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Saltwater and phosphorus drive unique soil biogeochemical processes in freshwater and brackish wetland mesocosms

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Abstract. Coastal ecosystems are exposed to saltwater intrusion but differential effects on biogeochemical cycling are uncertain. We tested how elevated salinity and phosphorus (P) individually and interactively affect microbial activities and biogeochemical cycling in freshwater and brackish wetland soils. In experimental mesocosms, we added crossed gradients of elevated concentrations of soluble reactive P (SRP) (0, 20, 40, 60, 80 µg/L) and salinity (0, 4, 7, 12, 16 ppt) to freshwater and brackish peat soils (10, 14, 17, 22, 26 ppt) for 35 d. We quantified changes in water chemistry [dissolved organic carbon (DOC), ammonium (NH_4^+), nitrate + nitrite (N + N), SRP concentrations], soil microbial extracellular enzyme activities, respiration rates, microbial biomass C, and soil chemistry (%C, %N, %P, C:N, C:P, N:P). DOC, NH_4^+, and SRP increased in freshwater but decreased in brackish mesocosms with elevated salinity. DOC similarly decreased in brackish mesocosms with added P, and N + N decreased with elevated salinity in both freshwater and brackish mesocosms. In freshwater soils, water column P uptake occurred only in the absence of elevated salinity and when P was above 40 µg/L. Freshwater microbial EEAs, respiration rates, and microbial biomass C were consistently higher compared to those from brackish soils, and soil phosphatase activities and microbial respiration rates in freshwater soils decreased with elevated salinity. Elevated salinity increased arylsulfatase activities and microbial biomass C in brackish soils, and elevated P increased microbial respiration rates in brackish soils. Freshwater soil %C, %N, %P decreased and C:P and N:P increased with elevated salinity. Elevated P increased %C and C:N in freshwater soils and increased %P but decreased C:P and N:P in brackish soils. Freshwater soils released more C and nutrients than brackish soils when exposed to elevated salinity, and both soils were less responsive to elevated P than expected. Freshwater soils became more nutrient-depleted with elevated salinity, whereas brackish soils were unaffected by salinity but increased P uptake. Microbial activities in freshwater soils were inhibited by elevated salinity and unaffected by added P, but brackish soil microbial activities slightly increased with elevated salinity and P.

Key words: Florida Coastal Everglades; microbial extracellular enzyme activities; saltwater intrusion; sea-level rise; subsidy-stress.

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Introduction

Disturbances are discrete events that alter ecosystem structure and function (Pickett and White 1985, White and Jentsch 2001). According to perturbation theory, beneficial inputs or subsidies enhance ecosystem function at low levels of exposure, but can diminish function at higher levels of exposure, whereas toxic inputs or stressors have immediate adverse effects on

ecosystem function (Odum et al. 1979). Ecosystem processes are continuously influenced by the presence of stressors and subsidies (Odum et al. 1979), and climate change is increasing the intensity, frequency, duration, and exposure of ecosystems to novel combinations of subsidies and stressors leading to uncertain ecosystem responses (Turner 2010, Trumbore et al. 2015). Although ecosystems have some capacity to recover structure and function (Holling 1973), disturbances often elicit changes in ecosystem state (Scheffer et al. 2001, Peters et al. 2011, Grimm et al. 2017). Therefore, legacies of disturbance interactions influence how ecosystems respond to long-term presses and short-term pulses of subsidies and stressors (Odum et al. 1979, Odum et al. 1995, Kominoski et al. 2020).

Legacies of previous exposure to disturbance interact to shape ecosystem response to subsequent disturbances (Franklin et al. 2000). Ecological memory, the information, and materials that persist after disturbance influence ecosystem responses to future disturbances (Padisak 1992, Johnstone et al. 2016). Disturbance legacies often manifest through altered soil properties, as soil microorganisms are sensitive to environmental perturbations and impacts to soils are often persistent (Gunderson and Pritchard 2002, Herbert et al. 2015, Newman et al. 2017). Three main hypotheses have been presented for how ecological disturbances can affect soil microbial communities (Herbert et al. 2015). The first hypothesis predicts that perturbations can result in shifts in species composition without a change in microbial function and processing rates if there is high functional redundancy among species (Hobbie 1988, Hart and Lovvorn 2003). Second is that microbial community composition will remain unchanged but the function will be altered as individual species adapt, become dormant, modify gene expression, and display functional plasticity (Edmonds et al. 2009, Nelson et al. 2015). The third hypothesis is that microbial community structure and function will change in tandem with response to perturbations (Jackson and Vallaire 2009). These three hypotheses are not mutually exclusive and provide insight into how microbial structure and function may respond differently to subsidies and stressors. Extracellular enzyme activities can be used to quantify how the soil microbial community is responding to environmental perturbations. Soil microorganisms contribute to ecosystem function by driving C and nutrient cycling through the release of extracellular enzymes to meet metabolic demands for C and nutrients (Dick et al. 1994, Sinsabaugh et al. 2002, Penton and Newman 2007). Saltwater intrusion can change microbe-mediated biogeochemical cycling in coastal wetlands (Herbert et al. 2018). Enzyme activities are often suppressed when exposed to elevated 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). Phosphorus enrichment studies find an inverse relationship with phosphatase enzyme activities (Spiers and McGill 1979, Wright and Reddy 2001, Morrison et al. 2016) and positive relationships with other enzymes activities (Rejmánková and Sirova 2007). The effects of simultaneous exposure to osmotic stress and increased nutrient availability on microbial function are unclear.

