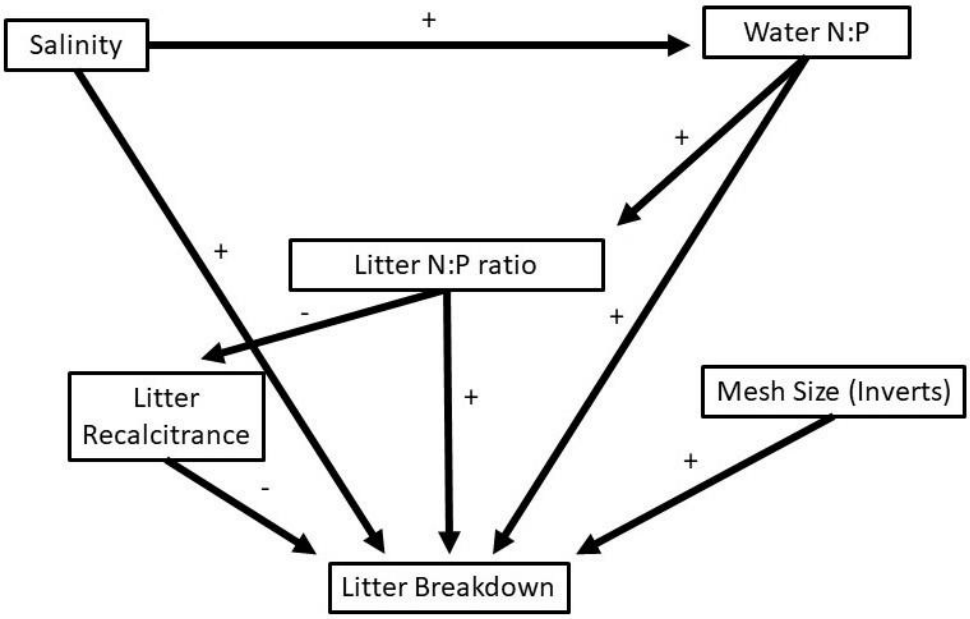

Figure 1. Conceptual model showing hypothesized drivers of leaf litter breakdown in oligotrophic coastal wetlands.

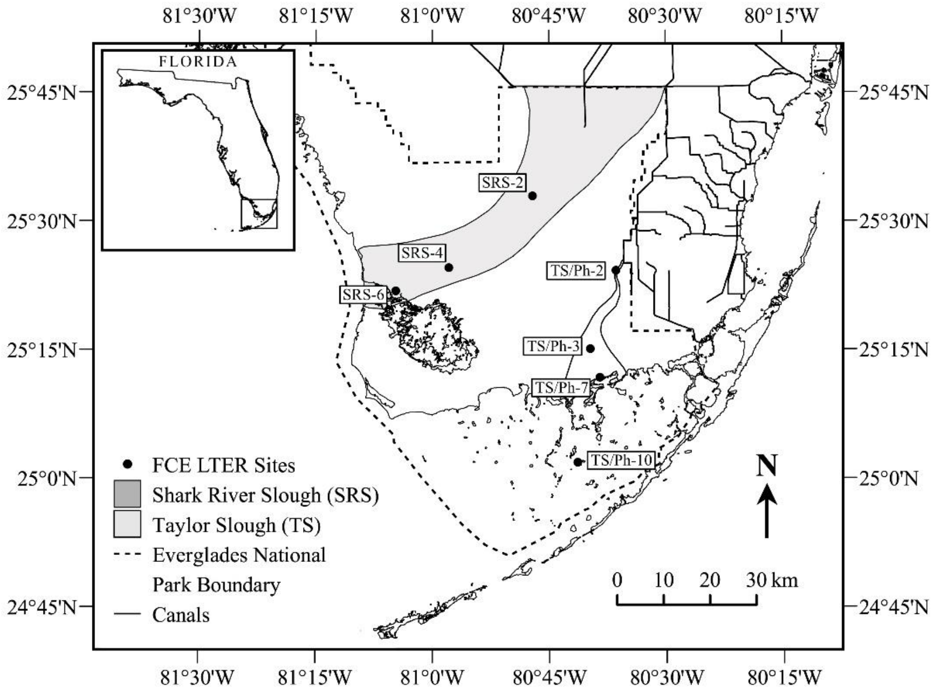

Figure 2. Location of study sites in the Florida Coastal Everglades (FCE), Everglades National Park (ENP), South Florida, USA. We deployed litterbags at marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in a seagrass meadow in Florida Bay (TS/Ph-10). All sites are part of the FCE Long Term Ecological Research (FCE-LTER) program.

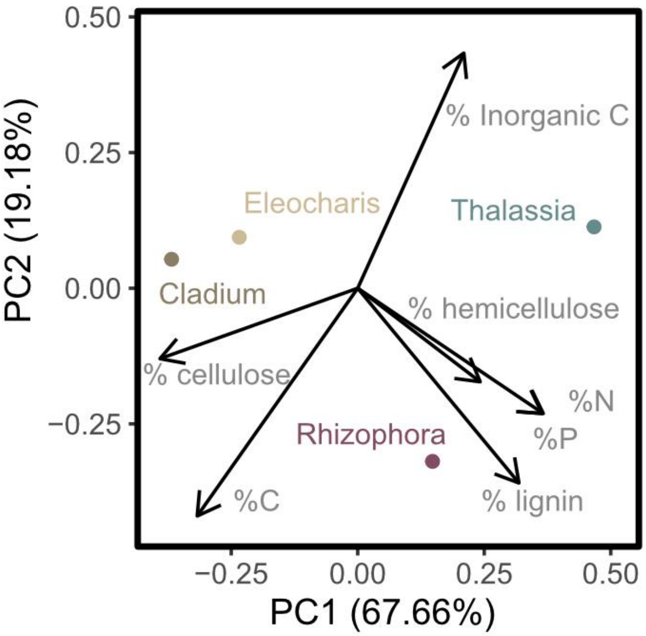

Figure 3. Principal component analysis of initial chemistry of four litter species prior to incubation. We constructed principal components from seven measured parameters: percent carbon (%C), percent phosphorus (%P), percent nitrogen (%N), and estimates of carbon quality measured by ramped pyrolysis: % hemicellulose, % cellulose, % lignin, % inorganic carbon.

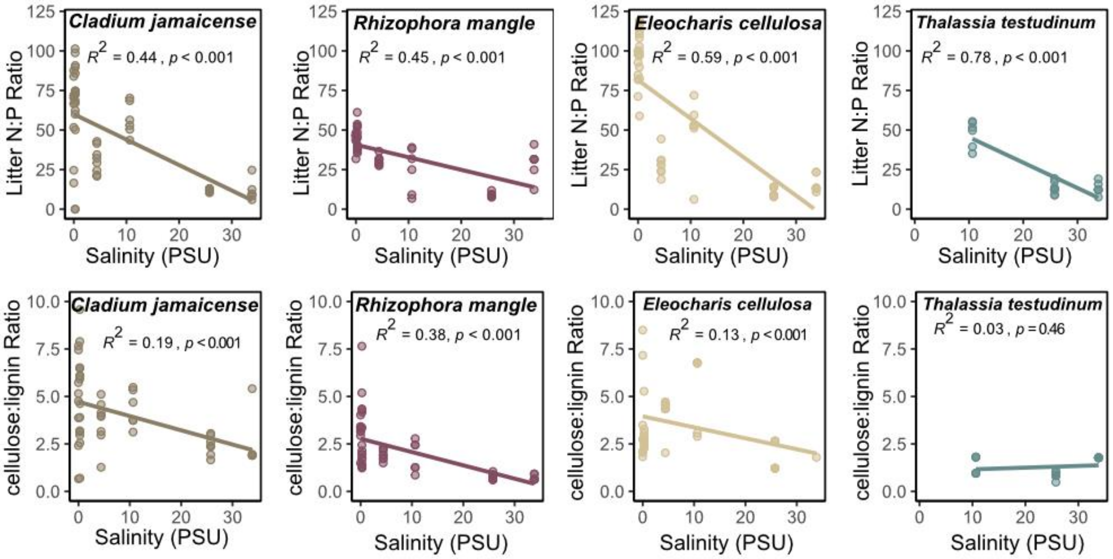

Figure 4. Linear relationships between average site surface water salinity and litter N:P ratio, after 6 months of incubation.

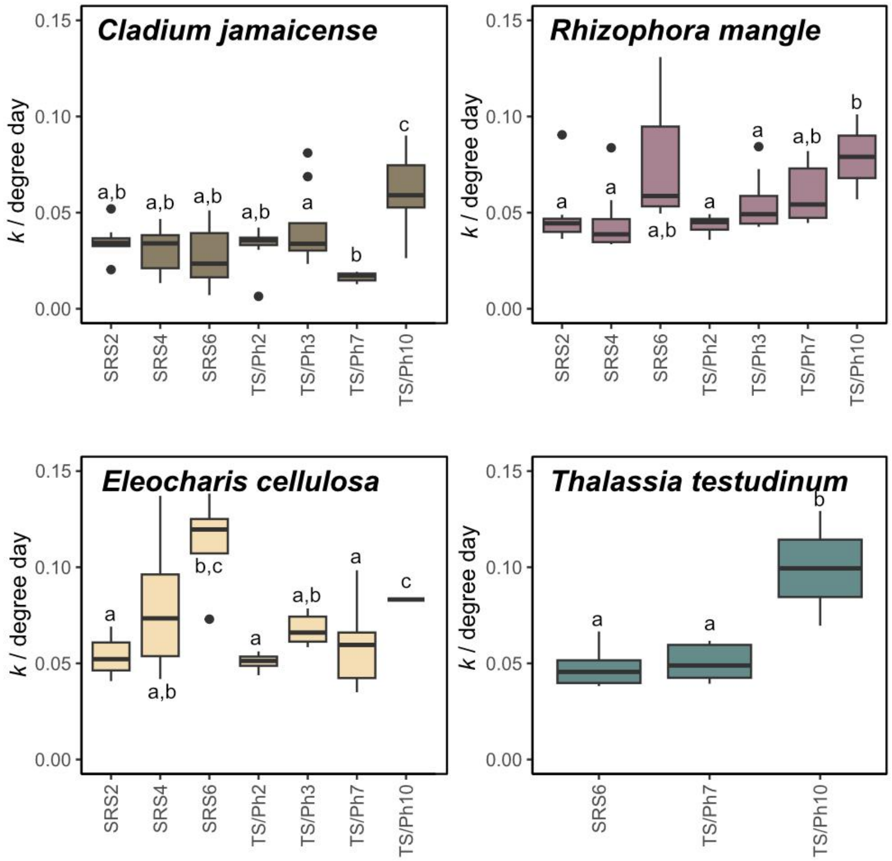

Figure 5. Litter breakdown rate (k / degree day) after 10 months incubation. We deployed litterbags at marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in a seagrass meadow in Florida Bay (TS/Ph-10).

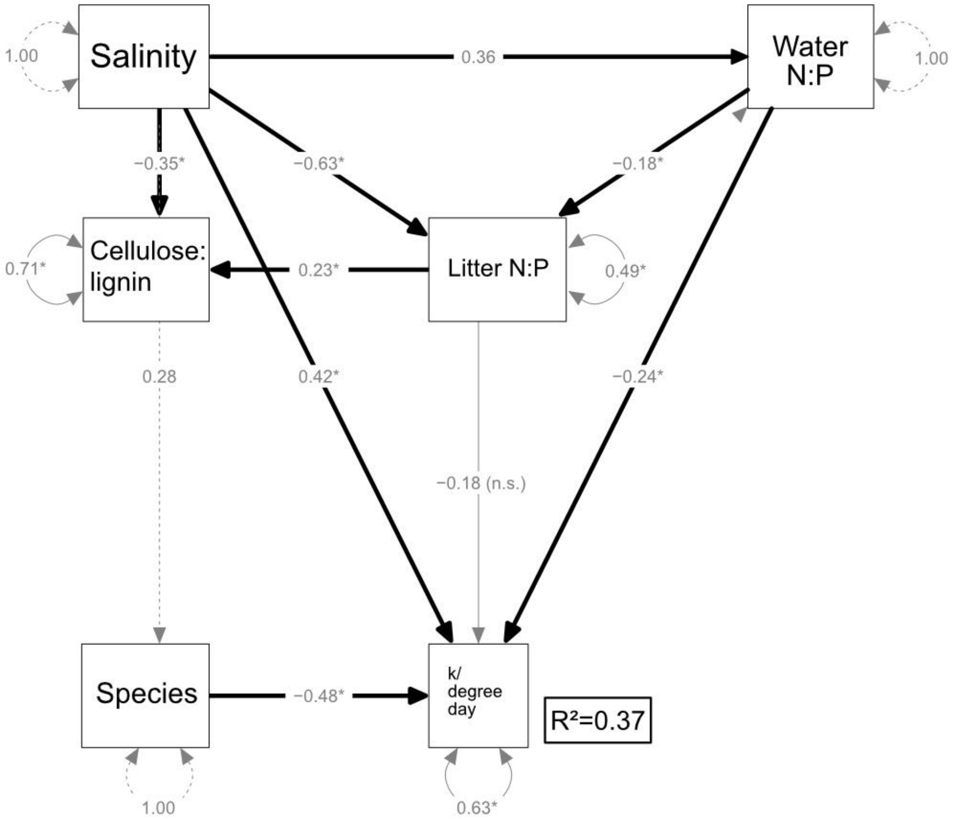

Figure 6. Best supported model for 10-month litter breakdown (k/degree day). Reported numbers are standardized path coefficients, where positive values indicate a positive relationship between variables. The best supported model explains 37% of the variation in litter breakdown rates. Bolded arrows indicate significant path coefficients ($P < 0.05$). Dotted lines indicate significant correlations.

Table 1. Average (± SD) litter chemistry after 6 months of incubation in marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in Florida Bay (TS/Ph-10). Litter chemistry from 10 months is not presented because there were many samples without enough mass remaining for all measurements. Measurements of percent total carbon (TC), percent total nitrogen (TN), and percent total phosphorus (TP) are presented. Carbon structure was estimated using ramped pyrolysis for the percent of carbon that is hemicellulose, cellulose, lignin, and inorganic carbon.

A. *Eleocharis cellulosa*

Site	% TC	% TN	% TP	% hemicellulose	% cellulose	% lignin	% inorganic C
SRS2	45.51 (1.07)	0.86 (0.04)	0.02 (0)	0.14 (0.21)	0.67 (0.16)	0.19 (0.13)	0.002 (0.001)
SRS4	44.09 (1.03)	1.03 (0.31)	0.03 (0.02)	0.11 (0.05)	0.72 (0.09)	0.17 (0.05)	0.001 (0.001)
SRS6	32.24 (3.22)	0.8 (0.07)	0.06 (0.02)	0.17 (0.12)	0.58 (0.13)	0.22 (0.04)	0.031 (0.023)
TS/Ph2	43.1 (0.38)	1.07 (0.1)	0.02 (0)	0.24 (0.17)	0.65 (0.14)	0.11 (0.06)	0.003 (0.001)
TS/Ph3	41.78 (0.99)	1.05 (0.12)	0.02 (0.01)	0.18 (0.07)	0.7 (0.06)	0.1 (0.02)	0.008 (0.002)
TS/Ph7	38.85 (1.79)	1.15 (0.25)	0.03 (0.01)	0.22 (0.08)	0.59 (0.05)	0.19 (0.04)	0.003 (0.009)
TS/Ph10	28.46 (2.82)	0.77 (0.16)	0.03 (0.02)	0.13 (0.07)	0.52 (0.1)	0.29 (0.05)	0.069 (0.079)

B. *Rhizophora mangle*

Site	TC	TN	TP	hemicellulose	cellulose	lignin	inorganic C
SRS2	48.6 (1.4)	2.32 (0.29)	0.05 (0.03)	0.09 (0.06)	0.61 (0.07)	0.29 (0.05)	0.015 (0.003)
SRS4	47.95 (1.61)	2.58 (0.35)	0.12 (0.02)	0.12 (0.07)	0.59 (0.13)	0.28 (0.09)	0.008 (0.004)
SRS6	29.81 (3.39)	1.59 (0.22)	0.1 (0.01)	0.07 (0.04)	0.4 (0.02)	0.45 (0.02)	0.081 (0.029)
TS/Ph2	44.33 (2.02)	2.5 (0.23)	0.08 (0.01)	0.27 (0.08)	0.53 (0.06)	0.18 (0.04)	0.013 (0.002)
TS/Ph3	44.62 (1.53)	2.27 (0.35)	0.08 (0.01)	0.23 (0.08)	0.55 (0.05)	0.21 (0.06)	0.018 (0.014)
TS/Ph7	46.15 (1.49)	2.83 (0.18)	0.1 (0.01)	0.15 (0.05)	0.58 (0.07)	0.27 (0.06)	0.001 (0.001)
TS/Ph10	33.14 (1.79)	2.11 (0.24)	0.07 (0.01)	0.1 (0.03)	0.38 (0.08)	0.42 (0.04)	0.095 (0.029)

C. *Cladium jamaicense*

Site	TC	TN	TP	hemicellulose	cellulose	lignin	inorganic C
SRS2	48.25 (1.18)	0.65 (0.13)	0.01 (0)	0.04 (0.01)	0.7 (0.09)	0.25 (0.1)	0.002 (0.001)

SRS4	48.35 (1.34)	0.99 (0.16)	0.03 (0.02)	0.09 (0.07)	0.76 (0.07)	0.14 (0.05)	0.001 (0.002)
SRS6	37.61 (2.66)	0.64 (0.12)	0.04 (0.01)	0.22 (0.08)	0.59 (0.09)	0.18 (0.02)	0.02 (0.009)
TS/Ph2	45.72 (0.5)	1.1 (0.2)	0.02 (0.01)	0.24 (0.17)	0.64 (0.13)	0.12 (0.07)	0.002 (0.001)
TS/Ph3	45.49 (0.53)	0.93 (0.17)	0.02 (0)	0.18 (0.05)	0.69 (0.04)	0.13 (0.05)	0.006 (0.009)
TS/Ph7	43.91 (1.1)	0.89 (0.2)	0.02 (0)	0.14 (0.04)	0.63 (0.08)	0.23 (0.1)	0.001 (0.001)
TS/Ph10	35.55 (3.22)	0.7 (0.15)	0.02 (0.01)	0.1 (0.03)	0.57 (0.06)	0.28 (0.08)	0.046 (0.031)

D. *Thalassia testudinum*

Site	TC	TN	TP	hemicellulose	cellulose	lignin	inorganic C
SRS6	26.55 (3.33)	1.49 (0.25)	0.07 (0.01)	0.08 (0.05)	0.38 (0.16)	0.47 (0.18)	0.078 (0.038)
TS/Ph7	35.3 (6.03)	2.6 (0.42)	0.06 (0.01)	0.13 (0.04)	0.53 (0.14)	0.32 (0.13)	0.019 (0.042)
TS/Ph10	21.52 (3.02)	0.96 (0.21)	0.05 (0.02)	0.02 (0.02)	0.39 (0.31)	0.22 (0.2)	0.22 (0.153)

Table 2. Effect coefficients for variables affecting litter *k* after 10 months incubation across seven sites in the Florida Coastal Everglades.

Variable	*Direct Effects*	*Indirect Effects*	*Total Effects*
Salinity	0.42	0.1134	0.5334
Litter N:P	-0.18	Not modelled	-0.18
Site N:P	-0.24	0.0324	-0.2076
Species	-0.48	Not modelled	-0.48

Table S1. Average (± SD) surface water physicochemistry data for the 10 months litter bags were deployed from marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in Florida Bay (TS/Ph-10).

	Total Nitrogen (μmol/L)	*Total Phosphorus (μmol/L)*	*Dissolved Organic Carbon (μmol/L)*	*Temperature (°C)*	*Salinity (PSU)*
SRS-2	80.10 (19.14)	0.90 (0.28)	2003.25 (1304.13)	25.70 (2.68)	0.28 (0.08)
SRS-4	64.21 (9.48)	0.75 (0.15)	1857.05 (1413.42)	24.93 (3.01)	4.40 (4.44)
SRS-6	36.70 (8.12)	0.82 (0.11)	1077.50 (926.50)	24.93 (3.01)	25.76 (8.03)
TS/Ph-2	41.62 (5.46)	0.60 (0.16)	604.72 (58.19)	26.03 (3.24)	0.18 (0.03)
TS/Ph-3	86.93 (30.18)	0.72 (0.33)	866.57 (237.37)	27.07 (1.33)	0.25 (0.07)
TS/Ph-7	62.13 (8.99)	0.54 (0.09)	1006.02 (320.51)	27.07 (1.33)	10.64 (8.55)
TS/Ph-10	40.45 (11.08)	0.27 (0.05)	906.84 (940.51)	27.21 (2.54)	35.47 (4.64)

Table S2. Effect coefficients for variables affecting litter *k* for A) *Cladium jamaicense*, B) *Eleocharis cellulosa*, C) *Rhizophora mangle* after 10 months incubation across seven sites in the Florida Coastal Everglades.

A) *Cladium jamaicense*

Variable	Direct Effects	Indirect Effects	Total Effects
Salinity	0	0.26	0.26
Litter N:P	-0.51	Not modelled	-0.51
Site N:P	-0.29	Not modelled	-0.29
Cellulose:lignin	0.29	Not modelled	0.29

B) *Eleocharis cellulosa*

Variable	Direct Effects	Indirect Effects	Total Effects
Salinity	0.62	0.05	0.67
Litter N:P	-0.12	Not modelled	-0.12
Site N:P	Not modelled	0.01	0.012
Cellulose:lignin	-0.03	Not modelled	-0.03

C) *Rhizophora mangle*

Variable	Direct Effects	Indirect Effects	Total Effects
Salinity	0.9	-0.36	0.54
Litter N:P	0.46	Not modelled	0.46
Site N:P	Not modelled	-0.11	-0.11

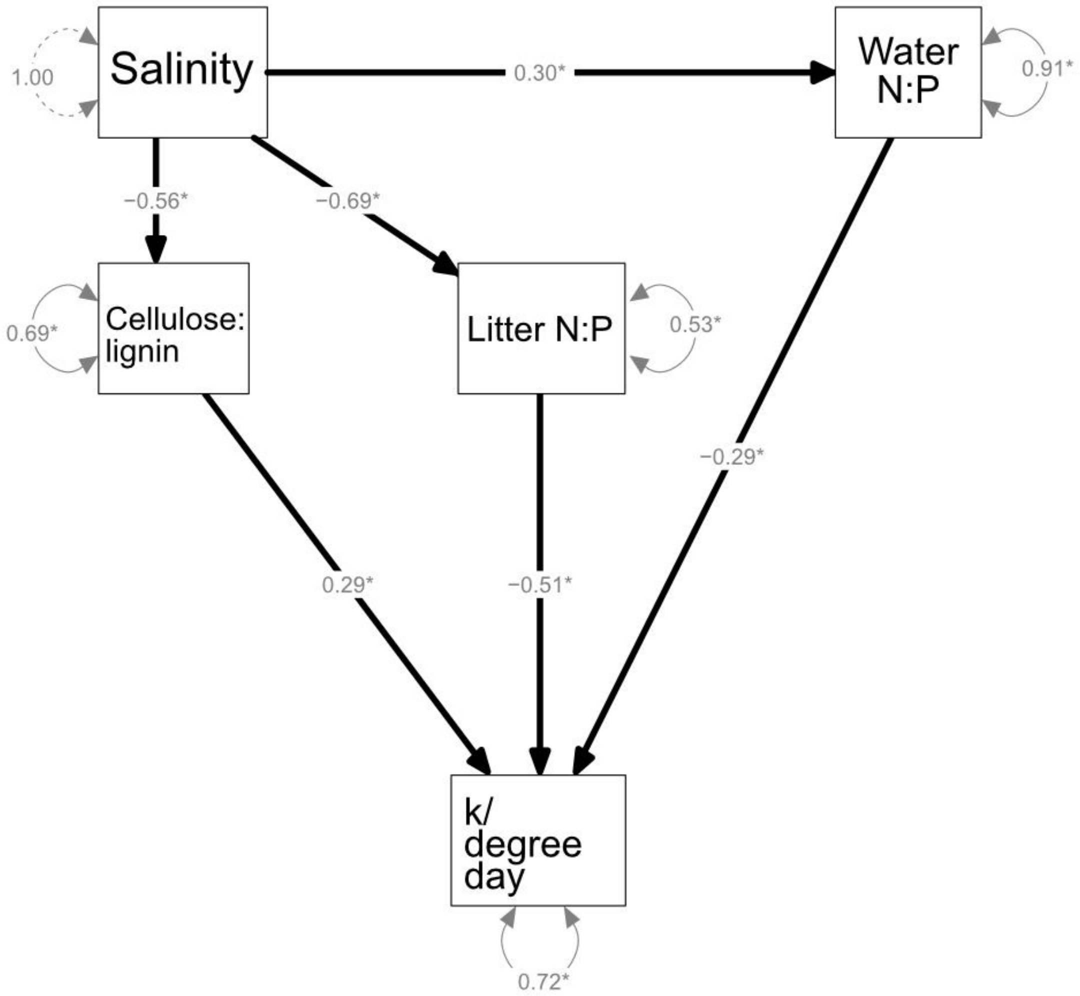

Figure S1. Best supported model for 10-month litter breakdown (k/degree day) of *Cladium jamaicense*. Reported numbers are standardized path coefficients, where positive values indicate a positive relationship between variables. The best supported model explains 28% of the variation in litter breakdown rates. Bolded arrows indicate significant path coefficients ($P < 0.05$). Dotted lines indicate significant correlations.

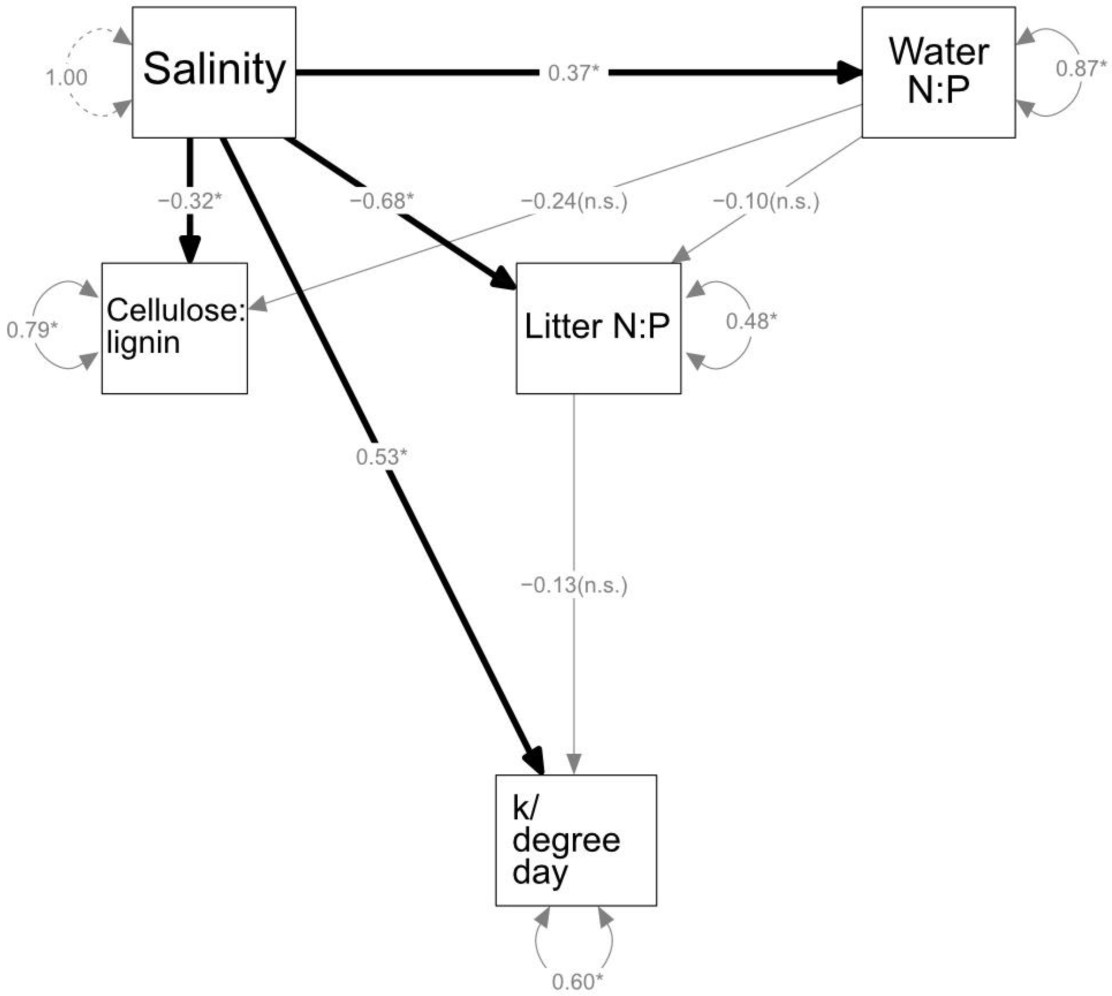

Figure S2. Best supported model for 10-month litter breakdown (k/degree day) of *Eleocharis cellulosa*. Reported numbers are standardized path coefficients, where positive values indicate a positive relationship between variables. The best supported model explains 28% of the variation in litter breakdown rates. Bolded arrows indicate significant path coefficients ($P < 0.05$). Dotted lines indicate significant correlations.

