Estuaries and Coasts
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Effects of Saltwater Pulses on Soil Microbial Enzymes and Organic Matter Breakdown in Freshwater and Brackish Coastal Wetlands

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Abstract

Coastal freshwater and brackish wetlands are exposed to pulses of saltwater during times of reduced freshwater flows (i.e., dry seasons, droughts), periodic storm surges, and increased tidal extent associated with rising seas. The effects of saltwater pulses on belowground processing rates of detrital organic matter as mediated by microbial activities are uncertain. Our objectives were to quantify whether and how pulses of saltwater (i) change soil porewater physicochemistry, (ii) change soil microbial extracellular enzyme activities, and (iii) change root litter breakdown over time in freshwater and brackish marshes. From 2014 to 2016, we simulated saltwater intrusion as monthly in situ pulsed additions of artificial seawater in experimental dosing chambers (1.4 m diameter) within freshwater and brackish marshes of Everglades National Park. At monthly intervals, we collected soil porewater chemistry, and measured microbial extracellular enzymes, elemental stoichiometric ratios, and breakdown rates (*k*) of incubated (0–30 cm depth) root litter and compared these responses over time. Saltwater pulses increased sulfate and nitrogen concentrations in porewater at the freshwater site. However, saltwater pulses generally decreased porewater constituents (e.g., dissolved organic carbon, dissolved nitrogen and phosphorus species) at the brackish site. One saltwater pulse increased root litter *k* by 1.25-fold in the brackish marsh. However, long-term (740 days) *k* in brackish wetlands, and both short- and long-term *k* in freshwater wetlands, were not affected by 24 monthly pulses of saltwater. Enzyme activities fluctuated with time and did not respond to multiple saltwater pulses. Our results suggest that detrital organic matter stocks and associated soil microbial activities are relatively resistant to single and multiple ($n = 24$) pulses of saltwater.

Keywords Saltwater intrusion · Extracellular enzyme activities · Peat collapse · Root litter breakdown · Everglades · Coastal wetlands

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Introduction

Organic matter breakdown is a fundamental ecosystem process that contributes to the regulation of carbon (C) storage and nutrient cycling (Hoorens et al. 2003). Ecosystem C storage at large spatial scales is dependent upon the transformation of C mediated by microbial and biochemical processes (Sinsabaugh and Follstad Shah 2012). It is important to understand how ecosystems maintain or lose stored C and how biogeochemical processes are altered by changing environmental conditions (Chambers et al. 2019). Across diverse ecosystems, a positive balance between inputs relative to losses of organic matter supports soil accretion and C storage (Chapin et al. 2006, Nyman et al. 2006, McKee 2011). This balance is important in wetland ecosystems where slow breakdown of organic matter resulting from submersion and burial in low oxygen promotes C accumulation in the soil (Mitsch and Gosselink 2007). Furthermore, the rate of soil organic matter breakdown relative to rates of autotrophic organic C inputs determines the rates of nutrient cycling and soil accretion or subsidence (Newman et al. 2001) and overall net ecosystem C storage (Chapin et al. 2006).

Organic-rich soils are prevalent throughout peat-based wetlands like the Florida Everglades (Craft and Richardson 2008), but loss of soil elevation is likely a landscape-scale feature of sea-level rise and saltwater intrusion (Wanless and Vlaswinkel 2005; Day et al. 2011; Wilson et al. 2018; Chambers et al. 2019). For example, historic peat collapse, or soil subsidence, likely led to the formation of coastal bays in the Everglades (Wanless and Vlaswinkel 2005). Recently, soil elevation loss in the Everglades has accelerated and has been linked to increased microbial breakdown (Chambers et al. 2015) and to reductions in fine root biomass associated with saltwater intrusion (Wilson et al. 2018; Charles et al. 2019). Additionally, reduced plant inputs of root and detritus into the soil organic matter pool have been hypothesized to transition wetlands from C sinks to C sources (Chambers et al. 2019). With accelerating rates of sea-level rise and reduced freshwater flows to the sea, coastal freshwater wetlands are increasingly threatened by novel exposure to saltwater which alters soil biogeochemistry (White and Kaplan 2017; Tully et al. 2019), thereby increasing microbial breakdown and stimulating organic C loss (Craft 2007; Chambers et al. 2011; Weston et al. 2011; Herbert et al. 2018). Despite increases in saltwater intrusion from drought, sea-level rise, and freshwater diversion (Herbert et al. 2015; Tully et al. 2019), few studies have directly tested the effects of saltwater additions on C cycling in coastal wetland soils (Servais et al. 2019).

Soil microbes are considered “first responders” and rapidly change community structure and function upon exposure to environmental stimuli (Chambers et al. 2016; Herbert et al. 2018). Soil microbial extracellular enzymes catalyze the reaction of high-molecular-weight organic matter into assimilable forms (Dick 1994, Schimel and Weintraub 2003), which enable microbial communities to obtain C and limiting nutrients (Burns 1982). The relationship between extracellular enzyme activities and the rate of breakdown of organic material in wetlands is well documented (Jackson et al. 1995, Freeman et al. 1996, Kang and Freeman 1999, Schimel and Weintraub 2003). Increased enzyme activities can increase the rate of organic matter breakdown, and previous studies have tested how changing environmental conditions can alter the relationship between enzyme activities and the breakdown of organic matter (Allison and Vitousek 2004; Rejmánková and Sirová 2007). However, it is unclear how environmental changes (e.g., saltwater pulses) affect the relationship between enzyme activities and organic matter breakdown. Both increases (Morrissey et al. 2014) and decreases (Servais et al. 2019) in extracellular enzyme activities have been reported with elevated salinity, but both studies reported decreased soil organic matter C with elevated salinity. Quantifying how microbial extracellular enzyme activities and organic matter breakdown rates change with exposure to saltwater pulses is needed to the potential mechanisms of microbe-mediated C loss in coastal wetlands undergoing saltwater intrusion (Chambers et al. 2015, 2016, 2019).

Here, we tested how short- and long-term responses of soil microbes to monthly pulsed saltwater additions affect soil C in freshwater and brackish wetlands. We used the Florida Coastal Everglades as a model system to investigate the effects of saltwater pulses on the breakdown of soil organic matter. The Everglades is particularly vulnerable to saltwater intrusion because of declines in seasonal freshwater delivery (McIvor et al. 1994, Odum et al. 1995, Sklar et al. 2002), a naturally low topographic incline (Ross et al. 2000), and rapidly increasing rates of sea-level rise (Wdowinski et al. 2016, Dessu et al. 2018). Our objectives were to test how both single (i.e., one pulse) and multiple (i.e., 24 pulses over two years) saltwater pulses affect microbial activities and in situ organic matter processing rates in freshwater compared to brackish marshes of Everglades National Park. We quantified effects of saltwater pulses on porewater chemistry, microbial extracellular enzyme activity associated with bulk soil and root litter, bulk soil, and root litter elemental stoichiometry, and root litter breakdown rates (*k*). We used a path analysis to identify how changes in *k* were explained by differences in porewater chemistry, extracellular enzyme activity, and organic matter stoichiometry at each marsh. We hypothesized that if soils are exposed to saltwater pulses, then extracellular enzyme activities would be inhibited. We also hypothesized that soils exposed to repeated saltwater pulses would have higher microbial extracellular enzyme activities that affect organic matter C and nutrient concentrations (i.e., increased soluble reactive P, total inorganic N, sulfate, and dissolved organic C [DOC]). We also predicted that microbe-mediated increases in root litter C and nutrient concentrations and decreases in C:nutrient ratios would lead to increased root litter breakdown rates (Fig. 1).

