2024

Can Multi-Species Plantings Alleviate Abiotic Stressors to Enhance Bald Cypress Restoration?

Victoria S. Ellis
Old Dominion University, vsmit006@odu.edu

Taylor M. Sloey
Old Dominion University, tsloey@odu.edu

Original Publication Citation
Ellis, V. S., & Sloey, T. M. (2024). Can multi-species plantings alleviate abiotic stressors to enhance bald cypress restoration? *Restoration Ecology*. 32(7), Article 14215. https://doi.org/10.1111/rec.14215

RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration

RESEARCH ARTICLE

Can multi-species plantings alleviate abiotic stressors to enhance Bald cypress restoration?

Victoria S. Ellis[1], Taylor M. Sloey[1,2]

Restoration researchers and practitioners alike advocate for novel restoration approaches, informed by ecological theories and principles, to enhance the likelihood of meeting restoration goals. Forested wetland restoration has historically focused on creating abiotic conditions that support the tolerance thresholds of desired species, but the stress gradient hypothesis provides guidance for potential new strategies that use biotic interactions to ameliorate stressful abiotic conditions. In this study, we tested whether multi-species planting approaches can be used to enhance the survival and growth of a target restoration tree species, Bald cypress, along multiple abiotic gradients. We conducted a fully factorial controlled greenhouse experiment which manipulated above- and belowground interactions between two species (Bald cypress and Soft rush), as well as light availability and depth of inundation. Our findings showed that co-planting Bald cypress seedlings with Soft rush did not increase tree biomass production or growth metrics (e.g. stem height and leaf area) under any exposed stress combination. Importantly, we found that full-sun irradiance negatively impacted functional traits associated with the tree seedlings' health and ability to photosynthesize. Our findings are important for consideration by practitioners as light is rarely the focus of wetland ecosystem restoration and degraded forested wetlands or restoration sites often have open canopies.

Key words: freshwater forested wetland, ***Juncus effusus***, restoration, stress gradient hypothesis, ***Taxodium distichum***

Implications for Practice

- Multi-species planting approaches, which aim to increase a target species' survival through facilitation by another species, have not been thoroughly tested as a restoration technique. Although facilitation was not observed between Bald cypress and Soft rush in this study, the potential of this novel approach deserves additional exploration.
- Hydrology is often the focal driver controlling plant communities in wetlands; however, light, specifically excessive irradiance, is an important limiting factor to health of Bald cypress seedlings.
- Plant functional traits associated with plant productivity may show opposite responses to environmental stressors compared to traits associated with plant health and nutritional status.
- Moderation of irradiance may be necessary to enhance Bald cypress seedling survival in disturbed or open-canopy restoration sites.

Introduction

Effective restoration of the world's degraded and destroyed forested ecosystems has been identified as a focal area of concern for the research community to combat climate change, alleviate the biodiversity crisis, and protect the services these ecosystems provide (United Nations Framework Convention on Climate Change 2010; United Nations 2023). Forested wetlands (e.g. bottomland forests, swamps, and mangroves) in particular are valued for their large contribution to carbon storage relative to their small area coverage (Laffoley & Grimsditch 2009; Donato et al. 2011), giving urgent impetus for the effective restoration of these systems. Several forest and wetland restoration efforts have documented complications that prevent projects from meeting restoration goals (Fagan et al. 2020). Complications may be due to the inability to recreate proper hydrological and abiotic conditions that support vegetation (Caldwell et al. 2011), the low survival of planted seedlings (Wodehouse & Rayment 2019), or resistance to new methods and approaches (Stanturf et al. 2001). Further, unsuccessful restoration projects are rarely documented, which limits the ability to effectively enact adaptive management or learn from previous mistakes (Lewis 2005).

Recent advances in the field of restoration ecology have placed a greater emphasis on the need for novel science-backed restoration approaches that use ecological theory to maximize

Author contributions: VSE, TMS conceptualized this study and experimental design; VSE led the collection and analysis of data; VSE, TMS wrote and edited the manuscript.

[1]Department of Biological Sciences, Old Dominion University, 5115 Hampton Boulevard, Norfolk, VA 23529, U.S.A.
[2]Address correspondence to T. M. Sloey, email tsloey@odu.edu

doi: 10.1111/rec.14215
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.14215/suppinfo

projects' ability to meet ambitious restoration goals. Examples of these novel approaches include the use of mixed-species plantings, which has been shown to accelerate the recovery of biodiversity and ecosystem function (Kanowski & Catterall 2010; Alexander et al. 2011; Gerber 2011). Also, the target plant concept emphasizes nursery practices that enhance seedling survival at out-planting sites (Dumroese et al. 2016). As the goal of many restoration projects is to ultimately create an ecosystem that is self-sustaining and does not require human maintenance, it is essential that restoration ecology focus on improving planting conditions to reduce the need for repeat species introductions and adaptive management action (Wagner et al. 2016). One potential strategy for improving vulnerable transplant survival is to use positive plant–plant interactions (facilitation) to ameliorate harsh abiotic conditions associated with degraded sites through the indirect modification of the environment (Gómez-Aparicio et al. 2004; Padilla & Pugnaire 2006; Ren et al. 2008).

Positive plant interactions have recently been documented as being critical to the recovery and success of ecosystems, particularly in stressful environments (Renzi et al. 2019). The stress gradient hypothesis (Bertness & Callaway 1994) states that as abiotic stress increases, the relationship between species can shift from competition (negative) to facilitation (positive) (Malkinson & Tielbörger 2010). Facultative relationships between plant species are typically observed in ecosystems characterized by high levels of abiotic stress, with much of this research based in deserts and dryland systems (Brooker et al. 2008). For example, in deserts with high amounts of light irradiance and low water availability, the Palo verde tree (*Parkinsonia florida*) serves as a nurse species for young Saguaro cacti (*Carnegiea gigantea*) by creating a microclimate with filtered sunlight, increased humidity, and protection from herbivores (Withgott 2000). Modified versions of the stress gradient hypothesis have demonstrated that these facultative interactions can collapse under extreme abiotic stress as the nurse species is pushed beyond its physiological tolerance threshold (Michalet et al. 2014). When considering a plant's tolerance threshold, it is important to recognize that tolerance to one abiotic driver may shift in the presence of other drivers/stressors (Maestre et al. 2009). For example, inundated plants may be more susceptible to photoinhibition (i.e. decreased photosynthetic capacity due to excessive irradiance) in the presence of another stressor like salt (Souther & Shaffer 2000; Murata et al. 2007). Tolerance thresholds shift throughout the ontogeny of a plant as well, with younger life stages typically most susceptible to mortality. Employing an ecological framework, such as the stress gradient hypothesis, provides opportunity to explore the nuance of species' abiotic thresholds in the presence of multiple abiotic stressors, ultimately improving understanding of plant ecological interactions and informing potential new strategies for restoration.