Coastal wetlands are increasingly exposed to saltwater intrusion, and the effects of legacies of salinity exposure and land use on wetland biogeochemistry are uncertain (Green et al. 2017, Tully et al. 2019). Changes in extracellular enzyme activities associated with elevated salinity and nutrients (nitrogen, N; phosphorus, P) may lead to long-term effects on C storage and nutrient removal capacity (Penton and Newman 2007). Here, we tested how simultaneous increases in salinity and nutrients on soil microbial extracellular enzyme activities, microbial biomass C, soil respiration, and soil elemental concentrations and stoichiometric ratios using experimental manipulations of crossed gradients in added concentrations of salinity and the limiting nutrient (P). Based on our previous findings (Servais et al. 2019), we predicted that (1) elevated salinity would reduce microbial activities (EEAs, microbial biomass C, and respiration rates) in freshwater marsh soils, (2) elevated P would increase microbial activities (expect phosphatase) in both freshwater and brackish soils, (3) brackish soils would have a lower sensitivity to elevated salinity and more responsive to P subsidies in the presence of salinity compared to freshwater soils due to ecological memory of saltwater exposure, (4) brackish soils and freshwater

soils exposed to elevated salinity would have lower %C, C:N, and C:P ratios than freshwater soils.

Methods

Soil sampling and preparation

In July 2015, we collected surficial (0–2 cm depth) peat soils (n = 100) from a freshwater wetland in the Florida Everglades (25°46′06.1″ N, 80°28′56.2″ W) and placed soils into 250-μm mesh plastic containers (6.5 cm length, 4.5 cm diameter). In June 2016, we again collected surficial (0–2 cm depth) peat soils (n = 100) from a brackish wetland in Everglades National Park (25°13′13.4″ N, 80°50′36.7″ W) and placed soils into 250-μm mesh plastic containers. In both years, we transported soils to an experimental outdoor mesocosm facility located in Key Largo, Florida, for a 35-d experiment that exposed peat soils to weekly pulsed additions of salinity and P (described below).

Experimental design

Soil samples collected from the field were homogenized and placed in 1-gallon incubation mesocosms exposed to crossed gradients of elevated salinity and P solutions. (Fig. 1). We placed four replicate soils in each mesocosm and collected replicates after 1, 2, 3, and 5 weeks of exposure. Elevated salinity solutions were made using Instant Ocean diluted with deionized water to achieve a gradient in salinity concentrations (freshwater: 0, 4, 7, 12, and 16 ppt; brackish: 10, 14, 17, 22, and 26 ppt). Elevated P solutions were made from concentrated phosphoric acid diluted with deionized water to achieve a gradient in P concentrations (0, 20, 40, 60, 80 μg/L).

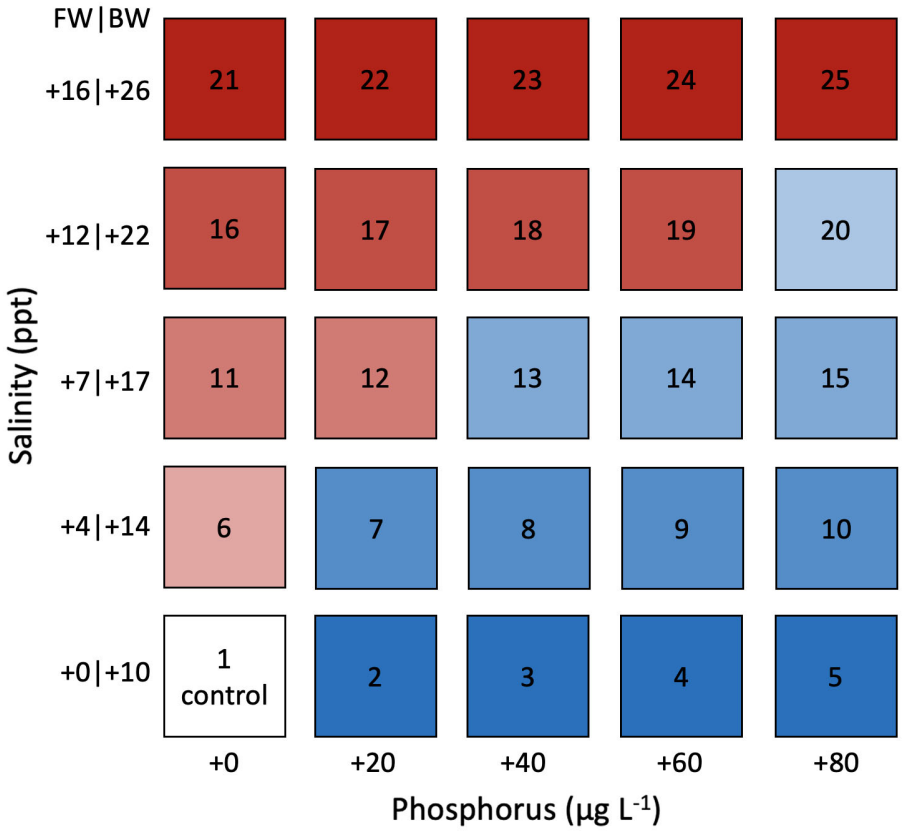

Fig. 1. Mesocosm experimental design of crossed gradients of added concentrations of salinity (freshwater: 0, 4, 7, 12, and 16 ppt; brackish: 10, 14, 17, 22, and 26 ppt) and phosphorus (0, 20, 40, 60, and 80 μg/L).