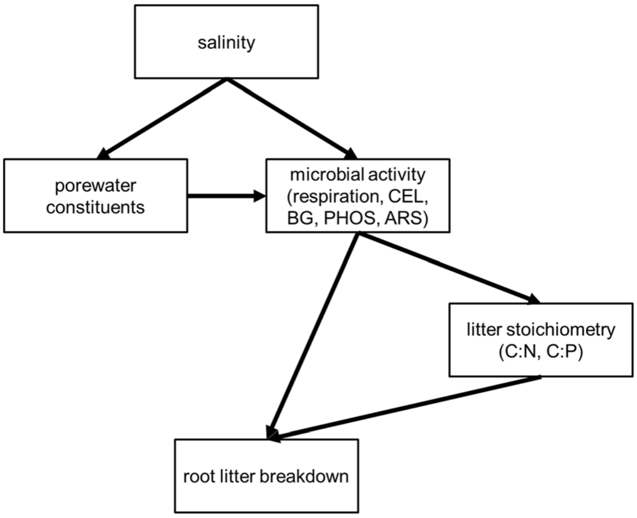


Fig. 1 Hypothesized path model describing how salinity affects root litter breakdown rates. Arrows indicate hypothesized causal links between variables. The structured set of linear equations that correspond to each response variable can be described based on the links associated with each variable (e.g., microbial activity ~ salinity + porewater constituents, porewater constituents ~ salinity). We hypothesized that microbial activity mediates effects of salinity on root litter breakdown because of the direct effects of salinity stress on microbial activity and indirectly through changes in concentrations of porewater constituents

Methods

Study Marshes

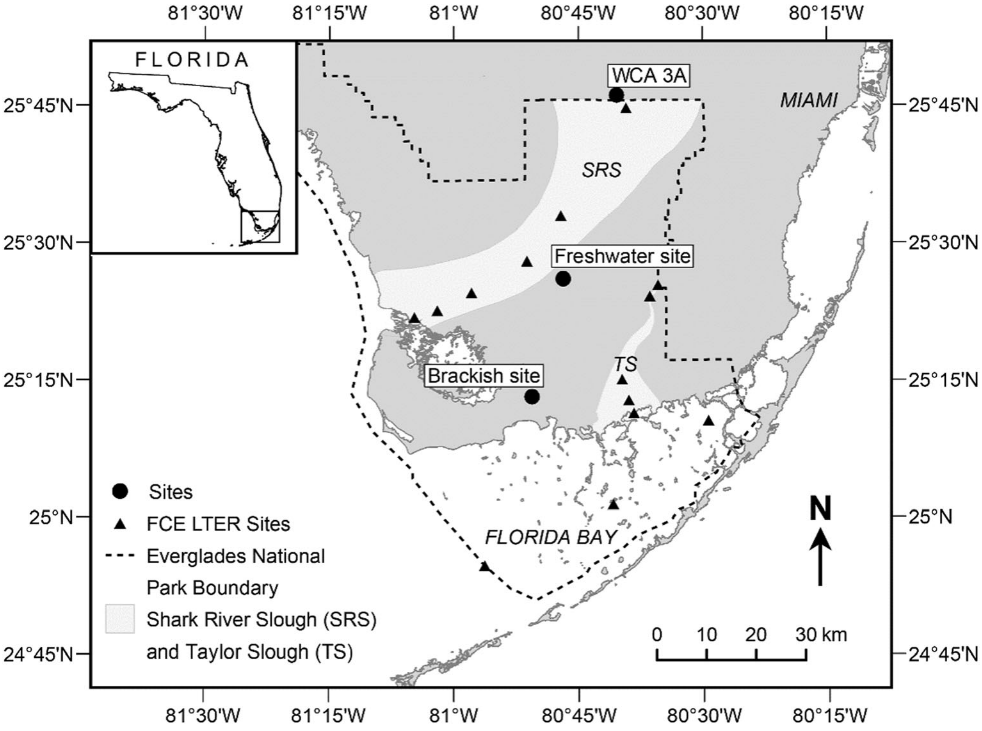
We identified two study marshes (one freshwater, one brackish) within Everglades National Park, Florida, USA (Fig. 2) to establish saltwater dosing experiments. To our knowledge, the freshwater marsh (25 26′ 6.11” N, 80 46′ 50.78” W) had not been exposed to saltwater intrusion, and the site was dominated by *Eleocharis cellulosa* and *Cladium jamaicense* macrophytes. The freshwater marsh had a long hydroperiod and water fell below the soil surface for only 39 days during our 2-year experiment (Wilson et al. 2018). The brackish marsh (25 13′ 13.38” N, 80 50′ 36.66’ W) was dominated by *C. jamaicense* with some dwarf mangroves (*Rhizophora mangle* and *Conocarpus erectus*) present. The brackish marsh was not tidally influenced, and water was below the soil surface for 132 days during our experiment (Wilson et al. 2018).

In September 2014, we installed 12 polycarbonate chambers (1.4 m diameter) along a constructed boardwalk (80 m long) at both freshwater and brackish marshes. Six chambers at each marsh were designated as ambient controls, and 6 were designated as saltwater treatments. Each chamber (embedded 30 cm into the soil) had a series of 10-cm holes drilled in the sides to facilitate water exchange and a collar that allowed these openings to be closed during dosing and sampling events (Stachelek et al. 2018). The saltwater treatments were established downstream from the topographic flow to ensure minimal contamination between saltwater treatment and control chambers.

Pulsed Saltwater Dosing

We began experimental pulsed additions of saltwater in October 2014 that continued monthly through November 2016. The 6 saltwater treatment chambers at each marsh were dosed monthly with artificial seawater (Instant Ocean, Spectrum Brands,

Fig. 2 Location of the freshwater and brackish marshes within Everglades National Park and WC3A where additional root litter was collected. The Florida Coastal Everglades Long Term Ecological Research (FCE LTER) sites are also plotted



Blacksburg, VA, USA; Atkinson and Bingman 1997) mixed with ambient water obtained at or near each study site. We dosed the equivalent amount of site water to the 6 control chambers at each marsh. Dosing mixtures for saltwater treatments were adjusted, to control for dilution, based on ambient conditions at the study marsh each month to account for seasonal changes in water level and surface water salinity at each marsh. We used a mass balance equation for the volume of dose-water to be added to each mesocosm as described in Stachelek et al. (2018). Our target was to double ambient porewater salinities at freshwater and brackish sites for 24 h post-dosing. Therefore, the target porewater salinity for the brackish marsh was 20 ppt, and the salinity of the dosing solution ranged from 30.7–65.0 ppt (Stachelek et al. 2018). The target porewater salinity for the freshwater marsh was 5 ppt, and the salinity of the dosing solution ranged from 26.8 to 68.0 ppt.

Before each dosing event, experimental dosing chambers were sealed to maintain treatment exposure. Artificial seawater solution was pumped through a hose to irrigate the surface of each treatment chamber. We were careful to not apply the dosing mixture directly on the vegetation and rinsed plants off with freshwater immediately following dosing. We kept the chambers closed for 24 h post-dosing to allow the dosing water to mix with the porewater; after sampling, we reopened the dosing chambers to allow for ambient exchange and to minimize any chamber effects.

Porewater Physicochemistry

Three porewater sippers with an aquarium aeration stone (4 cm long × 1 cm diameter) were inserted to a 15-cm depth within each chamber. Porewater was collected monthly, 24 h after each dosing event, using a 60-mL syringe by placing suction on the sipper and evacuating at least one sipper volume before sampling. Water was field filtered (0.7-µm GF/F) into clean HDPE bottles. Porewater was analyzed for temperature, salinity, and pH immediately after extraction using a YSI Model 600 XL (Xylem, Inc., Yellow Springs, OH, USA).

Porewater soluble reactive phosphorus (SRP), total dissolved P (TDP), and total dissolved N (TDN) were analyzed by the South Florida Water Management District Water Quality Laboratory on an Alpkem Flow Solution Analyzer (OI Analytical, College Station, TX, USA) following Standard Method 4500-P F (SRP) and Solorzano and Sharp (1980, TDP). Ammonium (NH_4^+) and dissolved inorganic N (DIN) were measured using a Lachat Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA) following Standard Method 4500-NH3 H (NH_4^+) or Standard Method 4500-N C (DIN). Sulfate (SO_4^{2-}) was analyzed using an ion chromatograph (Metrohm 881, Riverview, FL, USA). Dissolved organic C (DOC) was analyzed using a Shimadzu TOC-L analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA) following Standard Method 5310 B. Alkalinity and pH were determined using an automated titrator (Metrohm 855 Titrator, Herisau, Switzerland) following Standard Method 2320 B (Alkalinity) and a modification to Standard Method 4500 H^+ B (pH). Chloride (Cl^-) and sulfate (SO_4^{2-}) were measured using a Metrohm 881 Compact IC Pro System (Metrohm, Riverview, FL, USA) following Standard Method 4110 B. Porewater sulfide (HS^-) concentrations were quantified following the methods of McKee et al. (1988). Soil redox potential was measured using standard techniques (Faulkner et al. 1989). Briefly, three platinum-tipped probes were inserted to 15 cm depth in each plot and allowed to equilibrate for 30 min before measurement.

