Plant Ecol
https://doi.org/10.1007/s11258-020-01059-4

Influence of soil microbiota on *Taxodium distichum* seedling performance during extreme flooding events

Lorena Torres-Martínez · Mareli Sánchez-Julia · Elizabeth Kimbrough · Trey C. Hendrix · Miranda Hendrix · Richard H. Day · Ken W. Krauss · Sunshine A. Van Bael

Received: 28 July 2019 / Accepted: 10 July 2020

Abstract Plant associations with soil microbiota can modulate tree seedling growth and survival via mutualistic or antagonistic interactions. It is uncertain, however, whether soil microbiota influence seedling growth of coastal trees when exposed to extreme flooding regimes. We evaluated the role of soil microbes in promoting baldcypress (*Taxodium distichum*) seedling performance under different inundation scenarios and determined the influence of flooding on the colonization of *in planta* beneficial microbes. Seedlings reared in sterile and non-sterile soil were exposed to three different flooding regimes historically experienced in Louisiana swamps. Seedling growth was assessed, and the colonization by beneficial symbionts such as arbuscular mycorrizhal fungi (AMF), and dark septate endophytes (DSE) was evaluated in harvested roots. Seedlings grown in sterile soil had six times higher growth than seedlings reared in non-sterile soil. As a result, we evaluated pathogen load in the roots by assessing oomycete colonization. Flooding influenced the *in planta* colonization of DSE and oomycetes, but did not affect the colonization of mutualist AMF fungi. DSE and oomycetes were rarer in flooded conditions, while AMF remained abundant. Seedling biomass production was not correlated with *in planta* fungal

Communicated by Eleonora Egidi.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11258-020-01059-4) contains supplementary material, which is available to authorized users.

L. Torres-Martínez · M. Sánchez-Julia · E. Kimbrough · T. C. Hendrix · M. Hendrix · S. A. Van Bael
Department of Ecology and Evolutionary Biology, Tulane University, 6823 St. Charles Avenue, Boggs 400, New Orleans, LA 70118, USA

L. Torres-Martínez (✉)
Department of Evolution, Ecology and Organismal Biology, University of California, 5406 Boyce Hall, Riverside, CA 92521, USA
e-mail: lorenat@ucr.edu

T. C. Hendrix
Department of Ecology and Evolutionary Biology, Princeton University, 106A Guyot Hall, Princeton, NJ 08544, USA

R. H. Day · K. W. Krauss
Wetland and Aquatic Research Center, U.S. Geological Survey, 700 Cajundome Blvd., Lafayette, LA 70506, USA

Published online: 22 July 2020

colonization or pathogen load. Soil microbiota can negatively influence baldcypress seedling growth, and no growth benefit was evidenced from the root colonization of mutualist fungi. Flooding can modify baldcypress-fungal interactions by diminishing colonization of DSE. Overall, baldycpress seedlings were more sensitive to the presence of microbiota than flooding, and thus restoration efforts should focus on having a better understanding of plant–microbe interactions in swamps.

Keywords Arbuscular mycorrhizal fungi · Baldcypress · Dark septate endophytes · Hurricanes · Mutualism · Symbioses

Introduction

Global change has particularly affected coastal plant species, despite their adaptation to a periodically disturbed environment (Gabler et al. 2017; Kirwan et al. 2009). Sea level rise has increased threefold over the last decade, altering the physical conditions of coastal habitats (Dangendorf et al. 2017; Yi et al. 2017). Similarly, the intensity, duration, and frequency of Atlantic hurricanes have dramatically increased since the 1980s (Mendelsohn et al. 2012; Walsh et al. 2016), with more intense and less frequent tropical storms reaching the Gulf of Mexico (Buruyere and Coauthors 2017). As a consequence, extreme climate events leading to saltwater intrusion and flooding from storm surge and sea level rise have become the two main stressors directly affecting the survival and reproductive success of coastal plant species (Krauss et al. 2009).

In the southeastern USA, the native swamp tree, baldcypress (*Taxodium distichum)* has been severely affected by salt intrusion and floods where its range extends to coastal areas (Allen et al. 1996; Krauss et al. 2009; Shaffer et al. 2009). Adult trees of *T. distichum* are showing signs of stress and in many cases, extensive death due to the species' low tolerance for high salt concentrations (Conner et al. 1997; Krauss et al. 2009; Pezeshki et al. 1987). Additionally, in spite of *T. distichum*'s adaptations to deep brackish water (Anderson and Pezeshki 1999), prolonged flooding conditions are preventing population regeneration, as most seeds cannot germinate, and seedlings are not recruited in permanently flooded environments (Day et al. 2006; Demaree 1932; Langston et al. 2017; Newsham 2011; Souther and Shaffer 2000). Short-term, deeper floods, however, can have a positive influence on baldcypress germination and recruitment, since the presence of water and its movement promotes the distribution of seeds away from parent trees and into other suitable microhabitats (Schneider and Sharitz 1986). Studies have reported higher seed dispersal, germination and seedling establishment when swamps receive slow-moving water from riverine sources (De Steven and Sharitz 1997). This is mainly because seeds are carried away from the dense canopies of parent trees to open areas where they can establish once the water recedes (Schneider and Sharitz 1988). In addition, there was low survival for seedlings originating from seeds that did not disperse far from their parent trees (Schneider and Sharitz 1986, 1988). In contrast, excessive and fast moving floodwaters—such as those observed during storm surge events—result in a net movement of seeds out of the swamp (Schneider and Sharitz 1988).

Due to the reduced recruitment observed throughout the coastal range of *T. distichum* and the ecosystem services this species provides to the coast, there is a strong initiative to restore baldcypress tree populations (CPRA 2017). To date, these restoration efforts have rarely accounted for the role that soil biota, and in particular beneficial microbes, such as arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE), may play in swamp plant population dynamics. Recently, inoculation of native plants with endophytes (i.e., beneficial fungi and bacteria living inside plant tissues) that can confer resistance to abiotic stress has been proposed as a solution to increase the resilience of native plant species in the face of climate change (Fardella et al. 2014; Vasanthakumari et al. 2015; Zahn and Amend 2017). Investigating the symbiotic association of baldcypress with soil microbes could provide a mechanistic approach to improve seedling recruitment, and therefore current restoration efforts of *T. distichum* populations. A first step to use microbes as a restoration alternative in baldcypress is to explore whether soil microbiota can promote seedling growth under abiotic stress.

Extensive root colonization by beneficial symbionts, such as AMF and DSE has been reported in baldcypress (Kandalepas et al. 2010). AMF and DSE are symbiotic fungi known to confer plant survival

under nutrient poor and extreme environmental conditions (Compant et al. 2010; Kotilínek et al. 2017; Lenoir et al. 2016). AMF belong to the phylum Glomeromycota and can form different morphological structures inside the host plants, such as hyphae, arbuscules and vesicles (Cavagnaro et al. 2001; Schüβler et al. 2001). DSE are a polyphyletic group of non-clavicipitaceous endophytes characterized by forming intra-radical dark septate hyphae and microsclerotia (Marins and Carrenho 2017; Rodriguez et al. 2009). AMF and DSE fungi can act as mutualists in most terrestrial plants, exchanging soil-derived nutrients and water for photosynthates within the roots (Davison et al. 2015; Lenoir et al. 2016).

Plant abiotic stress caused by high salinity, temperature, and drought may be alleviated by symbiosis with AMF and DSE (Baltruschat et al. 2008; Bueno de Mesquita et al. 2018; Lenoir et al. 2016; Li et al. 2019b; Rodriguez et al. 2008; Santos et al. 2017). In comparison with AMF, DSE are often more abundant under stressful environmental conditions (Kotilínek et al. 2017; Marins and Carrenho 2017), but also have been identified as pathogens (Tellenbach et al. 2011). When exposed to flooding, recent findings have reported a change in the resource allocation of nutrients between AMF and host plants (Bao et al. 2019; Deepika and Kothamasi 2015), but to date no increase in host fitness or performance has been reported for plants forming symbiotic associations with either DSE or AMF when exposed to this type of abiotic stress [see Marins and Carrenho (2017) for an extensive review]. Experiments manipulating AMF and phosphorus concentrations under inundated conditions have observed no evidence of gained host performance by the presence of AMF (Deepika and Kothamasi 2015; Stevens et al. 2011), and no experimental approaches of this magnitude have been reported for DSE [but see Santos et al. (2017) under water deficit]. This knowledge is even more scarce in coastal wetlands, and particularly for baldcypress where the mutual benefits that the symbiotic association is providing are unknown. Therefore, it remains unclear to what extent these plant-fungal interactions will promote baldcypress persistence in the near future, as pulses of extreme flooding events intensify.

One way extreme flooding conditions could affect benefits from mutualist soil microbes is by shifting *in planta* root colonization. Some studies have suggested that flooding can reduce the initiation of mycorrhizal colonization in host wetland plants (Miller and Sharitz 2000), and that water depth can determine the type of AMF and DSE associations in wetlands (Miller and Bever 1999; Nobis et al. 2015). Other research, however, has shown no significant influence of soil moisture or redox status to mycorrhizal colonization (Brown and Bledsoe 1996). In fact, an absence of plant-fungal symbiotic associations in wetlands was initially hypothesized to exist due to the stress imposed to fungi by the low-oxygen availability in saturated soils (Khan 1974; Martin et al. 2018). Subsequent research has revealed that plants adapted to different types of wetlands can form associations with both AMF and DSE fungi (Cooke and Lefor 1998; Khan 1993; Liberta et al. 1983; Lodge 1989; Nobis et al. 2015; Weishampel and Bedford 2006). Several studies have observed greater colonization of AMF in wetter compared to drier soils (Lodge 1989; Martin et al. 2018; Rickerl et al. 1994). Others have observed the opposite pattern (Šraj-Kržič et al. 2006; Stevens et al. 2011). In many cases DSE and AMF have been observed co-existing in the roots of wetland plants, with lower AMF and higher DSE colonization in plants naturally growing in the deepest more flooded parts of wetlands (Šraj-Kržič et al. 2006). Consequently, the effect of permanent flooding conditions on the *in planta* colonization potential (i.e., percent colonization in roots) of mycorrhizal fungi in wetland plants remains inconclusive and actively debated.

