

RESEARCH ARTICLE

# Disentangling the effects of drought, salinity, and sulfate on baldcypress growth in a coastal plain restored wetland

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Because of their dominance in swamps of the southeastern United States, baldcypress (*Taxodium distichum*) trees are commonly used in wetland restoration. Though baldcypress are known to tolerate moderate flooding and salinity, their growth has been shown to decrease when they experience drought and high salinity. This study examined the effects of drought and elevated salinity on the growth of baldcypress seedlings and saplings. In a restored wetland in North Carolina, we examined the growth of 8-year-old baldcypress by measuring height and diameter at breast height (DBH) along salinity, nutrient, and flooding gradients. In a greenhouse, we placed 1-year-old baldcypress seedlings in either drought or saturated conditions and applied different water treatments: fresh water, sulfate, and artificial salt water (5 ppt [parts per thousand]). Over 26 weeks, we measured diameter at root collar, height, and biomass. In the field, chloride concentrations in soil solution had a negative effect on DBH and height (51 and 36% decrease, respectively); high water levels had a negative effect on height (47% decrease) and DBH (46% decrease). In the greenhouse, both drought and salinity decreased diameter growth (43 and 61% decline, respectively) and height (64 and 43% decline, respectively). Sulfate did not have a significant effect on diameter growth, but caused a 14% decrease in height. Our results suggest that both drought and salinity (even as low as 1 ppt) can lead to a 20–60% decline in baldcypress growth. Restoration practitioners should consider the negative consequences of both drought and increased salinity on baldcypress growth when planning for wetland restoration.

**Key words:** diameter, greenhouse, height, North Carolina, saltwater intrusion

## Implications for Practice

- Despite being considered a moderately salt tolerant species, even relatively low levels of salinity (1 ppt) can lead to 20–60% decline in baldcypress seedling and sapling growth.
- Hydrologic connectivity to saltwater bodies should be considered carefully when planning coastal wetland restoration sites.
- Restoration practitioners should expect slower growth of baldcypress trees in areas predicted to experience longer and more severe droughts.

## Introduction

Wetland restoration is an important tool for the recovery of ecosystem services such as habitat for water-dependent fauna, flood protection, and carbon sequestration (Zedler & Kercher 2005). Wetland restoration is increasingly being used as a tool for climate change adaptation (Temmerman et al. 2013). The success of a restoration project greatly depends on the establishment of wetland vegetation which has fairly exacting requirements associated with elevation, hydrologic, and salinity regimes (Middleton 2003). Thus, changes in hydrology due to climate change, sea-level rise, and human activities will affect the establishment of vegetation in restored wetlands. In the

southeastern United States, restored wetlands along the coastal plain are particularly vulnerable to changing hydrology and increasing salinity (Craft et al. 2009). It is unclear how wetland vegetation that is still establishing itself in restored sites will respond to changes in hydrology and salinity.

Baldcypress (*Taxodium distichum* (L.) Rich.) are foundational, dominant trees in the swamps of the southeastern United States (Shaler 1887; Mattoon 1915; Ellison et al. 2005), commonly used in wetland restoration (Krauss et al. 2007). Despite the amount of research on the effects of flooding and salinity on the growth of baldcypress in minimally impacted wetlands (Shanklin & Kozlowski (1985); Krinard & Johnson (1987); Conner et al. (1997); references therein), limited research has examined the response of baldcypress in restored wetlands to changes in flooding and salinity. Both climate change and sea-level rise will alter frequency of flooding and salinity along

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coastal areas (Conner et al. 1997), making it important to understand their individual and combined effects.

Baldcypress have been known to tolerate moderate flooding, but their growth decreases with deep (>1 m) or prolonged flooding (Brown & Montz 1986; Conner & Brody 1989). Symptoms of flooding stress in baldcypress include branch die-back, lack of fruiting, decreased shoot growth, and increases in vulnerability to predators and pathogens (Broadfoot & Williston 1973). Flood tolerant tree species are generally not drought tolerant; therefore, baldcypress may be just as, or more, sensitive to drought conditions than flood conditions (Magonigal & Day 1992). Drought stress can damage baldcypress shoots in approximately 4 hours, causing them to wilt or collapse (Dickson & Broyer 1972).

Salinity stress can cause decreases in growth and survival of baldcypress (Chapin 1991). Swamp forests may be exposed to periods of high salinity due to drought, storm surge, or weather fronts (Krauss et al. 2007). Under controlled settings, baldcypress can tolerate salinities of less than 2–3 parts per thousand (ppt) for more than 50% of the year (Brown & Montz 1986). If salinity levels are higher than 2–3 ppt for 50% of the year, baldcypress become severely stressed and eventually died, unless there is a freshwater input (Wicker et al. 1981; Brown & Montz 1986). In greenhouse experiments and in the field, baldcypress can survive with salinity of up to 3–4 ppt, but exhibit decreased growth (Allen et al. 1994; Krauss et al. 2007).

Much of the research on the effects of increased salinity on baldcypress growth and survival has focused on osmotic stress caused by increased salinity. The increase of marine salts can also cause increases in hydrogen sulfide (sulfate found in saline water is reduced to hydrogen sulfide in anaerobic soils), which is known to be toxic to plants and reduces biomass (Lamers et al. 2013; Hackney & Avery 2015). Smooth cordgrass (*Spartina alterniflora*) and maidencane (*Panicum hemitomon*), dominant marsh species along the Gulf Coast, had a significant lower total plant biomass when treated with sulfide in a greenhouse experiment (Koch & Mendelssohn 1989). A study that looked at fumigating seedlings of baldcypress with sulfate found sulfate did not have a significant effect on height; however, it did have a significant negative effect on number of needles and leaf area (Shanklin & Kozlowski 1985). To our knowledge, the potential negative effects of increasing sulfate and sulfide on baldcypress planted in a restoration site have not been previously studied.

We have previously reported that seasonal drought-induced saltwater incursion leads to increased nitrogen release from natural and restored wetlands in the coastal plain of North Carolina (Ardón et al. 2013). These major changes in water quality at a large-scale restored wetland have occurred without major changes in plant community composition (Hopfensperger et al. 2014). Here, we examined the effects of flooding and drought-induced salinity on baldcypress planted in 2004, in the same large-scale restored wetland as the previous studies. To disentangle the effects of drought from the effects of salinity, we also conducted a greenhouse experiment looking at the effects of drought and salinity on 1-year-old baldcypress seedlings. Furthermore, we were interested in isolating the effects of sulfate from other salt ions, so we included salinity treatments

with and without sulfate (Ardón et al. 2013). We predicted that (1) baldcypress growing in the field and experiencing drought-induced salinity would have the lowest growth; (2) baldcypress seedlings growing in the greenhouse under drought conditions would have lower growth than seedlings in saturated/flooding conditions; (3) baldcypress seedlings exposed to both drought and salinity would have the lowest growth and biomass; and (4) seedlings exposed to sulfate would show decreased growth rate, but the effect would be lower than salinity.