Root Litter Breakdown Rates

Root litter bags were deployed in all mesocosms to track root litter breakdown rates. In October 2014, we collected live *E. cellulosa* and *C. jamaicense* from the freshwater and brackish marsh and the Water Conservation Area 3A (25 46′ 09.3” N, 80 40’ 22.6” W). We rinsed the roots of sediment and dried in an oven at 60 °C. Litter bags (10 × 30 cm, containing three depth compartments) were constructed from 1-mm nylon mesh. We added a known amount (2.93 ± 0.02 g) of the dried root material to each mesh compartment. Litter bags deployed at the brackish marsh were filled with roots of *C. jamaicense* to represent the dominant vegetation. Litter bags used at the freshwater marsh were filled with either *E. cellulosa* or *C. jamaicense* roots; each saltwater treatment and control chambers was randomly assigned to *E. cellulosa*, or *C. jamaicense* to represent the dominant vegetation. Five litter bags were incubated within each chamber at the freshwater marsh, and 6 bags were incubated within each chamber at the brackish marsh in November 2014. Litter bags were inserted vertically into the soil profile so that the three compartments represented 0–10, 10–20, and 20–30 cm depths. We retrieved litter bags after 30, 105, 194, 383, 576, and 740 days (the 383-day collection occurred only at the brackish marsh). Directly following removal from the field, samples were placed on ice and transported to the laboratory for extracellular enzyme analysis. New root growth into the litter bags was easy to identify and removed during rinsing and not included in the analysis of the decomposing root material. Oven-dried (40 °C for 24 h) root litter was weighed to obtain dry mass and combusted at 550 °C for 4 h to estimate ash-free dry mass (AFDM). Breakdown rates were calculated for the first (30 days) and final (740 days) collections to distinguish between short- and long-term changes in root litter mass loss (Romero et al. 2005). We used the first-order, exponential loss decay model, $M_t = M_o e^{-kt}$, where M_t is the AFDM at time t (30 and 740 days), M_o is original dry mass, and k is degradation coefficient (d^{-1}; Olson 1963). We used AFDM data collected from all dates to estimate k at 740 days.

Root Litter Elemental Content and Stoichiometry

Root litter nutrient accumulation or loss was tracked by measuring elemental C, N, and P content and their ratios. We calculated the absolute mass as the product of dry mass remaining and C, N, or P content of litter following the methods of Davis III et al. (2003). After retrieval from the field, we rinsed the roots of sediment and dried them in an oven at 60 °C. Subsamples of root litter were ground to a homogeneous powder using a ball mill (Spex CertiPrep, Metuchen, New Jersey) and analyzed for %C, %N, and %P content. Root tissue %C and %N were analyzed using a Carlo Erba Elemental Analyzer (Carlo Erba, Milan, Italy), and %P was determined acid digestion of ground, combusted samples that were analyzed spectrophotometrically (Solórzano and Sharp 1980). Molar ratios (C:N, C:P, N:P) were calculated for all root samples. The elemental content of root litter was also measured on a subsample of the initial starting material. *E. cellulosa* was 45.0 %C, 0.35% N, and 0.02 %P. *C. jamaicense* was 45.8 %C, 0.3 %N, and 0.03 %P.

Extracellular Enzyme Potential

We measured extracellular enzyme activities on root litter, after it was rinsed of sediment, during each litter bag collection. We measured the fluorometric activities of the following extracellular enzymes with the substrate (in parentheses): acid phosphatase (4-MUF- phosphate), arylsulfatase (4-MUF- sulfate), β-1,4-glucosidase (4-MUF- β-D glucopyranoside), β-1,4-cellobiosidase (4-MUF- β-D cellobioside), and leucine aminopeptidase (L-Leucine-7-amido-4-methylcoumarin hydrochloride) using previously described methods (Saiya-Cork et al. 2002). Briefly, root litter subsamples (approximately 1 g) were taken from each root litter bag compartment, homogenized in 60 mL of 50 mM of sodium acetate buffer, and loaded onto a 96-well plate with the appropriate substrate (Servais et al. 2019). Fluorescence was read at 365 nm excitation and 450 nm emission using a Synergy H1 microplate reader (BioTek, Winooski, Vermont, USA). We incorporated blanks and controls within each microplate to account for autofluorescence and quenching.

Bulk Soil Properties

In addition to root litter bag collections, we collected soil cores (2.3 cm diameter × 30 cm deep) after 2 years of monthly experimental dosing. We collected one core from each chamber and sectioned it into three depths (0–10, 10–20, 20–30 cm). We analyzed soil at each depth section for extracellular enzyme potential and %C, %N, and %P, as well as C:N, C:P, and N:P molar ratios, as described above. To estimate the mass of the living microorganisms within the soil, we determined the microbial biomass C from soil subsamples from the 0–10 and 10–20 cm depths using chloroform fumigation and potassium sulfate extraction methods following Vance et al. (1987). Dissolved organic C samples were analyzed with a Shimadzu 5000 TOC Analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA). We calculated microbial biomass C as the difference in DOC between non-fumigated and fumigated samples.

Data Analyses

We calculated the average salinity, pH, alkalinity, DOC, TDN, NH_4^+, SRP, TDP, SO_4^{2-}, HS^-, temperature, and soil redox measured 24 h after each monthly dosing event for 2 years for each marsh site. We compared means of all biogeochemical parameters using Welch's two-sample *t* test. We compared enzyme potential (acid phosphatase, alkaline phosphatase, arylsulfatase, β-1,4-glucosidase, β-1,4-cellobiosidase, and leucine aminopeptidase), root liter proportion AFDM remaining, and root litter stoichiometry (%C, %N, %P, C:N, C:P, N:P) among control and saltwater treatment chambers using a two-way repeated measure analysis of variance (rmANOVA). For the brackish marsh, treatment ($n = 2$) and date ($n = 6$) were fixed factors, and chamber was a random factor. For the freshwater marsh treatment ($n = 2$), date ($n = 5$), and species of the litter were fixed factors, and chamber was a random factor. We analyzed each soil depth (0–10, 10–20, and 20–30 cm) separately. All two-way rmANOVA analyses were assessed for temporal differences using the least squared means (LSMEANS), with the date as a model effect (R package lsmeans, Lenth 2017). All proportion data (proportion AFDM root litter remaining, %C, %N, and %P) were transformed by taking the arcsine square root of each datum before the analysis. For soil extracellular enzyme activity measured at year two, we compared the saltwater treatment to control within each marsh using Welch's two-sample *t* test. All statistical analyses were performed using RStudio version 1.0.136 (R Core Team 2017).

We used a path analysis to estimate hypothesized correlations between variables (e.g., Manning et al. 2015). We constructed a path model with hypothesized links based on previous studies of how stoichiometry, salinity, and enzyme activities affect litter breakdown rates (e.g., Servais et al. 2019, Fig. 1). We used eleven predictor variables for root litter *k* when it was affected by our saltwater treatment: phosphatase, arylsulfatase, β-1,4-glucosidase, and β-1,4-cellobiosidase enzyme activities, salinity, litter C:N, litter C:P, porewater DOC, porewater TN and TDN, porewater SRP, and porewater SO_4^{2-}. We removed links from the model to improve model parsimony in cases where maintaining a specific link had a negligible impact on the overall model fit based on nonsignificant parameter estimates. We evaluated the support for each model based on Akaike's Information Criterion (AIC; Anderson and Burnham 2002). To increase the number of replicates in our path analysis, we used individual replicates of k, C:N, C:P, and extracellular enzyme activities from each depth.

Results

Porewater Physicochemistry

At the freshwater marsh, 24 h after dosing, porewater salinity concentrations were an average of 9× in our saltwater treatment chambers (Table 1). Saltwater pulses increased pH but had no effect on alkalinity (Table 1). Dissolved organic C, temperature, and soil redox were not different between saltwater treatment and control chambers (Table 1). Total dissolved N and NH_4^+ were 1.5× and 2.2× higher in the saltwater treatment chambers compared to the controls 24 h after each dosing event (Table 1). Both SRP and TDP were not different between control and saltwater treatment chambers 24 h after dosing (Table 1). Sulfate was 138× higher in the saltwater treatment chambers compared to the control chambers 24 h after dosing (Table 1). Sulfide was also higher in the saltwater treatment chambers compared to the controls (Table 1).

