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Saltwater intrusion and soil carbon loss: Testing effects of salinity and phosphorus loading on microbial functions in experimental freshwater wetlands

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ABSTRACT

Wetlands can store significant amounts of carbon (C), but climate and land-use change increasingly threaten wetland C storage potential. Carbon stored in soils of freshwater coastal wetlands is vulnerable to rapid saltwater intrusion associated with sea-level rise and reduced freshwater flows. In the Florida Everglades, unprecedented saltwater intrusion is simultaneously exposing wetlands soils to elevated salinity and phosphorus (P), in areas where C-rich peat soils are collapsing. To determine how elevated salinity and P interact to influence microbial contributions to C loss, we continuously added P (~0.5 mg P d^{-1}) and salinity (~6.9 g salt d^{-1}) to freshwater *Cladium jamaicense* (sawgrass) peat monoliths for two years. We measured changes in porewater chemistry, microbial extracellular enzyme activities, respiration rates, microbial biomass, root litter breakdown rates (*k*), and soil elemental composition after short (57 d), intermediate- (392 d), and long-term (741 d) exposure. After 741 days, both β-1,4-glucosidase activity ($P < 0.01$) and β-1,4-cellobiosidase activity ($P < 0.01$) were reduced with added salinity in soils at 7.5–15 cm depth. Soil microbial biomass C decreased by 3.6× at 7.5–15 cm ($P < 0.01$) but not 0–7.5 cm depth ($P > 0.05$) with added salinity and was unaffected by added P. Soil respiration rates decreased after 372 d exposure to salinity ($P = 0.05$) and did not change with P exposure. Root litter *k* increased by 1.5× with added P and was unaffected by salinity exposure ($P > 0.01$). Soil %C decreased by approximately 1.3× after 741 days of salinity exposure compared to freshwater controls ($P < 0.01$). Elevated salinity and P accelerated wetland soil C loss primarily through leaching of DOC and increased root litter *k*. Our results indicate that freshwater wetland soils are sensitive to short- and long-term exposure to saltwater intrusion. Despite suppression of some soil microbial processes with added salinity, salt and P exposure appear to drive net C losses from coastal wetland soils.

1. Introduction

Wetlands are critical carbon (C) reservoirs and store disproportionate amounts of C relative to the total land area, with some capable of storing up to 50 times more C than tropical forests (Mcleod et al., 2011). External stressors associated with climate change have the potential to degrade wetlands and drastically alter ecosystem function (Green et al., 2017). In particular, coastal freshwater wetlands at the interface of terrestrial and marine environments and are increasingly exposed to rapid saltwater intrusion resulting from reduced freshwater deliveries and increased sea levels (White and Kaplan, 2017). Saltwater intrusion is the intrusion of marine-origin water comprised of many salt-forming ions (i.e. Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}). Increased rates of saltwater intrusion pose a threat to wetland C storage, a globally important ecosystem service driven by historical and current environmental conditions that promoted carbon dioxide (CO_2) uptake and its sequestration as organic C within the soil and plant biomass. Further, exposure of coastal freshwater marshes to unprecedented rates of saltwater intrusion affects the biogeochemical cycles that support C storage through the introduction of marine ions which can potentially cause these systems to transition from net C sinks to net C sources (Weston et al., 2011).

Altered biogeochemical conditions with elevated salinity affect soil microbial processing and consequently the rate of C cycling in wetlands

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Table 1
Enzymes analyzed, corresponding Enzyme commission number (EC), substrate used in assay, enzyme function, and the predicted response to additions of salinity and phosphorus (P).

Enzyme	EC	Substrate	Function	Predicted salt effect	Predicted P effect	Predicted salt + P
Acid and Alkaline Phosphatase	3.1.3.2	4-MUF-phosphate	Hydrolyzes phospholipids and phosphosaccarides	↓	↓	↓
β-1,4-Cellobiosidase	3.2.1.91	4-MUF-β-D cellobioside	Hydrolyzes linkages in cellulose and cellotetraose	↓	↑	↔
β-1,4-Glucosidase	3.2.1.21	4-MUF β-D-glucopyranoside	Hydrolyzes glucose	↓	↑	↔
Arylsulfatase	3.1.6.1	4-MUF-sulfate	Hydrolyzes sulfoester bonds	↓	↔	↓

(Weston et al., 2006). Anaerobic conditions in wetland ecosystems slow biogeochemical processing rates and organic matter decomposition which promotes C storage (Helton et al., 2015). Increases in salinity can change redox potential (Rietz and Haynes, 2003; Van Ryckegem and Verbeken, 2005), increase electron acceptor availability (Helton et al., 2015), increase osmotic stress, and change organic substrate quantity and quality (Neubauer, 2013). Consequentially, saltwater intrusion can potentially increase microbial respiration, stimulating organic C loss from wetland soils. The effects of salinity on microbe-mediated biogeochemical processes can occur within days to weeks (Craft, 2007; Weston et al., 2006; Weston et al., 2011; Neubauer, 2013; Chambers et al., 2014). Salinity increases (3–15 ppt) can affect the physiochemical characteristics of freshwater wetland soils (Berner and Berner, 2012; Flower et al., 2017), and the species composition of microbial communities is linked to environmental gradients like salinity and nutrients (Ikenaga et al., 2011). However, the interactive effects of abrupt elevations in salinity and limiting nutrient availability on soil microbial functions, like extracellular enzyme activities are largely uncertain (but see Jackson and Vallaire, 2009). Further, how short- and long-term exposure to coupled additions of salinity and nutrients may differentially impact soil microbial processes that contribute to C gains or losses requires explicit testing.

Saltwater intrusion can also change biogeochemical cycling by altering concentrations of dissolved nutrients within soil porewater. For example, karstic coastal wetlands found in the Florida Everglades and throughout the Caribbean are extremely limited by phosphorus (P) supplied from marine water inputs (Fourqurean et al., 1993; Boyer et al., 1999; Noe et al., 2001; Childers et al., 2006). When saltwater infiltrates the porous limestone bedrock of the Everglades, P adsorbed to calcium carbonate is released (Price et al., 2006; Price et al., 2010; Flower et al., 2017). In freshwater wetlands of the Everglades, saltwater intrusion represents both a stress caused by elevated salinity and a resource subsidy in the form of P release, but the combined effects of elevated salinity and P on soil microbial functioning are unclear. Recent observations of peat collapse, rapid soil subsidence, have been observed within the Everglades (Wanless and Vlaswinkel, 2005; Day et al., 2011). Rapid and persistent marine intrusion into previously freshwater wetlands is hypothesized to increase soil susceptibility to collapse, by altering porewater chemistry, microbial processing rates, and plant productivity.

