Running Head: Saltwater effects in freshwater wetlands

Title: Phosphorus alleviation of salinity stress: effects of saltwater intrusion on an Everglades freshwater peat marsh

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Abstract

Saltwater intrusion and salinization of coastal wetlands around the world is becoming a pressing issue due to sea level rise. Here, we assessed how a freshwater coastal wetland ecosystem responds to saltwater intrusion. In wetland mesocosms, we continuously exposed *Cladium jamaicense* Crantz (sawgrass) plants and their peat soil collected from a freshwater marsh to two factors associated with saltwater intrusion in karstic ecosystems: elevated loading of salinity and phosphorus (P) inputs. We took repeated measures using a 2 • 2 factorial experimental design (n = 6) with treatments composed of elevated salinity (~9 ppt), P loading (14.66 μmol P day^{-1}), or a combination of both. We measured changes in water physicochemistry, ecosystem productivity, and plant biomass change over 2 years to assess monthly and two-year responses to saltwater intrusion. In the short-term, plants exhibited positive growth responses with simulated saltwater intrusion (salinity +P), driven by increased P availability. Despite relatively high salinity levels for a freshwater marsh (~9 ppt), gross ecosystem productivity (GEP), net ecosystem productivity (NEP), and aboveground biomass were significantly higher in the elevated salinity+P treated monoliths compared to the freshwater controls. Salinity stress became evident after extended exposure. Although still higher than freshwater controls, GEP and NEP were significantly lower in the elevated salinity+P treatment than the +P-only treatment after two years. However, elevated salinity decreased live root biomass regardless of whether P was added. Our results suggest that saltwater intrusion into karstic freshwater wetlands may initially act as a subsidy by initially stimulating aboveground primary productivity of marsh plants. However, chronic exposure to elevated salinity results in plant stress, negatively impacting belowground peat soil structure and stability through a reduction in plant roots.

Key Words

sea level rise, Florida Everglades, phosphorus, carbon cycling, coastal marsh, peat collapse

Introduction

Wetlands store 20-30% of global soil C, despite occupying only ~5-8% of the earth's surface, because of their high productivity and low decomposition rate (Mitsch and Gosselink 2007, Nahlik and Fennessy 2016). Wetland functions that determine C storage, however, are highly susceptible to perturbations, such as changes in hydrology, water chemistry, and vegetation (Deegan et al. 2012, Webster et al. 2013, Bernal et al. 2017). As sea level rise (SLR) is expected to accelerate (USGCRP 2017), one perturbation likely to become more frequent in coastal freshwater wetlands is saltwater intrusion. Saltwater intrusion is defined here as the intrusion of marine-origin water comprised of many salt-forming ions (i.e., Cl^-, NO_3^--N, SO_4^{2-}, PO_4^{3-}), also referred to as marine water intrusion. Saltwater can be a stressor for freshwater wetland vegetation and has been associated with decreased species richness (Sharpe and Baldwin 2012, Neubauer 2013), gross ecosystem productivity (GEP; Neubauer 2013, Herbert et al. 2018, Wilson et al. 2018), and primary production (Delaune et al. 1987, Spalding and Hester 2007, Herbert et al. 2015). In non-tidal, non-riverine, peat-dominated wetlands that do not receive an allochthonous sediment supply, a decline in productivity of freshwater plants can be coupled with a decrease in soil organic matter inputs, which can result in marsh instability (Delaune et al. 1994). In some areas, the current rate of saltwater intrusion is much greater than in the past, potentially affecting the ability for natural coastal transgression to occur (Kirwan and Megonigal 2013, Wdowinski et al. 2016, Yao and Liu 2017). Little work has been done to examine how ecosystem C processing in freshwater coastal peat marshes respond to saltwater intrusion, and

60 whether elevated salinity makes these marshes more vulnerable to collapse of peat soils that
61 support them (Wanless and Vlaswinkel 2005).
62 Saltwater intrusion into coastal wetlands occurs through two main pathways: overland
63 incursion through tides or storm surge and groundwater upwelling (Herbert et al. 2015). In
64 karstic systems, such as the Florida Everglades, porous limestone creates a conduit for large-
65 scale groundwater upwelling, and, in coastal areas, groundwater upwelling of saline water is the
66 most likely mechanism of saltwater intrusion into inland marshes, particularly in the dry season
67 (Price et al. 2006). This upwelling can have a significant influence on the chemical composition
68 of groundwater. Although marine water typically has higher P concentrations than surface water
69 in the Everglades (Childers et al. 2006a), P commonly adsorbs to calcium carbonate bedrock,
70 and an influx of saline groundwater can react with the limestone, causing P desorption and
71 further increasing water soluble reactive P (SRP) concentrations (Price et al. 2006, Flower et al.
72 2017). In oligotrophic wetlands, this newly available SRP can act as a subsidy to growth in P-
73 limited wetlands. Microbial community structure and processes can respond rapidly to nutrient
74 subsidies (Corstanje et al. 2007). For example, in oligotrophic soils, P enrichment has been
75 shown to stimulate microbial C processing and soil CO_2 production, which leads to a greater
76 amount of C breakdown and faster turnover (Amador and Jones 1993, DeBusk and Reddy 1998,
77 Wright and Reddy 2007, Medvedeff et al. 2015). Additionally, P enrichment can stimulate plant
78 growth and biomass, thus providing organic inputs to the soil, although this response is usually
79 much slower than the microbial response and can take years to manifest (Chiang et al. 2000, Noe
80 et al. 2002). Areas of the northern Everglades that have high P enrichment rates tend to have
81 higher peat accretion due to the stimulation of cattail growth (Craft and Richardson 2008).
82 However, areas of higher nutrient availability can also change biomass allocation within wetland

83 plants, generally causing more shoot relative to root production (Poorter and Nagel 2000) and the
84 development of less fine roots compared to areas of low nutrient availability (Castaneda-Moya et
85 al. 2011). Although nitrogen additions in N-limited marshes have been shown to decrease
86 belowground biomass and stimulate marsh collapse (Deegan et al. 2012), nutrient additions have
87 also been shown to stimulate root growth (Daoust and Childers 2004, Li et al. 2010). More work
88 is needed to investigate how nutrient additions may influence root growth and their effect on
89 marsh stability.

90 Saltwater intrusion into coastal, karstic P-limited freshwater wetlands is unique given that
91 exposure to saltwater is expected to act as both a subsidy (increased P availability) and a stress
92 (elevated salinity) on marsh vegetation within these ecosystems. Although numerous studies on
93 the effects of nutrient loading or elevated salinity individually on coastal wetlands have been
94 conducted, very few have looked at the interaction of subsidies and stressors that can occur with
95 saltwater intrusion (Macek and Rejmankova 2007, Rejmankova and Macek 2008). The goal of
96 this study was to determine how ecosystem productivity, biogeochemical cycling, and the
97 greenhouse gas carbon balance respond to simulated saltwater intrusion into an oligotrophic
98 freshwater karstic wetland. We chose the Florida Coastal Everglades as our study site because
99 60% of Everglades National Park (ENP) is at or below 0.9 m in elevation, a region highly
100 susceptible to saltwater intrusion because of depleted freshwater delivery and SLR (Pearlstine et
101 al. 2010). We hypothesized that increased salinity in a freshwater peat marsh system would
102 initially stimulate soil CO_2 efflux, but that continuous exposure to elevated salinity would
103 suppress soil CO_2 efflux over time due to stress on microbial communities. Conversely, we
104 hypothesized that P addition would increase soil CO_2 efflux by stimulating microbial activity.
105 We also hypothesized that increased salinity would reduce gross ecosystem productivity (GEP)

and net ecosystem productivity (NEP) due to stress on marsh plants, whereas increased P availability would increase GEP and NEP through alleviation of P-limitation. We hypothesized that the interaction of salinity and P would offset lower GEP and NEP from increased salinity, resulting in no change in GEP and NEP compared to controls. Lastly, we hypothesized that the decrease in CH_4 efflux as a result of elevated salinity would be greater than the increase in CH_4 efflux with added P, shifting the greenhouse gas carbon balance.

Methods

Study site and experimental facilities

The Florida Everglades are classified as a sub-tropical wetland with distinct wet and dry periods. The region annually receives 1380 mm of rainfall on average (Davis and Ogden 1994). Abundant freshwater availability and long hydroperiods allowed vast peatlands to form (Davis and Ogden 1994). However, with sea level rise, saltwater has begun intruding into these freshwater marshes (Saha et al 2011).

In July 2014, twenty-four plant-peat soil monoliths (intact plugs containing marsh peat soil and plants, 60 cm L x 40 cm W x 30 cm H) were collected from a freshwater marsh in the Everglades near Water Conservation Area 3B (25°46'07.4"N, 80°28'56.7"W) that was dominated by a dense stand of *Cladium jamaicense* (sawgrass). This location was ~24 km from the coast and had not experienced saltwater intrusion. Phosphorus load in this area is comparable to or lower than P loading into Everglades National Park and does not represent a nutrient enriched area (Xue 2018). Sawgrass biomass at time of collection was 534.1 ± 262.0 (mean ± SD) g dry weight m^{-2}. We extracted the peat monoliths using shovels to cut out a piece of marsh larger than a mesh-lined container (50 cm L × 40 cm W × 30 cm H). We then shaved the edges of the peat

129 monoliths to the size of the container and placed each into mesh-lined containers filled with an
130 array of 2.5 cm diameter holes to allow for water exchange but keeping soil structure intact.
131 Monoliths were then transported to an outdoor mesocosm facility at the Florida Bay Interagency
132 Science Center in Key Largo, FL (Wilson et al. 2018).