At the brackish marsh, we increased porewater salinity an average of 1.5× in our saltwater treatment chambers 24 h after dosing events (Table 1). Porewater temperatures were not different between control and saltwater treatment wetland chambers (Table 1). Saltwater additions lowered porewater pH and alkalinity (Table 1). After 24 h, TDN, NH_4^+, SRP, and TDP were all reduced by approximately 50% with added saltwater (Table 1). Sulfate (SO_4^{2-}) concentrations were 2.4× higher with added saltwater within 24 h after dosing (Table 1), whereas sulfide (HS^-) was 2.3× lower in saltwater treatment

Table 1 Average (± 1 SE) of porewater salinity, pH, alkalinity, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), ammonium (NH_4^+), soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), sulfate (SO_4^{2-}), sulfide (HS^-) temperature, and soil redox potential from the brackish and freshwater marshes over the 2-year duration of the study. Porewater samples were taken at 15 cm depth from the ambient water (control $n = 6$ at each site) and saltwater (treatment $n = 6$ at each site) addition plots. Salinity is reported in ppt. Alkalinity, DOC, TDN, NH_4^+, and SO_4^{2-} are mg L^{-1}. SRP, TDP, and HS^- are μmol L^-. Temperature is reported in °C. Soil redox is reported in mV. Controls and saltwater treatments were compared for each constituent using Welch's t test

	Control	Treatment	Significance
Freshwater marsh			
Salinity	0.28 (0.01)	2.61 (0.10)	$P < 0.00001$; $t_{(126.4)} = 22.61$
pH	7.45 (0.03)	7.58 (0.03)	$P = 0.005$; $t_{(225.1)} = 2.83$
Alkalinity	174.26 (3.15)	179.79 (4.37)	$P = 0.31$; $t_{(205.7)} = 1.03$
DOC	27.79 (1.92)	23.59 (1.16)	$P = 0.06$; $t_{(187.0)} = 1.87$
TDN	0.96 (0.03)	1.41 (0.03)	$P < 0.00001$; $t_{(208.5)} = 10.72$
NH_4^+	0.36 (0.02)	0.80 (0.03)	$P < 0.00001$; $t_{(209.5)} = 10.69$
SRP	0.06 (0.00)	0.06 (0.01)	$P = 0.86$; $t_{(239.3)} = 0.18$
TDP	0.30 (0.01)	0.32 (0.01)	$P = 0.37$; $t_{(227.0)} = 0.90$
SO_4^{2-}	0.85 (0.25)	177.12 (7.81)	$P < 0.00001$; $t_{(114.2)} = 22.55$
HS^-	0.00 (0.00)	0.05 (0.01)	$P < 0.00001$; $t_{(53.6)} = 5.26$
Temperature	27.76 (0.38)	28.18 (0.38)	$P = 0.44$; $t_{(251.0)} = 0.77$
Soil redox	147.68 (7.50)	135.06 (9.73)	$P = 0.32$; $t_{(170.19)} = 1.00$
Brackish marsh			
Salinity	10.76 (0.16)	15.55 (0.18)	$P < 0.00001$; $t_{(281.0)} = 19.51$
pH	7.62 (0.02)	7.53 (0.02)	$P = 0.0003$; $t_{(269.1)} = 3.51$
Alkalinity	537.30 (10.76)	305.18 (6.03)	$P < 0.00001$; $t_{(213.8)} = 18.82$
DOC	138.99 (1.56)	97.37 (1.56)	$P < 0.00001$; $t_{(273.0)} = 18.90$
TDN	7.83 (0.15)	4.36 (0.07)	$P < 0.00001$; $t_{(193.4)} = 20.61$
NH_4^+	4.34 (0.13)	1.97 (0.06)	$P < 0.00001$; $t_{(200.2)} = 16.78$
SRP	4.18 (0.18)	1.98 (0.10)	$P < 0.00001$; $t_{(220.3)} = 11.01$
TDP	6.66 (0.28)	3.03 (0.14)	$P < 0.00001$; $t_{(211.5)} = 9.71$
SO_4^{2-}	370.28 (13.81)	899.45 (20.56)	$P < 0.00001$; $t_{(237.9)} = 21.34$
HS^-	2.82 0.13)	1.25 (0.07)	$P < 0.00001$; $t_{(155.1)} = 9.71$
Temperature	29.03 0.33)	29.26 (0.34)	$P = 0.64$; $t_{(286.0)} = 0.47$
Soil redox	42.41 (11.92)	95.32 (10.75)	$P = 0.0006$; $t_{(229.7)} = 3.27$

Table 2 Average (± standard deviation) root breakdown rates (k d^{-1}) after 30 and 740 days for both freshwater and brackish marshes. Saltwater treatment k and control k were compared using a Welch two-sample t test at each time point and for both *E. cellulosa* and *C. jamaicense*

	Control	Treatment	Significance
Freshwater marsh			
E. cellulosa			
30 days	0.0100 (0.0002)	0.0094 (0.0005)	$P = 0.25$; $t_{(11.9)} = 1.19$
740 days	0.0004 (0.0000)	0.0003 (0.0001)	$P = 0.40$; $t_{(10.6)} = 0.88$
C. jamaicense			
30 days	0.0086 (0.0003)	0.0121 (0.0017)	$P = 0.08$; $t_{(8.7)} = 2.00$
740 days	0.0005 (0.0002)	0.0002 (0.0001)	$P = 0.19$; $t_{(12.5)} = 1.38$
Brackish marsh			
C. jamaicense			
30 days	0.0096 (0.0003)	0.0120 (0.0005)	$P = 0.00021$; $t_{(28.9)} = -3.97$
740 days	0.0005 (0.0000)	0.0005 (0.0001)	$P = 0.43$; $t_{(30.2)} = -0.79$

compared to control chambers (Table 1). Soil redox was approximately 50 mV higher in the saltwater treatment compared to the control chambers (Table 1).

Root Litter Breakdown Rates

At the freshwater marsh, neither short-term (30 days) nor long-term (740 days) k were different between the control and saltwater treatment chambers for either *E. cellulosa* or *C. jamaicense* (Table 2). At the brackish marsh, short-term k was 1.25× higher in the saltwater treatment compared to the controls (Table 2). However, long-term k was not different between controls and saltwater treatment chambers (Table 2).

At the freshwater marsh, saltwater treatment and days of incubation had an interactive effect on root litter %AFDM remaining within the 0–10 cm depth, whereby saltwater treatment chambers showed higher %AFDM remaining on the third collection compared to the controls indicating potential salt inhibition (Supplemental Table 1). There were no effects of the treatment, date, or litter species on %AFDM remaining in the 10–20 cm depth (Supplemental Table 1). There was an effect of species and an interaction between date and species on %AFDM remaining in the 20–30 cm depth (Supplemental Table 1, Fig. 3). *E. cellulosa* root litter from the first collection (30 days, December 2014) had lost approximately 27% AFDM and only lost an additional 5% AFDM by the last collection (740 days, November 2016) across all depths and treatments. *C. jamaicense* root litter from the first collection (30 days, December 2014) had lost approximately 28% AFDM and only lost an additional 1% AFDM by the last collection (740 days, November 2016) across all depths in both saltwater treatment and control.

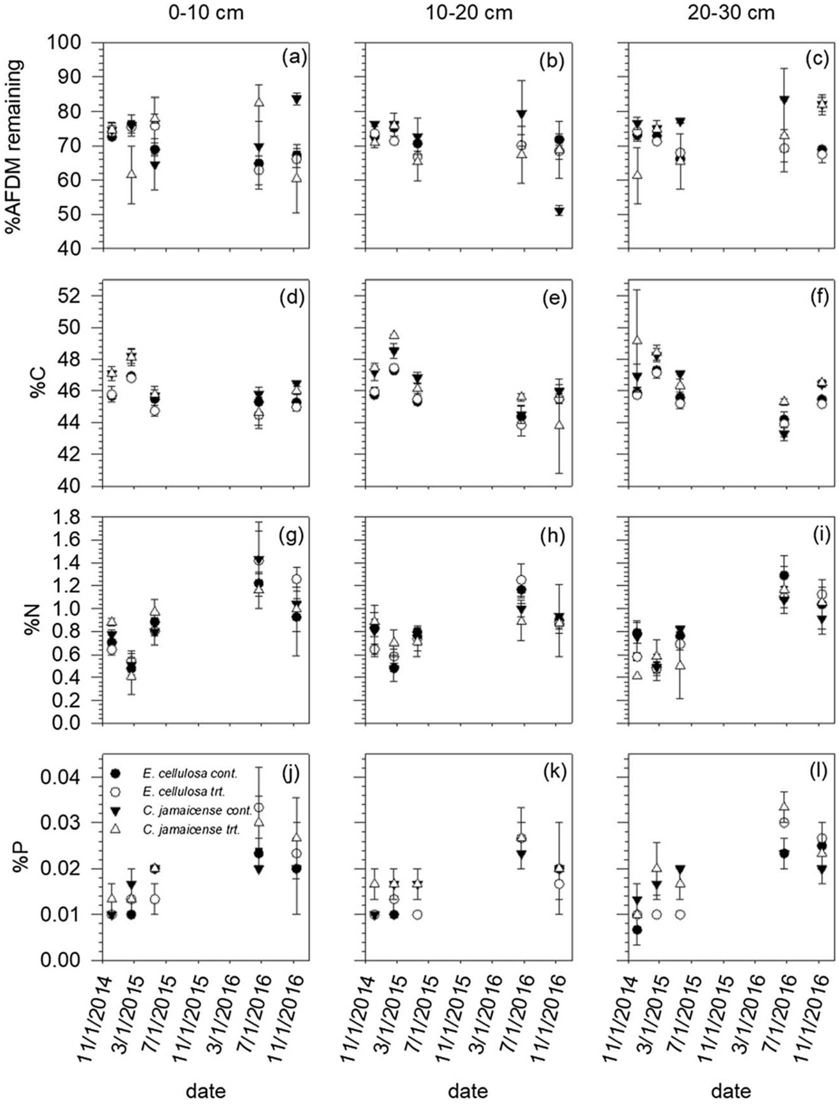
At the brackish marsh, root litter % AFDM remaining decreased over time at all depths (Supplemental Table 2, Fig. 4a, b, c). Root litter from the first 30 days lost approximately 27% AFDM, and only an additional 9% was lost after 740 days across all depths in both saltwater treatment and control. The saltwater treatment alone did not affect percent AFDM remaining. However, there was an interaction between date and saltwater treatment that resulted in differences in % AFDM remaining on the 576-day collection (June 2016) within the 0–10 cm depth.

