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Chronic but not acute saltwater intrusion leads to large release of inorganic N in a tidal freshwater marsh

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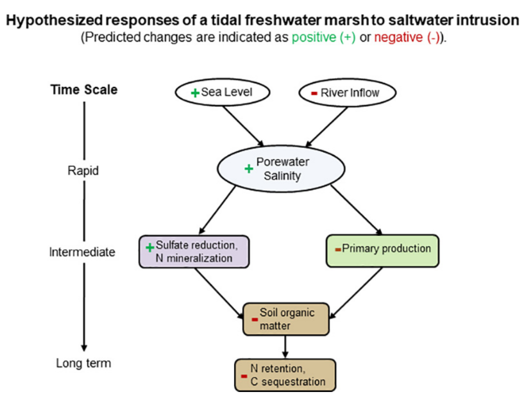
HIGHLIGHTS

- Three years of saltwater intrusion increased porewater Cl, SO_4, HS and inorganic N.
- Porewater NH_4 increased 50× and NO_3 increased 2× relative to untreated plots.
- Plant biomass decreased by 50–90% with N storage decreasing from 27 to 11 g N/m^2.
- Pulse (2 mo/yr) of brackish water had no effect on porewater N or plant N storage.
- N released by saltwater intrusion may exacerbate eutrophication of coastal waters.

GRAPHICAL ABSTRACT



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ABSTRACT

Sea level rise is expected to increase inundation and saltwater intrusion into many tidal freshwater marshes and forests. Saltwater intrusion may be long-term, as with rising seas, or episodic, as with low river flow or storm surge. We applied continuous (press) and episodic (pulse) treatments of dilute seawater to replicate 2.5 × 2.5 m field plots for three years and measured soil attributes, including soil porewater, oxidation-reduction potential, soil carbon (C), and nitrogen (N) to investigate the effects of continuous and episodic saltwater intrusion and increased inundation on tidal freshwater marsh elemental cycling and soil processes. Continuous additions of dilute seawater resulted in increased porewater chloride, sulfate, sulfide, ammonium, and nitrate concentrations. Plots that received press additions also had lower soil oxidation-reduction potentials beginning in the second year. Episodic additions of dilute seawater during typical low flow conditions (Sept.-Oct.) resulted in transient increases in porewater chloride and sulfate that returned to baseline conditions once dosing ceased. Freshwater additions did not affect porewater inorganic N or soil C or N. Persistent saltwater intrusion in freshwater marshes alters the N cycle by releasing ammonium-N from sorption sites, increasing nitrification and severely reducing N storage in macrophyte biomass. Chronic saltwater intrusion, as is expected with rising seas, is likely to shift tidal

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Soil carbon
Sea level rise

freshwater marshes from a sink to a source of N whereas intermittent intrusion from drought may have no long term effect on N cycling.

1. Introduction

Tidal freshwater marshes are highly productive ecosystems located upriver of brackish marshes in coastal estuaries. They are tidally influenced, experiencing high and low tides every day, but have salinities <0.5 ppt (Hopkinson, 1992; Barendregt et al., 2009). Tidal freshwater marshes provide valuable ecosystem services such as carbon (C) sequestration and removal of nitrogen (N) and phosphorus (P) from the water column (Neubauer and Craft, 2009). The high biological productivity that ensures provisioning of these services is dependent upon delivery of fresh water to these ecosystems (Neubauer and Craft, 2009).

Sea level is expected to rise 0.4 to 1.2 m by 2100 due to global climate change (Horton et al., 2014), leading to saltwater intrusion into freshwater wetlands (Ensign and Noe, 2018). Tidal freshwater marshes are especially vulnerable to the effects of seawater intrusion due to their location just upriver of the freshwater-saltwater interface (Park et al., 1989; Neubauer and Craft, 2009) and their low elevation relative to salt and brackish marshes in the Altamaha estuary. The effects of sea level rise (SLR) may be compounded by reduced precipitation and river flow (Nijssen et al., 2001), resulting in reduced delivery of freshwater to tidal freshwater marshes that allows salinity to encroach into freshwater reaches. In addition to its effects on sea level rise, climate change is expected to increase the frequency and intensity of hurricanes, leading to increased incidence of saltwater storm surge in coastal wetlands (Michener et al., 1997).

Seawater intrusion can occur as a continuous, or press, disturbance such as occurs from SLR or as an episodic, or pulse, disturbance as may occur during a hurricane-induced storm surge or drought-induced low river flow (Gardner et al., 1992; Michener et al., 1997; White Jr and Kaplan, 2017). Two measures of ecosystem stability in the face of disturbance are resistance, or how impervious the ecosystem is to change, and resilience, or how quickly the ecosystem returns to prior conditions after the disturbance ends (Donohue et al., 2016). The nature of a disturbance, including its intensity and duration, affects the resistance and resilience of an ecosystem (Ratajczak et al., 2017). Ecosystems exposed to a longer-term saltwater intrusion event are expected to be less resilient (i.e., recover more slowly when the disturbance ceases) than when exposed to short-term saltwater intrusion (Ratajczak et al., 2017). The length of recovery time is also correlated with the intensity of the disturbance (van Belzen et al., 2017).

Seawater intrusion can cause profound changes in natural biogeochemical cycles and ecosystem structure and function. Herbert et al. (2015) conducted a review of wetland salinization studies and identified potential biogeochemical consequences of soil salinization, including increased ammonium release and increased generation of hydrogen sulfide. Longer-term, saltwater intrusion can lead to reduced plant productivity, declining species richness and reduced N retention and C sequestration (Herbert et al., 2015; White Jr and Kaplan, 2017).

Salinization affects N cycling by increasing mineralization rates (Weston et al., 2006; Jun et al., 2013; Noe et al., 2013; Zhou et al., 2017), stimulating release of ammonium through cation exchange effects (Blood et al., 1991; Weston et al., 2010; Ardon et al., 2013; van Dijk et al., 2015), inhibiting denitrification (Joye and Hollibaugh, 1995; Rysgaard et al., 1999; Giblin et al., 2010; Wang et al., 2018), decreasing plant uptake of N due to plant stress (Gardner et al., 1992), and increasing dissimilatory nitrate reduction to ammonia (Brunet and Garcia-Gil, 1996; Senga et al., 2006). These effects combine to reduce N removal through denitrification and increase ammonium release from wetlands experiencing saltwater intrusion, reducing their ability to provide the important function of N removal. Increased ammonium release from coastal wetlands is of particular concern because excess N can lead to eutrophication in estuaries (National Research Council, 2000; Anderson et al., 2002; Howarth and Marino, 2006; Jordan et al., 2008; Howarth and Paerl, 2008).

Based on the large sulfate:oxygen ratio of seawater, seawater sulfate is the more readily available electron acceptor in seawater, and sulfate supply is typically the rate-limiting factor in sulfate reduction in freshwater wetlands (Capone and Kiene, 1988; Lamers et al., 2001). Added sulfate, such as that contained in seawater, increases sulfate reduction and leads to increased sulfide concentrations (Lamers et al., 2002). Elevated porewater salinity and sulfide concentrations create a stressful environment for plants, leading to declining biomass or death over time (Reddy and DeLaune, 2008). The reduced plant uptake of ammonium due to plant stress or death further adds to potential eutrophication concerns (Anderson et al., 2002).