Water within mesocosms was replaced with treatment solutions of salinity and P every other day with 0.5 L of the corresponding solution.

Water chemistry

We collected water samples (filtered) weekly from each mesocosm ($n = 25$). Water samples were collected in a plastic syringe and filtered onsite through a 0.7-μm glass fiber filter (GF/F) 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. Samples were analyzed for dissolved organic C (DOC), dissolved inorganic nitrogen (NO_3^-, NO_2^-, NH_4^+), and soluble reactive P (SRP). Dissolved inorganic N and SRP parameters were analyzed on an Alpkem RFA 300 auto-analyzer (OI Analytical, College Station, Texas, USA), and DOC was analyzed with a Shimadzu 5000 TOC Analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA). Salinity concentrations were measured using a handheld water quality sonde (YSI Model 600 XL, Xylem Inc., Yellow Springs, Ohio, USA).

Soil elemental concentrations and stoichiometric ratios

We dried all soil samples at 60°C until mass stabilized to determine dry mass. Samples were then ground using an 8000-D ball mill (Spex SamplePrep, Metuchen, New Jersey, USA). Ground soils from week 2 and 5 of the experiment were analyzed for organic matter content and chemistry. Subsamples of soils were oven-dried (60°C) for 48 h, weighed, combusted (550°C for 4 h), and re-weighed to determine ash-free dry mass (AFDM). Carbon and N content were analyzed using a Carlo Erba NA 1500 CHN Analyzer (Carlo Erba, Milan, Italy). Phosphorus content was analyzed using the ash/acid extraction method followed by spectrophotometric analysis using the ascorbic acid method (APHA 1998). We estimated elemental composition (%C, %N, and %P) and stoichiometry (C:N, C:P, and N:P). All elemental compositions were calculated from the molar mass.

Soil microbial extracellular enzyme activities

Extracellular enzyme activities were measured on initial, week 2, and week 5 fresh soil samples. We measured the fluorometric activities of extracellular phosphatase, arylsulfatase, β-1,4-glucosidase, β-1,4-cellobiosidase, and leucine aminopeptidase using the substrates described in Servais et al. (2019). Soil microbial enzyme activities were assayed using previously described methods (Saiya-Cork et al. 2002). Briefly, soil sub-samples were collected (approximately 1 g) from each soil container, homogenized in 60 mL of 50 mmol/L 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.

Soil microbial biomass carbon and respiration rates

To estimate the mass of the living microorganisms within the soil, we determined the microbial biomass C using chloroform fumigation and potassium sulfate extraction methods following Vance et al. (1987). We measured microbial biomass C on soil samples from week 2 and week 5 collections. Dissolved organic C samples were analyzed with a Shimadzu 5000 TOC Analyzer (Shimadzu Scientific Instruments, Columbia, Maryland, USA). We calculated microbial biomass C as the difference in DOC between non-fumigated and fumigated samples.

Microbial respiration rates on soils collected during week 2 and week 5 were measured as dissolved oxygen (DO) consumption. Approximately 2.5 g of weighed wet soils were placed in 40-mL glass vials. Vials were filled with treatment solutions of added salinity and P from the mesocosms, the headspace within each vial was removed, and vials were incubated at room temperature (24°C) for approximately 2 h. Vials filled only with treatment solutions (no added soil) served as controls. Initial and final DO were recorded before and after incubations using YSI ProOBOD meters (Xylem Inc., Yellow Springs, Ohio, USA). Oxygen consumption was determined as the slope of the regression of DO concentration over time minus the slope of the control, and respiration rates were expressed per gram ash-free dry mass (AFDM) per hour.

Data analyses

We used simple linear regression models to test effects of elevated P or elevated salinity on changes in water chemistry, soil elemental concentrations and ratios, and soil microbial responses (EEAs, respiration rates, microbial biomass C) and from freshwater and brackish marsh soils. Models included mean values of soil microbial responses collected during week 2 and week 5 of incubation in analyses, mean values of water chemistry collected from weekly measurements, and soil chemistry data collected after week 5 of incubation. We analyzed treatment responses separately from freshwater and brackish marsh soil mesocosms. All analyses were performed in R Studio (version 1.3.1093, R Core Team 2021).

Results

Water chemistry

Salinity concentrations were maintained at treatment concentrations based on weekly measurements. Concentrations of DOC were higher in freshwater mesocosms and increased with added salinity compared to lower DOC concentrations in brackish mesocosms that decreased with added salinity and P (Fig. 2A, B). Concentrations of N + N decreased with added salinity in both freshwater and brackish mesocosms and were unaffected by added P (Fig. 2C, D). Ammonium (Fig. 2E, F) and SRP concentrations (Fig. 2G, H) similarly increased with salinity in freshwater but decreased with salinity in brackish mesocosms, and added P did not impact concentrations of either in freshwater or brackish mesocosms.