Root Litter Elemental Content and Stoichiometry

At the freshwater marsh, differences in litter stoichiometry were attributed to litter species and sampling date (Supplemental Table 1). Within the 0–10 cm depth, root litter %C, %N, and %P changed over time (Supplemental Table 1, Fig. 3d, g, j). *E. cellulosa* root litter had lower %C than *C. jamaicense* root litter within the 0–10 cm depth (Supplemental Table 1). Within the 10–20 cm depth, root litter %C, %N, and %P varied over time (Supplemental Table 1, Fig. 3e, h, k). Within the 20–30 cm depth, there was an interaction between date and root litter species for %P, and effect of date alone on %C, %N, %P (Supplemental Table. 1, Fig. 3f, i, l). Within the 20–30 cm depth, *E. cellulosa* had lower %P than *C. jamaicense* after 30, 105, and 194 days of incubation (December 2014, February 2015, and May 2015), but the differences in %P declined over time (Fig. 3). Root litter %C for both species were similar throughout breakdown and remained above 45% during the first 6 months of breakdown and dropped below 45% after that (Fig. 3). Root litter %N and %P for both species were lower after 30, 105, and 194 days of incubation and tended to be higher in the 576-day and 740-day collections (June and November 2016) across all depths (Fig. 3). In the freshwater marsh, changes in root litter C:N, C:P, and N:P varied over time and by root litter species. Overall, C:N and C:P was highest at the second collection before declining after a year of incubation, whereas N:P generally decreased across sampling dates.

At the brackish marsh, the majority of changes in litter stoichiometry were attributed to sampling date. At 0–10 cm depth, root litter %C was unaffected by pulsed saltwater or

Fig. 3 Percent ash-free dry mass (AFDM) remaining, % carbon (C), % nitrogen (N), and % phosphorus (P) of root litter material collected from the control (cont.) and treatment (trt.) litterbags at the freshwater marsh from five retrieval dates and three depths (0–10, 10–20, 20–30 cm). Results from the repeated measures ANOVA can be found in Supplemental Table 1



days of incubation (Fig. 4d). The %N was typically higher in the saltwater treatment root liter and increased over time for both saltwater treatment and control chambers during the incubation period (Fig. 4g). The %P increased over time during the incubation period but was not affected by pulsed saltwater (Fig. 4j; Supplemental Table 2). At 10–20 cm depth, root litter %C decreased between the first and fifth collection and increased slightly between the June and November 2016 collections (Fig. 4e). At 10–20 cm depth, %N and %P increased over time (Fig. 4h, k). At 20–30 cm depth, there was an effect of time on %C which decreased over time with the lowest %C measured during the June 2016 collection (Supplemental Table 2; Fig. 4f). The %N increased over time with the highest %N estimated during the June 2016 collection (Supplemental Table 2; Fig. 4i). The %P was low during the first three collections and higher during the final three collections (Supplemental Table 2; Fig. 4l). At the brackish marsh, changes in root litter C:N, C:P, and N:P were only affected by date for all three depths and tended to decrease during the 2-year experiment (Supplemental Table 2).

Extracellular Enzyme Potential

At the freshwater marsh, there were no direct effects of pulsed saltwater on extracellular enzyme activity measured on root litter material. Phosphatase activities were low across sampling dates, except for the June 2016 collection when it was highest. Phosphatase was different between species within the 0–10 and 20–30 cm depths with *E. cellulosa* generally having higher phosphatase activities (Supplemental Table 3). There was also an interaction between species and sampling collection within the 0–10 cm depth where *E. cellulosa* had higher activities on the 576-day collection (Supplemental Table 3; Fig. 5a, b, c). There were no effects of saltwater treatment,

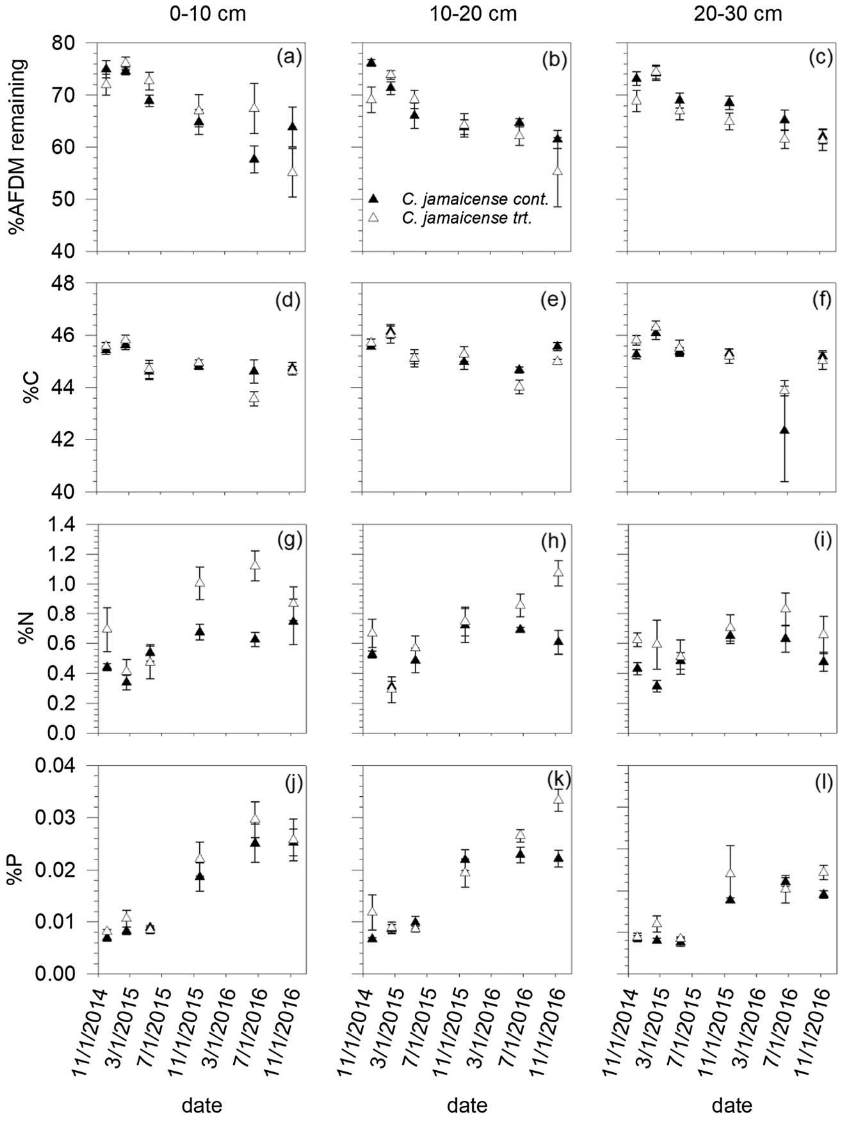
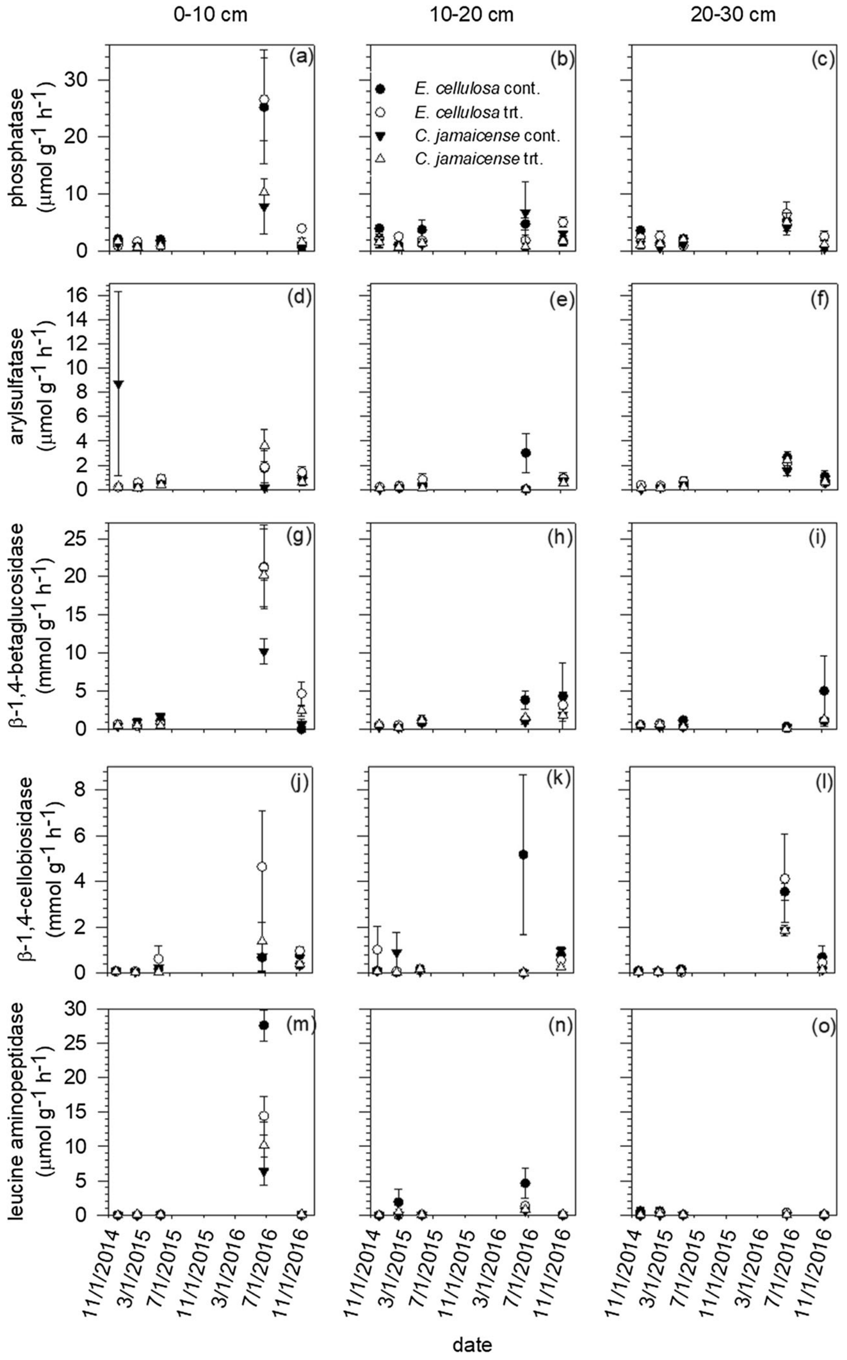