Here we assessed the ecological role of soil microbiota in promoting baldcypress seedling growth under different flooding regimes. We performed a manipulative experiment in controlled growth chamber conditions. We exposed young seedlings reared in sterile and non-sterile swamp soil to three different flooding regimes experienced by baldcypress trees in nature during hurricane and non-hurricane seasons. We addressed four specific questions: (1) Can the soil microbiota provide growth benefits to baldcypress seedlings when exposed to extreme flooding conditions? (2) Are these growth benefits provided by mutualistic interactions with soil microbes, such as AMF and DSE? (3) Is the colonization of beneficial microbes in host seedlings affected by flooding?, if so (4) Does flooding differentially affect the colonization of AMF and DSE?. We predicted that soil microbiota would promote seedling growth under flooding conditions present in swamps during the occurrence of a

hurricane. We expected this positive host response with abiotic stress to be associated with the *in planta* colonization of beneficial symbionts, such as AMF and DSE. However, we also hypothesized that AMF and DSE root colonization should decrease in extreme flooding conditions as a result of the anoxic environment characteristic of permanently inundated conditions, which ultimately results in a negative growth response in the seedlings. Alternatively, AMF and DSE might respond differently to extreme flooding conditions due to dissimilarities in their sensitivity to anoxic conditions.

Materials and methods

Seed source and sterilization

Baldcypress seeds were obtained from the Louisiana Forest Seed Company. This company collects fallen baldcypress seeds that have drifted to the edge of lakes and slow-moving streams (e.g., bayous) around central and north Louisiana, and thus provides a seed pool with a diverse set and large number of different native genotypes. To account for differences in maternal allocation to offspring among the seeds, we weighed and sorted a total of 2267 seeds in three main groups based on initial dry weight: small (0.02–0.05 g), intermediate (0.06–0.11 g), and large (0.12–0.17 g). Then, seeds were surface sterilized to remove any microbial contamination that could bias our experimental design. Seeds were submerged in 95% ethanol for 2 min followed by a 60-min bath in 10% Clorox and three subsequent washes with sterile water. After this sterilization procedure, seeds were immediately planted in seedling trays filled with sterile vermiculite and grown in controlled growth chamber conditions (model CMP6050, CONVIRON, Winnipeg, Canada). Baldcypress seeds germinate at the end of spring and early summer. Therefore, we simulated the average temperature and humidity over the summer in Louisiana by imposing a 14 h day at 27 °C ($\sim$ PAR = 400 mcmols) and 10 h night cycle at 25 °C at 90% humidity in our growth chamber. Germinated seedlings were raised in these same conditions for 2 months.

Soil provenance

We collected local swamp soil in early September of 2017 to obtain the microbial community and the natural physicochemical soil conditions available for baldcypress seedlings during the Atlantic hurricane season (NOAA 2018). The soil was extracted from the base of mature baldcypress trees growing on the natural levee of Bayou Chevreuil, a typical, coastal-associated freshwater swamp of southern Louisiana (Fig. 1a). This site has historically experienced several extreme flooding events due to storm surge from hurricanes (Fig. 1b). The natural levee of Bayou Chevreuil is slightly elevated above the forest floor and is flooded for $\sim$ 20% of the year, while lower areas in the swamp are completely flooded for $>$ 90% of the year. Thus, baldcypress trees and soil microbial communities present on the natural bayou levee experience more terrestrial-like conditions over the course of the year than the swamp floor, but were exposed to extreme flooding conditions during hurricanes Katrina and Rita in 2005 (Fig. 1b).

The soil was collected in buckets and processed the same day. The soil was mixed, sieved (2 mm mesh), and split in two equal portions. One portion was sterilized from all the native microbes (i.e., sterile soil treatment) by steaming the soil up to a temperature of 121 °C for 45 min, and the other portion was used as our live soil (i.e., non-sterile soil treatment; see Fig. 2). The next day each soil treatment—sterile or non-sterile—was independently mixed in a 1:1 ratio with previously prepared and sterilized soil composed of topsoil (Scotts® Premium Topsoil). The previously prepared topsoil had been pre-mixed with sand in a 3:1 ratio before sterilization. The complete mix was then added into individual mini-tree pots of 17.8 cm height (MT297, Stuewe & Sons). Pots were filled up to 16 cm height. Then, a total of 30 1-month-old seedlings were randomly assigned and transplanted from the vermiculite into each soil treatment (i.e., sterile and non-sterile soil; Fig. 2). To allow the establishment of soil microbiota inside the plant, the seedlings grew for a month in the new soil conditions before exposing them to any of the flooding treatments (see below). Over the course of the establishment time, the seedlings were watered every 3 days with 500 mL of deionized water to keep the soil moist but not over-saturated.

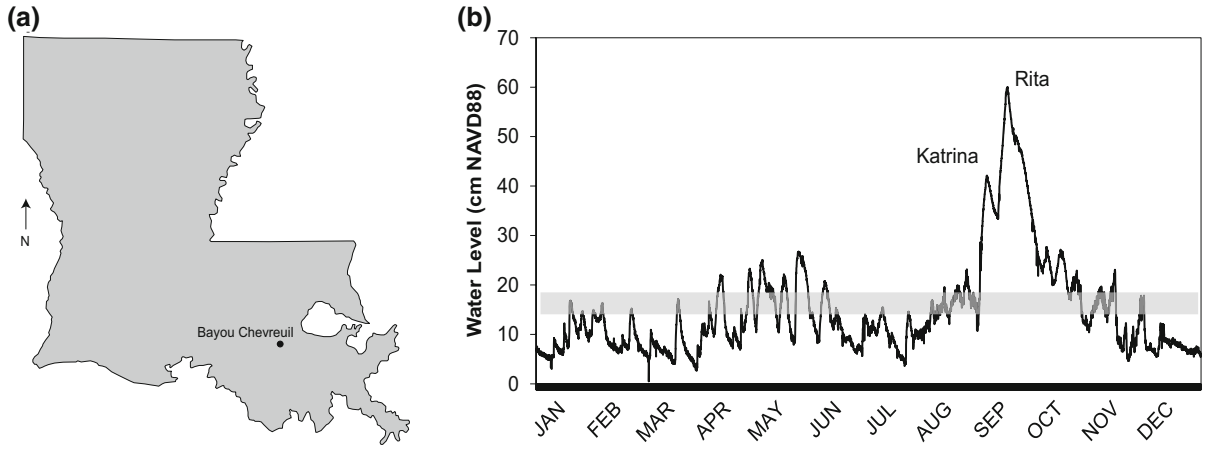

Fig. 1 **a** Location of Bayou Chevreuil in Louisiana, USA from where soil was collected for inoculation. **b** Hydroperiod of the swamp at Bayou Chevreuil during 2005 indicating the timing of water levels experienced by adult baldcypress trees over the two most extreme hurricanes observed in Louisiana over the last decades (Katrina and Rita). The gray line represents the base of baldcypress trees on the natural levee of Bayou Chevreuil

Flooding regimes

In controlled growth chamber conditions, we exposed the inoculated 3-month-old seedlings to three different flooding regimes experienced by baldcypress trees in nature. To calibrate our treatments, we used water level data from Bayou Chevreuil swamps recorded over the last two decades (Figs. 1b and 2). Based on these data, the flooding regimes were imposed by subjecting the seedlings to three different levels of soil saturation. The treatments were maintained for over a month which is the maximum amount of time recorded where baldcypress trees experience $>$ 40 cm of water after an extreme event, such as, in hurricanes Rita and Katrina (Figs. 1b and 2). Thus, to simulate an extreme flooding event, we over-saturated the soil and completely submerged the seedlings in water. This provided an approach where the seedlings experienced $>$ 40 cm of water from their base (Fig. 3). A typical precipitation regime was simulated by keeping water levels to the soil saturation point and facilitating the interchange of water and ions in the roots. This treatment represented our typical flooding regime. We enacted no flooding conditions (hereafter called non flooding) by maintaining water levels in each pot below soil saturation. Only 500 mL of water was added at the beginning of the experiment to the seedlings in this treatment group.

To simulate each of these flooding regimes in common garden conditions, each inoculated mini-tree pot was placed inside individual five-gallon buckets and randomly assigned to the watering regimes (Fig. 2). Buckets had a lateral hole at the height where the soil saturation point would be reached. A hose was connected to this hole to be able to slowly remove the standing water every week without imposing an additional stress to the seedlings. The hose was kept upright with tape at the top of the buckets (Fig. 2). The same day water was removed from the buckets, new, fresh deionized water was added to each bucket. Water levels were maintained throughout the experiment by visiting the treatments twice per week, checking if any evaporation had led to a change in the water levels initially set up and filling with additional water those buckets where some evaporation had occurred. A 5 mL amount of fertilizer (Miracle-gro® water soluble all-purpose plant fertilizer) was added at the beginning of the experiment. A total of 20 seedlings were exposed to each of the three treatments, out of which ten seedlings were grown in sterile or non-sterile soil before assigning them to the flooding regimes. Therefore, our experimental set up was a factorial design with ten replicates for each factor combination and a total of 60 plants. The growth chamber temperature, light cycle and humidity simulated summer conditions in Louisiana.