Soil elemental concentrations and stoichiometric ratios

Initial elemental concentrations (mean ± 1SE) of freshwater soils collected for the experiment were 39.6 ± 0.5%C, 3.4 ± 0.4%N, and 0.03 ± 0.00%P. Initial elemental composition of the brackish soils collected for the

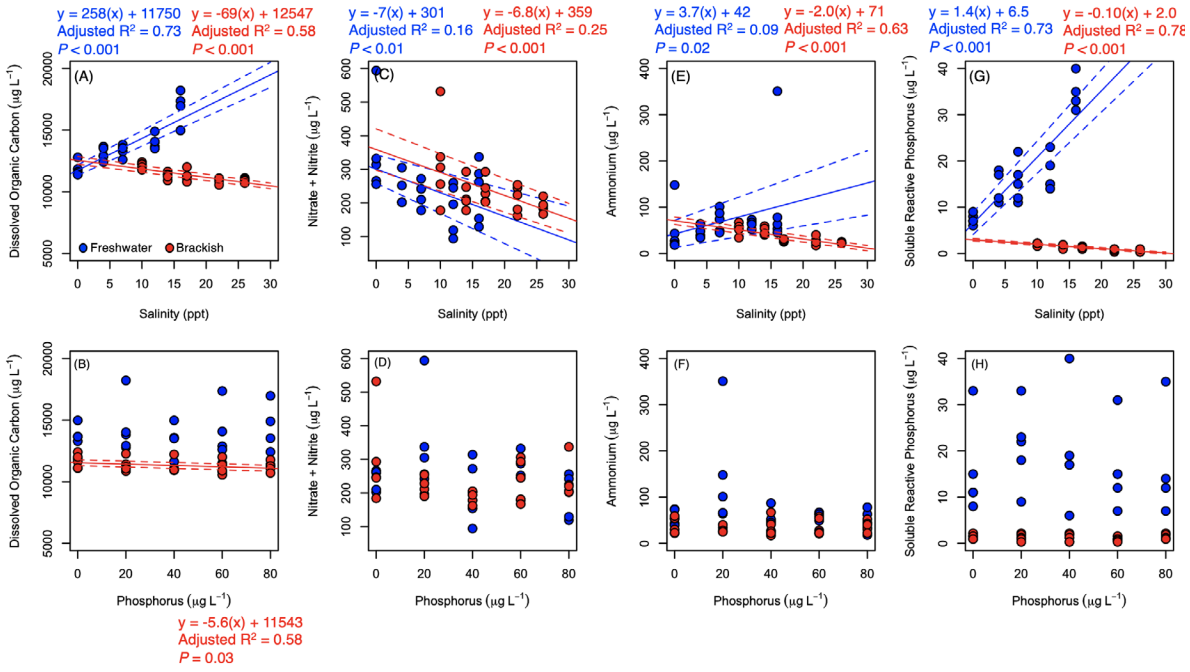

Fig. 2. Water chemistry concentrations (µg/L) along crossed gradients of elevated salinity and phosphorus: (A, B) dissolved organic carbon, (C, D) nitrate + nitrite, (E, F) ammonium, (G, H) soluble reactive phosphorus. Data are means of weekly measurements (n = 5) collected from each mesocosm (n = 25). Lines are linear regressions with 95% confidence intervals. Error bars are not included for ease of visibility.

experiment was 11.1 ± 2.3%C, 0.6 ± 0.1%N, and 0.01 ± 0.00%P.

Soil %C decreased with elevated salinity and increased with elevated P in freshwater soils, and brackish soils were unchanged by gradients of salinity of P (Fig. 3A, B). Soil %N decreased with elevated salinity in freshwater soils but was unaffected by elevated P (Fig. 3C), and soil %N from brackish soils did not respond to increases in salinity or P (Fig. 3D). Soil %P decreased with elevated salinity in freshwater soils and increased with elevated P in brackish soils (Fig. 3 E, F).

Soil C:N increased with added P in freshwater soils, but otherwise was unchanged by elevated salinity or P (Fig. 4A, B). Soil C:P increased with elevated salinity in freshwater soils and decreased with elevated P in brackish soils (Fig. 4C, D). Similarly, soil N:P increased with elevated salinity in freshwater soils and decreased with elevated P in brackish soils (Fig. 4E, F).

Soil microbial extracellular enzyme activities

All EEAs were consistently higher in freshwater soil controls compared to brackish soil controls after 2 and 5 weeks of incubations (Table 1). Microbial EEAs from freshwater and brackish soils did not respond to any concentration of added P. Phosphatase activities decreased with elevated salinity for freshwater soil microbes but not for brackish soil microbes (Fig. 5A). For every 1 ppt increase in salinity, phosphatase activity decreased by 1.30 μmol·g^{-1}·h^{-1} ($P < 0.01$).