Soil microbes contribute to rates of C and nutrient cycling within soils (Penton and Newman, 2007). Extracellular enzymes are important drivers of microbe-mediated biogeochemical cycling, and enzyme-catalyzed biochemical reactions are considered the rate-limiting step in organic matter degradation (Chróst and Rai, 1993; Dick, 1994). When bioavailable C or nutrients limit microbes, they release extracellular enzymes into soils to meet metabolic demands. Therefore, measurements of extracellular enzymes can provide information on the quality of organic soils, nutrient cycling, and microbial elemental demand (Sinsabaugh et al., 2002). Enzyme activities are often suppressed when exposed to salinity (Frankenberger and Bingham, 1982; Jackson and Vallaire, 2009), as microbes divert resources to the production of osmolytes and consequentially reduce production of extracellular enzymes (Kempf and Bremer, 1998). However, in natural salinity gradients positive relationships between salinity and enzyme activity have been reported (Morrissey et al., 2014). In contrast, P enrichment in P-limited ecosystems generally reduces phosphatase activities (Spiers and McGill, 1979; Wright and Reddy, 2001; Morrison et al., 2016) and increases other enzymes activities (Rejmánková and Sirova, 2007). The effects of simultaneous exposure to osmotic stress and increased availability of limiting nutrients on microbial function and the evolution of microbial responses over time are unknown despite the importance of soil microbes in determining ecosystem C storage potential. In the Everglades and other coastal freshwater wetlands with organic-rich soils, changes in extracellular enzyme activities may lead to long term-effects on soil collapse and accumulation of C-storing peat soils

(Penton and Newman, 2007).

Here, we used P-limited, freshwater peat soils from the Florida Everglades to test how microbial extracellular enzyme activity, soil elemental content, root litter breakdown rates (*k*), and porewater chemistry responded to continuous exposure to elevated salinity and P and measured net effects on soil C (gains or losses). We hypothesized that (1) elevated salinity would cause increases in C and nutrients in the porewater and become available to soil microbes for metabolism (Ardón et al., 2013); (2) elevated salinity would cause microbial communities to invest more resources to maintaining cell turgor and consequentially have fewer resources devoted to the production of P- and sulfur (*S*)-acquiring enzymes (Aristi et al., 2016); (3) salinity induced P release would cause microbial communities to increase demand for energy from C and consequentially soil microbes would devote more resources to the production of C-acquiring enzymes (Table 1) (Benfield, 2006); (4) the greatest effects of salinity would occur during early exposure as the microbial community transitioned from a freshwater community to a salt-adapted community (Benfield, 2006); (5) P addition would increase potential activity for C- and sulfur (*S*)-acquiring enzymes and decrease in potential activity for P-acquiring enzymes because the release from P-limitation would increase C and other nutrient demands while lowering P demands (Table 1) (Berner and Berner, 2012); (6) salinity and P would interact to decrease P and S-acquiring enzymes and result in similar activity levels of C-acquiring enzymes relative to the freshwater control (Boyer et al., 1999); (7) soil microbial respiration rates, biomass C, and root litter *k* would be highest in the P treatment salinity (Caravaca et al., 2005). Understanding how microbial functions changes with salinity exposure and nutrient enrichment is increasingly important as coastal freshwater wetlands become more exposed to marine water supplies.

2. Materials and methods

2.1. Study area and experimental wetland facility

We collected twenty-four vegetated sawgrass-peat cores from a freshwater marsh in the Florida Everglades (25°46′06.1″N 80°28′56.2″W) in July 2014. We removed the plant-soil monoliths using shovels to excise the marsh and trimmed the excess soil and roots to fit within each mesh lined containers (0.3 m D × 0.4 m W × 0.5 m L). Monoliths were transported to the Florida Bay Interagency Science Center in Key Largo, FL USA. We randomly assigned each plant-soil monolith to one of six concrete containers (0.7 m D × 0.8 m W × 2.2 m L) and placed inside separate, open-top polycarbonate mesocosms (0.5 m D × 0.5 m W × 0.7 m L). We allowed the monoliths to equilibrate in fresh water from a nearby canal (C-111; 25°17′31.74″N, 80°27′21.59″W) for approximately seven months before beginning experimental manipulations.

2.2. Experimental design and salinity and phosphorus loading

We used a 2 × 2 factorial design with two factors, salinity and P, for a total of four treatments: (i) freshwater, (ii) freshwater + P, (iii) salinity, (iv) and salinity + P. Each concrete container was designated as receiving either freshwater or elevated salinity source waters to each mesocosm. To minimize P contamination, we isolated P treatments to one half of each concrete container, such that two mesocosms received P and the other two did not. Phosphorus was continuously added to each P treatment monolith individually by delivering 2.25 mg L^{-1} diluted phosphoric acid (H_3PO_4) at 0.14 mL min^{-1} using two multichannel peristaltic pumps, with six lines each (n = 12, Watson Marlow, Wilmington, Massachusetts, USA). The freshwater treatment (fresh) consisted of twice-weekly manual additions of fresh water collected from the nearby C-111 canal to maintain the submerged soil surfaces of each monolith. To achieve target porewater salinity concentrations (7–10 ppt) for the salinity treatment, we mixed water from the freshwater treatment with seawater from a nearby site in Florida Bay. The salinity treatment consisted of twice-weekly manual additions of the saltwater mixture to maintain submerged of soil surfaces of each monolith. Cumulative phosphorus loads were measured for each mesocosm as the daily added molar mass of P delivered to P treatment mesocosms plus the added molar mass of P from freshwater and elevated salinity source waters (based on monthly measures and the total volume of source water added to each mesocosm). Cumulative salinity loads were measured for each mesocosm as the added molar mass of chloride based on weekly salinity measures and the total volume of elevated source water added to each salinity treatment mesocosm.

Filtered and unfiltered grab samples were collected monthly from 1/6/2016 to 12/11/2016 within the freshwater and saltwater mixtures. The source water samples analyzed for TN, TP, TOC, DOC, DIN, NO_3^-, NO_2^-, NH_4^+ and SRP following the same methods described above for water samples collected from each monolith.