Freshwater forested wetland restoration provides an ideal habitat to test the stress gradient hypothesis, as these ecosystems are typically species-limited and influenced by multiple abiotic environmental drivers including intermittent inundation, light availability, and anaerobic soil conditions that range from suitable to stressful for plant life (Trettin et al. 2019). Additionally, early life history stages typically used in restoration plantings have more narrow tolerance thresholds to abiotic drivers compared to their adult counterparts, which makes them ideal for testing the application of the stress gradient hypothesis (Maestre et al. 2009). This study used Bald cypress seedlings as a case study for understanding the role of interspecies facilitation in forested wetland communities.

It is well recognized that abiotic and biotic filters are drivers of species' establishment and community assembly (Hobbs & Norton 2004). However, candidate forested wetland restoration sites often have altered abiotic conditions which no longer match the physiological niche of the transplanted target species, in this case Bald cypress. For example, changes to local hydrology through irrigation, elevation change, or degradation upstream, may result in drier or more inundated soils. Establishing typical wetland hydrology has historically received dominant focus for wetland restoration (Mitsch & Gosselink 2015), but light is a critical co-variable that impacts plant growth patterns and has been shown to have a positive relationship with understory species diversity in freshwater swamps (Conner et al. 1981). Disturbances such as fires or storms may destroy the canopy, resulting in increased irradiance, change in understory vegetation composition (Souther & Shaffer 2000), and potentially unfavorable, or stressful, conditions for the establishment of seedlings of desired species. Although ecologically informed restoration practice should consider species' physiological constraints to abiotic parameters, the physiological limitations determined for earlier (germinating seeds) or more mature (adult) life stages which dominate the existing literature, may not be representative of transplants used in restoration plantings (Anderson et al. 2009). Previous research has provided important information on the limiting effects of flooding, drought, salinity, macronutrients, sulfates, plant competition, and herbivory on Bald cypress seedling growth (Myers et al. 1995; Powell et al. 2016). Field experiments investigating plant interactions found that Bald cypress seedlings allocated more growth to girth than height when surrounding vegetation was managed versus unmanaged (Myers et al. 1995). Other community-level studies showed that the use of selective herbicides can reduce plant competition, thus enhancing Bald cypress seedling survival (Osiecka & Minogue 2012). Although much of the foundational research in Bald cypress forests has focused on competition dynamics, multi-species plantings with potential nurse species may have the ability to improve restoration successes and accelerate succession (Mayence & Hester 2011). The ability to increase the breadth of suitable conditions for a target species by strategically plantings with a companion nurse species has not been widely explored.

In this study, we used a controlled greenhouse experiment to test if biological interactions between two freshwater forested wetland species, Bald cypress (*Taxodium distichum*) and Soft rush (*Juncus effusus*) shifted along two abiotic gradients (light and hydrologic inundation). Bald cypress was selected as it is a target species of forested wetland restoration efforts (Carmichael & Smith 2016). Soft rush was selected as a potential nurse species because of its presence in both closed-canopy and disturbed open-canopy freshwater marshes, its co-occurrence with Bald cypress (Kruse & Groninger 2003; personal observation Ellis & Sloey, June 2021, Old Dominion University) and known contribution to soil oxidation through radial oxygen loss (Sorrell 2000). Importantly, we chose an herbaceous graminoid species, rather than tree or shrub, to capture growth habits representative of disturbed or early successional habitats (e.g. candidate restoration or habitat creation sites). Using the principles of the stress gradient hypothesis, we hypothesized that: (1) under moderate abiotic conditions Soft rush will compete with Bald cypress, but will facilitate Bald cypress under harsh conditions by ameliorating irradiance and anoxia stress through aboveground shade provision and root radial oxygen loss respectively. However, (2) when both light and inundation are stressful conditions (e.g. full irradiance and deepest inundation), the interaction between species will collapse as one species is pushed beyond its physiological threshold and dies.

Methods

Experimental Design

Bald cypress seedlings and mature Soft rush plugs were purchased from American Native Plants Nursery (Middle River, MD, U.S.A.). Approximately, 1 m^3 of soil was collected from the top 40 cm of soil from mature Bald cypress-dominated forested wetlands within the Great Dismal Swamp (Suffolk, VA, U.S.A.) and transported to the Old Dominion University campus, where it was homogenized and used to fill plastic planting pots (23 cm diameter × 22 cm height). Each pot was filled with the same volume of soil (approximately 0.01 m^3) and soil mass was standardized to achieve similar soil bulk density across experimental units (soil bulk density ranged between 0.55 and 0.92 g/cm^3). Each pot had four 1-cm perforations on the bottom and was perforated six times along the sides at the soil level to allow for water exchange. The pots were placed in white buckets (30 cm diameter × 35.5 cm height), which were filled with water, depending on treatment, and served as individual water reservoirs for the pots to be placed inside.

Experimental plantings consisted of each of four plant interaction treatments to isolate aboveground and belowground interactions (BGs). Treatments consisted of: (1) no interaction (NI), which contained Bald cypress but no Soft rush; (2) aboveground interaction only (AG) in which Bald cypress and Soft rush were separated belowground using a corrugated plastic partition; (3) BG only, in which Soft rush stems were trimmed to 5 cm height weekly to prevent AGs while still allowing for photosynthesis to take place; and (4) full above- and belowground interaction of both species (AGBG) (Fig. 1). The Bald cypress seedlings were planted in the center of the pot for the NI treatment units. For all other biotic interaction treatments, one individual of each species was planted 8 cm from the side of the pot (and 7 cm apart from the other plant sharing the pot). After the initial transplantation, all plants acclimated for 21 days in full sun with water levels filled to the soil surface level. At the initiation of the experiment, all Bald cypress seedling stems were trimmed to 50 cm, and all Soft rush plugs were trimmed to a maximum of 60 stems to standardize initial plant biomass.