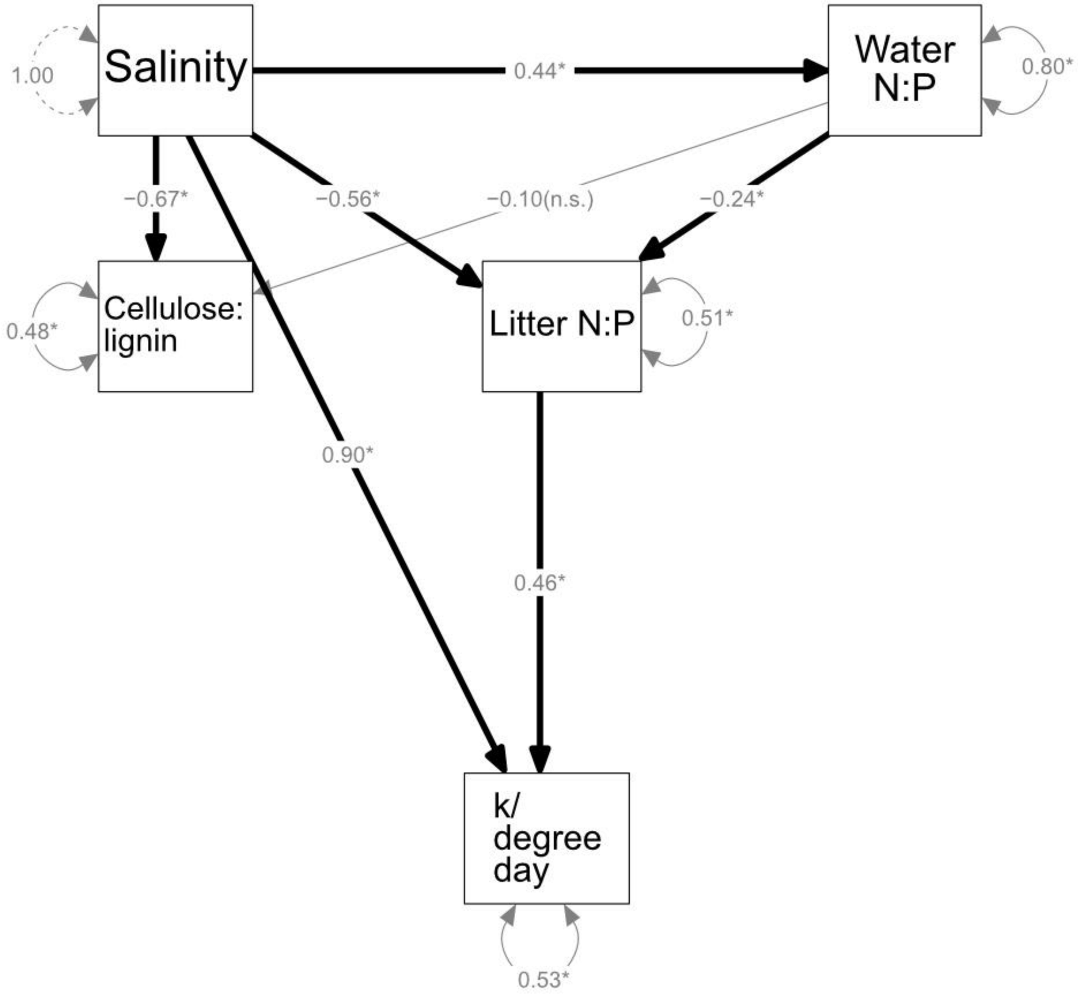

Figure S3. Best supported model for 10-month litter breakdown (k/degree day) of *Rhizophora mangle*. Reported numbers are standardized path coefficients, where positive values indicate a positive relationship between variables. The best supported model explains 28% of the variation in litter breakdown rates. Bolded arrows indicate significant path coefficients ($P < 0.05$). Dotted lines indicate significant correlations.

CHAPTER III

CHARACTERIZING THE RELATIVE EFFECTS OF CARBON AND NUTRIENT LIMITATION OF WETLAND MICROBIAL COMMUNITIES ALONG FRESHWATER TO MARINE GRADIENTS

ABSTRACT

Leaf litter in coastal wetlands lays the foundation for carbon storage, and the creation of coastal wetland soils. As climate change alters the biogeochemical conditions, and macrophyte composition of coastal wetlands, managers require a better understanding of the drivers of coastal litter breakdown to predict how carbon stores will be altered in changing wetlands. Coastal wetlands are dynamic systems with shifting biogeochemical conditions, with both tidal and season variation redox conditions, and marine subsidies to inland habitats. Here we use microbial expression of genes associated with various redox pathways to understand how changing conditions are affecting the microbial communities responsible for litter breakdown along coastal wetland gradients. We performed a reciprocal transplant of four leaf litter species along freshwater to marine gradients in the Florida Coastal Everglades, tracking changes to environment and litter biogeochemistry, as well as microbial RNA expression of 8 genes associated with varied redox conditions, carbon degradation, and phosphorus acquisition. Microbial transcriptomes indicated that surface soils are more anaerobic than adjacent litter, but that both express enzymes involved in both aerobic and anaerobic processes. We found both seasonal and spatial variation in microbial gene expression, where genes associated with carbon degradation were less expressed in marine sites. Additionally, we found seasonal variation in sulfate cycling, where sulfate acquisition was higher at sites with higher marine influence during the dry season, but dissimilatory use of sulfate was highest at intermediate sites when water levels increased. This work provides a starting point for understanding conditions that limit microbial communities breaking down litter in rapidly changing environments.

INTRODUCTION

Wetland ecosystems have a disproportionately large role in the global carbon cycle and are responsible for the storage of up to 70% of terrestrial organic carbon (Mitra et al. 2005). Wetlands are critical stores of organic carbon because they create anoxic benthic environments in highly productive ecosystems, leading to slow decomposition, and net accumulation of carbon (Bridgham et al. 2006, Jackson et al. 2014). In these environments, carbon processing is driven by a combination of environment and organic matter chemistry, and changes in chemistry can determine the development, or breakdown of organic rich soils (Bridgham et al. 2006, Hodgkins et al. 2018, Byun et al. 2019). Understanding carbon processing can be enhanced by considering multiple ecological frameworks, including stoichiometric, metabolic, and redox constraints to capture both assimilatory and dissimilatory nutrient limitations of microbial decomposers (Brown et al. 2004, Allen and Gillooly 2009, Helton et al. 2015). The limitations imposed on decomposers is a key topic of study in wetland restoration, as the combination of organic matter and environmental chemistry will determine the degree of organic matter storage as compared to mineralization, controlling the development (or loss) of wetland soils (Kayranli et al. 2010, Chambers et al. 2019).

Macrophyte and microbial communities change along freshwater to marine gradients, which can lead to significant changes in the quality and quantity of organic matter deposited on and contributing to wetlands soils (McKee 2011, Smith et al. 2019, Servais et al. 2020, Zhao et al. 2023). When considering energetic limitations to microbial communities, the bioavailability of carbon can play an important role, occasionally leaving communities in carbon rich systems to become limited not by total carbon, but by the proportion of labile carbon (Berggren et al. 2015, Soares et al. 2017). Additionally, recalcitrant carbon can be bound to other limiting nutrients, which can prime organic matter mineralization for the purpose of releasing bound nutrients (Guenet et al. 2010, Howard-Parker et al. 2020). This should especially occur during the breakdown of particulate organic matter where labile carbon is quickly utilized, leaving behind largely recalcitrant carbon (Sinsabaugh and Shah 2011). However, the breakdown of organic matter often relies on specific communities specialized to certain litter species which can lead to a “home-field advantage” where certain litter types are common (Yeung et al. 2019). Recent shifts in microbial communities and chemistry along freshwater to marine gradients, raise questions about the consequences to the mineralization of organic matter (Mobilian et al. 2023).

Saltwater intrusion exposes microbial decomposers to a series of novel stressors and subsidies that have been linked to increases in the breakdown of organic matter in coastal wetlands (Morrissey et al. 2014b, Chambers et al. 2015, Neubauer et al. 2019). Changing redox conditions, which are especially important in anaerobic wetland soils, can constrain microbial activity when highly reducing environments cause the availability of terminal electron acceptors to become limiting (Falkowski et al. 2008, Tully et al. 2019). Changing redox conditions can be ecologically important to the function of wetlands, such as when systems are shifted towards, or away from the production of methane, which plays an important role in the global carbon cycle, or the use of sulfates as alternate electron acceptors (Bachoon and Jones 1992, Helton et al. 2014, Dean et al. 2018). Similarly, dissolved organic carbon (DOC) and nitrates are tightly linked in wetlands because of the combination of assimilatory and dissimilatory uses of nitrates by aquatic microbes (Taylor and Townsend 2010, Helton et al. 2015). Better understanding of how and when assimilatory and dissimilatory uses of nutrients are limiting is key to understanding how microbial communities will change their processing of carbon in changing ecosystems.

Understanding how the relative availabilities of carbon, nutrients, and redox conditions affect microbial communities will be important for predicting how changing conditions will change the processing of carbon, and where limitations will change for microbial communities. In this study, we investigated the relative importance of carbon quality, litter stoichiometry, and environmental stressors and subsidies to the function and composition of microbial communities, and their role in the mineralization of organic matter. The Florida Coastal Everglades is the ideal environment for this study, as it has macrophytes that produce litter of variable quality, is experiencing sea-level rise that is changing redox conditions where seawater is intruding, and has two major drainages that vary in elemental stoichiometry. We use the Florida Coastal Everglades as a model ecosystem to study how variations in organic matter quality drives the composition and function of microbial communities across freshwater to marine gradients. To better understand the drivers of the mineralization of organic matter, and the structure and function of microbial communities we asked three questions: (1): How does anaerobic processing of carbon change across litter species? 2): How does anaerobic processing of carbon change along freshwater to marine gradients? 3): How does anaerobic processing of carbon vary seasonally during peak freshwater input, and the lowest freshwater input? We hypothesized that higher salinity sites would have more anaerobic activity, in response to increased sulfate availability, more recalcitrant litter would have more anaerobic processing, and we hypothesized that we would observe stronger responses in Shark River Slough because it is less phosphorus limited, and expected more anaerobic processing in Taylor Slough because it has lower flow, and less tidal exchange.

METHODS

Site Description and Experimental Design

We deployed litterbags along freshwater to marine gradients in wetlands in Everglades National Park (Florida, USA), an International Biosphere Reserve, a World Heritage Site, and a Ramsar Wetland of International Importance. The Everglades begins at Lake Okeechobee in central Florida and flows from there to Florida Bay at the southern tip of the state. Everglades wetlands are highly oligotrophic and heterogeneous with wide variation in hydrology, productivity, and relative nutrient limitation (Noe et al. 2001, Childers et al. 2003, Castañeda-Moya et al. 2013). Sea-level rise currently introduces both subsidies and stressors into the ecosystem, which are shaping and altering macrophyte communities along coastal gradients (Tully et al. 2019). One major subsidy is phosphorus, which is higher in the marine waters of Florida Bay and the Gulf of Mexico than that of the extremely oligotrophic freshwater wetlands further inland (Fourqurean and Zieman 2002, Boyer 2006). Marine water additionally brings subsidies of sulfates, which have shaped soil microbial communities and can be used to alleviate redox constraints in the anaerobic soils of wetlands (Pester et al. 2012, Zhao et al. 2023).

We performed a reciprocal transplant of litter across seven FCE-LTER sampling sites within the two major drainages of the Everglades: Shark River Slough (SRS) and Taylor Slough/Panhandle (TS/Ph). SRS is a high productivity, long-hydroperiod wetland that transitions from sawgrass dominated ridge and slough peat marshes, to tidal riverine mangroves (Childers et al. 2006, Ewe et al. 2006, Castañeda-Moya et al. 2013). TS/Ph is a lower productivity, short hydroperiod wetland that transitions from sawgrass and periphyton dominated marl prairies to microtidal mangrove scrub forests. SRS has higher concentrations of phosphorus, and in the ecotone and mangrove forests has significant diurnal exchanges of marine water (Cawley et al. 2014). TS/Ph has a more seasonal pattern of marine inputs into the mangrove ecosystems (Anderson et al. unpublished data). We deployed litterbags at 3 sites in each drainage corresponding to marsh (SRS-2, TS/Ph-2), ecotone (SRS-4, TS/Ph-3), and mangrove (SRS-6, TS/Ph-7) and a single site in the seagrass meadows in Florida Bay (TS/Ph-10).

Surface Water Physicochemistry

We averaged data collected every 3 days from ISCO autosamplers for the month that litter was collected at all sites except for TS/Ph-10 for concentrations of, TN, TP, and salinity (Gaiser and Childers 2022, Troxler 2022a, 2022b). At TS/Ph-10 we averaged data collected from monthly grab samples during the month that litter was collected (Briceno 2020). TP was measured following the method of Solórzano and Sharp (1980). TN and TC were measured using an Antek TN analyzer (Antek Instruments, Houston, Texas, USA). All water chemistry analyses were conducted by the CREST CAChE Nutrient Core Facility who are NELAC Certified for non-potable water-General Chemistry under State Lab ID E76930.

Litter Breakdown and Chemistry

*Eleocharis cellulosa (*spikerush) and *Cladium jamaicense* (sawgrass) are the dominant species in Everglades freshwater marshes, *Rhizophora mangle* (red mangroves) dominates at mangroves and coincides with higher salinity, and *Thalassia testudinum* (seagrass) is the dominant species in seagrass meadows of Florida Bay. We collected litter to be deployed as live stems from SRS-2 (sawgrass and spikerush), SRS-6 (red mangrove), and TS/Ph-10 (seagrass). All litter was air dried for at least one week and weighed prior to being sealed into litterbags except for seagrass. Seagrass was not dried as drying can alter the chemical makeup. The wet mass of seagrass was measured for each litterbag and we oven dried a subset of seagrass to calculate the ratio of dry to wet mass of litter deployed in each litterbag. All deployed seagrass was collected within 48 h of being deployed and was stored at 4°C until being deployed.

We deployed litterbags with litter of a single species inside coarse mesh (5 mm) bags with spikerush, sawgrass, or mangrove litter at each of the 7 sites, or seagrass at each mangrove site (TS/Ph-7, SRS-6) and in Florida Bay (TS/Ph-10). We deployed four litterbags at two sub-sites for each site for a total of n = 12 litterbags of each species deployed to each site. We collected 2 litterbags from each subsite at 1 and 4 months after deployment. For each recovered litterbag we rinsed the litter with deionized water and removed a subset of litter for transcriptomics, or productivity measurements. We measured the wet mass of both the subset and remaining coarse mesh litter to correct for the total dry mass. After rinsing and taking subsets, we oven-dried all litter samples at 45°C for at least 3 days. After measuring the dry mass remaining, we ground the dry litter using a ball mill. We calculated the ash free dry mass (AFDM) for each sample by combusting a subsample at 550°C and measuring the mass remaining. We calculated the breakdown rate (*k*) for each sample as: $k = \ln(\mathrm{AFDM}_t/\mathrm{AFDM}_0)$ / days incubated. We calculated degree-days as: degree-days = summed daily mean temperature °C / number of days deployed. We then scaled breakdown rate (*k*) by temperature as: *k* / degree-day.

We measured total nitrogen (TN) and total carbon (TC) for each litter sample using a CE Flash 1112 Elemental Analyzer. We measured total phosphorus (TP) using a UV-2101 Shimadzu Spectrophotometer using a modified colorimetric method (Solórzano and Sharp 1980). We measured the recalcitrance of each litter sample with ramped pyrolysis, combusting at a series of thermal intervals associated with the loss of different qualities of carbon. We combusted at 4 intervals: T1: 180°C (hemicellulose), T2: 300°C (cellulose) T3: 400°C (lignin), 550°C (inorganic carbon), using thermal intervals validated by Trevathan-Tackett et al. (2017). After each combustion we measured the mass lost, and the percentage of the remaining material that was carbon using a CE Flash 1112 Elemental Analyzer (Manufacturer, City, Country). We calculated the percent carbon lost at each thermal interval as the mass of carbon post-combustion divided by the mass of carbon pre-combustion.

Bacterial Productivity

We measured the maximal bacterial productivity (BP) of each litter type as the uptake of tritiated thymidine, using a modification of the method presented by Wetzel and Likens (2013). All litter samples were returned from the field on ice and were processed with 24 h of collection. We homogenized a known mass of each litter sample in 200 mL deionized water using a small bullet blender. We added 10 mL of the homogenized litter into four clean falcon tubes, immediately fixing one with 2% bacteria free formalin, for $n = 3$ live replicates and a killed control sample. We added tritiated thymidine to each sample for a final concentration of 10 nM and incubated for 1 h. After incubation, we halted microbial activity with the addition of 50% trichloroacetic acid, and incubated samples at 0°C for 15 min. After the final incubation we filtered each sample and after 24 h, ran on a liquid scintillation counter (Beckman Model 3801, (Manufacturer, City, Country)). The values for the blanks were subtracted from each sample to calculate the mols of thymidine taken up per gram of material in the hour incubation.

Microbial Molecular Analyses

For each site and litter pair we collected a subset of 2-3 g wet mass of litter and a grab sample of soil for each site. All subsamples were preserved at -20□C until extraction. Samples were sent to Novogene (Novogene Co. Ltd., Beijing, China) for the total RNA extraction followed by metatranscriptome sequencing. Briefly, the total RNA was extracted using TRIzol reagent (Rio et al. 2010) and the quality and quantity of the RNA were assessed using the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and Nanodrop ND-1000 (ThermoScientific, Waltham, MA, USA), respectively. After the total RNA samples passed the quality check, cDNA libraries were constructed and sequenced by paired-end (PE) sequencing (PE 2 × 150 bp) using an Illumina NovaSeq 6000 platform (NovaSeq Reagent Kits, Illumina, Inc., San Diego, CA, USA).

Raw reads were processed using the Simple Annotation of Metatranscriptomes by Sequence Analysis 2.0 (SAMSA2) pipeline (Westreich et al. 2018) with slight modification. Briefly, low quality bases were trimmed using Trimmomatic v0.39 (Bolger et al. 2014) and overlapping paired-end reads were merged into single sequences using PEAR v0.9.11 (Zhang et al. 2014). Ribosomal RNA reads were removed with SortMeRNA v2.1 (Kopylova et al. 2012) and the cleaned transcripts were annotated by DIAMOND v0.9.36 (Buchfink et al. 2021) against the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database (O'leary et al. 2016) for taxonomic and functional characterization. The resulting annotation files were aggregated and merged with custom Python and R scripts included in the SAMSA2 pipeline (Westreich et al. 2018).

We selected n = 8 focal enzymes to investigate which are important to the breakdown of organic matter: acid phosphatase (associated with the release of phosphorus from organic molecules), alkaline phosphatase (associated with the release of phosphorus from organic molecules), arylsulfatase, (associated with the release of sulfates from organic molecules), leucine aminopeptidase (associated with nitrogen acquisition), β-1,4-glucosidase (associated with the breakdown of cellulose), β1,4-cellobiosidase (associated with the breakdown of cellulose), b-D-xylosidase (associated with the breakdown of cellulose), phenol oxidase (associated with the breakdown of lignin) (Morrissey et al. 2014b, 2014a, Servais et al. 2020, 2021).

Statistical Analysis

Principal component analysis was used to reduce the dimensionality of litter chemistry for initial litter. We used both one and two-way analysis of variance (ANOVA) to test the effects of site and species on metrics of litter chemistry and breakdown rates, differences between groups were determined by a post-hoc Tukey HSD test. Linear regressions were used to test significant correlations between metrics of litter chemistry, site chemistry, and breakdown rates. All analyses were performed using R version 4.2.0 (R Core Team 2022). All plots were constructed with the ‘ggplot2’ package (Wickham 2009).

RESULTS

Surface Water Physicochemistry

January total phosphorus concentrations in surface waters were high in Shark River Slough (SRS), with averages (± SD) ranging from 0.84 (± 0.11) µmol/L to 1.74 (± 2.09) µmol/L, and lower in Taylor Slough/Panhandle (TS/Ph) with averages ranging from 0.37 (± 0.11) µmol/L to 0.67 (± 0.05) µmol/L. April phosphorus concentrations were generally lower than January in Shark River Slough (SRS), ranging from 0.70 (± 0.30) µmol/L to 1.80 (± 1.03) µmol/L, and Taylor Slough/Panhandle (TS/Ph) ranging from 0.27 (± 0.03) µmol/L to 0.56 (± 0.22) µmol/L. Florida Bay had the lowest total phosphorus with an average of 0.27 (±0.05) µmol/L (Table S1). Salinity significantly increased moving from freshwater to more marine sites, and was significantly higher in SRS ecotone and mangrove sites than TS/Ph (ANOVA, F(5,54) = 38.86, $P < 0.001$; Table S1). Temperature was not significantly different among sites (ANOVA, F(5,54) = 1.41, $P = 0.24$).

Litter Chemistry and Breakdown

We decomposed metrics of litter chemistry using principal component analysis of seven measured parameters: total carbon (%TC), percent phosphorus (%P), percent nitrogen (%N), and estimates of carbon quality measured by ramped pyrolysis: % hemicellulose, % cellulose, % lignin, % inorganic carbon (Figure 2). The first principal component explained 51% of the variation, driven equally by %N (22%), % cellulose (20%), % lignin (19%), % inorganic C (19%) and %P (19%). The second principal component explained 22% of the variation and was primarily driven by % hemicellulose (53%), and %TC (23%).

Percent carbon was highest in *Cladium* (45.3±3.0) and *Rhizophora* (44.6±4.8), followed by *Eleocharis* (40.9±5.5), and then *Thalassia* (32.2±4.9; ANOVA, $F_{(3,183)} = 53.37$, $P < 0.001$). Percent nitrogen was higher in *Thalassia* (2.0 ± 0.6) and *Rhizophora* (2.0 ± 0.4) compared to *Cladium* (0.7 ± 0.2) and *Eleocharis* (0.8 ± 0.1; ANOVA, $F_{(3,183)} = 219.5$, $P < 0.001$). Percent phosphorus was highest in *Rhizophora* (0.08 ± 0.02), followed by *Thalassia* (0.06 ± 0.02), and then *Cladium* (0.02 ± 0.00) and *Eleocharis* (0.03 ± 0.02; ANOVA, $F_{(3,183)} = 189.7$; $P < 0.001$).

Species explained breakdown rates better than site after 1 month of breakdown, however both species (ANOVA, $F_{(3,167)} = 140.35$, $P < 0.001$) and site (ANOVA, $F_{(6,167)} = 20.98$, $P < 0.001$) were significant predictors of breakdown (Table 1a). After 6 months of breakdown the explanatory power of both species (ANOVA, $F_{(3,167)} = 28.58$, $P < 0.001$) and site (ANOVA, $F_{(6,132)} = 4.10$, $P < 0.001$) became more similar, and both were still significant predictors (Table 1b).

Bacterial Productivity

We found no significant difference in maximal bacterial productivity of litter between either species (ANOVA, $F_{(2,37)} = 1.90$; $P = 0.16$), or sites (ANOVA, $F_{(6,33)} = 1.41$; $P = 0.24$) after the first or sixth month of litter breakdown. There was no significant linear relationship between BP and environmental variables including salinity ($R^2 = 0.00$, $P = 0.94$), total nitrogen ($R^2 = 0.05$, $P = 0.18$), total phosphorus ($R^2 = 0.01$, $P = 0.45$), or dissolved organic carbon ($R^2 = 0.01$, $P = 0.68$).

Relative Gene Abundance

We detected the presence of all of our genes of interest except for methyl coenzyme m reductase (Table 2). Most genes related to anaerobic pathways were more abundant in soils as compared to litter and periphyton, including sulfatases (ANOVA, $F_{(2,30)} = 3.58$, $P < 0.05$) and formylmethanofuran (ANOVA, $F_{(2,30)} = 13.72$, $P < 0.001$; Figure 3). Dioxygenases, which indicate aerobic pathways were more abundant in litter as compared to soil (ANOVA, $F_{(2,30)} = 3.11$, $P < 0.05$), as were nitrogen reductases (ANOVA, $F_{(2,30)} = 18.09$, $P < 0.001$).

After the first month of breakdown there was a significant positive correlation between salinity and sulfatase ($R^2 = 0.64$, $P < 0.001$) gene abundance, and a significant negative correlation between salinity and nitrite reductase ($R^2 = 0.34$, $P < 0.01$), acid phosphatase ($R^2 = 0.24$, $P < 0.005$), glucosidase ($R^2 = 0.24$, $P < 0.01$), and cellobiosidase ($R^2 = 0.19$, $P < 0.05$) gene abundance (Figure 4). There was no significant relationship between salinity and alkaline phosphatase ($R^2 = 0.02$, $P = 0.40$), xylosidase ($R^2 = 0.04$, P

= 0.29), phenol oxidase (R^2 = 0.01, P = 0.64), sulfite reductases (R^2 = 0.02, P = 0.46), dioxygenase (R^2 = 0.00, P = 0.72), or formylmethanofuran (R^2 = 0.09, P = 0.09).

After four months of breakdown there was a significant negative correlation between salinity and glucosidase (R^2 = 0.47, $P < 0.001$) and xylosidase (R^2 = 0.18, $P < 0.05$). There was a significant non-linear relationship between salinity and sulfite reductase with a peak at intermediate salinity (R^2 = 0.68, $P < 0.001$). There was a weak positive correlation between salinity and sulfatase (R^2 = 0.09, P = 0.09), and a weak negative correlation between salinity and phenol oxidase (R^2 = 0.10, P = 0.07). There was no significant relationship between salinity and dioxygenase (R^2 = 0.03, P = 0.33), formylmethanofuran (R^2 = 0.04, P = 0.28), nitrite reductase (R^2 = 0.00, P = 0.80), cellobiosidase (R^2 = 0.07, P = 0.15), alkaline phosphatase (R^2 = 0.03, P = 0.33), and acid phosphatase (R^2 = 0.03, P = 0.32).

Genes specifically associated with redox reactions had relatively low expression, however sulfite reductases were more than twice as abundant as methane production genes (Figure 5). Gene expression of carbon degrading enzymes were somewhat higher on *Eleocharis* and *Rhizophora* and were generally higher on litter as compared to soils (Figure 6).

Microbial Community Composition

We investigated organismal abundance for six monophyletic anaerobic microbial groups: sulfite reducers, sulfite oxidizers, methane oxidizers, methanogens, nitrite oxidizers, and ammonia oxidizers. We found significantly higher abundance of sulfite reducers (ANOVA, $F_{(2,63)} = 6.28$, $P < 0.01$), methane oxidizers (ANOVA, $F_{(2,63)} = 7.88$, P

< 0.001), methanogens (ANOVA, $F_{(2,63)} = 6.86$, $P < 0.01$), nitrite oxidizers (ANOVA, $F_{(2,63)} = 15.99$, $P < 0.001$), and ammonia oxidizers (ANOVA, $F_{(2,63)} = 44.36$, $P < 0.001$) in soils as compared to litter. There was no significant difference in abundance between litter and soils for sulfite oxidizers (ANOVA, $F_{(2,63)} = 0.49$, $P = 0.61$).

Of the anaerobic microbial taxa we investigated, relative gene expression of sulfite reducers after both one (11.64-0.31) and four months (16.23-0.03) had the highest gene abundances but were highly variable across sites (Figure 7). Methane oxidizers for one month (2.56-1.02) and four months (2.93-0.43) and nitrite oxidizers for one month (0.31-4.28) and four months (0.06-3.13) were also abundant but more even across sites (Figure 7). Ammonia oxidizers (0.007-1.10), sulfite oxidizers (0-0.76), and methanogens (0-0.03) had lower relative gene expression.

