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Salinity legacy: Foliar microbiome's history affects mutualist-conferred salinity tolerance

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Abstract

The rapid human-driven changes in the environment during the Anthropocene have placed extreme stress on many plants and animals. Beneficial interactions with microorganisms may be crucial for ameliorating these stressors and facilitating the ecosystem services host organisms provide. Foliar endophytes, microorganisms that reside within leaves, are found in essentially all plants and can provide important benefits (e.g., enhanced drought tolerance or resistance to herbivory). However, it remains unclear how important the legacy effects of the abiotic stressors that select on these microbiomes are for affecting the degree of stress amelioration provided to their hosts. To elucidate foliar endophytes' role in host-plant salt tolerance, especially if salinity experienced in the field selects for endophytes that are better suited to improve the salt tolerance of their hosts, we combined field collections of 90 endophyte communities from 30 sites across the coastal Everglades with a manipulative growth experiment assessing endophyte inoculation effects on host-plant performance. Specifically, we grew >350 red mangrove (*Rhizophora mangle*) seedlings in a factorial design that manipulated the salinity environment the seedlings experienced (freshwater vs. saltwater), the introduction of field-collected endophytes (live vs. sterilized inoculum), and the legacy of salinity stress experienced by these introduced endophytes, ranging from no salt stress (0 parts per thousand [ppt] salinity) to high salt stress (40 ppt) environments. We found that inoculation with field-collected endophytes significantly increased mangrove performance across almost all metrics examined (15%–20% increase on average), and these beneficial effects typically occurred when the endophytes were grown in saltwater. Importantly, our study revealed the novel result that endophyte-conferred salinity tolerance depended on microbiome salinity legacy in a key coastal foundation species. Salt-stressed mangroves inoculated with endophyte microbiomes from high-salinity environments performed, on average, as well as plants grown in low-stress freshwater, while endophytes from freshwater environments did not relieve host salinity stress. Given the increasing salinity stress imposed by sea level rise and the importance of foundation species like mangroves for ecosystem services, our results indicate that consideration of endophytic associations and their salinity legacy may be critical for the successful restoration and management of coastal habitats.

KEYWORDS
endophytes, Everglades, legacy effects, mangroves, mutualism, salinity legacy, salt tolerance, sea level rise

INTRODUCTION

The rapid human-driven changes in the environment during the Anthropocene have placed extreme stress on many organisms (Allen et al., 2015; Wake & Vredenburg, 2008), requiring them to adapt or move to avoid extinction (Feeley et al., 2012). Beneficial species interactions provide an avenue for overcoming these environmental stressors and maintaining resilience within communities (Putnam et al., 2017; Waqas et al., 2012). Increasingly, research has shown that microbes that reside within and on organisms can be crucial to proper functioning of a wide range of plants and animals (Harris, 2009; Ngumbi & Kloepper, 2016; Turner et al., 2013) and can play important roles in ameliorating stresses (Iannone et al., 2017; Song et al., 2015; Yu et al., 2019) and allowing persistence in otherwise untenable conditions (Afkhami et al., 2014; David et al., 2019; Malinowski & Beleskey, 2000). Therefore, partnerships with microbes may serve as a well from which organisms can draw for in situ ecological adaptation to the stresses of the changing world (van der Putten et al., 2016). Here, we examine the role of microorganisms in the resilience of plants to human-driven stress by testing whether stress-experienced foliar endophyte microbiomes increase the stress tolerance of a foundation species—*Rhizophora mangle* (red mangrove)—to the increased salinity that accompanies sea level rise in the Everglades.

Salinity stress represents a massive challenge for organisms in the Anthropocene. Many plants in coastal areas are increasingly suffering from excessive salt stress owing to natural and anthropogenic disturbances, such as sea level rise, storm surge, hurricanes, and tidal inundation, that increase soil and water salinity (Saha et al., 2011; Woodruff et al., 2013). Importantly, long-term groundwater salinity records in coastal areas show that seawater intrusion inland is increasing. Sea level is currently rising at a rate of ∼2.6–2.9 mm year^{-1} globally (Watson et al., 2015) and is expected to rise at an accelerating rate, increasing by a cumulative 46 cm during the 2010–2060 period (Obeysekera et al., 2015). As the salinity of soil pore water increases with sea level rise (Subedi et al., 2020; Ross et al., 2021), increased saline stress is expected to lead to decline, endangerment, and, in some cases, even extinction of many taxa (Maschinski et al., 2011; Ross et al., 2016). For instance, an estimated 21 species have been threatened in Everglades National Park owing to increasing salt stress (Saha et al., 2011). The loss or decline of primary producers will be especially detrimental to coastal ecosystem health and function if foundation species that underpin these environmental properties are affected.