133 Once on site, monoliths were placed into polycarbonate boxes (69 cm L x 51 cm W x 53
134 cm H) and randomly assigned to one of four treatments (n = 6) interspersed among six large
135 concrete tanks (2.2 m L x 0.8 m W x 0.7 m H; Appendix S1: Fig. S1). Each monolith was
136 contained within its own water source and did not interact with any surrounding monoliths. The
137 monoliths were allowed to acclimate for 7 months under inundated freshwater conditions before
138 treatment manipulations and measurements began. The experiment lasted a total of 2 years (Feb
139 2015-2017). This was a 2 x 2 factorial experiment with repeated measures. The four treatments
140 were Fresh (freshwater, no P), Fresh+P (freshwater with added P), Salt (elevated salinity, no P),
141 and Salt+P (elevated salinity with added P). A partition was inserted between the no P and +P
142 treatments to avoid possible contamination of water from one monolith splashing into another
143 (Appendix S1: Fig. S1). We chose our salinity and P targets based on both field observations
144 and previous experimental data (Noe et al. 2001, Gaiser et al. 2005). Our salinity was targeted at
145 ~9 ppt because this was the ambient porewater salinity at a historically freshwater field site
146 where we are observing peat collapse and are monitoring conditions continually (Mazzei et al.
147 2018, Wilson et al. 2018).

148 Because the Everglades is P limited, most added P above ambient levels is quickly taken
149 up by the biota and can be hard to detect in surface waters. The evidence on saltwater causing P
150 desorption from limestone is clear (Price et al. 2006, Flower et al. 2017), however, the magnitude
151 of desorption is likely to be variable depending on initial conditions and the rate of saltwater

intrusion. There is a myriad of studies within the Everglades that examine how increased P loading affects ecosystem functioning (e.g., Newman et al. 1996, Noe et al. 2003, Gaiser et al. 2005). Because any P concentration over 5 ppb can offset P limitation to organisms in the Everglades (Noe et al. 2003), we chose to target our P load at ~ 1 g P m^{-2} yr^{-1}, which is similar to the loading rate of other wetland P enrichment experiments and is likely to elicit a response from the biota (Craft et al. 1995, Daoust and Childers 2004, Gaiser et al. 2005, Macek and Rejmankova 2007).

In February 2015, water in the salt treatments was added in gradually incrementing amounts of salinity over 2 months to hit our target salinity of ~9 ppt, while P (72.65 µmol L^{-1} diluted phosphoric acid) was pumped to the appropriate treatments at a rate of 0.14 mL^{-1} min^{-1} (14.66 µmol P day^{-1}). Dosing concentrations of salinity were adjusted monthly depending on porewater salinity in order to maintain a treatment level of ~7-10 ppt. Salinity was controlled by mixing water weekly to desired experimental salinity concentrations from four 7,570-liter head tanks, two with freshwater and two with saltwater. Two liters of this water was manually added 2-3 times per week to each box in order to keep the monoliths completely inundated. Freshwater was collected from a nearby canal (C-111; 25°17'31.74" N, 80°27'21.59" W) and had similar nutrient concentrations to freshwater wetlands of the Everglades. Saltwater head tanks were equipped with a pump to draw water from adjacent Florida Bay. Nutrient concentrations of water added to the fresh and salt monoliths are reported in Appendix S1: Table S1. Notably, the only significant difference between the two source waters was that bay water had higher salt concentrations.

Cumulative salt loads were measured for each monolith as the total volume of source water added and the molar mass of chloride in the source water, based on weekly salinity

175 measurements. Cumulative P loads were measured for each monolith as the daily added molar
176 mass of P delivered to P treatment monoliths plus the added molar mass of P from source waters
177 (based on monthly measures and the total volume of source water added to each monolith) (Fig.
178 S2).
179
180 Water physicochemistry and soil redox
181 Surface water in each monolith was collected monthly (25 collections total) using a 60-
182 mL syringe and placed into new, acid-washed plastic bottles after rinsing with sample water.
183 Prior to the experiment, a porewater sipper with an air stone (4-cm long x 1-cm diameter) was
184 inserted to 15-cm depth near the middle of each monolith. Porewater was collected monthly (26
185 collections total) using a 60-mL syringe by placing suction on the sipper and evacuating at least
186 1 sipper volume before sampling. Filtered samples were run through a 0.7-µm glass fiber filters
187 (GFF) before being placed into a separate bottle. At the time of collection, temperature (°C),
188 salinity (ppt), and pH were measured on samples of freshwater source, saltwater source, and
189 monolith surface water using a YSI Model 600 XL (Xylem, Inc., Yellow Springs, OH, USA).
190 All water samples were stored at -20°C until analysis at the Southeast Environmental Research
191 Center Nutrient Analysis Laboratory at Florida International University. Unfiltered surface water
192 was analyzed for total N (TN), total P (TP), and total organic C (TOC). Filtered porewater and
193 filtered surface water samples were analyzed for dissolved organic C (DOC), dissolved inorganic
194 nitrogen (DIN; NO_3^--N, NO_2^--N, NH_4^+-N), and soluble reactive P (SRP). DIN, TN, TP, and SRP
195 samples were analyzed on a Alpkem RFA 300 auto-analyzer (OI Analytical, College Station,
196 TX, USA); TOC and DOC were analyzed with a Shimadzu 5000 TOC Analyzer (Shimadzu

197 Scientific Instruments, Columbia, MD, USA). Sulfide (S^{2-}) was measured using standard
198 methods (McKee et al. 1988).
199 Oxidation-reduction potential was measured monthly (25 collections total) using standard
200 techniques (Faulkner et al. 1989). Briefly, three platinum-tipped probes were inserted to 5-cm
201 depth in each monolith and allowed to equilibrate for 30 minutes until measurements were taken.
202 Electrode potentials were corrected using a reference electrode and standard electrode potentials.
203 Soil bulk density was calculated by taking one core (2.4 cm^2 x 30 cm depth) from each monolith
204 at the end of the experiment. The core was separated into 10-cm sections, dried at 60°C, and
205 weighed. The bulk density for each soil depth was calculated as the dry weight divided by the
206 volume of the core segment.
207
208 Plant biomass and elemental stoichiometry
209 Sawgrass aboveground net primary productivity (ANPP) was measured every two
210 months beginning in April 2015 (10 measurements total) non-destructively following methods
211 described in Daoust & Childers (1998). Briefly, sawgrass plants were tagged and measured every
212 other month for the number of live and dead leaves, shoot height, and culm diameter. Change in
213 sawgrass aboveground biomass and ANPP were calculated using previously derived allometric
214 relationships between plant height, culm diameter, and biomass (Childers et al. 2006b). Live
215 belowground biomass was determined by taking one core (2.4 cm^2 x 30 cm depth) from each
216 monolith at the end of the experiment, separating the core into 10-cm sections, and storing at 4°C
217 until processing (within two weeks). The live roots, those which floated in water and were not
218 visibly dark and dead, were separated by washing over a 1-mm sieve, dried at 60°C, and
219 weighed. Sawgrass leaf (year 1 & 2) and root (year 2) samples for each monolith were dried,

220 ground, and subsampled for analysis of C, N, and P. Carbon and N was combusted in a Flash
221 1112 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Zimmermann and
222 Keefe 1997), and P was determined using the molybdate method and run on a UV-2101PC
223 spectrophotometer (Shimadzu Corp., Kyoto, Japan) (Solorzano and Sharp 1980).
224
225 Soil CO_2 and CH_4 fluxes
226 One 10-cm diameter PVC collar was installed 5-cm into the soil of each peat monolith
227 for soil C efflux measurements. Soil CO_2 efflux (n = 6 per treatment) was measured monthly
228 near noon between Feb 2015 and Sep 2016 (20 measurements total) on all 24 monoliths using a
229 portable infrared gas analyzer (LI-8100, LI-COR, Lincoln, NE, USA) equipped with a 10-cm
230 diameter chamber. Each flux measurement was taken for 120s. The flux was calculated as the
231 linear slope of CO_2 concentration over time.
232 Soil CH_4 efflux was measured monthly between Feb and Nov 2015 (7 sampling events)
233 from a subset (n = 4 per treatment) of monoliths using the LI-8100 modified to collect a subset
234 of air for trace gas sampling. The chamber was sealed and, immediately following closure, 25-
235 mL of gas was withdrawn using a 60-mL syringe from a port in-line with the instrument. After
236 15 min, another gas sample was collected. The gas was sealed in a 20-mL evacuated glass vial
237 and transported back to the lab for analysis. Samples were run within 2 days of collection on a
238 gas chromatograph (Shimadzu Scientific Instruments GC 8A, Columbia, MD, USA) fitted with a
239 flame ionization detector (FID). Methane flux was calculated as the slope of CH_4 concentration
240 over time. No soil gas flux measurements were taken in March, April, and December 2015, or
241 January 2016 because of equipment failure.
242