Fig. 4 Percent ash-free dry mass (AFDM) remaining, % carbon (C), % nitrogen (N), and % phosphorus (P) of root litter material collected from the control (cont.) and treatment (trt.) litterbags at the brackish marsh from five retrieval dates and three depths (0–10, 10–20, 20–30 cm). Results from the repeated measures ANOVA can be found in Supplemental Table 2

sampling date, or root litter species on arylsulfatase activity within the 0–10 cm depth (Fig. 5d). However, at the 10–20 cm depth, there was a difference between the litter species, an interaction between sampling date and saltwater treatment, and an interaction between species, date, and saltwater treatment (Fig. 5e). There was only an effect of sampling date on arylsulfatase within the 20–30 cm depth (Fig. 5f). β-1,4-glucosidase activity varied across sampling dates within all soil depths but was unaffected by saltwater treatment or the species of the litter (Fig. 5g, h, i). β-1,4-cellobiosidase varied across sampling date within the 0–10 and 20–30 cm depth, and there was an interaction between date and species within the 20–30 cm depth (Fig. 5j, k, l). However, within the 10–20 cm depth, there were no effects of saltwater treatment, date, or root litter species on β-1,4-cellobiosidase activity. Leucine aminopeptidase activity was low and often undetectable at the freshwater marsh. Within the 0–10 cm depth, leucine aminopeptidase was affected by species, sampling date, and an interaction between saltwater treatment and date. The highest leucine aminopeptidase activities were measured on *E. cellulosa* and were highest for both species after 576 d of incubation (Fig. 5m). Leucine aminopeptidase was only different across sampling dates in the 10–20 cm depth with the June 2016 collection having the highest measured activities and unaffected by saltwater treatment, date, and species in the 20–30 depth (Supplemental Table 3; Fig. 5n, o).

At the brackish marsh, there was no direct effect of pulsed saltwater on extracellular enzyme activities measured on root litter material; however, there were interactions between saltwater treatment and sampling date. Across all samples, phosphatase activities varied across sampling collections within all depths and generally increased over time with highest activities occurring at 576 days before dropping slightly on the final collection at 740 days (Fig. 6a, b). Arylsulfatase activities also varied with sampling date for all depths. There was also an

Fig. 5 Extracellular enzyme activities of phosphatase, arylsulfatase, β-1,4-glucosidase, β-1,4-cellobiosidase, and leucine aminopeptidase measured on the control (cont.) and treatment (trt.) litterbag material at the freshwater marsh. Full statistical results from the repeated measures ANOVA are reported in Supplemental Table 3



interaction between date and saltwater treatment for arylsulfatase within the 10–20 cm depth where on the final sampling collection (Fig. 6h, 11/20/2016), there was a difference between saltwater treatment and control chambers (Supplemental Table 4). β-1,4-glucosidase activities also varied across sampling collections within all depths, and highest activities were measured during the 576- and 740-day collections. There were also interactions between saltwater treatment and sampling date, within the 10–20 and 20–30 cm depth, whereby β-1,4-glucosidase activity at 740 days was 2.0× and 3.25× higher on the root litter incubated in saltwater treatment chambers compared to controls, respectively (Supplemental Table 4; Fig. 6g, h, i). β-1,4-cellobiosidase activities increased across sampling collections within all depths with highest activities measured in the 0–10 cm depth on the 383-day collection (November 2016; Fig. 6j, k, l). Leucine aminopeptidase activities were low and often undetectable within the 10–20 and 20–30 cm depths. However, leucine aminopeptidase activities varied

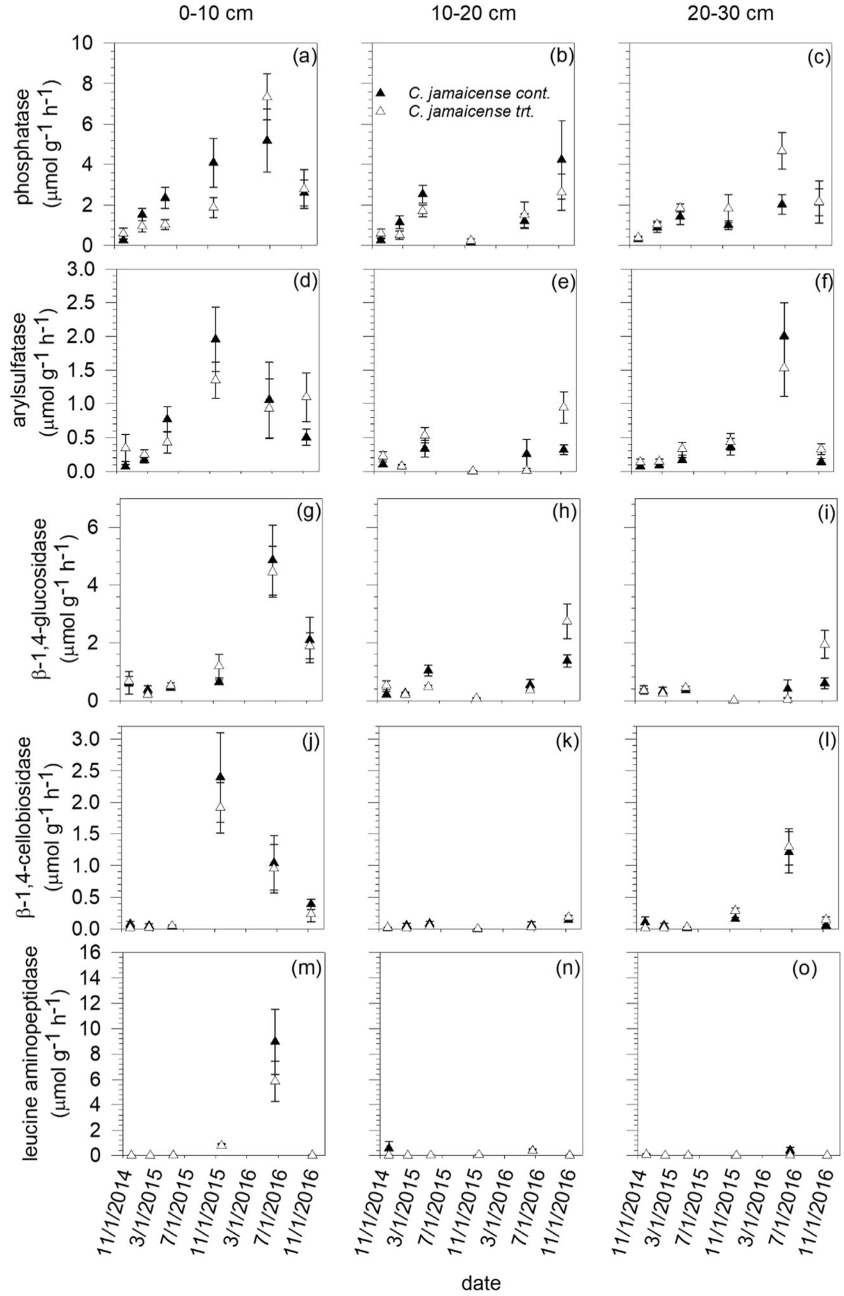


Fig. 6 Extracellular enzyme activities of phosphatase, arylsulfatase, β-1,4-glucosidase, β-1,4-cellobiosidase, and leucine aminopeptidase measured on the control (cont.) and treatment (trt.) litterbag material at the brackish marsh. Full statistical results from the repeated measures ANOVA are reported in Supplemental Table 4

across sampling collections within the 0–10 cm depth, whereby the highest activities were measured during the 576-day collection (Supplemental Table 4; Fig. 6m, n, o).