The effects of seawater intrusion on tidal freshwater marshes have been documented through studies of natural seawater intrusion (Weston et al., 2010; Ardon et al., 2013; Kiehn et al., 2013) and in vitro incubations with soil cores (Edmonds et al., 2009; Weston et al., 2011). However, only a few studies of in situ manipulations of salinity have been conducted (see Neubauer, 2013; Herbert et al., 2018; Mazzei et al., 2018; Stachelek et al., 2018; van Dijk et al., 2019). We aim to build upon this work, especially Herbert et al.'s (2018) study of carbon cycling at the same experimental site, to elucidate the effects of seawater intrusion on the biogeochemical cycling of N in tidal freshwater marshes. Our study is novel because of its spatial and chronological scale (replicate 2.5 by 2.5 m plots, treatments applied for three full years) and the fact that we include both press and pulse seawater addition treatments. In this paper, we use a combination of in situ soil measurements and sample-based measurements of porewater and soil to investigate seawater intrusion's impacts on N cycling in a tidal freshwater marsh. We are interested in how porewater and soil chemistry respond to press and pulse disturbance, including the sequence in which individual attributes respond. We also are interested in how resilient the marsh is, i.e. how quickly measured attributes return to baseline once a pulsed seawater addition ends.

2. Methods

2.1. Experimental design

The Seawater Addition Long-Term Experiment (SALTEx) was established in a tidal freshwater marsh on the Altamaha River near Darien, GA, USA (31°20′16″ N, 81°27′52″ W) to investigate the effects of press vs. pulse seawater intrusion. The experiment is located 50 m from the main (south) channel of the Altamaha River and approximately 15 km upstream of the mouth of the river where the vegetation consists of primarily freshwater plant species. The plant community is dominated by giant cutgrass (*Zizaniopsis miliacea* Michx.), with lesser amounts of creeping primrose-willow (*Ludwigia repens* J.R. Forst.), smartweed (*Polygonum hydropiperoides* Michx.), and pickerelweed (*Pontederia cordata* L.). The marsh is flooded twice daily by astronomical tides, with an average high tide depth of 0.24 m over the soil surface, and the porewater salinity does not change appreciably with the rise and fall of the tides (porewater salinity data for a well installed 1.13 m below the soil surface at SALTEx can be found at: https://portal.lternet.edu/nis/mapbrowse?packageid=knb-lter-gce.656.6). Though the river water that floods the site is typically fresh (< 0.1 ppt), this location naturally experiences periodic, brief levels of increased salinity with some regularity, during hurricane storm surges and king tides in the fall (see Appendix Fig. A.1).

Plots are 2.5 m on a side and separated from one another by a buffer of 2.5 m (Fig. 1). Treatment plots have plastic siding to hold the treatment water in during dosing. The siding extends 15 cm deep into the soil and 15 cm above the soil and contains holes at the soil surface to

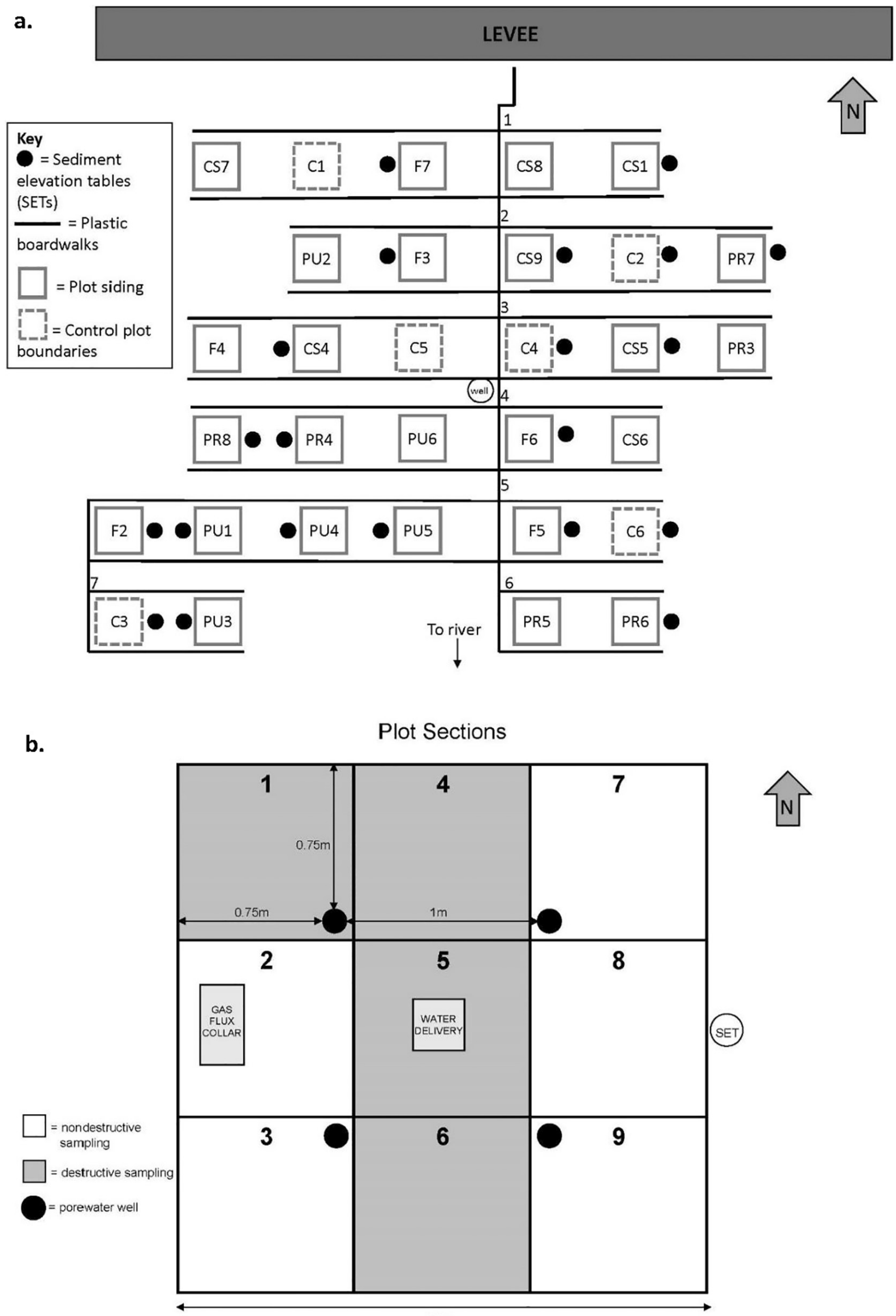

Fig. 1. (a) Plot layout map for the SALTEx site. Plot names (treatment abbreviation [C = control, CS = control with sides, F = fresh, PR = press, PU = pulse] + replicate) are indicated within the boundaries of each plot. (b) Sampling locations within the individual plots.

allow exchange of surface water. These holes are plugged during dosing events, which occur at low tide so the water has an opportunity to infiltrate prior to high tide. The holes are opened after all of the treatment water has infiltrated, approximately 30 min after the water is added.