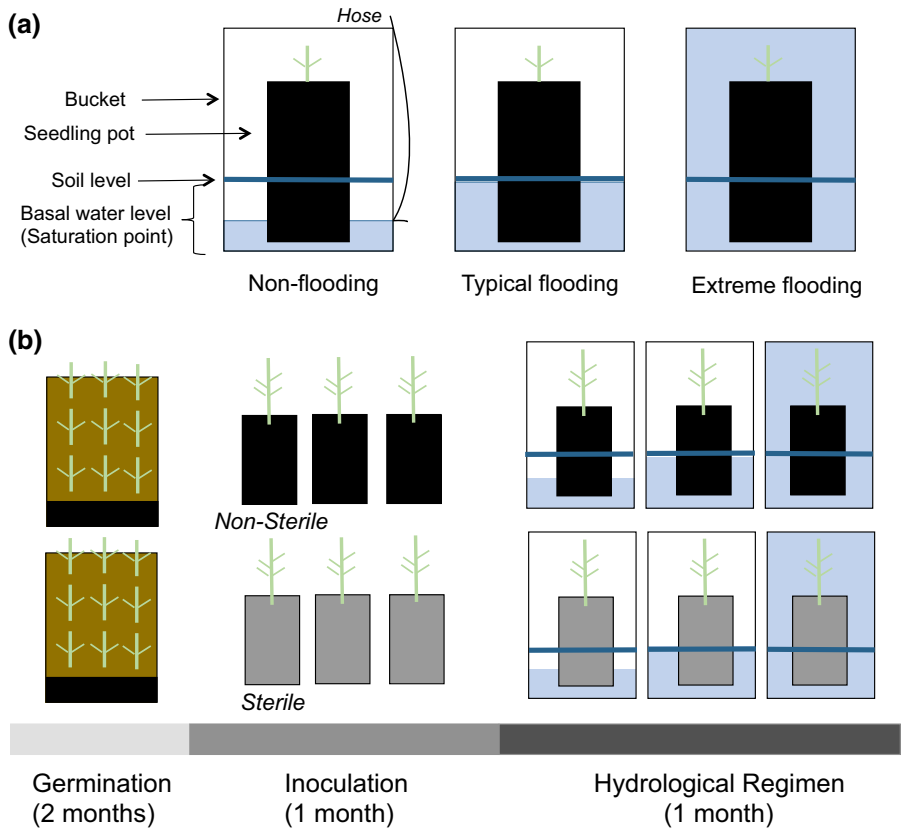

Fig. 2 Set up and timeline of experimental design implemented. **a** Three flooding regimes were simulated by manipulating soil saturation levels. Mini-tree pots were located inside five-gallon buckets to impose each saturation treatment. A non-flooding treatment was imposed by keeping the soil in the pots below saturation. An average precipitation regime was imposed by saturating the soil (typical), and an extreme flooding event was simulated by over saturating the soil of the pots and submerging the majority of the shoot of young seedlings. Each bucket had a side opening with a hose connected and maintained upright to be able to slowly release the water every week. **b** The timeline of the experiment started with the germination of seedlings over a 2-month period followed by the transplant of seedlings into pots with either non-sterile (black pots) or sterile (gray pots) soil inoculant. Young seedlings grew for 1 month after inoculation and then were exposed to the flooding regimes for a month

Soil analyses

We evaluated the effects of sterilization on the nutrient availability profile of the soil as well as the influence of the different flooding treatments. Soil samples of both sterile and non-sterile treatments were analyzed before and after transferring the seedlings into the treatments. A bulk sample of sterile and non-sterile soil was collected before distribution into each of the seedlings (hereafter named as soil before treatments). After the seedlings were exposed to all three flooding regimes, and during our harvest day we collected soil samples from five different replicates that were assigned into our sterile and non-sterile soil treatments. All soil samples were analyzed by the Soil Testing and Plant Analysis Lab at Louisiana State University where 2 g of soil were suspended in 20 mL of Mehlich 3 solution (Mehlich 1953, 1984) and subsequently measured in a inductively coupled plasma spectrophotometer. The soil pH, available phosphorus, nitrogen, carbon percentages, and the composition of other micro and macro nutrients were analyzed.

Plant growth and performance

We evaluated the effect of soil microbiota (i.e., addition of non-sterile soil) and influence of symbionts on the tolerance of baldcypress seedlings to flooding by measuring plant growth, total dry biomass, specific

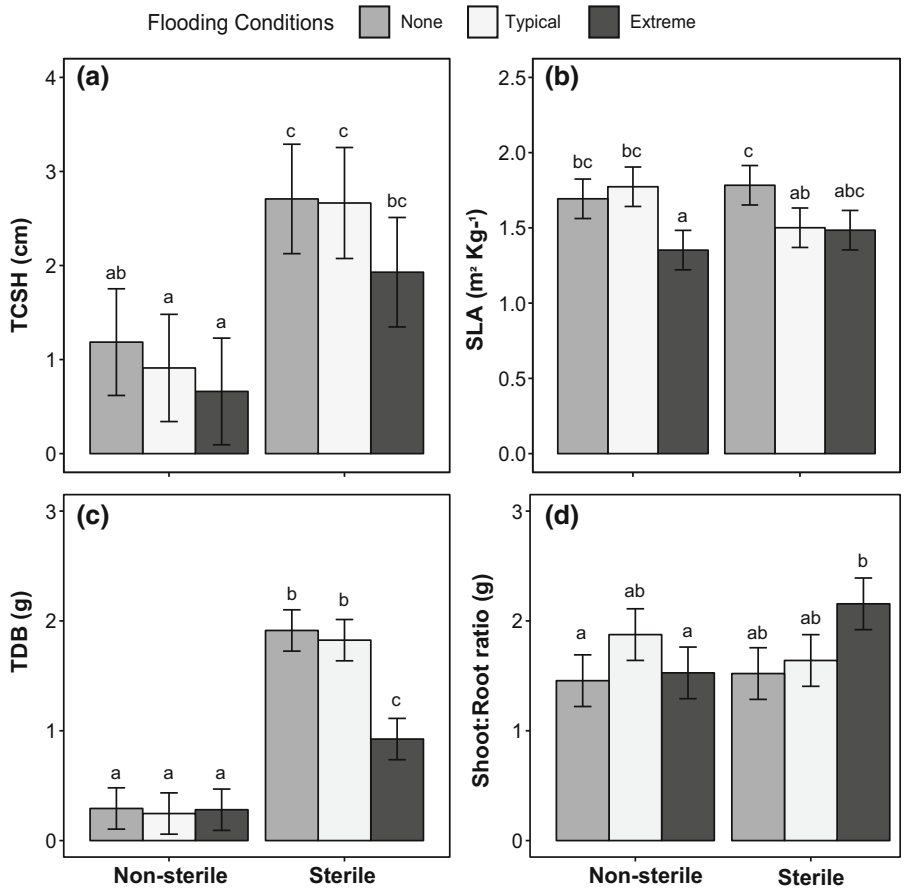

Fig. 3 *Taxodium distichum* mean seedling growth (top row) and performance (bottom row) in the presence (non-sterile) and absence (sterile) of soil microbes in response to different flooding regimes. Plant growth was measured as **a** mean change in height (TCSH) and **b** specific leaf area (SLA), while plant performance was evaluated in terms of **c** total dry biomass production (TDB) and **d** shoot:root dry biomass ratio. Bars represent back-transformed least square means. Error bars represent ± one standard error. Tukey's honest significant differences among treatments are represented by distinct letters at the 5% level

leaf area and shoot to root ratio. Plant growth was estimated by measuring seedling height throughout the duration of the experiment. Height was measured for the seedlings once per week over the course of 2 months. To track changes in plant height from the beginning to the end of the experiment, we placed a mark under the first leaves that appeared on the plant using white paint. Initial seedling height was then measured. We used a flexible measuring tape and reported the observed height from the white mark on the seedling to the top of the plant. We defined the top of the plant as the tallest observable part with a woody stem. If a woody stem in the plant was not visible we measured the height from the white mark to the highest point of the green stem.

We calculated the total change in seedling height (hereafter, TCSH) relative to the initial height of the plants as the difference between the seedling height recorded at the end of the experiment, final height (FH), and the height observed before exposing the seedlings to the inoculation treatment, initial height (IH), divided by the initial height of the seedlings: TCSH = (FH – IH)/IH.

To understand the strategies of resource acquisition for the seedlings when exposed to different flooding regimes in the presence of live and sterile soil, we estimated the specific leaf area for the first leaf produced by each seedling at the end of the experiment. Specific leaf area was measured as the ratio of leaf area to leaf dry mass (Garnier et al. 2001). Freshly

collected leaves were pressed and flattened on a white board and a picture was taken for each separate leaf. Leaf area was calculated by analyzing the pictures in ImageJ (Schneider et al. 2012). Leaves were then saved in individual coin envelopes to be dried in an oven at 65 °C for over a month and weighed to estimate their dry mass.

Plant performance was evaluated by the total dry biomass produced in stems, leaves and roots. Aboveground biomass and belowground biomass were harvested after 1 month of exposing the inoculated seedlings to each of the flooding regimes. Soil from each pot was washed and the complete root system was cut from the main stem. We recorded the wet weight from stems, leaves and roots separately. A small portion ($\sim$ 0.3 g) of the root system was placed aside in vials for further evaluation of AMF and DSE. We stored the harvested biomass in paper envelopes that were placed in a drying oven at 65 °C for over a month. Then, we recorded the dry weight of the biomass obtained from stems, leaves and roots separately. Total dry biomass was then estimated as the added weight of below and above ground biomass. The allocation of resources to root versus shoot to the total biomass produced was estimated by the shoot to root dry biomass ratio (A:B ratio) by dividing the total aboveground over the belowground dry biomass. All biomass measurements were recorded to the 0.001 g.

Quantification of symbiont in planta colonization

A subsample of five seedlings per treatment combination was used to quantify root colonization of symbionts such as AMF and DSE (n = 30). Root processing was initiated immediately after experimental harvest. Approximately 0.3 g of root wet weight was cleared with 10% KOH, first at room temperature (22 °C) for 30 min and then at 35 °C for 1.5 h using a water bath. Roots where acidified in 2% HCl for 1 h, then stained with 3% Trypan Blue for 15 min at 35 °C (Giovannetti and Mosse 1980). Stained roots were rinsed thoroughly with deionized water and placed in acidified glycerol until quantification (Phillips and Hayman 1970). For each of the 30 root samples, five root strands measuring 4 cm in length were mounted along a microscope slide in acidified glycerol. A Nikon Eclipse Ci-L (Nikon Corporation, Tokyo, Japan) microscope was used at × 200 magnification for data collection, and images were taken with an iPhone 6 at × 400 magnification for proper structure identification.

Fungal root colonization was quantified using a modified magnified intersections method (McGonigle et al. 1990), with 100 total intersections assessed per sample. Total colonization was calculated as the percentage of root length containing either AMF or DSE fungal structures in 100 fields of view. AMF colonization was identified on the basis of three fungal morphological structures (i) hyphae growing intracellularly with arbuscules terminally in cortical cells (e.g., *Paris-type* Am morphology, here after ARB); (ii) as hyphae growing intercellularly without the production of arbuscules or vesicles hereafter HYP); (iii) forming storage structures such as vesicles (hereafter VES). If both hyphae and arbuscules were present at an intersection, only the arbuscule was counted. For DSE colonization, only dark septate hyphae (hereafter SHYP) and microsclerotia (hereafter MS) were counted as DSE due to their distinct morphology (Jumpponen 2001; Kandalepas et al. 2010). If both AMF and DSE were present at a single root intersection, both structures were counted. After discovering a negative effect of soil microbiota, we evaluated if structures from known plant pathogens such as oomycetes were present. An oomycete was counted if it had a perfectly rounded form or diamond shaped, thick cell wall, and staining dark blue (Díez-Navajas et al. 2007; William and Grünwald 2010). Pathogen load was estimated as the percent colonization of oomycetes using the same methodology described above.