2.3. Source and porewater physicochemistry

We measured water temperature, pH, and salinity monthly using a YSI Model 600 XL (Xylem, Inc., Yellow Springs, OH, USA) in porewater in each of the 24 individual containers housing the sawgrass-peat monoliths. We collected monthly soil porewater (filtered) samples monthly from each plant-soil monolith (n = 24). Porewater was collected from a sipper with an air stone installed through the center of each sawgrass-peat monolith at a depth of 15 cm from the soil surface. Porewater was filtered using a 0.7-μm GF/F and transferred into a 60 mL HDPE sample bottle. All water samples were stored at −20 °C until analyzed at the Southeast Environmental Research Center, Nutrient Analysis Laboratory. Filtered porewater samples were analyzed for dissolved organic C (DOC), dissolved inorganic nitrogen (DIN, NO_3^-, NO_2^-, NH_4^+), and soluble reactive P (SRP). Dissolved inorganic N and SRP parameters were analyzed on an Alpkem RFA 300 autoanalyzer (OI Analytical, College Station, TX, USA) and DOC was analyzed with a Shimadzu 5000 TOC Analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA).

Freshwater was added to the salinity cores starting in June of 2016 to prevent salinity treatment cores from becoming hypersaline through evaporation. The sources of freshwater and saltwater were used to replenish water within each bin to maintain water levels and to reach target salinity treatment conditions.

We measured oxidation-reduction potential (hereafter redox) every other month (starting in June 2016) in each plant-soil monolith at approximately 15-cm depth, using platinum-tipped copper probes and a pH voltmeter (Faulkner et al., 1989).

2.4. Soil elemental composition

We collected soil samples after 57, 392, and 741 days of exposure to treatment conditions to assess short-, intermediate-, and long-term responses. We implemented two approaches to measure bulk soil responses: 1) we deployed surface soil pouches in the water column after short-term exposure to treatment conditions, and 2) we collected soil cores after intermediate- and long-term exposure to treatment conditions. Surface soil pouches were used to minimize oversampling of monoliths. We incubated three replicate surficial soil pouches, containing soils collected from the same location as the monoliths placed in 125-μm nylon mesh. Soils were incubated within a subset of each treatment mesocosm by suspending the soil pouch in the water column (n = 12). For the later collections, soil cores were taken from the sawgrass-peat monolith after 392 and 741 d of continuous exposure to the treatments (2 cm diameter × 15 cm depth) and sectioned into two depths (0–7.5 and 7.5–15 cm, n = 48). We dried all soil samples in an oven at 60 °C for 48 h. Ground soil material was subsampled, oven-dried (60 °C) for 48 h, weighed, combusted (550 °C for 4 h), and reweighed to determine ash-free dry mass (AFDM). Carbon and N content were analyzed using a Carlo Erba NA 1500 CHN Analyser (Carlo Erba, Milan, Italy). Phosphorus content was analyzed using the ash/acid extraction method followed by spectrophotometric analysis using the ascorbic acid method (Allen 1974, APHA 1998). We estimated elemental composition (%C, %N, and %P) in the 57 d surficial soil and at two soil depths (0–7.5 cm and 7.5–15 cm) for the 392 and 741 d collections.

2.5. Soil and root litter extracellular enzyme activities

On days 57, 392, and 741 of the experiment we measured the fluorometric activities of extracellular acid phosphatase, arylsulfatase, β-1,4-glucosidase, and β-1,4-cellobiosidase from soil sub-samples. Using the substrates described in Table 1, soil microbial enzyme activities were assayed using previously described methods (Saiya-Cork et al., 2002). Soil sub-samples were collected (approximately 1 g) from each sawgrass-peat monolith, homogenized in 60 mL of 50 mM sodium acetate buffer, and loaded onto a 96-well plate with the appropriate substrate. 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 auto fluorescence and quenching. We also calculated enzyme activities (phosphatase, arylsulfatase, β-1,4-glucosidase, and β-1,4-cellobiosidase) associated with decomposing root litter using the same method for soil enzyme activities.

2.6. Soil microbial respiration rates and biomass carbon

We measured microbial respiration in soil samples harvested at 57 d and from the two soil depths on 392 and 741 d collections. Approximately 2.5 g of wet-weighed soils were placed in respiration chambers (60 mL) within 2 h of retrieval. The chambers were filled with either the freshwater or saltwater sources depending on the sample's assigned treatment to remove headspace and incubated at room temperature (24 °C) for 2 h. Chambers filled only with the source water served as blanks. Oxygen concentrations were measured at the start and end of the incubation period to determine the rate of oxygen consumption. Soil respiration was determined by subtracting the change in oxygen concentrations in control chambers from the change in oxygen consumption in the samples to account for respiration in the water.

To estimate the mass of the living microorganisms within the soil, we determined the microbial biomass C from cores collected at the two soil depths on the 392 and 741 d collections using chloroform fumigation and potassium sulfate extraction methods following Vance et al. (1987). Soil microbial biomass C was only measured after intermediate- and long-term exposure because we expected changes in microbial biomass to be integrated over time. 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.

2.7. Root litter breakdown

Within each sawgrass-peat monolith, nylon mesh (1 mm) root litter bags (10 cm W × 15 cm L) were deployed at two depths (0–7.5 and 7.5–15 cm). We determined mass loss and quantified enzyme activities on the root litter bag enzyme activities. Each bag ($n = 24$) was filled with dried sawgrass root material of known, constant mass (0.954 ± 0.005 g). We retrieved all liter bags after 361 d of incubation to quantify the mass loss. We estimated the proportion of mass loss within each litter bag by rinsing sediments from remaining litter material, drying it to a constant mass, and calculating the proportion of mass that was lost over the 361 d incubation. We estimated breakdown rate, *k*, by first transforming by the natural log the proportion of AFDM remaining. We then produced a linear regression of the ln-transformed data versus time (Benfield, 2006). The model was $M_{361} = M_0 \times e^{-k361}$, where M_0 is the initial litter mass on day 0, M_{361} is the litter mass on day 361.

2.8. Data analyses

We used R (R Core Team, 2017) to perform all statistical analyses. We used a Student's *t*-test to compare the porewater constituents (DOC, NO_3^-, NO_2^-, NH_4^+ and SRP) of the fresh and salinity source water added to the monoliths.

We used linear mixed effects models (Package "nlme") to determine differences in porewater constituents (DOC, NO_3^-, NO_2^-, NH_4^+ and SRP), enzyme activities (phosphatase, arylsulfatase, β-1,4-glucosidase, and β-1,4-cellobiosidase) from 392 and 741 d, microbial biomass C, soil respiration, and soil elemental composition (C, N, and P) between treatments. We designated treatment and date as fixed factors and plant-soil monolith number as a random factor.