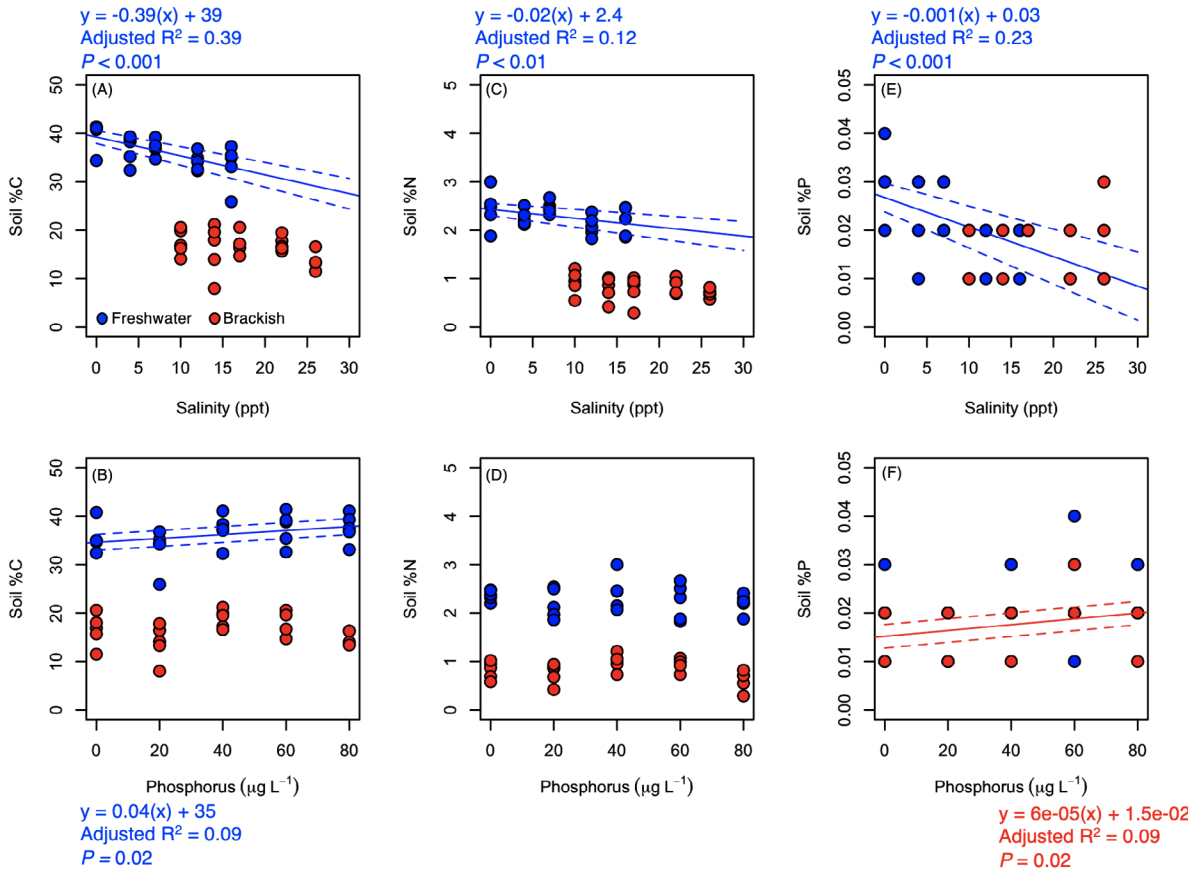

Fig. 3. Soil (A, B) carbon (C), (C, D) nitrogen (N), and (E, F) phosphorus (P) concentrations (%) along crossed gradients of elevated salinity and phosphorus. Data are individual measurements ($n = 1$) collected on week 5 from each mesocosm ($n = 25$). Lines are linear regressions with 95% confidence intervals.