Statistical analyses

We evaluated the influence of native soil microbiota on the growth and performance of baldcypress seedlings in response to three flooding regimes by performing mixed-model ANOVAs for plant growth, specific leaf area, shoot:root ratio and total dry biomass. Each model included soil treatment, flooding regime and their interaction as fixed effects. Seed size group was considered our random effect to account for the variability associated with maternal effects of the sampled seeds (i.e., resource allocation of each seed). Seed size was assumed to be a random factor because seedlings originating from seeds with different sizes were randomly assigned to our treatments. All analyses were performed in R version 3.6.1 (R Core Team

2019) with deviation coding ("contr.sum") for categorical variables. All mixed models were conducted using restricted maximum likelihood (REML) in the package *lme4* (Bates et al. 2015). For all the models we tested significance of fixed effects by doing marginal likelihood ratio tests in all the terms of the model using the *Anova* function in the *car* package (Fox and Weisberg 2019). Random factors were evaluated by comparing models with and without the factor and performing likelihood ratio tests between the models with the function *anova* from the *R Stats* package. When treatments exhibited significant interactions between the main effects, we conducted *t*-student post hoc tests to identify the specific treatment levels driving the overall differences using the R packages *lsmeans* (Lenth 2016) and *multcomp* (Hothorn et al. 2008). When testing for significant pairwise differences between each of the treatment levels we used a Tukey–Kramer adjustment to account for multiple comparisons. The residuals of total relative change in height, total, above and belowground biomass revealed strong deviations from homoscedasticity and normality, and data were log-transformed prior to analysis.

We evaluated if beneficial fungi within the soil microbiota provided growth benefits to baldcypress seedlings by assessing if total plant biomass was related to the total colonization percentage of fungi using a liner regression. This analysis was performed for the non-sterile soil treatment across flooding regimes. We also tested if the colonization percentage of each fungal group influenced seedling growth through separate linear regressions between AMF, and DSE colonization percentages with total dry biomass. Similarly, we evaluated if pathogen load influenced plant performance and growth by performing linear regressions between the colonization percentage by oomycetes and both total dry biomass and mean change in plant height. For oomycetes we were particularly interested in their effects on plant height because it has been reported that some Oomycota taxa can cause stunted growth in conifers (e.g., *Pythoptora* spp. Preuett et al. 2013; William and Grünwald 2010).

We assessed if different flooding regimes affected the association of *T. distichum* with root fungi (i.e., *in planta* colonization) by analyzing the total percent of fungal colonization for seedlings grown in non-sterile soil conditions. We tested if flooding differentially affected the colonization of AMF and DSE using a two-way ANOVA with fungal group, flooding regimen and their interaction as main effects. Initially we used a mixed model with total fungal colonization percent as our response variable, seed size as our random factor and flooding treatment, fungal group and their interaction as fixed effects. After performing a log-likelihood ratio test between our mixed model and a linear model that excluded seed size, we did not find evidence that this factor significantly explained the variation in our data and it was discarded from our model. We tested for significant pairwise differences between each of the treatment levels using Tukey–Kramer adjustment. Additionally, we evaluated if the abundances of the different fungal structures varied with flooding to understand potential changes in the physiology of the fungi under different flooding regimes. To this end, we performed separate ANOVAs for each fungal structure with percentage colonization as our response variable and flooding as our main effect.

To test if pathogen load varied with flooding we performed a one-way ANOVA with flooding treatment as our fixed factor and percent colonization of oomycetes as our response variable. Furthermore, to evaluate if the colonization of oomycetes varied at similar levels as root fungi we performed a two-way ANOVA with flooding treatment and microbial group (e.g., DSE, AMF, oomycetes) as our fixed factors and percent colonization as our response variable. After finding a significant interaction between flooding and microbial group we performed t-student post hoc tests.

The soil nutrient data were analyzed using separate linear models for each nutrient with flooding treatment, soil sterilization treatment and their interaction as main factors. Because we did not observe an overall interaction between flooding treatments and soil sterilization treatment we then simplified our model and evaluated pairwise differences among the levels of each factor using Tukey tests.

Results

Plant growth and performance

No seedling mortality was observed in our experiment. Soil sterilization treatment had a strong effect on the growth and total biomass production of *T. distichum* seedlings (Table 1; Figs. 3a, b, S1). At the end of the

Table 1 Results of linear mixed models evaluating the effects of soil sterilization and flooding treatments on the relative total change in seedling height (TCSH), specific leaf area (SLA), total dry biomass (TDB) and root to shoot biomass ratio (shoot:root ratio)

Factor		TCSH		SLA		TDB		Shoot:root ratio	
	d.f	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Fixed factors									
Flooding treatment	2	3.27	0.20	7.03	0.03	9.96	0.01	1.63	0.44
Soil	1	21.07	< 0.01	0.07	0.80	100.30	< 0.01	1.44	0.23
Flooding × Soil	2	0.80	0.67	3.86	0.15	8.31	0.02	2.21	0.33
Random factor									
Seed size	1	0.54	0.46	0	1	3.89	0.05	0	1

experiment, plants grown in sterile soil conditions were significantly taller than plants grown in non-sterile conditions for all three flooding regimes (Figs. 3a, S1). Extreme flooding conditions had no effect on seedling height (Table 1; Figs. 3a, S1). Seedlings reared in the same soil sterilization treatment had similar overall plant growth for the typical and extreme flooding regimes (Fig. 3a).

Specific leaf area (SLA), which is a measure of resource allocation and photosynthesis activity, changed with flooding regimen (Table 1; Fig. 3b). Seedlings exposed to extreme flooding conditions showed on average 1.34 and 1.25 times lower SLA than those submitted to non and typical flooding regimes, respectively (Fig. 3b). Soil sterilization treatment did not influence seedling specific leaf area (Table 1; Fig. 3b). All seedlings showed similar SLA values in sterile and non-sterile soils (Fig. 3b).

Soil sterilization treatments and flooding regimes affected seedling performance differently (Table 1; Figs. 3c, d, S2). Seedlings had greater total dry biomass production in sterile compared to non-sterile soil conditions. Total dry biomass production was five times greater in sterile compared to non-sterile soil conditions (Figs. 3c, S2). Seedlings produced higher biomass under non and typical flooding regimes compared to extreme flooding conditions (Fig. 3c). Soil sterilization treatment influenced seedling performance in response to the different flooding regimes (significant Hydrology × Soil, Table 1; Fig. 3c). Plants grown in sterile soil conditions had reduced biomass production for extreme flooding conditions in comparison with typical and non-flooding regimes, while all seedlings reared in non-sterile soil had on average equal biomass production in each flooding treatment (Fig. 3c, Tables 1 and S2). Seedling resource allocation to shoot and roots was similar across all soil and flooding regimes (Fig. 3d; Table 1). A greater shoot:root ratio was observed in seedlings reared in sterile than in non-sterile conditions when exposed to extreme flooding, while in the absence and typical flooding treatments similar ratios were observed (Fig. 3d). Seed size was not a predictor of the physiological responses of baldcypress seedlings to soil microbiota and flooding (Table 1).

Influence of root fungi colonization in plant growth

Total root fungi colonization did not predict plant total dry biomass ($F_{(1,30)} = 0.16$, $P = 0.69$, Fig. 4a), indicating that beneficial fungi did not provide a significant growth benefit to baldcypress across flooding treatments. Seedlings with higher AMF colonization percentages tended to show high plant performance (Fig. 4b). This tendency was not significant ($F_{(1,14)} = 1.26$, $P = 0.28$, Fig. 4b). DSE colonization percentages did not have any tendency with respect to plant performance ($F_{(1,14)} = 0.01$, $P = 0.97$, Fig. 4b). Root colonization by oomycetes had no effect on plant growth ($F_{(1,14)} = 0.05$, $P = 0.82$, Fig. 4c). For mean plant height, oomycete root colonization tended to have a negative influence, though not significant ($F_{(1,14)} = 0.55$, $P = 0.47$, Fig. 4d).

Influence of flooding in fungal root colonization and pathogen load

The percentage of root fungal colonization in baldcypress seedlings varied between the two fungal groups (Fungal group, $F_{(1,26)} = 38.20$, $P < 0.01$), and this

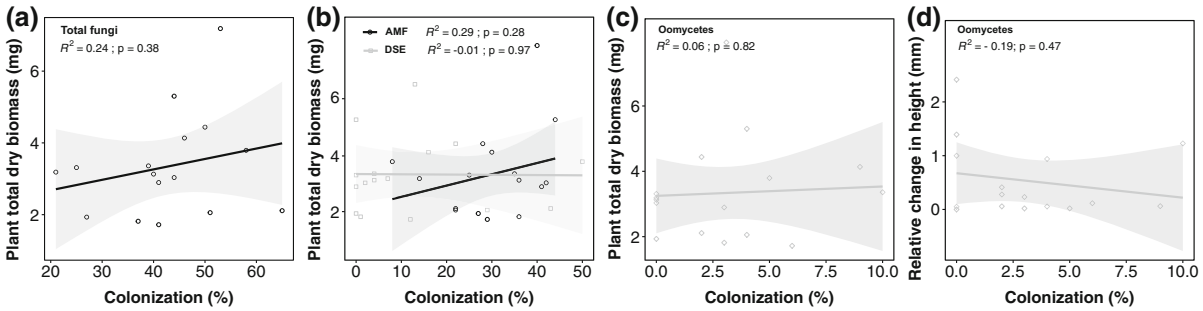

Fig. 4 Relationships between fungal root colonization and *T. distichum* seedling performance. **a** Relationship between total fungal colonization and total dry plant biomass (TDB; $R^2 = 0.24$, $P = 0.38$). **b** Separate relationships were also estimated for AMF (dark gray line; $R^2 = 0.29$, $P = 0.28$), and DSE (light gray line; $R^2 = -0.01$, $P = 0.97$) root colonization with plant performance. **c** Relationship of oomycete root colonization with plant dry biomass ($R^2 = 0.06$, $P = 0.82$) and **d** mean change in height (TCSH; $R^2 = -0.19$, $P = 0.47$). Lines represent the approximate linear relationship. *P*-values indicate the significance of each relationship

difference was dependent on the flooding treatment imposed (Fungal group x Flooding, $F_{(2,26)} = 4.52$, $P = 0.02$). On average, AMF colonization was three times higher than DSE, with DSE showing three times lower colonization than AMF when seedlings were exposed to extreme flooding and almost no colonization under typical flooding conditions (Fig. 5a). Total fungal colonization in baldcypress roots was not affected by the flooding regimen (Inundation, $F_{(2,31)} = 0.36$, $P = 0.69$, Fig. 5a).