For the soil pouches collected at 57 d we tested the effect of salinity and P on the enzymes using a two-way ANOVA, followed by a Tukey's HSD post hoc test to determine differences between treatments. We tested the normality of data visually inspecting plotted residuals and, when necessary, we log-transformed the data. We set a significance factor of $\alpha = 0.05$ for all statistical analysis. We tested the effect of salinity and P on root litter *k* and the extracellular enzyme activities measured on root litter using a two-way ANOVA followed by a Tukey HSD test to determine differences between treatments.

3. Results

3.1. Salinity and phosphorus loading

In total, 25,480 ± 515.1 g m^{-2} of salt was added to each salinity treated sawgrass-peat monolith and 1.85 ± 0.00 g m^{-2} of P was added to each P treated sawgrass-peat monolith. The cumulative daily load of salt was 6,440, 22,065, and 25,480 g m^{-2}. The cumulative daily load of P was 0.14, 0.98, and 1.85 g m^{-2} of P after 57, 392, and 741 d respectively.

3.2. Source and porewater physicochemistry

The source water for the fresh and salinity treatments was not different in their pH, DOC, DIN, NO_3^-, NO_2^-, NH_4^+, and SRP (all $P > 0.05$; Supplemental Table 1). However, the salinity was higher in the salinity treatment source water compared to the freshwater treatment source water ($P < 0.05$, Supplemental Table 1).

Porewater constituents prior to initiating the experimental conditions were as follows: DOC (1343 ± 265 μg L^{-1}), DIN (0.28 ± 0.29 μg L^{-1}), NO_3^- (0.25 ± 0.25 μg L^{-1}), NO_2^- (0.08 ± 0.08 μg L^{-1}), NH_4^+ (10.34 ± 7.82 μg L^{-1}), and SRP (0.04 ± 0.02 μg L^{-1}). During the experiment, the average porewater salinity for the freshwater and freshwater + P treatment was 0.44 ± 0.01 and 0.47 ± 0.01 ppt, respectively. The average porewater salinities for the salinity and salinity + P treatments were 9.03 ± 0.28 and 8.63 ± 0.26 ppt, respectively. Porewater NO_2^-, NH_4^+, DOC, and SRP were higher in the salinity treatments ($P < 0.05$) regardless of P addition ($P > 0.05$; Supplemental Table 2).

3.3. Soil elemental composition

Soil %C was not different among treatments at 57 nor 392 d, however, at 741 d soil %C was reduced by 1.36 and 1.33× with salinity treatments compared to the fresh treatment and at 0–7.5 cm and 7.5–15 cm depths, respectively (Table 4). Soil %N increased in P treatments at 57 d ($F = 13.03$; $P < 0.01$) but was not different among treatments at 392 and 741 d (Table 2). Soil %P was higher with added P at 57 d in surficial soil pouches ($F = 26.83$; $P < 0.01$) and at 392 d at

Table 2

Soil % organic ash free dry mass carbon (C), nitrogen (N), and phosphorus (P) for the four treatments: fresh, fresh + P, salt, salt + P. Soil elemental composition was measured at 57-d for surficial soil, and at 392 and 741-d at two depths (0–7.5 and 7.5–15 cm) Treatments were compared using a two-way ANOVA. P-values < 0.05 were considered significant. Values are reported as percentages (± standard error).

	Treatment				
	Fresh	Fresh + P	Salt	Salt + P	Significance
57-d					
% C	37.02 (1.18)	39.25 (1.16)	36.78 (1.29)	36.41 (0.60)	NS
% N	2.17 (0.00)	2.43 (0.01)	2.22 (0.10)	2.38 (0.06)	P
% P	0.028 (0.00)	0.033 (0.00)	0.028 (0.00)	0.030 (0.00)	P, salt × P
392-d					
0–7.5 cm					
% C	32.21 (2.23)	32.17 (1.41)	34.43 (3.98)	31.40 (2.47)	NS
% N	1.83 (0.09)	1.79 (0.06)	1.78 (0.18)	1.82 (0.15)	NS
% P	0.03 (0.01)	0.04 (0.00)	0.03 (0.00)	0.03 (0.00)	NS
7.5–15 cm					
% C	32.68 (2.23)	31.84 (1.41)	34.51 (3.98)	32.01 (2.47)	NS
% N	1.88 (0.14)	1.83 (0.08)	1.70 (0.10)	1.72 (0.13)	NS
% P	0.03 (0.00)	0.04 (0.00)	0.03 (0.00)	0.03 (0.00)	P
742-d					
0–7.5 cm					
% C	47.12 (5.2)	42.06 (1.61)	34.68 (0.74)	35.37 (1.16)	Salt
% N	1.94 (0.26)	2.00 (0.17)	1.73 (0.11)	1.53 (0.17)	NS
% P	0.04 (0.01)	0.05 (0.00)	0.03 (0.00)	0.06 (0.01)	P
7.5–15 cm					
% C	47.91 (4.91)	43.19 (1.22)	35.92 (0.78)	36.57 (0.86)	Salt
% N	2.10 (0.34)	1.93 (0.19)	2.02 (0.14)	1.56 (0.22)	NS
% P	0.06 (0.02)	0.04 (0.00)	0.03 (0.01)	0.04 (0.000)	NS

7.5–15 cm soil depth ($F = 5.07$; P = 0.04). However, soil %P at 0–7.5 depth at 392 d was not different among treatments (Table 2). Soil %P was higher in the salinity + P treatment than the freshwater and freshwater + P treatments at 741d (Table 2).

3.4. Soil and root litter extracellular enzyme activities

Short-term exposure (57 d) to salinity suppressed most soil enzyme activities except for β-1,4-cellobiosidase (Supplemental Table 3; Fig. 1). Phosphatase activity in the salinity treatment was 2.9× lower compared to the freshwater treatment (Fig. 1a), arylsulfatase activity in the salinity treatment was 2.4× lower compared to the freshwater treatment (Fig. 1d), and β-1,4-glucosidase activity in the salinity treatment was 2.8× lower compared to the freshwater treatment (Fig. 1g). Phosphatase activities in the salt + P treatment were 1.8× lower compared to the freshwater treatment. However, arylsulfatase and β-1,4-glucosidase activities in the salt + P treatment were not different from the freshwater treatment (Fig. 1).