## Methods

### Field Measurements

The Timberlake Observatory for Wetland Restoration (TOWeR) site is located in Tyrrell County, NC, U.S.A. (35°54'32.835''N, 76°9'36.054''W) (Fig. 1). This area was formerly comprised of pocosins (freshwater wetlands in higher elevations) and baldcypress swamps until it was logged extensively in the mid-1800s and cleared for agriculture production in the 1970s and 1980s (Carter 1975; Richardson 1983). In 2004, restoration began with 750,000 seedlings of eight different tree species planted throughout the site. Approximately 60% of the planted trees

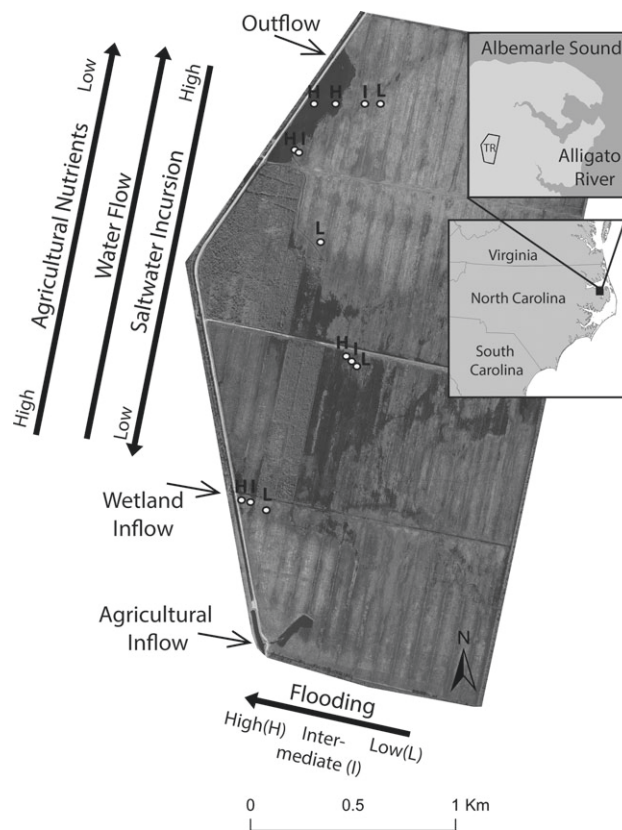


Figure 1. Inset shows location of TR (Timberlake Observatory Wetland Restoration) in eastern North Carolina. Map of Timberlake Observatory Wetland Restoration Site (TOWeR) with location of inventoried plots. Arrows represent direction of water flow, agricultural nutrients, saltwater incursion and flooding.

were baldcypress. The baldcypress were purchased from ArboGen Inc. (Ridgeville, SC, U.S.A. <http://arborgen.us/>) and were from the Northern Louisiana seed source (R. Needham 2014, personal communication). Currently, agricultural fields to the south drain into the site, providing seasonal inputs of fresh water containing high nitrogen levels (up to 4 mg/L  $\text{NO}_3\text{-N}$ , (Ardón et al. 2010)).

Since the seedlings were planted, the site has been subjected to inundation and saltwater incursion, creating flooding and salinity gradients (Ardón et al. 2013). Water flow at the site depends on precipitation and wind direction and can exhibit bidirectional flow (Ardón et al. 2010). Water levels across the site have increased since the initial flooding in 2007 and continue to increase (Ardón et al. 2013). One of the original goals of the restoration project was to allow hydrologic reconnection to Albemarle Sound, providing additional spawning habitat for river herring (*Alosa aestivalis* and *Alosa pseudoharengus*) (Needham 2006). River herring are anadromous fishes whose populations are at historical lows due to overfishing and habitat loss (Hall et al. 2012). Hydrologic reconnection to allow access to anadromous fishes has meant that during periods of drought, salt water has entered the site from the north due to wind tides from the Alligator River, causing salinity to increase from 0 to a maximum of 6 ppt (Ardón et al. 2013).

To study the effects of flooding, salinity, and water nutrient concentrations, we conducted an inventory of baldcypress in 13 circular plots with a diameter of 20 m (area = 314 m<sup>2</sup>; 0.03 ha) across five transects spanning elevation (flooding), salinity, and nutrient gradients (Fig. 1). Within each plot, all baldcypress with a diameter at breast height (DBH) greater than 1 cm were tagged and measured (height and DBH). For trees that were taller than 5.3 m, true height was recorded as greater than 5.3 m. At the end of the study, only 33 stems out of 253 exceeded this height, limiting underestimation of heights by plot. We also measured standing water depth at each tree.

**Soil Solution Water Chemistry.** Between 2007 and 2012, soil solution was sampled from 15 cm piezometers at bimonthly to quarterly frequency. Samples were collected in high density polyethylene (HDPE) plastic bottles, filtered in the field (Whatman GF/F, 0.7 mm), and frozen until analyses for nitrate ( $\text{NO}_3\text{-N}$ ), ammonium ( $\text{NH}_4\text{-N}$ ), total dissolved nitrogen (TDN),  $\text{PO}_4\text{-P}$  (measured as soluble reactive phosphorus [SRP]), chloride ( $\text{Cl}^-$ ), and sulfate ( $\text{SO}_4^{2-}$ ) (Ardón et al. 2010) were done.  $\text{NO}_3\text{-N}$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$  were measured using a Dionex ICS-2000 ion chromatograph with an AS-18 column (Dionex Corporation, Sunnyvale, CA, U.S.A.).  $\text{NH}_4\text{-N}$  was measured using the phenate method on a Lachat QuickChem 8000 automated system (Lachat Instruments, Milwaukee, WI, U.S.A.). SRP was measured using the ascorbic acid and molybdenum blue method also with a Lachat QuickChem 8000. TDN was measured on a Shimadzu TOC-V total carbon analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.).