The plots have an average elevation of 0.718 m relative to NAVD88. In 2015, a representative year in terms of river flow, the plots were inundated 95% of the time based on water levels measured every 15 min at a well in the center of the experiment. The average depth of flooding at high tide was 25 cm which is considerably above the 15.5 cm height of the plot siding. Most high tides flood above the level of the plastic siding - 69% of high tides in 2015 were >15 cm above the soil surface. The treatment water volume (53,000 L/plot/year) is approximately 5% of the mean tidal volume for 2015 (1,056,000 L/plot/year), calculated by summing the plot area multiplied by the maximum tide height for each tide in 2015. The contribution of groundwater is unknown but likely is negligible relative to the large volume of surface flooding from tidal inundation (>1 million liters per plot per year).

2.1.1. Treatment additions

Treatments consist of three dosing treatments (press, pulse, and fresh) and two controls, each replicated six times: press plots receive additions of dilute seawater approximately four days/week throughout the year, pulse plots receive additions of dilute seawater four days/week during September and October and fresh river water (4/week) the rest of the year, and fresh plots receive additions of fresh river water (4/week) throughout the year. The two control treatments consist of replicated plots with and without sides. The press treatment is meant to simulate the effects of chronic saltwater intrusion, maintaining porewater salinity of 2–5 ppt, while the pulse treatment is meant to simulate relatively brief, episodic saltwater intrusion (such as during a drought, which would typically occur in the fall). The challenge of maintaining a constant salinity under tidally inundated field conditions inevitably leads to variability in ion delivery and displacement which is why we chose a range of target concentrations, 2–5 ppt. Our robust experimental design (i.e. six replicates per treatment) enables us to identify biogeochemical and ecological responses to low salinity intrusion. The freshwater treatment is used to separate the effects of increased water additions (i.e. greater submergence) from increased salinity. Similarly, when not dosed with saltwater, the pulse treatment receives river water to keep the volume of water added constant between treatments.

Saline treatment water consists of a mixture of seawater collected from a tidal creek in Meridian, GA and river water collected from the Altamaha River adjacent to SALTEx. Analysis of the water sources, conducted concomitantly with five porewater sampling dates between January 2016 and March 2017, (Table 1) indicates that the seawater is higher in salinity (22 ppt) and sulfate (1900 mg/L) than the river water (0.09 ppt and 36 mg/L, respectively). River water is higher in ammonium, nitrate, total N, and dissolved organic C than seawater, and the brackish treatment water is intermediate in chemical composition to seawater and river water. The treatment water consists of equal volumes of seawater and river water. Seawater and river water are filtered (50 μm nominal pore size) before being mixed to ~15 ppt salinity (approximately 9000 mg chloride/L) in opaque tanks. The addition of 15 ppt brackish water is not appreciably different from peak salinities that naturally occur in surface water at that part of the estuary. The difference is that the high(er) salinities in the press and pulse plots occur several (4) times a week rather than once a month or more in nature (Appendix Fig. A.1). Fresh treatment water is filtered, but otherwise unaltered, Altamaha River water. All water addition treatments apply approximately 265 L of treatment water/day.

Plots were established in March 2013 and treatments were initiated on April 14, 2014. Treatments have continued for nearly three years, through March 2017, with annual interruptions for approximately 2–3 weeks during late December-early January due to freezing temperatures. After one year (in March 2015) five plots (two control with sides, one fresh, and two press) were no longer dosed due to consistent leakage from the plots leading to ineffective treatment application (press plots not maintaining high salinity and fresh or control plots becoming saline). These plots were excluded from the statistical analyses.

2.2. Porewater

Soil porewater was collected seasonally for three years from four porewater wells situated in a 1 m by 1 m square in the middle of the plot (Fig. 1). The wells consist of porous plastic cups (nominal pore size 40 μm) topped with PVC pipe and an airtight cap, with the porous cup extending between 10 and 35 cm below the soil surface. The wells were accessed with Tygon tubing that extended to the side of the plot and were pumped with a peristaltic pump.

Porewater was consistently sampled two tidal cycles (~24 h) after the last treatment addition from two randomly selected wells within each plot. The wells were purged (for five minutes or until they ran dry) and allowed to re-fill for approximately 30 min prior to sampling. Samples from the two wells were combined in a 500 mL acid-washed Nalgene bottle to form a composite sample. Sulfide samples were immediately collected from the 500 mL composite bottle by removing 20 mL of water with a syringe and transferring it to a 50-mL centrifuge tube containing 20 mL of Orion sulfide antioxidant buffer (SAOB). The remaining composite was then divided into three 125-mL acid-washed Nalgene bottles for N, C, and ion chromatography analyses (two bottles for N and one for C and IC analysis). All samples were placed on ice immediately after collection and frozen at the end of the day. Two bottles from each plot were shipped frozen on dry ice to the US EPA Office of Research and Development (Cincinnati, OH) for N analysis. The remaining bottles were shipped frozen to Indiana University (Bloomington, IN) for ion chromatograph (IC) and dissolved organic carbon (DOC) analysis. Samples remained frozen until analysis.

Depth-specific porewater samples were collected in July 2017 to construct porewater profiles. We collected two 35- to 40-cm deep soil cores from each press and control with sides plot using a 7.62-cm diameter acetate corer. Soil cores were extruded in the field and sectioned at 5 cm intervals. Soil sections were placed into labeled Ziploc bags and transported back to UGAMI on ice. The soils were stored at -80 °C until processing, which occurred within approximately 30 days of collection. The sections were thawed prior to processing and the porewater was extracted by hand squeezing the soils (which typically included a substantial amount of roots and rhizomes) with a DI-cleaned potato ricer. Porewater from duplicate sections was composited and centrifuged at 6200 rpm at 18 °C for 20 min. Centrifuged porewater was decanted into sample bottles and frozen at -80^{o} C until analyzed as described below for chloride and sulfate.

Table 1
Chemical composition of seawater, fresh river water, and brackish treatment water. Seawater and river water are mixed to make press and pulse treatment water. Means ± SE (number of samples analyzed).