Intermediate- (392 d) and long-term (741 d) exposure to salinity suppressed most enzyme activities except for phosphatase (Supplemental Table 4; Fig. 1). Phosphorus exposure only directly affected phosphatase activity which was lower with added P. Time had a significant effect on all enzymes at both soil depths, whereby enzyme activities measured on 741 d were much lower than enzyme activities measured on 392 d (Supplemental Table 4; Fig. 1).

Neither phosphatase activity or β-1,4-cellobiosidase measured on the roots were different among treatments at any depth (Fig. 2b). Arylsulfatase activity within the 0–7.5 cm depth was significantly lower in the salinity + P treatment compared to the fresh and was 2.9× lower compared to the freshwater treatment (Fig. 4c). Similarly, β-1,4-glucosidase activity for the salinity treatment was significantly lower, within the 0–7.5 cm depth, and was 1.7× lower compared to the freshwater treatment (Fig. 2d).

3.5. Soil microbial respiration rates and biomass carbon

Soil microbial respiration rates were enhanced by the main effect of salinity at 57 d, however only the F-test was significant (P = 0.04, Supplemental Table 3), the Tukey post hoc test was not (Fig. 3). There was no effect of salinity or P on soil microbial respiration rates at 392 and 741 d (Supplemental Table 4). Soil microbial biomass C was lower within the salinity treatment, but only on the 392 d collection (Supplemental Table 4; Fig. 4). There was no effect of P or time or any interactions among treatments on soil microbial biomass C (Supplemental Table 4).

3.6. Root litter breakdown

Root litter k within the 0–7.5 cm soil depth was higher in the freshwater + P and salinity + P treatments and was 1.5× greater for both when compared to the freshwater treatment (Fig. 5). However, within the 7.5–15 cm depth root litter k was only significantly higher in the freshwater + P treatment which was 1.7× greater compared to the freshwater treatment.

4. Discussion

We quantified the response of microbially mediated soil organic matter processing, soil elemental composition, and porewater chemistries to short-, intermediate-, and long-term changes in salinity and P exposure. Exposure to salinity generally decreased enzyme activities throughout the two-year experiment and supported our original hypothesis that nutrient acquiring enzymes would be suppressed. However, the suppression of C-acquiring enzymes with increased salinity did not support our predictions of how C loss occurs following saltwater intrusion. Our prediction that P exposure would increase soil respiration and root litter k was partially supported by our results. Phosphorus addition increased root litter k but had little effect on soil microbial biomass or respiration rates. We measured decreased soil C, attributed to increased salinity, and enhanced breakdown of root liter, attributed to increased P. Microbial responses, like extracellular enzyme activities, biomass C, and soil respiration do not appear to be the direct cause of soil C losses following saltwater intrusion in the Everglades.

Salinity exposure generally increased porewater C and nutrients. Previous studies have documented how saltwater intrusion increases