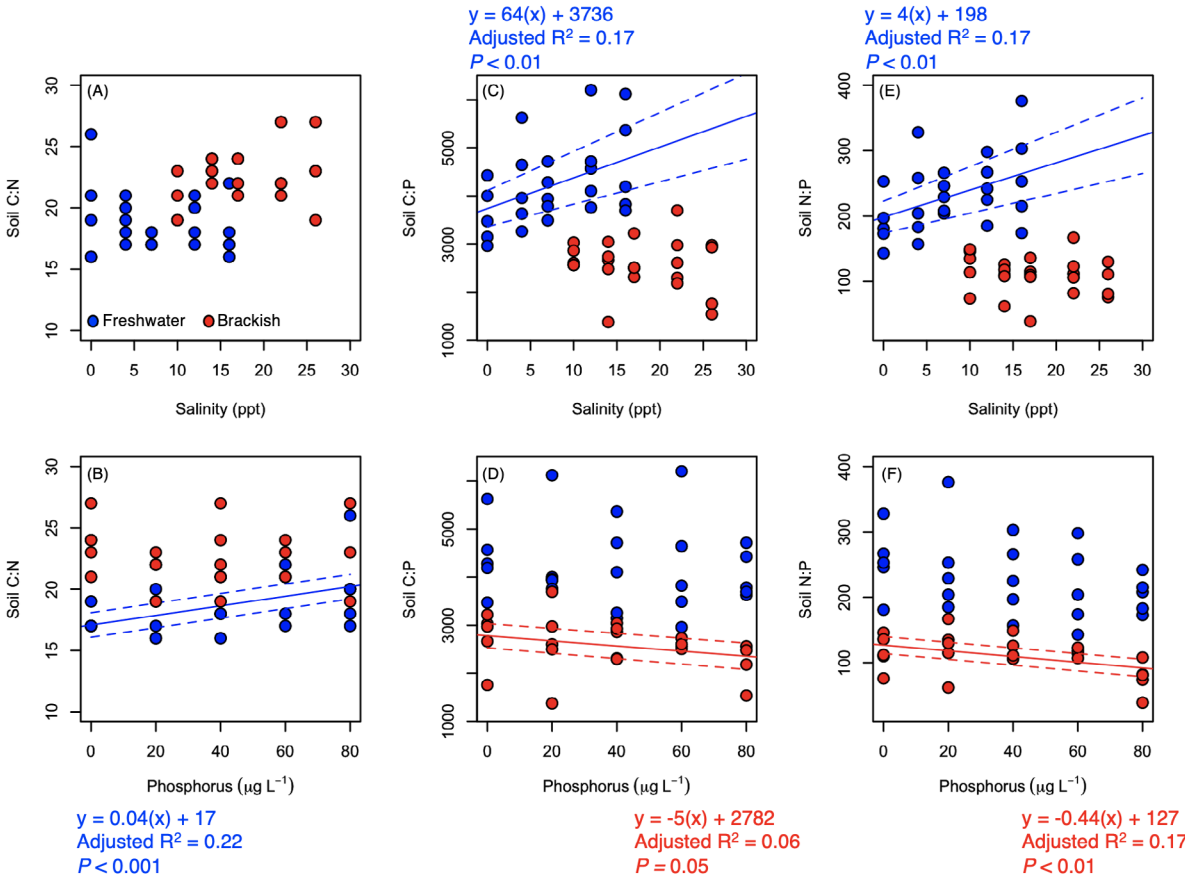

Fig. 4. Soil (A, B) carbon to nitrogen (C:N), (C, D) carbon to phosphorus (C:P), and (E, F) nitrogen to phosphorus (N:P) molar ratios along crossed gradients of elevated salinity and phosphorus. Data are individual measurements ($n = 1$) collected on week 5 from each mesocosm ($n = 25$). Lines are linear regressions with 95% confidence intervals.

Phosphatase activities in both freshwater and brackish soils did not respond to elevated P (Fig. 5B). Arylsulfatase activity in brackish soils increased with elevated salinity but activities did not change with added salinity in freshwater soils (Fig. 5C). For every 1 ppt increase in salinity, arylsulfatase activity increased by 0.02 μmol·g^{-1}·h^{-1} ($P < 0.01$). Arylsulfatase activities in both freshwater and brackish soils did not respond to elevated P (Fig. 5D).

Soil microbial biomass carbon and respiration rates

Soil microbial biomass C was higher in freshwater than brackish soils. Freshwater soil microbial biomass C did not respond to elevated salinity or P, and brackish soils had higher microbial biomass C with elevated salinity (Fig. 5E, F). Similarly, microbial respiration rates were higher in freshwater than brackish soils. However, elevated salinity substantially reduced respiration rates in freshwater but not brackish soils, and brackish soils had higher respiration rates with added P that was not detected in freshwater soils (Fig. 5G, H).

Discussion

We identified distinct differences in how freshwater and brackish soils responded to elevated salinity and P. We discovered soils from freshwater wetlands released more C and nutrients than

Table 1. Enzyme activities measured on initial, week 2, and week 5 soil samples.

Enzyme activities	Freshwater marsh soils	Brackish marsh soils
Initial		
Phosphatase	0.15 (0.13)	2.76 (1.24)
Arylsulfatase	5.97 (1.35)	2.02 (0.50)
β-1,4-glucosidase	2.99 (0.66)	2.81 (0.36)
β-1,4-cellobiosidase	0.56 (0.10)	0.53 (0.06)
Leucine aminopeptidase	0.30 (0.09)	Non-detectable
Week 2		
Phosphatase	54.16	0.00
Arylsulfatase	3.73	0.25
β-1,4-glucosidase	3.77	0.19
β-1,4-cellobiosidase	0.80	0.03
Leucine aminopeptidase	3.09	0.00
Week 5		
Phosphatase	38.87	0.01
Arylsulfatase	4.54	0.26
β-1,4-glucosidase	3.85	0.12
β-1,4-cellobiosidase	0.51	0.00
Leucine aminopeptidase	0.00	0.00

Note: Units for extracellular enzyme activity are $\mu mol \cdot g^{-1} \cdot h^{-1}$ and represent the average of $n = 6$ initial samples (± standard error) and control ($n = 1$) samples for week 2 and 5.

brackish soils when exposed to elevated salinity, and both soils were less responsive to elevated P than expected (Fig 3A–F). In general, freshwater soil chemistry became more nutrient-depleted with elevated salinity, whereas brackish soil chemistry was unaffected by salinity but increased P uptake (Fig. 4A–F). Microbial activities in freshwater soils were inhibited by elevated salinity and unaffected by added P, but brackish soil microbial activities slightly increased with elevated salinity and P (Fig. 5A–H). We predicted that salinity would decrease microbial EEAs, microbial biomass C, and respiration rates and that added P would enhance microbial EEAs, soil respiration, and microbial biomass C. Although microbial EEAs in freshwater and brackish soils were similar initially (Table 1), phosphatase and arylsulfatase activities were consistently higher in freshwater compared to brackish soils for control soils (Table 1)—illustrating higher ambient nutrient limitation in freshwater soils compared to brackish soils—and across all treatment levels (Fig. 5A–D). Increases