Plant–microbial interactions may play a crucial role in mitigating the salinity stress plants experience. Mutualistic microbes have been shown to confer a diversity of benefits on plants, such as tolerance to drought (Afkhami et al., 2014; Oberhofer & Leuchtmann, 2013; Song et al., 2015), heavy metals (Zhang et al., 2010), or thermal stress (Khan et al., 2012), resistance and tolerance to herbivores and pathogens (Iannone et al., 2017; Kuldau & Bacon, 2008; Xia et al., 2016; Yu et al., 2019), and enhanced growth and nutrient/water acquisition (Read, 1999). A growing body of work has documented that some microbes can also reduce salinity stress. For example, some endophytes from coastal plants can increase salt tolerance of their wild hosts and even confer this benefit on agricultural plants when human-inoculated (Rho et al., 2018; Rodriguez et al., 2008; Soares et al., 2016). However, these salinity-mitigating effects are not universal, even within a host species (Gernns et al., 2001; Hardoim et al., 2008; Kogel et al., 2006; Lata et al., 2018; Rho et al., 2018; Rodriguez et al., 2008; Schulz & Boyle, 2005; Yu et al., 2019), and it is unclear what underpins this variability. One possibility is that the legacy of past environmental stress may select for endophyte communities better able to provide benefits. Yet it remains unclear how important the legacy of salinity stress is for microbiomes' abilities to confer salinity tolerance of host plants, especially for foundation species that undergird community structure and productivity.

Understanding what factors determine when microbiome mediation of salinity stress occurs is important for developing a predictive framework for primary producer responses to sea level rise. Of particular interest is the impact of salinity stress and microbial interactions on foundation species that underpin a great deal of ecosystem and community functions. Mangroves are commonly foundation species in coastal ecosystems where salinity stress is especially prevalent and are arguably the most important coastal plants in terms of productivity, structural importance for resident plant and animal communities, and ecosystem goods and services. Mangroves protect shorelines, reduce the devastating impact of natural disasters (tsunamis and hurricanes), provide breeding and nursing grounds for marine species, and serve as sources of food, medicine, fuel, and building materials for humans (Barbier et al., 2008; Vo et al., 2012). However, mangrove forests have been declining at an alarming rate (McFadden et al., 2007; Ross et al., 2001)—even more rapidly than some of the classically considered bellwether ecosystems like inland tropical forests and coral reefs (Duke et al., 2007)—and much of what remains is in degraded condition (Valiela et al., 2001; Wilkie et al., 2003).

Because of the role mangroves play in sustaining coastal ecosystems, their distribution across a wide range of salinity conditions, and the rise of microbiome research, there has been increasing interest in studying mangrove–microbe interactions. Studies have documented a range of endophytic fungi and bacteria associated with mangrove trees (Ananda & Sridhar, 2002; Costa et al., 2012; Debbab et al., 2013; Soldan et al., 2019) and substantial variation in endophyte communities among mangrove species (Ali et al., 2017; de Souza Sebastianes et al., 2013; Jiang et al., 2018; Nicoletti et al., 2018). The majority of these microbe–mangrove studies have focused on the morphology and taxonomy of endophytes (Ho et al., 1990; Sarma & Hyde, 2001; Xing & Guo, 2010; Zhou et al., 2018), while few studies—mainly focused on bacterial endophytes—have provided insight into the fitness consequences of these associations (e.g., Soldan et al., 2019). Of particular interest are the possibilities that beneficial endophytes may help this group of coastal foundation species to tolerate or resist salt stress, allowing them to persist in their saline environments, and that legacy effects may play a role in the degree of microbial mitigation. Because red mangroves (*R. mangle*) are found across a wide salinity gradient, from freshwater inland systems (0 parts per thousand [ppt] salinity) to intertidal environments in which salinity may exceed 40 ppt, it is an ideal species to study the role of endophytes in mangrove salinity tolerance.

To elucidate foliar endophytes' role in mangrove salt tolerance, especially if salinity experienced in the field (salinity legacy) selects for endophytes that are better suited to improve the salt tolerance of their hosts, we combined field collections of 90 endophyte communities from 30 sites across the coastal Everglades with a manipulative growth experiment in which endophyte effects on red mangrove performance were assessed. Specifically, we grew >350 *R. mangle* seedlings in a factorial experiment that manipulated the salinity the seedlings experienced (freshwater vs. saltwater), the introduction of field-collected endophytes (live vs. sterilized inoculum), and the salinity origin of these introduced endophytes (salinity legacies ranging from 0 to 40 ppt). This experimental design allowed us to pose the following questions: (1) Does inoculation with field-collected endophytes affect mangrove growth? (2) Do endophyte inoculation effects depend on the host's salinity environment? (3) Do endophytes from high-salinity environments confer greater salt tolerance on their mangrove hosts?