**Statistical Analysis.** Total basal area was calculated for each plot. Dry weight was calculated using the equation:

$\text{BM} = \text{Exp}(\beta_0 + \beta_1 \ln(\text{DBH}))$ , where  $\beta_0 = -2.0336$  and  $\beta_1 = 2.2592$  (Jenkins et al. 2003). Once the woody biomass was obtained, we assumed that 50% of the woody biomass was carbon. We separated the plots into three categories using water level: low (<10 cm,  $n = 4$ ), intermediate (10–30 cm,  $n = 5$ ), and high (>30 cm,  $n = 4$ ). To compare means of water nutrients, DBH, height, stem density, basal area, and total carbon among water level categories, we used analyses of variance followed by post hoc Tukey and adjusted the  $p$  values for false discovery rate (Verhoeven et al. 2005; Pike 2011). We used linear regression to examine relationships between water chemistry parameters and water depth and DBH and height of baldcypress measured in the field. We were unable to conduct multiple linear regression model selection procedures due to the low sample size ( $n = 13$  plots) and a high degree of intercorrelation between variables. We compared the different models using Akaike's information criteria (AIC) and the corrected AIC (AICc) for small sample size (McQuarrie & Tsai 1998). Regression analyses were performed in R 2.13.0 (R Core Team 2012) using the leaps and SME packages and lm() and the AICc() commands.

### Greenhouse Measurements

A greenhouse experiment was conducted to isolate the effects of drought and salinity on growth of seedlings and to separate the effects of sulfate from other salt ions (chloride, sodium, magnesium). Dormant, 1-year-old, bare root seedlings (North Carolina Forestry Service, New Bern) were potted and kept at East Carolina University, Department of Biology greenhouse for the duration of the experiment (35°36'18.7236N, 77°21'49.6182W). One seedling was planted per pot (5.68 L) and watered with 1 L of fresh water biweekly until treatments began 10 weeks after potting. Treatments and measurements continued for the next 26 weeks.

We used two hydrological treatments, drought and saturated, with 32 seedlings in each treatment. Within the hydrological treatments, there were four water chemistry treatments: (1) fresh water as a control, (2) only sulfate ( $\text{SO}_4^{2-}$ ), (3) artificial salt water without  $\text{SO}_4^{2-}$ , and (4) artificial salt water with  $\text{SO}_4^{2-}$  (target 5 ppt) (as in Ardón et al. (2013)), with eight seedlings in each water chemistry treatment. All seedlings were watered biweekly, once with 500 mL of the appropriate treatment, and once with 500 mL of fresh water (to avoid excess salinity build up in the soils), for the first 7 weeks. Starting week eight, seedlings were watered twice a week, once with 500 mL of fresh water and on alternating weeks either 500 mL of the appropriate treatment or 500 mL of fresh water. Artificial salt water was made with a modified version from Kester et al. (Kester et al. 1967) as described in Ardón et al. (2013). Seedlings in the drought treatment were watered and allowed to drain. Seedlings in the saturated treatment were placed in trays where the water level was maintained at 7.5 cm deep for the duration of the experiment.

Height, diameter at root collar (here-after "diameter"), and water conductivity for the different treatments were measured weekly (YSI Model 556, Yellow Spring, OH, U.S.A.). At the end of the experiment, seedlings were divided into leaves,

stems, and roots, and dried at 70°C for 48 hours to measure biomass. Additionally, water was collected every other week from a subset of pots using microlysimeters (Rhizon MOM, 5 cm, Sunvalley Solutions Inc., Sunny Isles Beach, FL, U.S.A.). Water samples were analyzed for Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> using ion chromatography (Dionex ICS-2100, Dionex Corporation).

**Statistical Analyses.** Height and diameter data were log transformed and analyzed using repeated measures multivariate analysis of variance (MANOVA), and the difference between means was considered significant when *p* values were less than 0.05 (version 10, JMP Software, Cary, NC, U.S.A.). Factors in the MANOVA were date (26 weeks), hydrology (saturated and

drought), sulfate (present and absent), salt (present and absent), and all interactions.

Biomass data, also log transformed, were analyzed using analysis of variance (ANOVA) (JMP 10). Factors in the ANOVA were hydrology (saturated and drought), sulfate (present and absent), salt (present and absent), and their interactions.

## Results

### Field Measurements

Nutrient concentrations in soil solution varied across the site (Fig. 2). Only NH<sub>4</sub>-N and TDN concentrations were