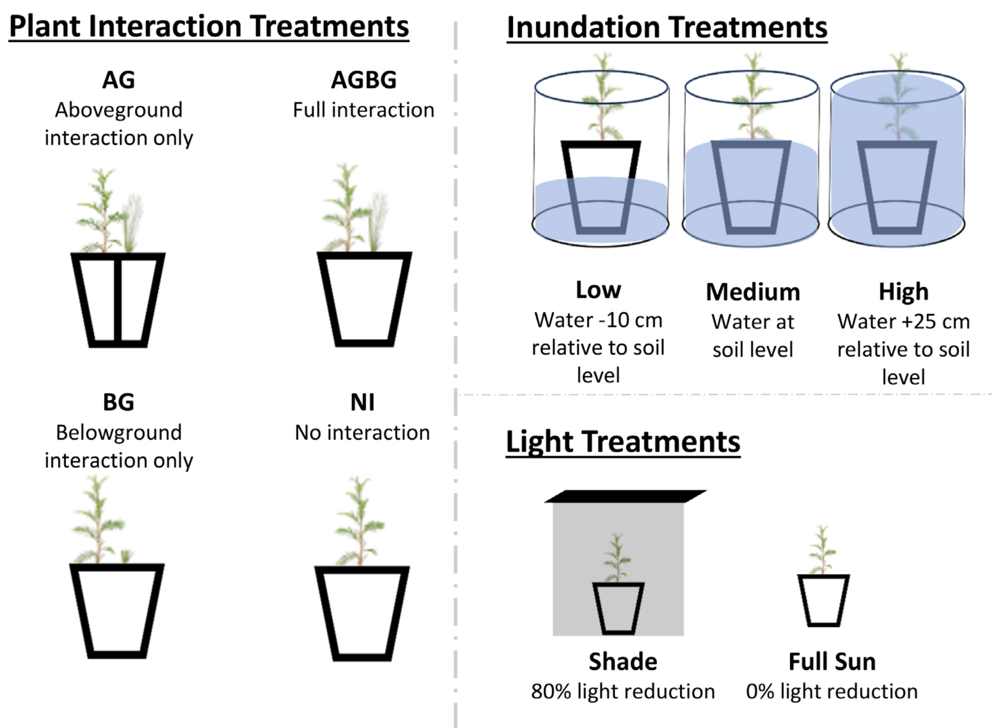


Figure 1. Graphical depictions of experimental treatments for plant interaction, inundation, and light exposure.

Planted pots were placed inside the water reservoirs and reservoirs filled with fresh tap water to achieve each of three inundation treatments: low (water level at −10 cm relative to soil level), medium (saturated with water level equal to soil level), and high (flooded with water level + 25 cm relative to soil level) (Fig. 1). Inundation treatments were selected to capture a range within the suitable establishment hydrology for a mature Bald cypress forest, which may range from dry soil to inundations of more than 1 m (Wilhite & Toliver 1990; Carmichael & Smith 2016). Maximum flooding depth in this study (25 cm) was based on the overhead space of the water reservoirs and previous studies, which found flooding approximately 30 cm was stressful to Bald cypress seedlings (Neufeld 1983; Souther & Shaffer 2000). Each treatment combination was also exposed to each of two light treatments: closed-canopy forest (20% incidental light) and open-canopy (100% incidental light), representing a mature forest where natural regeneration occurs and an open-canopy restoration site, respectively. Light conditions for the closed-canopy treatment were determined by measuring the percent light penetration within a mature forest stand at the Great Dismal Swamp using HOBO Photosynthetically Active Radiation (PAR) sensors (Onset Computer Corporation, Bourne, MA, U.S.A.) and HOBO light pendant sensors (Onset Computer Corporation, Bourne, MA, U.S.A.). Shade cloth (Fotmishu, China) obstructing approximately 80% of light was hung 120 cm above the base of each bucket and adjusted higher as necessary throughout the experiment to prevent growing plants from touching. Six replicates of all treatment combinations were randomized within each of three blocks oriented parallel to the greenhouse's east–west light gradient, with shade cloth covering half of each block.

Experimental treatments and observation took place for 12 weeks, from 06 June to 17 September 2022 to capture the peak growing season for Bald cypress (Carmichael & Smith 2016). Measurements of growth (survival, stem height, stem density, and stem diameter) were recorded for each species every 2 weeks. Survival was determined as alive or dead based on the presence of green photosynthetic tissue. Stem density was measured as the count of stems. Stem diameter was measured for Bald cypress at 10 cm above the soil level using a digital caliper (Mitutoyo America Corp.). Water was changed every 4 weeks to reduce algal growth. Two PAR sensors were placed within each light treatment to measure light and temperature continuously throughout the study. At the conclusion of the experiment, final growth metrics (survival, stem height, stem density, stem diameter, and total leaf count) were measured. A subset of four mature leaves were haphazardly selected from each Bald cypress and measured for leaf functional traits (leaf length, leaf area, leaflet length, leaflet count, specific leaf area [SLA], and leaf dry mass). These traits were selected for both their commonness as measured plant functional traits and their importance for providing information about plant growth allocation and stress responses (Sloey et al. 2023). Leaves were scanned using a photo scanner (Epson v850 Pro, Vienna Scientific, Austria) and chlorophyll content was estimated using red (R), green (G), and blue (B) numerical values derived from four randomly selected points on each leaf using ImageJ. Estimated chlorophyll content was determined using the equation: $0.55 + 11.4((G - B)/(G + B)) - 12.5((R - B)/(R + B)) + 9((R - G)/(R + G))$ (Sánchez-Sastre et al. 2020). Soil redox potential (Eh) (i.e. a measure of the electron availability in soil indicating how oxidized or reduced the soil is) was measured at 10 cm depths prior to harvest (16 September 2022) using platinum electrodes and a handheld ORP tester (Oakton Instruments). Redox potential was measured on both sides of the partition for AG interaction treatments. All plants were harvested, and aboveground and belowground plant material was separated into live and dead biomass; fresh biomass was measured within 24 hours of harvest then all biomass was dried to a constant weight in a convection oven at 60°C and dry mass was then measured. Root-to-shoot ratio was calculated from dried biomass by dividing the total belowground biomass by the total aboveground biomass production.

Data Analysis

All data were analyzed using R version 4.2.1 (R Core Team 2022). A three-way analysis of variance was used to test the effect of all independent variables (hydrology, light, and plant interaction treatment) and all interactions on all final growth metrics. Response variables include height, diameter, stem count, leaf count, leaflet count, leaflet length, root-to-shoot ratio, chlorophyll content, and above- and belowground biomass. Although the data exhibited mild departures from normality and homoscedasticity, determined using a histogram and Bartlett's test, respectively, analysis of variance (ANOVA) is robust with respect to minor violations of these assumptions (Neter et al. 1996). Block was treated as a random effect and removed as it was not statistically significant in any measured variables. Tukey honest significant difference post hoc test was used to assess the significance of differences between all treatment combination means. To show multivariate relationships between response variables, we conducted a principal components analysis (PCA) of all *Bald cypress* response metrics.

Results

Abiotic Conditions

Air temperature in the greenhouse ranged from 22.8 to 35°C throughout the experiment. Shade cloth reduced light irradiance by approximately 88.6% and temperature by 2.61%. Soil redox potential was lower in the high inundation treatments ($p < 0.0001$), and lower in shade treatments compared to sun treatments ($p = 0.0263$) (Table S1), but showed no effect of plant interaction treatment. All Bald cypress seedlings survived except for one individual under sun-medium inundation-NI treatment.