MATERIALS AND METHODS

Field sample collection

Thirty red mangrove (*R. mangle*) communities were selected along the coast of the Everglades, including areas in Everglades National Park and along Florida Bay in southern Miami-Dade County (Figure 1). These sites spanned much of the southern Everglades and a large water salinity range of 0–40 ppt (Appendix S1: Table S1) and were accessed through a combination of foot, kayak, air boat, and helicopter travel. All sampled sites were continuous red mangrove forests along the coastal fringe or on tree islands in coastal Everglades wetlands, with populations containing morphologically similar trees at least 10 m in height.

During December 2018 and January 2019, we collected five leaves from each of three individual red mangrove trees per site that were at least 10 m apart from one another. For all the sampled trees, outer canopy (i.e., sun-exposed), fully grown leaves with no visible damage were collected from the south-facing, second-order branches in the top third of the trees' crowns to minimize the leaf age variation. Leaves from each tree at each site were stored separately in sterile zip-locked bags and transported on ice to the laboratory within 6 h of collection. Soil pore water salinity was determined for each site during the peak dry season prior to our microbiome collection (between April and May 2018) (Ross et al., 2019) with the goal of quantifying salinity stress experienced by each microbiome when most intense in its area of origin (i.e., quantifying the strongest salinity stress the endophyte microbiome would have recently experienced). To obtain pore water, a soil core (5.5 cm diameter, 30 cm length) was removed from a representative area in the sampling site and allowed to fill with water. Three replicates of pore water samples were

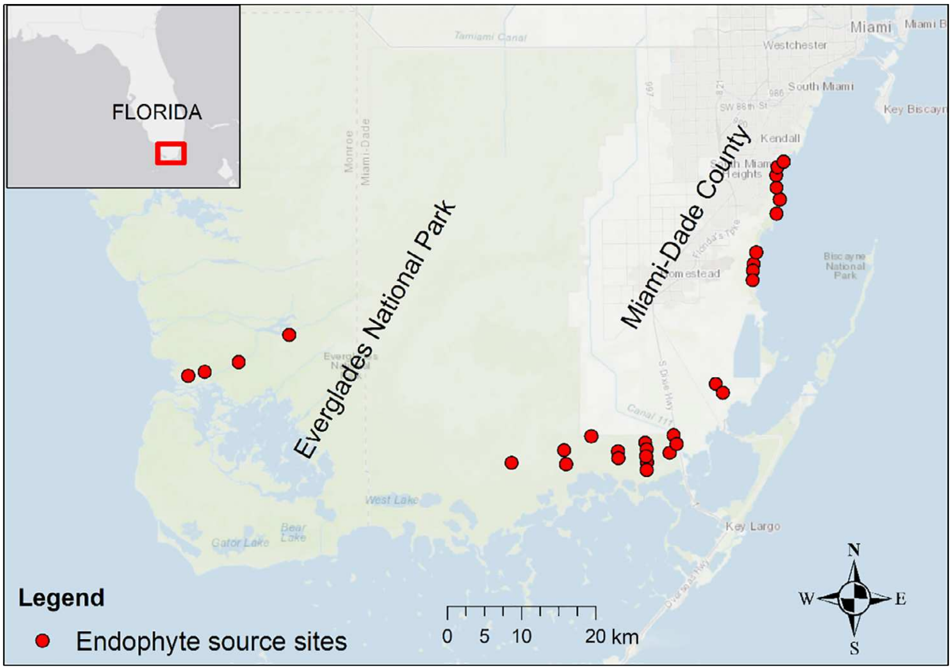

FIGURE 1 Study area showing locations of red mangrove microbiome collections along coastal area of Florida Bay

collected 1 m apart at each site. Soil pore water salinity was measured using a calibrated salinity and conductivity meter (YSI 20, Ohio, USA) in parts per thousand and averaged over the three samples collected per site.

Experimental design, setup, and data collection

To determine how leaf endophytes from across the southern Everglades affected red mangroves performance and whether the environmental salinity legacy of the microbiome affected endophyte–plant interactions, we grew red mangrove seedlings in a factorial experiment that manipulated (1) the introduction of field-collected leaf endophytes ("live" vs. "sterilized" leaf inoculum), (2) the salinity legacy of these introduced endophytes (a measure of salinity at each microbiome's origin, ranging from 0 to 40 ppt), and (3) the salinity of the water in which seedlings were grown (freshwater vs. saltwater). In total, our experiment included 360 pots (2 inoculation treatments × 30 sites that vary in salinity legacy × 2 water salt treatments × 3 replicates).