Root colonization by oomycetes varied with flooding (Flooding, $F_{(2,11)} = 4.95$, $P = 0.03$, Fig. 5a), with four times lower colonization under extreme and typical flooded conditions than in the absence of flooding (Fig. 5a). DSE, AMF and oomycetes differed in their percent of root colonization (Microbial group, $F_{(2,33)} = 39.79$, $P < 0.01$), and flooding influenced these differences (Flooding × Microbial group, $F_{(4,33)} = 4.01$, $P < 0.01$). Oomycetes were present in baldcypress roots at lower percentages than AMF at each flooding treatment, and at similar percentages of DSE in both typical and none flooded conditions. In the most extreme flooding treatment all three microbial groups differed, with AMF showing the highest colonization percentage, followed by DSE, while oomycetes had the lowest percentage.

A diversity of fungal structures that characterize AMF and DSE fungi were identified in seedlings grown in non-sterile soil conditions among the flooding treatments (Figs. 5b, S3). In terms of AMF fungal structures, arbuscules were present at higher colonization percentages in baldcypress roots under typical flooding conditions than in either extreme flooding or non flooding ($F_{(2,12)} = 8.91$, $P < 0.01$), while hyphae were more abundant under non flooding ($F_{(2,12)} = 6.93$, $P < 0.01$) and vesicles were present at lower percentages (0–5%) across all three flooding treatments ($F_{(2,12)} = 2.75$, $P = 0.10$, Fig. 5b). From the two characterized DSE structures, only the percentages of dark septate hyphae (SHYP) differed among the flooding treatments ($F_{(2,12)} = 4.15$, $P = 0.04$). SHYP were present at higher percentages under either non or extreme flooded conditions. Microsclerotia were present at very low percentages (0–5%) in all treatments ($F_{(2,12)} = 1.88$, $P = 0.19$).

Soil nutrient composition

Prior to any allocation of our two soil batches into each of our flooding treatments, we observed no differences in the nutrient profile between the portion that was sterilized and the portion that was non-sterile (Fig. 6). We did observe differences in the content of macronutrients such as carbon, nitrogen, phosphorus and sulfur between our sterile and non-sterile soil at the end of our flooding regimes, while the content of copper, zinc and the soil pH were similar between the treatments (Table 2, Fig. 6). The concentration of carbon, nitrogen, sulfur, and magnesium were between one to two times higher under the non-sterile soil than in sterile soil, while phosphorus was higher under the sterile soil (Fig. 6). Only the concentrations of sulfur, magnesium and copper differed among the flooding treatments when averaged over the soil sterilization treatments. Sulfur and copper were two and three times higher, respectively, in the absence of flooding (non-flooding

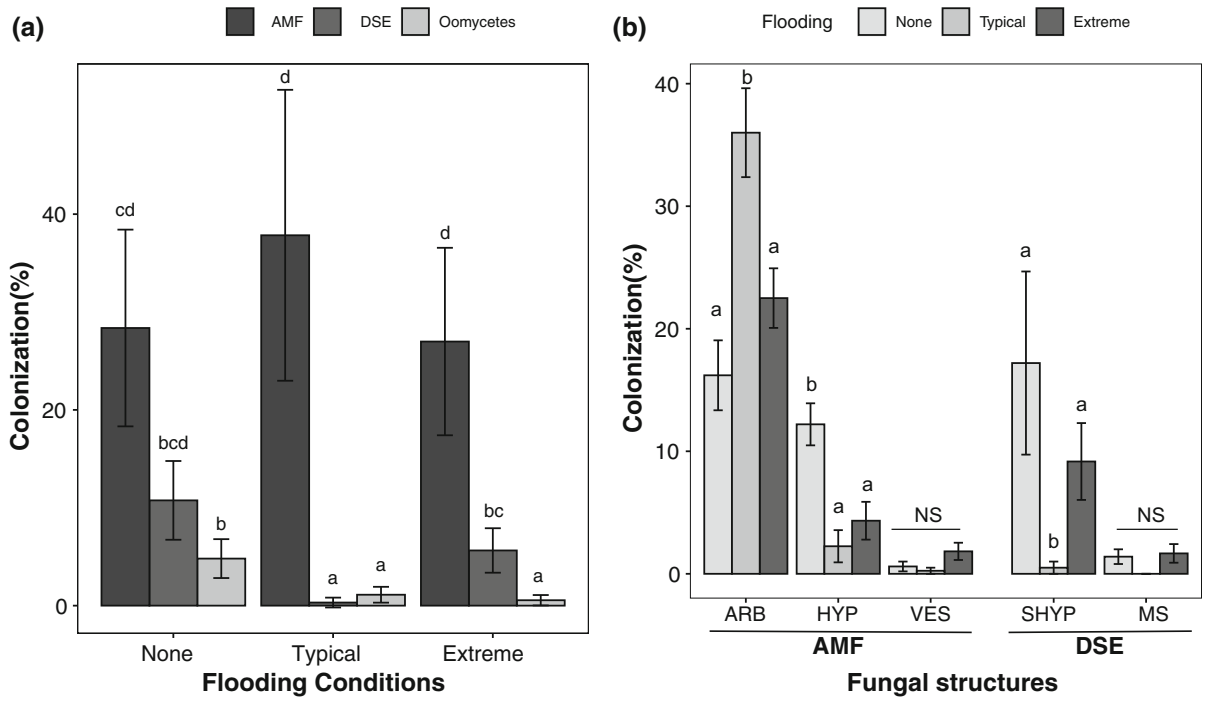

Fig. 5 Fungal colonization of *Taxodium distichum* seedling roots after exposure to three different flooding treatments. **a** Mean root colonization percentages of arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) in response to extreme, typical and non-flooding conditions. Error bars represent ± 1 standard error. Tukey's honest significant differences among treatments are represented by distinct letters at the 5% level. **b** Percentage colonization of fungal structures observed in the roots of baldcypress seedlings grown in non-sterile soil. Arbuscules (ARB), vesicles (VES) and hyphae (HYP) are characteristic of AMF fungi, while septate hyphae (SHYP) and microsclerotium (MS) are structures of DSE. Error bars represent ± 1 standard error. Tukey's honest significant differences among treatments are represented by distinct letters at the 5% level

treatment) than in either of typical or extreme flooding. Magnesium was one time higher in the absence of flooding than under extreme flooding. Nutrient concentrations of each soil did not dependent on the flooding treatment imposed (no significant Soil-by-Flooding treatment interaction, Table 2, Fig. 6).

Discussion

Plant-microbiota associations have been poorly explored in Louisiana swamps, where the combined effects of landscape modification and increased climate variability have resulted in more permanent flooded conditions (see CPRA 2017; Shaffer et al. 2009). Because submergence and soil saturation are stressful for young baldcypress saplings, these flooding changes have affected baldcypress seedling establishment, and, thus, population regeneration and growth. Our results confirmed the slower growth of baldcypress seedlings when exposed to flooded conditions, and a tenuous link to resilience (sensu Chapin 1991), but suggest a negative influence of soil microbes—collected near adult baldcypress trees—in plant biomass production and growth. Despite this observed decreased growth in the presence of soil microbes, seedlings showing association with well-known mutualist groups of root fungi had similar biomass production when exposed to extreme flooding compared to typical and an absence of flooding. However, this response was not correlated with the colonization percentages of AMF and DSE in baldcypress roots, which were influenced by flooding.

Effects of flooding in baldcypress seedlings

Flooded conditions affected the performance and resource allocation of seedlings (e.g., SLA), but not

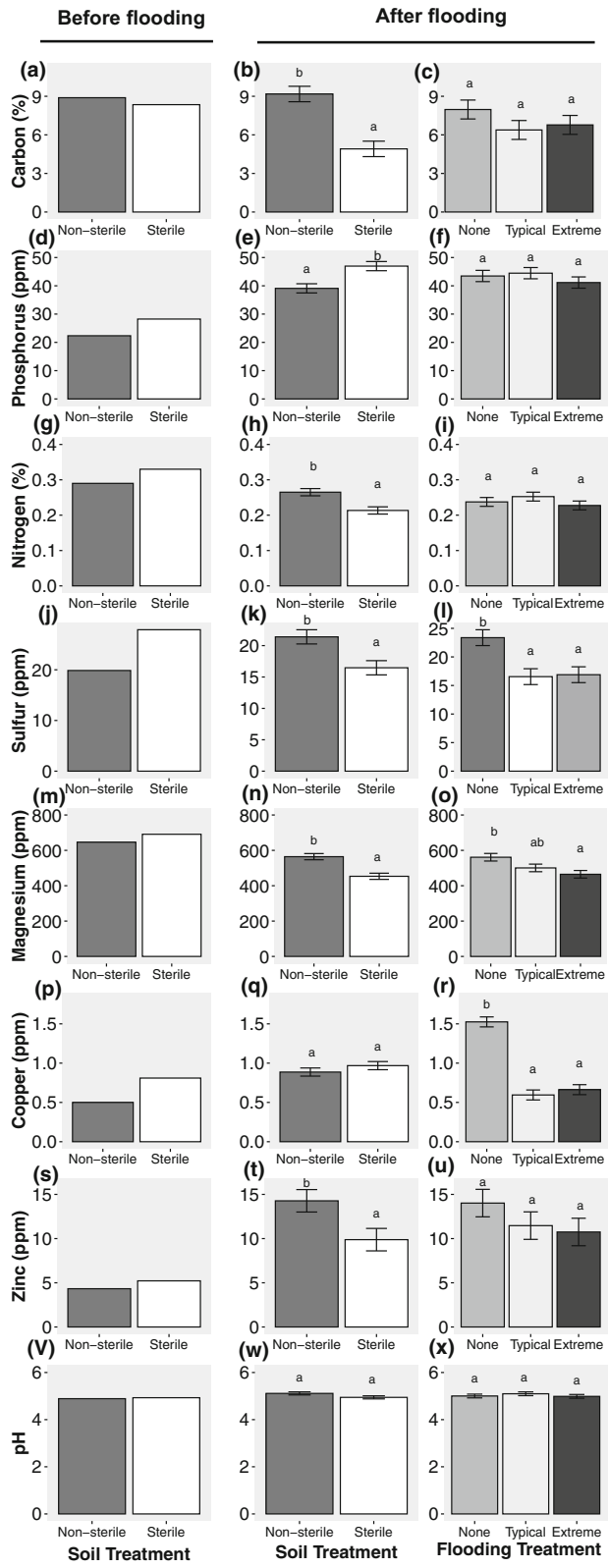

Fig. 6 Soil macro (**a**–**o**) and micronutrient (**p**–**u**) composition of our sterile and non-sterile treatments before and after flooding exposure. Soil pH was also measured in soil samples collected before (**v**) and after (**w**–**x**) exposure to our flooding treatments. Error bars represent ± one standard error. Tukey's honest significant differences between sterile and non-sterile treatments (upper left at each panel) are represented by distinct letters at $\alpha = 0.05$