Water type	Salinity (ppt)	Sulfate (mg-S/L)	Dissolved organic C (mg-C/L)	Nitrate/nitrite (μg-N/L)	Ammonium (μg-N /L)	Total N (μg-N/L)
Sea water	21.88 ± 1.93 (5)	1914 ± 12 (2)	6.12 ± 0.56 (5)	17.2 ± 1.6 (5)	15.0 ± 5.0(5)	673 ± 98 (5)
River water	0.09 ± 0.03 (5)	36 ± 6.9 (2)	11.13 ± 2.17 (5)	194.6 ± 42.5 (5)	52.6 ± 21.2 (5)	1186 ± 254 (5)
Treatment water	16.24 ± 0.08 (5)	1546 ± 116 (2)	7.33 ± 0.88 (5)	95.5 ± 21.2 (5)	28.8 ± 2.6 (5)	700 ± 44 (5)

Porewater chloride and sulfate were measured on a Dionex ICS-2000 Ion Chromatograph (Sunnyvale, CA) with an AS11-HC analytical column (method detection limit [MDL] = 0.5 mg chloride/L and 0.5 mg sulfate/L). Porewater sulfide was measured using an Orion Model 9616 Sure-Flow Combination Silver/Sulfide Electrode with Optimum Results B filling solution (Orion Cat. No. 900062; Orion Research, Inc., 1997). Porewater N was analyzed with a Lachat QuikChem 8500 Flow Injection Analysis system (Hach Company, Loveland, CO, USA) using QuikChem method 10-107-06-1-B for ammonium (indophenol blue complex, MDL = 7 μg N/L) and QuikChem method 10-107-04-1-J for nitrate/nitrite (cadmium reduction/EDTA red complex, MDL = 3 μg N/L).

2.3. Soils

2.3.1. Oxidation-reduction (redox) potential

Soil redox potential was measured in situ quarterly starting in July 2016. Four platinum electrodes (APHA, 1998) were placed in each plot to a depth of 10 cm and allowed to equilibrate for ~15 min. Redox potential was measured using a double junction Ag^{+}/AgCl electrode with a glass outer body and ceramic junction (Fisher Scientific Catalog No. 13-620-273), placed in the plot between the platinum electrodes, connected to an Orion Model 250A Portable pH/ISES meter. A correction factor of +236 mV was added to the field values to correct for the difference between the potential of the reference electrode and the standard hydrogen electrode.

2.3.2. Soil sampling

Pre-treatment soils were collected March 26, 2014 and analyzed for bulk density, total C and N, and extractable N. Post-treatment soils were collected December 14, 2016 for bulk soil properties. A 7.62-cm diameter by 20 cm deep core was collected from each plot. Cores were sectioned in the field into 0–5, 5–10, and 10–20 cm increments and stored in separate water-tight bags with as much of the air squeezed out as possible. Soils were put on ice in the field and stored at 4 °C at the end of the day. Soils were transported on ice to Indiana University for analysis. They were stored at 4 °C until analysis.

2.3.3. Labile nitrogen

A 20 g subsample of field-moist soil was weighed into a 50-mL centrifuge tube for N extraction. Labile N was extracted by adding 30 mL of 2 M KCl to each tube and shaking on a longitudinal shaker table for 1 h at 180 shakes per minute (Mulvaney, 1996). The extracts were then centrifuged for 1 h at 3400 rpm (2171 g). After centrifuging, the supernatant was filtered through Whatman Grade 5 filter paper into clean centrifuge tubes. The extracts were kept frozen until analysis. They were analyzed for nitrate/nitrite and ammonium on a Lachat QuikChem 8500 Flow Injection Analysis system (Hach Company, Loveland, CO, USA) using QuickChem methods 10-107-06-1-J for ammonium (indophenol blue complex) and 10-107-04-1-A for nitrate (cadmium reduction/EDTA red complex).

2.3.4. Bulk density and total carbon and nitrogen

The remaining soil was air-dried, weighed for bulk density, and ground and sieved through a 2-mm mesh sieve. Bulk density was measured by dividing the air-dried weight of the sample, corrected by the ratio of air-dried weight to oven-dried weight, by the core increment volume (Blake and Hartge, 1986).

A subsample of the ground soil was dried in an oven at 70 °C for at least 48 h for use in total C and N analyses. Soils were analyzed for total C and N by combustion on a Perkin Elmer 2400 CHN analyzer (PerkinElmer, Waltham, MA; MDL = 0.001 mg C and N). Recoveries of C and N (internal marsh soil standard; 6.106% C, 0.365% N) were 105% and 88%, respectively.

2.4. Vegetation sampling

In July 2016, we collected 50 *Zizaniopsis miliacea* individuals, 54 *Polygonum hydropiperoides* individuals, and 95 *Pontederia cordata* leaves from a marsh adjacent to the study site. The heights of the stems or leaves were measured on site, and then the plant material was dried at 60 °C to constant mass. We used these data to create allometric relationships between biomass and height for these species. The heights of all plants in one section (0.69 m^2) of the plot were measured in August 2016 and the allometric equations were used to calculate aboveground biomass for each species, which were summed to calculate total aboveground biomass. In March 2017 we collected a 7.62-cm diameter by 20 cm deep core from each plot, washed away the soil and dead plant material, and dried the roots to constant mass in a 70 °C drying oven to estimate belowground biomass. Leaf and root samples were analyzed for total N by combustion on a Perkin Elmer 2400 CHN analyzer (PerkinElmer, Waltham, MA; MDL = 0.001 mg N).

2.5. Statistical analyses

Porewater and redox data were log-transformed to meet assumptions of normality, but are back-transformed for presentation purposes. A three-way ANOVA of porewater data with treatment, elevation block, and sampling date as factors revealed a significant effect of sampling date, but not elevation block, for all analytes. Subsequently, log-transformed porewater and redox data were analyzed separately for each sampling date via a one-way ANOVA based on treatment and means were separated via Tukey's honestly significant difference test. Data that did not meet the assumption of homogeneity of variance (indicated by a Levene's test $p < 0.10$) were analyzed via Welch's robust test of equality of means followed by the Games-Howell nonparametric post-hoc test. Porewater profile data were log-transformed and compared between treatments with independent sample t-tests. For soil data, we used a two-way ANOVA based on treatment and sampling depth. Tukey's honestly significant difference test was used to compare treatment averages. Spearman's rank-order correlation analysis was used to explore associations between porewater, soil, and aboveground biomass data. All tests of significance were conducted at $\alpha = 0.05$ unless otherwise noted. Statistical analyses were conducted with IBM SPSS Statistics version 24 (IBM Corp., 2016).

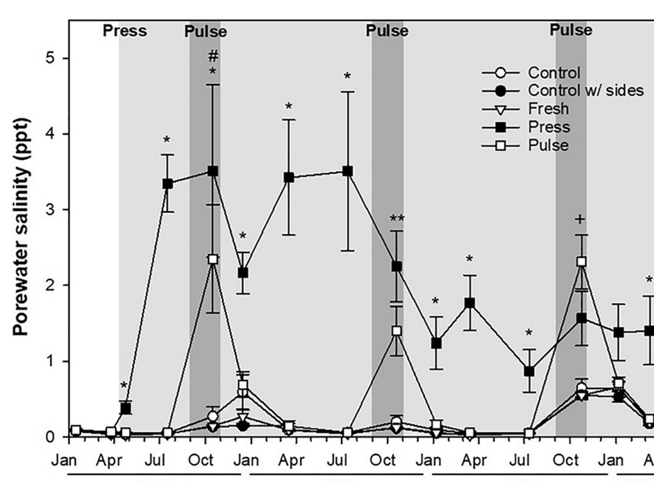

Fig. 2. Porewater salinity (means ± SE) of all treatments beginning in January 2014 and continuing through March 2017. Light gray shading indicates the duration of the press treatment, while the darker gray shading indicates the duration of the pulse treatments. * = press > others ($p < 0.05$); ** = pulse and press > others ($p < 0.05$); # = press > others except pulse; + = pulse greater than others except press.