To generate our endophyte inoculum, we surface sterilized all field-collected leaves with 0.5% sodium hypochlorite (2 min) and 70% ethanol (2 min) and washed with sterile water (Arnold et al., 2000, 2003; Moore et al., 2006). We then homogenized each surface-sterilized leaf individually in 25 ml of sterile water (Zahn & Amend, 2017). Half of the homogenized solution from each leaf was autoclaved (3× at 121°C for 45 min) to make it microbially inactive and to allow us to generate two types of inoculants: (1) a sterile, sham inoculant composed of 100% autoclaved solution (for the endophyte-free inocula treatment [I−]) and (2) a microbially active inoculant composed of 25% live solution (not autoclaved) and 75% autoclaved solution (for the endophyte-present inocula treatment [I+]) (David et al., 2018, 2019). Note that in the I+ treatment, the small amount (25%) of microbially live solution was included to introduce field-collected endophytes, while the 75% autoclaved solution was included to account for any nonmicrobial effects of autoclaving on inocula (David et al., 2018, 2019).

The mangrove propagules used in our experiment were collected from coastal wetlands adjacent to Biscayne Bay (with the salinity of the area ranging from 0 to 32 ppt), which was not one of the 30 sites used for endophyte inoculant source. Those propagules were then planted in pots of organic commercial soil (Nature's Care Organic Garden Soil, Miracle-Gro Lawn Products Inc., Marysville, OH, USA) and were assigned to random locations to grow within a shade house common garden for ~1 year on the Biscayne Bay campus of Florida International University in a freshwater environment. The common garden approach helped to ensure all seedlings began the experimental phase of the research in a similar physical condition and with a similar background microbiome since they experienced the same potential microbial sources and growth conditions for a year before they were used for the experiment. Then 400 approximately year-old seedlings were transplanted into ~1.1-L pots (Growers Solution, Cookeville, TN) containing the same organic soil (Nature's Care Organic Garden Soil). The seedlings were then grown in freshwater (~3 cm deep) for around a month in a growth room (12 h:12 h light: dark cycle) before applying treatments (Appendix S1: Figure S1). The initial height and number of leaves were recorded for all plants prior to treatment exposure. Seedlings were randomly assigned to salinity and endophyte treatments, and both initial height (25–35 cm) and number of leaves (8–12 leaves) did not differ significantly among treatments (Appendix S1: Figure S2). We then manipulated the endophytic community by inoculating seedlings with endophytes collected from one of the 30 field sites that varied in salinity, from fresh (no salt) to high salt (40 ppt). To do this, we gently abraded all seedling leaves with sterilized (three times at 121°C) quartz sand (Red Devil 580 Silica Sand, Meeco Mfg Co. Inc., Seattle, WA, USA) and treated with either I+ or I− inoculum applied with sterile cotton gauze to all leaves on the adaxial and abaxial leaf surfaces (the number of leaves ranged from 8 to 12) (Oh et al., 2021; Xu et al., 1997). In total, there were 90 different endophyte communities applied—3 endophyte communities from each of the 30 sites (i.e., 1 community came from each of the 3 trees sampled). In a previous study (Lin & Sternberg, 1992), a salinity level greater than 15 ppt was shown to have significant negative effects on photosynthesis in *R. mangle* seedlings. Thus, 90 I+ and 90 I− treated seedlings (1 per microbial community) were grown in stressful saltwater conditions (maintained between 15 and 20 ppt), and the other 90 I+ and 90 I− seedlings were grown in freshwater conditions (maintained at <2 ppt). In the I+ treatment, salinity was increased to 15 ppt over the course of a week, the salinity level was checked twice per week using a salinity and conductivity meter (YSI 20, Ohio, USA), and the saltwater treatment was adjusted by adding freshwater or sea salt (nitrate- and phosphate-free Instant Ocean sea salt for marine aquariums, Instant Ocean, Cincinnati, OH, USA) as needed (Lin & Sternberg, 1992, 1993). The saltwater treatment induced plant stress compared to the freshwater treatment. Both the overall mangrove performance and many individual metrics showed declines in saltwater, although these effects often depended on whether the plants had been inoculated with field-collected endophytes and the endophytes with which they had been inoculated (discussed in more detail in the "Results" section).

To evaluate legacy-specific endophyte effects and their interactions with water salinity, we measured seedling height, stem diameter, number of nodes, branches, and leaves for all plants in our experiments 7 months following treatment application, which was sufficient time to see significant responses in these performance metrics at a crucial life history stage for this species. Measured traits were selected based on previous work showing them to be important in field studies of these mangroves. For instance, Ross et al. (2001) showed that leaf production, node growth, and branch number were good predictors of productivity in south Florida populations of red mangrove. Changes in the endophyte communities were not examined in present study.