Table 2 Results of ANOVAs testing for differences in the soil macro and micronutrients concentrations before and after exposure to the flooding treatments

Factor		Macronutrients										Micronutrients				pH	
		Carbon		Phosphorus		Nitrogen		Sulfur		Magnesium		Copper		Zinc			
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Flooding treatment	3	1.68	0.27	0.61	0.57	1.21	0.36	5.81	0.04	4.18	0.07	59.29	0.00	1.25	0.35	0.63	0.56
Soil	1	25.26	< 0.01	9.78	0.02	15.25	0.01	7.14	0.04	16.30	0.01	1.10	0.33	6.14	0.05	3.76	0.10
Flooding × Soil	3	2.01	0.21	0.34	0.72	1.78	0.25	0.02	0.98	0.24	0.79	0.56	0.60	1.10	0.39	1.53	0.29

their primary growth (i.e., height or the vertical extension and reproduction of cells in the primary meristem; Fig. S2, Table S3). Several studies have reported reduced biomass production, less leaf area and also reduced height in seedlings exposed to lasting flooding conditions (e.g., Conner and Day 1976; Allen et al. 1996). However, in other instances, differences in the physiological responses to flooding have been observed among genotypes. Allen (1994) reported marked patterns of leaf loss among 15 genotypes, where more tolerant seedlings tended to retain healthy younger leaves at the top of the stem and remain alive at the top of the plant, while less tolerant genotypes exhibited partial stem dieback and refoliation along the lower portion of the stem. The seedling provenance for this study was across a wide range of baldcypress trees, and as a result we observed substantial differences in the height of individual seedlings as a response to flooding (see S.E. in Fig. 2a). Some seedlings developed younger, healthier leaves at the top of the plant and tended to extend their apical growth even though 90% of their stem was submerged, and others tended to lose their top leaves and exhibited signs of senescence. This variation in the growth response of seedlings to flooding led to statistical similarities with respect to the relative changes in height observed in seedlings exposed to none and typical conditions. On average, seedlings exposed to the extreme flooded treatment had less change in height than those exposed to typical and none flooding conditions, but the observed phenotypic variation in all treatments was substantial.

SLA values were lower under both flooded conditions in the absence of soil microbiota, and under the extreme flooding conditions in the presence of soil microbiota, suggesting that plants were experiencing resource allocation and osmotic stress (Herrera 2013; Kimball et al. 2002). Usually lower values of SLA indicate thicker leaves and an increment in reallocation of biomass to the leaves (i.e., higher leaf mass than leaf area) in response to water deficit (Vile et al. 2005). In flooded environments the expectation is that SLA should be higher because thinner leaves would promote gas exchange and submergence tolerance (Colmer et al. 2011; Mommer et al. 2004, 2005). This behavior will depend on the acclimation stage to flooding (Herrera 2013), and the alternative adaptations to submergence [e.g., production of new leaves under water (Colmer et al. 2011)]. Contrary to the expected SLA under flooded environments, baldcypress seedlings were thus able to re-allocate more CO_2 to the leaves under submergence than under non-flooded conditions. This could have been caused by a decrease in root water absorption and stomatal closure, as a first acclimation response to the new flooded environment experienced by the seedlings (Herrera 2013).

Soil microbiota growth benefits in response to flooding

Soil microbiota had a negative impact on baldcypress seedling growth and biomass production independent of the flooding regimen to which they were exposed. Two different hypotheses could explain this pattern: (i) high presence of pathogens and other antagonistic organisms in comparison with beneficial symbionts in the swamp soil or (ii) symbiotic associations formed in the non-sterile soil were too costly to the plants. Our experimental soil was collected below adult trees in the field. For trees, theory has predicted and empirical data have revealed that the accumulation of species-specific enemies (i.e., pathogens) around adults can limit the recruitment of conspecific seedlings relative to heterospecific seedlings as a negative-frequency selection force that helps maintain plant diversity in forests (Connell 1970; Janzen 1970; Mangan et al. 2010). In tropical tree species, seedling growth was reduced when grown with conspecific soil relative to soil from heterospecifics (e.g., Mangan et al. 2010). Previous studies in baldcypress have observed low seedling recruitment from gravity-mediated dispersed seeds that stay below adult trees (Schneider and Sharitz 1986, 1988). In the absence of floods, baldcypress seeds that do not disperse far from parent trees tend to not germinate and the ones that do are short-lived (Schneider and Sharitz 1986, 1988; Souther and Shaffer 2000). Furthermore, the colonization of oomycetes in baldcypress roots of seedlings grown on this type of soil suggest the presence of pathogens. Although a significant decline in plant height was not correlated with oomycete colonization, these observations suggest that conspecific soil biota collected around adult trees can reduce seedling growth in baldcypress seedlings, providing support for our first hypothesis. However, a further experiment growing baldcypress seedlings with conspecific and heterospecific soil could confirm this hypothesis.

An alternative explanation is that symbiosis was costly for baldcypress seedlings. Nutrient uptake by the plant and carbon soil deposition would be an indication of this cost (Grayston et al. 1997). Carbon percentages were higher under non-sterile than in sterile soil at the end of our experiment, with the opposite trend for phosphorus concentrations, the main limiting nutrient in aquatic systems and a benefit provided via symbiosis with AMF and DSE. These data suggest that, in exchange for phosphorus, plants were diverting a lot of carbon into the soil. However, other important macronutrients such as nitrogen, sulfur, and magnesium were present at higher concentrations in the non-sterile soil than in sterile soil, indicating that other processes (e.g., microfauna or pathogens present in the soil) might be affecting nutrient exchange between the soil and the plant. Therefore, even though our data suggest an interchange of nutrients between root fungi and baldcypress seedlings, we do not have enough support to consider this association either beneficial or costly.

It is uncertain if beneficial soil microbiota enhanced the performance of baldcypress seedlings when exposed to extreme flooding conditions. In spite of the reduced growth and biomass production observed in seedlings reared in non-sterile compared to seedlings reared in sterile soil, we observed similar biomass production across the three different flooding regimes in seedlings grown in non-sterile soil. In contrast, seedlings reared in the absence of soil microbes showed decreased biomass production in extreme flooding compared to typical and non-flooding regimes (See Fig. 3). This pattern suggests that the presence of soil microbiota has a positive growth effect on baldcypress seedlings when exposed to extreme flooding conditions. However, the non-significant association of plant performance with the colonization of beneficial symbionts (but see Bao et al. 2019 for a similar pattern), the reduced SLA under extreme flooding conditions, and the fact that the seedling growth in the non-sterile treatment was equally stunted also suggest this pattern could be the result of a higher stress affecting all plants equally, such as the presence of other antagonistic microbes or microfauna not measured in this study (Kut'áková et al. 2018). It has been hypothesized that some stress combinations can negate the effect of each other and lead to a net neutral or positive impact on plant performance (Pandey et al. 2017). Our experiment conditioned the plants to the soil microbiota before exposing them to the flooding treatments. It is possible that under the presence of biotic stress, abiotic stress due to flooding was thus negated and led to a neutral plant performance under these conditions. Single-inoculations of both DSE and AMF will be needed to test this hypothesis and corroborate the positive effect of mutualist fungi in baldcypress tolerance to flooding.

Influence of flooding in the colonization of beneficial microbes

Total fungal colonization was not influenced by flooding. Instead the colonization percentages of AMF, and DSE varied with flooding. Several studies have previously reported no effect in the colonization success of AMF and DSE across different flooding treatments in wetland plant species (Deepika and Kothamasi 2015; Martin et al. 2018; Stevens et al. 2011). Instead, changes in AMF and DSE diversity have been reported (Deepika and Kothamasi 2015; Stevens et al. 2010; Wang et al. 2011). These studies and our results suggest that flooding does not affect the potential for root fungal colonization but it does modify fungal community composition. Here we observed that AMF colonization in baldcypress roots was higher than DSE colonization under typical and extreme flooding conditions, but we did not observe this difference in the drier conditions (non-flooding treatment). Our findings support previous observations on the sensitivity and prevalence of DSE and AMF under flooded conditions (e.g., Lodge 1989; Martin et al. 2018; Rickerl et al. 1994). Furthermore, we observed that each fungal group showed differences in the structures formed under each flooding treatment, indicating changes in the physiology of fungi when seedlings were exposed to different inundation regimes. Despite underestimating AMF hyphae, a predominance of this structure was observed under drier conditions than in either flooded treatment, suggesting a higher fungal growth rate under non-flooded conditions, while a higher percentage of arbuscules under typical flooding conditions suggests that nutrient exchange between the host plant and the fungi was promoted in the inundated situations that baldcypress commonly experiences (Smith 1995). DSE tend to form dark hyphae and microsclerotia during later stages of colonization or in periods of plant stress (Barrow and Aaltonen 2001; Jumpponen

and Trappe 1998; Mandyam and Jumpponen 2005). Both of these structures were predominantly observed under the two stress-induced scenarios of the flooding gradient, suggesting that stressful rather than typical inundation scenarios in swamps might promote DSE colonization of baldcypress seedlings. This osmotic tolerance of DSE has been reported mostly in arid ecosystems (Li et al. 2019a) and has been attributed to the rigid structure of their cell walls composed of phenolic and indolic monomers (Berthelot et al. 2017; Bloomfield and Alexander 1967). Our study is reporting a similar tolerance of DSE to flooded conditions. Since extreme climate events and sea level rise are projected to bring more permanent flooded conditions to coastal wetlands, our results indicate that fungal root diversity will be disrupted and selected to maintain plant symbiotic associations with specialized AMF and DSE phyla adapted to flooded environments.