243 Ecosystem CO_2 flux

244 Measurements of ecosystem CO_2 exchange were conducted every other month from Dec
245 2015 to Jan 2017 (8 sampling events) on a subset (n = 4 per treatment) of monoliths. Prior to
246 each measurement, a polycarbonate collar (67 L x 49 W x 58 H cm) was inserted between the
247 container holding the monolith and the box containing the monolith and surrounding water. The
248 collar was fitted with a foam platform on which the chamber could sit while enclosing both the
249 plants and soil. The clear polycarbonate chamber (53 L x 38 W x 150 H cm) was placed onto the
250 foam platform and sealed using bungee cords to ensure an airtight seal during measurements. A
251 pump sent air from the chamber to an infrared gas analyzer (LI-840, LI-COR) and back to the
252 chamber. The chamber was allowed to equilibrate for 2 mins, then CO_2 concentration was
253 measured every second for 3 mins in both full light and in the dark (Wilson et al. 2018). The flux
254 was calculated as the linear slope of CO_2 concentration over time. Net ecosystem productivity
255 (NEP) was measured in full light, whereas ecosystem respiration of CO_2 (ER_{CO2}) was measured
256 in the dark immediately after light measurements by covering the chamber with a dark cloth that
257 blocked out all sunlight. Gross ecosystem productivity (GEP) was calculated from NEP and
258 ER_{CO2} as:

259 $$-GEP = -NEP - ER_{CO2} \quad (1)$$

260 where NEP is instantaneous CO_2 flux (μmol m^{-2} s^{-1}) in light and ER_{CO2} is the CO_2 flux in the
261 dark. Ecosystem flux measurements were not taken from February to November 2015 because
262 the experimental setup had not yet been equipped to handle these kinds of measurements.

263

264 Global greenhouse gas carbon balance contributions

The contribution of experimental peat-sawgrass monoliths to greenhouse gas C balance, and how the balance changes with added salinity and P, was estimated by comparing the overall mean NEP rates to overall mean soil CH_4 efflux (mol mol^{-1}) using the sustained-flux global warming potential (SGWP) of 45 for efflux and the sustained-flux global cooling potential (SGCP) of 203 for uptake for a 100 year time frame (Neubauer and Megonigal 2015). A new corrected NEP based on the SGWP (overall efflux) or SGCP (overall uptake) was calculated as either:

$$Corrected\ \ NEP = NEP - (CH_4 * SGWP) \quad (2)$$

or

$$Corrected\ \ NEP = NEP - (CH_4 * SGCP) \quad (3)$$

Statistical Analyses

Statistical analyses were performed using R (R Core Team 2017). Differences in porewater physicochemistry, C flux, sawgrass biomass, and sawgrass ANPP among treatments were determined through linear mixed effects models (R package *nlme*, Pinheiro *et al.* 2017). Salinity, P, and Date (and their interaction) were set as fixed factors, and monolith number was set as a random factor. Following the mixed effects model, within-date differences in C flux, biomass, and ANPP were assessed with a standard least squares ANOVA, with date as a model effect (R package *lsmeans*, Lenth 2017). Covariance structure was calculated using "variance components," "compound symmetry," and "first order autoregressive," and "variance components" was found to give the lowest AIC. The effect of salinity and P on the overall mean C flux, biomass, ANPP, surface and source water physicochemistry, soil bulk density, and belowground biomass was determined using a two-way ANOVA; for each factor, we then

assessed mean differences among treatments using a least square means multiple comparison test adjusted to Tukey's HSD. Normality and homoscedasticity were tested by visually inspecting plotted residuals, and data were log-transformed to increase heteroscedasticity when necessary. All analyses used a significance factor of $\alpha = 0.05$. All data are presented as mean ± standard error.

Results

Water physicochemistry and soil redox

Phosphorus concentrations in the source waters were 2-3 orders of magnitude lower than that of our P-added treatment, meaning that the difference in the P concentration between the fresh and salt source water likely did not influence our results (Appendix S1: Table S1). Mean porewater salinity in the salinity treatments (both Salt and Salt+P) over the duration of the experiment was 8.83 ± 0.27 ppt (Table 1). In total, 84.9 ± 1.7 kg m^{-3} of salt were added to each salinity treated sawgrass-peat monolith and 6.17 ± 0.01 g m^{-3} of P were added to each P treated sawgrass-peat monolith. Surface water DOC, TOC, NO_2^--N, and TN were all higher in the saltwater amended monoliths (Appendix S1: Table S2; ANOVA, $P<0.05$). Surface water SRP and TP were higher with added P only in the saltwater monoliths (Appendix S1: Table S2). Porewater NO_2^--N, NH_4^+-N, DOC, SRP, and S^{2-} were all higher in the saltwater amended monoliths (ANOVA, $P<0.05$) regardless of P addition (ANOVA, $P>0.05$; Table 1, Appendix S1: Table S3). Overall, there was a Salinity*Date interaction for DOC, NH_4^+-N, NO_2^--N, NO_3^--N, SRP, and S^{2-} and a P*Date interaction for DOC, NO_2-N^-, SRP, and S^{2-} and a Salinity*P*Date interaction for S^{2-} (ANOVA, $P<0.05$, Appendix S1: Table S3). Soil bulk density was lower in the Fresh+P and Salt+P monoliths (0.089 ± 0.012 and 0.079 ± 0.011 g cm^{-3}, respectively) compared

311 to the Fresh and Salt monoliths (0.102 ± 0.012 and 0.110 ± 0.011 g cm^{-3}, respectively) (ANOVA,
312 $F_{(1,60)}$=4.27, P=0.043), whereas salinity had no effect (ANOVA, $F_{(1,60)}$=0.01, P=0.907). We
313 found a significant effect of salinity (ANOVA, $F_{(1,20)}$=10.12, P=0.004) and Salinity*Date
314 (ANOVA, $F_{(10,728)}$=3.91, P<0.001) on soil redox potential. Averaged over the duration of the
315 experiment, soil redox potential at 15-cm depth was +185.1 ± 6.5, +180.4 ± 6.5, +169.9 ± 8.2
316 mV, and +147.5 ± 8.1 mV in the Fresh, Fresh+P, Salt, and Salt+P treatments, respectively.
317
318 Plant biomass and elemental stoichiometry
319 Sawgrass aboveground biomass remained relatively constant over the two-year study
320 period in both the Fresh and Salt treatments, whereas aboveground biomass increased over time
321 in the Fresh+P and Salt+P treatments (Fig. 1). On average, P had a significant effect on
322 aboveground biomass (ANOVA, $F_{(1,20)}$=8.54, P=0.008) and ANPP (ANOVA, $F_{(1,20)}$=11.46,
323 P=0.002). Beginning in June 2016, we found higher aboveground biomass in the Fresh+P
324 treatment (LSMEANS, P<0.05). We found no difference among treatments in mean ANPP
325 during the first year (Fig. 2, Tukey HSD, P>0.05). During the second year, ANPP was higher in
326 both Fresh+P and Salt+P amended treatments, but this effect was only significant in the Fresh
327 treatment (Tukey HSD, P=0.007). Sawgrass leaf stoichiometry was relatively consistent across
328 treatments and years (Appendix S1: Table S4). Leaf C (ANOVA, $F_{(3,20)}$=0.39, P=0.756) and N
329 (ANOVA, $F_{(3,20)}$=0.71, P=0.556) content did not vary across treatments, and leaf P content was
330 significantly higher in the Salt+P treatment compared to the Fresh treatment only during the first
331 year (Tukey HSD, P=0.002). We found an interactive effect of P and Date on both aboveground
332 biomass (ANOVA, $F_{(10,200)}$=7.45, P<0.001) and ANPP (ANOVA, $F_{(9,180)}$=3.25, P=0.001).

Belowground biomass collected at the end of the study was significantly affected by elevated salinity (ANOVA, $F_{(1,250)}$=9.25, P=0.002), added P (ANOVA, $F_{(1,250)}$=39.31, P<0.001), and salinity*P (ANOVA, $F_{(1,250)}$=4.24, P=0.045). Elevated salinity decreased live root biomass by 32 and 53% in the Salt and Salt+P treatments, respectively (Fig. 3). The ratio of above- to below-ground biomass increased with both elevated salinity and added P (Fig. 3).

Soil CO_2 and CH_4 fluxes

Soil CO_2 efflux was dynamic across time but tended to be higher during warmer months (Fig. 4). Elevated salinity had a significant effect on soil CO_2 efflux (ANOVA, $F_{(1,20)}$=44.92, P<0.001) and, on average, decreased soil CO_2 efflux by 41% and 61% within the Salt and Salt+P monoliths, respectively (Table 2). There was also an interactive effect of salinity and P on soil CO_2 efflux (ANOVA, $F_{(1,20)}$=4.38, P=0.049) with greater soil CO_2 loss from the Fresh+P monoliths compared to the Salt+P monoliths (Table 2). There was also a Salinity*Date interaction for soil CO_2 efflux (ANOVA, $F_{(12,238)}$=3.68, P<0.001), where soil CO_2 efflux was greater with ambient salinity except for the first month. Soil CH_4 efflux showed a seasonal trend with greater efflux occurring during the warmer summer and fall months (Fig. 5). Elevated salinity had a significant effect on CH_4 efflux (ANOVA, $F_{(1,12)}$=8.32, P=0.013) which decreased by 100% and 96% in the Salt and Salt+P monoliths compared to the Fresh and Fresh+P monoliths, respectively (Table 2). Adding P increased soil CH_4 efflux by 403% in the Fresh+P monoliths, although this result was not significant due to high variability (ANOVA, $F_{(1,12)}$=4.41, P=0.057). There was an interactive effect of Salinity and Date on soil CH_4 efflux (ANOVA, $F_{(6.72)}$=4.12, P=0.001) where there was more efflux from the Fresh treatment compared to the Salt treatment in July 2016, regardless of P (Fig. 5).