Data analysis

Because we measured multiple performance traits, we first analyzed the effect of our treatments on the composite plant performance using a multivariate analysis of covariance (MANCOVA), with the saltwater treatment, endophyte inoculation treatment, salinity of endophyte source, and their interactions as explanatory variables and the five plant performance measures (stem diameter, node number, branch number, height, and leaf number) as response variables. After significant overall effects were identified in the MANCOVA ($\propto = 0.05$), we examined effects on individual response variables using analysis of covariance (ANCOVA) using the same explanatory variables. All the statistical analyses were conducted in R version 3.5.3 (R Core Team, 2019).

RESULTS

Endophyte inoculation effects on mangrove performance under salt stress

Our analysis of the overall mangrove performance (i.e., MANCOVA of all five performance response variables measured) showed that the main effect of endophyte inoculation on plant performance was significant ($F_{5,305} = 6.14$, $p < 0.01$) (Table 1) such that plants inoculated with field-collected endophytes (I+) performed better on average than noninoculated plants (I−). In follow-up analyses, we found beneficial effects of endophyte inoculation across all individual plant performance metrics examined (with the exception of height; Appendix S1: Table S2). I+ plants had significantly greater seedling stem diameters and numbers of nodes, branches, and leaves (Appendix S1: Table S2; Figure 2a–d). For instance, inoculated mangroves had 14.6% more leaves ($p < 0.01$; Figure 2a) and 15.8% more nodes ($p < 0.01$; Figure 2b) than noninoculated

TABLE 1 Effects of endophyte, salinity, and salinity legacy on overall mangrove performance

Explanatory variables	F	Pr(>F)
Salt water treatment	1.16	0.33
Endophyte treatment	6.14	<0.01
Salinity legacy (of endophyte source)	3.15	0.01
Salt water × endophyte	1.85	0.10
Salt water × salinity legacy	0.48	0.79
Endophyte × salinity legacy	0.90	0.48
Salt water × endophyte × salinity legacy	2.94	0.01

Note: Multivariate analysis of covariance (MANCOVA) was used to determine the effect of our treatments and their interactions on the composite plant performance. Saltwater treatment, endophyte inoculation treatment, salinity of endophyte source (salinity legacy), and their interactions were the explanatory variables, and the five plant performance measures (stem diameter, node number, branch number, height, and leaf number) were the response variables. The numerator and denominator degrees of freedom for each of the explanatory variables were 5 and 305, respectively.

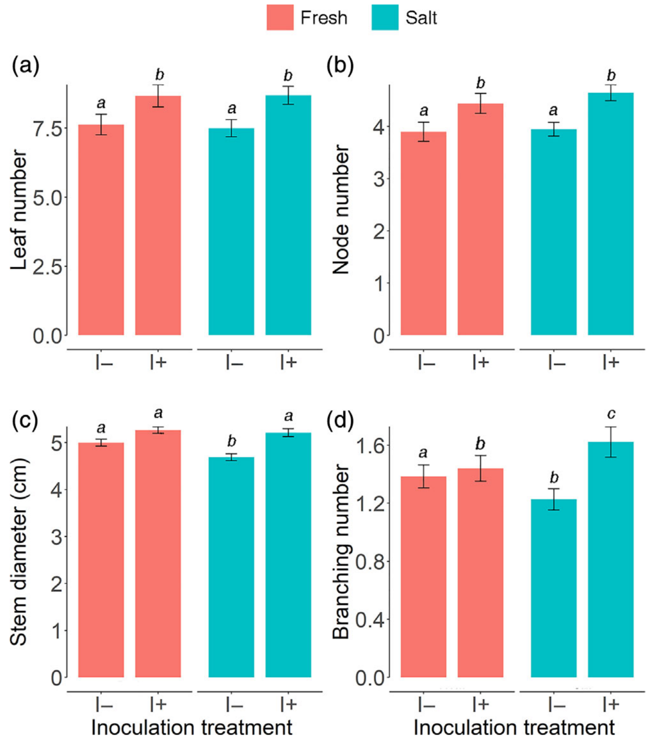

FIGURE 2 Endophyte inoculation increased mangrove performance and conferred resistance to salinity stress. The endophyte inoculation treatment (I+ vs. I−) was found to significantly increase the number of (a) leaves and (b) nodes. Endophyte inoculation and seedling growth conditions (freshwater vs. saltwater) significantly interacted to affect (c) stem diameter and (d) number of branches, such that positive endophyte effects on mangroves were only observed in saltwater conditions. Different lowercase letters are assigned for significantly different treatment effects in post hoc tests ($p < 0.05$)