After the first month of breakdown there was a significant positive correlation between salinity and relative gene expression of sulfite oxidizers ($R^2 = 0.71$, $P < 0.001$) and ammonia oxidizers ($R^2 = 0.25$, $P < 0.05$) on leaf litter. There was no correlation between salinity and relative gene abundance of sulfite reducers ($R^2 = 0.05$, $P = 0.32$), methane oxidizers ($R^2 = 0.14$, $P = 0.10$), methanogens ($R^2 = 0.12$, $P = 0.09$), or nitrite oxidizers ($R^2 = 0.08$, $P = 0.18$) on leaf litter or soil. After four months of breakdown there was a significant positive correlation between salinity and relative gene expression of sulfite oxidizers ($R^2 = 0.26$, $P < 0.05$) on leaf litter. After four months there was no correlation between salinity and relative gene abundance of ammonia oxidizers ($R^2 = 0.00$, $P = 0.76$), sulfite reducers ($R^2 = 0.00$, $P = 0.93$), methane oxidizers ($R^2 = 0.04$, $P = 0.36$), methanogens ($R^2 = 0.00$, $P = 0.75$), or nitrite oxidizers ($R^2 = 0.00$, $P = 0.81$) on leaf litter or soil.

DISCUSSION

Our goal was to understand how site specific, and litter specific drivers interact to drive microbial processing of litter, with a specific focus on salinity, and phosphorus. We found that microbial transcription and community composition largely varies by site as opposed to litter species. Despite finding that litter species explained litter breakdown better than site, we found that variation in both microbial activity and community were primarily controlled by site. This suggests that microbial contributions may become more influential over time, where breakdown rate becomes more influenced by site over time. Early litter breakdown is often dependent on leachable proportions of litter, explaining early breakdown rates (Berg and Staaf 1981, Benner and Hodson 1985). After that initial leaching microbial communities play a major role in determining breakdown, and those communities are shaped by both environmental chemistry and litter quality (Aerts and de Caluwe 1997, Ball and Drake 1997, Bradford et al. 2016). Our data suggests that early breakdown of litter is suppressed by salinity, with negative relationships between carbon degrading enzymes and salinity. Salinity is often found to inhibit enzyme activity, including carbon degrading enzymes (Tripathi et al. 2007, Jackson and Vallaire 2009, Yun et al. 2010). The impact of salinity however, is still unclear with reports of both increases (Weston et al. 2006, Saviozzi et al. 2011, Wang et al. 2018) and decreases to carbon processing in response to increasing salinity (Rejmánková and Houdková 2006, Roache et al. 2006, Neubauer 2013).

Marine subsidies and stressors associated with salinity can vary across sites, and our work highlights the need for a more in-depth understanding of the microbial community’s contribution to mineralization of carbon in changing coastal wetlands.

Salinity often is coupled with increasing sulfate concentrations, which can shift dominant anaerobic pathways to the more efficient sulfate production from methanogenesis, potentially leading to increases in organic matter mineralization (Neubauer et al. 2005, Weston et al. 2011, Neubauer 2013). We found that sulfatases, which are commonly used as an indicator of microbial sulfate acquisition, were strongly correlated with salinity during January when water levels are higher compared to April when there was no correlation, and lower water levels (Morrissey et al. 2014b). We found no relationship in January between sulfate reductase activity and salinity, however in April there was high activity at intermediate salinity sites indicating higher dissimilatory use of (Morrissey et al. 2014b, Servais et al. 2021). This may indicate that sulfate acquisition is highest when water level is low and marine influence is high, but that sulfate is being actively used for dissimilatory reduction when water depths increase and freshwater pushes out marine water. This suggests that sulfate acquisition and sulfite reduction are decoupled, and further study is necessary to fully understand the dynamics controlling microbial metabolism. The difference in expression of sulfate acquisition and reduction enzymes could also be related to the relative redox conditions at our sites. Sulfatase activity was significantly higher in our soils than litter, while dioxygenases (which require the presence of oxygen) were higher, suggesting that while not associated with the expression of sulfite reductase, sulfatases are being produced by more anaerobic communities.

Our data suggest that marine subsidies lead to the uptake of sulfates that contribution to litter mineralization, but also suggests that marine phosphorus subsidies do not play a major role in regulating the microbial community. We found that alkaline phosphatase was nearly 10 times more expressed than acid phosphatase, likely because the Everglades landscape is underlain by limestone and therefore buffered by bicarbonate. Additionally acid phosphate was negatively correlated with salinity, but alkaline phosphatase had no relationship with salinity, this is likely explained by decreasing acidity in more marine environments. The lack of a relationships between alkaline phosphatase and environmental total phosphorus is possibly because Everglades environments are extremely phosphorus limited and phosphatase expression may be saturated even at relatively higher phosphorus sites. While phosphorus plays a role in the breakdown of litter our data may suggest that redox conditions and carbon limitation play similarly important roles in the breakdown of leaf litter.

When observing the composition of anaerobic microbial communities we found high abundance of sulfite reducers, which were up to 6% of the microbial community. Sulfite reducer abundance was highest in marine sites and was especially high at TS/Ph-7, which is both stagnant and shaded; likely one of the most anoxic sites. The combination of communities along with the variation in gene expression suggests that Everglades surface soils and leaf litter experience periodic anaerobic conditions that sulfate reducers exploit where sulfate is available, but also oxygen is frequently available on the soil surface. This is further supported by clear gradients of sulfite reducers at these same sites but in deeper soils (Ikenaga et al. 2010, Zhao et al. 2023). Everglades mangroves experience both seasonal and diurnal variation in water flows, with changing dynamics of both fresh and marine water, each of which has the potential to change redox conditions across the landscape (Rivera-Monroy et al. 2007, Smith et al. 2016). This highly dynamic environment is likely to continue changing redox, and nutrient conditions leading to shifting microbial communities over time as well as the spatial variation that we see (Cheung et al. 2018, Maietta et al. 2020).

Our data show that microbial communities in surface soils and litter are a mix of aerobic and anaerobic microbes, and that specifically sulfate cycling likely plays an important role in the breakdown of litter. Seasonal shifts play a major role in coastal wetland carbon dynamics and shifting microbial activity and communities as marine stressors and subsidies are pushed back by incoming freshwater (Malone et al. 2014, Wilson et al. 2018). An in depth understanding of microbial community, and functional changes will allow us to better predict how increasing sea-level rise will change the processing of carbon within coastal wetlands, as both substrate, and environmental chemistry are changing (Kirwan et al. 2013, Smoak et al. 2013). This data provides a starting point for further study on how sea-level rise, and resultant changes to macrophyte community structure will change the deposition, and breakdown of organic matter across wetlands, highlighting lingering questions about how seasonal changes to environmental chemistry and water depths, and changing litter quality will further affect the structure and function of coastal wetland decomposers.

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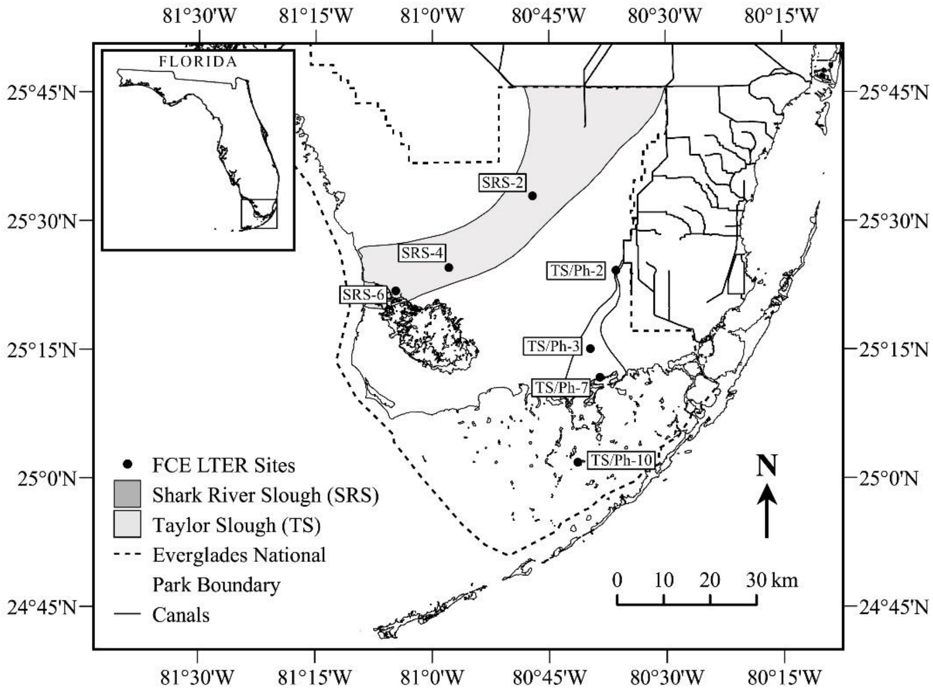

Figure 1. Location of study sites in the Florida Coastal Everglades (FCE), Everglades National Park (ENP), South Florida, USA. We deployed litterbags at marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in a seagrass meadow in Florida Bay (TS/Ph-10). All sites are part of the FCE Long Term Ecological Research (FCE-LTER) program.

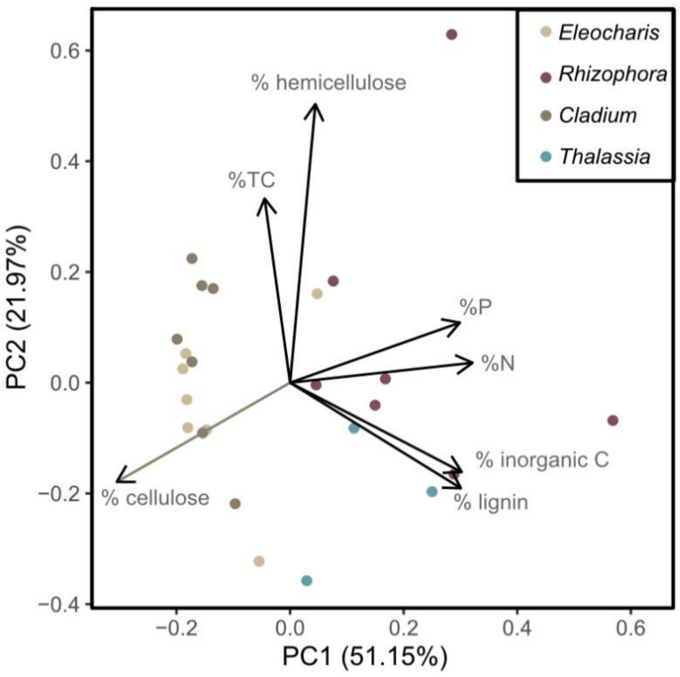

Figure 2. Principal component analysis of litter chemistry of four litter species (*Eleocharis cellulose, Rhisophora mangle, Cladium jamaicense,* and *Thalassia testudinum*) after one month of incubation. We constructed principal components from seven measured parameters: percent total carbon (%TC), percent phosphorus (%P), percent nitrogen (%N), and estimates of carbon quality measured by ramped pyrolysis: % hemicellulose, % cellulose, % lignin, % inorganic carbon.

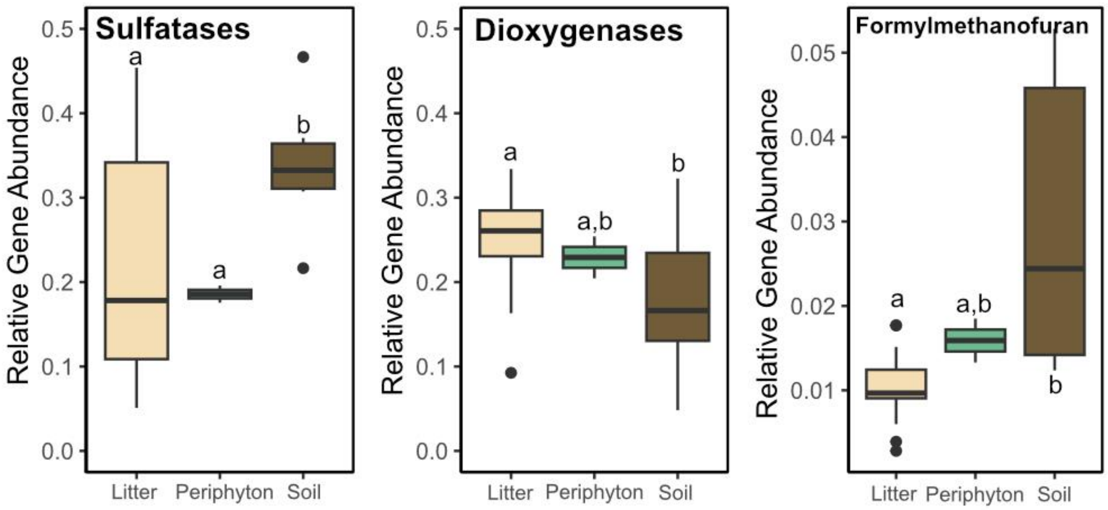

Figure 3. Gene expression for sulfatases, dioxygenases, and formylmethanofuran across leaf litter, periphyton, and soil along Everglades freshwater to marine gradients. Boxplots represent the interquartile range for each variable, and the solid line is the median. Error bars represent the 95% confidence intervals. Letters above boxplots indicate significant ($\alpha \leq 0.05$) differences among groups using one-way ANOVA.

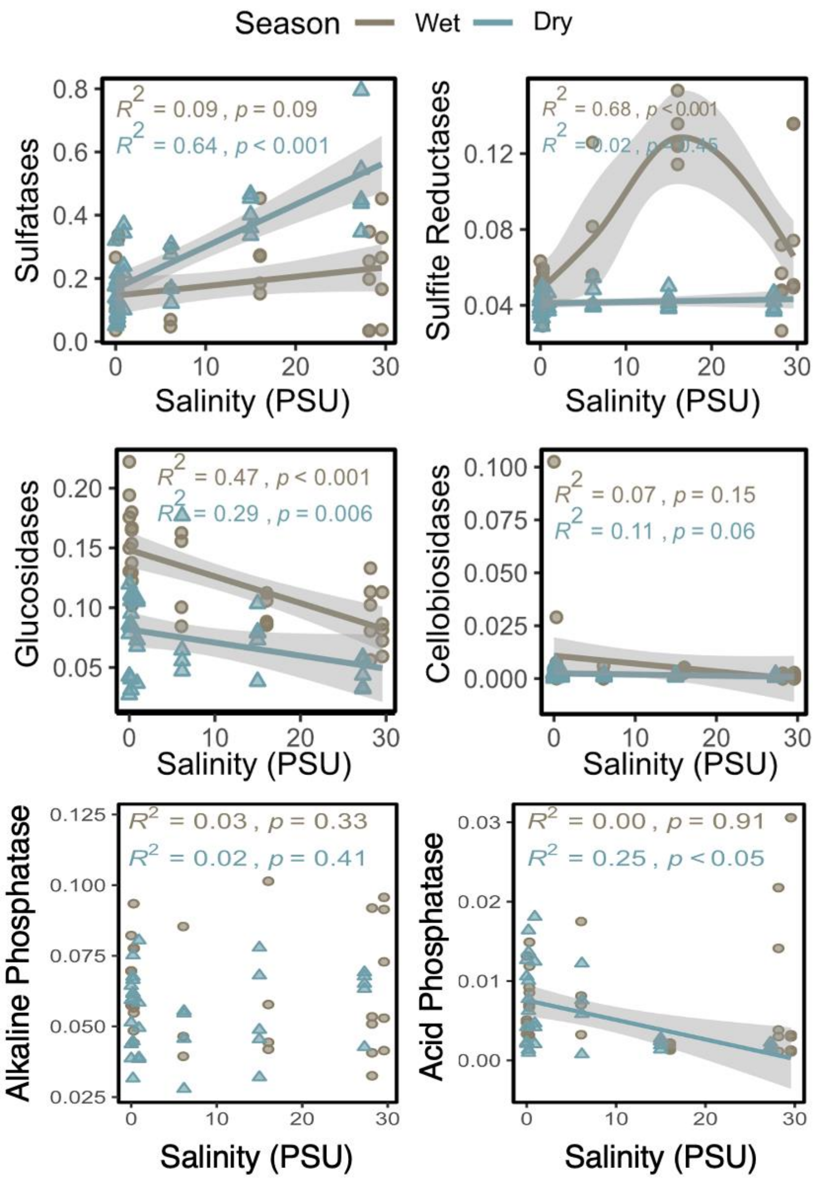

Figure 4. Linear regressions comparing surface water salinity and relative gene abundance of four focal genes, along Everglades freshwater to marine gradients. The shaded area indicates 95% CI.

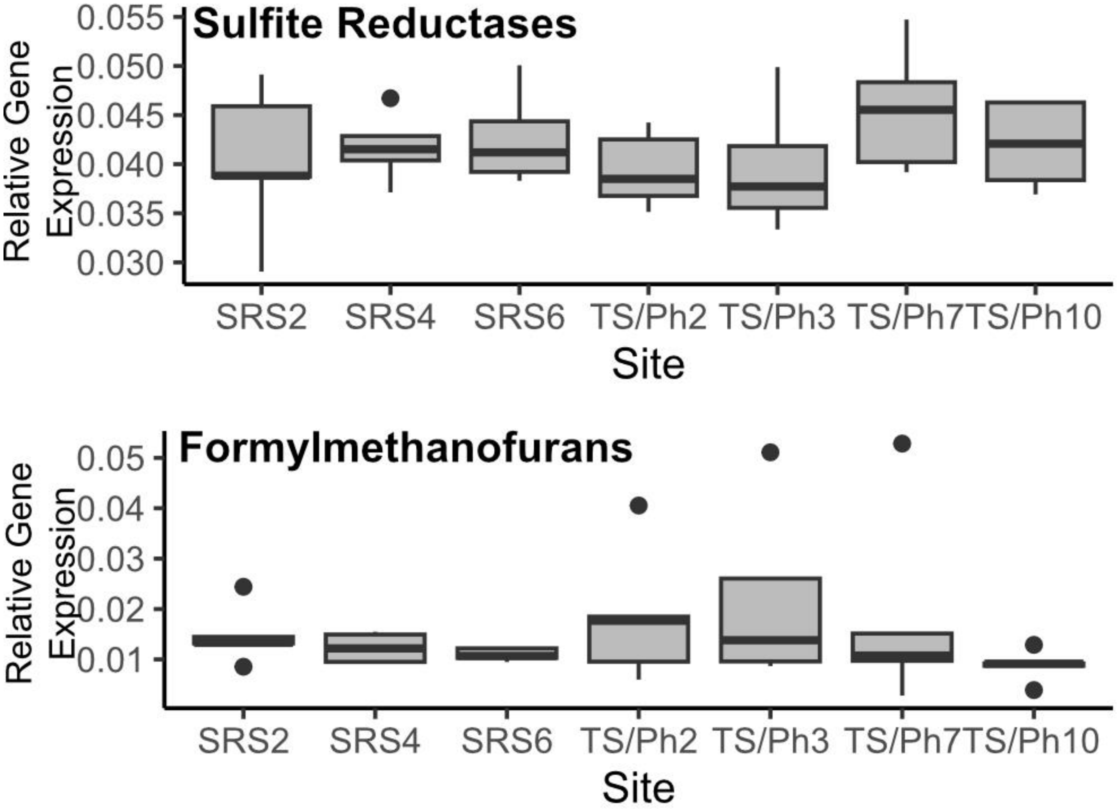

Figure 5. Relative gene expression of sulfite reduction and formylmethanofurans on litter along freshwater to marine gradients. Boxplots represent the interquartile range for each variable, and the solid line is the median. Error bars represent the 95% confidence intervals. Significance is not reported because there was no significant ($\alpha \leq 0.05$) difference between sites.

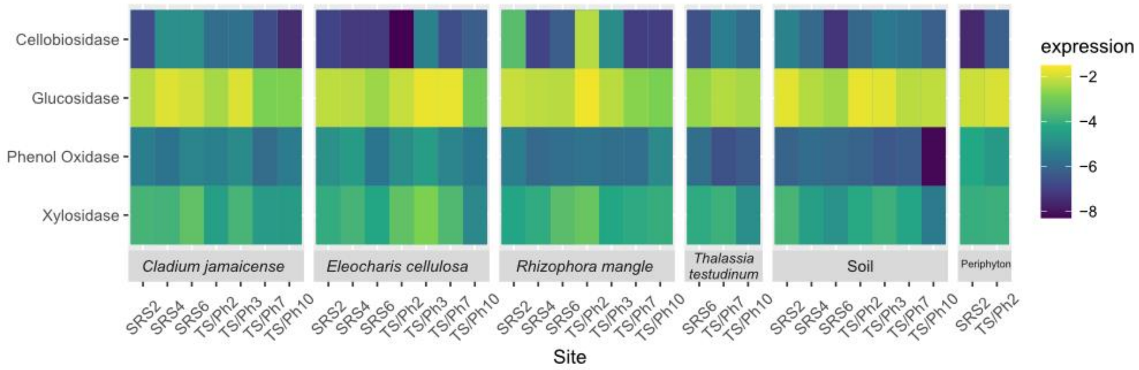

Figure 6. Heatmap of log gene expression of four carbon degrading enzymes across the four leaf litter species, soil, and periphyton.

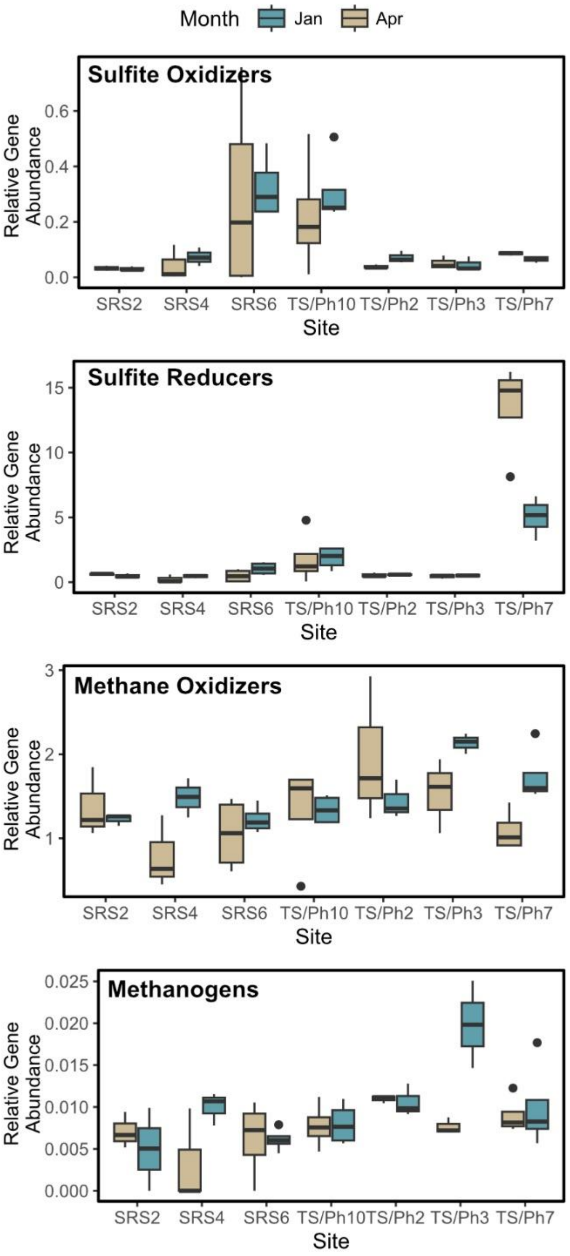

Figure 7. Relative gene abundance for four anaerobic microbial taxa along Everglades freshwater to marine gradients. Boxplots represent the interquartile range for each variable, and the solid line is the median. Error bars represent the 95% confidence intervals.

Table 1. Litter breakdown rate (k / degree day) after A) 1 and B) 4 months of incubation. We deployed litterbags at marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in a seagrass meadow in Florida Bay (TS/Ph-10).

A. One month (January)

	SRS-2	**SRS-4**	**SRS-6**	**TS/PH-2**	**TS/PH-3**	**TS/PH-7**	**TS/PH-10**
Eleocharis cellulosa	0.021 (0.002)	0.023 (0.005)	0.016 (0.004)	0.021 (0.001)	0.016 (0.001)	0.014 (0.003)	0.019 (0.003)
Rhizophora mangle	0.023 (0.002)	0.022 (0.002)	0.018 (0.004)	0.025 (0.003)	0.02 (0.005)	0.019 (0.002)	0.028 (0.009)
Cladium jamaicense	0.015 (0.003)	0.011 (0.005)	0.008 (0.003)	0.015 (0.002)	0.012 (0.002)	0.006 (0.003)	0.010 (0.004)
Thalassia testudinum	ND	ND	0.03 (0.005)	ND	ND	0.023 (0.003)	0.045 (0.012)

B. Four months (April)

	SRS-2	**SRS-4**	**SRS-6**	**TS/PH-2**	**TS/PH-3**	**TS/PH-7**	**TS/PH-10**
Eleocharis cellulosa	0.028 (0.003)	0.032 (0.012)	0.031 (0.011)	0.033 (0.005)	0.026 (0.003)	0.028 (0.006)	0.029 (0.030)
Rhizophora mangle	0.034 (0.004)	0.031 (0.003)	0.024 (0.006)	0.033 (0.004)	0.029 (0.005)	0.037 (0.008)	0.042 (0.011)
Cladium jamaicense	0.021 (0.005)	0.019 (0.005)	0.011 (0.004)	0.024 (0.004)	0.019 (0.001)	0.010 (0.003)	0.015 (0.007)
Thalassia testudinum	ND	ND	0.027 (0.013)	ND	ND	0.045 (0.030)	0.073 (0.046)

Table 2. We selected *n* = 12 focal enzymes to investigate, which are important to the breakdown of organic matter. We selected enzymes involved in 5 metabolic pathways: oxygen metabolism, sulfate metabolism, methane metabolism, carbon degradation, and phosphorus acquisition.

Gene of Interest	*Microbial Usage*
Dioxygenases	Aerobic Respiration
Sulfatases	Releases sulfates from complex molecules
Sulfite Reductases	Sulfite Reduction
Methyl Coenzyme M Reductase	Methanogenesis
Formylmethanofuran	Methanogenesis
Nitrite Reductases	Nitrite Reduction
Cellbiosidase	Cellulose breakdown
Glucosidase	Cellulose breakdown
Xylosidase	Cellulose breakdown
Phenol Oxidase	Lignin Breakdown
Acid Phosphatase	Phosphate acquisition in acid environment
Alkaline Phosphatase	Phosphate acquisition in basic environment

Table S1. Average (± SD) surface water physicochemistry data for the months of A) January and B) April where litter bags were deployed from marsh, ecotone, and mangrove sites along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7), and in Florida Bay (TS/Ph-10). Data for all site except TS/Ph-10 were collected ever 3 days via autosamplers, TS/Ph-10 was collected as a single grab sample.