3. Results

3.1. Porewater

Porewater salinity increased in the press plots shortly after the treatments were initiated in April 2014, and elevated concentrations were maintained throughout the experiment (Fig. 2). Sulfate concentrations also increased in the press plots beginning in April 2014 (Fig. 3a), and led to increased sulfate reduction as evidenced by elevated porewater sulfide concentrations (Fig. 3b) beginning at least by October 2014 when measurements were first made. In the pulse plots, salinity and sulfate concentrations increased in October of each year when the (pulse) treatment was applied (Figs. 2 and 3a), but decreased to background levels within 2–3 months once treatments ceased.

Porewater ammonium concentrations increased in the press plots within 4 months of the start of treatments and remained elevated throughout the three years of dosing (Fig. 4a). Though not as pronounced as ammonium, porewater nitrate concentrations also increased in the press treatments within four months of treatment additions and remained elevated, especially in summer months, for the three year duration of dosing (Fig. 4b). We did not observe any statistically significant differences in porewater ammonium or nitrate in the pulse plots relative to the controls. Porewater DOC did not differ between treatments during the three year experiment (data not shown). Porewater chemistry in the fresh plots also did not differ from the controls throughout the duration of the experiment (Figs. 2, 3, and 4).

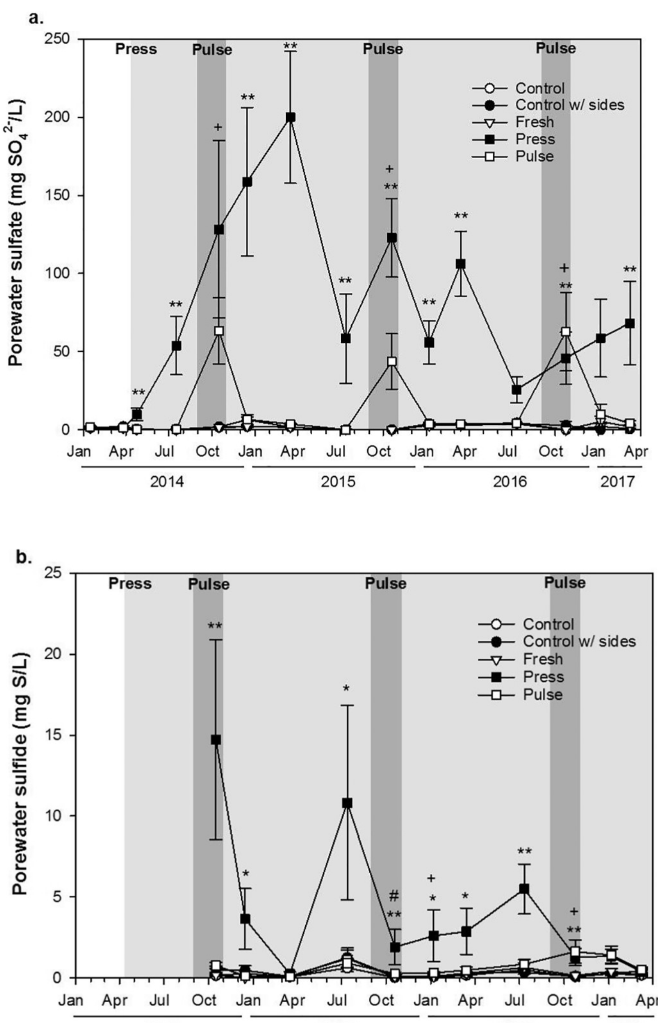

Fig. 3. Porewater (a) sulfate and (b) sulfide concentrations (means ± SE) of all treatments since measurements began. Light gray shading indicates the duration of the press treatment, while the darker gray shading indicates the duration of the pulse treatments. ** = press > others ($p < 0.05$); * = press > others ($p < 0.10$); # = press > others except pulse; + = pulse greater than others except press.

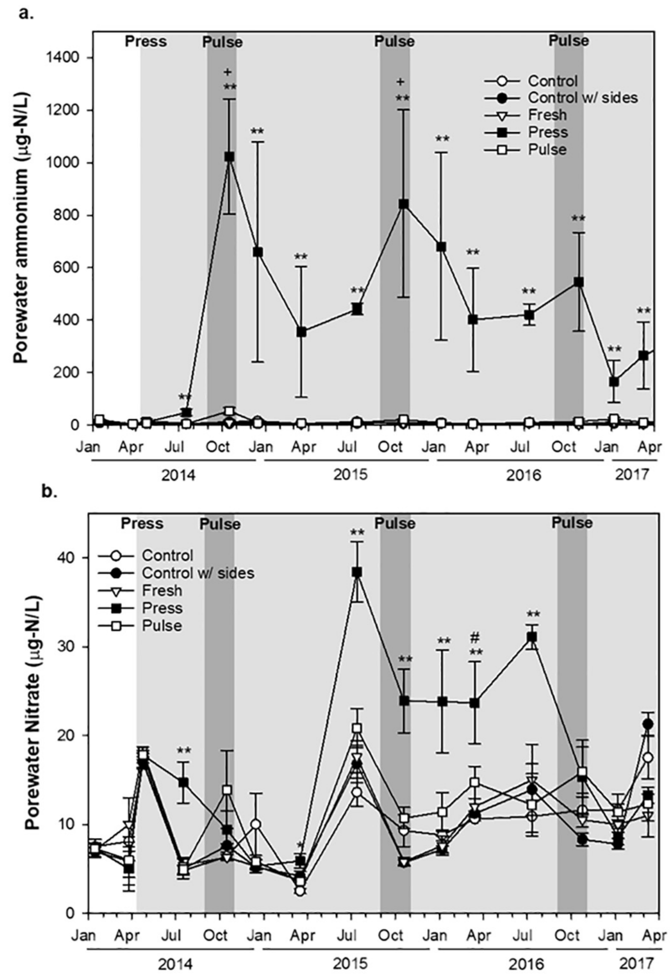

Fig. 4. Porewater (a) ammonium and (b) nitrate concentrations (means ± SE) of all treatments beginning in January 2014 and continuing through March 2017. Light gray shading indicates the duration of the press treatment, while the darker gray shading indicates the duration of the pulse treatments. ** = press > others ($p < 0.05$); * = press > others except control with sides ($p < 0.05$); # = press > others except pulse; + = pulse greater than others except press. An outlier (press replicate 5, which was an order of magnitude larger than other replicates) was excluded from ammonium analyses for July 2015.

Profiles of porewater chloride and sulfate with depth differed between press and control with sides plots. Chloride concentrations were significantly higher in press plots ($p < 0.0005$) but sulfate concentrations were not significantly different between treatments ($p = 0.398$). The chloride:sulfate ratio increased with depth in the press plots ($y = 35.16 \times \ln(\text{depth}) - 80.37$, $r^2 = 0.73$, $p = 0.02$; Fig. 5), indicating sulfate depletion relative to chloride at deeper depths. Chloride and sulfate concentrations and the chloride:sulfate ratio in the control with sides plots did not show strong trends with depth.