Ecosystem CO_2 flux

When ecosystem flux measurements began in December 2015, GEP and NEP were higher in the P amended treatments (LSMEANS, $P<0.001$) with no difference with added salinity (LSMEANS, $P=0.612$), whereas we found no effect of either salt or P on ER_{CO2} (LSMEANS, $P>0.05$; Fig. 6). By the end of the two-year experiment, we found no difference in GEP, NEP, or ER_{CO2} in the Salt treatment (LSMEANS, $P>0.05$), yet adding P caused GEP, NEP, and ER_{CO2} to be higher in both the Fresh+P and Salt+P monoliths (LSMEANS, $P<0.05$). Over the course of the entire experiment, added P had a significant effect on NEP (ANOVA, $F_{(1,12)}=95.96$, $P<0.001$), GEP (ANOVA, $F_{(1,12)}=68.24$, $P<0.001$), and ER_{CO2} (ANOVA, $F_{(1,12)}=17.36$, $P=0.001$). Cumulatively, added P increased GEP by 138% and 67%, ER_{co2} by 121% and 92%, and NEP by 136% and 62% in the Fresh+P and Salt+P monoliths, respectively (Table 2). Together, we found an interactive effect of salinity and P on NEP (ANOVA, $F_{(1,12)}=10.14$, $P=0.007$) and GEP (ANOVA, $F_{(1,12)}=6.09$, $P=0.029$), but not ER_{CO2} (ANOVA, $F_{(1,12)}=0.41$, $P=0.531$). Mean NEP was significantly greater in the Fresh+P monoliths than the Salt+P monoliths (Tukey's HSD, $P = 0.027$). Overall, we found an interaction between P and Date and Salinity, P, and Date for NEP, GEP, and ER_{CO2} (ANOVA, $P<0.05$, Appendix 1: Table S3). This is because there is a strong separation between the Fresh+P and Salt+P treatments in the final two sampling events (Fig. 6).

Contributions to global greenhouse carbon balance

Changes in contributions to the global greenhouse C balance occurred with the addition of both salinity and P. The $CH_4 : CO_2$ ratio was much smaller in the Salt and Salt+P monoliths compared to the Fresh and Fresh+P monoliths (Table 3). Elevated P greatly increased the CH_4 :

380 CO_2 ratio in the freshwater monoliths, but had no effect in the elevated salinity monoliths. As a
381 result, although the Fresh+P treatment had the highest measured NEP, increased CH_4 emissions
382 associated with elevated P offset the increase in CO_2 uptake based on the SGWP (Table 3). Net
383 ecosystem productivity, calculated incorporating the SGWP of CH_4 (CO_2 eq), showed that the
384 Salt+P monoliths had the greatest NEP among all treatments.
385
386 **Discussion**
387 Saltwater intrusion and salinization of coastal, freshwater wetlands is typically viewed as
388 having a negative influence on ecosystem productivity and stability (Neubauer 2013, Herbert et
389 al. 2015, Herbert et al. 2018). However, when saltwater intrudes into karstic ecosystems, such as
390 the Everglades, freshwater wetlands are simultaneously exposed to both a physiological stressor
391 in the form of elevated salinity and direct supply and desorption of a limiting-nutrient, P (Price et
392 al. 2006, Flower et al. 2017). Understanding how marsh vegetation responds to the coupled
393 subsidy-stress saltwater intrusion is crucial given that vegetation in these ecosystems directly
394 control organic matter input and determine the stability of the marsh (Delaune et al. 1994,
395 Nyman et al. 2006, Wilson et al. 2018, Wilson et al. In Press). In our study, the sawgrass marsh
396 responded in different ways to the two aspects of saltwater intrusion. Aboveground, elevated
397 salinity had little effect on plant biomass or ecosystem C exchange, but higher P availability
398 increased both. However, belowground, elevated salinity reduced live root biomass whereas
399 higher P availability had little effect. These results show that although saltwater intrusion into
400 karstic, freshwater wetlands may initially stimulate ecosystem productivity, peat soil stability
401 may become compromised. Below, we further examine the effects and implications of saltwater
402 intrusion into freshwater wetlands.

403

404 *Effects of salinity on plant biomass and C flux*

405 The effects of elevated salinity on soil CO_2 and CH_4 efflux from freshwater marsh
406 ecosystems vary in magnitude, duration, and direction (Setia et al. 2010, Chambers et al. 2011,
407 Weston et al. 2011, Marton et al. 2012). We found that elevated salinity reduced soil CO_2 and
408 soil CH_4 efflux an average of 52% and 98%, respectively (Table 2). Most saltwater manipulation
409 studies on freshwater marsh soils to date have shown stimulatory effects of elevated salinity on
410 soil CO_2 efflux (Weston et al. 2011, Marton et al. 2012). This response is usually attributed to an
411 increase in SO_4^{2-} availability, which adds an electron acceptor for microbial metabolism and
412 stimulates sulfate reduction (Capone and Kiene 1988). However, we did not see this stimulatory
413 effect. This could be because previous studies only examined low level salinity increases (~5
414 ppt; (Weston et al. 2011, Marton et al. 2012), although salinity in our study was slightly higher
415 (~9 ppt). Elevated salinity has been shown to cause osmotic stress for microbial communities
416 and can even result in cell lysis (Wichern et al. 2006, Chambers et al. 2011). Given enough time,
417 microbial communities adapted to higher salinities should replace those not adapted. In a similar
418 mesocosm study, Wilson et al. (2018) found that raising salinity from 10 to 20 ppt had no effect
419 on soil CO_2 efflux, but the mean overall flux was even lower (0.20-0.24 μmol CO_2 m^{-2} s^{-1}) than
420 fluxes measured at elevated salinity in the current study (0.48 – 0.55 μmol CO_2 m^{-2} s^{-1}). It is
421 possible that as freshwater marshes transition to brackish conditions, organic matter inputs to the
422 soil decrease, resulting in less substrate for microbial communities to use for respiration. Then,
423 as salt-tolerant vegetation becomes established, organic matter inputs increase and concurrently,
424 so does microbial respiration. Another possible factor contributing to lower soil CO_2 efflux with
425 elevated salinity is a reduction in root respiration, which can account for anywhere between 22

426 and 81% of overall soil respiration (Wang et al. 2006, Li et al. 2016). As freshwater vegetation
427 becomes more stressed with elevated salinity, a reduction in root respiration is possible.
428 For many freshwater wetland plants, elevating salinity causes osmotic and ionic stress
429 that can decrease aboveground biomass (Macek and Rejmankova 2007, Troxler et al. 2014),
430 decrease root production (S. Charles, Florida International University, unpublished data), and
431 shift the composition of species persistence in the marsh (Neubauer 2013). Although sawgrass is
432 typically characterized as a freshwater wetland plant, sawgrass can persist under brackish water
433 conditions (Wilson et al. 2015), extending into areas of the coastal Everglades with mean annual
434 surface water salinity up to 16.4 ppt (Troxler et al. 2014). In our study, we found that elevating
435 salinity ~9 ppt above ambient (0 ppt) did not affect aboveground biomass after two years of
436 continuous exposure (Fig. 3). However, Wilson et al. (2018) showed that elevating porewater
437 salinity from 10 to 20 ppt in a flow-through, continuous dosing mesocosm experiment
438 significantly decreased sawgrass GEP and ANPP. These results suggest a salinity "tipping point"
439 between 10 to 20 ppt in which sawgrass productivity is significantly affected. In a field survey
440 within the estuarine ecotone of the Everglades, Troxler *et al.* (2014) found that the number of
441 days in which surface water salinity exceeded 30 ppt was significantly correlated with decreased
442 sawgrass ANPP, but when the marsh was flushed with low salinity water (< 5 ppt), ANPP was
443 unaffected by previous exposure to high salinity. The results of our study and others (Troxler et
444 al. 2014) suggest that flushing by low salinity water is crucial for maintaining high productivity
445 in sawgrass within the estuarine ecotone of the Everglades.
446 Although we found no decrease in GEP or ANPP with salinity elevated to ~9 ppt, we
447 found a significant decrease in live root biomass after two years of elevated salinity exposure,
448 suggesting that elevated salinity is affecting root production. Elevated salinity in coastal