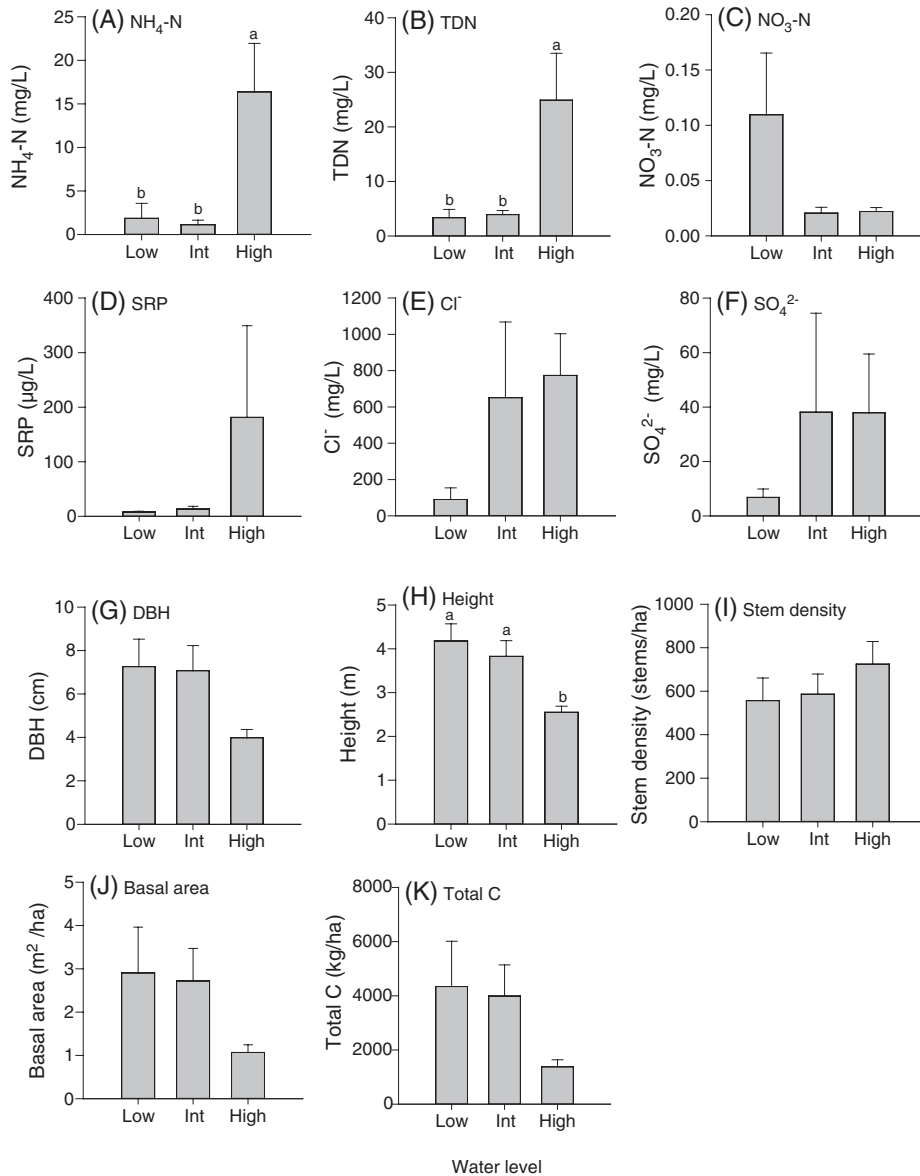


Figure 2. Mean ammonium (A), total dissolved nitrogen (B), nitrate (C), soluble reactive phosphorus (SRP, D), chloride (E), sulfate (F), diameter at breast height (G), height (H), stem density (I), basal area (J), and total C (K) across TOWeR based on water depths (low <10 cm, intermediate 10–20 cm, high >20 cm) (+ SE). Letters denote significant differences from post hoc Tukey test for significantly different comparisons adjusted for false discovery rate.



significantly different among water level categories, with both being higher in the sites with high water level ( $\text{NH}_4\text{-N}$   $F_{[2,10]}=7.51$ ,  $p=0.010$ ,  $FDR$  adjusted  $p=0.04$ , TDN  $F_{[2,10]}=6.71$ ,  $p=0.014$ ,  $FDR$  adjusted  $p=0.04$  Fig. 2A & 2B). In contrast,  $\text{NO}_3\text{-N}$  concentrations were highest in the low water level sites, but due to high variability, the difference was not significant.  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  were also higher in higher water levels, but due to high variability, the differences were not significant (Fig. 2E & 2F).

For all 246 tagged trees, DBH, height, basal area, and total C were all lower in the high water level plots, but the difference was only significant for height ( $F_{[2,10]}=6.34$ ,  $p=0.0160$ ,  $FDR$  adjusted  $p=0.04$ , Fig. 2G, 2H, 2J, & 2K). We estimated that increased salinity led to an 80% decrease in C standing stocks. Stem density was higher in the high water level plots, but the difference was not significant (Fig. 2I). DBH and height were negatively correlated to  $\text{Cl}^-$  concentrations in soil solution (51 and 36% decrease, respectively; Table 1; Fig. 3A & 3C).  $\text{NH}_4\text{-N}$  concentrations and TDN were negatively related to height, but not to DBH (Table 1). DBH and height declined with water level (46 and 47% decrease, respectively; Table 1; Fig. 3B & 3C). The linear regression models suggest that salinity was a better explanatory variable than water depth (higher  $r^2$  and

**Table 1.** Significant linear regression models relating average DBH and tree height for the 13 plots versus water chemistry and water depth. AIC, Akaike's Information Criteria;  $\text{AIC}_c$ , Akaike's information criteria adjusted for small sample size.

	$r^2$	$p$	Slope	AIC	$\text{AIC}_c$
<b>DBH</b>					
Water depth	0.30	0.051	-0.06	22.16	63.72
$\text{Cl}^-$	0.37	0.027	-0.002	20.83	62.38
<b>Height</b>					
Water depth	0.51	0.005	-0.03	-7.96	33.59
$\text{Cl}^-$	0.38	0.020	-0.0008	-4.99	36.56
$\text{NH}_4\text{-N}$	0.34	0.035	-0.05	-4.07	37.48
TDN	0.31	0.045	-0.03	-3.58	37.97

lower  $\text{AIC}_c$ ) for DBH (Table 1). The models also suggest that water depth was better at explaining the variability in height (higher  $r^2$  and lower  $\text{AIC}_c$ ) than  $\text{Cl}^-$ ,  $\text{NH}_4\text{-N}$ , and TDN (Table 1).

### Greenhouse Measurements

Water conductivity (mS/cm) differed across the treatment: fresh water  $0.2 \pm 0.02$ ,  $\text{SO}_4^{2-}$  only  $1.0 \pm 0.01$ , salt water without  $\text{SO}_4^{2-}$   $9.1 \pm 0.4$ , and salt water with  $\text{SO}_4^{2-}$   $9.4 \pm 0.1$ . As

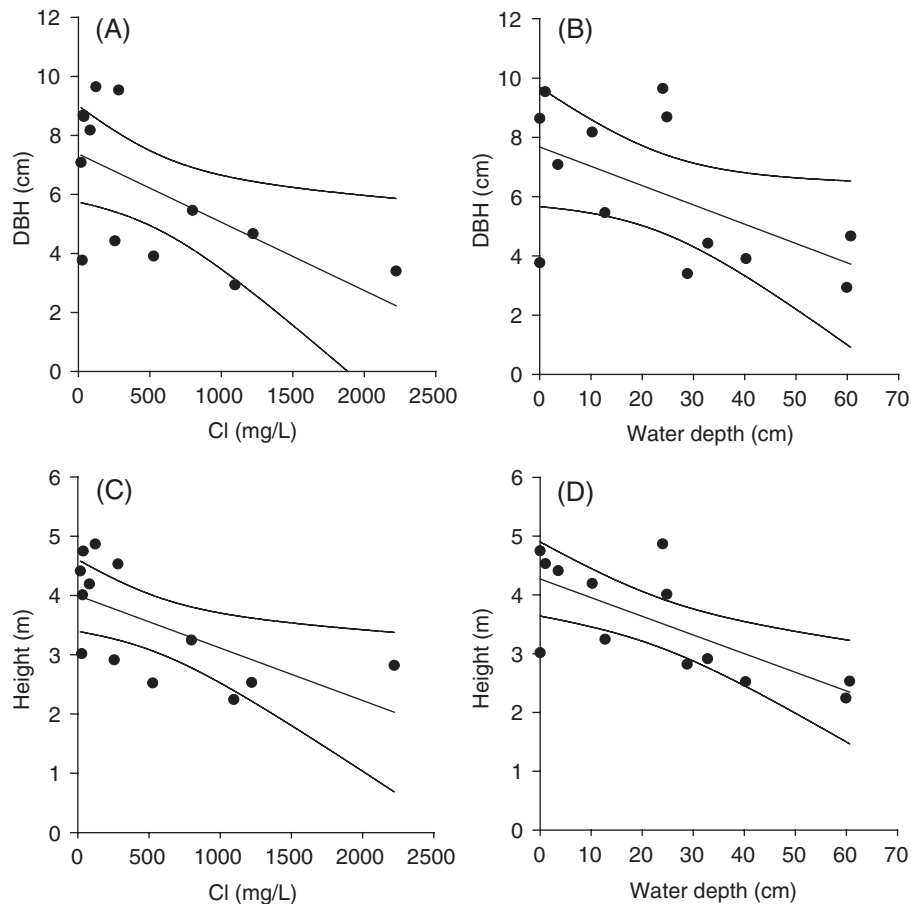


Figure 3. Linear relationships between DBH and chloride ( $r^2=0.37$ ,  $p=0.027$ , A); DBH and water depth ( $r^2=0.30$ ,  $p=0.051$ , B); tree height and chloride ( $r^2=0.38$ ,  $p=0.020$ , C); and tree height and water depth ( $r^2=0.51$ ,  $p=0.005$ , D).