Bulk Soil Properties

At the freshwater marsh, there were no differences in bulk soil enzyme activities between saltwater treatment and control chambers, except for arylsulfatase activities in the 10–20 cm depth (Supplemental Table 5). There were no differences in soil enzyme activities between saltwater treatment and control at the brackish marsh (Supplemental Table 6). Overall, soil enzyme activities were highest in the 0–10 cm depth at both marshes (Supplemental Tables 5 and 6). Soil %C at 0–10 cm depth at the freshwater marsh was 15% lower in saltwater treatment than control chambers (Supplemental Table 7). However, there were no differences in %C at 10–20 cm or 20–30 cm depths attributed to saltwater treatment at the freshwater marsh (Supplemental Table 7). Soil %N at the freshwater marsh was not different between saltwater treatment and control at any depth (Supplemental Table 7). Soil %P was higher within the saltwater treatment chambers, but only in the 10–20 cm depth (Supplemental Table 7). At the brackish marsh, both soil %C and %P were the same between saltwater treatment

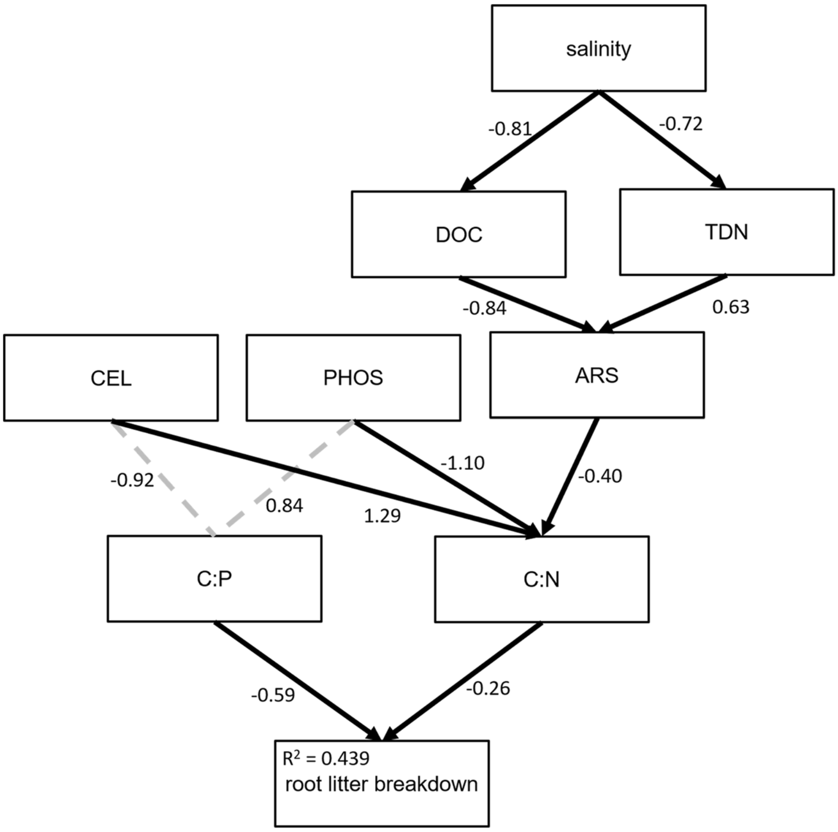


Fig. 7 The best-supported model for 30-day litter breakdown (k d^{-1}) for the brackish marsh. Standardized path coefficients are reported, and the sign of the path coefficient indicates the direction of the correlation between variables. The best-supported model explains 44% of the variation in litter breakdown rates. Dashed arrows indicate nonsignificant path coefficients ($P > 0.05$)

and control for all depths (Supplemental Table 7). Soil %N was only different in the 10–20 cm depth and was lower in the saltwater treatment chambers (Supplemental Table 7). Microbial biomass C was not different between saltwater treatment and control at either marsh (Supplemental Tables 5 and 6).

Path Analysis

We used a path analysis to test for short-term (30 days) root litter *k* from the brackish marsh, where *k* was 1.25× higher in saltwater treatment compared to control chambers (Table 2). The best-supported model predicted 44% of the variation in root litter *k*. Both C:P (−0.59) and C:N (− 0.26) were directly and negatively correlated with *k* (Fig. 7). β-1,4-cellobiosidase (1.29), phosphatases (− 1.10), and arylsulfatase (− 0.40) enzyme potential were correlated with C:N. DOC (− 0.84) and TDN (0.63) were correlated with arylsulfatase enzyme potential (Fig. 7). Salinity was negatively correlated with DOC (− 0.81) and TDN (− 0.72; Fig. 7).

Discussion

The combined effects of sea-level rise and freshwater diversion have accelerated saltwater exposure into coastal freshwater marshes. In this study, the effects of saltwater pulsing varied between the single and multiple pulses and between freshwater and brackish marshes in the Florida Everglades. We detected changes in porewater chemistry and enhanced short-term root litter *k* in brackish marsh soils. When short-term root litter *k* was affected by saltwater dosing, it was mediated by P-, S-, and C-acquiring enzymes that led to stoichiometric changes within decomposing root litter (Fig. 7). However, repeated ($n = 24$, 2 years of monthly dosing events) simulated pulses of saltwater did not affect the long-term breakdown of root litter *k* in peat soils or alter microbial enzyme activities. After 2 years of experimental exposure to saltwater pulses, soil C content was reduced at the freshwater marsh while brackish marsh soil C was unaffected.

Saltwater pulses had an immediate effect on porewater chemistry that varied between the freshwater and brackish marshes. However, the repeated saltwater pulses did not cause a build-up of salt in the soils or water column. In the freshwater marsh, saltwater additions resulted in increased porewater SO_4^{2-} and HS^-, likely because the saltwater solution used for dosing had higher concentrations of sulfate (Table 1; Atkinson and Bingman 1997) that led to increased sulfate reduction (Table 1). Saltwater additions also increased porewater N concentrations (TDN and NH_4^+) in the freshwater marsh. We attribute N increases to the release of adsorbed N compounds from soils into the porewater (Weston et al. 2006, Ardón et al. 2013). Salt exposure increases the concentrations of marine-derived dissolved ions causing desorption of nutrient ions from exchange sites (Rosenfeld 1979, Liu and Lee 2007, Ardón et al. 2013, Tully et al. 2019). In the brackish marsh, pulsed saltwater decreased dissolved porewater constituents, namely DOC, TDN, NH_4^+, SRP, TDP, and HS^-. The brackish marsh, previously exposed to seawater, may have already exported desorbed nutrients from previous saltwater addition and, when exposed to pulses of saltwater, experienced a dilution of porewater C and nutrients (Table 1). Additionally, microbe and plant communities within the brackish marsh may be better adapted to using available nutrients and C following saltwater exposure compared to freshwater species whose nutrient use may be inhibited by saltwater stress. Although we predicted that saltwater exposure would increase porewater dissolved C and nutrients, changes in porewater chemistry were only detected in the freshwater marsh which was not previously exposed to saltwater. Within the freshwater marsh, saltwater addition increased porewater N and S, but not C, whereas within the brackish marsh, saltwater addition decreased dissolved nutrients and C (Table 1). The porewater results indicate that saltwater exposure alters porewater constituents, but the effect is site-specific and may depend on legacies of previous saltwater exposure.