449 wetlands has been shown to have a disproportionately larger effect on root rather than shoot
450 growth (Rozema and Blom 1977, Janousek and Mayo 2013). Root death with added salinity
451 likely liberated C and created a new source of DOC (Hansson et al. 2010) and may explain why
452 porewater DOC was much higher in the saltwater-amended monoliths than the freshwater
453 monoliths. As roots die, DOC can be leached abiotically out of the soil and usually provides an
454 additional energy source for bacteria (Pinney et al. 2000). However, we saw a decrease in soil
455 CO_2 efflux with increased salinity, which means that the microbial community was likely too
456 stressed to use this newly available energy source (Schimel et al. 2007, Servais 2018). In
457 addition, we saw higher DOC content in the surface water, indicating that this newly produced
458 belowground DOC may also be diffusing or leaching into the water column (Appendix S1: Table
459 S2). Therefore, saltwater intrusion has the potential to increase DOC export to downstream
460 estuaries. This finding runs counter to other studies that show either no change or a decrease in
461 porewater DOC and export with saltwater intrusion into freshwater wetlands (Weston et al. 2011,
462 Ardon et al. 2016). Soil core experiments, like those performed in Weston et al. (2011) and
463 Ardon et al. (2016), likely contain little to no live roots, and therefore an increase in DOC
464 leaching from dying plant roots would not have been observed.
465
466 *Effects of P on plant biomass and C flux*
467 In coastal wetlands, additional nutrient loading usually leads to an increase in soil
468 respiration (Morris and Bradley 1999, Wigand et al. 2009). In the Everglades, P is the main
469 limiting nutrient (Noe et al. 2001). In the northern Everglades, P from fertilizer runoff has been
470 shown to stimulate soil CO_2 efflux by 36% (Wright and Reddy 2007) and accelerate peat
471 decomposition (Amador and Jones 1993, Qualls and Richardson 2008). We expected that an

472 increase in P load would stimulate soil CO_2 efflux. However, although there was a significant
473 effect of P and Salinity*P on soil CO_2 efflux, this was only significant when comparing the
474 Fresh+P to the Salt and Salt+P treatments. If only looking at the effect of P across Fresh and Salt
475 treatments, P had no effect (Appendix S1: Table S3). In a similar P enrichment study within the
476 Everglades, Noe et al. (2003) also found that soils were slow to respond to added P (Gaiser et al.
477 2005). The absence of cattail could have also explained why we did not see a stimulatory effect
478 of added P on soil CO_2 efflux. Cattail aerates the soil at a greater rate than sawgrass (Chabbi et
479 al. 2000), so increased production from increased P availability could stimulate aerobic
480 respiration. However, even though cattail thrive in higher nutrient load conditions (Li et al.
481 2010), a concurrent increase of both salinity and P would not encourage cattail invasion because
482 of their low tolerance to salinity (Beare and Zedler 1987).
483 In P-limited freshwater wetlands, such as the Everglades, high nutrient levels have also
484 typically been correlated with higher CH_4 production (Drake et al. 1996, Wright and Reddy
485 2001). We found that increased P loading stimulated soil CH_4 by 96% in the freshwater
486 monoliths, although because of high monthly variability, this effect was not significant. These
487 results are similar to those by Holmes et al. (2014) who found a fourfold increase in soil CH_4
488 efflux in a P enriched site compared to a nearby unenriched site. In this study, we may have
489 underestimated the total CH_4 flux from the marsh because we did not measure CH_4 loss from the
490 vegetation. Despite previous work suggesting that sawgrass does not have active gas transport
491 through its aerenchyma (Chabbi et al. 2000), loss of CH_4 through sawgrass stems has been
492 measured in the Everglades and could account for a substantial portion of total CH_4 efflux from
493 the marsh (Steven Oberbauer, Florida International University, pers. comm.)

494 Inputs of a limiting nutrient in oligotrophic wetlands can have both positive and negative
495 effects in regard to marsh stability. Additions of limiting nutrients can stimulate both shoot and
496 root production, increasing organic matter inputs into the soil (Macek and Rejmankova 2007).
497 However, addition of nutrients may also shift resource ratios and alter biomass allocation
498 towards less root production, which in turn decreases organic input into the soil and could
499 potentially leave the marsh vulnerable to collapse (Tilman 1985, Castaneda-Moya et al. 2011,
500 Deegan et al. 2012). We found that an increase in P loading significantly increased aboveground
501 biomass, GEP, ER, and NEP (Appendix S1: Table S3), but these results were not seen until the
502 second year of P addition (Fig. 1, Fig. 2). Given our finding of a significant interaction between
503 P and date, cumulative P loading is likely the cause for the delayed response. In a similar study,
504 Macek and Rejmankova (2007) found that P addition had no effect on sawgrass growth;
505 however, their study lasted only 2 months. In another P-enrichment study, Daoust and Childers
506 (2004) found that P addition (0.396 g m^{-2} yr^{-1}) stimulated both aboveground and belowground
507 sawgrass growth, with the greatest results not seen until two years after addition. Sawgrass
508 response to P enrichment is often slow, which is what typically allows cattails to outcompete
509 sawgrass in the P-enriched northern Everglades (Lorenzen et al. 2001, Webb and Zhang 2013).
510 We did not see an increase in macrophyte TP content after two years, which is consistent with
511 results from a long-term P addition study in the Everglades (Daoust and Childers 2004). Gaiser
512 et al. (2005) found that *Eleocharis cellulosa* increased leaf TP only after 3-4 years of continuous
513 low-level P, because most available P was taken up by the microbial community and rapidly
514 transported downstream (Noe et al. 2003). Live belowground root biomass after two years was
515 higher in the P amended freshwater monoliths (Fig. 3), and root ingrowth doubled with P
516 addition, regardless of the presence of salinity (Charles 2018). However, added P shifted the

517 shoot to root ratio towards more shoots and less roots in both the freshwater and saltwater
518 monoliths. Deegan et al. (2012) found a similar shift in biomass allocation in a New England salt
519 marsh with nitrogen additions, a result they suggested caused increased erosion and collapse of
520 the marsh. In addition to a shift in biomass allocation, we found a reduction in soil bulk density
521 with added P, indicating that P additions alone could begin to destabilize the marsh.
522
523 *Saltwater intrusion: a subsidy or a stress?*
524 While, in the previous two sections, we discussed the individual effects of either
525 saltwater or P on freshwater peat marsh ecosystem function, our ultimate goal was to look at the
526 effects of saltwater intrusion as a whole, which, in karstic marshes, means comparing freshwater
527 (Fresh) conditions to those exposed to both elevated salinity and P (Salt+P). Here, we were able
528 to determine the responses of some ecosystem functions to saltwater intrusion over short-term
529 (monthly repeated measures) and chronic (2-year manipulation) exposure. In our study, we found
530 that the main forcing factor with saltwater intrusion (elevated salinity or increased P) depended
531 on the response or function variable measured (Fig. 7). There were several instances where there
532 was a significant interaction between Salinity and P (Table S3). For example, NEP and GEP was
533 higher in the Fresh+P and Salt+P compared to the Salt and Fresh treatment, respectively. Net
534 ecosystem productivity was greatly enhanced with added P, mainly because of increased plant
535 productivity, whereas elevated salinity had little effect on both NEP and ANPP, leading to the
536 Salt+P treatment to have significantly higher NEP than the Fresh treatment. However, two years
537 may not have been long enough to detect the effect of salinity on primary productivity. We found
538 a significant effect of Salinity, P, and Date on NEP, GEP, and ER_{CO2}. During the final months of
539 measurements, NEP in the Salt+P treatment was significantly less than the Fresh+P treatment

(Fig. 6c), potentially indicating that NEP had already plateaued and was trending downward because of continued salt stress as perturbation duration increased (Odum et al. 1979). Wilson et al. (*in press*) also found that it took two years for sawgrass in a brackish marsh to have a negative response of exposure to elevated salinity. However, in the same study, sawgrass in a freshwater marsh did not respond to elevated salinity (Wilson et al. In Press). In this study, salt had a much stronger influence than P on soil CO_2 and CH_4 efflux, yet there was a significant interaction between Salinity and P on soil CO_2 efflux in that flux was lower in the Salt+P treatment compared to the Fresh treatment. The response of sawgrass to salinity is complex and depends on the duration, concentration, and initial salinity conditions of the marsh where the salt is applied.

The contribution to greenhouse gas C balance accounts for not only the instantaneous uptake of CO_2, but also the long-term radiative forcing of CH_4 emitted to the atmosphere (Neubauer and Megonigal 2015). In our study, salinity strongly suppressed CH_4 efflux, whereas increased P loading stimulated CH_4 efflux, but only when salt was not added to freshwater monoliths. Thus, saltwater intrusion, when coupled with increased P loading, had greater calculated NEP, and therefore a larger sink of CO_2-eq C, than the freshwater control when accounting for the SGWP (Table 3). Although saltwater intrusion may initially increase the C flux to the atmosphere and alter greenhouse gas C balance in a positive way, the overall health and survival of the marsh does not only depend on aboveground productivity (see below).