3.2. Soils

Soil redox potential, which we began to measure in July 2016, generally was lower in the treatments that receive water additions (fresh, press, and pulse; Fig. 6). Redox potential measured in press plots was consistently significantly lower than in control with sides plots, but was only statistically different from all other treatments in January 2017. Redox potential also was lower in the pulse plots relative to

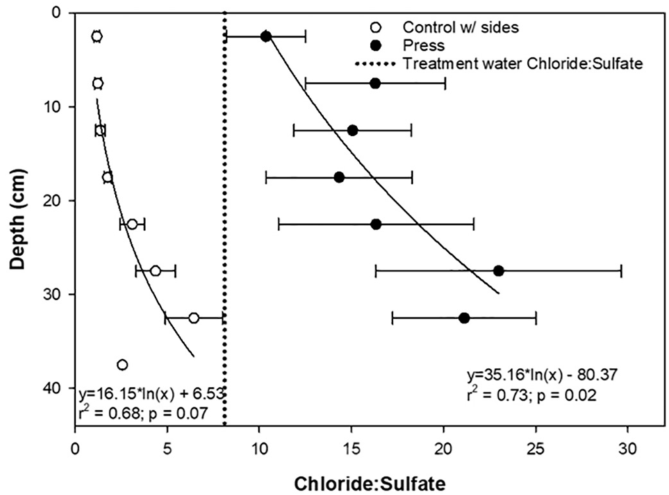

Fig. 5. Porewater chloride:sulfate ratios with depth and exponential decay curves of the control with sides and press plots sampled in July 2017.

controls in October, the season (Sept.-Oct.) of dosing. We also saw seasonal differences, with all treatments generally being more reduced in summer than the rest of the year.

Bulk density, organic C and total N did not differ by treatment in March 2014 (pre-treatment; see Appendix Table A.1) or December 2016, after three years of treatment (Table 2). They also did not differ between pre- and post-treatment samples or by sampling depth. Available ammonium-N was significantly higher in the 0–5 cm depth increment compared to the 5–10 cm and 10–20 cm depth increments ($p < 0.05$) but was not significantly different between treatments ($p > 0.05$). Available nitrate-N was below method detection limits for most (80%) of the samples, and did not differ by treatment.

3.3. Correlations

Porewater sulfate was positively correlated with porewater chloride ($p < 0.01$), sulfide ($p < 0.01$), ammonium ($p < 0.01$) and nitrate ($p < 0.01$; Fig. 7). Redox potential was negatively correlated with porewater chloride ($p = 0.008$), sulfate ($p = 0.04$), sulfide ($p = 0.047$) and ammonium ($p = 0.019$) ($p = 0.03$; Table 3). Total aboveground biomass was negatively correlated with porewater chloride, sulfate, sulfide, ammonium, and nitrate and positively correlated with soil redox potential.

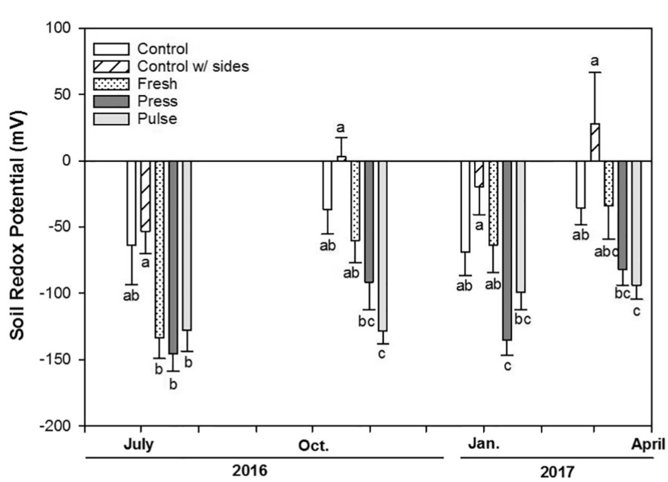

Fig. 6. Soil oxidation-reduction potential (means ± SE) measured July 2016–March 2017. Means with the same letter are not significantly different ($p > 0.05$) according to Tukey's test.

4. Discussion

4.1. Effectiveness of treatments

We successfully elevated porewater salinity to ~2 ppt in the press plots during the first two years of the study period and in the pulse plots during the pulse treatment months (Sept.-Oct.; Fig. 2). However, there was some leakage from the plots during later study months that led to slightly lower porewater salinities of 1–2 ppt in the press plots starting in January 2016. The study site also experienced two natural saltwater intrusion events that resulted in porewater salinities of 0.5 ppt or higher in all of the study plots: one in December 2014 when river flow was below the 7-day, 10-year annual low-flow threshold due to drought (USGS Station ID 0226000, Doctortown, GA) and another in October 2016–January 2017, following the storm surge of Hurricane Matthew that pushed waters of 20 ppt into the tidal freshwater marshes of our study site (Appendix Fig. A.1). Still, the press plot salinities were always significantly higher than those of the control and fresh plots except during two sampling events: October 2016 and January 2017. The two saltwater intrusion events observed during the study period indicate that the marsh at this location experiences acute saltwater intrusion similar to our pulse treatment with some regularity, which potentially explains why we did not see a large effect of the pulse treatment on soil chemistry.

The inundation only (fresh) treatment did not result in significant changes in porewater chemistry, soil redox potential, or bulk soil properties relative to the controls. The lack of a substantial effect from the fresh treatment indicates that the changes we observed in the press and pulse treatments are a result of the increased porewater salinity rather than increased submergence. Neubauer (2013) and Neubauer et al. (2013) also did not find a significant effect of freshwater additions on porewater conductivity or soil C and N content.

4.2. Soil and porewater chemistry

Continuous (press) seawater additions led to persistent and extensive changes in porewater and soil attributes, while episodic (pulse) seawater additions led to mostly transient effects that disappeared when the dosing ceased. Similar to what other researchers, including Weston et al. (2006), Chambers et al. (2011), Neubauer (2013), and Ardon et al. (2013) observed in other saltwater intrusion studies, we found increased salinity, sulfate, and sulfide concentrations in the press plots relative to the controls (Figs. 2 and 3).

We found ample evidence of sulfate reduction occurring in response to the seawater additions. For example, elevated sulfide concentrations in the press plots (Fig. 3b) indicates that the added sulfate is being reduced in the soil. Furthermore, Fig. 5 shows the rate of change in chloride:sulfate with depth (i.e. the slope) of the press treatment is twice that of the control with sides, indicating substantial sulfate reduction, especially in the deeper depths. Over the course of the experiment, on average 3% of the “missing” sulfate was found as dissolved sulfide in the porewater. Lamers et al. (2001) also observed an increase in sulfate reduction (based on measurements of sulfate depletion) when they added sulfate to freshwater marsh soil cores in a mesocosm experiment. The increase in sulfate reduction likely affects other marsh processes, such as inhibiting plant growth due to the toxic effects of hydrogen sulfide on plants (Reddy and DeLaune, 2008) and inhibiting methanogenesis, as observed by Herbert et al. (2018).