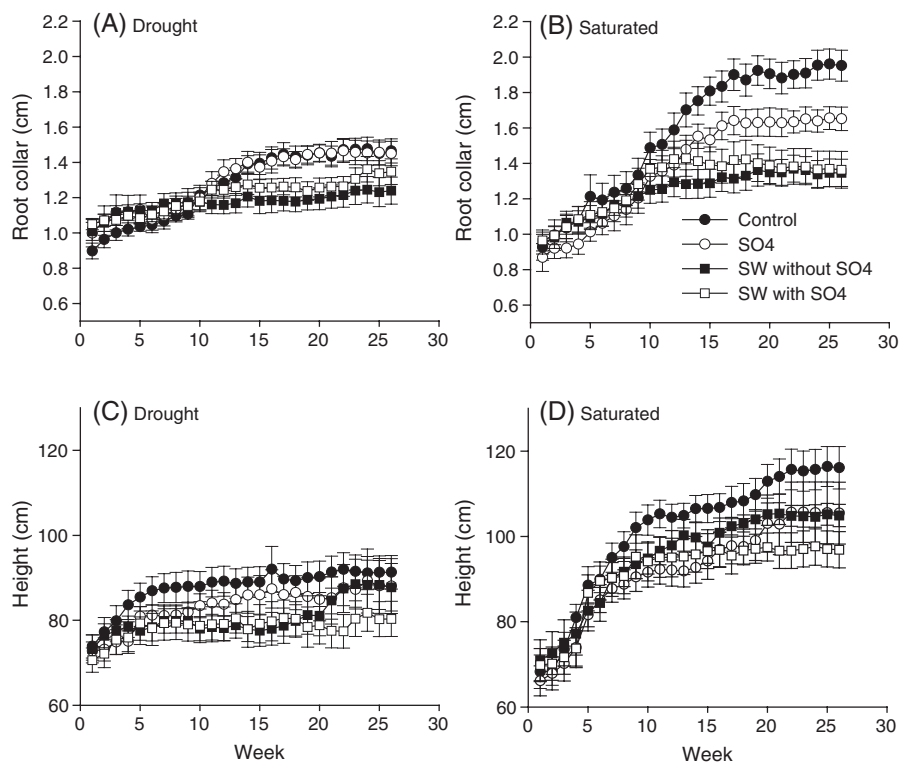


Figure 4. Mean diameter at root collar (cm) for drought (A) and saturated (B) and mean height (cm) for drought (C) and saturated treatments (D) of the seedlings over 26 weeks in the greenhouse experiment. The symbols represent the different water chemistry treatments (fresh water, sulfate only, salt water without sulfate, and salt water with sulfate) ( $n = 8$ ,  $\pm$  SE).

expected, the salt water treatments had a higher  $\text{Cl}^-$  concentration than the fresh water treatments (salt water = 332 mg Cl/L compared to fresh water = 4.49 mg Cl/L drought; salt water = 481 mg Cl/L compared to fresh water = 31 mg Cl/L saturated, Table S1, Supporting Information).  $\text{SO}_4^{2-}$  concentrations were higher in the  $\text{SO}_4^{2-}$  only treatment (48 mg/L in drought and 129 mg/L saturated) compared to fresh water treatments (6 mg/L, drought; 84 mg/L saturated, Table S1).

Time had a significant effect on how salinity, water level, and their interaction altered diameter (Table S2). Time also had a significant effect on how hydrology affected the height of the seedlings (Table S2). Salt water had a significant negative effect on diameter (Table S2; Fig. 4A & 4B) and height (Table S2; Fig. 4C & 4D). The presence of  $\text{SO}_4^{2-}$  did not have a significant effect on diameter (Table S2; Fig. 4A & 4B), but had a significant negative effect on height (Table S2; Fig. 4C & 4D).

Regardless of hydrology, seedlings watered with salt water had significantly lower biomass (leaf  $p = 0.042$ , stem  $p = 0.045$ , and root  $p = 0.002$  [Table S3]), while seedlings in the saturated control treatment had higher biomass (Fig. 5A–5D).  $\text{SO}_4^{2-}$  did not have a significant effect on biomass (Table S3). By the end of the experiment, 8 of the 64 seedlings had died, all from salt water treatments: drought salt water with  $\text{SO}_4^{2-}$  (2), drought salt water without  $\text{SO}_4^{2-}$  (1), saturated salt water with  $\text{SO}_4^{2-}$  (3), and saturated salt water without  $\text{SO}_4^{2-}$  (2).

To examine if the magnitude of the effects of salinity on growth was similar between the field and the greenhouse

experiment, we plotted the percent change of growth versus chloride concentration. For the field data, we calculated the percent change as the percent difference in growth between each site and the average of the three sites with the lowest chloride concentrations (109, 601, and 603), assuming that those values represent a low salinity baseline condition. This means that a negative value would indicate sites that grew more than the average of those three sites. At the end of the greenhouse experiment, we estimated the percent difference in growth between the fresh water and the salinity treatments (with and without sulfate) and plotted it against the maximum salinity measured (Table S1). Plotting the data this way suggests that both adult trees and seedlings suffer declines in DBH and height growth when chloride concentrations are higher than 500 mg/L (Fig. 6). Fitting a logarithmic curve explained 60 and 66% of the variation in DBH and height changes in growth respectively (Fig. 6).