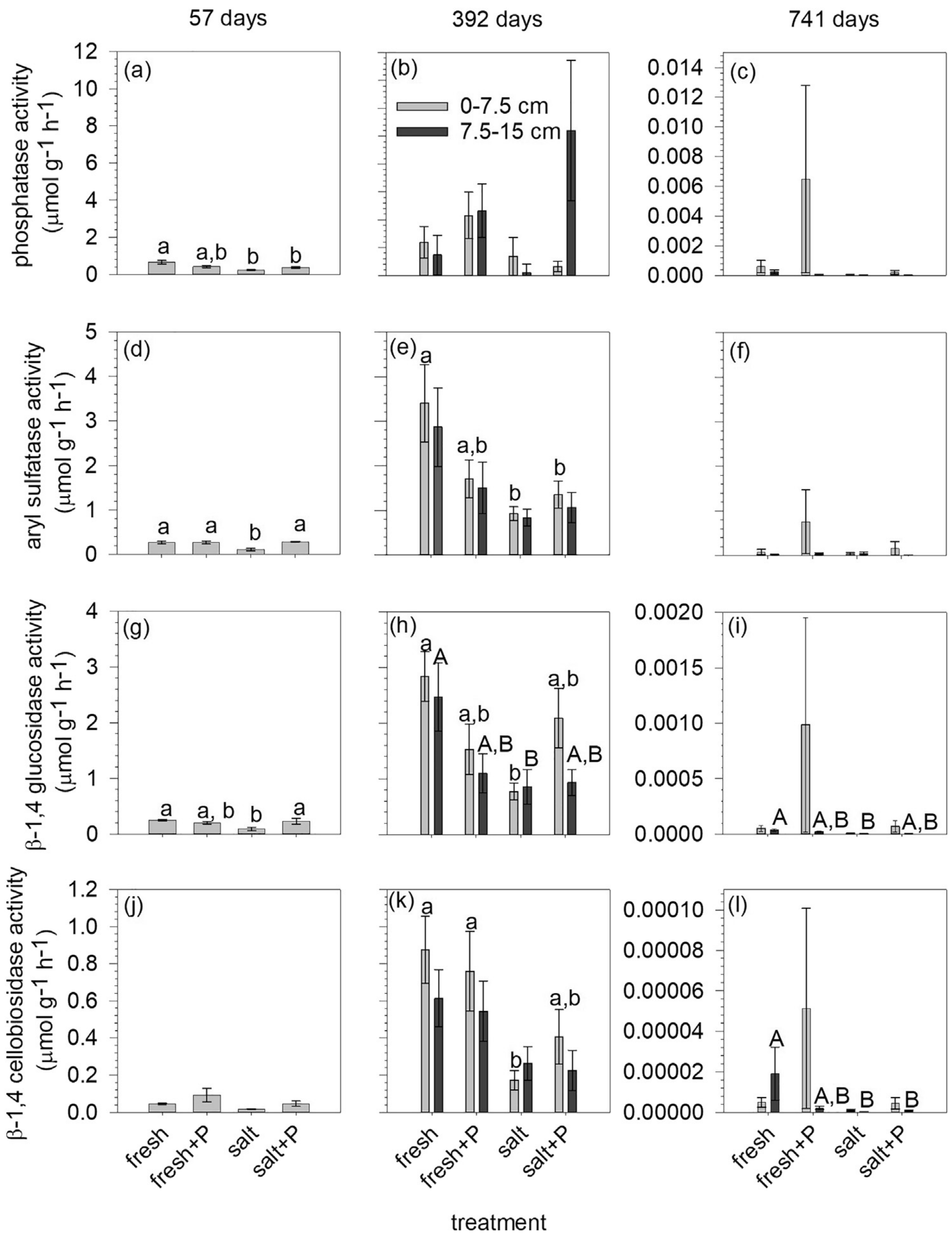

Fig. 1. Microbial extracellular enzyme activities from surficial soils (0–7.5 cm) after 57 days and from soil at 0–7.5 cm and 7.5–15 cm depths at 392 and 741 days. Enzyme activities (phosphatase, aryl sulfatase, β-1,4-glucosidase, and β-1,4-cellobiosidase) were assayed from experimental mesocosm wetlands exposed to four treatments: freshwater (fresh), freshwater with added phosphorus (fresh + P), elevated salinity (salt), and elevated salinity with added phosphorus (salt + P). Responses were compared using a two-way ANOVA followed by a Tukey HSD for comparison. P-values < 0.05 were considered significant. Lower case significance indicators (a, b) are reported within the 0–7.5 cm depth and uppercase significance indicators (A, B) are reported within the 7.5–15 cm depth.

the concentrations of dissolved ions causing desorption of organic compounds from exchange sites and making them available within the soil porewater (Liu and Lee, 2007). We think the adsorption and desorption dynamics in our experiment were altered with exposure to salinity resulting in early changes in porewater constituents that varied over time. We measured increased porewater DOC from soils exposed to increased salinity at intermediate- and long-term exposure. Short-term exposure did not result in increases in DOC, indicating small (~1.3 ppt) increases in salinity do not elicit releases of DOC. Similarly, NH_4^+ and SRP can be desorbed following the addition of other cations in saline waters (Rosenfeld, 1979; Ardón et al., 2013). In our study, we saw evidence of potential short-term desorption of porewater NH_4^+ and SRP within our salinity treatment. However, intermediate- and long-term exposure to salinity led to reductions in NH_4^+ indicating plants may have removed available ammonium. Total N content in the aboveground biomass of the sawgrass in the salinity treatment was only slightly higher than the freshwater controls and was not significantly different (B. Wilson, *in revision*). Previously adsorbed nutrients released following saltwater intrusion would then be available for uptake by plants and periphyton or be exported from the marsh to the estuary.

The response of soil P to added P varied with soil depth and exposure duration. Exposure to elevated P increased soil P within the surface soil pouches where we believe algal and plant competition for P was reduced compared to soil collected from the monoliths. Increased soil P was not evident at the 392 d collection in the shallow (0–7.5 cm) soil depth, but was significantly higher in deeper soil at 392 d and both depths after longer-term exposure, particularly in the salinity + P treatment. The delayed increase in soil P from monoliths compared to surface soil pouches suspended in the water column is likely caused by greater surface area exposed to P-enriched water. Suspended soil subsamples maximized exposure and prevented plant and algal competition for porewater P. Soil subsamples taken directly from sawgrass cores were exposed to lesser amounts of P because this nutrient is rapidly assimilated by algal mats on the soil surface and by sawgrass roots (Mazzei et al. *in review*; Wilson et al. *in review*). Algal mats can act as a short-term P sink during P loading until saturated; once these organisms are no longer P-limited, excess P can accumulate in the soil as we saw after long-term exposure (Richardson and Craft, 1993; Reddy et al., 1999; McCormick et al., 2006). Coastal wetlands with underlying calcium carbonate bedrock will likely see increased P following saltwater

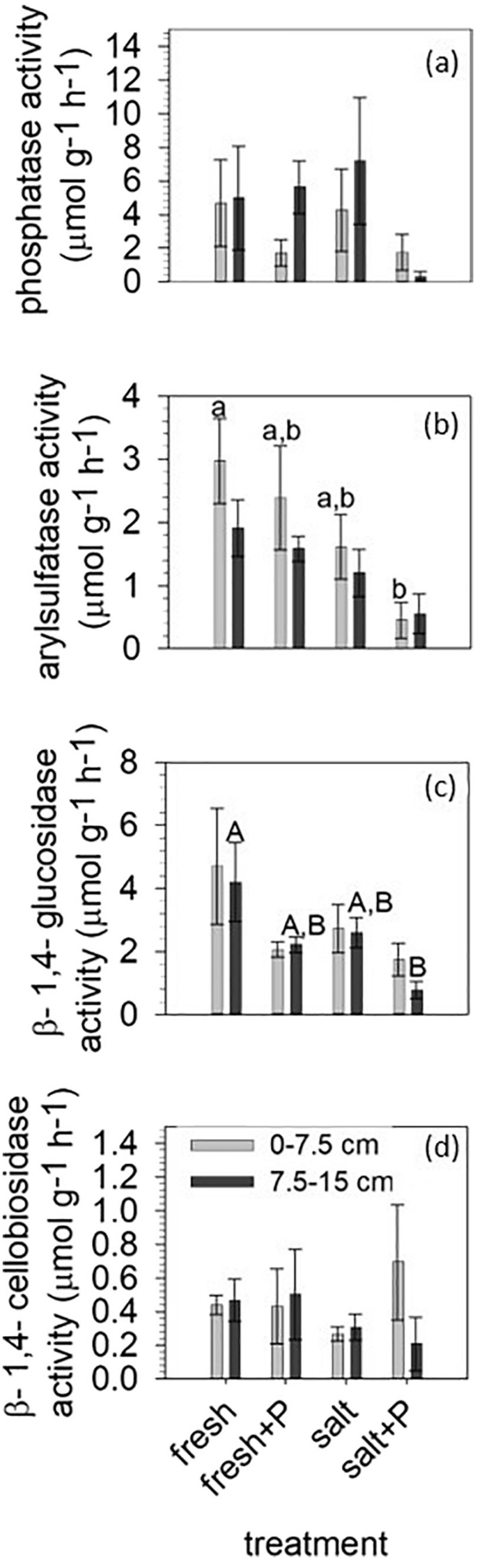

Fig. 2. Enzyme activities for phosphatases (a), aryl sulfatase (b), β-1,4-glucosidase (c), and β-1,4-cellobiosidase (d) are reported in µmol g^{-1} h^{-1} measured on root litter upon retrieval at 356 days. Enzyme activity (phosphatase, aryl sulfatase, β-1,4-glucosidase, and β-1,4-cellobiosidase) were calculated from experimental mesocosm wetlands exposed to four treatments: freshwater (fresh), freshwater with added phosphorus (fresh + P), elevated salinity (salt), and elevated salinity with added phosphorus (salt + P). Responses were compared using a two-way ANOVA followed by a Tukey HSD for comparison. P-values < 0.05 were considered significant. Lower case significance indicators (a, b) are reported within the 0–7.5 cm depth and uppercase significance indicators (A, B) are reported within the 7.5–15 cm depth.

intrusion, and understanding the phases of biological responses to P loading will inform how nutrient limitation may change (Flower et al., 2017). Despite adding P at 2× the natural rate in the Everglades, we did not see large changes in microbial enzyme activities, biomass C, or respiration. It is likely that soil microbial communities are less able to use added P than algal and macrophyte communities due to reduced oxygen conditions of wetland soils (Helton et al., 2015).