Saltwater intrusion into freshwater coastal wetlands is a current pressing issue because of the potential for elevated salinity to suppress ecosystem productivity in the short term. In non-tidal marshes without allochthonous sediment inputs, such as the Everglades, peat soils are formed primarily through organic matter inputs (Nyman et al. 2006). If saltwater disrupts the processes that form peat, the marsh platform may become unstable and peat may collapse

563 (Chambers et al. 2015). Although we found little impact of GEP or ANPP with continuous
564 exposure to elevated salinity, we found a decrease in live root biomass, although this effect was
565 not significant when comparing the Salt+P to the Fresh treatment. This result supports other
566 experiments in the Everglades that show that live root biomass in the soil significantly decreases
567 with elevated salinity (Wilson 2018, Wilson et al. In Press). A decrease in live roots could result
568 in less root binding of soil and less live root mass to replace senesced roots and overall
569 compression of the soil matrix causing the soils to begun to slump and collapse (Delaune et al.
570 1994). Another study conducted on the same mesocosm monoliths used in this study found that
571 there was a ~2-cm in elevation loss after only one year in the Salt+P compared to the Fresh
572 treatment (Wilson 2018). Therefore, even though aboveground productivity and NEP increased
573 with Salt+P, soil structure and integrity appear to be negatively affected by salt and thus
574 vulnerable to collapse. This finding would explain the presence of live sawgrass "pedestals" in
575 the brackish portions of the Everglades (ambient salinity ~10 ppt), where it appears that the
576 surrounding soil has collapsed (Wilson et al. In Press). Although saltwater intrusion into
577 freshwater karstic wetlands may initially stimulate primary productivity through a P subsidy, the
578 trade-off of P subsidy and stress of elevated salinity on root productivity and soil structure may
579 ultimately be what matters to the survival or collapse of these marshes. Given the highly
580 managed character of Everglades marsh hydrology, more available freshwater delivery may help
581 to offset the stress of elevated salinity observed with saltwater intrusion.
582

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837

838 **Tables**

839 Table 1. Porewater temperature, salinity, pH, and dissolved constituents of experimental marsh mesocosms exposed to elevated

840 salinity and P.

Treatment	Temp (°C)	Salinity (ppt)	pH	DOC	NO_2^--N	NO_3^--N	NH_4^+-N	SRP	S^{2-}
Fresh	26.1 ± 0.3	0.44 ± 0.01[a]	6.81 ± 0.01[a]	1862 ± 83[a]	0.11 ± 0.006[a]	0.46 ± 0.08	6.60 ± 0.77[a]	0.15 ± 0.03[a]	0.010 ± 0.001[a]
Fresh+P	26.6 ± 0.3	0.47 ± 0.01[a]	6.80 ± 0.01[a]	1981 ± 92[a]	0.12 ± 0.011[a]	0.58 ± 0.14	8.80 ± 2.05[a]	0.20 ± 0.06[a]	0.007 ± 0.001[a]
Salt	26.8 ± 0.3	9.03 ± 0.28[b]	6.71 ± 0.01[b]	3281 ± 132[b]	0.27 ± 0.022[b]	0.32 ± 0.06	36.55 ± 4.01[b]	0.38 ± 0.03[b]	0.270 ± 0.023[b]
Salt+P	27.1 ± 0.4	8.63 ± 0.26[b]	6.70 ± 0.02[b]	3268 ± 106[b]	0.25 ± 0.013[b]	0.27 ± 0.03	28.60 ± 3.45[b]	0.39 ± 0.02[b]	0.209 ± 0.019[b]

841 Data represent mean (n = 6 replicates) ± 1 standard error of temperature, salinity, pH, and dissolved porewater constituents averaged

842 across treatments and over the duration of the experiment. DOC, NO_2^--N, NO_3^--N, NH_4^+-N, and SRP are in µmol L^{-1}, S^{2-} is in mM.

843 Letters represent significant differences among treatments from a multiple comparison test (LSMEANS, Tukey adjusted).

844 *DOC* dissolved organic carbon, *NO_2^--N* nitrate, *NO_3^--N* nitrite, *NH_4^+-N* ammonium, *SRP* soluble reactive phosphorus, *S^{2-}* sulfide.

845

846 Table 2. Averaged carbon flux measured of experimental marsh mesocosms exposed to elevated salinity and P over the experimental
847 period.

Treatment	NEP	GEP	ER_{CO2}	Soil CO_2	Soil CH_4
Fresh	5.3 ± 0.5	6.6 ± 0.5	1.4 ± 0.2	0.93± 0.08	9.3 ± 5.0
Fresh+P	12.5 ± 2.1	15.8 ± 2.9	3.2 ± 0.7	1.23 ± 0.12	35.2 ± 12.9
Salt	6.0 ± 0.8	7.4 ± 1.1	1.4 ± 0.3	0.55 ± 0.07	-0.01 ± 0.77
Salt+P	9.6 ± 1.3	12.3 ± 1.7	2.7 ± 0.5	0.48 ± 0.07	1.4 ± 0.6

848

849 Data represent the mean (n = 6) ± 1 SE of net ecosystem productivity (NEP), gross ecosystem productivity (GEP), ecosystem
850 respiration of CO_2 (ER_{CO2}), soil CO_2 (all in μmol CO_2 m^{-2} s^{-1}); and mean (n = 4) ± 1 SE of soil CH_4 flux (nmol CH_4 m^{-2} s^{-1}) from each
851 treatment over the duration of the experiment.

852

853 Table 3. Calculated sustained-flux global warming potential (SGWP) of experimental marsh mesocosms exposed to elevated salinity
854 and P over a 100-year period and corrected net ecosystem productivity (NEP) accounting for the contribution of methane (CH_4) to the
855 global greenhouse carbon balance.

	CH_4	SGWP (CO_2-eq)	NEP	Corrected NEP	CH_4 : CO_2
Fresh	0.024 ± 0.013	1.11 ± 0.59	3.81 ± 0.39	2.7 ± 0.49	0.291
Fresh+P	0.092 ± 0.034	4.18 ± 1.53	8.99 ± 1.53	4.8 ± 1.53	0.466
Salt	0.000 ± 0.000	-0.01 ± 0.10	4.28 ± 0.54	4.28 ± 0.32	-0.001
Salt+P	0.003 ± 0.001	0.16 ± 0.07	6.91 ± 0.95	6.74 ± 0.51	0.024

856 Values of CH_4 and NEP are converted from Table 2 to mg C m^{-2} min^{-1}. SGWP is calculated as 45 times the measured soil CH_4 flux
857 when CH_4 was emitted, and as 203 times soil CH_4 flux when we was uptake (Salt) in CO_2 equivalents (CO_2-eq) (Neubauer and
858 Megonigal 2015). Corrected NEP is calculated as the difference between NEP and SGWP.
859

860 **Figure Legends**

861 Figure 1. Change in sawgrass aboveground live biomass over time from experimental marsh
862 mesocosms exposed to elevated salinity (cumulative load = ~ 22 kg salt m^{-2} y^{-1}) and P
863 (cumulative load = ~ 1 g P m^{-2} y^{-1}). Increased P loading significantly increased biomass, and P
864 increased biomass over time. Points represent the monthly mean (n = 6 replicates per treatment)
865 ± 1 SE.

866

867 Figure 2. Measured aboveground net primary productivity (ANPP) from experimental marsh
868 mesocosms exposed to elevated salinity and P separated by treatment and year. Letters represent
869 the results of a Tukey's post-hoc analysis of a fixed effects two-way ANOVA performed
870 separately for each year. Points represent the annual mean (n = 6 replicates per treatment) ± 1
871 SE.

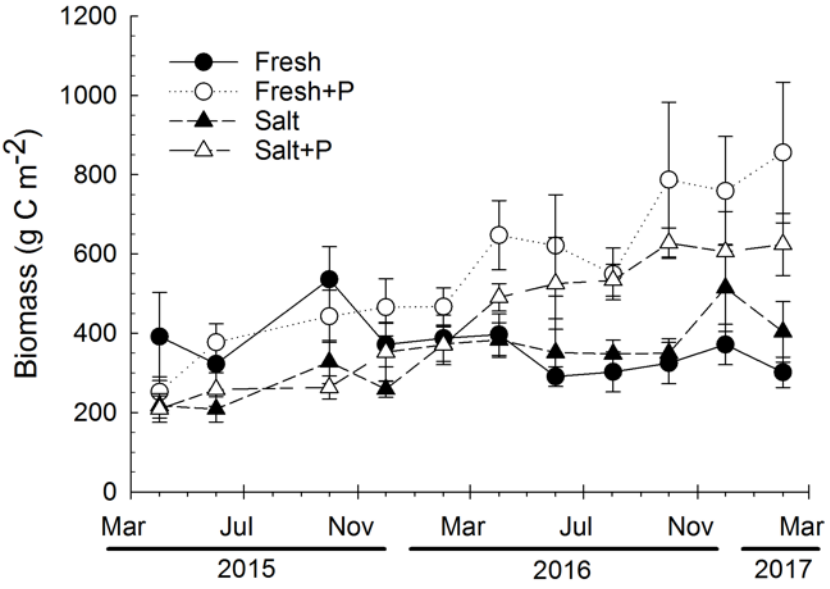
872

873 Figure. 3. Live aboveground (AG) and belowground (BG) biomass from experimental marsh
874 mesocosms exposed to elevated salinity and P after two years. Aboveground biomass was
875 calculated allometrically, whereas belowground biomass was measured through soil coring down
876 to 30-cm depth. Subscripted letters represent differences among treatments from a Tukey's HSD
877 post-hoc test of a fixed effects two-way ANOVA. Bars represent the mean (n = 6 replicates per
878 treatment) ± 1 SE in grams dry weight of material per meter squared. The number at the top of
879 each treatment represents the ratio of aboveground to belowground biomass.