## Discussion

In both the field and the greenhouse experiment, we found support for our initial hypothesis that increased salinity leads to decreased growth of baldcypress. Our results suggest that salinities as low as 1 ppt ( $\text{Cl}^- = 600$  mg/L) can lead to decreased growth of baldcypress in restored wetlands. Our results agree with a recent study that showed that salinities as low as 1 ppt cause stress and a decrease in baldcypress survival, ultimately

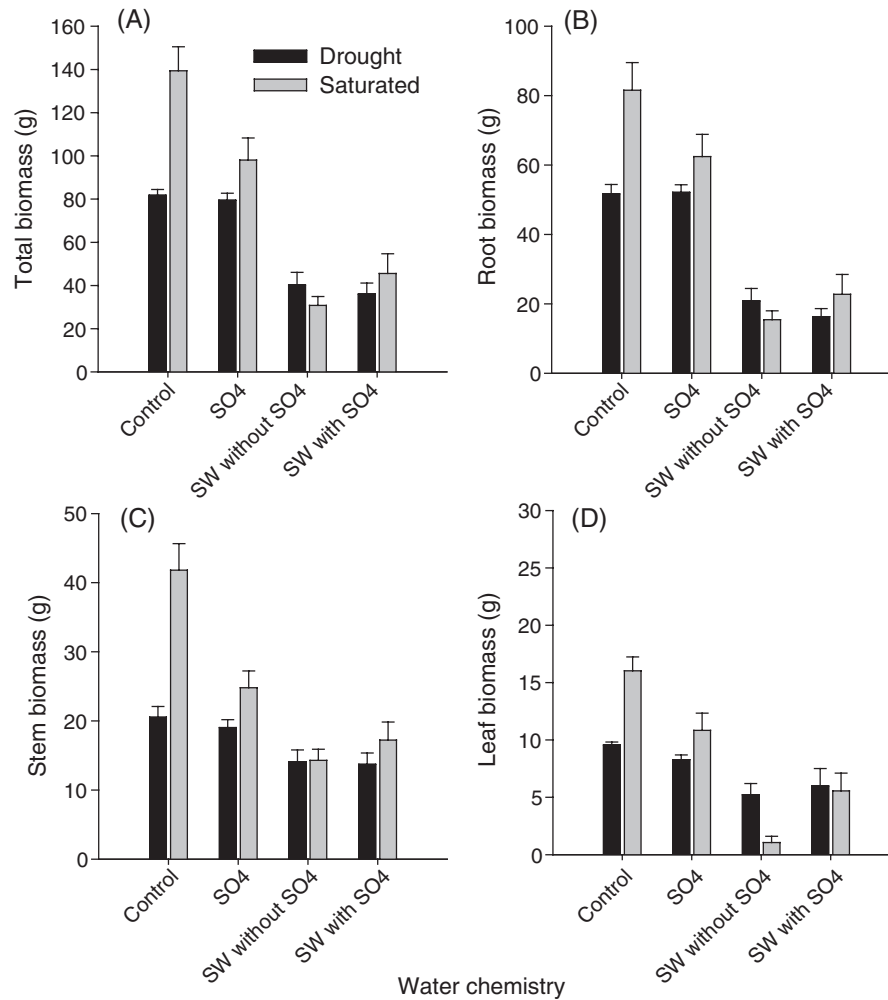


Figure 5. Mean biomass across treatments for total biomass (leaves, stems, plus roots) (A), roots (B), stems (C), and leaves (D) ( $n = 8$ , + SE). Drought treatments are in black bars, saturated in gray bars.

converting swamps into marshes (Hackney & Avery 2015). We also found that baldcypress are sensitive to water level variations, with saplings in the field declining in growth due to flooding, while seedlings in the greenhouse experiment had lower growth under drought conditions. Our results will be helpful to restoration practitioners by elucidating the sensitivity of a commonly used tree species to water level and salinity, both environmental drivers changing due to climate change.

#### Field Measurements

Our results illustrate that wetland restoration practitioners may have to deal with a variety of trade-offs, such as decreased tree growth in areas that have experienced drought-induced salinity. One of the original goals of restoration was to reconnect the site to the Albemarle Sound to allow migration of anadromous fishes; this same hydrologic reconnection has allowed the incursion of salt water during drought periods (Ardón et al. 2013). When planting baldcypress on a restoration site, land managers should take note of any possible paths in which saltwater

incurion could enter the site. Given that models suggest that droughts are likely to become more common in the southeastern United States (Carter et al. 2014), salinization of former freshwater areas is likely to increase. Our results suggest that periodic salinization events even with low levels of salinity can decrease growth of baldcypress by 20–60%.

Previous studies have differentiated between acute and chronic exposure to salinity (Krauss et al. 2007). Acute salinity sources include storm surges and droughts, which expose plants to a short duration (<30 days) of higher salinity. Baldcypress swamp forests have been shown to tolerate salinities as high as 18.5 ppt as long as the salinity is quickly flushed with fresh water from rainfall or upstream river discharge (Conner & Inabinette 2005). On the other hand, chronic events (>90 days) that lead to prolonged exposure to salinity can often lead to complete mortality and a transformation of forested wetlands to marshes or open water (McCarron et al. 1998; Krauss et al. 2009). In the TOWeR site, we estimate that the trees in the sites with the highest salinity concentrations in soil solution had experienced at least 846 days of salinities over 0.5 ppt since

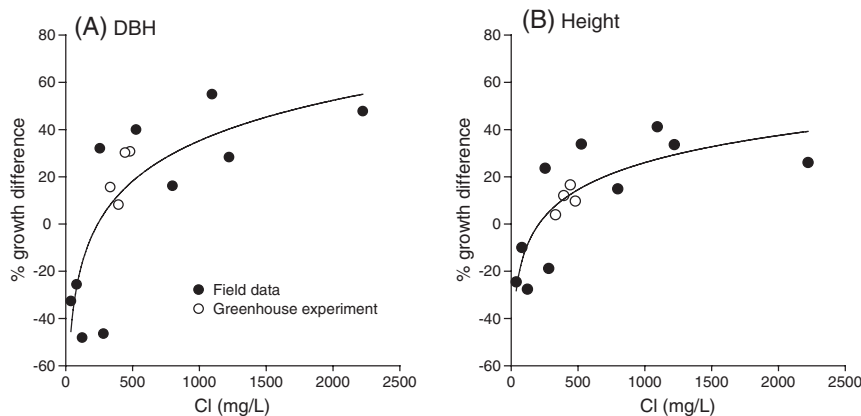


Figure 6. Percent difference in growth in DBH (A) and tree height (B) for baldcypress measured in the field and a greenhouse experiment versus chloride concentration. See text for explanation of percent difference calculations. Line represents best fit logarithmic line ( $r^2 = 0.60$ ,  $p < 0.05$  for DBH and  $r^2 = 0.66$ ,  $p < 0.05$  for height).

hydrologic reconnection in 2007 (Ardón et al. 2013). These periods of high salinity were interrupted by periods of low salinity due to increased precipitation (Ardón et al. 2013). However, it is clear from our field results that even these relatively low levels of salinity were enough to decrease growth of baldcypress, and thus the C storage capacity of this site. Our estimates of C standing stocks were similar (range 300–7,000 kg/ha) to previously reported estimates for this site using field and remote sensing methods (30–8,400 kg/ha, Riegel et al. (2013)).