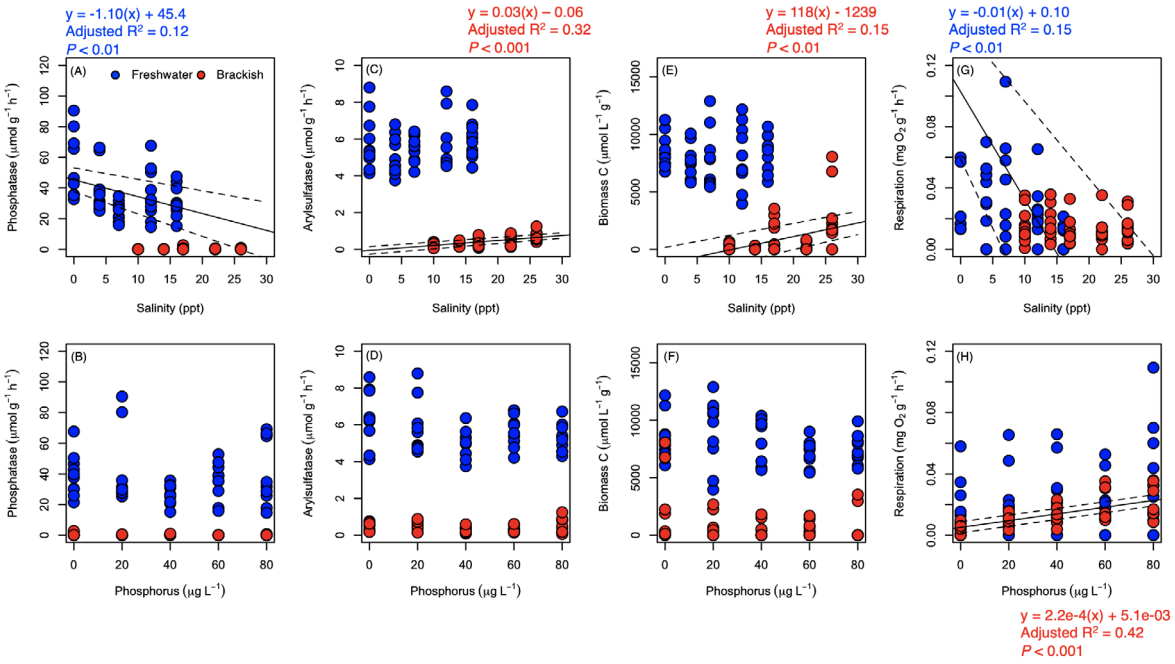

Fig. 5. Freshwater and brackish soil microbial (A, B) phosphatase, (C, D) arylsulfatase, (E, F) microbial biomass carbon (C), and (G, H) microbial respiration rates measured along crossed gradients of elevated salinity and phosphorus. Data are means from weeks 2 and 5 of treatment exposure from each mesocosm ($n = 25$). Lines are linear regressions with 95% confidence intervals. Error bars are not included for ease of view.

in freshwater soil EEAs relative to brackish soil EEAs suggest that nutrient-limited conditions were greater in the freshwater mesocosms than that of the field from which the freshwater soils were collected. Elevated salinity and P concentrations had no impact on C- or N-associated EEAs for either soil type. Exposure of soils to elevated salinity and P concentrations increased biogeochemical changes in brackish microbes more than those in freshwater soils. Overall, our results suggest that effects of saltwater intrusion and nutrient additions in coastal wetlands are fundamentally linked to legacies of past exposure to stressors and subsidies.

The effects of salinity and P concentrations on the water chemistry within mesocosms depended upon treatment exposure level and soil type. Soils, especially those exposed to salinity and nutrients, can be sources of dissolved nutrients and C to the water column (Ardón et al. 2013, 2016, Flower et al. 2017, Servais et al. 2019). Water collected from mesocosms containing freshwater marsh soils had higher NH_4^+, SRP, and DOC compared to water collected from the brackish mesocosms (Fig. 2A–H). Within freshwater mesocosms, we identified a P concentration threshold at 20 μg/L when salinity was at 0 ppt. Phosphorus dissolved in the water column was not used by freshwater soil microbes until it had reached a threshold at 40 μg/L. Concentrations of SRP were greatest in freshwater and lowest in brackish soil mesocosms exposed to the highest salinity concentrations, illustrating physicochemical release of P (soil ion exchange) at higher salinities in freshwater soils compared to microbial uptake of P in brackish soils (Fig. 2 G). Dissolved organic C showed similar trends as SRP specific to each soil type; DOC was released to the water column as salinity increased in freshwater mesocosms but was removed from the water column in brackish mesocosms (Fig. 2 A, B). Lower %C and %P in brackish marsh soils from legacy saltwater exposure may have limited C and P soil-water fluxes from brackish soils during treatment salinity exposure (Fig. 3A, E). Elevated salinity and P have been shown to alter dissolved nutrients and organic C within the surface waters of wetlands (Charles et al. 2019, Servais et al. 2019).

Saltwater intrusion into Caribbean coastal wetlands exposes soils to both salinity stress and P subsidies causing changes in ecosystem function. These extremely P-limited ecosystems receive P from marine sources (Fourqurean et al. 1993, Boyer et al. 1999, Noe et al. 2001, Childers et al. 2006), as P is adsorbed from calcium carbonate during exposure of limestone to saltwater (Price et al. 2006, Price et al. 2010, Flower et al. 2017). Therefore, we had expected strong responses to our experimental P concentration gradient. The highest concentration of SRP measured within mesocosms on any sampling date was 58.3 μg/L (average was 18.0 μg/L) which is higher than the threshold concentration for P of 12.0 μg/L recommended for the Everglades (Richardson et al. 2007). Previous P enrichment studies in Everglades' soils have shown P accumulation within the soil to take more than a year to be detectable (Servais et al. 2019). Microbial utilization of P is likely constrained by anoxic conditions and salinity stress (Helton et al. 2015).