mangroves. Interestingly, for some components of mangrove performance, the effect of endophyte inoculation on the metric depended on whether plants were growing in freshwater versus saltwater (Figure 2c,d). For instance, for both seedling stem diameter (Figure 2c) and number of branches (Figure 2d), noninoculated plants showed declines in plant performance when grown in saltwater versus freshwater, indicating that saltwater was stressful, but endophyte inoculation significantly increased mangrove performance in the high-salinity treatment. Under high-salinity conditions, I+ plants displayed a 19.1% increase in the stem diameter and a 17.1% increase in the number of branches compared to I− plants (Figure 2c,d). Similarly, I+ plants were 12.08% taller than I− plants in saltwater ($F_{1,311} = 3.64$, $p = 0.05$; Appendix S1: Table S2). In the case of stem diameter, inoculation with endophytes restored the performance of salt-treated plants to that of plants grown in freshwater, while in the case of branching, I+ plants in high-salinity environments actually had greater branching than plants grown in freshwater.

Importance of salinity legacy in endophyte effects on mangroves

Our analysis of the overall mangrove performance (i.e., MANCOVA of all five performance metrics) also showed that the salinity legacy of endophytes had a significant effect on mangrove performance that depended on the salinity environment the host plant was currently experiencing (significant three-way interactive effect of the water salinity treatment, the endophyte inoculation treatment, and the salinity of the endophyte source on composite performance; $F_{5,305} = 2.94$, $p = 0.01$; Table 1; Figure 3). I− seedlings growing in saltwater had reduced performance compared to I− seedings growing in freshwater (Figure 3, comparing panels b and d), indicating salinity stress for noninoculated mangroves. When mangroves were grown in the freshwater environment, their composite performance was similar for the I− (noninoculated) and the I+ treatment plants, regardless of the salinity legacy of the endophytes (Figure 3, panel c vs. d). Interestingly, when mangroves were grown in saltwater conditions, the salinity legacy of the endophytes became important for mangrove performance. In saltwater, the composite performance of I+ and I− mangroves was similar when the inoculum was derived from freshwater sites (endophytes with freshwater legacy), but crucially I + plants performed much better than I− plants when grown in saltwater if the endophytes originated from a high-salinity environment (endophytes with salinity