Bald Cypress Response

Bald cypress stem height in shaded treatments was greater than that in light treatments ($p < 0.001$), excluding low inundation NI treatments, where heights were taller under full-sun treatments ($p < 0.001$) (Tables S1 & S2). Stem diameter was generally larger in sun treatments compared to shade ($p < 0.001$) and in high inundation treatments ($p < 0.001$). AGBG treatments showed the lowest stem diameter compared to other biotic interaction treatments ($p < 0.001$) (Tables S1 & S2). Leaf count was generally greater in shade treatments ($p < 0.001$) and low inundation ($p < 0.001$), although there was no significant difference between sun and shade treatments in leaf counts for NI biological treatment (Table S1; Fig. 2D). SLA was significantly lower in all full-sun treatments ($p < 0.001$), regardless of inundation or plant interaction treatment (Table S1; Fig. 2E). Chlorophyll content estimation showed significant effects of all main effects, with higher chlorophyll content in shade treatments ($p < 0.001$) and low inundation treatments ($p = 0.0134$). Chlorophyll content was higher in biotic interaction treatments where there was no aboveground interaction (BG and NI) compared to other biotic interaction treatments ($p < 0.001$) (Table S1; Fig. 2F).

Aboveground biomass exhibited an interaction between light and biotic interaction treatment ($p = 0.012$), with sun treatments showing greater aboveground biomass compared to shade in AG and NI biotic interaction treatments. Overall, aboveground biomass was greatest in NI biotic interaction treatments and lowest in AGBG treatments ($p < 0.001$; Table S1; Fig. 2A). Belowground biomass showed significant interactions between light and inundation ($p = 0.040$) and light and plant interaction treatment ($p = 0.025$), with belowground biomass being lower in high inundation treatments than in low treatments ($p < 0.001$). Plants in the shade treatment had lower belowground biomass compared to sun treatments, regardless of biotic interaction ($p < 0.001$). NI treatments showed the largest belowground biomass in light treatments, whereas shade NI treatment did not differ from other biotic interaction treatments ($p < 0.001$). Overall, belowground biomass was lowest in high-inundation shade treatments and highest in low inundation sun treatments (Table S1; Fig. 2B). Consequently, the root-to-shoot ratio was lower in shade treatments compared to light treatments ($p < 0.001$). Root-to-shoot ratio decreased as inundation increased ($p < 0.001$) and fully interacting plants (AGBG) had the highest root-to-shoot ratio values, whereas NI had the lowest ($p < 0.001$) (Table S1; Fig. 2C).

A biplot of PCA1 versus PCA2 (Fig. 3) showed that the two main principal components of variability in the dataset explained 90% of the variance. Principal component 1 (58.5%) had large positive associations with SLA and chlorophyll and negative associations with root-to-shoot ratio, indicating PC1 is a measurement in relation to light availability and plant health. Principle component 2 (31.5%) showed a large positive association with stem diameter and aboveground biomass, indicating PC2 is a measurement of growth and productivity. The ordination results of sample plots by PCA (Fig. 3) show the most evident clustering by light treatment. Effects of inundation and plant interaction treatments showed more dispersive patterns, though full sun-NI treatment combinations showed distinctive clustering in the lower right corner.

Soft Rush Response

Soft rush showed a significant interaction between light and plant interaction treatment ($p < 0.0001$) and all significant main effects on both aboveground and belowground biomass. Biomass production was greater in full-sun treatments except for in BG treatments in which stems were clipped (Table S1; Fig. 2G & 2H). High inundation further reduced Soft rush biomass production in fully interacting biological treatments (AGBG). Soft rush stem count showed a significant three-way interaction between all main effects ($p = 0.035$), with stem count being reduced in high inundation treatments and lowered by shade in BG (Table S1; Fig. 2I). Other measured parameters that showed nonsignificant main effects are summarized in Table S2.

Discussion

Testing the Stress Gradient Hypothesis

We tested the stress gradient hypothesis to understand if interactions between two freshwater swamp species, Bald cypress and Soft rush, would shift from competition to facilitation under increasing light and inundation stress. We isolated aboveground and belowground biotic interactions between plants to understand the interactive effects of irradiance stress and inundation stress, respectively. Contrary to our predictions that increased irradiance and inundation would promote facilitation in aboveground and BGs, respectively, the interactions between the two species remained competitive regardless of biological interaction treatment. Bald cypress growth metrics, such as stem diameter and biomass production, was greatest in treatments that lacked interaction with Soft rush. Plant interactions also exacerbated the stressful impact of light on some traits. For example, the negative impact of light on leaf count and chlorophyll content was more apparent when plants were interacting, particularly belowground. These findings give evidence that stressful abiotic conditions were achieved, but facilitation did not occur. Thus, our findings show that Soft rush is not a facilitator of Bald cypress seedlings under the light and flooding gradients tested, and the stress gradient hypothesis was not supported by this study.

We also hypothesized a collapse of interspecies interactions under high stress levels for both abiotic drivers. We did observe this collapse in high inundation—BG interaction treatment combinations where aboveground biomass of Soft rush was trimmed. Higher mortality and reduced biomass production belowground of Soft rush under these treatments suggest that clipping has not only served to isolate BGs, but also introduced a third stressor (herbivory) on Soft rush. Wetland plants that are unable to emerge stems above the water level, such as trimmed Soft rush in high inundation treatment, experience additional abiotic stress from flooding than due to soil inundation alone as total submergence limit convective and diffusive gas flow (Sorrell & Brix 2013). Regardless, the two species did not exhibit positive interactions. However, these findings should not conclude an abandonment of exploration for using multi-species plantings to facilitate target species establishment in restored sites.