A. January

Site	***Total Phosphorus***	***Total Nitrogen***	***Salinity***
SRS-2	0.84 (0.12)	68.65 (3.53)	0.25 (0.05)
SRS-4	0.86 (0.18)	69.13 (6.46)	0.89 (0.19)
SRS-6	1.74 (2.09)	55.31 (22.03)	14.99 (1.85)
TS/Ph-2	0.60 (0.12)	44.76 (5.05)	0.20 (0.00)
TS/Ph-3	0.67 (0.05)	63.33 (3.60)	0.20 (0.00)
TS/Ph-7	0.37 (0.11)	49.70 (5.50)	6.17 (3.34)
TS/Ph-10	0.33 (ND)	47.85 (ND)	33.83 (ND)

B. April

Site	***Total Phosphorus***	***Total Nitrogen***	***Salinity***
SRS-2	0.74 (0.30)	97.33 (4.75)	0.38 (0.04)
SRS-4	0.70 (0.07)	73.60 (7.50)	6.09 (2.15)
SRS-6	1.80 (1.03)	50.02 (8.66)	28.17 (2.11)
TS/Ph-2	0.27 (0.03)	47.95 (7.92)	0.20 (0.00)
TS/Ph-3	0.45 (0.08)	214.52 (49.78)	0.28 (0.04)
TS/Ph-7	0.56 (0.22)	65.37 (12.83)	16.04 (1.90)
TS/Ph-10	0.31 (ND)	36.81 (ND)	33.84 (ND)

CHAPTER IV

DISSOLVED ORGANIC MATTER IN PEAT AND MARL MARSHES VARY WITH NUTRIENT ENRICHMENT AND RESTORED HYDROLOGY

ABSTRACT

Dissolved organic matter (DOM) drives biogeochemical processes in aquatic ecosystems. Yet, how hydrologic restoration in nutrient-enriched ecosystems changes DOM and the consequences of those changes for the carbon cycle remain unclear. To predict the consequences of hydrologic restoration on carbon cycling in restored wetlands, we need to understand how local environmental factors influence production, processing, and transport of DOM. We collected surface water samples along transects in restored peat (organic-rich, macrophyte-dominated) and marl (carbonate, periphyton-dominated) freshwater marshes in the Everglades (Florida, USA) that varied in environmental factors [water depth, phosphorus (P) concentrations (water, macrophytes, periphyton, and soil), and primary producer biomass] to understand drivers of dissolved organic carbon (DOC) concentrations and DOM composition. Higher water depths led to a “greening” of DOM, due to increasing algal contributions, with decreasing concentrations of DOC in peat wetlands, and a “browning” of DOM, due to increasing humic contributions, with increasing DOC concentrations in marl wetlands. Soil total P was positively correlated with DOC concentrations and microbial contributions to DOM in peat wetlands, and periphyton total P was positively correlated with algal contributions to DOM in marl wetlands. Despite large variations in both vegetation biomass and periphyton biovolume across transects and sites, neither were predictors of DOC concentrations or DOM composition. Hydrologic restoration differentially alters DOM in peat and marl marshes and interacts with nutrient enrichment to shift proportions of green and brown contributions to surface water chemistry, which has the potential to modify wetland food webs, as well as the processing of carbon by micro-organisms.

IMPLICATIONS FOR PRACTICE

- Freshwater restoration can alter DOM, increasing DOC in organic-poor, carbonate marl wetlands and diluting high concentrations of DOC in organic-rich, peat marshes.
- Changing sources of DOM can alter the base of aquatic food-webs and modify how carbon flows through oligotrophic ecosystems.
- Understanding how carbon sources and nutrient limitation impact carbon cycling is critical for managers to predict how hydrologic restoration and nutrient legacies can affect carbon storage in ecosystems.

INTRODUCTION

Dissolved organic carbon (DOC) is the most ubiquitous form of carbon in aquatic systems (Wetzel 1995). DOC controls biogeochemical processing and is an important regulator of the microbial loop (Amon & Benner 1996; Boyer et al. 1997; Qualls & Richardson 2003). The composition of DOC, which is a main portion of dissolved organic matter (DOM), is a function of organic matter production, transport, and transformation in aquatic ecosystems (Osborne et al. 2007; Fellman et al. 2010). Changing hydrology and nutrient availability drive the production and processing of DOM in aquatic ecosystems, subsequently changing its composition, quality, and bioavailability (Marschner & Kalbitz 2003; Shen et al. 2015, Graeber et al. 2021). Variation in carbon sources contribute to differences in DOM composition among ecosystems, which further influences its transport and transformation (Graeber et al. 2021).

Freshwater ecosystems play a major role in the global carbon cycle (Battin et al. 2009), but it is unclear how the composition of DOM interacts with environmental drivers (i.e., hydrology, nutrient enrichment, vegetation composition and biomass) to determine its fate (Berggren et al. 2022). The combination of environmental and compositional (the chemical makeup of DOM) drivers determines how DOC is subject to biodegradation, photo-oxidation, or flocculation (Anderson et al. 2019). During wetland ecosystem restoration, which typically involves increasing water depths, shifting ecosystems from oxic to anoxic states, and maintaining stores of carbon (Christen et al. 2016; Evans et al. 2005; Moreno-Mateos et al. 2012; Venterink et al. 2002; Zerbe et al. 2013), DOC is rarely managed for directly, largely because of a lack of consistent data on the interactions between DOC and ecosystem restoration (Stanley et al. 2012). However, recent studies have found that increases in water depth are also responsible for increases in lateral DOC fluxes which play a significant role in the net ecosystem carbon balance, potentially offsetting reductions in emissions from wetlands (D′Acunha et al. 2019; Strack et al. 2011). Although DOC fluxes are rarely managed for, a global trend of “browning” (referring to increased concentrations of DOC that increase the coloration of waters), has received attention for its potential to both increase monetary costs for drinking water purification and reducing fish production in lakes (van Dorst et al. 2019; Kritzberg et al. 2020; Lavonen et al. 2013). The potential for restoration to both mobilize DOC (reducing the carbon storage potential of wetlands) and contribute to browning of freshwater highlights the importance of understanding sources and fates of DOC in restored wetlands.

The Florida Everglades is undergoing the world's largest restoration effort to reconnect more than a century of altered hydrology and restore wetland ecosystem functions (Sklar et al. 2005). Maintaining carbon storage capacity is a major goal of Everglades restoration, and achieving that goal requires a thorough understanding of the local drivers of carbon processing and their interactions with drivers and legacies of disturbance in recovering ecosystems (Herrick et al. 2006; Meli et al. 2014). Fluxes of DOC throughout the Everglades are closely linked to hydrology, whereby water management and precipitation patterns control DOC transport (Regier et al. 2016). Increasing water depths with hydrologic restoration have the potential to change DOC production, transport, and photo- and biological degradation, which could also change the fate of carbon in restored areas (Chen & Jaffé 2016). As water depths increase, the microtopography of Everglades habitats are also shifting, and understanding how those shifts impact the production and transport of DOC is important for predicting how restoration will alter the fates of carbon (Choi & Harvey 2016). The fate of DOC has consequences for carbon storage potential of wetlands, as well as the potential to shift the balance of "green" and "brown" food webs within restored ecosystems (Belicka et al. 2012).

In the Everglades, restoration is primarily achieved through water management, with the goal of increasing hydroperiods (i.e., total number of days in a year with water above the soil surface) and reducing eutrophication caused by excess phosphorus loading from upstream agriculture. This is accomplished by redirecting water that was previously diverted through canals, back in wetlands of the Everglades (Juston & DeBusk 2011). However, early evidence suggests that bound phosphorus is being mobilized from legacy hotspots as a collateral effect of increased freshwater flows and hydroperiods (Sarker et al. 2020). Phosphorus enrichment is an important driver of flocculant material respiration, and periphyton composition (Gaiser et al. 2006; Lu et al. 2003; Pisani et al. 2018), and has the potential to shift the relative contributions of algal and detrital basal resources of food webs (Belicka et al. 2012). Increasing water depths and the mobilization of legacy phosphorus are both contributing to increased DOC concentrations, which can also increase detrital resources to microbial consumers (Regier et al. 2016). Carbon processing within restored aquatic ecosystems is driven by autochthonous and allochthonous water and carbon sources (Anderson et al. 2019; Berggren et al. 2022). Everglades restoration moves water from upstream peatlands to downstream peat and marl wetlands, so restored wetlands are receiving increases in peat-derived DOM as more fresh water is delivered.

Here, our goal was to understand how restoration of water depths and variations in relative nutrient enrichment affect the concentration of DOC and the composition of DOM in oligotrophic Everglades wetlands, ranging from ridge and slough habitats in long-hydroperiod peat to short-hydroperiod marl marshes. Past studies have shown that there are significant differences in both DOC concentrations and DOM composition in peat and marl marshes (Regier et al. 2020). Compared to past studies, we added sampling along nutrient-enrichment gradients in peat and marl marshes that vary in water depth, phosphorus (P) concentrations (water, macrophytes, periphyton, and soil), and primary producer biomass to understand how these drivers affect DOC concentrations and DOM composition. We hypothesized that freshwater restoration would increase DOC concentrations in areas with higher P enrichment, and that DOM composition would change based on periphyton and macrophyte biomass. In addition, we expected that DOC in peat marshes would be less altered by hydrologic restoration than DOC in marl marshes because of novel allochthonous peat-derived DOC subsidies entering the marl wetland ecosystems.

METHODS

Study Sites

The Greater Everglades was originally a continuous wetland flowing from north of Lake Okeechobee to Florida Bay. Prior to hydrologic alteration, most of the water moved as sheet flow in the wet season (May – November), during which the Everglades receives 70% of its total rainfall. Hydrologic alteration began in the early 1900s with the construction of drainage canals, intended to create agricultural areas, and drainage of areas for urban development. The construction of canals for drainage has caused a compartmentalization of the system, with the creation of Water Conservation Areas (WCAs). Although a major conservation goal is the restoration of sheet flow in much of the Everglades, these compartments are still primarily linked along canal boundaries (Kominoski et al. 2019).

Sample collection for our experiment was performed at the boundary of Everglades National Park (ENP) along its two major drainages: Shark River Slough (SRS) and Taylor Slough (TS) (Figure 1). These two drainages sit at different elevations, causing a difference in hydroperiod between the two sloughs. This difference has led SRS, the longer-hydroperiod wetland, to develop deep peat soils, whereas TS, the short-hydroperiod slough, is primarily characterized by marl soils. The boundary where upstream water enters Shark River Slough is the L-29 Canal, which runs east to west along the northern boundary of ENP and is the connection between ENP and the WCAs. The long hydroperiod of Northeast Shark River Slough (NESRS) has allowed for the formation of deep peat soils, characterized by high carbon and P concentrations (Osborne et al. 2011). Phosphorus enrichment in these soils originates in the Everglades Agricultural Area (EAA) along the border of Lake Okeechobee. Runoff from the EAA from the 1960s to present led to the buildup of phosphorus along the borders of ENP (DeBusk et al. 1994, 2001). We collected surface water samples along the boundary where overland flow is increasing in response to the Modified Water Deliveries (MOD) project (McLean 2015), with 4 transects (~1 km in length), perpendicular to the upstream 1.6-km bridge, with a longer history of modified flow, and 4 transects (~1 km in length), perpendicular to the upstream 4.2-km bridge, with a shorter history of modified flow.

In Taylor Slough, our second focal area, upstream water enters a portion of the ENP boundary along the L-31W/Aerojet Canal, which runs north to south. Through the Aerojet Canal, water is delivered from upstream wetlands towards Taylor Slough (Kotun & Renshaw 2014). This region has lower hydrologic connectivity with upstream Everglades wetlands and is an ecosystem largely characterized by marl soils, supporting a mix of prairie and marsh vegetation communities. Marl wetlands have relatively low organic carbon concentrations in soils, made from a mix of decaying calcareous periphyton and the limestone base. This ecotype is extremely oligotrophic and highly phosphorus limited, in part because the available phosphorus is rapidly adsorbed by the limestone (Osborne et al. 2011). The Taylor Slough region is also characterized by the accumulation of phosphorus at canal boundaries and seasonal mobilization of phosphorus mineralized during dry conditions (Sullivan et al. 2014). We collected surface water samples along the boundaries of marl wetlands, with three transects in a marsh with a history of higher phosphorus loading as it is situated at the end of the L-31W Canal (where canal overspill increased phosphorus loading during high flow) and three transects further North in a marsh with a history of lower phosphorus loading, away from the overspill zone (see Nocentini et al. 2022).

Water Physicochemistry

In September (wet season) of 2020 and March (dry season) of 2021, along each sampling transect, we collected two surface water samples at each site, one filtered and one unfiltered. Samples were filtered in the field using cellulosic 0.45-μm filters (GVS MicronSep, Zola Predosa, Bologna, Italy) and stored in 60-mL acid-washed amber HDPE bottles. Unfiltered grab water samples were collected in 250-mL acid-washed amber HDPE bottles. All samples were transported to the laboratory on ice and then stored at 4°C until analyzed. Filtered samples were analyzed for dissolved organic carbon (DOC) on a Shimadzu TOC-V total organic carbon analyzer, after acidification to remove inorganic carbon. Unfiltered samples were analyzed for total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP). TOC was analyzed using a Shimadzu TOC Analyzer (Shimadzu Corporation, Columbia, MD, USA), TN was measured with an Antek TN analyzer (Antek Instruments, Houston, TX, USA), and TP was analyzed following Solórzano and Sharp (1980).

Fluorescence spectroscopy was performed on all filtered samples to determine the chemical composition of the DOM of each sample. Fluorescence excitation-emission matrices (EEMs) were measured on a Horiba Aqualog (Jobin Yvon Horiba, France). Samples were measured at room temperature at 3 nm wavelengths, over excitation wavelength intervals between 240 and 455 nm and an emission wavelength range of λex + 10 nm to λex + 250 nm in a 1 cm quartz cuvette. EEMs were corrected, Raman normalized, and blank subtracted using MATLAB R2019a (Mathworks, Natick, MA, USA).

We measured water depth as an average of three measurements within each 1 × 1 m quadrat during each sampling. We calculated the average depth between the 3 quadrats at each site for each time point.

Macrophytes, Periphyton, Flocculant Organic Matter, and Soils

Along each sampling transect, we collected vegetation, organic matter, and periphyton in 1 × 1 m quadrats, and we collected 3 replicate quadrats at each site. We recorded percent cover of vegetation, canopy height, and the total number of plant stems in each quadrat. From the number of stems and the canopy height, we calculated species-specific mass corrections to estimate the total live macrophyte biomass in each quadrat (Childers et al. 2006, Nocentini et al. 2022). We measured floc depth and periphyton biovolume from each quadrat. We collected grab samples of soil, periphyton, sawgrass leaves, and floc for nutrient analysis. All grab samples were oven-dried at 40°C for 72 h, and ground using an 8000D ball mill (SPEX SamplePrep, Metuchen, NJ, USA). Then, ground samples were analyzed for TC and TN by high-temperature dry combustion using a Carlo-Erba NA-1500 CNS Analyzer (Nelson & Sommers 1996), and TP was measured by oxidation (dry combustion) and hydrolysis of the P-containing compounds in the sample to soluble forms using $MgSO_4/H_2SO_4$ and HCl (Solórzano & Sharp, 1980).

Data Analyses

We processed absorbance and fluorescence data using the DrEEM 3.0 toolbox in MATLAB R2019a and calculated five common metrics of DOM fluorescence: fluorescence index as an indicator of microbial versus terrestrial sources of DOM (FI; McKnight et al. 2001), humification index as an indicator of higher molecular weight and refractory DOM (HIX; Zsolnay et al. 1999), biological index as an indicator of new autochthonous inputs to DOM (BIX; Huguet et al. 2009), aromatic index as an indicator of aromaticity of DOM ($SUVA_{254}$; Weishaar et al. 2003), and slope ratio as an indicator of molecular weight of DOM (SR; Helms et al. 2008).

To test how legacies of hydrologic and phosphorus enrichment alter the composition and concentrations of dissolved organic matter in peat and marl wetlands, we grouped sites by soil type (peat, marl) and phosphorus legacy impact (P-enriched, P-limited). In peat wetlands of NESRS, the P-enriched sites were located downstream of the bridge constructed in 2012, and the P-limited sites were located downstream of the bridge constructed in 2018. In marl wetlands along the Aerojet Canal, the P-enriched sites were located at the end of the canal, whereas the P-limited sites were located along the middle section and perpendicular to the canal. We used one-way Analysis of Variance (ANOVA) to test for significant ($\alpha \leq 0.05$) effects of soil type, season, and nutrient legacy impact on fluorescence metrics, emergent macrophyte biomass, periphyton biovolume, and water and particulate biogeochemistry. Although not in the objectives of the study, we analyzed these variables as they mediated the combined effects of hydrologic restoration and relative P limitation on DOC concentrations and DOM composition.

To better understand the drivers of carbon fluorescence, we used Pearson's bivariate correlation (*r*) and linear regression analysis (R^2) to test significant relationships between fluorescence metrics, emergent macrophyte biomass, periphyton biovolume, and water and particulate biogeochemistry. To investigate how hydrology, emergent macrophyte biomass, periphyton biovolume, and phosphorus enrichment differentially drive DOC concentration and DOM composition in marl and peat wetlands, we used a series of covariates corresponding to each potential driver, created a series of linear models, and performed model selection (see information below). We used water depth as a covariate corresponding to hydrology, macrophyte biomass and periphyton biovolume as covariates corresponding to primary producers, and TN:TP ratios for sawgrass, soil, and water as measures of relative phosphorus enrichment. We constructed a series of linear models including all combinations of the three covariate groups (Table S1). We removed sawgrass TN:TP ratios from the peat models a priori, in order to fit all models to the same number of observations after finding that sawgrass TN:TP ratios did not have a significant relationship with DOC concentration or DOM composition (Figure S1).

We calculated Akaike's Information Criterion (AIC_c) for small sample size scores for each model and selected the best-fitting model as the one with the highest adjusted R^2 score that had a ΔAIC less than or equal to 2 (Burnham & Anderson 2002). We calculated coefficient estimates for each explanatory variable in the best-fitting models and tested for significant relationships between coefficients and response variable for each model. All analyses were performed using R version 4.2.0 (R Core Team 2022). All plots were constructed with the ‘ggplot2’ package (Wickham 2009).

RESULTS

Water Physicochemistry

Peat wetlands had deeper water depths than short-hydroperiod marl wetlands (ANOVA, $F_{(3,109)} = 115.3$, $P < 0.001$, Figure 2a). In peat wetlands, the P-enriched transects had higher water depths (Figure 2a), and there was no change in water depth between seasons, as freshwater restoration and water management maintained similar water levels year round (ANOVA, $F_{(1,109)} = 0.115$, $P = 0.74$). In contrast, marl wetlands were deeper in the wet season than in the dry season (ANOVA, $F_{(1,109)} = 50.60$, $P < 0.001$).

Water chemistry varied across sites and seasons. Surface water total phosphorus (TP) concentrations were similar between peat and marl wetlands during the dry season, but during the wet season surface water TP in peat wetlands decreased while marls wetlands did not (ANOVA, $F_{(1,109)} = 5.993$, $P = 0.02$. Total nitrogen (TN) was higher in peat than in marl wetlands regardless of season (ANOVA, $F_{(3,109)} = 11.93$, $P < 0.001$), and TN increased during the dry season across all wetlands (ANOVA, $F_{(1,109)} = 11.22$, $P < 0.001$).

Primary Producer Biomass and Soil Chemistry

Phosphorus-enriched wetlands had less macrophyte biomass compared to P-limited wetlands, and macrophyte biomass was higher in peat than marl wetlands

(ANOVA, $F_{(3,109)} = 10.678$, $P < 0.001$; Figure 2b). Biomass of emergent macrophytes and periphyton biovolume were characterized by high spatial variability in peat wetlands, and by low spatial variability in marl wetlands . High biovolumes of periphyton were ubiquitous in marl wetlands. In contrast, periphyton was highly variable, ranging from absent to complete coverage, in peat wetlands. Overall, marl wetlands had higher biovolume of periphyton than peat wetlands (ANOVA, $F_{(3,109)} = 26.04$, $P < 0.001$; Figure 2c), and while not statistically significant there was a trend of periphyton biovolumes increasing in the wet season for both wetland types (ANOVA, $F_{(3,109)} = 3.66$, $P = 0.058$).

Soil TC concentrations were higher in peat than marl wetlands (ANOVA, $F_{(3,109)} = 62.197$, $P < 0.001$), and did not vary between dry and wet season in either wetland type. Soil TN concentrations, on the other hand, were lower in marl wetlands during the dry season compared to the wet season, and wet season soil TN was similar to both wet and dry season values for the peat wetlands (ANOVA, $F_{(1,33)} = 12.898$, $P = 0.001$). Soil TP concentrations were higher in all peat wetland sites than marl wetland sites (ANOVA, $F_{(3,109)} = 3.083$, $P = 0.03$) and were marginally lower during the dry than wet season across all sites (ANOVA, $F_{(3,109)} = 3.249$, $P = 0.074$).

Trends in DOC Concentrations and DOM Fluorescence

Mean DOC concentrations in peat wetlands ranged between 13.6 mg C L^{-1} in P-enriched wetlands and 20.4 mg C L^{-1} in P-limited wetlands and were higher in the dry than in the wet season (ANOVA, $F_{(3,109)} = 109.6$, $P < 0.001$; Table 1). Mean DOC concentrations in marl wetlands were lower than those of peat wetlands and more variable between seasons, with mean concentrations of 9.5-9.6 mg C L^{-1} in the marl and

10-12 mg C L^{-1} in the peat, for the dry and wet seasons, respectively (Table 1). Mean HIX values in peat wetlands ranged from 12.7 in P-enriched to 14.0 in P-limited wetlands, indicating highly humic DOM, whereas marl wetlands mean HIX values were significantly lower (ANOVA, $F_{(3,109)} = 241.4$, $P < 0.001$) and ranged from 6.9 at P-enriched sites to 9.4 at P-limited sites. HIX values varied seasonally at both sites, but in opposite directions; in marl wetlands HIX increased in the wet season (ANOVA, $F_{(1,40)} = 19.75$, $P < 0.001$), while in peat wetlands HIX decreased in the wet season (ANOVA, $F_{(1,75)} = 10.20$, $P < 0.001$).

Mean FI values ranged from 1.49 to 1.55 across wetland type, nutrient enrichment, and season and indicated a moderate level of microbial influence on DOM for both marl and peat wetlands (Table 1). FI values were higher in marl than peat wetlands. They were highest at P-enriched sites of marl wetlands during the dry season (ANOVA, $F_{(1,109)} = 8.404$, $P = 0.004$), and were lower at P-enriched sites in both wetland types (ANOVA, $F_{(3,109)} =14.55$, $P < 0.001$).

Mean BIX values range from 0.71 in marl to 0.64 in peat wetlands. BIX values indicated low autochthonous productivity in both peat and marl wetlands, but were significantly higher in marl than in peat wetlands, and were higher in P-enriched sites of both wetland types (Table 1; ANOVA, $F_{(3,109)} = 294.3$, $P < 0.001$). BIX values were generally higher in the dry season, except at the P-limited sites of marl wetlands, where they did not vary seasonally (ANOVA, $F_{(1,49)} =2.69$, $P = 0.107$).

$SUVA_{254}$ values ranged from 2.1 in marl to 3.2 in peat wetlands. $SUVA_{254}$ values indicated low carbon complexity in the DOM of both peat and marl wetlands, although they were significantly higher in peat compared to marl wetlands (Table 1; ANOVA,

$F_{(3,109)} = 41.49$, $P < 0.001$). $SUVA_{254}$ values did not vary seasonally except at the P-enriched marl wetland sites (ANOVA, $F_{(1,109)} = 5.282$, $P < 0.02$).

Mean SR values ranged from 0.99 in peat to 1.21 in marl wetlands, indicating high molecular weight DOM in both wetland types. They were significantly higher in the P-enriched marl than peat wetland sites (ANOVA, $F_{(3,109)} = 3.076$, $P = 0.01$). Mean SR values did not differ between seasons (ANOVA, $F_{(1,109)} = 1.268$, $P = 0.28$).

In summary, our analyses of the optical properties of fluorescence showed that DOM in peat wetlands was highly concentrated, humic, minimally influenced by autochthonous productivity, and more aromatic than the DOM of marl wetlands. In marl wetlands, DOM was more influenced by autochthonous productivity (composed of less humic and less aromatic compounds), especially in P-enriched marl wetlands, where we detected more autochthonous components in DOM (Figure 3).

Drivers of DOC Concentration and DOM Composition

DOC concentrations were best explained by the combined effects of hydrology and phosphorus enrichment in peat wetlands (Table S1), where both increasing depth and soil TN:TP ratio correlated with decreasing concentrations of DOC (Table 2; Figure 4). Surface water TN:TP did not have a significant effect on the model. In marl wetlands, hydrology, emergent macrophyte biomass, and periphyton biovolume best explained DOC concentrations (Table S1). Although water depth was the only covariate that showed a significant, positive correlation with DOC concentrations (Table 2; Figure 4), the inclusion of macrophyte biomass (negative relationship with DOC) and periphyton biovolume (positive relationship with DOC) improved the overall model fit (Table S1).

In both peat and marl wetlands, FI was best explained by the combined effects of hydrology and phosphorus enrichment (Table S1), but the relationships between hydrology and FI trended in opposite directions in the two wetland types (Table 2; Figure 4). In peat wetlands, FI increased with increasing water depth and increasing soil TN:TP ratios. In marl wetlands, FI decreased with water depth, although it was only a marginally significant predictor ($P = 0.06$). Marl wetlands soil nutrient stoichiometric ratios did not correlate significantly with FI, but their inclusion improved the overall model fit.

The full model best explained BIX in peat wetlands (Table S1), whereby increasing water depth and surface water TN:TP ratios corresponded to an increase in BIX (Table 2; Figure 4). Increasing periphyton biovolume ($P = 0.07$), macrophyte biomass ($P = 0.56$), and soil TN:TP ratio ($P = 0.47$), all corresponded to decreasing BIX, but none showed a significant correlation. In marl wetlands, BIX was best explained by the combined effects of hydrology and phosphorus enrichment (Table S1). BIX decreased as water depth increased, but it increased as surface water TN:TP ratios increased (Table 2; Figure 4). There was no significant response of BIX to soil or sawgrass TN:TP ratios in peat wetlands, but there was a significant relationship in marl wetlands between BIX and sawgrass TN:TP ratios ($R^2 = 0.11$, $P =0.046$; Figure 5c). Periphyton stoichiometric ratios were excluded from models, as they were collected only in the dry season from marl wetlands. However, there was a strong, positive correlation between BIX collected in marl wetlands and periphyton TN:TP ratio ($R^2 = 0.59$, $P < 0.001$; Figure 5a). Conversely, BIX in peat wetlands did not correlate with periphyton TN:TP ratio (Figure 5b).

HIX in peat wetlands was not well explained by the model; the best model included the effects of hydrology, emergent macrophyte biomass and periphyton biovolume, but had an R^2 of only 0.10 (Table S1). The best-fitting model in marl wetlands included the effects of hydrology, emergent macrophyte biomass, and periphyton biovolume (Table S1). Periphyton nutrient concentrations were excluded from models because they were collected only in the dry season for marl sites; however, in marl wetlands HIX decreased with increasing periphyton TN:TP ratio ($R^2 = 0.32$, $P = 0.01$). Conversely, HIX in peat wetlands did not correlate with periphyton TN:TP ratio (Figure 5b).

Models did not explain $SUVA_{254}$ or S_R well in either peat ($R^2 = 0.02, 0.001$) or marl ($R^2 = 0.001, 0.19$) wetlands (Table S1). There was no significant correlation between $SUVA_{254}$ or S_R and the covariates in either of the best fitting models (Table 2).

DISCUSSION

The results presented here support our hypothesis that freshwater restoration would have different effects on DOM based on its interactions with local drivers. We found that hydrology and phosphorus are the major drivers of changes to DOM in both peat and marl wetlands. However, our data also showed that the source of a carbon in water used to restore an ecosystem can determine the direction of its interactions with local environmental conditions, such as water depth and nutrient enrichment. We found that increasing water depths caused a “greening” of DOM in peat wetlands (increasing BIX and FI, decreasing concentration of DOC), but a “browning” of DOM in marl wetlands (decreasing BIX and FI, increasing HIX). We found that soil phosphorus enrichment in peat wetlands enhanced microbial production of peat DOM, while in marl wetlands, we found that phosphorus shifted DOM from "brown" to "green" sources based on BIX and FI, suggesting an increase in algal and periphyton contributions. BIX and FI correspond to algal and microbial contributions to DOM respectively and their increases of both indicate a growing influence of periphyton as a conglomeration of algal and microbial organisms contributing to both metrics. Despite high variation within and between wetland types, macrophyte biomass, and periphyton biovolume were not significant predictors of DOC concentrations, or DOM composition. Our data highlights the importance of water depth and phosphorus as the major drivers of DOM in oligotrophic Everglades wetlands, where the direction of change depends on their interactions with different types of carbon.

Hydrologic Controls on Wetland DOM

We found that DOM in peat and marl wetlands had an opposite relationship with water depth, which is a master variable in wetland ecosystems, especially the Everglades (Jackson et al. 2014; Regier et al. 2016). Increasing water depths caused a "greening" of DOM in peat (decreasing concentrations, and increasing BIX which indicates algal influence), but a "browning" of DOM in marl wetlands (increasing concentrations and increasing HIX which indicates humic influence). A fundamental difference in hydroperiod between peat and marl wetlands likely explains this difference. Peat wetlands are characterized by longer hydroperiods that maintain anoxic conditions and allow for the buildup of organic matter to create peat soils (Fenner & Freeman 2011; Ritson et al. 2017). In contrast, marl wetlands are characterized by ephemeral organic matter pools, where carbon stored as floc is rapidly mobilized following the annual dry down (Pisani et al. 2013). This difference in carbon sources explains why increasing water depths dilute peat-derived DOM, but mobilize ephemeral marl wetland carbon and import upstream humic DOM.