Soil microbial enzymes are sensitive to short- and long-term exposure to low-concentration salinity. All enzymes that we measured, except β-1,4-cellobiosidase, were suppressed after short-term exposure to elevated (7 ppt) salinity, a finding that is supported by other studies that manipulated salinity effects on non-wetland soils (Pathak and Rao, 1998; Saviozzi et al., 2011). Short-term reduction of enzyme activities is likely a result of microbial communities diverting resources from the production of extracellular enzymes to the production of osmolytes to maintain cell turgor (Kempf and Bremer, 1998). Interestingly, for arylsulfatase and β-1,4-glucosidase, salinity suppression was absent with added P, indicating that nutrient subsidies can mitigate short-term effects of stressors on nutrient acquiring enzymes. After 392 d both C acquiring enzymes (β-1,4-glucosidase and β-1,4-cellobiosidase) was decreased within the salinity treatment. However, there appears to be a continuation of the mitigating effect with added P as salt + P did not have suppressed C acquiring enzymes. Previous studies that have documented how nutrient exposure can alleviate the stress effects of contaminants indicating simultaneous exposure to salinity and P may elicit different microbial responses than salinity alone (Aristi et al., 2016). Enzyme activities in the lower soil depth were mostly unaffected by salinity and P treatments, except for β-1,4-glucosidase activity which followed the same pattern as the surficial soil. After intermediate-term exposure to salinity and P, phosphatase activities were no longer different among treatments and were likely less susceptible to sustained exposure to environmental stressors and indicates the microbial demand for P was similar across treatments. Long-term effects of exposure to salinity and P on enzyme activities were similar to intermediate-term responses. On the 741 d collection, both C-acquiring enzymes (β-1,4-glucosidase and β-1,4-cellobiosidase) were suppressed. Salinity suppression of C-acquiring enzymes within deeper soil may have occurred as result of the movement of denser saltwater lower into the soil profile increasing osmotic stress to microbes at lower soil depths (Empadinhas and da Costa, 2008). Salinity suppression of C enzymes appears to be sustained long-term, while nutrient-acquiring enzymes are only affected after short to intermediate-duration exposure. We stopped adding salinity to the salt treatment between the 392 and 741 d sampling to avoid conditions from becoming hypersaline. Therefore, the salinity did not change as much and may have allowed more microbial community adaptation which contributed to fewer responses in enzyme activity on the 741 d sampling.

Microbial respiration rates were not affected by added salinity or P. Saltwater, and nutrient exposure can enhance soil respiration rates leading to net losses of ecosystem C stores (Howarth and Fisher, 1976; Robinson and Gessner, 2000; Chambers et al., 2011). We detected short-term increases in soil respiration, but intermediate- and long-term respiration was not enhanced by increased salinity. It is possible that we were unable to capture changes in soil respiration and that time

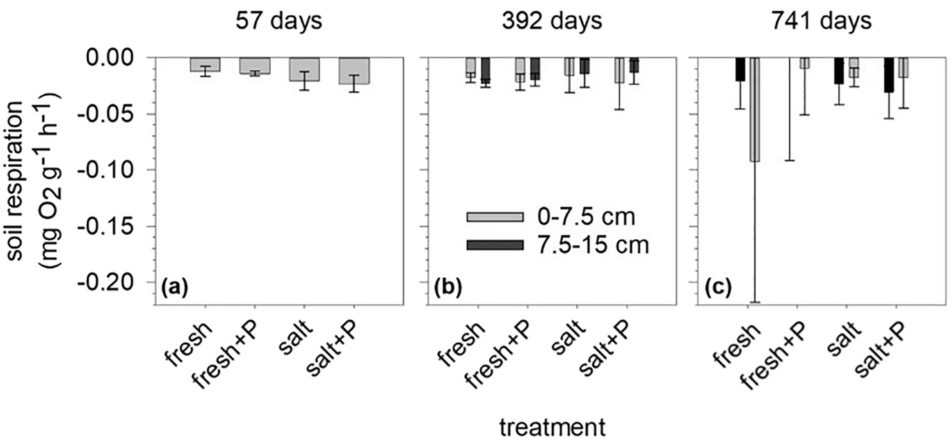

Fig. 3. Soil respiration rates at (a) 57-d, (b) 392-d, and (c) 741-d. Respiration was measured at 0–7.5 cm samples on all dates and at 7.5–15 cm samples on the 392 and 741-d events. For each date and soil depth the four treatments fresh, fresh + P, salt, salt + P were compared using a two-way ANOVA followed by a Tukey HSD for comparison. P-values < 0.05 were considered significant.

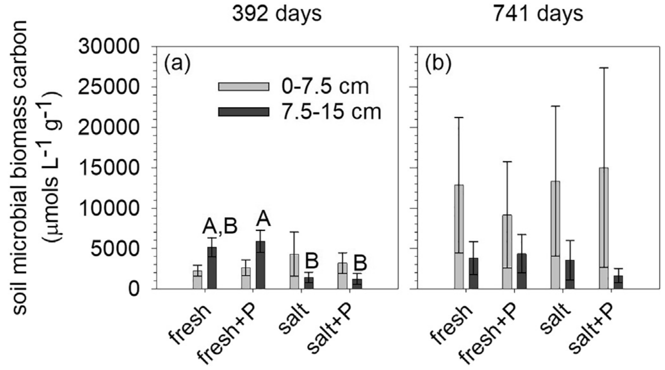

Fig. 4. Soil microbial biomass at (a) 392-d and (b) 741-d. Soil microbial biomass was measured at 0–7.5 cm and 7.5–15 cm samples on the 392 and 741-d events. For each date and soil depth the four treatments fresh, fresh + P, salt, salt + P were compared using a two-way ANOVA followed by a Tukey HSD for comparison. P-values < 0.05 were considered significant. Uppercase significance indicators (A, B) are reported within the 7.5–15 cm depth.