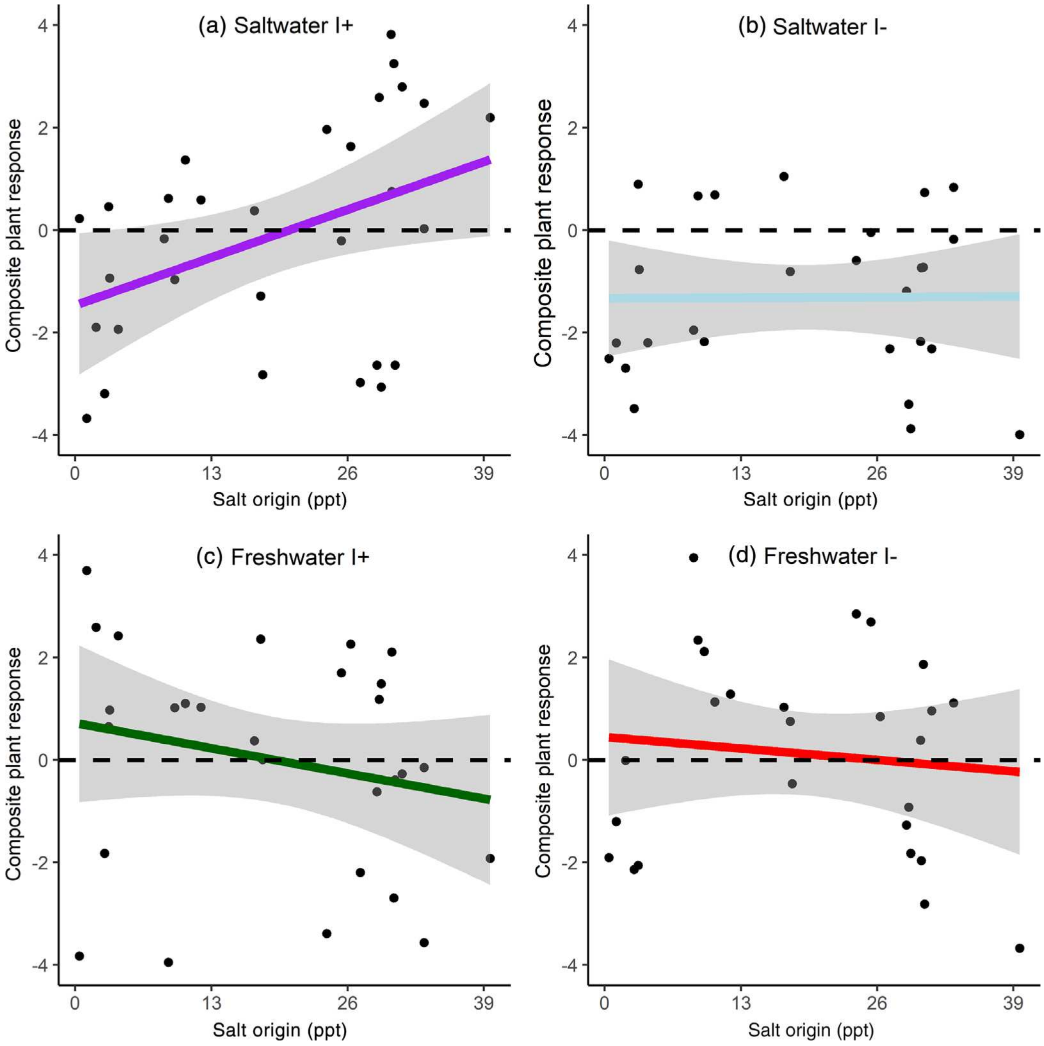

FIGURE 3 Composite plant performance response along endophyte salinity legacy gradient showed an interaction between saltwater treatment, endophyte treatment, and endophyte salinity legacy. Endophyte inoculation was most beneficial for plants grown in saltwater (panel a) and inoculated with endophytes collected from sites with high salinity (i.e., endophytes with a salinity legacy). To visualize the multivariate analysis of covariance (MANCOVA) results, the composite performance index (graphed here) was calculated by equally weighting each of the five normalized performance metrics (mean centered with SD = 1), and lines were fit with a linear regression of the composite plant performance on the endophyte source salinity gradient for each treatment combination. Data shown are from the following treatment combinations: (a) endophyte inoculation (I+) and saltwater treatment, (b) no endophyte inoculation (I−) and saltwater treatment, (c) endophyte inoculation (I+) and freshwater treatment, and (d) no endophyte inoculation (I−) and freshwater treatment. Dashed line: mean composite fitness across all treatments

legacy) (Figure 3, panel a vs. b). In fact, inoculation with endophytes originating from the highest salinity habitats enhanced the composite performance of mangroves grown in saltwater to the point where they were comparable to (or even exceeded) that of mangroves grown in freshwater (Figure 3, panel a vs. panels c and d). In follow-up analyses, a significant three-way interaction between the salinity legacy of the symbiont, current host salinity environment, and symbiont inoculation was also detected for node number ($F_{1,311} = 5.01$; $p = 0.03$; Appendix S1: Figure S3) with a similar pattern in leaf number ($F_{1,311} = 2.84$, $p = 0.09$).

DISCUSSION

Our results showed how inoculation with foliar endophytes could meaningfully reduce salinity stress in a key foundation species of the Everglades. In particular, this study revealed that the ability of the endophytic microbiome to confer salinity tolerance on host plants depends on the salinity legacy of these endophytes in a coastal foundation species. This result not only elucidates a factor underpinning when microbes can mitigate a growing environmental stress, it emphasizes the need for further exploration into how microbiomes can mitigate plant response to sea level rise. In what follows, we discuss salinity stress and endophytic mitigation of this challenge, the role of salinity legacy in endophyte-driven effects on stress relief, and fruitful directions for future investigation sparked by our findings.

Endophyte inoculation improves foundation species performance under salt stress

High salinity poses major challenges to many plants in coastal environments (DeLaune et al., 1987; Ross et al., 2009) and represents a growing problem with sea level rise, causing submergence and increased flooding of coastal lands as well as saltwater intrusion of surface water and groundwater (Nicholls & Cazenave, 2010). A relatively small proportion of plant species can prosper in brackish/saline environments (Himabindu et al., 2016), but some plant species—halophytes—have adapted to these conditions using a wide range of physiological, metabolic, and ecological mechanisms (Himabindu et al., 2016; Volkmar et al., 1998). In our study system of red mangroves (*R. mangle*), previous research showed that a key mechanism of salinity resistance is the regulation of salt's entry into plants using ultrafiltration across the root cortex and that a key mechanism of tolerance is sequestration of salt ions in the hypodermal water storage tissue of leaves (Tomlinson, 2016; Werner & Stelzer, 1990). However, in high-salt environments, even halophytes may suffer from osmotic stress initially (reflecting a water deficit) and subsequently experience ion-specific stress resulting from altered ion concentrations (Blumwald et al., 2000; Shabala & Mackay, 2011). Exceeding critical cytoplasmic Na^+ and Cl^+ concentrations can cause damage in halophytes, resulting in a reduction of the net photosynthesis rate through stomatal closure (Himabindu et al., 2016). Mangrove leaves, for example, accumulate salt as they mature (Cram et al., 2002) to levels up to 12 times higher compared to freshwater plants (Dissanayake & Amarasena, 2009). Furthermore, experiments have shown that the salt content in the leaves of red mangroves growing in high-salt environments is significantly higher than the leaf salt content of plants in low-salt environments (Lin & Sternberg, 1992). Like many halophytes, high salinity has negative consequences for metabolic processes in mangroves (Ball, 1988), and mangroves often grow more slowly and have lower productivity in high-salinity environments (Cintron et al., 1978), indicating that plant mechanisms for tolerating (sequestration) and resisting (filtration) salinity stress may be costly to maintain or only partially effective at reducing salinity stress.