The production and release of DOM from humic wetland ecosystems (e.g., “browning”) is increasing globally in response to changes in climate and precipitation (Freeman et al. 2001; Worrall et al. 2004). Our data show a similar pattern where increasing water flows from peat-rich upstream wetlands increased humic DOM (both in terms of HIX and concentration), overwhelming the limited local production of DOM in marl wetlands (Duan et al. 2017). Droughts commonly stimulate microbial activity in peat, increasing breakdown and leading to a feedback loop where breakdown-derived increases in pH enhance anaerobic breakdown (Fenner and Freeman 2011; Ritson et al. 2017). In contrast, high water depths can shift conditions from aerobic to anaerobic breakdown of peat as benthic oxygen decreases, leading to slower breakdown and lower concentrations of humic DOM. As water levels increase and potentially suppress peat production of DOM, DOM can become diluted and transported downstream, decreasing its concentrations (Hornberger et al. 1994; Wen et al. 2020).

Phosphorus Enrichment as a Driver of DOM

Phosphorus enrichment (included in every best fitting model) contributed to both the composition and concentration of DOM produced in peat and marl wetlands. Nutrient enrichment is a critical factor affecting many ecological processes, including the storage, processing, and release of carbon (Deegan et al. 2012, Rosemond et al. 2015, Kominoski et al. 2018). Our finding that DOC concentration decreases in response to increasing soil

TP and FI (which indicates microbial processing) increases in response to soil TP increases, may reflect a characteristic pattern to the Everglades: primary producer biomass decreases with P enrichment (up to a certain threshold) and wetlands become open water sloughs with more labile vegetation. Low phosphorus in these wetlands typically corresponds to more dense and recalcitrant vegetation (such as *Cladium jamaicense*), which likely explains the negative correlation between DOC concentration and soil TP. As restoration mobilizes legacy phosphorus through the Everglades (Sarker et al. 2020), it is essential to continue monitoring how carbon storage and processing interact with nutrient cycling during long-term hydrologic restoration efforts. Given that local variation in P availability and hydroperiods shape the ridge and slough landscapes (Watts et al. 2014), understanding interactions among hydrology, legacy nutrients, and carbon processing will improve predictions of structure and function of primary producers in restored Everglades marshes.

Dominant carbon sources and relative labilities interact with nutrient enrichment to drive patterns of DOC concentrations and DOM composition. Nutrient enrichment often has higher magnitude effects on the breakdown of recalcitrant organic matter (Manning et al. 2015; Rosemond et al. 2015), which may explain how P enrichment increased DOC concentrations in peat wetlands. In marl wetlands, instead of controlling the concentration of DOC, elevated periphyton TP shifted the sources of DOM to higher autochthonous algal contributions. In oligotrophic wetlands like the Everglades, periphyton has a unique response to eutrophication and nutrient loading, where increasing nutrients lead to losses of biomass (Gaiser et al. 2006). Our finding of increasing algal derived DOM (BIX) with increasing periphyton phosphorus concentrations suggests that losses in periphyton biomass due to P-enrichment may be detected in DOM. In many areas of the Everglades, periphyton is the dominant primary producer, and understanding how nutrients will shift its production and contribution to DOM is necessary to predict how restoration will impact periphyton assemblages and the DOM derived from them (Childers et al. 2003).

Wetland Type Determines Carbon Sources of DOM

We did not find evidence that primary producer biomass influenced DOM, but restoration in the Everglades, that alters the structure and composition of vegetation communities across the landscape (Sah et al. 2020), is also both mobilizing and creating stores of carbon (Sarker et al. 2020). Much of the data currently available on DOM has focused on long term data from just a few locations, which misses out on the spatial heterogeneity and microtopographic variations that have been highlighted as key variables to the success of restoration of Everglades wetlands (Harvey et al. 2017). Our findings highlight the variability of carbon production across water depths which drive the variation between ridge and slough habitats across the Everglades. As water depths increase, our findings showing a “greening” of “browner” peatlands, and a “browning” of “greener” marl wetlands suggests that DOM will become more similar between wetlands, with marl wetlands receiving increasing inputs of peat DOM as well as an increase in production of DOM with the accumulation of peat associated with increasing water depths., and peat wetlands producing more algal DOM. Our data suggests that peat in the Everglades significantly contributes to DOC concentrations, more than leaf litter being produced by macrophytes, similar to other peat wetlands (Ritson et al. 2017). In agreement with previous findings (Chen et al. 2013), higher BIX in DOM of marl wetlands, as compared to peat, is best explained by increased periphyton contributions to DOM. Autochthonous marl DOM is likely entering the water column through floc, which, in Everglades marl wetlands, is primarily formed by periphyton and submerged aquatic vegetation (Pisani et al. 2013). Seasonal dry down of marl sites explains increases of BIX in the wet season, as autochthonously derived floc is mobilized to the water column with re-wetting of the ecosystem (Pisani et al. 2013).

As increasing water depths increase hydrologic connectivity throughout Everglades wetlands, and both peat and marl wetlands become dominated by more humic DOM, carbon and nutrient cycling in the water column of peat and marl wetlands are changing (Dessu et al. 2018; Kominoski et al. 2020). Humic DOM (i.e., higher HIX) reduces photo-degradation of DOM, is more recalcitrant than microbial DOM sources, less bioavailable to microbes, and commonly reduces ecosystem productivity (Moran & Hodson 1990; Qualls & Richardson 2003). A shift from more labile to more recalcitrant carbon could reduce microbial activity, and lead to higher export of DOC from freshwater wetlands into Everglades estuaries (Tranvik 1992; Raymond et al. 2016). This export caused by increases in carbon recalcitrance can lead to undetected fluxes of carbon between ecosystems (D′Acunha et al. 2019; Strack et al. 2011).

The “greening” or “browning” of carbon sources has the potential to alter the path of ecosystem food-webs based on the interactions between consumers and carbon (Cole et al. 2006). Understanding food-web pathways is a major concern in Everglades restoration and we show that measurements of DOM provide a window into potential changes in the base of the food-web in response to restoration activities. Further study is needed to understand how those changes in detrital and algal carbon sources may affect energy flow to the base of the food web (Moore et al. 2004; Sobczak et al. 2005). This is especially relevant in Everglades food-webs that are closely tied to periphyton mats that are ubiquitous throughout the Everglades; our results suggest that increasing water depths could homogenize food-webs across the landscape, increasing detrital resources in marl marsh food-webs, and increasing algal resources in peat marsh food-webs (Rader 1999; Sargeant et al. 2010, Sanchez & Trexler 2016).

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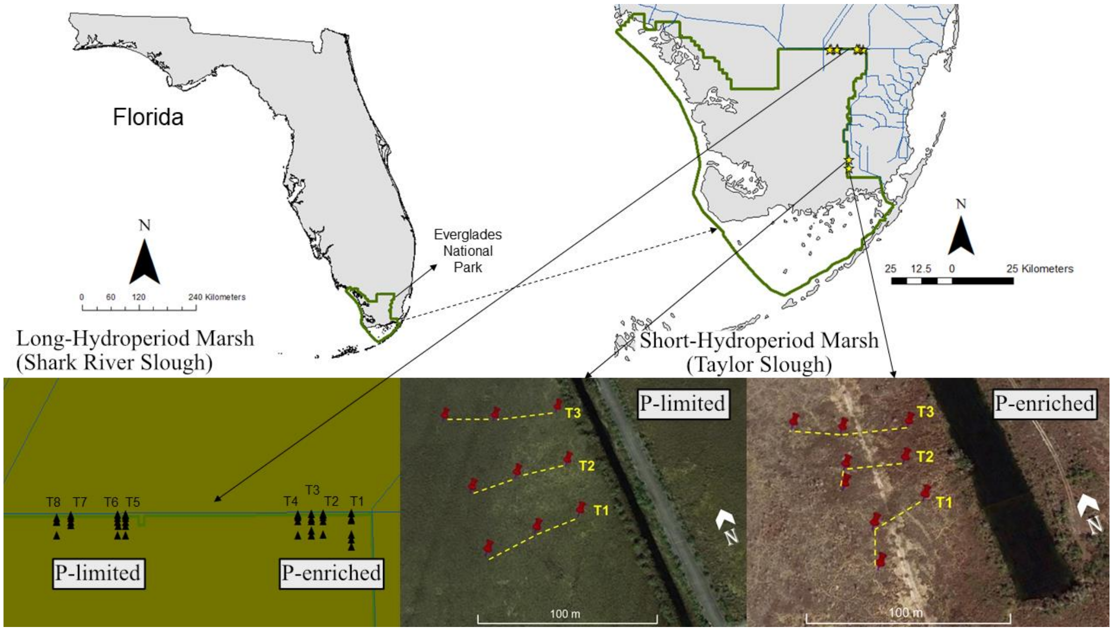

Figure 1. Location of the study sites along the boundaries of the Florida Coastal Everglades (FCE), Everglades National Park (ENP) in South Florida, USA. We sampled along the L29 canal in the long-hydroperiod Shark River Slough drainage and along the L-31W/Aerojet Canal in the short-hydroperiod drainage Taylor Slough.

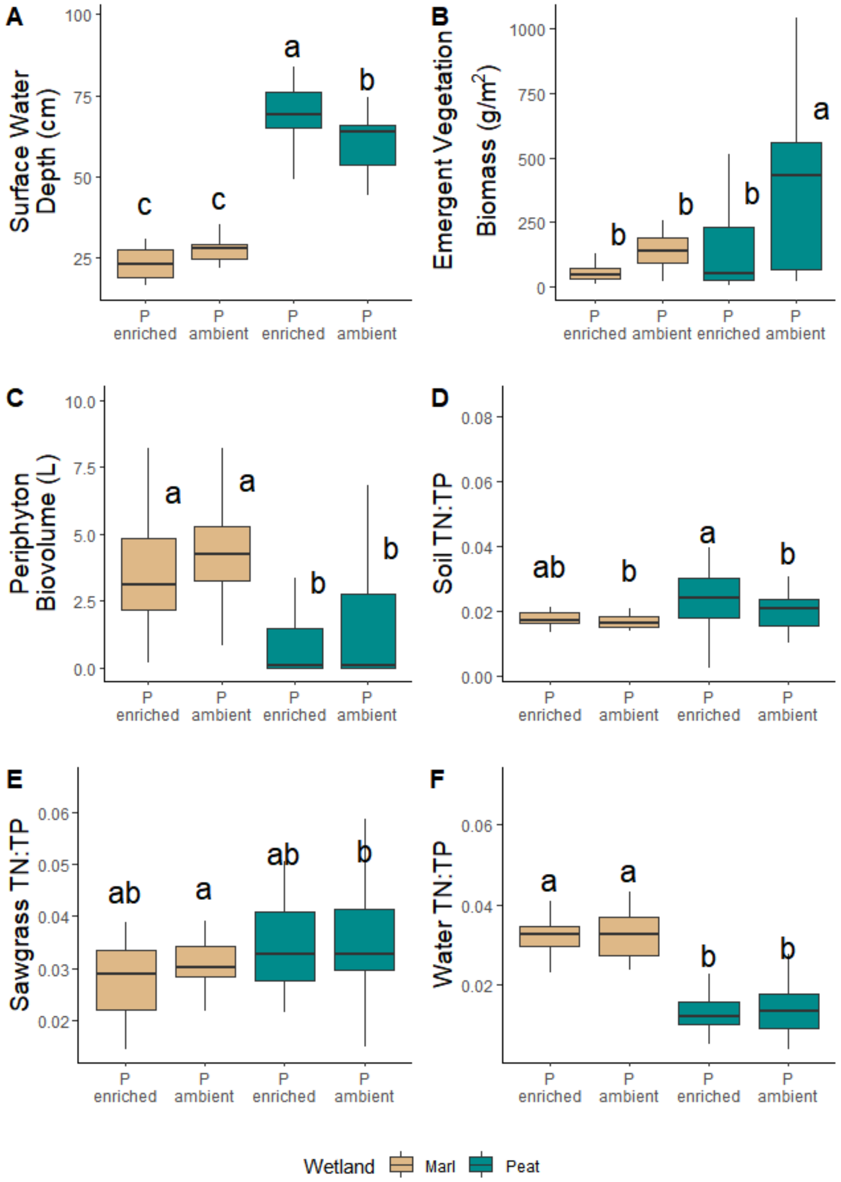

Figure 2. Comparison of hydrology, primary producers, and nutrient availability between marl and peat wetlands, across P-enriched and P-ambient sites in the Everglades (Florida, USA). Boxplots represent the interquartile range for each variable, and the solid line is the median. Error bars represent the 95% confidence intervals. Letters above boxplots indicate significant (alpha ≤ 0.05) differences among groups using one-way ANOVA.

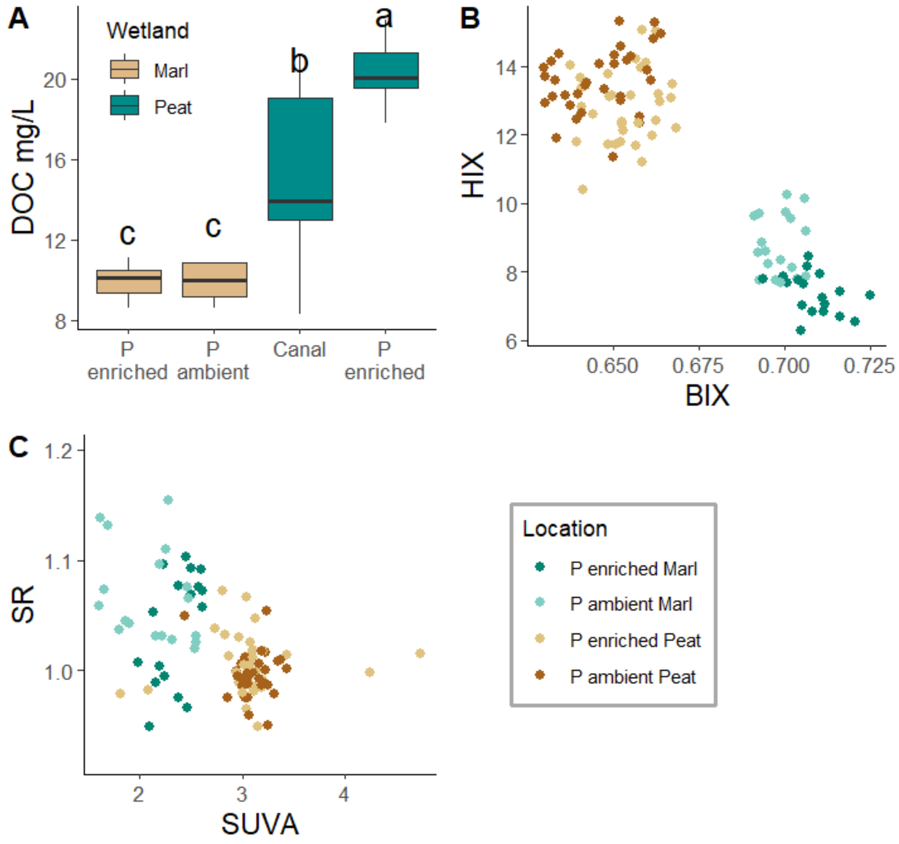

Figure 3. Dissolved organic carbon (DOC) concentrations and dissolved organic matter (DOM) composition across habitats in peat and marl wetlands. A) Humification index (HIX) and biological index (BIX) across ecosystem compartments. Increasing HIX indicates increasing humic influence to DOM, while increase BIX indicates increasing autochthonous influence to DOM. B) Concentrations of DOC across ecosystem compartments. Boxplots represent the interquartile range for each variable; the solid line represents the median. Error bars represent the 95% confidence intervals. Letters above boxplots indicate significant (alpha ≤ 0.05) differences among groups using one-way ANOVA. C) Slope Ratio (SR) and Aromaticity (SUVA) across ecosystem compartments. Increasing S_R indicates decreasing molecular weight, while increasing $SUVA_{254}$ indicates increasing aromaticity.

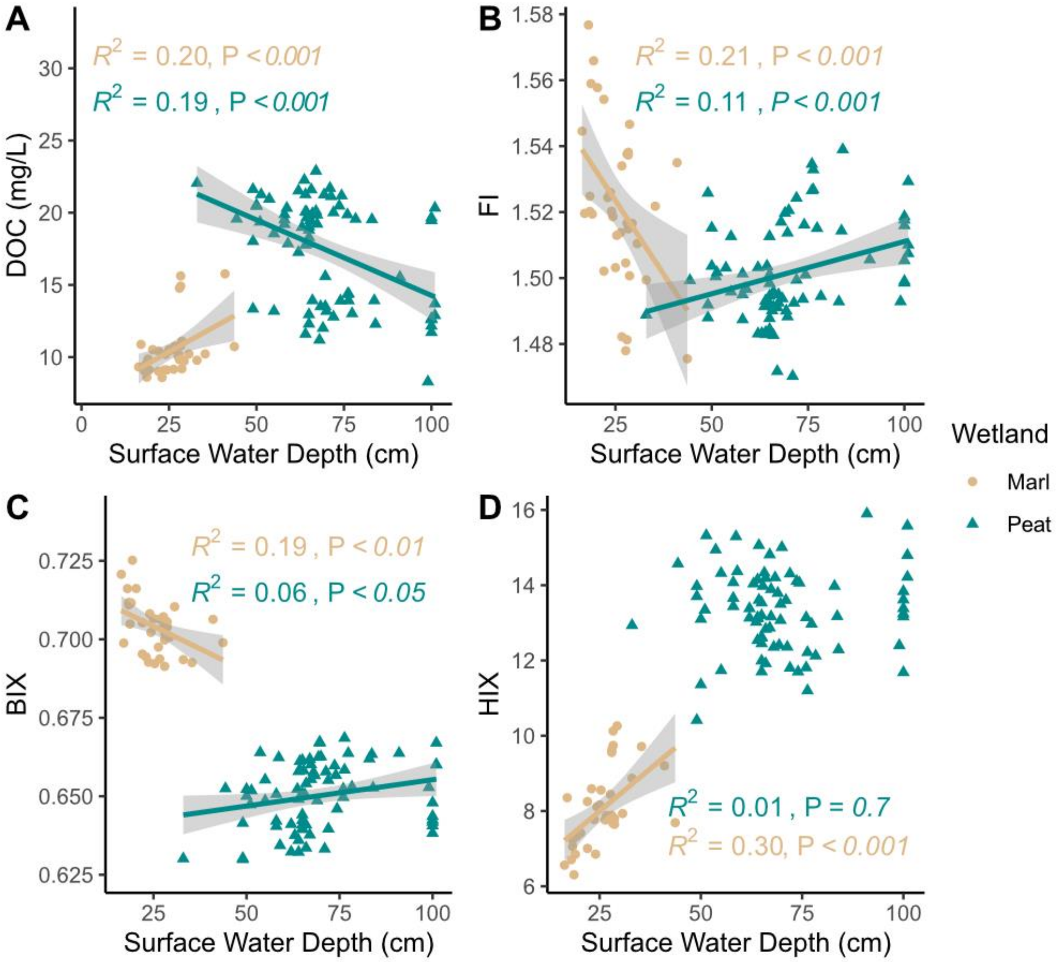

Figure 4. Linear regressions comparing effects of surface water depths on dissolved organic carbon (DOC) concentrations (A), and fluorescence indices of dissolved organic matter (DOM) composition (B-D) between peat and marl wetlands. (B) Increasing FI (Fluorescence Index) indicates increasing microbial processing of DOM. (C) Increasing BIX (Biological Index) indicates increasing autochthonous influence on DOM. (D) Increasing HIX (Humification Index) indicates increasing humic influence to DOM. The shaded area indicates the 95% confidence interval. Data was collected along a series of transects and for each wetlands includes data from areas with both high and low historic impact of restoration water.

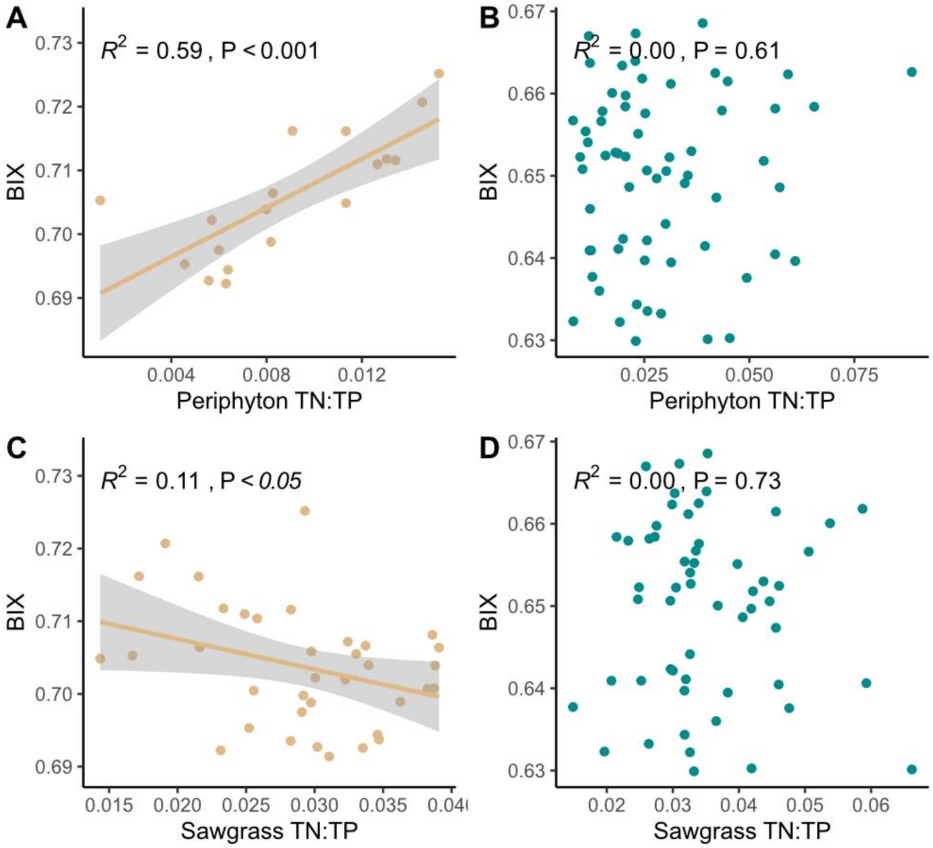

Figure 5. Linear regressions comparing the relationship between BIX (Biological Index) and dominant primary producer nutrient stoichiometric ratios (indicating relative nutrient limitation) of the major primary producers between marl (A and C) and peat (B and D) wetlands. Increasing BIX (Biological Index) indicates increasing autochthonous influence to dissolved organic matter. The shaded area indicates the 95% confidence interval.

Table 1. Average ± standard deviation of dissolved organic carbon (DOC) concentrations and fluorescence indices of dissolved organic matter (DOM) composition across ecosystem compartments and seasons. Indices calculated are FI (Fluorescence index), BIX (Biological Index), HIX (Humification index), SUVA$_{254}$ (Specific Ultraviolet Absorbance at 254 nm wavelength), and S$_{R}$ (Slope Ratio).

		Marl				***Peat***			
		P-enriched		**P-limited**		**P-enriched**		**P-limited**	
Covariate	*Range/Units*	***Dry Season***	***Wet Season***	***Dry Season***	***Wet Season***	***Dry Season***	***Wet Season***	***Dry Season***	***Wet Season***
DOC	mg L^{-1}	9.50 (±0.64)	10.40 (±0.37)	9.59 (±0.86)	12.37 (±2.78)	16.20 (±3.00)	13.65 (±2.99)	20.42 (±0.86)	19.92 (±1.53)
FI	1.2 (terrestrial) - 1.9 (microbial)	1.55 (±0.02)	1.50 (±0.02)	1.52 (±0.01)	1.52 (±0.02)	1.51 (±0.01)	1.51 (±0.02)	1.50 (±0.01)	1.49 (±0.01)
BIX	0.6 (low productivity) - >1 (high autochthonous productivity)	0.71 (±0.01)	0.70 (±0.01)	0.70 (±0.01)	0.70 (±0.01)	0.66 (±0.00)	0.65 (±0.01)	0.66 (±0.01)	0.64 (±0.01)
HIX	2 (mineral soils) - 16 (fulvic acids)	6.94 (±0.37)	7.79 (±0.43)	8.12 (±0.33)	9.42 (±0.77)	13.48 (±1.31)	12.67 (±0.95)	13.99 (±1.06)	13.27 (±0.58)
SUVA$_{254}$	0.5 (fulvic acid) - 5.3 (humic acid)	2.21 (±0.14)	2.60 (±0.25)	2.11 (±0.20)	2.13 (±0.46)	3.04 (±0.12)	3.26 (±0.78)	3.07 (±0.09)	3.14 (±0.22)
S_R	0.7 (blackwater) - 10 (open ocean)	1.00 (±0.05)	1.21 (±0.38)	1.06 (±0.05)	1.07 (±0.04)	1.01 (±0.03)	1.00 (±0.03)	0.99 (±0.01)	1.00 (±0.03)

Table 2. Estimate effect sizes for best fitting models for dissolved organic matter (DOM) metrics [dissolved organic carbon (DOC) concentration; FI (fluorescence index); BIX (Biological index); HIX (Humification index); SUVA$_{254}$ (Specific UV absorbance at 254 nm wavelength); and SR (Slope Ratio)] from A. peat, and B. marl wetlands. Covariates not included in the best model are marked as NA.

A. Peat

	DOC		**FI**		**BIX**		**HIX**		**SUVA$_{254}$**		**SR**	
Covariate	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***
Water Depth	-4.20	< 0.001	2.665	0.010	3.50	< 0.001	-1.50	0.14	1.03	0.31	NA	NA
Periphyton Biovolume	NA	NA	NA	NA	-1.86	0.07	-1.64	0.11	NA	NA	1.68	0.10
Macrophyte Biomass	NA	NA	NA	NA	-0.59	0.56	-0.17	0.86	NA	NA	0.11	0.92
Soil TN:TP	-2.73	0.009	2.83	0.01	-0.73	0.47	NA	NA	NA	NA	1.09	0.28
Water TN:TP	0.307	0.760	1.24	0.22	3.96	< 0.001	NA	NA	NA	NA	1.06	0.30

B. Marl

	DOC		**FI**		**BIX**		**HIX**		**SUVA$_{254}$**		**SR**	
Covariate	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***	**t**	***P***
Water Depth	2.97	0.01	-1.889	0.068	-2.84	0.01	3.40	0.00	-0.99	0.33	NA	NA
Periphyton Biovolume	0.71	0.48	NA	NA	NA	NA	1.58	0.12	NA	NA	NA	NA
Macrophyte Biomass	-1.59	0.12	NA	NA	NA	NA	-0.09	0.93	NA	NA	NA	NA
Soil TN:TP	NA	NA	0.25	0.81	0.497	0.62	NA	NA	-0.18	0.86	1.27	0.21
Water TN:TP	NA	NA	1.25	0.22	2.92	0.01	NA	NA	0.39	0.70	0.07	0.95
Sawgrass TN:TP	NA	NA	-1.10	0.28	-0.06	0.96	NA	NA	1.92	0.06	2.58	0.01

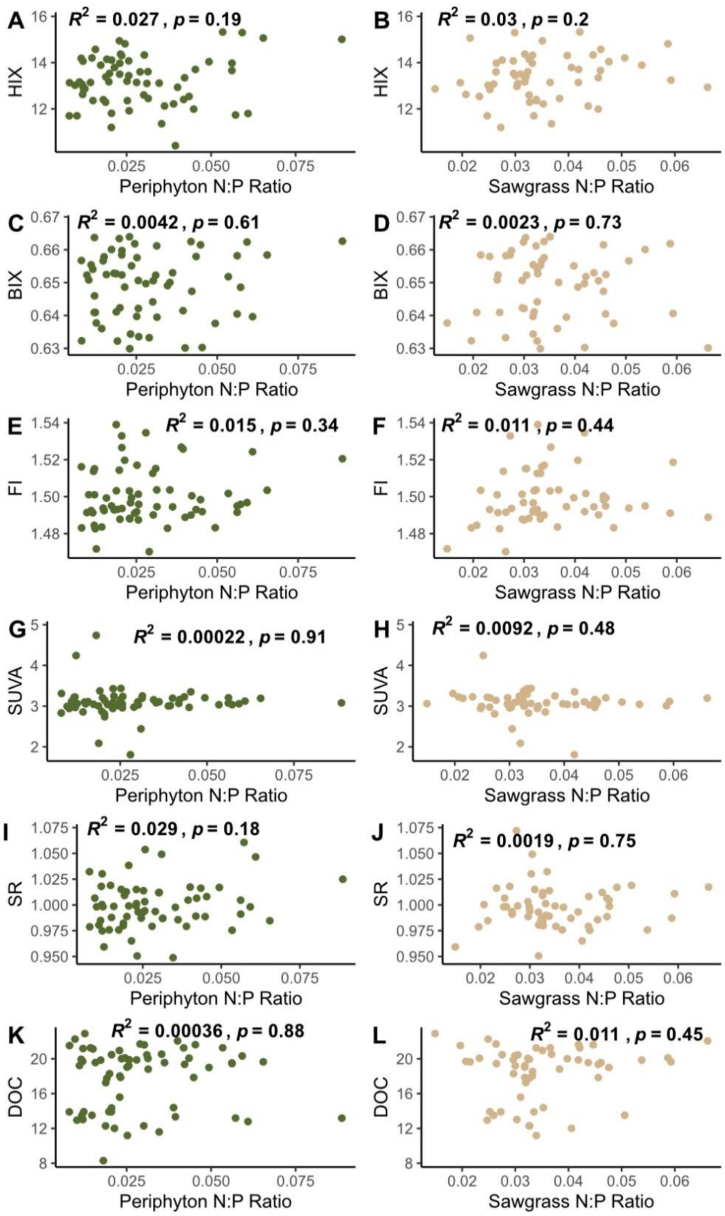

Figure S1. Peat wetland linear relationships between fluorescence indices, and periphyton and sawgrass TN:TP. In the peat wetlands there were no significant relationships between periphyton or sawgrass TN:TP, so we removed them from future modelling processes. Indices calculated are FI (Fluorescence index), BIX (Biological Index), HIX (Humification index), $SUVA_{254}$ (Specific Ultraviolet Absorbance at 254nm), and SR (Slope Ratio).