We were surprised by the lower growth rates of baldcypress associated with higher concentrations of ammonium and TDN. We expected that higher nitrogen concentrations would lead to greater growth. Chloride, ammonium, and TDN were higher in the higher water level sites, making it challenging to separate the effects of nutrients, salt, and water level. The linear regression models show that both water depth and salinity were important explanatory variables of DBH and height variability across the site. We think the negative relationships with increased nitrogen were superseded by the negative effect of higher salinity and higher water level in those sites. These results illustrate the challenges in understanding field observations and is one of the main reasons we designed a greenhouse experiment to isolate the effects of water depth and salinity.

Baldcypress in areas with flowing water can withstand flooding longer than trees in areas with standing water due to the higher concentrations of oxygen associated with water flow (Brown & Montz 1986). The lack of a significant negative relationship between water depth and DBH of baldcypress across the site could be because water level has been increasing since the initial flooding. If water levels continue to rise, we suspect that trees in the sites with deeper water will start showing signs of stress.

#### Greenhouse Measurements

We found that seedlings in the greenhouse saturated treatments had greater root, stem, and leaf biomass than seedlings in the drought treatments. Previous work has shown that under drought

conditions, a hormonal signal from roots causes reduced leaf growth (Chapin 1991). A similar study found that moderate drought decreased dry mass of stem and leaves in baldcypress, while severe drought significantly reduced dry mass (Nash & Graves 1993). In our study, seedlings watered with salt water had a significantly lower total biomass compared with the other treatments, due to the majority of their leaves dying and falling off, and smaller diameters and heights. By week seven, seedlings watered with salt water (with or without sulfate) started showing signs of stress. By week nine, the majority of leaves were dying and some seedlings started putting on new growth and continued to put on new growth for the duration of the experiment. In another study, plants that were flooded with addition of salt had an 8% reduction in biomass when compared with flooding alone (Pezeshki 1992).

Watering with sulfate solution had a significant negative effect on height, but was not toxic to the seedlings. Our results of the effects of sulfate must be interpreted with caution because we did not directly measure hydrogen sulfide or redox potential. However, two lines of evidence suggest that there was production of hydrogen sulfide in the treatments that received sulfate. The first was that we could smell the characteristic smell of “rotten egg” from hydrogen sulfide (Mitsch & Gosselink 2007) in the sulfate and salt water with sulfate saturated treatments. The second is that in a similar previous study using soil cores with the same water chemistry treatments used here (Ardón et al. 2013), we measured hydrogen sulfide concentrations of around 30  $\mu\text{mol/L}$  (Ardón unpublished data), which can cause stress in freshwater plant species (Lamers et al. 2013). At this point, it is unclear if the decline in height we observed with sulfate was due to changes in the osmotic balance of the seedlings due to the added sulfate, or due to the toxicity of sulfide from sulfate reduction. Shanklin and Kozłowski (Shanklin & Kozłowski 1985) found that seedlings fumigated with sulfate showed a decrease in number of leaves, as well as surface area, which would lead to a lower leaf biomass. Contrary to our results, Shanklin and Kozłowski (1985) found that seedlings fumigated with sulfate experienced no change in height growth. The



difference between fumigation and watering directly with sulfate solution may account for the effect on height. Sulfate alone did not significantly affect the biomass of the leaves, stems, or roots. In areas where there is an increase in saltwater intrusion, sulfate will also increase—which may have a significant impact on the height of baldcypress. Our previous work showed that as salt moves into TOWeR, the spatial extent of increased chloride might be greater than the spatial extent of increased sulfate (Ardón et al. 2013). Chloride ions move conservatively upstream, while sulfate is consumed by sulfate-reducing microbes. Thus, it is important to understand the separate and combined effects of sulfate and chloride. More research should be conducted examining at what levels sulfate and sulfide may be harmful for the growth and/or survival of baldcypress seedlings. The concentration of iron in the soil is also an important determining factor of the production of sulfide in wetland soils (Schoepfer et al. 2014). Even though baldcypress may be able to tolerate the current salinity levels, higher sulfate and sulfide concentrations could have a greater impact on the growth.

Overall, we found that both water level and salinity consistently affect the growth of baldcypress in the field and greenhouse. Our results suggest that salinity levels as low as 1 ppt can lead to decreased growth of baldcypress. Restoration practitioners should consider the potential for increased salinization under a changing climate when planning restoration sites. Information on the growth and functioning of baldcypress seedlings in response to flooding in natural wetland settings are scarce (Flynn 1986; Pezeshki 2001). Future studies should examine how both the duration and the magnitude of salinity exposure affect growth of baldcypress and other species commonly used in coastal wetland restoration.

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## LITERATURE CITED

Allen JA, Chambers JL, McKinney D (1994) Intraspecific variation in the response of *Taxodium distichum* seedlings to salinity. *Forest Ecology and Management* 70:203–214

Ardón M, Morse JL, Colman BP, Bernhardt ES (2013) Drought-induced saltwater incursion leads to increased wetland nitrogen export. *Global Change Biology* 19:2976–2985

Ardón M, Morse JL, Doyle MW, Bernhardt ES (2010) The water quality consequences of restoring wetland hydrology to a large agricultural watershed in the southeastern Coastal Plain. *Ecosystems* 13:1060–1078

Broadfoot WM, Williston HL (1973) Flooding effects on southern forests. *Journal of Forestry* 71:584–587

Brown CA, Montz GN (1986) Baldcypress: the tree unique, the wood eternal. Claitor's Publishing Division, Baton Rouge, Louisiana

Carter LJ (1975) Agriculture: a new frontier in coastal North Carolina. *Science* 189:271–275

Carter LM, Jones JW, Berry L, Burkett V, Murley JF, Obeysekera J, Schramm PJ, Wear D (2014) Ch. 17: Southeast and the Caribbean. Pages 396–417. In: Melillo JM, Richmond TC, Yohe GW (eds) *Climate change impacts in the United States: The Third National Climate Assessment*. U.S. Global Change Research Program, doi: 10.7930/J0N-P22CB

Chapin FS III (1991) Integrated responses of plants to stress. *BioScience* 41:29–36

Conner WH, Brody M (1989) Rising water levels and the future of southeastern Louisiana swamp forests. *Estuaries* 12:318–323

Conner WH, Inabinette LW (2005) Identification of salt tolerant baldcypress (*Taxodium distichum* (L.) Rich) for planting in coastal areas. *New Forests* 29:305.312

Conner WH, McLeod KW, McCarron JK (1997) Flooding and salinity effects on growth and survival of four common forested wetland species. *Wetlands Ecology and Management* 5:99–109