The press treatment also led to increased ammonium in the porewater starting within 4 months of the initiation of treatments and continuing for the duration of the experiment (Fig. 4a). Weston et al. (2010), Ardon et al. (2013), and Jun et al. (2013) also observed increased ammonium release from soils experiencing saltwater intrusion, which they explained by increased salt cations that displace ammonium-saturated cation exchange sites in the sediments through their mass action effect. Our findings suggest that higher levels of

Table 2
Depth-weighted means ± standard error of bulk density, organic C, total N, and labile NH_4-N of soils collected in December 2016. The means were not significantly different among treatments ($p > 0.05$).

	Control ($n = 6$)	Control w/sides ($n = 4$)	Fresh ($n = 5$)	Press ($n = 4$)	Pulse ($n = 6$)
Bulk density (g/cm^3)	0.106 ± 0.007	0.110 ± 0.005	0.108 ± 0.004	0.113 ± 0.016	0.093 ± 0.003
Organic C (mg/cm^3)	21.89 ± 1.32	24.37 ± 1.83	21.96 ± 2.01	22.40 ± 1.99	21.25 ± 0.69
Total N (mg/cm^3)	1.16 ± 0.05	1.28 ± 0.09	1.19 ± 0.09	1.13 ± 0.08	1.10 ± 0.04
Labile NH_4-N (μg/cm^3)	0.933 ± 0.158	1.131 ± 0.042	0.976 ± 0.074	1.244 ± 0.263	0.892 ± 0.142

porewater ammonium are the result of displacement of ammonium from cation exchange site and reduced nitrogen uptake by the loss of vegetation in the press plots (see discussion below).

As with ammonium, we observed a significant increase in porewater nitrate concentrations within 4 months of initiating treatments and continuing for the duration of the experiment (Fig. 4b). Our findings are in contrast to lab experiments (Weston et al., 2006) and field surveys (Ardon et al., 2013) that reported no increase in nitrate with increasing salinity. Weston and Ardon suggested that nitrification was inhibited by salinity and perhaps hydrogen sulfide in their non-tidal laboratory (sediment flow reactors; Weston et al., 2006) and field (no tidal inundation; Ardon et al., 2013) studies. Our field-based tidal freshwater marsh experiment, however, receives twice daily tidal inundation alternating with short periods (hours) where surface water is absent. Thus, there are short but frequent periods where nitrification may occur. Porewater nitrate was highest in summer months (Fig. 4b)

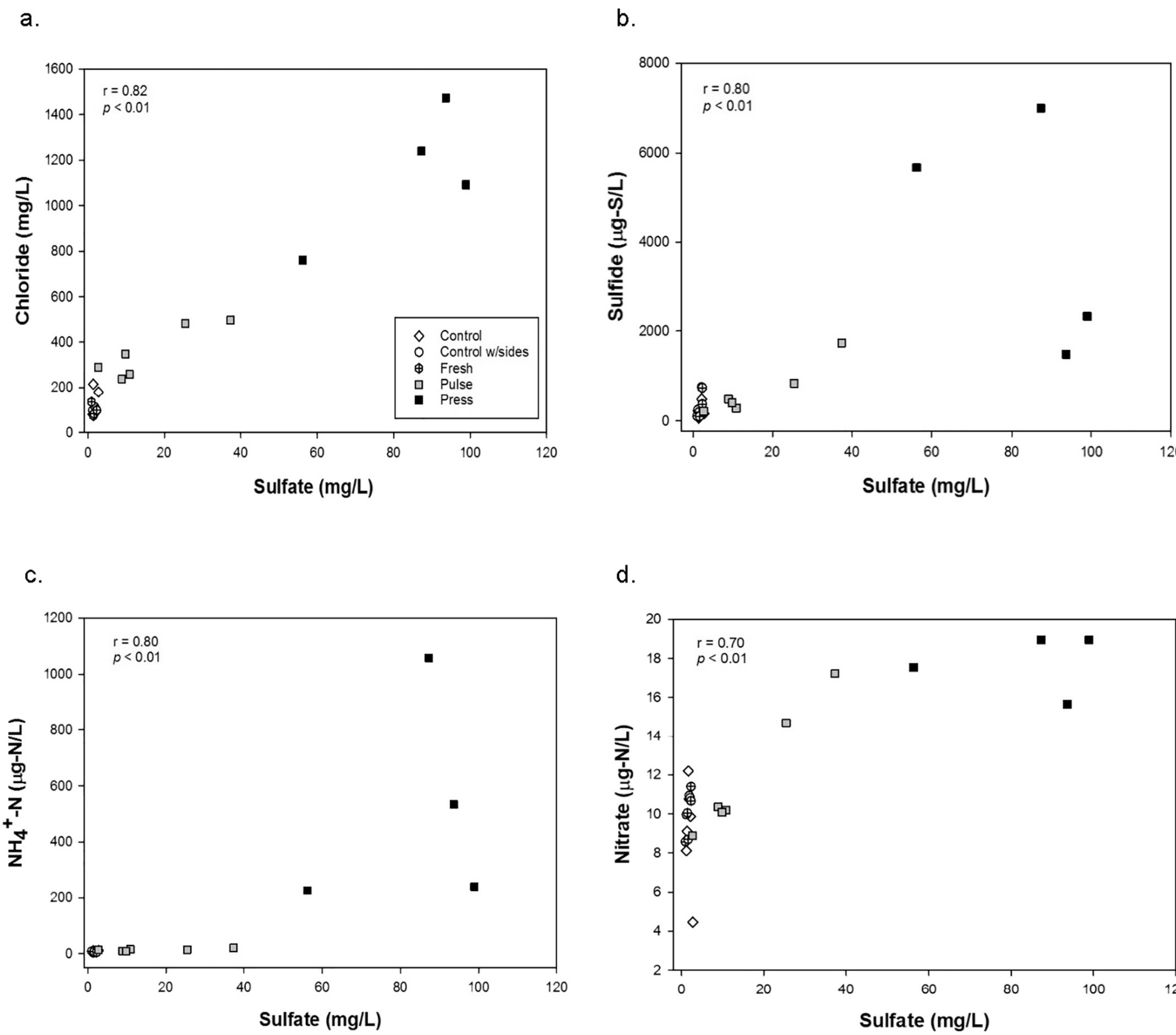

Fig. 7. Porewater sulfate plotted against (a) porewater chloride, (b) porewater sulfide, (c) porewater ammonium, and (d) porewater nitrate concentrations with Spearman's rho (ρ) correlation coefficients and p values.

Table 3
Spearman's rho (ρ) correlation coefficients for relationships between porewater sulfate, sulfide, ammonium (NH_4^+) and nitrate, soil redox potential and total aboveground biomass. Superscripts indicate significance: $^* = p < 0.05$ and $^{**} = p < 0.01$.