Although we are confident that abiotic conditions stressful for Bald cypress were achieved in this experiment and that Soft rush did not ameliorate that stress, it is possible that Soft rush may serve as a facilitator in the presence of stressors other than what

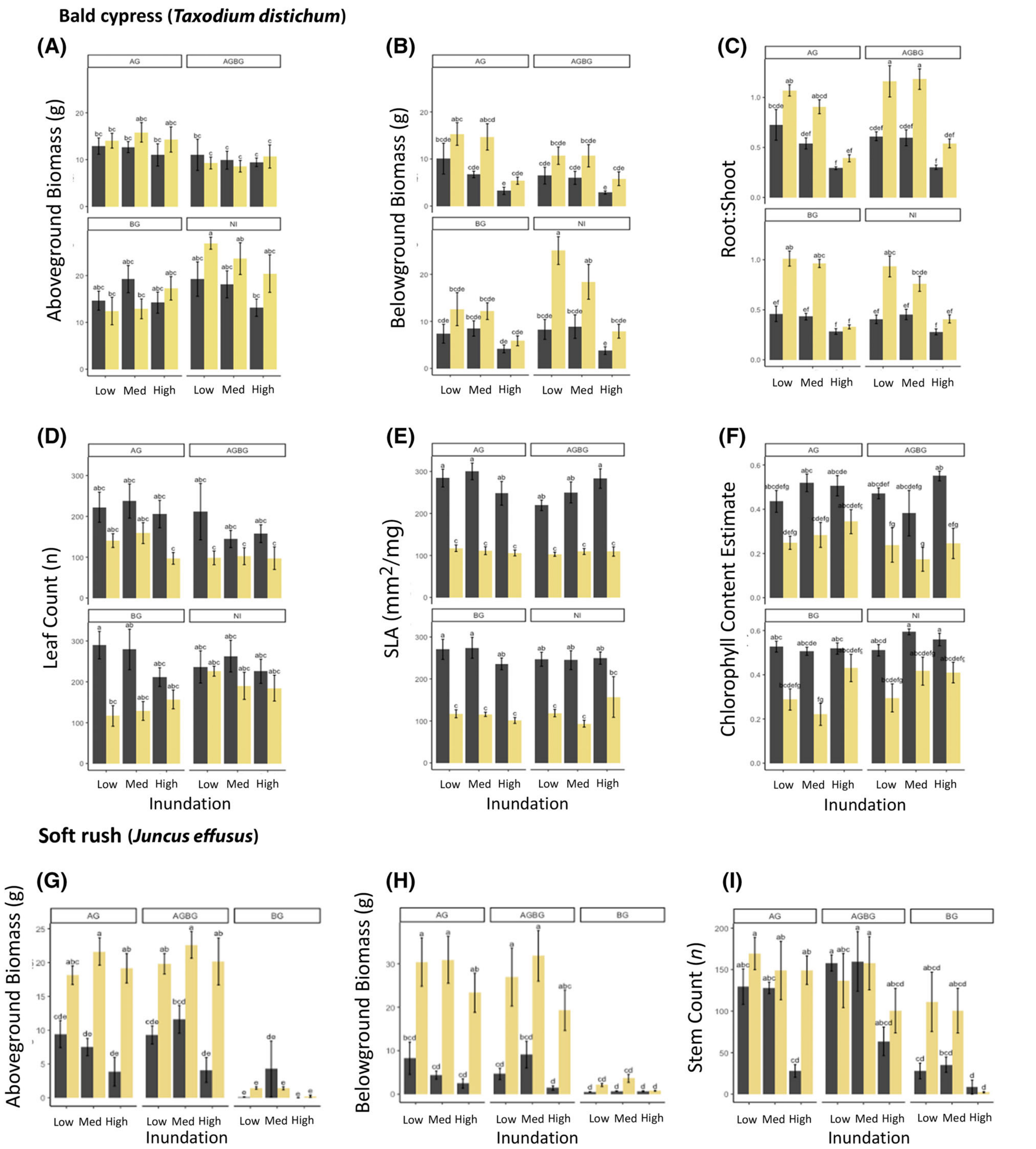
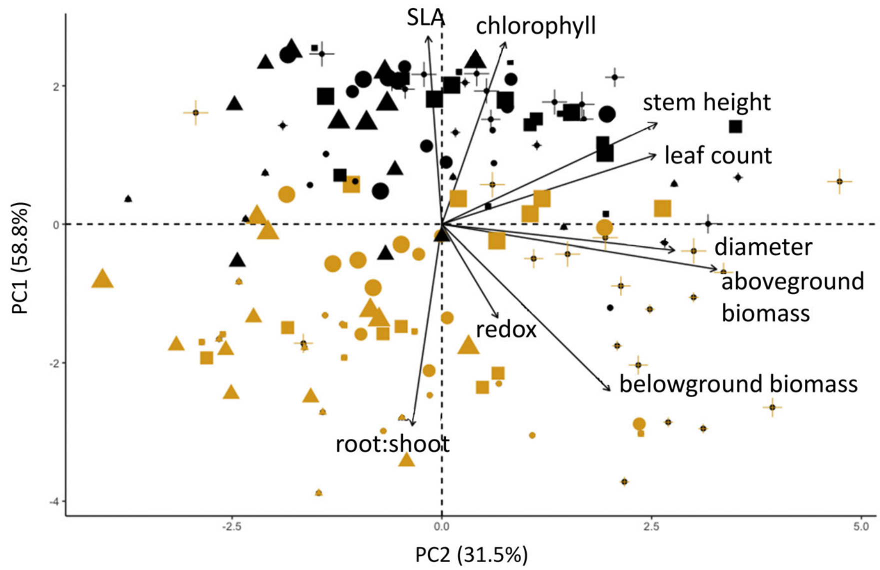


Figure 2. Bald cypress (target species) (A–F) and Soft rush (nurse species) (G–I) responses to fully factorial combination of plant interaction treatments (AG, BG, AGBG, and NI), inundation treatments (low, medium, and high), and light treatments (full sun [yellow] and 80% light reduced shade [black]) after 12 weeks of growth. All bars show mean ± SE. Means demarked by the same superscript letter were not statistically different (α >0.05), as determined by Tukey post hoc analysis.

we tested. For example, Soft rush root system may contribute to soil strength by reducing erosion and the loss of cypress seedlings along banks and ditches. Additionally, diverse plant communities have been noted to reduce herbivory pressure by insects on a particular species (Ristok et al. 2022).

Multi-species Restoration Approach

Restoration is still a relatively new field of science, and new techniques, such as multi-species plantings, are understudied (McAfee et al. 2020). Yelenik et al. (2015) state that ecologists have overlooked nurse species for years and that they are much



Variable	Comp 1	Comp 2
Stem Height	0.24516426	0.39868439
Stem diameter	-0.06964611	0.57752479
Leaf count	0.16713877	0.30061832
Aboveground biomass	-0.11739571	0.53320852
Belowground biomass	-0.41550503	0.20735143
Root:shoot	-0.49746628	-0.20250096
Redox	-0.23204339	-0.10849437
SLA	0.46617420	-0.19222960
Chlorophyll	0.44949654	0.01042466