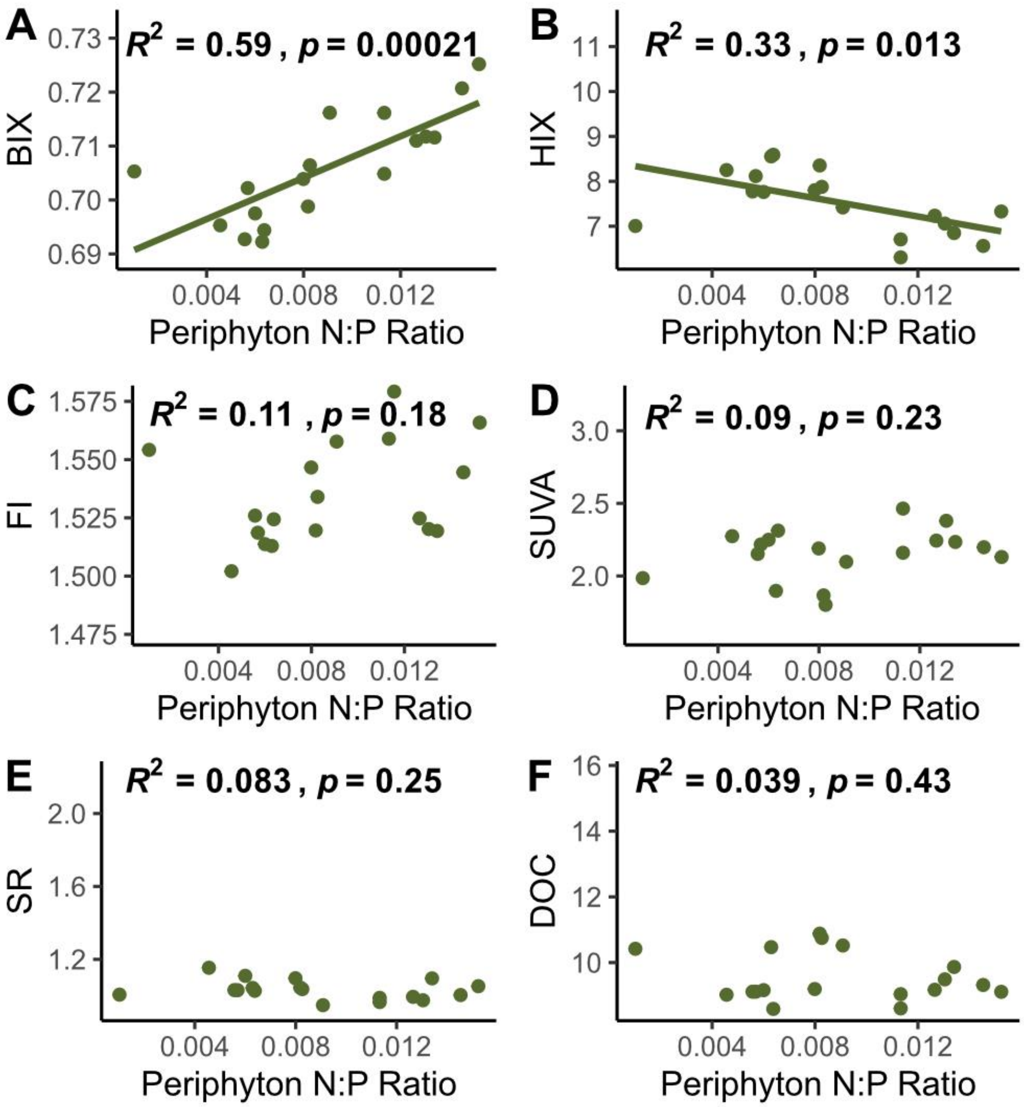

Figure S2. Relationships between marl wetland fluorescence indices and periphyton TN:TP. We found no significant relationships with TN:TP except for BIX and HIX. We removed periphyton from modelling because we were missing data on periphyton TN:TP from one season which would not allow us to fit the models to the same number of observations. Indices calculated are FI (Fluorescence index), BIX (Biological Index), HIX (Humification index), $SUVA_{254}$ (Specific Ultraviolet Absorbance at 254nm), and SR (Slope Ratio).

Table S1. Model selection on factors explaining DOM metrics: DOC concentration, FI (fluorescence index), BIX (Biological index), HIX (Humification index), SUVA (Specific UV absorbance), and SR (Slope Ratio). Factors are separated into hydrology (water depth; H), Primary producers (total macrophyte biomass and periphyton biovolume; P), Nutrient limitation (Soil and water N:P ratio; N), and combinations of the three categories (Full). We calculated Akaike's Information Criterion (AIC_c) and log-likelihood for each model and selected the best-fitting model as the one with the highest adjusted R^2 score that has a ΔAIC less than or equal to 2.

A. DOC

	Peat						**Marl**				
Model	**df**	**AIC_c**	**$\Delta\ AIC_c$**	**logLik**	**R^2**	***Model***	**df**	**AIC_c**	**$\Delta\ AIC_c$**	**logLik**	**R^2**
H+N	5	308.28	0.00	-149.14	0.29	*H+P*	5	144.75	1.23	-67.37	0.26
Full	7	310.49	2.20	-148.24	0.31	*H*	3	143.51	0.00	-68.76	0.20
H	3	311.76	3.47	-152.88	0.19	*H+N*	6	148.66	5.15	-68.33	0.22
H+P	5	312.20	3.92	-151.10	0.24	*Null*	2	149.59	6.08	-66.88	0
P	4	318.02	9.74	-155.01	0.13	*Full*	8	149.77	6.26	-66.89	0.28
P+N	6	319.16	10.88	-153.58	0.17	*N*	5	151.49	7.98	-70.75	0.11
Null	2	322.65	14.37	-159.32	0.00	*P*	4	151.51	8.00	-71.76	0.06
N	4	322.72	14.44	-157.36	0.06	*P+N*	6	152.04	8.53	-70.02	0.14

B. FI

	Peat						**Marl**				
Model	**df**	**AIC_c**	**$\Delta\ AIC_c$**	**logLik**	**R^2**	***Model***	**df**	**AIC_c**	**$\Delta\ AIC_c$**	**logLik**	**R^2**
Full	7	-337.58	1.27	175.79	0.24	*N*	5	-167.45	1.59	88.72	0.26
H+N	5	-338.85	0.00	174.42	0.20	*H*	3	-169.04	0.00	87.52	0.21
H	5	-336.14	2.72	173.06	0.17	*H+N*	6	-167.05	2.00	89.52	0.29
H+P	4	-336.12	2.73	172.06	0.14	*Full*	8	-166.39	2.65	91.20	0.35
P	6	-335.13	3.72	173.56	0.18	*P+N*	5	-165.12	3.92	87.56	0.21
P+N	4	-333.69	5.16	170.84	0.10	*P*	6	-164.04	5.00	89.02	0.27
N	3	-332.34	6.51	169.16	0.05	*Null*	2	-162.64	6.40	83.32	0.00

Null	2	-331.22	7.63	167.61	0.00	*H+P*	4	-161.39	7.65	84.69	0.07

C. BIX

	Peat						**Marl**				
Model	**df**	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**	***Model***	**df**	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**
Full	7	-395.09	0.19	204.54	0.38	*H+N*	6	-249.48	0.00	130.74	0.38
H+N	5	-395.28	0.00	202.64	0.34	*Full*	8	-247.37	2.11	131.68	0.41
P+N	6	-384.80	10.48	198.39	0.24	*P+N*	6	-242.36	7.11	128.18	0.28
H+P	5	-383.52	11.76	196.75	0.20	*H+P*	5	-246.07	3.41	128.03	0.28
N	4	-382.68	12.60	195.33	0.16	*N*	5	-243.13	6.34	126.57	0.22
H	3	-381.01	14.27	193.50	0.11	*H*	3	-245.91	3.57	125.95	0.19
P	4	-378.26	17.02	193.13	0.10	*P*	4	-241.95	7.53	124.98	0.14
Null	2	-376.20	19.08	190.10	0.00	*Null*	2	-240.39	9.09	122.19	0.00

D. HIX

	Peat						**Marl**				
Model	**df**	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**	***Model***	**df**	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**
H+P	5	184.32	0.98	-87.16	0.10	*H+P*	5	97.46	1.01	-43.73	0.35
H	3	183.34	0.00	-88.66	0.06	*H*	3	96.45	0.00	-45.22	0.30
P	4	184.67	1.33	-88.33	0.07	*P*	4	106.58	10.13	-49.29	0.12
Null	2	184.92	1.58	-90.46	0.00	*H+N*	6	101.47	5.03	-44.74	0.31
H+N	5	184.96	1.63	-88.48	0.06	*Full*	8	102.78	6.33	-43.39	0.36
Full	7	185.61	2.27	-85.80	0.14	*N*	5	105.27	8.83	-47.64	0.19
N	4	185.73	2.39	-88.86	0.05	*P+N*	6	105.49	9.04	-45.74	0.27
P+N	6	186.17	2.84	-87.08	0.11	*Null*	2	107.05	10.07	-51.52	0.00

E. SUVA

						Marl				
	Peat	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**	***Model***	**df**	**AIC_c**	**Δ**	**logLik**	**R^2**

									AIC_c		
Model	**df**	54.15	0.00	-23.72	0.00	*Null*	3	28.31	0.00	-12.15	0.00
Null	2	55.06	0.91	-24.53	0.02	*H*	3	30.25	1.94	-12.13	0.00
H	3	57.40	1.25	-24.69	0.01	*P*	4	30.87	2.56	-11.43	0.04
P	4	58.04	2.07	-24.02	0.03	*H+P*	5	32.75	4.44	-11.37	0.04
H+P	5	58.13	2.15	-25.06	0.00	*N*	5	31.30	2.99	-10.65	0.08
N	4	58.94	2.97	-24.46	0.02	*H+N*	6	32.18	3.87	-10.09	0.11
H+N	5	61.27	5.30	-24.63	0.01	*P+N*	6	33.43	5.12	-9.72	0.13
P+N	6	61.46	5.48	-23.72	0.04	*Full*	8	34.63	6.32	-9.31	0.15
Full	7										

F. SR							**Marl**				
	Peat	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**	***Model***	**df**	**AIC_c**	**Δ AIC_c**	**logLik**	**R^2**
Model	**df**	-271.58	0.00	137.79	0.00	*N*	5	-11.99	0.00	11.00	0.19
Null	2	-269.72	1.86	137.85	0.00	*H+N*	6	-11.22	0.77	11.61	0.21
H	3	-269.34	2.24	138.67	0.03	*Null*	2	-10.54	1.45	7.27	0.00
P	4	-268.58	3.00	138.28	0.02	*P+N*	6	-9.27	2.72	11.64	0.22
N	4	-268.08	3.50	140.04	0.07	*H+N*	3	-8.72	3.28	7.36	0.00
P+N	6	-267.35	4.23	138.67	0.03	*Full*	8	-8.11	3.89	12.05	0.23
H+P	5	-267.05	4.53	138.52	0.02	*P*	4	-7.83	4.16	7.92	0.04
H+N	5	-266.48	5.06	140.24	0.08	*H+P*	5	-6.14	5.86	8.07	0.04
Full	7										

CHAPTER V

SHIFTING SOURCES AND FATES OF CARBON WITH INCREASING HYDROLOGIC PRESSES AND PULSES IN COASTAL WETLANDS

ABSTRACT

Coastal ecosystems are rapidly shifting due to changes in hydrologic presses (e.g., sea-level rise) and pulses (e.g., seasonal hydrology, disturbances, and restoration of degraded wetlands). Changing water depths can alter subsidies and stressors to coastal ecosystems, and responses likely depend on relative changes in dissolved organic carbon (DOC) concentrations and dissolved organic matter (DOM) composition. We analyzed 20 years of surface water biogeochemistry data from peat and marl wetlands (marshes, ecotones, mangroves) of the Florida Everglades to understand how changing hydrologic drivers are affecting DOC and DOM. Both water depths and total phosphorus concentrations increased throughout the Everglades, but total nitrogen and bacterioplankton productivities were variable among sites. Dissolved organic carbon concentrations decreased with water depths in peat marshes and increased with water depths in marl marshes and across mangroves. Sources of DOM shift seasonally from algal to detrital sources in freshwater marshes, from detrital marsh to detrital mangrove sources in the ecotone, and from detrital mangrove to algal marine sources in downstream mangroves. As climate change and anthropogenic drivers alter water levels in coastal wetlands, integrating spatial and temporal measurements of DOC concentrations and DOM compositions is essential to better budget the production and export of carbon across ecosystems.

INTRODUCTION

The importance of presses and pulses as drivers of ecosystem structure and function has a long history in the field of ecology (Clements 1916; Connell 1978; Pulsford et al.

2016; Jentsch and White 2019). Pulses are defined as abrupt changes in ecological parameters (abiotic or biotic) that alter ecosystem dynamics and vary in frequency, magnitude, and duration (Jentsch and White 2019). Studying the interaction between duration and magnitude of pulses is important for understanding their long-term effects (Ratajczak et al. 2017). The frequency, magnitude, and duration of pulses determine if they are a usable subsidy or whether at a certain threshold they will transition to being a stressor to the system (Odum et al. 1979).

Sea-level rise and its associated subsidies and stressors (including nutrients and salinity) present a fundamental threat to coastal wetland ecosystems due to a persistent press of marine water. These subsidies and stressors, acting on ecosystems that in many cases are already degraded, have the potential to rapidly change important role of coastal wetlands in carbon storage (Mcleod et al. 2011; Pendleton et al. 2012). The combination of climate change–driven sea-level rise and restoration of degraded coastal wetlands is driving a series of hydrologic presses and pulses, and understanding how these pulses are affecting the production, processing, and storage of carbon is needed to protect coastal waters (Harris et al. 2018). Coastal waters are unique in that they receive hydrologic pulses from both fresh and marine water. Fresh and marine water pulses have distinct biogeochemical signatures that allow for the use of chemical tracers to understand their relative contributions to ecosystem carbon transfer (Odum et al. 1979; Jaffé et al. 2004; Hobbs et al. 2007; Smith et al. 2023). Many aquatic ecosystems are highly pulse-driven but have been engineered to be less so, leading to recent efforts to restore aquatic ecosystems, especially coastal wetlands (Poff et al. 2007).

Restoration of aquatic ecosystems often aims to reestablish the original hydrologic regime of a system, which typically increases water depths and connectivity between ecosystem compartments and can have significant impacts on the productivity of the ecosystem (Junk et al. 1989; Sarker et al. 2020). Changes to the pulse dynamics of ecosystems can alter the synchrony of nutrient and carbon dynamics across systems, either increasing or decreasing synchrony between compartments (Kominoski et al. 2020). This can occur at multiple scales including local scales with fluxes between sediments and the water column or regional scale with fluxes between connected regions. The press of sea-level rise often has unforeseen functional impacts on coastal ecosystems, like transporting nutrients and organic matter which drive landscape scale processing and storage of carbon (Saha et al. 2011; Wilson et al. 2019). At the same time, restoration of degraded systems is increasing water depths and reducing light attenuation and oxygen availability to the benthos, all of which change relative contributions and production of autochthonous and allochthonous carbon (Cory et al. 2014; Howard-Parker et al. 2020). This combination of changes in carbon production and connectivity from both marine and terrestrial sources is dependent on the specific patterns of pulses and presses affecting coastal wetland ecosystems, and the management of these systems relies on a clearer understanding of how pulses are driving changes to carbon processing in coastal wetlands.

Dissolved organic matter (DOM) connects terrestrial carbon with aquatic ecosystems and is an important component of the hydrologic and carbon cycles (Battin et al. 2009). After being produced in terrestrial systems, DOM is transported through aquatic ecosystems where it is responsible for a variety of biogeochemical processes, including regulating nutrient cycling, light attenuation, and controlling the microbial loop (Amon and Benner 1996; Qualls and Richardson 2003). The composition of DOM is determined by its source and determines its availability as a substrate for microbes (Medeiros et al. 2017). The composition of DOM, which varies by carbon source, can alter bacterial processing of organic matter and be indicate by DOM fluorescence (Jaffé et al. 2004; Osburn et al. 2019a). Pulse events can rapidly change the composition of DOM, and discrete events such as hurricanes have been shown to modify regional carbon cycling for months following pulse events, leading to large functional shifts in microbial processing of DOM in disturbed areas (Osburn et al. 2019b). Changing water depths, in response to both pulse events and hydrologic presses, play a strong role in controlling the transport and production of DOM in coastal wetlands, but it is unclear how that relationship is responding to the combination of hydrologic changes caused by restoration and sea-level rise (Regier et al. 2020).

Our main goal in this study is to understand the interactions between hydrology, nutrients, salinity, bacterioplankton, and organic matter to better predict how freshwater restoration projects interact with the press of sea-level rise and how that has affected the composition and concentration of dissolved organic matter in Everglades wetlands. Here, we used long-term DOM fluorescence, bacterioplankton productivities, and concentrations of dissolved organic carbon (DOC), total nitrogen, and total phosphorus collected along two coastal wetland gradients in the Florida Everglades, that have different carbon sources, to better understand how increasing water depths from upstream restoration and downstream sea-level rise are altering production, processing, and transport of DOC. Nutrient concentrations and bacterioplankton productivity are becoming more synchronized in the Florida Coastal Everglades, but DOC concentrations are not (Kominoski et al. 2020). Dissolved organic carbon instead is controlled by location and sources of carbon between peat and marl wetlands and along the freshwater to marine gradient, but we still do not fully understand the drivers of DOC concentration and DOM composition as these ecosystems are being repeatedly affected by changes to both their freshwater and marine inputs (Regier et al. 2020).

We asked two major questions: 1) How does spatiotemporal variability in water depth affect DOC concentrations and DOM composition along gradients of phosphorus and salinity in peat and marl coastal wetlands? 2) How do changes in DOC concentrations and DOM composition interact with bacterioplankton productivities along phosphorus and salinity gradients in peat and marl coastal wetlands? We used time-series analyses and explorations of the fluorescent properties of carbon to test how hydrologic pulses of fresh and salt water, and their associated resource subsidies and stressors, are altering the sources, production, processing, and movement of carbon in the Florida Coastal Everglades. We utilized 20 years of data to investigate the relationship between water depth and DOC concentrations at endmember freshwater marshes and mangrove wetlands in higher and lower productivity peat and marl drainages. We then used 10 years of data to understand how the composition of DOM varies with water depth across freshwater, ecotone, and mangrove ecosystems in higher and lower productivity ecosystems. We hypothesized that increases in water depth in freshwater marshes would increase allochthonous DOM and decrease autochthonous DOM, which would then be mobilized to ecotone and marine sites. We predicted that the amount of DOM exported would be determined by the extent of seasonal increases in allochthonous phosphorus, salinity, and water from marine sources in the ecotone and mangroves. Finally, we predicted that seasonal increases in marine-derived allochthonous DOM would drive increases in bacterioplankton productivity.

METHODS

Site Description and Experimental Design

We analyzed long-term data from the Florida Coastal Everglades, an International Biosphere Reserve, World Heritage Site, and Ramsar Wetland of International Importance. The Everglades ranges from Lake Okeechobee in central Florida to Florida Bay at the southern tip of the state. It consists of a series of highly oligotrophic, diverse, and heterogeneous wetlands, with variation in hydrology, productivity, and relative nutrient limitation (Noe et al. 2001; Castañeda-Moya et al. 2013). The hydrology of the Everglades was radically altered starting in the early 1900s with the construction of drainage canals which created agricultural and inundated areas (Light and Dineen 1994). Current restoration is focused on restoring sheet flow across the Everglades during the wet season, when the area receives over 70% of its total rainfall. As part of restoration efforts, phosphorus from upstream farming has been removed from water coming into Everglades National Park by a series of water management areas. However, freshwater restoration appears to be mobilizing stores of legacy phosphorus from hotspots in degraded wetlands, leading to increasing phosphorus concentrations where restorative freshwater is entering the system (Sarker et al. 2020). Phosphorus also enters the system from marine water; phosphorus in Florida Bay and the Gulf of Mexico are extremely low in concentration (0.25–0.65 μmol/L; Fourqurean and Zieman 2002), but they still have slightly higher concentrations than the extremely oligotrophic coastal mangroves and inland freshwater wetlands. This dynamic means that pulses of both fresh and marine water leads to increasing phosphorus delivery to interior ecosystems of the Everglades, making it an ideal location to study how those pulses can change biogeochemical cycling.

We analyzed data from the FCE-LTER sampling sites within the two major drainages of the Everglades: Shark River Slough (SRS) and Taylor Slough (TS/Ph). We focused our analysis on six sites: one freshwater, one ecotone, and one mangrove environment for each slough (with the best data coverage for each type): SRS-2, SRS-4, SRS-6, TS/Ph-2, TS/Ph-3, TS/Ph-7 (Figure 1). We also included data from the full range of inland FCE-LTER sites (SRS 1–6, TS/PH 1–7). Shark River Slough is a wide, deep, and higher-productivity drainage of long-hydroperiod wetlands (inundated for >9 months of the year), which transition from sawgrass-dominated, ridge and slough peat marshes to tidal riverine mangrove forests connecting to the Gulf of Mexico (Childers et al. 2006; Castañeda-Moya et al. 2013). Taylor Slough is a smaller, shallower, and lower-productivity drainage of short-hydroperiod wetlands (inundated for <9 months of the year), which transition from sawgrass- and periphyton-dominated marl prairies to microtidal scrub mangrove forests draining into the shallow Florida Bay seagrass estuary. Productivity in Taylor Slough is highest in the ecotone, where marine groundwater upwelling brings subsidies of phosphorus inland. When moving from freshwater to marine ecosystems in Shark River Slough, DOC decreases in concentration and becomes less influenced by decaying organic matter (humic) and more microbially derived. Taylor Slough has very low concentrations of DOC in the freshwater and derives most of its

DOM from the mangroves, becoming more humic as mangrove densities increase (Kominoski et al., 2020; Regier et al., 2020).

History of Disturbance and Restoration

The Everglades has experienced a series of important disturbances and restoration efforts over the past 20 years while the FCE-LTER has monitored the Everglades. Both Everglades drainages have been impacted by major hurricanes (2005, 2017), cold snaps (2010, 2011), fire (2008), drought (2010, 2015, 2021), and flooding (2015). In terms of restoration, the Comprehensive Everglades Restoration Plan (CERP) was authorized by the U.S. Congress in 2000 as a multi-agency plan and is one of the biggest restoration efforts in the world. Major aspects of this plan have been implemented, especially in the past 10 years. A series of joint plans, including the Combined Operational Plan (COP) and the Modified Water Deliveries (MOD) plan, have been working to create new canals, reservoirs, and culverts to move water into and through Everglades National Park. In 2012, a series of canals and pumping stations, including the C-111 and Aerojet canals, were constructed to transport more water into Taylor Slough. A year later in 2013, a 1.6-km bridge was constructed along Tamiami Trail to allow more water to enter Shark River Slough. In 2015 and 2017, flow was increased (under the Combined Operational Plan Test Phase I and II; USACE 2020) to transport water from outside of the park to both Shark River and Taylor Slough. In 2019, another 3.7-km bridge was completed along Tamiami Trail, and the combination of the two bridges increased the total water entering Everglades National Park by nearly 80% (McLean 2015).

Surface Water Physicochemical Properties

Average monthly measurements of surface water depth were collected from 2001–2021 at water level stations for marsh (Kominoski et al. 2022; Troxler and Childers 2022) and mangrove sites (Castañeda-Moya and Rivera-Monroy 2021). Any data gaps were filled by auxiliary data gathered by the U.S. Geological Survey Everglades Depth Estimation Network (EDEN) at the closest station to the focal site collected (Shark River Slough [NP201, P36, MO-215, TE, Gunboat Island, SH3]; Taylor Slough/Panhandle [NTS1, TSB, E146, UTR, TRM]). We created linear models between water depth and EDEN water level and used those models to predict water depth where there were data gaps (USGS/EDEN 2018). The linear model fit and the percentage of data gaps filled by this method for each site was as follows: SRS-2 (R^2=0.89, 11%), SRS-4 (R^2=0.52, 24%), SRS-6 (R^2=0.47, 15%), TS/Ph-2 (R^2=0.76, 1%), TS/Ph-3 (R^2=0.59, 3%), TS/Ph-7 (R^2=0.73, 54%).

Monthly surface water grab samples were collected from 2001–2021 at all sites for DOC, total nitrogen, total phosphorus, and salinity concentrations (Briceño 2020, Gaiser and Childers 2021, Troxler 2021, Troxler and Childers 2021). Total phosphorus was measured following the method of Solórzano and Sharp (1980). Total nitrogen was measured using an Antek total nitrogen analyzer (Antek Instruments, Houston, Texas, USA). Dissolved organic carbon concentrations were measured using filtered water samples (0.7-µm GF/F filters; Whatman, Maidstone, UK) with a Shimadzu TOC Analyzer (Shimadzu Corporation, Columbia, Maryland, USA). All water chemistry analyses were conducted by the CREST CAChE Nutrient Core Facility, which is NELAC Certified for non-potable water-General Chemistry under State Lab ID E76930. Bacterial productivity from monthly grab samples at each site was measured as the uptake of tritiated thymidine (Briceño 2018). Fluorescence spectroscopy was performed on filtered samples from 2011–2021 at our 6 focal sites (SRS-2, SRS-4, SRS-6, TS/Ph-2, TS/Ph-3, TS/Ph-7) and from 2019–2021 at the remaining sites (SRS-1, SRS-4, SRS-5, TS/Ph-1, T/Ph-6) to determine the chemical composition of the dissolved organic matter (DOM) of each sample. Fluorescence excitation-emission matrices (EEMs) were measured on a Horiba Aqualog (Jobin Yvon Horiba, France). Samples were measured at room temperature at 3 nm wavelengths over excitation wavelength (λex) intervals between 240 and 455 nm and an emission wavelength (λem) range of λex + 10 nm to λex + 250 nm in a 1 cm quartz cuvette. EEMs were corrected for inner filtering effect, Raman normalized, and blank subtracted using in-house processing codes written in MATLAB R2019a (Mathworks, Natick, MA, USA).

Data Analyses

DOM fluorescence was processed using the drEEM 3.0 toolbox in MATLAB R2019a to calculate five common metrics of DOM fluorescence: Fluorescence Index (FI; McKnight et al. 2001), Humification index (HIX; Zsolnay et al. 1999), Biological Index (BIX; Huguet et al. 2009), Specific Absorbance at 254 ($SUVA_{254}$; Weishaar et al. 2003), Slope ratio (S_R; Helms et al. 2008).