880

881 Figure 4. Monthly soil CO_2 efflux over time from experimental marsh mesocosms exposed to
882 elevated salinity and P. There was a significant Salinity, Salinity*P, and Salinity*Date response
883 (ANOVA, $P<0.05$). Points represent the monthly mean ($n = 6$ replicates per treatment) ± 1 SE.
884
885 Figure 5. Measured soil methane (CH_4) efflux over the first year of the experiment from
886 experimental marsh mesocosms exposed to elevated salinity and P. Salinity significantly reduced
887 CH_4 efflux (ANOVA, $F_{(1,12)}=8.32$, $P=0.013$). There was also a Salinity*Date response (ANOVA,
888 $F_{(6.72)}=4.12$, $P=0.001$). Points represent the monthly mean ($n = 4$ replicates per treatment) ± 1
889 SE.
890
891 Figure 6. Instantaneous flux of gross ecosystem productivity (a; GEP), ecosystem respiration of
892 CO_2 (b; ER), and net ecosystem productivity (c; NEP) from experimental marsh mesocosms
893 exposed to elevated salinity and P. GEP and NEP each had a significant Salinity, P and
894 Salinity*P response, while ER_{CO2} only significantly responded to P (ANOVA, $P<0.05$). There
895 was a significant P*Date and Salinity*P*Date for GEP, NEP, and ER_{CO2} (ANOVA, $P<0.05$).
896 Points represent the monthly mean ($n = 4$ replicates per treatment) ± 1 SE.
897
898 Figure. 7. Conceptual summarization of how a freshwater, karstic wetland responds to saltwater
899 intrusion given elevated salinity and a higher P load. Responses include changes in carbon
900 dioxide (CO_2) cycling and aboveground and belowground vegetation. The flux arrows are drawn
901 to scale based on the results in Table 2, but the responses of above and belowground vegetation
902 are not drawn to scale.
903

904 **Figures**



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906 Figure 1.

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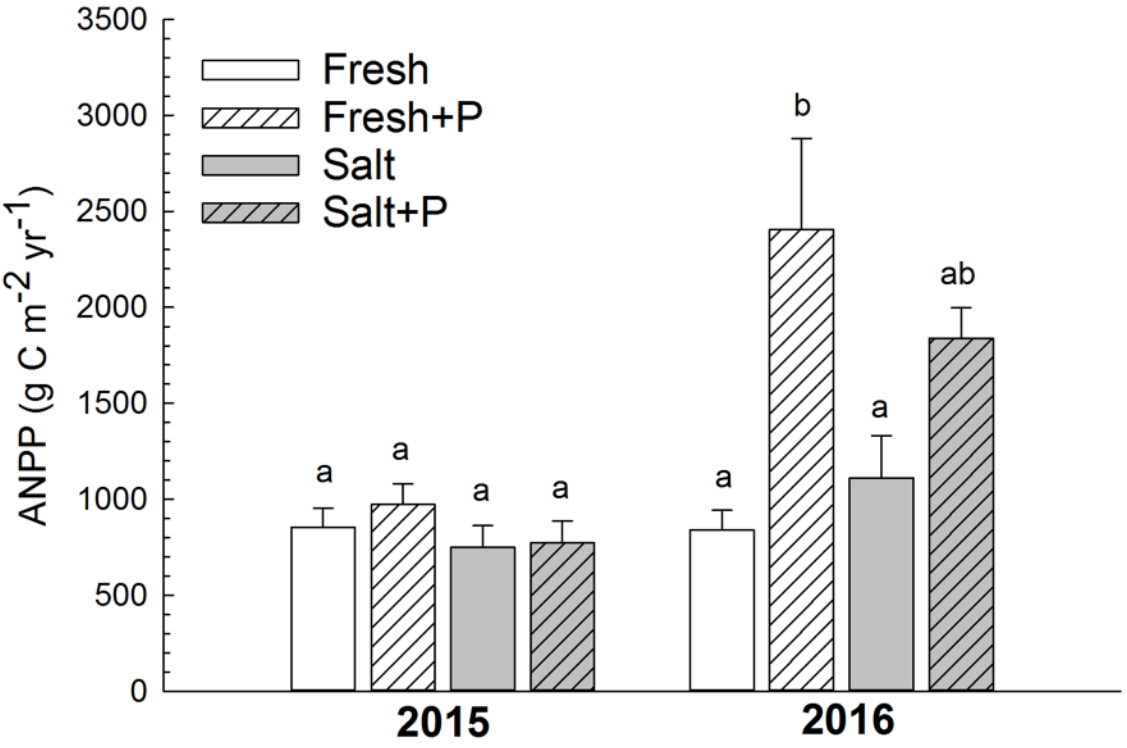

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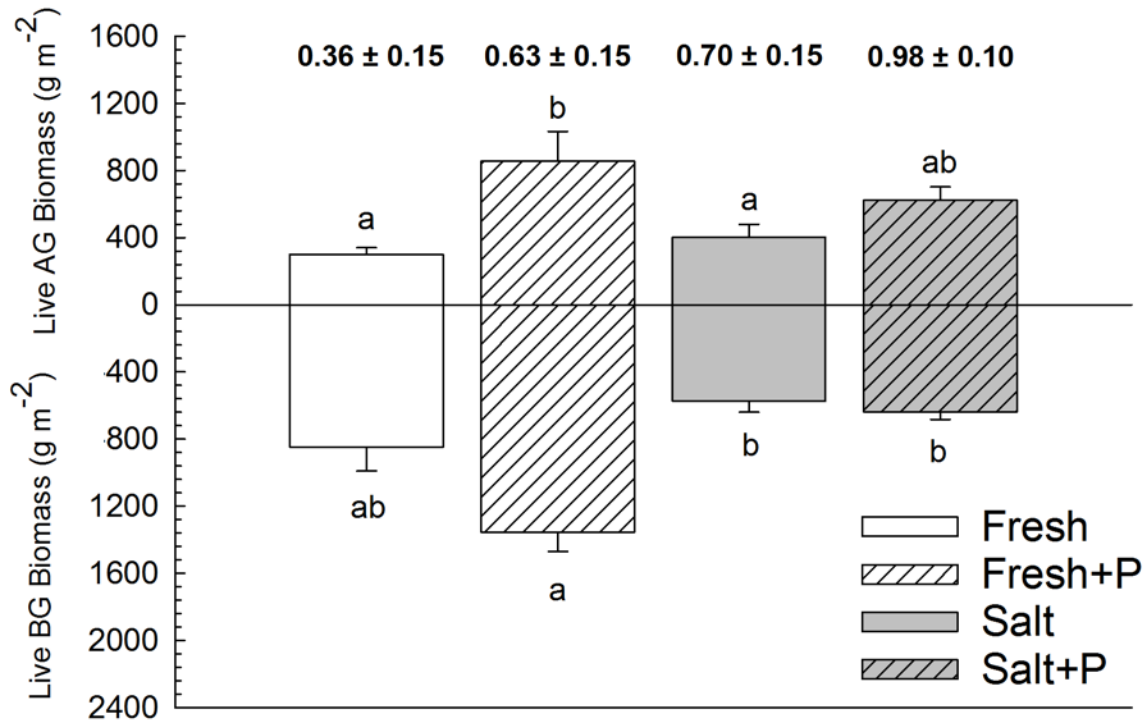

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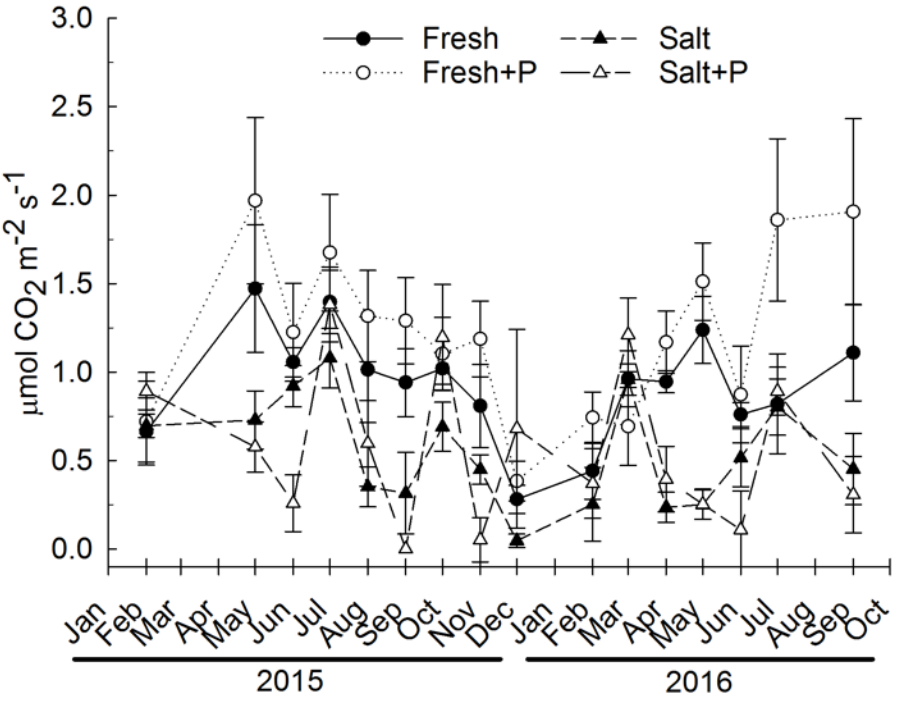