Craft C, Clough J, Ehman J, Joye S, Park R, Pennings S, Guo H, Machmuller M (2009) Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. *Frontiers in Ecology and the Environment* 7:73–78

Dickson RE, Broyer TC (1972) Effects of aeration, water supply, and nitrogen source on growth and development of tupelo gum and bald cypress. *Ecology* 53:626–634

Ellison AM, Bank MS, Clinton BD, Colburn EA, Elliott K, Ford CR, et al. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3:479–486

Flynn KM (1986) Growth and metabolic response of *Taxodium distichum* (L.) Rich. (baldcypress) seedlings to different flooding regimes. Thesis. Louisiana State University, Baton Rouge, Louisiana

Hackney CT, Avery GB (2015) Tidal wetlands community response to varying levels of flooding by saline water. *Wetlands* 35:227–236

Hall CJ, Jordaan A, Frisk MG (2012) Centuries of anadromous forage fish loss: consequences for ecosystem connectivity and productivity. *BioScience* 62:723–731

Hopfenperger KN, Burgin AJ, Schoepfer VA, Helton AM (2014) Impacts of saltwater incursion on plant communities, anaerobic microbial metabolism, and resulting relationships in a restored freshwater wetland. *Ecosystems* 17:792–807

Jenkins JC, Chiojnacky DC, Heath LS, Birdsey RA (2003) National-scale biomass estimators for United States tree species. *Forest Science* 49: 12–35

Kester DR, Duedall IW, Connors DN, Pytkowicz RM (1967) Preparation of artificial seawater. *Limnology and Oceanography* 12:176–179

Koch MS, Mendelssohn IA (1989) Sulphide as a soil phytotoxin: differential responses in two marsh species. *Journal of Ecology* 77:565–578

Krauss KW, Chambers JL, Creech D (2007) Selection for salt tolerance in tidal freshwater swamp species: advances using baldcypress as a model for restoration. Pages 385–410. In: Conner WH, Doyle TW, Krauss KW (eds) *Ecology of tidal freshwater forested wetlands of the Southeastern United States*. Springer, Dordrecht, The Netherlands

Krauss KW, Doyle TW, Howard RJ (2009) Is there evidence of adaptation to tidal flooding in saplings of baldcypress subjected to different salinity regimes? *Environmental and Experimental Botany* 67:118–126

Krinard RM, Johnson RL (1987) Growth of 31 – year-old baldcypress plantation. USDA Forest Service Research Note SO-339. Southern Forest Experiment Station, New Orleans, Louisiana

Lamers LPM, Govers LL, Janssen ICJM, Geurts JJM, Van der Welle MEW, Van Katwijk MM, Van der Heide T, Roelofs JGM, Smolders AJP (2013) Sulfide as a soil phytotoxin – a review. *Frontiers in Plant Science* 4:1–14

Matton WR (1915) The southern cypress. Bulletin No. 272. United States Department of Agriculture, Washington, D.C.

McCarron JK, McLeod KW, Conner WH (1998) Flood and salinity stress of wetland woody species, buttonbush (*Cephalanthus occidentalis*) and swamp tupelo (*Nyssa sylvatica* var. *biflora*). *Wetlands* 18:165–175

McQuarrie ADR, Tsai CL (1998) Regression and time series model selection. World Sci, London, United Kingdom

Megonigal JP, Day FP (1992) Effects of flooding on root and shoot production of bald cypress in large experimental enclosures. *Ecology* 73:1182–1193

- Middleton BA (2003) Soil seed banks and the potential restoration of forested wetlands after farming. *Journal of Applied Ecology* 40:1025–1034
- Mitsch WJ, Gosselink JG (2007) *Wetlands*. Wiley and Sons, Hoboken, New Jersey
- Nash LJ, Graves WR (1993) Drought and flood stress effects on plant development and leaf water relations of five taxa of trees native to bottomland habitats. *Journal of American Society for Horticultural Science* 118:845–850
- Needham R (2006) Implementation plan for agricultural restoration at Timberlake farms. Needham Environmental Inc., Wilmington, North Carolina
- Pezeshki SR (1992) Response of *Pinus taeda* L to soil flooding and salinity. *Annales des Sciences Forestières* 49:149–159
- Pezeshki SR (2001) Wetland plant responses to soil flooding. *Environmental and Experimental Botany* 46:299–312
- Pike N (2011) Using false discovery rates for multiple comparisons in ecology and evolution. *Methods in Ecology and Evolution* 2:278–282
- R Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/> (accessed 25 Sept 2015)
- Richardson CJ (1983) Vanishing wastelands or valuable wetlands? *BioScience* 33:626–633
- Riegel JB, Bernhardt ES, Swenson J (2013) Estimating above-ground carbon biomass in a newly restored coastal plain wetland using remote sensing. *PLoS One* 8:e68251
- Schoepfer VA, Bernhardt ES, Burgin AJ (2014) Iron clad wetlands: soil iron-sulfur buffering determines coastal wetland response to salt water incursion. *Journal of Geophysical Research, Biogeosciences* 119:2209–2219
- Shaler NS (1887) Notes on the *Taxodium distichum* or bald cypress. *Memoirs of the Museum of Comparative Zoology* 16:1–15
- Shanklin J, Kozlowski TT (1985) Effect of flooding of soil on growth and subsequent responses of *Taxodium distichum* seedlings to SO<sub>2</sub>. *Environmental Pollution* 38:199–212
- Temmerman S, Meire P, Bouma TJ, Herman PMJ, Ysebaert T, De Vriend HG (2013) Ecosystem-based coastal defense in the face of global change. *Nature* 504:79–83
- Verhoeven KJF, Simonsen KL, McIntyre M (2005) Implementing false discovery rate control: increasing your power. *Oikos* 108:643–647
- Wicker KM, Davis D, DeRouen M, Roberts D (1981) Assessment of extent and impact of saltwater intrusion into the wetlands of Tangipahoa Parish, Louisiana. Coastal Environments, Inc., Baton Rouge, Louisiana
- Zedler JB, Kercher S (2005) Wetland resources: status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources* 30:39–7

## Supporting Information

The following information may be found in the online version of this article:

**Table S1.** Mean and maximum chloride and sulfate concentrations over the duration of the greenhouse experiment ( $\pm$ SE).

**Table S2.** Results for repeated measures MANOVA for log-transformed diameter at root collar and height of 64 baldcypress seedlings over 26 weeks looking at the different treatments. Factors were hydrology (drought or saturated), saltwater (present or absent), and sulfate (present or absent).

**Table S3.** ANOVA results of leaf, stem, and root biomass of 64 baldcypress seedlings. Factors were hydrology (drought or saturated), saltwater (present or absent), and sulfate (present or absent).

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