Figure 3. Principle components analysis (PCA) of trait variation for Bald cypress (*Taxodium distichum*) seedlings exposed to variable combinations of inundation, light, and plant interaction treatments. Points represent individual treatments. Symbol size indicates inundation treatment (low, medium, and high), color indicates light treatment (shade or full sun), and shape indicates plant interaction treatment (AG, aboveground interaction only; AGBG, above- and belowground interaction; BG, belowground interaction only; NI, no interaction). Traits are: *Stem Height* (cm); *Diameter*, stem diameter (mm); *Leaf count* (*n*); *Aboveground and Belowground biomass* (g); *Root:Shoot*, root-to-shoot ratio of dry mass (g); *Redox*, soil redox potential (mv); *SLA* leaf specific area (cm^2/g); *Chlorophyll*, chlorophyll content estimate. PC1 and PC2 loadings for each variable are shown in the adjacent table.

more common than initially thought. Although Soft rush was not an evident nurse species for Bald cypress seedlings in this experiment, there is some evidence that graminoid and forb species can facilitate shrub and tree species in wetlands. For example, Hacker and Bertness (1995) found that the presence of Black needle rush (*Juncus gerardi* Loisel) created a more favorable environment for High tide bush (*Iva frutescens* L.) in saline ecosystems, extending the realized niche of High tide bush beyond its typical distribution. McKee et al. (2007) found that coastal succulent forbs can ameliorate temperature and salinity stress on coastal mangrove propagules and seedlings. It is possible that another graminoid species or growth form could facilitate Bald cypress seedlings, but this requires additional exploration.

Our findings emphasize the importance of ameliorating the stress of full-sun irradiance to enhance Bald cypress seedling growth. Mature trees have been shown to promote seedling establishment of other tree species through the provision of shade in harsh environments. For example, mature oak trees are associated with increased pine seedling establishment in sand dunes (Kellman & Kading 1992) and canopy shade is considered one of the main determinants of seedling establishment in humid savannahs (Vadigi & Ward 2013). However, in open-canopy settings such as disturbed restoration or habitat creation sites, we suggest future inquiries explore Bald cypress's competition/facilitation dynamics with better shade-producing early successional species typical of freshwater forests. Bald cypress forests contain diverse assemblages of trees, understory shrubs, and herbaceous vegetation (Devall 1998; McWilliams et al. 1998). Potential facilitating species may include taller co-occurring graminoids, such as Cypress-knee sedge (*Carex decomposita*), broadleaf forbs, such as False nettle (*Boehmeria cylindrica*), short-statured shrubs such as Buttonbush (*Cephalanthus occidentalis*), or fast growing and rapidly colonizing tree species, such as Red maple (*Acer rubrum*) and Sweetgum (*Liquidambar styraciflua*). Perhaps even invasive non-native trees that thrive in remedial soils and full sun, such as Chinese tallow (*Triadica sebifera*) in the southeastern United States, may serve a purpose to ameliorate harsh light for Bald cypress.

Finally, using multiple-species planting approaches may have greater potential to ameliorate hypoxic soils if plants are more densely and closely configured. Greater belowground biomass of Soft rush would likely have a greater impact on local soil physiochemistry through radial oxygen loss en masse. Perhaps a more successful way to test this relationship in a multiple-species restoration approach would be to plant Bald cypress seedlings in an established graminoid patch rather than transplanting a plug of Soft rush adjacent to the tree.

Importance of Historically Overlooked Drivers

Very few studies have focused on light as a driver in Bald cypress ecology. Previous research has shown strong positive relationships between Bald cypress sapling growth and canopy gaps (i.e. filtered light) (deGravelles et al. 2014), or has documented Bald cypress competition for limited light resources (Pietrzykowski et al. 2015). Although nearly all seedlings survived the observed growing season, long-term survival and growth are expected to be reduced in seedlings exposed to full light. The greater root-to-shoot ratios observed in full-sun treatments may be due to stressful irradiance reducing aboveground growth or slowed carbon assimilation allocated belowground in shaded treatments (Megonigal & Day 1992). The reduction of chlorophyll associated with full-sun treatments suggests a physiological breakdown of photosynthetic machinery, or photoinhibition, due to high irradiance (Carmichael & Smith 2016). Neufeld (1983) documented that maximum net photosynthesis occurred at lower light levels compared to full sun. This study also found that the light compensation point was slightly higher in Bald cypress saplings grown in full sunlight compared to those acclimated to shade. Extreme temperatures, often associated with irradiance, can also contribute to photoinhibition. In this study, temperatures were not strongly impacted by shading ($\Delta < 3\%$); but combined irradiance and temperature effects may have stronger impacts in a field setting, particularly in regions that may experience prolonged elevated temperature spikes due to a warming climate.

The differences in trait responses to drivers observed in our study are important for determining which indicators best characterize a habitat's condition (Gann et al. 2019; Yando et al. 2021). Selecting appropriate attributes is critical for monitoring an ecosystem's restoration trajectory. As seen in our study, rarely measured traits such as leaf area and color may be as informative of seedling health as biomass is of growth. Further research is needed to understand the relationships between plant traits, the leaf economic spectrum, and environmental drivers in forested wetland species. Addressing this research gap would complement a growing global body of work exploring plant trait stress trade-offs (Onoda et al. 2017). Importantly, restoration of Bald cypress forests in open-canopy or disturbed landscapes should consider techniques to ameliorate the stress associated with excess light availability, perhaps through continued experimentation with facilitator plant species, shade cloth, or other novel approaches.

Acknowledgments

Funding for this study was supported by the Paul W. Kirk, Jr. Wetland Research Award. Data collection and greenhouse maintenance were supported by the Great Dismal Swamp National Wildlife Refuge, S. Hildebrandt, K. Carr, Z. Rebolledo-Sanchez, K. Overstreet, O. Dubovyk, C. Wei, A. Amrhein, and D. Sloey. Early review of this manuscript was provided by P. Lysko and Drs. L. Wallace, E. Walters, and E. Yando. Finally, we thank Drs. Amaral, Wang, and the anonymous reviewers for their thoughtful comments and suggestions on this manuscript.

LITERATURE CITED

Alexander S, Nelson CR, Aronson J, Lamb D, Cliquet A, Erwin KL, et al. (2011) Opportunities and challenges for ecological restoration within REDD+. Restoration Ecology 19:683–689. https://doi.org/10.1111/j.1526-100X.2011.00822.x

Anderson JT, Landi AA, Marks PL (2009) Limited flooding tolerance of juveniles restricts the distribution of adults in an understory shrub (*Itea virginica*; Iteaceae). American Journal of Botany 96:1603–1611. https://doi.org/10.3732/ajb.0800366

Bertness M, Callaway RM (1994) Positive interactions in communities. Trends in Ecology & Evolution 9:191–193. https://doi.org/10.1016/0169-5347(94)90088-4

Brooker RW, Maestre FT, Callaway RM, Lortie CL, Cavieres LA, Kunstler G, et al. (2008) Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96:18–34. https://doi.org/10.1111/j.1365-2745.2007.01295.x