Loss of soil C has been increasingly well documented among wetlands exposed to saltwater intrusion. We measured initial %C in brackish marsh soils that was 3.5× lower than that of freshwater marsh soils, indicating that previous exposure to saltwater likely reduces soil C storage (Chambers et al. 2013, Neubauer et al. 2013). Lower initial %C likely influenced how the brackish soils in our study responded to salinity and P treatments. As we predicted, freshwater soils were more susceptible to C loss with added salinity compared to brackish soils. Elevated salinity can result in desorption of organic particles and lead to C export from soils (Servais et al. 2019, Servais et al. 2020). In addition, C inputs into soils from plant root biomass have been shown to be inhibited by increases in salinity in other studies (Wilson et al. 2018, Charles et al. 2019, Solohin et al. 2020). The brackish soils collected for our experiment were taken from a brackish marsh that has document soil subsidence or peat collapse (Wilson et al. 2018, Servais et al. 2019). In the Everglades and other coastal wetlands, peat collapse is attributed to decreases in freshwater water and increased in saltwater intrusion (Wanless and Vlaswinkel 2005, Chambers et al. 2019). Lower overall soil %C within the brackish soils and the negative relationship between salinity and %C within the freshwater soils indicate salinity as a fundamental driver of C loss from wetland soils exposed to saltwater

intrusion. Although there has been inconclusive evidence about the overall effect of saltwater intrusion on carbon losses (Herbert et al. 2015), our findings add support to recent mechanistic studies that consistently show C loss from peatlands exposed to elevated salinity (Weston et al. 2011, Herbert et al. 2018, Wilson et al. 2018, Charles et al. 2019, Servais et al. 2019, Servais et al. 2020).

Microbial respiration rates associated with incubated freshwater marsh soils were more sensitive to the stress of salinity, whereas respiration rates associated with brackish marsh soils were most sensitive to subsidies of P. Previous experiments in Everglades soils have shown no effect (Chambers et al. 2013), suppression of soil C mineralization (Chowdhury et al. 2011, Servais et al. 2019), and increased rates of soil C mineralization (Chambers et al. 2011, Wilson et al. 2018, Charles et al. 2019). The positive relationship between P and brackish soil respiration rates remained significant throughout the study. Phosphorus addition to brackish soils likely stimulates the microbial use of dissolved C and which has also been correlated with increased arylsulfatase activity (Klose et al. 2011). Freshwater soil respiration rates were negatively correlated with salinity at week 5 indicating there may be a lag in freshwater soil response to salinity exposure which accumulated over time. Salinity exposure leads to osmotic stress and can cause changes in microbial assemblages (Ikenaga et al. 2010) and the diversion of microbial resources from the production of extracellular enzymes to the creation of osmolytes (Killham 1994, Kempf and Bremer 1998) which is energy-intensive (Oren 2001). Microbial biomass decreases with salinity (Malik and Azam 1980) and increases with P (Liu et al. 2012). Therefore, we predicted combinations of the low salinity and high P levels to result in the greatest increase in microbial biomass. However, there was no direct effect of P on microbial biomass C in either soil type. Our study found a positive relationship between microbial biomass C and salinity within the brackish soils which may have resulted from a faster adaption in microbial assemblages to higher salinity in these already exposed soils. Previous research suggests that some microbes within any soil can retain function and increase biomass despite high salinities (Yan and Marschner 2012).

Legacies of saltwater intrusion will likely have persistent effects on biogeochemical cycles in coastal ecosystems. Carbon and nutrient pathways in soils are mediated by microbial activities, and these critical ecosystem functions in wetlands may remain altered following saltwater intrusion. Several studies have reported decreases in soil microbial EEAs with elevated salinity (Jackson and Vallaire 2009, Neubauer et al. 2013, Servais et al. 2019), suggesting that salinity regulates microbial community metabolic processes (Garcia-Pinchel et al. 1999, Abed et al. 2007). Studies reporting increased enzyme activity had narrower salinity gradients (0 to 7 ppt; Morrissey et al. 2014), which were less than the difference in ambient salinity between our freshwater and brackish soils. Results from our experiment show that specific microbial functions, like the production of C- and N-acquiring enzymes may be resistant within each soil type to changes in salinity and P, while other functions like soil respiration, freshwater P-acquiring enzymes, and brackish S-acquiring enzymes are altered when exposed to salinity and P perturbations. Our results provide a better understanding of how microbial function changes with increased salinity and P; however, more work is necessary to elucidate the relationship between observed functional changes and microbial community diversity.

Understanding responses of ecosystem functions to stressors and subsidies requires experiments that manipulate multiple treatment levels to test for low-level and saturating responses that can inform both scientists and managers (Cottingham et al. 2005, Kominoski et al. 2015, this study). Management of coastal ecosystems must consider how legacies of land use and salinity interact to influence carbon storage and nutrient uptake capacity of wetlands exposed to increasing saltwater intrusion. Sea-level rise and increases in saltwater intrusion are occurring in coastal wetland ecosystems worldwide (Herbert et al. 2015). There is a growing need to better understand how the effects of sea-level rise and saltwater intrusion will have different impacts among coastal ecosystems that vary in structure and function, including variation in land-use legacies and exposure to salinity and nutrients (Tully et al. 2019). Direct effects of climate change on coastal ecosystems can result in loss of

wetland area and ecosystem function if and when rates of sea-level rise exceed the natural capacity of foundation species in both freshwater and brackish wetlands to adapt (Wilson et al. 2018, Charles et al. 2019). There is a critical need and a timely opportunity to coordinate experimental, observation, and modeling approaches to understand saltwater intrusion impacts in coastal wetlands that will inform effective policies and management. In order to better manage coastal ecosystems into the future, we need to understand how disturbance legacies interact with long-term environmental changes—such as sea-level rise and saltwater intrusion—to influence how ecosystems respond to long-term presses and short-term pulses of subsidies and stressors (Odum et al. 1979, Odum et al. 1995, Kominoski et al. 2020).

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