Flooding effects on pathogen load

While oomycetes are well-known to prevail in moist and damp conditions (William and Grünwald 2010), baldcypress root colonization by oomycetes declined with flooding and was significantly lower than the colonization by AMF and DSE, particularly under the most extreme flooding scenario. In other wetlands, colonization by the oomycete *Pythium phragmitis* has been higher within young host plants and after extreme flooding events (Wielgoss et al. 2009). It is thus unlikely that flooded conditions in our study prevented oomycete colonization. Instead, microbe-microbe interactions could be a pausible explanation, since these interactions are very context-dependent (Kemen 2014). An increase in antagonistic effects of AMF and DSE towards oomycetes under stressful conditions for the host could have led to a reduced oomycete colonization. In other conifers, DSE have been shown to protect against oomycetes (Tellenbach and Sieber 2012), and we observed a decline of oomycetes associated with an increase in DSE colonization under extreme flooding conditions. Furthermore, AMF have also been observed to protect against other fungal pathogens (Lewandowski et al. 2013). In our study, AMF colonization remained relatively constant across all flooding regimes, with higher colonization than DSE and oomycetes under the most extreme scenario of flooding. This environment-dependent colonization by beneficial fungi and oomycetes should be further studied to fully acknowledge a protective role of AMF and DSE against oomycetes. Our findings further suggest that the lack of significant differences in seedling performance across flooding regimes could be due to these observed context-dependent microbe-microbe interactions within the host plant.

Conclusions

Prolonged flooding presents a stressful environment for baldcypress seedlings, but extensive physiological variation in response to this condition exists among individuals in nature. Unexpectedly, we observed evidence that soil microbiota collected around adult trees can have an overall negative influence on seedling growth, suggesting the potential role of negative-plant feedbacks in regulating seedling recruitment for baldcypress. The extent of plant-fungal associations was not affected by flooding, instead the prevalence of AMF with respect to DSE, and of beneficial fungi over pathogens were altered with flooding. Further studies should investigate whether these environment-dependent interactions are due to a protective role of AMF and DSE towards oomycetes, and/or the role of microbe-microbe interactions in promoting plant resilience. Together our results suggest that it is more important for baldcypress seedlings to be surrounded by a beneficial microbiota than to be dry or flooded, and thus restoration efforts should focus on having a better understanding of plant–microbe interactions in swamps.

Acknowledgements We thank Andrew From, Steve Formel, Peter Tellez, Emma Tower, Demetra Kandalepas and Emma Darr for their technical support conducting the experiment. We thank Camille Stagg and Christina Birnbaum for edits and suggestions in an early version of the manuscript. We are thankful for the comments and suggestions of Jeremiah A. Henning and one anonymous reviewer that helped us improve the final version of this manuscript. This work was partially supported by a Faculty Mentored Undergraduate Research Award from CELT-Tulane University by Tulane's School of Science and Engineering, the provost office of Tulane University, and the U.S. Geological Survey Land Change Science Research and Development Program (RHD, KWK). Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Author contributions LTM and SV conceived and designed the experiment. LTM, TCH, MH, MSJ and LK implemented the experiment. MSJ, LK, RHD and KWK assisted in field sampling. RHD and KWK provided hydrological models and data collection, as well as facilitating soil collection. MSJ collected symbiont colonization data from the roots. LTM analyzed the data and wrote the first draft of the manuscript. All authors contributed to editing the final manuscript.

Data availability Associated data and R codes are deposited in figshare (https://figshare.com/s/ae73837e2c63536502c3).

References

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

Allen JA, Pezeshki SR, Chambers JL (1996) Interaction of flooding and salinity stress on baldcypress (*Taxodium distichum*). Tree Physiol 16:307–313

Anderson PH, Pezeshki SR (1999) The effects of intermittent flooding on seedlings of three forest species. Photosynthetica 37:543–552

Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Barna B, Gullner G, Janeczko A, Kogel K-H, Schäfer P, Schwarczinger I, Zuccaro A, Skoczowski A (2008) Salt tolerance of barley induced by the root endophyte Piriformospora indica is associated with a strong increase in antioxidants. New Phytol 180:501–510

Bao X, Wang Y, Olsson PA (2019) Arbuscular mycorrhiza under water—carbon-phosphorus exchange between rice and arbuscular mycorrhizal fungi under different flooding regimes. Soil Biol Biochem 129:169–177

Barrow J, Aaltonen R (2001) Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. Roots by dark septate fungi and the influence of host physiological activity. Mycorrhiza 11:199–205

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:48

Berthelot C, Perrin Y, Leyval C, Blaudez D (2017) Melanization and ageing are not drawbacks for successful agro-transformation of dark septate endophytes. Fungal Biol 121:652–663

Bloomfield BJ, Alexander M (1967) Melanins and resistance of fungi to lysis. J Bacteriol 93:1276–1280

Brown AM, Bledsoe C (1996) Spatial and temporal dynamics of mycorrhizas in *Jaumea carnosa*, a tidal saltmarsh halophyte. J Ecol 84:703–715

Bueno de Mesquita CP, Sartwell SA, Ordemann EV, Porazinska DL, Farrer EC, King AJ, Spasojevic MJ, Smith JG, Suding KN, Schmidt SK (2018) Patterns of root colonization by arbuscular mycorrhizal fungi and dark septate endophytes across a mostly-unvegetated, high-elevation landscape. Fungal Ecol 36:63–74

Buruyere CL, Coauthors (2017) Impact of climate change on Gulf of Mexico hurricanes. NCAR technical note NCAR/TN-535+STR: 165

Cavagnaro TR, Gao LL, Smith FA, Smith SE (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. New Phytol 151:469–475

Chapin F (1991) Integrated responses of plants to stress. A centralized system of physiological responses. Bio Sci 41:29–36

Colmer TD, Winkel A, Pedersen O (2011) A perspective on underwater photosynthesis in submerged terrestrial wetland plants. AoB PLANTS. https://doi.org/10.1093/aobpla/plr030

Conner WH, Day JW (1976) Productivity and composition of a baldcypress-water tupelo site and a bottomland hardwood site in a Louisiana swamp. American J Bot 63:1354–1364

Compant S, Heijden MGAVD, Sessitsch A (2010) Climate change effects on beneficial plant–microorganism interactions. FEMS Microbiol Ecol 73:197–214

Connell JH (1970) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Den Boer PJ, Gradwell GR (eds) Dynamics of population. Centre for Agricultural Publishing and Documentation, Wageningen

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

Cooke JC, Lefor MW (1998) The mycorrhizal status of selected plant species from Connecticut wetlands and transition zones. Restor Ecol 6:214–222

CPRA (2017) Louisiana's comprehensive master plan for a sustainable coast. Coastal Protection and Restoration Authority, Baton Rouge, LA, p 93

Dangendorf S, Marcos M, Wöppelmann G, Conrad CP, Frederikse T, Riva R (2017) Reassessment of 20th century global mean sea level rise. Proc Natl Acad Sci 114:5946–5951

Davison J, Moora M, Öpik M, Adholeya A, Ainsaar L, Bâ A, Burla S, Diedhiou AG, Hiiesalu I, Jairus T, Johnson NC, Kane A, Koorem K, Kochar M, Ndiaye C, Pärtel M, Reier Ü, Saks Ü, Singh R, Vasar M, Zobel M (2015) Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. Science 349:970–973

Day RH, Doyle TW, Draugelis-Dale RO (2006) Interactive effects of substrate, hydroperiod, and nutrients on seedling growth of *Salix nigra* and *Taxodium distichum*. Environ Exp Bot 55:163–174

De Steven D, Sharitz RR (1997) Differential recovery of a deepwater swamp forest across a gradient of disturbance intensity. Wetlands 17:476–484

Deepika S, Kothamasi D (2015) Soil moisture—a regulator of arbuscular mycorrhizal fungal community assembly and symbiotic phosphorus uptake. Mycorrhiza 25:67–75

Demaree D (1932) Submerging experiments with *Taxodium*. Ecology 13:258–262

Díez-Navajas AM, Greif C, Poutaraud A, Merdinoglu D (2007) Two simplified fluorescent staining techniques to observe infection structures of the oomycete *Plasmopara viticola* in grapevine leaf tissues. Micron 38:680–683

Fardella C, Oses R, Torres-Diaz C, Molina-Montenegro MA (2014) Antarctic fungal endophytes as tool for the reintroduction of native plant species in arid zones. Bosque 35:235–239

Fox J, Weisberg S (2019) An R companion to applied regression. Sage, Thousand Oaks, CA

Gabler CA, Osland MJ, Grace JB, Stagg CL, Day RH, Hartley SB, Enwright NM, From AS, McCoy ML, McLeod JL (2017) Macroclimatic change expected to transform coastal wetland ecosystems this century. Nat Clim Change 7:142

Garnier E, Shipley B, Roumet C, Laurent G (2001) A standardized protocol for the determination of specific leaf area and leaf dry matter content. Funct Ecol 15:688–695

Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Grayston SJ, Vaughan D, Jones D (1997) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl Soil Ecol 5:29–56

Herrera A (2013) Responses to flooding of plant water relations and leaf gas exchange in tropical tolerant trees of a blackwater wetland. Front Plant Sci 4:106–106

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363

Janzen DH (1970) Herbivores and the number of tree species in tropical forests. Am Nat 104:501–528

Jumpponen A (2001) Dark septate endophytes—are they mycorrhizal? Mycorrhiza 11:207–211

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Kandalepas D, Stevens KJ, Shaffer GP, Platt WJ (2010) How abundant are root-colonizing fungi in southeastern Louisiana's degraded marshes? Wetlands 30:189–199

Kemen E (2014) Microbe–microbe interactions determine oomycete and fungal host colonization. Curr Opin Plant Biol 20:75–81

Khan AG (1974) The ocurrence of mycorrizas in halophytes, hydrophytes, and xerophytes and of endogene spores in adjacent soils. J Gen Microbiol 81:7–17

Khan AG (1993) Occurrence and importance of mycorrhizae in aquatic trees of New South Wales, Australia. Mycorrhiza 3:31–38