Caldwell PV, Vepraskas MJ, Gregory JD, Skaggs RW, Huffman RL (2011) Linking plant ecology and long-term hydrology to improve wetland restoration success. Transactions of the ASABE 54:2129–2137. https://doi.org/10.13031/2013.40662

Carmichael MJ, Smith WK (2016) Growing season ecophysiology of *Taxodium distichum* (L.) rich. (bald cypress) saplings in a restored wetland: a baseline for restoration practice. Botany 94:1115–1125. https://doi.org/10.1139/cjb-2016-0147

Conner WH, Gosselink JG, Parrondo RT (1981) Comparison of the vegetation of three Louisiana swamp sites with different flooding regimes. American Journal of Botany 68:320–331. https://doi.org/10.1002/j.1537-2197.1981.tb06369.x

de Gravelles WW, Hutchinson S, Conner WH (2014) Survival and growth of suppressed bald cypress reproduction in response to canopy gap creation in a North Carolina, U.S.A. swamp. Wetlands 34:79–87. https://doi.org/10.1007/s13157-013-0484-z

Devall MS (1998) An interim old-growth definition for cypress-tupelo communities in the southeast. General Technical Report SRS-19. U.S. Department of Agriculture, Forest Service, Southern Research Station.Stoneville, Mississippi.

Donato DC, Kauffman JB, Murdiyarso D, Kurnianto S, Stidham M, Kanninen M (2011) Mangroves among the most carbon-rich forests in the tropics. Nature Geoscience 4:293–297. https://doi.org/10.1038/NGEO1123

Dumroese KR, Landis TD, Pinto JR, Haase DL, Wilkinson KW, Davis AS (2016) Meeting forest restoration challenges: using the target plant concept. Reforesta 1:37–52. https://doi.org/10.21750/REFOR.1.03.3

Fagan ME, Reid JL, Holland MB, Drew JG, Zahawi RA (2020) How feasible are global forest restoration commitments? Conservation Letters 13:e12700. https://doi.org/10.1111/conl.12700

Gann GD, McDonald T, Walder B, Aronson J, Nelson CR, Jonson J, Dixon K (2019) International principles and standards for the practice of ecological restoration. Restoration Ecology 27:S1–S46. https://doi.org/10.1111/rec.13035

Gerber JF (2011) Conflicts over industrial tree plantations in the south: who, how, and why? Global Environmental Change 21:165–176. https://doi.org/10.1016/j.gloenvcha.2010.09.005

Gómez-Aparicio L, Zamora R, Gómez JM, Hódar JA, Castro J, Baraza E (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. Ecological Applications 14:1128–1138. https://doi.org/10.1890/03-5084

Hacker SD, Bertness MD (1995) Morphological and physiological consequences of a positive plant interaction. Ecology 76:2165–2175. https://doi.org/10.2307/1941690

Hobbs RJ, Norton DA (2004) Ecological filters, thresholds, and gradients in resistance to ecosystem reassembly. In: Temperton VM, Hobbs RJ, Nuttle T, Halle S (eds) Assembly rules and restoration ecology: bridging the gap between theory and practice. Island Press, Washington, D.C.

Kanowski J, Catterall CP (2010) Carbon stocks in above-ground biomass of monoculture plantations, mixed species plantations and environmental restoration plantings in north-east Australia. Ecological Management and Restoration 11:119–126. https://doi.org/10.1111/j.1442-8903.2010.00529.x

Kellman M, Kading M (1992) Facilitation of tree seedling establishment in a sand dune succession. Journal of Vegetation Science 3:679–688. https://doi.org/10.2307/3235836

Kruse BS, Groninger JW (2003) Vegetative characteristics of recently reforested bottomlands in the lower Cache River watershed, Illinois, U.S.A. Restoration Ecology 11:273–280. https://doi.org/10.1046/j.1526-100X.2003.00178.x

Laffoley D, Grimsditch GD (2009) The management of natural coastal carbon sinks. World Conservation Union, Gland, Switzerland

Lewis RR (2005) Ecological engineering for successful management and restoration of mangrove forests. Ecological Engineering 24:403–418. https://doi.org/10.1016/j.ecoleng.2004.10.003

Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97:199–205. https://doi.org/10.1111/j.1365-2745.2008.01476.x

Malkinson D, Tielbörger K (2010) What does the stress-gradient hypothesis predict? Resolving the discrepancies. Oikos 119:1546–1552. https://doi.org/10.1111/j.1600-0706.2010.18375.x

Mayence CE, Hester MW (2011) Assessment of a multi-species planting approach for restoring thick-mat floating marsh, Louisiana, U.S.A. Restoration Ecology 19:216–224. https://doi.org/10.1111/j.1526-100X.2009.00557.x

McAfee D, Larkin C, Connell SD (2020) Multi-species restoration accelerates recovery of extinguished oyster reefs. Journal of Applied Ecology 58:286–294. https://doi.org/10.1111/1365-2664.13719

McKee KL, Rooth JE, Feller IC (2007) Mangrove recruitment after forest disturbance is facilitated by herbaceous species in the Caribbean. Ecological Applications 17:1678–1693. https://doi.org/10.1890/06-1614.1

McWilliams WH, Tansey JB, Birch TW, Hansen MH (1998) Taxodium-Nyssa (cypress-tupelo) forests along the coast of the Southern United States. Pages 257–270. In: Laderman AD (ed) Coastally restricted forests. Oxford University Press, New York

Megonigal JP, Day FP (1992) Effects of flooding on root and shoot production of bald cypress in large experimental enclosures. Ecology 73:1182–1193. https://doi.org/10.2307/1940668

Michalet R, Le Bagousse-Pinguet Y, Maalouf JP, Lortie CJ (2014) Two alternatives to the stress-gradient hypothesis at the edge of life: the collapse of facilitation and the switch from facilitation to competition. Journal of Vegetation Science 25:609–613. https://doi.org/10.1111/jvs.12123

Mitsch WJ, Gosselink JG (2015) Wetlands. 5th ed. John Wiley & Sons, Inc., Hoboken, New Jersey

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photoinhibition of photosystem II under environmental stress. Biochimica et Biophysica Acta (BBA) – Bioenergetics 1767:414–421. https://doi.org/10.1016/j.bbabio.2006.11.019

Myers RS, Shaffer GP, Llewellyn DW (1995) Baldcypress (*Taxodium distichum* (L.) rich.) restoration in southeast Louisiana: the relative effects of herbivory, flooding, competition, and macronutrients. Wetlands 15:141–148. https://doi.org/10.1007/BF03160667

Neter J, Kutner MH, Nactsheim CJ, Wasserman W (1996) Applied linear statistical models. McGraw-Hill, Chicago, Illinois