	Porewater .					
	Sulfate	Sulfide	NH_4^+	Nitrate	Soil Redox	Aboveground Biomass
Chloride	0.818**	0.599**	0.903**	0.554**	−0.520**	−0.588**
Sulfate		0.798**	0.797**	0.702**	−0.414*	−0.564**
Sulfide			0.594**	0.756**	−0.401*	−0.571**
NH_4^+				0.542**	−0.467*	−0.493*
Nitrate					−0.211	−0.398**
Redox						0.418*

when coupled nitrification-denitrification typically is the highest of the year (Hamersley and Howes, 2005). We postulate that the observed increase in porewater nitrate is linked to increased porewater ammonium-driven nitrification in the press treatment. In a long-term (10 year) fertilization experiment in this marsh, Herbert et al. (2019) reported that porewater nitrification potential was significantly and positively correlated with porewater ammonium concentrations ($\rho = 0.971$, $p < 0.0001$).

We also found evidence for decreased plant uptake of N due to plant stress/death (Gardner et al., 1992; Noe et al., 2013; Alldred et al., 2016). By August 2016, press plots had lost >90% of their aboveground biomass relative to control plots (Table 4) and by March 2017 press plots had roughly half as much living belowground biomass as control plots (Li, 2017 and C. B. Craft unpubl.). Based on this decline in biomass, decreased plant N uptake would result in approximately 16 g N/m^2/year of additional N in the porewater, assuming all biomass N is accumulated in one year. We are unable to say how this excess N compares to the excess N found in the press plot porewater without better measurements of porewater turnover at the site.

We found less support for other potential mechanisms of N release, including increased dissimilatory nitrate reduction to ammonium (did not see a concurrent reduction in nitrate) and increased mineralization (more below). Despite the observed increases in porewater ammonium and nitrate, the labile and total N measured in press plot soils after three years of dosing were not significantly different from the other treatments (Table 2). Our findings suggest that these N pools, especially organic N which is 95% of the total N in wetland soils (Craft, 1997), may be relatively resistant to low level salinity pulses.

Prior research (Weston et al., 2006; Weston et al., 2010; Noe et al., 2013) has suggested that seawater intrusion accelerates organic matter mineralization in fresh and brackish marshes. However, evidence from this study does not support that hypothesis. Herbert et al. (2018) measured extracellular enzyme activity (EEA) in our experimental plots during the first year of the study and found that C-acquiring EEA, which would be expected to increase if organic matter mineralization increased, declined in press plots in concert with the decline in vegetation. Further, we did not see a decrease in soil total organic C (Table 2) or a consistent effect on porewater DOC (Herbert et al., 2018), adding further evidence that organic matter mineralization was not accelerated by the saltwater additions. Our field-based measurements of soil C are in contrast to Weston et al. (2011), who observed a reduction in soil C in soil cores (10 cm diameter by 25 cm deep) exposed to 5 ppt artificial seawater for one year.

Table 4
Plant biomass, percent N, and total biomass N for control and press plots as measured in August 2016 (aboveground biomass) and March 2017 (belowground biomass).

	Control	Press
Aboveground biomass (g/m^2)	656	53
Leaf N (%)	1.16	1.38
Belowground biomass (g/m^2)	2930	1630
Root N (%)	0.66	0.66
Total biomass N (g N/m^2)	27	11

The decline in vegetation and macrophyte N storage in press plots is linked to freshwater vegetation's sensitivity to increased porewater sulfide, which was on the order of 0.06 to 0.45 mM/L (Fig. 3b). For example, porewater concentrations of 0.36 to 1.02 mM sulfide/L dramatically reduced culm, root and rhizome biomass of the freshwater emergent *Panicum hemitomon* Schult. (Koch and Mendelssohn, 1989). In contrast, the saline marsh species *Spartina alterniflora* exhibited only minor mortality of culms, roots and rhizomes at sulfide concentrations double that (1.13 mM) observed for *Panicum* (Koch and Mendelssohn, 1989).

Soil redox potential tended to be lower in all watering treatments, and was positively correlated with plant biomass across all the plots. There are two likely explanations for these results. First, regularly watering the plots, whether with fresh or saline water, may have limited oxygen penetration into the soil (Reddy and DeLaune, 2008). This is the most likely explanation for the low redox potential in the fresh treatment in July 2016 and the pulse treatment on many dates. Second, plants actively oxidate the rhizosphere as a consequence of transporting oxygen from shoots to roots (Howes et al., 1981; Armstrong et al., 1985), and the almost complete loss of plant biomass in the press treatment by 2016 (F. Li and S. C. Pennings, personal observations) likely also contributed to the low redox potential in this treatment on all sampling dates.

4.3. Disturbance, resilience, and recovery

Along with intensity and duration, timing can be an important factor in determining the effects of a disturbance (Michener et al., 1997). In this study, we found that the timing of the pulse of saline water, which we chose based on the natural drought cycle, coincided with the beginning of autumnal plant senescence, limiting the effect of the saline pulse on vegetation (F. Li et al. unpubl.). Applying the pulse treatment at a different time of year (e.g., spring) might have more of an effect on vegetation, though it seems clear that this ecosystem regularly receives episodic saltwater intrusion (see Appendix Fig. A.1).

Though the marsh shows resilience to a fall seawater pulse, the press of saline water caused extensive changes in the marsh soil characteristics including increased chloride, sulfate, sulfide, ammonium and nitrate in the porewater, and a significant decrease in aboveground biomass. The concentration of ammonium-N in the press plot porewater increased by 30 times relative to control plots, indicating the potential for eutrophication of the marsh itself and waters downstream of the salinizing marshes, both of which are N-limited in this system (Frost et al., 2009; Howarth and Paerl, 2008; Ket et al., 2011). Without knowledge of porewater exchange between the marsh and the river, we cannot estimate the magnitude of N export. However, large-scale, long-term saltwater intrusion has the potential to result in considerable N export from tidal forests and marshes (Ardon et al., 2013). Porewater nitrate concentrations were also elevated in press plots (20–40 μg N/L) relative to other treatments (5–20 μg N/L) but were much less than ammonium in the press plots (200–1000 μg N/L). However, the press treatment did not have a significant effect on organic C or total N in the soil after three years of dosing, likely due to the fact that C and N pools in soils (22 mg C/cm^3, 1100 μg N/cm^3) are much larger than the porewater C (0.011 mg DOC/cm^3) and N pools (0.38 μg N/cm^3). The effects of long-term press saltwater intrusion include changes in the plant community (brackish and saline species replace freshwater vegetation) that alter primary production and ecosystem services such as N retention and C sequestration.

In conclusion, press additions of dilute seawater in a tidal freshwater marsh resulted in considerable changes in porewater and soil chemistry, including increased ammonium, nitrate, and toxic sulfides in the porewater as well as reduced plant productivity and N storage in vegetation. Pulse additions had transient effects that dissipated when the seawater additions ceased. These findings highlight the potential for negative effects (i.e., eutrophication) downstream of permanently salinizing wetlands due to the loss of N sorption sites in soil and the loss of the plant community and its capacity to assimilate N. On a more positive note, we see the potential for resilience of tidal freshwater marshes in the face of temporary (pulse) seawater intrusion.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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