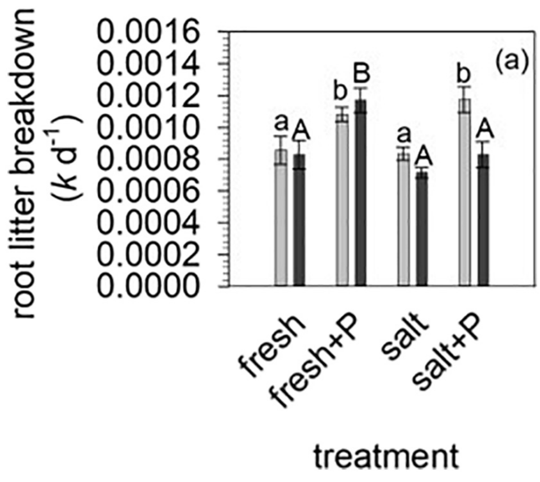

Fig. 5. Proportion of mass loss during 356 d incubation of root litter within soil at 0–7.5 (light grey) and 7.5–15.0 (dark grey) cm depths.

attenuated the response of soil microbial respiration to long-term exposure to increases in salinity and P as communities adapt to environmental conditions. Negative relationships between increased salinity and microbial biomass have been observed in experimental manipulations (Malik and Azam, 1980; Egamberdieva et al., 2010), other studies found that increased salinity resulted in higher microbial biomass (Wong et al., 2008). Here, we observed a change in microbial biomass after intermediate-term exposure to salinity in soils at 7.5–15 cm depth. The reduction in biomass after intermediate-term salinity exposure is likely a result of increased exposure to salinity at lower depths because of higher density saltwater settling towards the bottom of treatment monoliths and higher biomass in freshwater soils with added P. Suppression of enzyme activities in inundated marsh soils has previously been attributed to smaller, less active, microbial communities (Caravaca et al., 2005; Huang and Morris, 2005), but in our study we found reduced microbial biomass C and enzyme activities with added salinity. Microbial communities and their biomass C, respiration rates, and extracellular enzyme activities may be constrained by inundation and less sensitive to changes in salinity and P.

Saltwater intrusion results in net losses in soil C. Long-term exposure to salinity resulted in decreases in soil C at 741 d for both soil depths. The reduction of C after long-term exposure to salinity is one potential pathway towards peat collapse and may represent the source of DOC within the porewater. However, increases in intermediate- and long-term porewater DOC losses do not appear to be connected with the changes in microbial community function. We would have expected the released DOC to be used by the water column and soil microbial community for metabolism and result in increased respiration, which may explain short-term responses (Liu and Lee, 2007; Weston et al., 2011). However, the loss of C from the soil and increased concentrations of TOC and DOC in the water after intermediate- and long-term exposure to salinity do not appear to be biologically controlled by the soil microbial community. Enzymes associated with C acquisition, microbial biomass C, and soil respiration were never enhanced and appear to be disconnected from the loss of soil C and DOC release. If C losses were dominated by microbial processing, we would expect increased soil respiration and CO_2 efflux (Weston et al., 2006). However, within our study, soil CO_2 efflux measured monthly captured treatment effects on soil and root respiration and showed salinity consistently reduced soil CO_2 efflux (Wilson et al. *in review*). Changes in root biomass and root respiration drive integrated rates of respiration from soils. Wilson et al. measured changes in belowground plant biomass and found that salinity decreased belowground biomass (*in review*). The decrease of soil CO_2 measured in our study is likely dominated by the reduction in belowground biomass as opposed to soil microbial respiration which is likely controlled by inundation.

Accelerated rates of sea-level rise are fundamentally altering biogeochemical cycles in coastal wetlands with uncertain effects on net C gains or losses (DeLaune et al., 1994; Chambers et al., 2011). In our study, P addition enhanced C losses by increasing root litter *k*. In nutrient-poor soils, plants often have high nutrient resorption resulting in poor litter quality and slow decomposition rates (Aerts and Chapin, 1999; Rejmánková, 2005). Previous studies have shown that breakdown increases with nutrient addition (Howarth and Fisher, 1976; Robinson and Gessner, 2000) and salinity exposure (Weston et al., 2011). Increased root litter k with P is a potential mechanism of peat collapse with lower levels of salinity because in the salinity + P treatment k was also increased, but only within the 0–7.5 cm depth (Fig. 4a). In the Everglades, marine intrusion and P inputs are coupled (Price et al., 2006), and the direct effects of P on breakdown will affect organic C accumulation in the soil. Even low levels of saltwater intrusion through the limestone bedrock of the Everglades has the potential to release adsorbed P into the water column (Flower et al., 2017). Despite changes in root litter k being attributed to P exposure, enzyme activities measured on root were either unaffected by treatments or were reduced with salinity exposure. Among the treatments, the potential activity phosphatase, arylsulfatase, β-glucosidase, and cellulase enzymes resulted in similar breakdown efficiencies.

5. Conclusions

Despite reductions in microbial biomass C and enzyme activities, elevated salinity and P accelerated wetland soil C loss through leaching of DOC and increased root litter k. Our results indicate that freshwater wetland soils are sensitive to saltwater intrusion, leading to C loss after both short- and long-term exposure; however, salinity appears to suppress mechanisms behind C processing like soil respiration and enzyme activities. Climate and land-use changes are altering the supplies of water and nutrients to coastal wetland ecosystems (Weston et al., 2011; Deegan et al., 2012; Ardón et al., 2013). Previous conditions promoting wetland C storage are rapidly altered by multiple interacting stressors, such as saltwater intrusion and P enrichment (Weston et al., 2006; Weston et al., 2011). Microbial community function can be used as an indicator of ecosystem health; we saw that microbial extracellular enzyme activity in freshwater soils is particularly sensitive to salinity exposure. Our results suggest that enhanced microbial processing of C is not the primary mechanism behind C losses from wetland soils following salinity and P exposure. However, the link between changing microbial function and C loss needs further work. To understand how saltwater intrusion will affect the fate of wetland microbial biogeochemical pathways, more information is needed to determine the threshold for these responses and to assess how plant responses contribute to peat collapse. Phosphorus exposure has the potential to offset some of the effects of salinity and understanding how different ratios of subsidy to stress inputs affect ecosystem responses will help inform future resilience of coastal wetlands.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.11.013.

Declaration of conflicts of interest

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