To analyze changes to the composition of DOM, we performed a Principal Component Analysis (PCA) for each site in R. We created principal components of DOM using concentrations of DOC, total nitrogen, and total phosphorus, as well as fluorescence measures FI, HIX, BIX, $SUVA_{254}$, and S_R. Total nitrogen and total phosphorus are both measures of total unfiltered nutrients, but we grouped them with fluorescence measures; Everglades phosphorus is so rapidly taken up that concentrations of soluble reactive phosphorus are frequently undetectable. Total nitrogen and total phosphorus give a more reliable measure of nutrients available in the water column. All variables were centered and standardized to the same scale before the creation of principal components using the scale function in R.

To understand both seasonal and pulse dynamics of DOC, water depth, and bacterioplankton productivity, we computed both single and cross-wavelet power spectra by applying the Morlet wavelet, using the package WaveletComp in R (Roesch and Schmidbauer 2022). We performed these analyses at freshwater and mangrove endmember ecosystems for Shark River and Taylor Slough (SRS-2: peat freshwater, SRS-6: peat riverine mangrove, TS/Ph-2: marl freshwater, TS/Ph-7: marl shrub mangrove). Wavelet analysis requires continuous data, so we gap-filled any missing values for DOC and bacterioplankton productivity with the median value for the site to provide the most conservative estimate for a specific date. The percent data gap filled for DOC at each site is as follows: SRS-2 (6%), SRS-6 (1%), TS/Ph-2 (25%), TS/Ph-7 (3%). The percent data gap filled for bacterioplankton productivity at each site is as follows: SRS-2 (12%), SRS-6 (8%), TS/Ph-2 (32%), TS/Ph-7 (17%). We used cross-wavelet power spectra to analyze the coherence and time-lags between DOC and water depth over time, and we used single wavelet power spectra to understand how temporal bacterioplankton productivity has shifted over the past 20 years. We estimated the significance level of wavelet coherence using the Monte Carlo permutation test. For a more in-depth description of the use of wavelet analysis in ecological research, see Cazelles et al. (2008).

We constructed linear models to track the relationships between biogeochemical covariates, both for use independently and in comparison, and the wavelet analysis methods. All analyses were performed using R version 4.2.0, and all plots were constructed using the ggplot2 package (Wickham 2009; R Core Team 2022).

RESULTS

Biogeochemistry Along Coastal Wetland Gradients

Over the past 20 years, water depth increased at all sites except for TS/Ph2 (however discharge has increased there), total phosphorus increased at all sites except for TS/Ph7, and microbially processed DOM (FI) decreased at all sites (Table 1; Figure S1). In riverine mangroves, molecular weight of DOM decreased. In Shark River Slough freshwater peat marshes, humic contributions to DOM (HIX, $SUVA_{254}$) increased, while algal contributions (BIX) decreased (Table 1). In contrast, detrital contributions to DOM (HIX) in Taylor Slough freshwater marl marshes decreased (Table 1). In the marl ecotone marsh (TS/Ph-3), algal contributions to DOM (BIX) decreased, while the aromaticity of DOM increased ($SUVA_{254}$; Table 1).

Total phosphorus increased across the spatial gradient from freshwater to marine, with freshwater sites ranging from 0.26 µmol/L in Taylor Slough marl marshes to 0.37 µmol/L in Shark River Slough peat marshes and mangrove sites ranging from 0.40 µmol/L in Taylor Slough shrub mangroves to 0.71 µmol/L in Shark River Slough riverine mangroves (Table S1). Total nitrogen varied by drainage, with freshwater sites ranging from 34.50 µmol/L in Taylor Slough marl marshes to 70.34 µmol/L in Shark River Slough peat marshes and mangrove sites ranging from 51.15 µmol/L in Taylor Slough shrub mangroves to 34.40 µmol/L in Shark River Slough riverine mangroves (Table S1). Dissolved organic carbon followed opposite patterns in Shark River Slough and Taylor Slough; peat marshes had high concentrations (1600 µmol/L) that decreased by nearly half (814 µmol/L) closer to the coast. Dissolved organic carbon concentrations in Taylor Slough are lower in the marsh (607 µmol/L) and increased at the coast (1022 µmol/L; Table S1).

Dissolved organic matter (DOM) was highly humic in Shark River Slough, with HIX ranging from an average of 12.12 in the marsh to 12.41 in the riverine mangroves. In Taylor Slough, DOM was only moderately humic with HIX ranging from 8.35 in the marsh to 6.73 in the shrub mangroves. Algal contributions to DOM (BIX) were very low in Shark River Slough, with averages from 0.64 to 0.65, and only somewhat higher in Taylor Slough, with averages from 0.72 to 0.74. Aromaticity ($SUVA_{254}$) was higher in Shark River Slough and increased from marsh to mangroves, with an average of 2.85 in the marsh to 3.37 in the riverine mangroves. Taylor Slough was lower in aromaticity and did not vary across the slough, with averages from 2.58 in the marsh to 2.59 in the shrub mangroves. The molecular weight of DOM (S_R) was higher in Shark River Slough, ranging from an average of 0.97 in the marsh to 1.09 in the riverine mangroves, while Taylor Slough ranged from an average of 1.07 in the marsh to 1.23 in the shrub mangroves. Microbial contributions to DOM (FI) were even across Shark River Slough, ranging from 1.50 in the marsh to 1.52 in the mangroves, and were higher in Taylor Slough, where they decreased from 1.58 in the marsh to 1.53 in the mangroves.

We found seasonal shifts in DOM composition which appear to be driven by changing water sources. In Shark River Slough peat wetlands, wet-season DOM became more peat derived (HIX, $SUVA_{254}$) but lower in concentration, while in the dry season microbial and algal influence becomes more common and concentrations increase (BIX, FI; Figure 3). In Taylor Slough marl wetlands, freshwater upstream inputs increased concentrations of DOC and nutrient loading in the wet season, but increased in microbial processing (FI) in the dry season. At both Shark River Slough peat ($R^2 = 0.16$, $P < 0.001$) and Taylor Slough marl ($R^2 = 0.57$, $P < 0.001$) ecotone sites, HIX significantly increased with salinity, but there was no relationship at downstream mangroves (Figure 4). To better understand carbon movements in the ecotone we used the full complement of 11 FCE-LTER sites and found that salinity had a significant relationship with BIX at the most downstream mangroves at both Shark River Slough peat ($R^2 = 0.21$, $P < 0.001$) and Taylor Slough marl ($R^2 = 0.38$, $P < 0.001$) sites and at the upstream ecotone in the peat ($R^2 = 0.06$, $P < 0.05$), but not in the middle mangroves (SRS-5; Figure 4). At the Shark River Slough peat ecotone marsh and both riverine and shrub mangroves, we detected seasonal shifts in freshwater and marine water signals of DOM, whereby the freshwater signal (HIX, $SUVA_{254}$) was more dominant in the wet season and the marine signal (FI, BIX) was more prevalent in the dry season (Figure S2).

Water Depth as a Driver of DOC and DOM

Water depth was strongly negatively correlated with DOC concentration in all freshwater sites except TS/Ph-1 (Figure 5). There was no correlation between water depth and DOC concentration in Shark River Slough mangrove sites; however, in TS/Ph-7, there was a strong positive correlation between DOC concentration and water depth

(Figure 5). There was a strong negative correlation between water depth and BIX across Shark River Slough freshwater marshes and both Shark River Slough and Taylor Slough mangroves, as well as a positive correlation between water depth and HIX at Shark River Slough freshwater and Taylor Slough mangroves (Figure 5).

Cross-wavelet power spectra analysis was conducted to better understand both how correlation between DOC and water depth change over time, as well as the phase differences and time-lags between variables. We performed our analysis at freshwater and mangrove ecosystem endmembers for both high and low productivity wetlands. We chose to focus on the endmember sites as the sites that are furthest up and downstream, which are the most influenced by incoming pulses of fresh or marine water, respectively. Linear models of the relationship between water depth and DOC concentration grouped by ecosystem type (marsh vs. mangrove), justifying the selection of representative sites with the strongest relationships and the most data coverage. Significant wavelet power at a certain timepoint indicates that both variables were simultaneously changing at that time. Arrows on wavelet plots indicate the direction of the relationship between the two variables. Cross-wavelet power spectra showed significant wavelet power at the 12-month period (indicating that both DOC and water depth are going through an annual cycle at the given point in time where the 12-month period is significant) for all sites, but only Shark River Slough freshwater marsh and Taylor Slough scrub mangroves had significant wavelet power across the entire time series of 20 years. At mangrove sites, DOC concentration and water depth were largely in phase; at Shark River Slough freshwater sites, DOC and water depth were consistently out of phase. The Taylor Slough freshwater time series had no consistent phase relationship between DOC concentration and water depth (Figure 6). In Shark River Slough mangroves, we detected disturbance and pulse events had the most significant impacts on the relationship between DOC and water depth during the multiple periods in which Hurricane Irma increasing wavelet power.

Bacterioplankton Productivity

We found no correlation between either of the first two constructed principal components of DOM and bacterioplankton productivity at any site except for SRS-2 ($R^2 = 0.09$, $P < 0.001$), and TS/Ph-7 ($R^2 = 0.03$, $P = 0.03$). Wavelet power spectra showed that bacterioplankton productivity does not have a consistent annual pattern; it instead is associated with changing water depth only during disturbance pulse events (Figure S3).

DISCUSSION

Restoration of coastal wetlands in the Florida Coastal Everglades is occurring at the same time as sea-level rise and increasingly frequent disturbance events (Hobbs et al. 2007; Tully et al. 2019). As such, coastal nutrient subsidies come from both freshwater and marine sources, as restoration mobilizes legacy P from agriculturally enriched freshwater sources, and marine water has higher TP concentrations than the highly oligotrophic Everglades freshwater marshes (Sarker et al. 2020). At the same time, increasing salinity from marine water is a stressor to coastal ecosystems, leading to large shifts in ecosystem function and structure. This includes the collapse of carbon-rich peat stores (Chambers et al. 2019) and increasing subsidies of marine DOM to coastal ecosystems (Zeller et al. 2020). Predicting the fate of restored systems relies on an understanding of the interactions between multiple drivers of ecosystem trajectories

(Kominoski et al. 2018). Based on previous work showing hydrology as a strong driver of larger spatial scale patterns in carbon and nutrients (Regier et al. 2020; Kominoski et al. 2020), we predicted that the interaction between water depths and dominant carbon sources (i.e., peat, marl, mangrove) would explain pulsed changes in DOC concentrations and composition. We additionally predicted that the interaction between water depths and dominant carbon sources would explain changing bacterioplankton productivity at smaller spatial scales. Instead, we found that water depth is a strong and consistent driver of DOC only at locations where carbon is being produced (freshwater marsh of Shark River Slough, and shrub mangroves of Taylor Slough) and that increases in bacterioplankton productivity are not associated with any specific driver (including DOC concentration, composition, nutrient availability, water depth) but are associated with disturbance events (see also Kominoski et al. 2020).

Changes in water depth are responsible for ecologically relevant lateral fluxes of carbon between ecosystems (Strack et al. 2011; Wen et al. 2020). Our wavelet analysis showed a consistent relationship between water depth and DOC concentrations where DOC concentrations are the highest, agreeing with previous work showing that freshwater peatlands and marl mangroves are the major sources of DOM production in the Everglades (Regier et al. 2016). A shift from more labile (FI, BIX) to more recalcitrant (HIX, SUVA) DOM in the Everglades peat marsh over the study period suggests that restoration is importing more recalcitrant DOC from upstream marshes of the greater Everglades, as it also mobilizes stores of legacy phosphorus throughout the Everglades (Sarker et al. 2020). This is often seen in peatlands where increasing water flow flushes more recalcitrant DOM out of the system (Austnes et al. 2010).

Seasonal shifts in the composition of DOM and concentration of DOC can further clarify how water depths are changing the makeup of Everglades carbon. In peat marshes, high water depths correspond with lower DOC concentrations and BIX (algal DOM), but higher HIX (detrital DOM). This pattern suggests that high water levels in the wet season flush high concentrations of locally produced DOC to downstream ecosystems, reducing the algal influence on DOM, while low-concentration inputs of highly humic DOM from upstream peat marshes increase the detrital signal (HIX). The reduction in algal DOM may be a response to reduced light attenuation caused by higher concentrations of humic DOM (Karlsson et al. 2009). Similar to other systems, seasonal variation in water depth seems to shift the role of freshwater peatlands from producing and storing carbon to exporting it (Wen et al. 2020). In freshwater marl wetlands, changing water depth does not shift the contribution of either detrital (HIX) or algal (BIX) DOM, indicating that the dominant source of carbon does not change seasonally in the marl wetland. Water enters Taylor Slough through a series of canals and pumping stations, and the lack of a relationship between water depth and DOM composition in the last 10 years, alongside inconsistent phase relationships between DOC and water depth, suggests that marl wetland DOC concentrations are dominated by the contributions of allochthonous DOM imported through the canals. The opposite was found on the border of Taylor Slough in 1999, where marl marshes produced a significant amount of autochthonous DOM (Lu et al. 2003). This contrast could be explained either by restoration efforts within the past 20 years drastically increasing the amount of water and carbon entering Taylor Slough or could be indicative of variation in DOM production across the landscape that our data does not capture.

Mangrove ecosystems play a significant role in the production and export of DOM into marine ecosystems, and understanding the roles of restoration and sea-level rise in that dynamic is important for tracing the relationship of the Everglades to its surrounding waters (Dittmar et al. 2006; Sippo et al. 2017). Our optical data suggests that mangrove ecosystems seasonally shift between marine (higher BIX/FI values) and detrital mangrove (higher HIX/$SUVA_{254}$ values) sources of carbon, where higher water depths typically correspond to lower influence of marine carbon. This pattern is very strong in the shrub mangroves, where there are strong seasonal shifts in the extent of marine water intrusion. This is especially notable in comparison to the riverine mangroves of Shark River Slough, which are micro-tidal with diurnal changes in water levels where the relationship between water depth likely occurs on a smaller temporal scale than we measured in this study (Regier and Jaffé 2016). We show that BIX has a strong relationship with salinity in the most coastal mangroves, but they do not have the same relationship further inland, indicating that BIX at the most coastal sites is dominated by marine carbon imports. The extent of tidal variation is a strong driver of the amount of DOM being exported from mangroves in coastal wetlands (Bergamaschi et al. 2012).

In the riverine mangroves of Shark River Slough, $SUVA_{254}$ increases when mangrove influence outweighs marsh influence (Cawley et al. 2014; Regier et al. 2020). Our data showed increases in $SUVA_{254}$ during the wet season in the ecotone of Shark River Slough, but not in the downstream mangrove forests. This suggests that, during the dry season, upstream marsh DOM dominates the Shark River Slough ecotone as the mangrove forests dry down, while in the wet season, increasing water depths mobilize mangrove-derived DOM (Adame and Lovelock 2011). At the same time, dry-season

DOM appears to be more influenced by marine sources, as indicated by higher BIX and FI values associated with increasing salinity. At Shark River Slough mangroves, increases in freshwater flow appear to decrease the contributions of marine carbon. Our data showed that seasonal changes in riverine mangroves lead to a shift between BIX and HIX, where ecotone riverine mangroves seasonally shift between marsh and mangrove influence. During these seasonal shifts, salinity associated increases in HIX indicate further mangrove influence in the dry season. HIX does not vary seasonally in downstream riverine mangroves; instead, BIX varies, indicating seasonal shifts in the presence of marine carbon and consistent production of humic mangrove carbon. Mangrove-produced DOM has significant implications for the future health of Everglades wetlands and likely originates in declining soils before being flushed into Florida Bay (Charles et al. 2019; Arnaud et al. 2020).

The Everglades relies on the storage of carbon in soils to keep pace with sea-level rise, and one of the goals of restoration has been to maintain Everglades soils (Chambers et al. 2019). Tracking DOM sources allows for low impact measurements of indicators of both processing and sources of carbon within changing wetlands. Our long-term DOM data suggest that increasing water depths may be changing the microbial processing of carbon (decreasing FI) from Everglades soils. Regier et al. (2020) showed that FI increased across the same 6 sites from 2002–2014, whereas our data from 2012–2021 show that the trajectory of FI has flipped and is now decreasing. If changes to FI were happening in the water column, we would expect to see a similar change in bacterioplankton productivity, but we do not. This suggests that these changes to FI are occurring in the soil before carbon is released into the water column. Production of DOM in wetland soils is often controlled by water depth, where changing anaerobic conditions can reduce microbial activity on soil carbon and change the concentration and makeup of carbon being released from soils (Strack et al. 2008). The decrease in FI over the past 10 years may reflect a decrease in soil microbial activity in response to a reduction in aerobic conditions to Everglades soils. This is especially noteworthy because we would expect higher microbial activity as phosphorus concentrations increase across this highly oligotrophic landscape, if oxygen was not a limiting factor (Noe et al. 2001). The decrease we see in FI is relatively small and is not accompanied by changing HIX or BIX, which would be expected from large shifts in carbon sources. Additionally, Everglades aquatic productivity is highly variable and decoupled from overall ecosystem productivity (Malone et al. 2022). While this trend in FI is not conclusive, it is highly consistent across habitats and opposite the trend of the previous 10 years, highlighting the importance for continued monitoring of DOM as an indicator of changes to organic matter storage in coastal wetlands. A signal of productivity both in the soil and the water column, DOM is valuable long-term data for detecting changes to ecosystem trajectories in response to the interaction of restoration and sea-level rise across multiple compartments (Kominoski et al. 2018; Sarker et al. 2020).

An in-depth understanding of carbon processing in coastal wetlands is needed to better inform carbon storage restoration strategies. Peat wetlands are created by high water depths that create anoxic conditions and allow for the buildup of humic organic matter, and our data suggests that increased water depths from restoration are creating conditions that are more conducive to the buildup of peat (Fenner and Freeman 2011; Ritson et al. 2017). In marl wetlands, where floc is seasonally mineralized by microbes when oxygen becomes available, organic matter pools are largely ephemeral (Pisani et al. 2013), and our data suggest that restoration may be increasing their potential to store carbon; however, the decrease in microbial signals may also originate in upstream peat wetlands that dominate the DOM pool of short-hydroperiod freshwater wetlands. Rewetting of wetland soils typically increases their biomass by reducing microbial activity (Zerbe et al. 2013), but the microbial response depends on subsidies or stressors (such as salinity and phosphorus) delivered along with the water (Saha et al. 2011; Servais et al. 2019). Saltwater intrusion typically reduces carbon storage capacity and increases the leaching of DOM and the microbial breakdown of soils (Weston et al. 2011; Servais et al. 2019). Continued monitoring is needed to track how accelerating sea-level rise will change the production, processing, and movement of carbon throughout the Everglades, especially where legacies of salinity and phosphorus from marine water persist in affected soils (Lee et al. 2021).

Conclusions

Parsing how increasing pulse and press events will change ecosystems relies on an understanding of how sources of carbon are being shifted through ecosystems. We show that restoration is mobilizing detrital carbon, reducing microbial processing of carbon, increasing the connectivity between carbon sources, and synchronizing biogeochemical cycling across the Everglades (Kominoski et al. 2020). Our study highlights the mechanisms through which pulses and presses of freshwater (from restoration) and seawater (from sea-level rise) are driving lateral movements of organic matter between ecosystems. Tracing the dynamics of resources and organic matter is critical to understanding long-term ecosystem trajectories (Odum 1969; Kominoski et al. 2018). Understanding the specific drivers and dynamics of lateral movements of nutrients and organic matter allows for improved predictions and more effective management of organic matter and nutrients in ecosystems that are being exposed to novel pulses and presses around the world.

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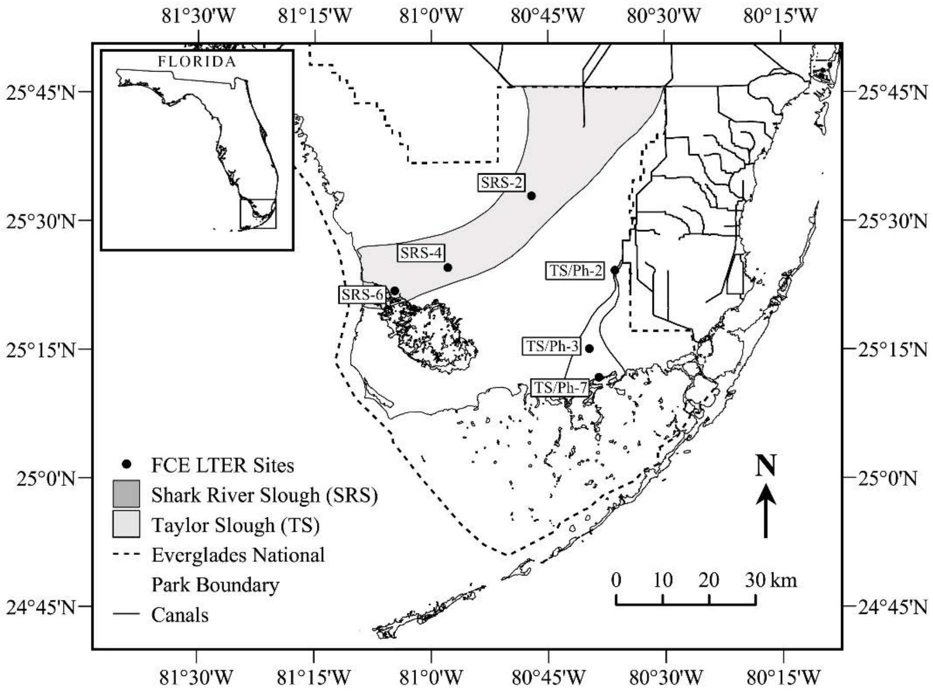

Figure 1. Location of the study sites in the Florida Coastal Everglades (FCE), Everglades National Park (ENP) in South Florida, USA. We sampled at a marsh, ecotone and mangrove site along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7). All sites are part of the FCE Long-Term Ecological Research (FCE-LTER) program.

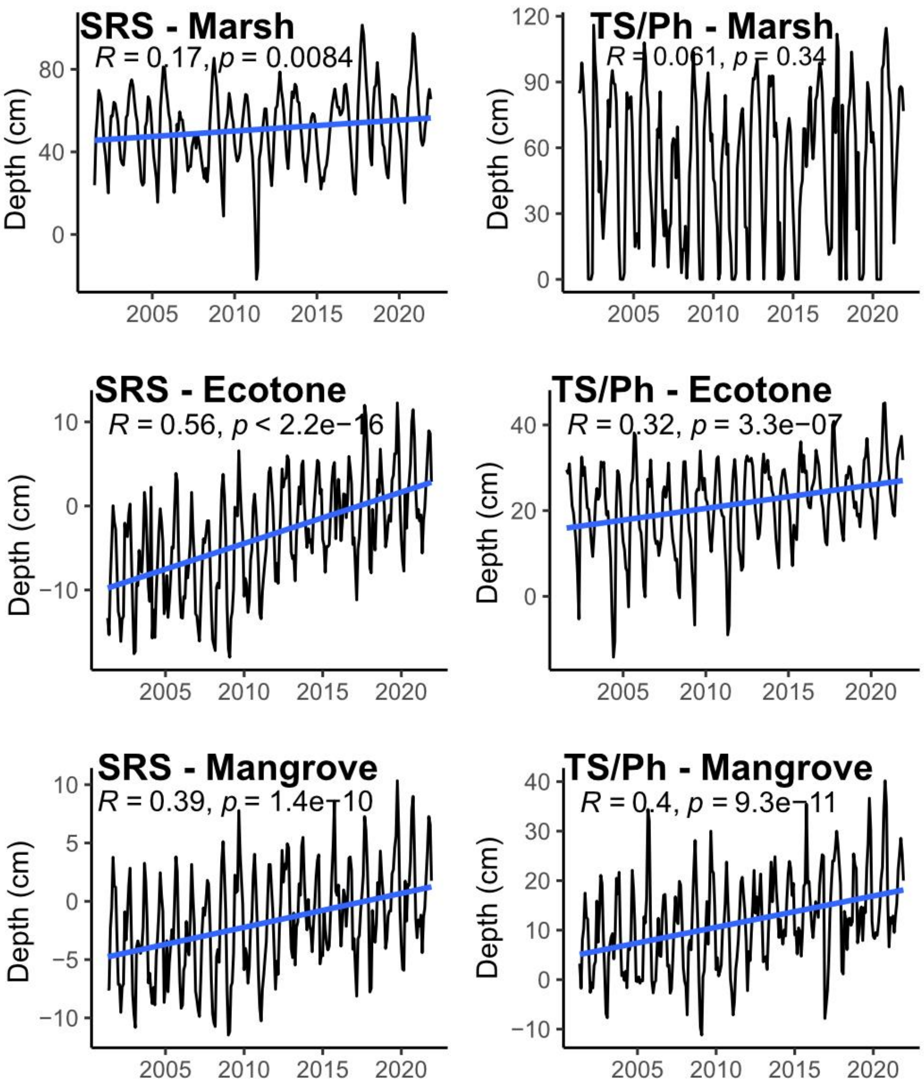

Figure 2. Changing water depth over time in marsh, ecotone and mangrove site along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7).

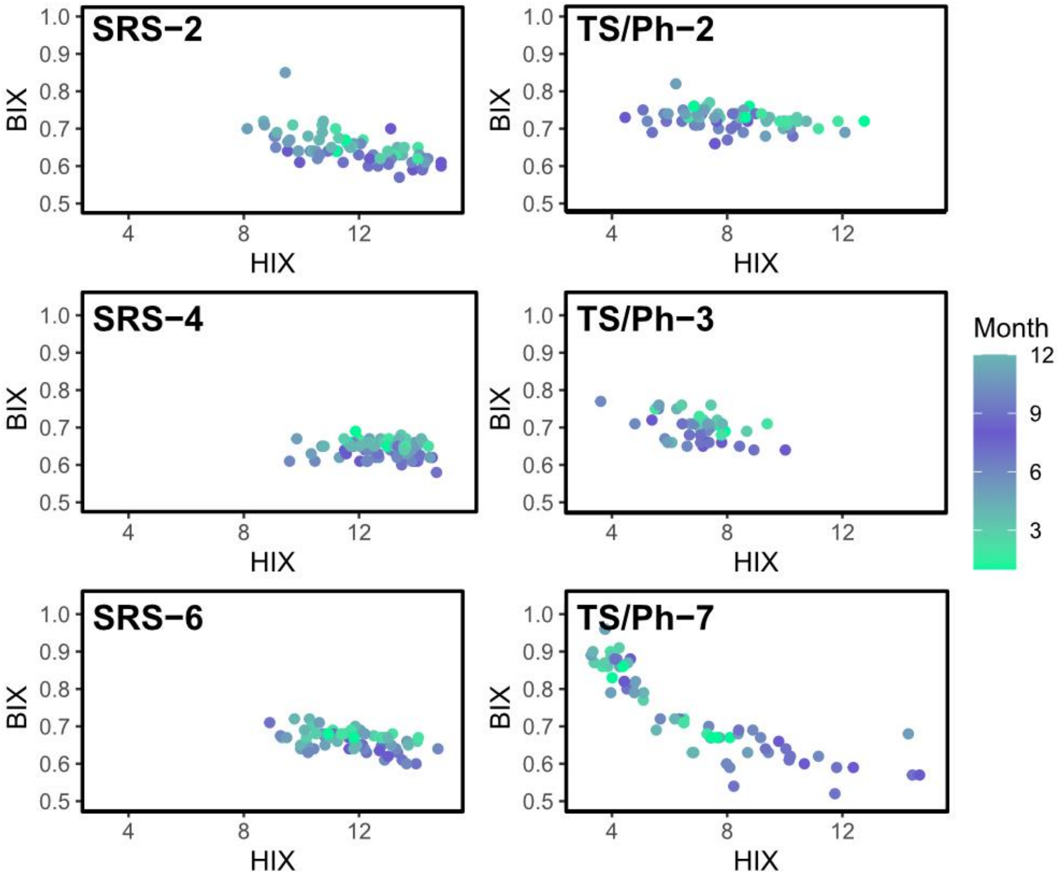

Figure 3. Seasonal changes in HIX and BIX in marsh, ecotone and mangrove site along the long-hydroperiod Shark River Slough (SRS-2, -4, -6), and the short-hydroperiod Taylor Slough (TS/Ph-2, -3, -7). Increasing BIX (Biological Index) indicates increasing autochthonous influence on DOM. Increasing HIX (Humification Index) indicates increasing humic influence on DOM.