Neufeld HS (1983) Effects of light on growth, morphology, and photosynthesis in baldcypress (*Taxodium distichum* (L.) rich.) and pondcypress (*T. Ascendens* Brongn.) seedlings. Bulletin of the Torrey Botanical Club 110:43–54. https://doi.org/10.2307/2996516

Onoda Y, Wright IJ, Evans JR, Hikosaka K, Kitajima K, Niinemets Ü, Westoby M (2017) Physiological and structural tradeoffs underlying the leaf economics spectrum. New Phytologist 214:1447–1463. https://doi.org/10.1111/nph.14496

Osiecka A, Minogue PJ (2012) Selective herbicides for bald cypress restoration and cultivation. Weed Technology 26:460–468. https://doi.org/10.1614/WT-D-11-00171.1

Padilla FM, Pugnaire FI (2006) The role of nurse plants in the restoration of degraded environments. Frontiers in Ecology and the Environment 4:196–202. https://doi.org/10.1890/1540-9295(2006)004[0196:TRONPI]2.0.CO;2

Pietrzykowski M, Daniels WL, Koropchak SC (2015) Microtopographic effects on growth of young bald cypress (*Taxodium distichum* L.) in a created freshwater forested wetland in southeastern Virginia. Ecological Engineering 83:135–143. https://doi.org/10.1016/j.ecoleng.2015.06.024

Powell AS, Jackson L, Ardón M (2016) Disentangling the effects of drought, salinity, and sulfate on baldcypress growth in a coastal plain restored wetland. Restoration Ecology 24:548–557. https://doi.org/10.1111/rec.12349

R Core Team (2022) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Ren H, Yang L, Liu N (2008) Nurse plant theory and its application in ecological restoration in lower subtropics of China. Progress in Natural Science 18:137–142. https://doi.org/10.1016/j.pnsc.2007.07.008

Renzi JJ, He Q, Silliman BR (2019) Harnessing positive species interactions to enhance coastal wetland restoration. Frontiers in Ecology and Evolution 7:131. https://doi.org/10.3389/fevo.2019.00131

Ristok C, Eisenhauer N, Weinhold A, Dam NM (2022) Plant diversity and soil legacy independently affect the plant metabolome and induced responses following herbivory. Journal of Ecology 111:412–427. https://doi.org/10.1111/1365-2745.14032

Sánchez-Sastre LF, Alte da Veiga NMS, Ruiz-Potosme NM, Carrión-Prieto P, Marcos-Robles JL, Navas-Gracia LM, Martín-Ramos P (2020) Assessment of RGB vegetation indices to estimate chlorophyll content in sugar beet leaves in the final cultivation stage. AgriEngineering 2:128–149. https://doi.org/10.3390/agriengineering2010009

Sloey TM, Ellis VS, Kettenring KM (2023) Using plant functional traits to inform wetland restoration. Wetlands 43:92. https://doi.org/10.1007/s13157-023-01741-z

Sorrell B (2000) Ecophysiology of wetland plant roots: a modelling comparison of aeration in relation to species distribution. Annals of Botany 86:675–685. https://doi.org/10.1006/anbo.2000.1173

Sorrell BK, Brix H (2013) Gas transport and exchange through wetland plant aerenchyma. Pages 177–196. In: DeLaune RD, Reddy KR, Richardson CJ, Megonigal JP (eds) Methods in biogeochemistry of wetlands. Soil Science Society of America, Inc. Madison, WI. https://doi.org/10.2136/sssabookser10.c11

Souther RF, Shaffer GP (2000) The effects of submergence and light on two age classes of baldcypress (*Taxodium distichum* (L.) Richard) seedlings. Wetlands 20:697–706. https://doi.org/10.1672/0277-5212(2000)020[0697:TEOSAL]2.0.CO;2

Stanturf JA, Schoenholtz SH, Schweitzer CJ, Shepard JP (2001) Achieving restoration success: myths in bottomland hardwood forests. Restoration Ecology 9:189–200. https://doi.org/10.1046/j.1526-100x.2001.009002189.x

Trettin CC, Jurgensen MF, Dai Z (2019) Effects of climate change on forested wetland soils. Developments in Soil Science 36:171–188. https://doi.org/10.1016/B978-0-444-63998-1.00009-4

United Nations (2023) United Nations decade on ecosystem restoration: 2021–2030. https://www.decadeonrestoration.org (accessed 07 Aug 2023)

United Nations Framework Convention on Climate Change (2010) Draft decision CP.16 outcome of the Ad hoc Working Group on long-term cooperative action under the convention: advance unedited version. http://unfccc.int/files/meetings/cop_16/application/pdf/cop16_lca.pdf (accessed 25 Mar 2011)

Vadigi S, Ward D (2013) Shade, nutrients, and grass competition are important for tree sapling establishment in a humid savanna. Ecosphere 4:1–27. https://doi.org/10.1890/ES13-00239.1

Wagner AM, Larson DL, DalSoglio JA, Harris JA, Labus P, Rosi-Marshall EJ, Skrabis KE (2016) A framework for establishing restoration goals for contaminated ecosystems. Integrated Environmental Assessment and Management 12:264–272. https://doi.org/10.1002/ieam.1709

Wilhite LP, Toliver JR (1990) *Taxodium distichum* (L.) Rich. var. distichum. In: Silvics of North America: volume I conifers. Agriculture handbook 654. U. S. Department of Agriculture Forest Service, Washington, D.C.

Withgott J (2000) Botanical nursing: from deserts to shorelines, nurse effects are receiving renewed attention. Bioscience 50:479–484. https://doi.org/10.1641/0006-3568(2000)050[0479:BN]2.0.CO;2

Wodehouse DC, Rayment MB (2019) Mangrove area and propagule number planting targets produce sub-optimal rehabilitation and afforestation outcomes. Estuarine, Coastal and Shelf Science 222:91–102. https://doi.org/10.1016/j.ecss.2019.04.003

Yando ES, Sloey TM, Dahdouh-Guebas F, Rogers K, Abuchahla GM, Cannicci S, Friess DA (2021) Conceptualizing ecosystem degradation using mangrove forests as a model system. Biological Conservation 263: 109355. https://doi.org/10.1016/j.biocon.2021.109355

Yelenik SG, DiManno N, D'Antonio CM (2015) Evaluating nurse plants for restoring native woody species to degraded subtropical woodlands. Ecology and Evolution 5:300–313. https://doi.org/10.1002/ece3.1294

Supporting Information

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

Table S1. ANOVA output for full model statements of all main effects.
Table S2. Mean and standard error for all treatment combinations for all dependent variables not displayed in figures.

Coordinating Editor: Guodong Wang

Received: 21 December, 2023; First decision: 24 January, 2024; Revised: 3 June, 2024; Accepted: 5 June, 2024