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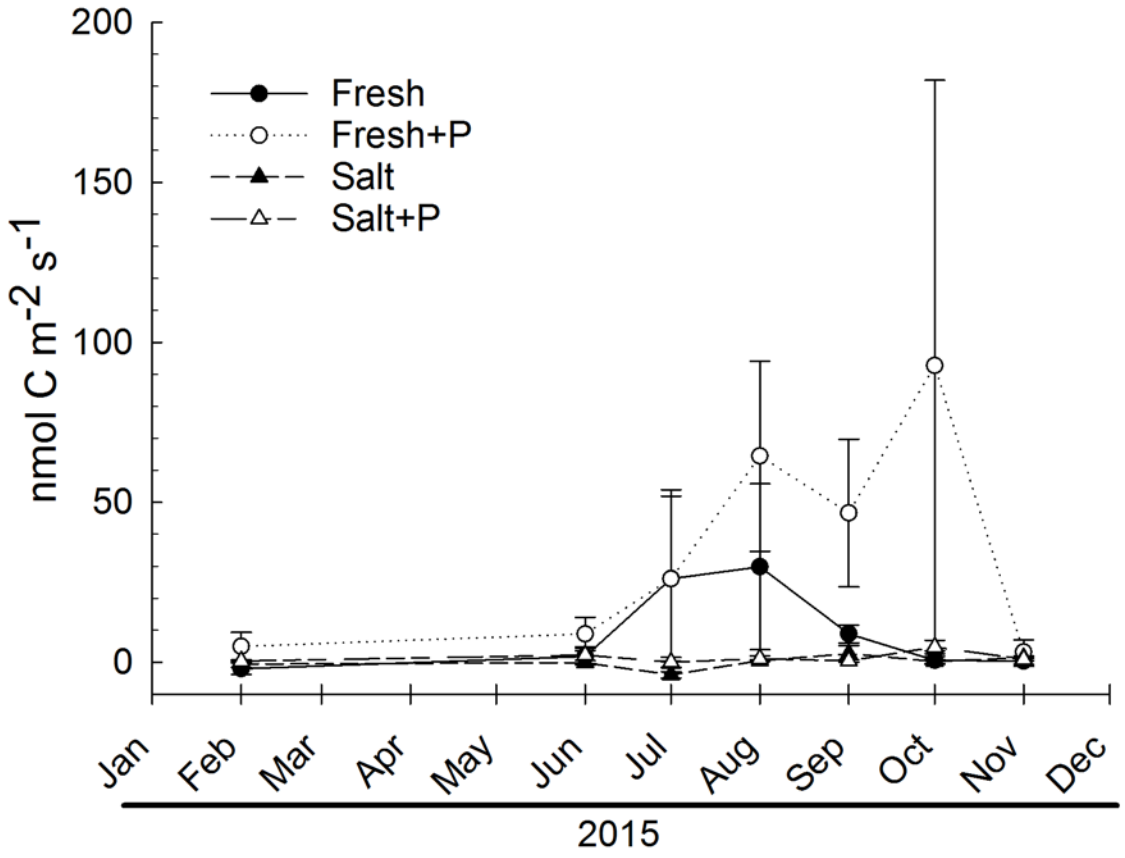
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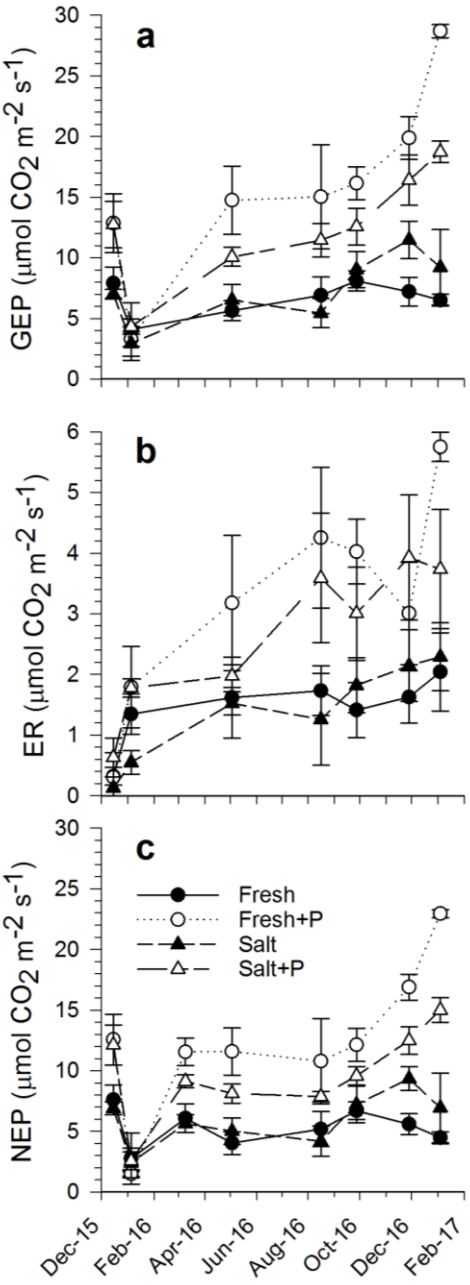
Figure 5.



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924 Figure 6.

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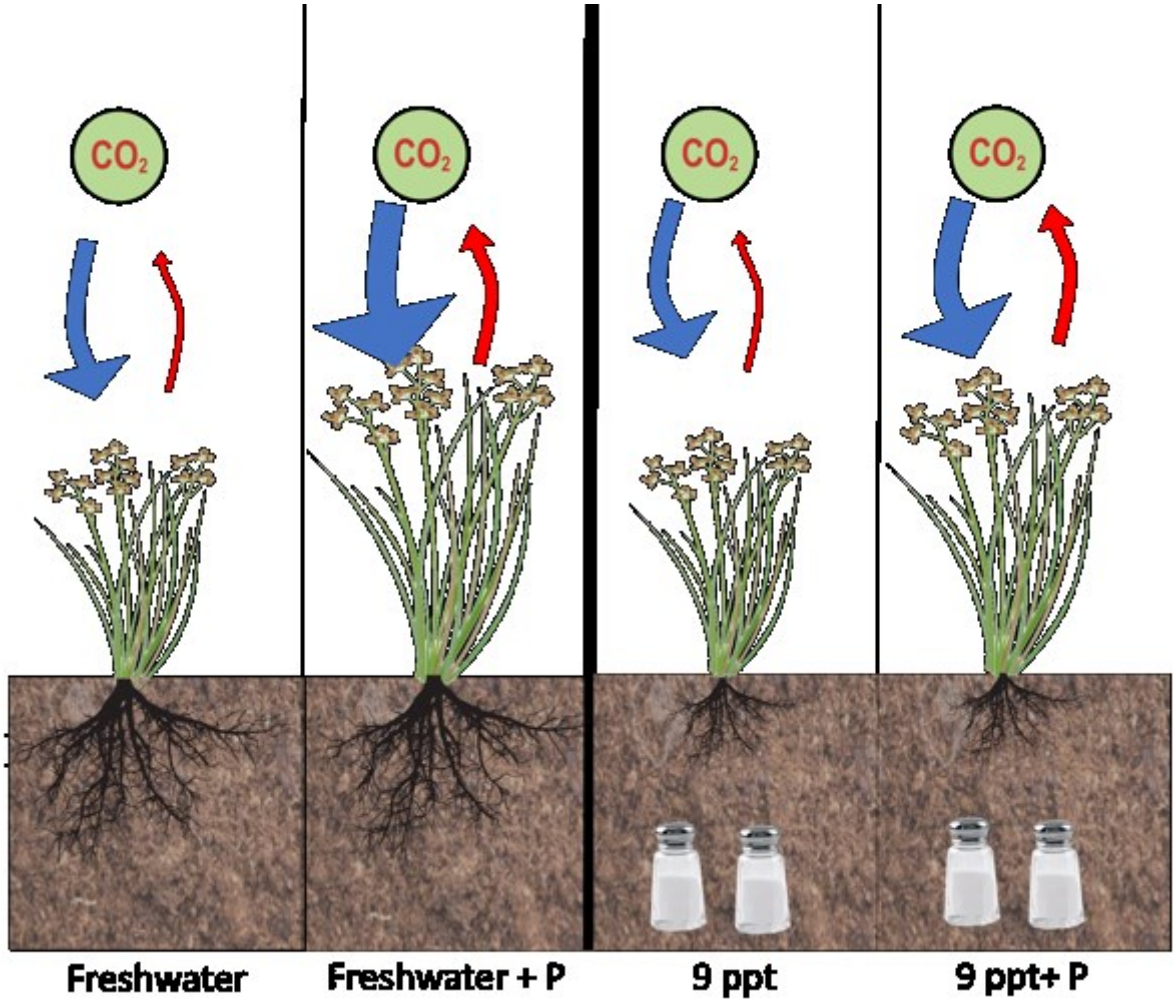

926

927 Figure. 7