We hypothesized that soil C loss would be associated with pulsed saltwater addition. However, we only detected changes in bulk soil organic C at the freshwater marsh where soil organic C was 15% lower within the saltwater treatment chambers after 2 years (Supplemental Table 2). The loss of soil organic C can be attributed to both the export of DOC in the porewater and to decreased C inputs from plant root growth (Wilson et al. 2018), as well as soil elevation loss (Charles et al. 2019). Results from previous studies are mixed and have reported both increases and decreases in soil C with saltwater additions (Weston et al. 2006; Weston et al. 2011; Neubauer et al. 2013). Adding to the variable responses of soil C to saltwater addition found in the literature, we did not observe changes in soil organic C at the brackish marsh. Freshwater marshes experiencing saltwater addition for the first time are likely more sensitive to effects of salt exposure relative to brackish marshes where the largest responses to saltwater may already have occurred. A recent study found that freshwater marshes exposed to continuous low-concentration salinity (~ 7 ppt) can lose 2.6 cm of soil elevation after 160 days (Charles et al. 2019). Environmental conditions at each marsh and legacies of salt exposure in brackish marshes may influence how saltwater addition affects soil organic C.

Despite the abrupt and maintained changes to soil and porewater chemistry, saltwater pulses did not affect long-term changes in the proportion of root litter remaining at both freshwater and brackish marshes. Root litter lost approximately 30% dry mass within the first 30 days and only an additional 5–10% by the end of the 740-day incubation at both marshes (Table 2). Previous breakdown studies within the Everglades report similar patterns of litter breakdown. For example, leaching accounts for about 33% of mass losses in *Rhizophora mangle* in the first 3 weeks of incubation (Newman et al. 2001; Davis III et al. 2003). However, breakdown studies from other wetlands have shown mixed effects with some studies showing increased decomposition with saltwater (Mendelssohn et al. 1999; Craft 2007) and others that found saltwater decreased decomposition (Mendelssohn et al. 1999, Davis III and Childers 2007). In our experiment, saltwater dosing only affected short-term breakdown at the brackish marsh where k increased 1.25× in the saltwater treatment chambers.

We characterized the variation in short-term root litter k at the brackish marsh using a path analysis. Exposure to one pulse of elevated saltwater resulted in a 1.25× increase in short-term root litter k at the brackish marsh. Both C:N and C:P were negatively correlated with root litter k, a result also found on decaying leaves in streams (Fig. 7, Manning et al. 2015). The inverse relationship between C:N and C:P and litter breakdown reflects lost C from the litter relative to N and P (Zechmeister-Boltenstern et al. 2015). Short-term breakdown rates at the brackish marsh were indirectly affected by changes in P-, S-, and C-acquiring enzyme activity that altered elemental stoichiometry. Salinity was important in determining S-acquiring enzyme activities which increased with decreasing concentrations of DOC and TDN within the porewater (Fig. 7). We were able to characterize 44% of the variation in short-term brackish k; therefore, we did not capture all the factors contributing variation in short-term k. For example, we anticipate that exposure to saltwater increases leaching of soluble materials during the initial stages of litter breakdown and may help explain why there is only a short-term effect of saltwater addition in our study. Typically, the leaching of soluble compounds dominates changes in the mass loss early on while microbe-mediated breakdown occurs at longer timescales (Valiela et al. 1985). In a leaf leaching experiment, Davis et al. (2006) found that abiotic leaching accounted for the greatest losses in C across four different Everglades plant species and may explain the remaining variation in short-term brackish k in this study. More work is necessary to understand how saltwater affects both abiotic and biotic processes contributing to root litter decomposition.

Anaerobic conditions in wetlands enable soil C storage by constraining microbial activities (Freeman et al. 2001, Tokarz and Urban 2015), as low oxygen concentrations limit many biogeochemical cycles (Helton et al. 2015). During our 2-year study, water levels infrequently fell below the soil surface at either freshwater or brackish marsh sites (freshwater 39 days, brackish 132 days); thus, near-continuous soil inundation at our experimental marsh sites maintained reduced soil conditions that served as the primary limitation to microbial activities. The non-detectable responses of microbial extracellular enzyme activities and long-term breakdown rates to pulsed saltwater exposure may be caused by oxygen limitation as soil inundation and waterlogging can result in decreases in the production of new enzymes and increased concentrations of enzyme inhibitors (Pulford and Tabatabai 1988; Freeman et al. 1996). Since the timing of the dry-down events overlaps the sampling of decomposing root litter, we cannot directly test effects of water level on *k* or enzyme activities. However, we have shown that dry-down events interact with salinity pulses to increase CO_2 release from brackish marshes (Wilson et al. 2018). In studies manipulating both water level and salinity, water level seems to contribute more to changes in C and N cycling (Liu et al. 2017). Pulses of saltwater nearly doubled brackish soil redox potential, yet soils remained reduced and there was little change in enzyme activities compared to the controls. Additionally, higher enzyme activities associated with roots and soils at shallower depths compared to those at lower depths provide further evidence that oxygen-limited conditions might have suppressed microbial activity in our study marshes and maintained slow breakdown rates despite changes in salinity as has been shown in other studies where reduced soil conditions and inundation limited microbe-mediated breakdown of organic matter (McKee and Seneca 1982; White and Trapani 1982).

Extracellular enzyme activities changed more with time than with treatment and generally increased during the first year (Figs. 5 and 6). However, we detected increased β-1,4-glucosidase activity on decomposing root litter after 2 years of pulsed salinity dosing at the brackish marsh. Previous studies have suggested that increases in β-1,4-glucosidase activity are explained by increases in cellobiose (Chröst 1991). β-1,4-glucosidase is a mediator of cellulose degradation and can be considered the rate-limiting step in the degradation of cellulose (Sinsabaugh et al. 1993; Alef and Nannipieri 1995). Previous salt dosing studies also found a negative effect of salinity on glucosidase activities in soils (Jackson and Vallaire 2009; Neubauer et al. 2013). However, in our study, we did not detect higher activities of β-1,4-glucosidase on the bulk soil and the increased β-1,4-glucosidase we did detect on root litter did not contribute to changes in *k*.

Our results indicate that microbial enzyme activities in reduced soils of coastal wetland ecosystems have low sensitivity to pulsed saltwater addition and are not a predominant biological factor explaining observed C losses (Wilson et al. 2018). Changes in environmental conditions may alter the composition of the biologically active microbial community that is producing extracellular enzymes (Kearns et al. 2016). Although the focus of this study was on how microbial functioning (i.e., extracellular enzymes) responded to repeated pulses of saltwater, but there is some evidence that our saltwater treatment affected microbial community structure at the freshwater marsh but not at the brackish marsh (Martens-Habbena, unpublished data). In an experimental wetland mesocosm study, freshwater marsh soils continuously exposed to saltwater had reduced extracellular enzyme activities (Servais et al. 2019). In mangrove soils of the Everglades, inundation has been shown be a stronger control on enzyme activities than salinity (Chambers et al. 2016). Other studies have documented how inundation controls microbial functioning. For example, Freeman et al. (1996) found that P-, S-, and C-acquiring enzymes activities were increased up to 70% within peat soils after water table drawdown. Therefore, we may expect drier conditions to increase enzyme activity and organic mineralization (Freeman et al. 1996) and drier soils to be more sensitive to pulsed saltwater exposure. Changes in microbe-mediated breakdown may only occur with sustained saltwater exposure, more extreme levels of salinity, or when other controls on microbial processing, like inundation, are also altered.

Conclusions

Coastal peat soils are susceptible to large-scale C loss from extended duration saltwater exposure (Wanless and Vlaswinkel 2005; Day et al. 2011). In the Florida Everglades, soil loss has occurred over the past 2000 years ago, leading to the formation of coastal lakes and lagoons (Wanless and Vlaswinkel 2005). Evidence from our experimental salinity dosing in a brackish marsh illustrates that C losses are occurring further inland as a result of saltwater exposure that is exacerbated by sea-level rise and drought events (Wanless and Vlaswinkel 2005; Wilson et al. 2018; Chambers et al. 2019). However, our results also suggest that increased breakdown of plant root-derived, detrital organic matter may not be the primary mechanism of soil elevation and C loss within the Everglades. The lack of strong microbe-mediated responses to pulsed saltwater in our study might also be because the salinity level was not high enough to elicit responses or that responses to saltwater have already been triggered. Additionally, inundation and reduced conditions in coastal wetlands limit the decomposing processes of soil microbial communities associated with organic matter and may explain the lack of strong responses to pulsed saltwater dosing we found in our study (Chambers et al. 2019). Future research priorities should focus on how environmental conditions (e.g., drought, temperature) interact with saltwater exposure to affect microbial community structure and function in coastal wetland soils. Understanding the underlying mechanisms controlling organic matter breakdown, such as microbial extracellular enzyme activities, is critical to understanding how biogeochemical cycling will be altered by climate change and land management (Sinsabaugh et al. 2002).

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