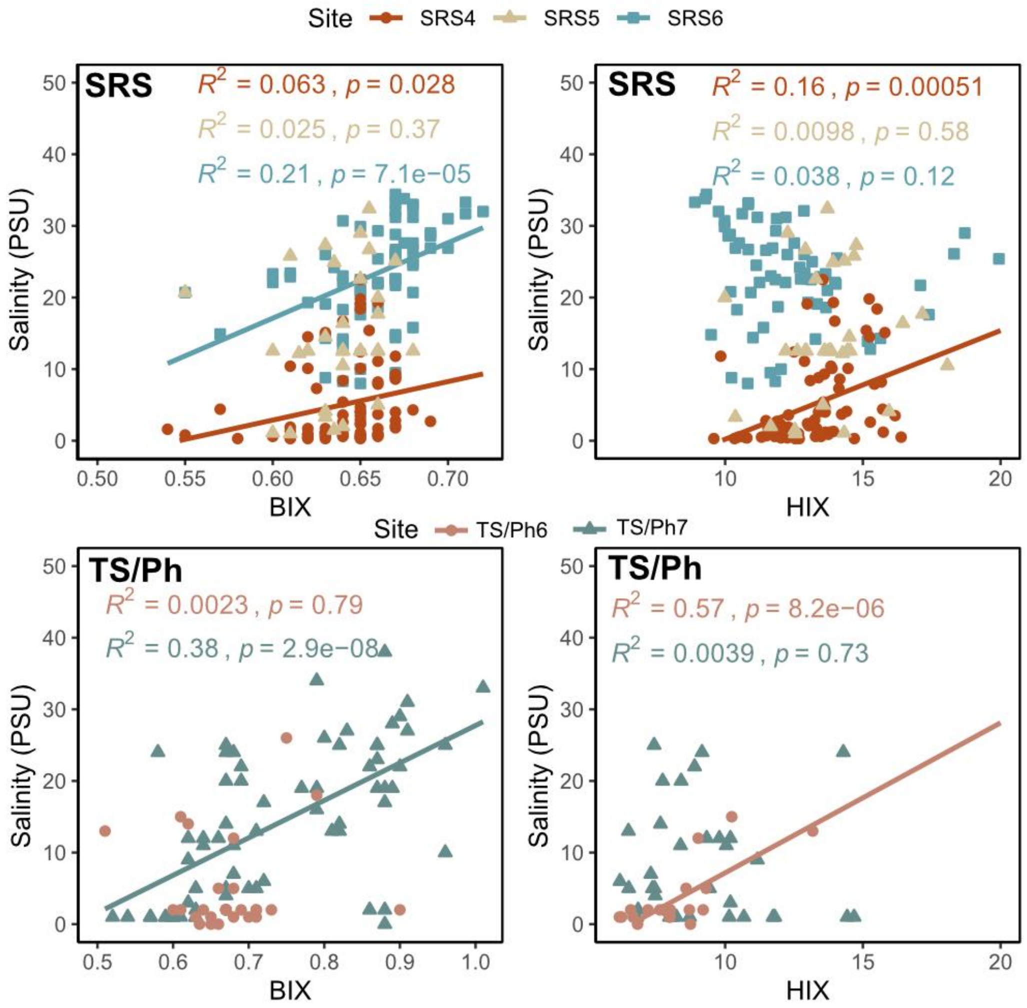

Figure 4. Salinity as a driver of BIX and HIX at mangrove sites in the long-hydroperiod Shark River Slough (SRS-4, -5, -6), and the short-hydroperiod Taylor Slough (TS/Ph-6, -7). Increasing BIX (Biological Index) indicates increasing autochthonous influence on DOM. Increasing HIX (Humification Index) indicates increasing humic influence on DOM.

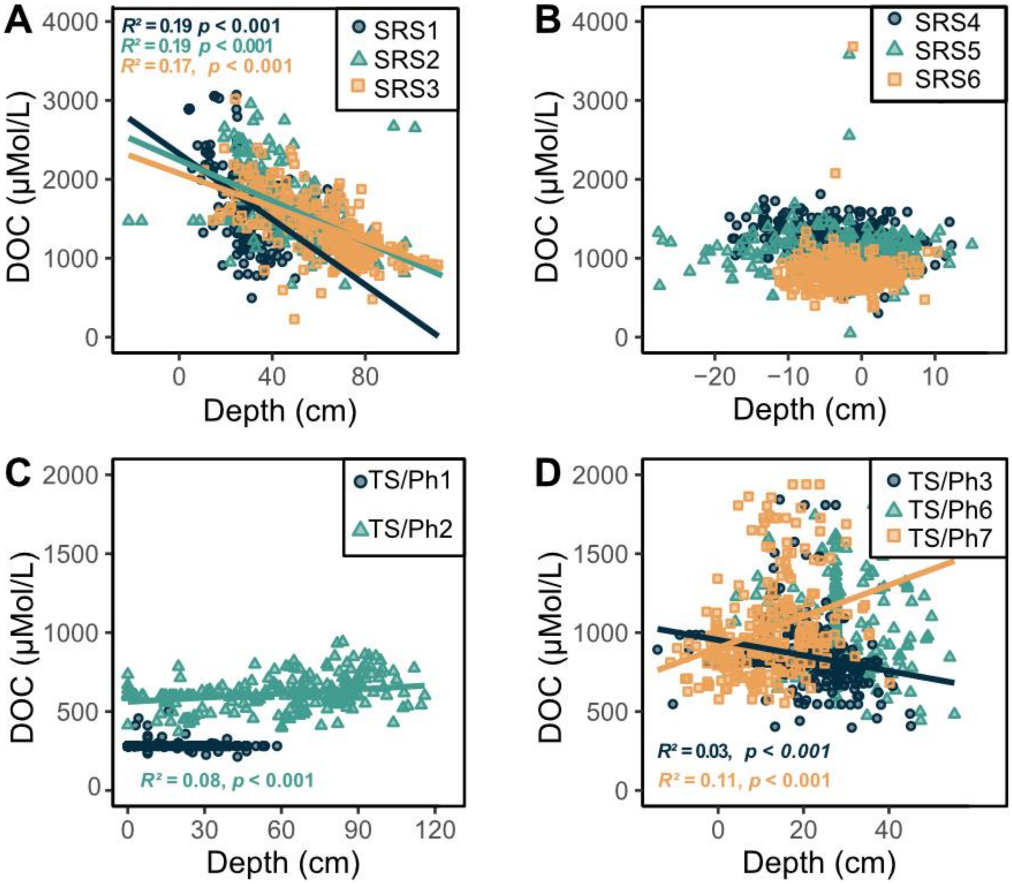

Figure 5. Linear relationships between DOC and water depth for all sites in the long-hydroperiod Shark River Slough (SRS-1:6), and short-hydroperiod Taylor Slough (TS/Ph-1:7).

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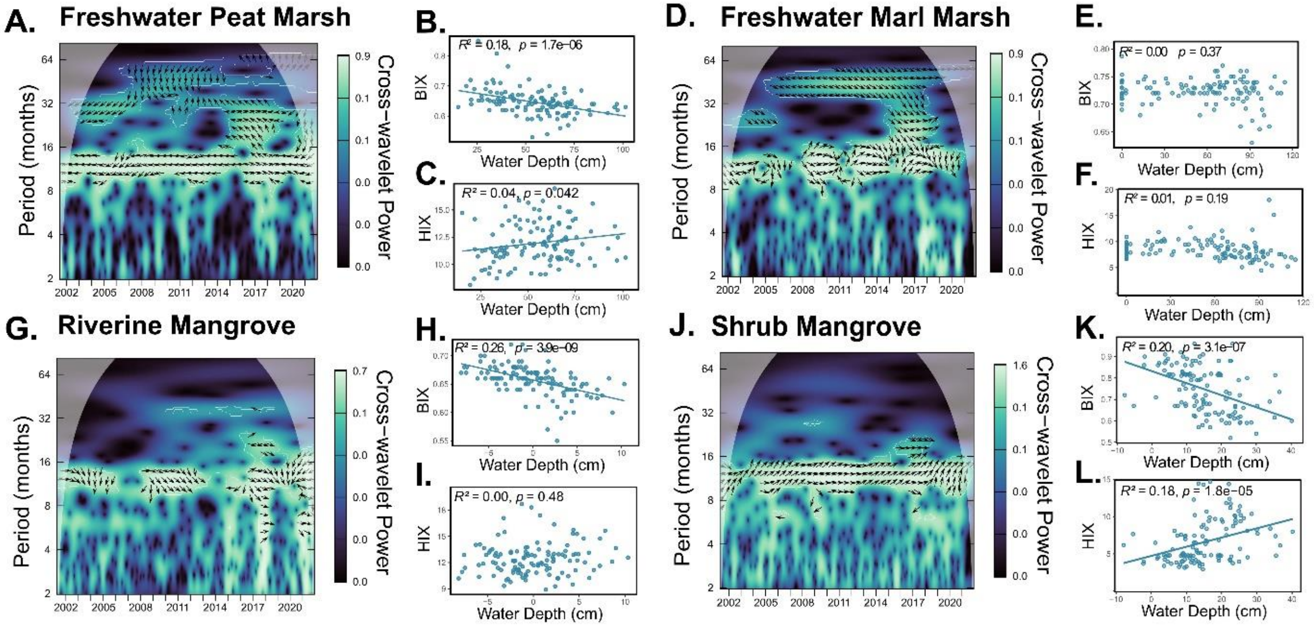

Figure 6. Dissolved organic carbon patterns in Everglades freshwater marsh (A-F) and mangrove (G-L) habitats. White contour lines wavelet plots (A, D, G, J) indicate statistically significant cross-wavelet power between DOC concentrations (µMol/L) and water depth (cm). Arrows are plotted on where wavelet power is significant, and the facing of arrows at each time point describes the relationship between the two variables. Right facing arrows indicate the two variables are in phase, while left indicates out of phase. Arrows facing up indicate that changes in depth lag after changes in DOC, while arrows facing down indicate that changes in DOC lag changes in depth. Scatterplots use two fluorescence metrics: BIX (B, E, H, K) and HIX (C, F, I, L). Increasing BIX indicates increasing algal influence on DOC, while increase HIX indicates increasing humic influence to DOC.

Table 1. Linear models of nine metrics of dissolve organic matter over time. Linear models were constructed for total nitrogen (TN, μMol/L), total phosphorus (TP, μMol/L), dissolved organic carbon (DOC, μMol/L), and 5 fluorescence metrics. The fluorescence metrics included are: Fluorescence Index (FI), Humification index (HIX), Biological Index (BIX), Specific Absorbance at 254 (SUVA254), Slope ratio (SR).

	SRS 2		**SRS 4**		**SRS 6**		**TS/PH 2**		**TS/PH 3**		**TS/PH 7**	
Variable	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*	*t*	*P*
Depth (cm)	2.066	0.041	3.035	<0.001	2.012	0.047	1.133	0.259	3.072	0.003	2.860	0.005
DOC (μMol/L)	0.718	0.474	1.300	0.196	1.321	0.189	-1.029	0.305	-2.004	0.047	1.216	0.226
TP (μMol/L)	5.393	<0.001	4.194	<0.001	2.078	0.040	3.082	0.003	3.211	0.002	-0.348	0.728
TN (μMol/L)	-0.121	0.904	1.923	0.057	4.544	<0.001	2.954	0.004	-0.037	0.970	3.850	<0.001
FI	-4.216	<0.001	-3.573	<0.001	-3.777	<0.001	-2.288	0.024	-2.376	0.020	-3.447	<0.001
BIX	-4.574	<0.001	-0.476	0.635	0.832	0.407	-0.129	0.897	-2.867	0.005	-0.596	0.553
HIX	5.788	<0.001	0.212	0.833	-0.294	0.77	-2.929	0.004	-0.652	0.515	-1.661	0.099
SUVA$_{254}$	2.114	0.0367	0.782	0.436	-0.433	0.666	-0.261	0.795	2.803	0.006	-0.754	0.452
SR	-1.219	0.225	2.731	0.0073	5.217	<0.001	1.704	0.091	0.843	0.401	-0.588	0.557

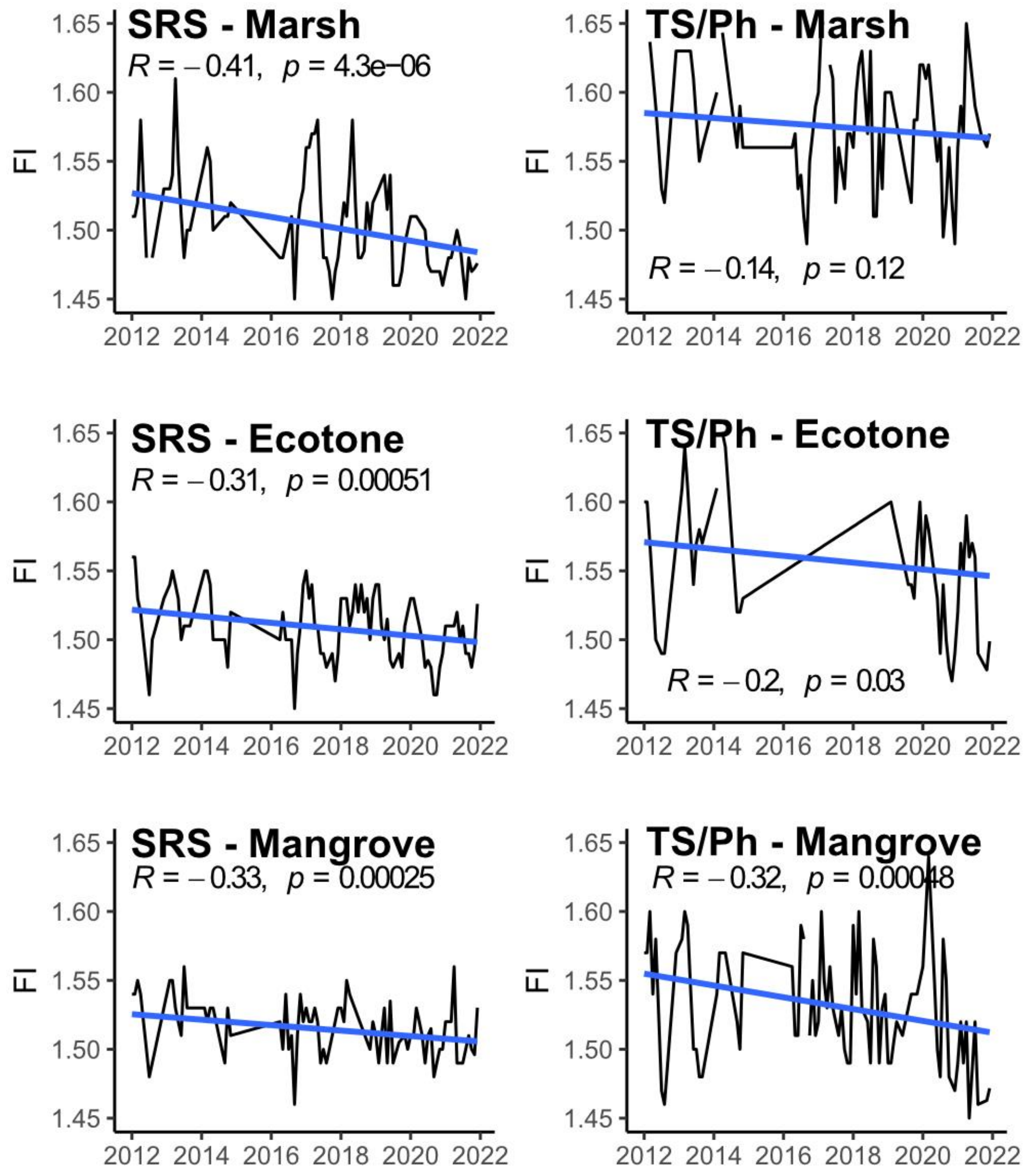

Figure S1. Changes to fluorescence index over time in Everglades freshwater, ecotone, and marine sites. FI ranges from high microbial influence at low values, to high terrestrial influence at higher values.

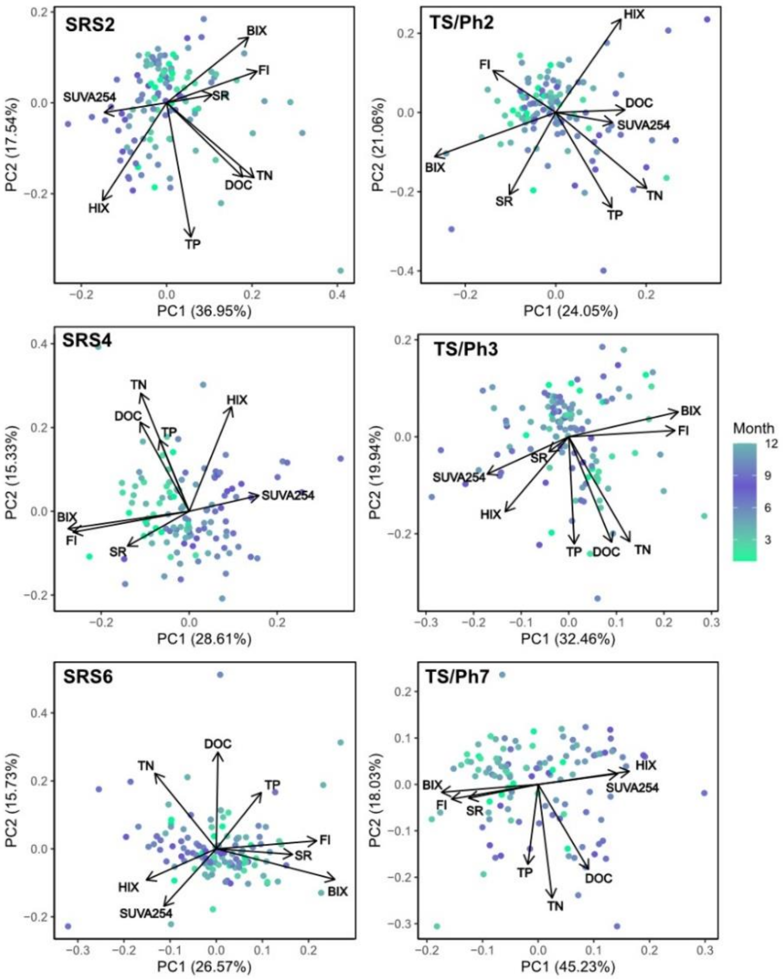

Figure S2. Principal component analysis of seasonal changes to total composition of DOM. PCAs were built with total nitrogen (TN, μMol/L), total phosphorus (TP, μMol/L), dissolved organic carbon (DOC, μMol/L), and 5 fluorescence metrics. The fluorescence metrics included are: Fluorescence Index (FI), Humification index (HIX), Biological Index (BIX), Specific absorbance at 254 (SUVA254), Slope ratio (SR).

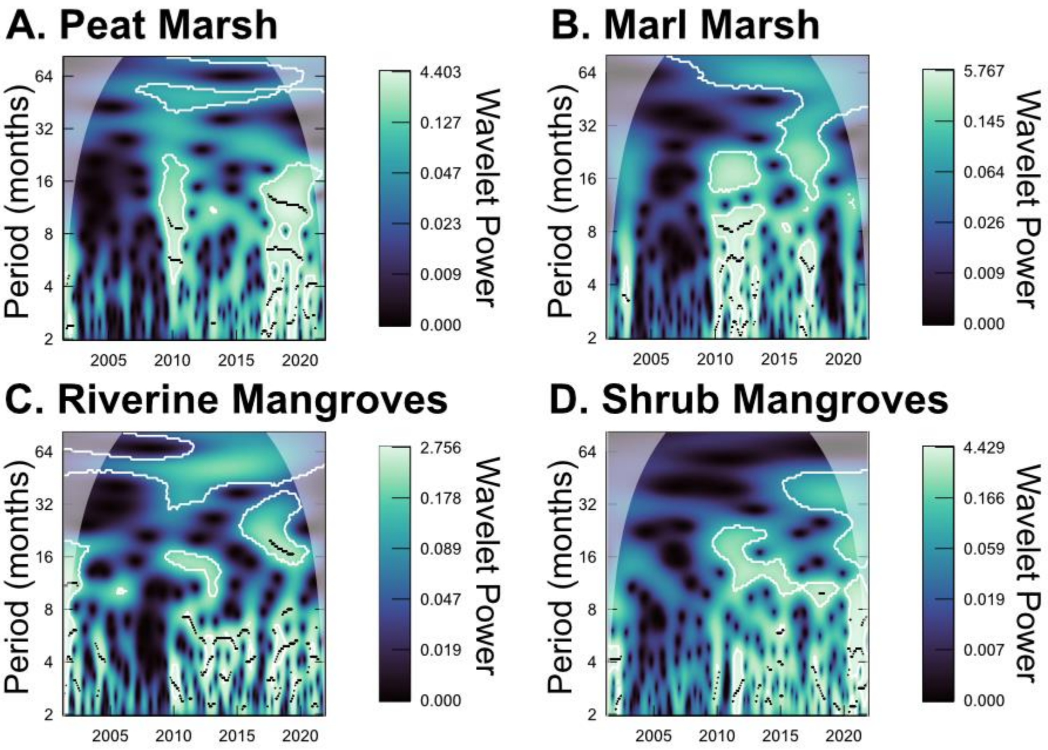

Figure S3. Wavelet power of bacterioplankton productivity (mg Thymidine/L/day) in Everglades marshes. Areas outlined in white indicate significant wavelet power ($P < 0.05$).

Table S1. Average and standard deviation of water column physiochemical parameters from the past 20 years (2001-2021) measured as monthly grab samples across eleven long-term ecological research sites in the Florida Coastal Everglades.

Site	*Total Nitrogen (μMol/L)*	*Total Phosphorus (μMol/L)*	*Bacterioplankton Productivity (mg Thymidine/L/day)*	*DOC (μMol/L)*	*Water Depth (cm)*
SRS1	76.42 (36.68)	0.39 (0.3)	8.36 (8.53)	1698.89 (590.06)	30.81 (14)
SRS2	70.34 (38.6)	0.36 (0.33)	10.58 (12.86)	1599.21 (543.35)	51.01 (18.73)
SRS3	61.76 (34.18)	0.4 (0.35)	18 (22.69)	1438.6 (515.35)	61.31 (20.46)
SRS4	46.48 (15.63)	0.47 (0.28)	14.89 (14.03)	1280.61 (380.03)	-3.47 (6.54)
SRS5	41.55 (14.22)	0.57 (0.3)	13.03 (13.34)	1086 (278.37)	-2.77 (7.7)
SRS6	34.37 (13.02)	0.71 (0.34)	9.81 (7.79)	814.71 (312.06)	-1.76 (4.44)
TS/Ph1	36.17 (12.21)	0.32 (0.14)	12.67 (9.59)	284.8 (26)	14.08 (14.71)
TS/Ph2	34.47 (13.75)	0.26 (0.2)	9.54 (12.49)	607.25 (101.66)	51.78 (33.83)
TS/Ph3	53.76 (28.64)	0.23 (0.19)	4.41 (6.26)	847.97 (224.5)	21.48 (10.08)
TS/Ph6	54.16 (23.95)	0.39 (0.21)	18.95 (18.32)	1014.43 (256.43)	27.83 (9.46)
TS/Ph7	51.15 (17.05)	0.39 (0.26)	13.45 (13.58)	1022.09 (312.69)	11.6 (9.54)

CHAPTER VI

CONCLUSION

Understanding the processes of organic carbon in ecosystems is essential for protecting carbon storage that drives ecosystem trajectories of coastal wetlands (Battin et al. 2009, Mcleod et al. 2011). Coastal wetlands rely on soil accretion mediated by litterfall to withstand climate change and food webs often have strong detrital sources as basal resources (Moore et al. 2004, Arnaud et al. 2020). Anthropogenic changes are rapidly altering these carbon-cycling pathways and monitoring of benthic and pelagic carbon gives insights into the specific microbial and biogeochemical cycles and pathways that are being affected (Morris et al. 2002, Gedan et al. 2011, Craft et al. 2016). The Florida Coastal Everglades is a model system to test questions about carbon cycling in coastal wetlands, within its two major drainages, giving contrast between higher- and lower-productivity ecosystems (Davis and Ogden 1994, Childers et al. 2006, Castañeda-Moya et al. 2013). My dissertation research provides evidence for how hydrologic pulses of fresh and marine water influence carbon cycling in the dynamic wetlands of the Florida Coastal Everglades.

In Chapter II, I investigated the extrinsic and intrinsic drivers of litter breakdown across coastal wetland gradients through a reciprocal transplant of four litter species along Everglades freshwater to marine gradients. I found variation in litter breakdown rates that were not well explained by site and litter nutrient availability, but by seawater availability which may indicate changing redox conditions as an essential predictor of litter breakdown. To further understand the role of redox conditions, in Chapter III I used microbial gene expression (RNA transcriptomics) to investigate the microbial pathways that were most relevant across the same litter species, and environmental conditions. I found that Everglades litter is a mix of aerobic and anaerobic microbial activity, and that there is a strong relationship between marine influence and sulfate uptake. This supports my results from Chapter II, indicating that marine subsidies of sulfate may play an important role in the breakdown of litter. In Chapter IV, I transitioned my focus to dissolved organic carbon, with a focus on freshwater ecosystems. I investigated the effects of restoration on carbon processing, where dissolved organic carbon acts as a signal of carbon processing happening across both allochthonous and autochthonous sources to indicate carbon processing across the ecosystem. I found that hydrologic restoration is altering the processing of carbon in peat vs marl ecosystems in opposite directions, causing a greening of peat systems, but a browning of marl systems. Overall, this opposite response is shifting the dissolved organic carbon of the two ecosystems to become more similar to each other as water levels increase. Although Chapter IV used spatial variation in water depths to test these relationships, in Chapter V I used long-term data from the FCE-LTER sites to investigate the drivers of dissolved organic carbon concentrations and composition over the past 20 years. I analyzed time series of water depth, DOC concentration, and DOM fluorescence to show that restoration is mobilizing detrital carbon, while highlighting water depth as an important driver of dissolved organic matter. My analyses showed that the role of water depth depends heavily on the environment, with increasing water depths decreasing DOC concentrations in higher-nutrient peat wetlands, but increasing DOC in lower-nutrient marl wetlands that are producing far less of their own DOC. I also showed the strong difference between mangrove habitats that are likely driven by differences in tidal exchange of soil carbon.

My research has provided new information of the drivers of carbon processing throughout oligotrophic coastal wetlands, and highlighted the importance of carbon processing as a major change that is controlled by hydrologic cycles that are rapidly changing in response to restoration and climate change. My work also highlights multiple further directions for future study: 1) Future research could specifically look at how the diurnal tidal exchange in the riverine mangroves of SRS influences the breakdown, and release of carbon into the water column. The conclusions of Chapters II, III, and V all suggest that diurnal tidal dynamics in mangroves play an important role in carbon cycling, but currently data are not collected on a scale necessary to study these dynamics. 2) Our data indicate that differences in litter quality play an important role in the breakdown of litter across Everglades wetlands, but our data on litter chemistry were not sufficient to explain those differences. Additional studies focusing on more physical and chemical metrics of leaf litter in Everglades wetlands would be useful to further explain the breakdown of litter. 3) Our transcriptomics dataset contains a wide array of information about microbial activity across Everglades wetlands, and while it is limited in its replication, there is a wealth of data available for use. Our results indicate that further in-depth study of the microbial pathways of organic matter processing is likely to show spatiotemporal differences that vary among microbial species.

Overall, my dissertation contributed to filling the knowledge gap of how carbon processing is responding (including the drivers of the response) to changing hydrologic pulses and presses in coastal wetlands. I illustrated the importance of water depths in the release, mobilization, and transport of detrital carbon, and showed that marine water has significant changes to both the composition of carbon (as subsidies of marine carbon) and the processing of detrital carbon (as marine subsidies of both phosphorus, and sulfates). The focus of my dissertation on uncovering the specific drivers of changing carbon makeup and concentrations will allow for more accurate predictions and management of how coastal ecosystems respond to restoration and climate change. My dissertation highlights the importance of long-term datasets in tracking these changes, as well as their importance as a tool to understand the drivers of those changes. The carbon cycle of coastal wetlands is one of the major factors determining their fate, and can be used to predict ecosystem trajectories, providing managers the tools they need to utilize these drivers of carbon processing to protect our stores of carbon for future generations.

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APPENDIX

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