Kimball BA, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO2 enrichment. In: Sparks DL (ed) Advances in agronomy. Academic Press, Cambridge, pp 293–368

Kirwan ML, Guntenspergen GR, Morris JT (2009) Latitudinal trends in *Spartina alterniflora* productivity and the response of coastal marshes to global change. Glob Change Biol 15:1982–1989

Kotilínek M, Hiiesalu I, Košnar J, Šmilauerová M, Šmilauer P, Altman J, Dvorský M, Kopecký M, Doležal J (2017) Fungal root symbionts of high-altitude vascular plants in the Himalayas. Sci Rep 7:6562

Krauss KW, Duberstein JA, Doyle TW, Conner WH, Day RH, Inabinette LW, Whitbeck JL (2009) Site condition, structure, and growth of baldcypress along tidal/non-tidal salinity gradients. Wetlands 29:505–519

Kut'áková E, Cesarz S, Münzbergová Z, Eisenhauer N (2018) Soil microarthropods alter the outcome of plant-soil feedback experiments. Sci Rep 8:11898–11898

Langston AK, Kaplan DA, Putz FE (2017) A casualty of climate change? Loss of freshwater forest islands on Florida's Gulf Coast. Glob Change Biol 23:5383–5397

Lenoir I, Fontaine J, Lounès-Hadj SA (2016) Arbuscular mycorrhizal fungal responses to abiotic stresses: a review. Phytochemistry 123:4–15

Lenth RV (2016) Least-squares means: the R package lsmeans. 2016 69:33

Lewandowski TJ, Dunfield KE, Antunes PM (2013) Isolate identity determines plant tolerance to pathogen attack in assembled mycorrhizal communities. PLoS ONE 8:e61329–e61329

Li X, He C, He X, Su F, Hou L, Ren Y, Hou Y (2019a) Dark septate endophytes improve the growth of host and non-host plants under drought stress through altered root development. Plant Soil 439:259–272

Li X, He X-L, Zhou Y, Hou Y-T, Zuo Y-L (2019b) Effects of dark septate endophytes on the performance of *Hedysarum scoparium* under water deficit stress. Front Plant Sci 10:903

Liberta AE, Anderson RC, Dickman LA (1983) Vesicular-arbuscular mycorrhiza fragments as a means of endophyte identification at hydrophytic sites. Mycologia 75:169–171

Lodge DJ (1989) The influence of soil moisture and flooding on formation of VA-endo- and ectomycorrhizae in *Populus* and *Salix*. Plant Soil 117:243–253

Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonising dark septate endophytic fungi. Stud Mycol 53:173–189

Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD (2010) Negative plant–soil feedback predicts tree-species relative abundance in a tropical forest. Nature 466:752

Marins JFD, Carrenho R (2017) Arbuscular mycorrhizal fungi and dark septate fungi in plants associated with aquatic environments. Acta Bot Bras 31(295):308

Martin JA, Gillevet PM, Torzilli AP (2018) Changes in mycorrhizal colonization and overall fungal communities across upland and wetland *Acer* forests of the Virginia Coastal Plain. Wetl Ecol Manag 26:547–561

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol 115:495–501

Mehlich A (1953) Determination of P, Ca, Mg, K, Na, NH4. Short Test Methods Used in Soil Testing Division, Department of Agriculture, Raleigh, NC

Mehlich A (1984) Mehlich 3 soil test extractant: a modification of Mehlich 2 extractant. Commun Soil Sci Plant Anal 15:1409–1416

Mendelsohn R, Emanuel K, Chonabayashi S, Bakkensen L (2012) The impact of climate change on global tropical cyclone damage. Nat Clim Change 2:205

Miller SP, Bever JD (1999) Distribution of arbuscular mycorrhizal fungi in stands of the wetland grass *Panicum hemitomon* along a wide hydrologic gradient. Oecologia 119:586–592

Miller SP, Sharitz RR (2000) Manipulation of flooding and arbuscular mycorrhiza formation influences growth and nutrition of two semiaquatic grass species. Funct Ecol 14:738–748

Mommer L, Pedersen O, Visser EJW (2004) Acclimation of a terrestrial plant to submergence facilitates gas exchange under water. Plant, Cell Environ 27:1281–1287

Mommer L, Pons TL, Visser EJW (2005) Photosynthetic consequences of phenotypic plasticity in response to submergence: *Rumex palustris* as a case study. J Exp Bot 57:283–290

Newsham KK (2011) A meta-analysis of plant responses to dark septate root endophytes. New Phytol 190:783–793

NOAA (2018) Tropical cyclone climatology. National Hurricane Center National Oceanic and Atmospheric Administration, Miami, FL

Nobis A, Błaszkowski J, Zubek S (2015) Arbuscular mycorrhizal fungi associations of vascular plants confined to river valleys: towards understanding the river corridor plant distribution. J Plant Res 128:127–137

Pandey P, Irulappan V, Bagavathiannan MV, Senthil-Kumar M (2017) Impact of combined abiotic and biotic stresses on plant growth and avenues for crop improvement by exploiting physio-morphological traits. Front Plant Sci 8:537–537

Pezeshki SR, Delaune RD, Patrick WH (1987) Response of baldcypress (*Taxodium distichum L. var.Distichum*) to increases in flooding salinity in Louisiana's Mississippi River deltaic plain. Wetlands 7:1–10

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55:158-IN118.

Preuett JA, Collins DJ, Luster D, Widmer TL (2013) Screening selected gulf coast and southeastern forest species for susceptibility to *Phytophthora ramorum*. Plant Health Prog 14:17

R Core Team (2019) R: a Language and Environment for Statistical Computing. Austria, Vienna. Available at: https://www.R-project.org/

Rickerl DH, Sancho FO, Ananth S (1994) Vesicular-arbuscular endomycorrhizal colonization of wetland Plants. J Environ Qual 23:913–916

Rodriguez RJ, White JF Jr, Arnold AE, Redman RS (2009) Fungal endophytes: diversity and functional roles. New Phytol 182:314–330

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Kim Y-O, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. ISME J 2:404–416

Santos SGD, Silva PRAD, Garcia AC, Zilli JÉ, Berbara RLL (2017) Dark septate endophyte decreases stress on rice plants Braz. J Microbiol 48(333):341

Schneider RL, Sharitz RR (1986) Seed bank dynamics in a southeastern riverine swamp. Am J Bot 73:1022–1030

Schneider RL, Sharitz RR (1988) Hydrochory and regeneration in a bald cypress water tupelo swamp forest. Ecology 69:1055–1063

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671

Schüβler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution* *Dedicated to Manfred Kluge (Technische Universität Darmstadt) on the occasion of his retirement. Mycol Res 105:1413–1421

Shaffer GP, Wood WB, Hoeppner SS, Perkins TE, Zoller J, Kandalepas D (2009) Degradation of baldcypress-water tupelo swamp to marsh and open water in southeastern Louisiana, USA: an irreversible trajectory? J Coast Res 54:152–165

Smith SE (1995) Discoveries, discussions and directions in mycorrhizal research. In: Varma A, Hock B (eds) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin, pp 3–24

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

Šraj-Kržič N, Pongrac P, Klemenc M, Kladnik A, Regvar M, Gaberščik A (2006) Mycorrhizal colonisation in plants from intermittent aquatic habitats. Aquat Bot 85:331–336

Stevens KJ, Wellner MR, Acevedo MF (2010) Dark septate endophyte and arbuscular mycorrhizal status of vegetation colonizing a bottomland hardwood forest after a 100 year flood. Aquat Bot 92:105–111

Stevens KJ, Wall CB, Janssen JA (2011) Effects of arbuscular mycorrhizal fungi on seedling growth and development of two wetland plants, *Bidens frondosa* L., and *Eclipta prostrata* (L.) L., grown under three levels of water availability. Mycorrhiza 21:279–288

Tellenbach C, Sieber TN (2012) Do colonization by dark septate endophytes and elevated temperature affect pathogenicity of oomycetes? FEMS Microbiol Ecol 82:157–168

Tellenbach C, Grünig CR, Sieber TN (2011) Negative effects on survival and performance of Norway spruce seedlings colonized by dark septate root endophytes are primarily isolate-dependent. Environ Microbiol 13:2508–2517

Vasanthakumari MM, Jadhav SS, Sachin N, Vinod G, Shweta S, Manjunatha BL, Kumara PM, Ravikanth G, Nataraja KN, Shaanker RU (2015) Restoration of camptothecine production in attenuated endophytic fungus on re-inoculation into host plant and treatment with DNA methyltransferase inhibitor. World J Microbiol Biotechnol 31:1629–1639

Vile D, Garnier E, Shipley B, Laurent G, Navas M-L, Roumet C, Lavorel S, Díaz S, Hodgson JG, Lloret F, Midgley GF, Poorter H, Rutherford MC, Wilson PJ, Wright IJ (2005) Specific leaf area and dry matter content estimate thickness in laminar leaves. Ann Bot 96(1129):1136

Walsh KJE, McBride JL, Klotzbach PJ, Balachandran S, Camargo SJ, Holland G, Knutson TR, Kossin JP, Lee TC, Sobel A, Sugi M (2016) Tropical cyclones and climate change. Wiley Interdisc Rev Clim Change 7:65–89

Wang Y, Huang Y, Qiu Q, Xin G, Yang Z, Shi S (2011) Flooding greatly affects the diversity of arbuscular mycorrhizal fungi communities in the roots of wetland plants. PLoS ONE 6:e24512

Weishampel PA, Bedford BL (2006) Wetland dicots and monocots differ in colonization by arbuscular mycorrhizal fungi and dark septate endophytes. Mycorrhiza 16:495–502

Wielgoss A, Nechwatal J, Bogs C, Mendgen K (2009) Host plant development, water level and water parameters shape *Phragmites australis*-associated oomycete communities and determine reed pathogen dynamics in a large lake. FEMS Microbiol Ecol 69:255–265

William F, Grünwald N (2010) Introduction to oomycetes. Plant Health Instructor. https://doi.org/10.1094/PHI-I-2010-1207-01

Yi S, Heki K, Qian A (2017) Acceleration in the global mean sea level rise: 2005–2015. Geophys Res Lett 44:11905–11913

Zahn G, Amend AS (2017) Foliar microbiome transplants confer disease resistance in a critically-endangered plant. Peerj 5:e4020