Another strategy for plants to improve tolerance and resistance to salinity stress is through interactions with beneficial microbiomes (Li, La, et al., 2021; Mateu et al., 2020; Qin et al., 2018). Microbial communities are composed of diverse bacteria and fungi with the needed machinery for a wide range of useful metabolic, molecular, and physiological functions, which can improve plant growth and health by alleviating harmful stress effects (Ahmed et al., 2021; Li et al., 2016; Sampangi-Ramaiah et al., 2020; Shahzad et al., 2017; Sun et al., 2020). Specifically, our experiments showed that a crucial foundation species of the Everglades ecosystem—red mangrove—benefits substantially from inoculation with field-collected endophytes, especially endophytes that experience salinity, in high-salinity conditions. Endophyte inoculation significantly increased mangrove seedling leaf number, node number, number of branches, and stem diameter, metrics that have been shown to be good estimators of the aboveground biomass and productivity of red mangroves in the field (Amarasinghe & Balasubrananiam, 1992; Fromard et al., 1998; Putz & Chan, 1986; Ross et al., 2001). These findings show that, although mangroves are known to tolerate a wide range of salinities, seedling performance—which can be restricted by both stomatal and nonstomatal (i.e., biochemical) limitations in high-salt environments—can be strongly affected by microbial interactions. Importantly, early seedling development is likely to be the most sensitive life stage for this and other halophytes (Krauss et al., 2008), and mangrove seedling growth plays an important role in determining red mangroves' establishment and competitive capabilities (e.g., canopy height and canopy area) in later years (e.g., Proffitt & Travis, 2010). Thus, our findings demonstrate that salinity-experienced endophyte effects on a critical life history stage in red mangroves—that is, the seedling stage—are likely important for population dynamics and functional roles of this foundational species in extreme salinity environments.

The fitness improvement due to endophyte inoculation could have resulted from several nonmutually exclusive physiological and metabolic mechanisms (Brader et al., 2014; Santoyo et al., 2016). First, the salinity-experienced microbiome could have benefited the mangroves by regulating oxidative stress. Owing to excessive accumulation of salt ions in the cytosol, plants produce reactive oxygen species that cause oxidative stress (Latef & Chaoxing, 2014; Rasool et al., 2013), thereby reducing their physiological and biochemical activities (Ahmad et al. 2014). When mangroves experience salinity stress, the endophytes may reduce oxidative stress on their host trees by enhancing detoxifying enzyme activity (Li et al., 2016) or through the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and osmotic regulators (Egamberdieva et al., 2017;

Jogawat et al., 2013). Second, the microbiome may have benefited the mangroves through other types of metabolic regulation. Abiotic stress, including high salinity, can increase hydrogen peroxide production in plants; while at low concentrations hydrogen peroxide typically acts as a signaling molecule, at higher concentrations it can provoke the onset of cell death (Gechev & Hille, 2005). When plants are under abiotic stress, proline is essential for plant recovery (Szabados & Savoure, 2010), and a relatively high accumulation of proline has been reported in salt-tolerant species (e.g., Chaum & Kirdmanee, 2009; Hameed & Ashraf, 2008; Hayat et al., 2011). Studies in other systems show marked decreases in hydrogen peroxide and increases in proline concentrations for endophyte-inoculated plants experiencing extreme salt stress (Ahmad et al., 2015; Egamberdieva et al., 2017), suggesting likely mechanisms for how endophytes, like those from high-salinity environments in our experiment, improved salinity tolerance in plants.

Salt-stressed legacy effects of endophytes on plant fitness

Our study demonstrated that the salinity legacy of the endophytic microbiome is important for whether the endophytes improved foundation species performance under high-salt stress. Specifically, we observed that endophytes with a legacy of experiencing salt stress (i.e., collected from mangroves in higher salinity sites in the Everglades) significantly increased mangrove performance in high-salinity experimental environments, while endophytes with a freshwater legacy did not. Interestingly, the benefits to mangroves of interacting with “salinity legacy endophytes” declined in the freshwater experimental environment, illustrating that the match between endophyte legacy and the salinity environment host plants experience is a crucial part of endophyte-mediated stress amelioration in this foundation species. Several studies of native and invasive grasses have made important strides toward understanding habitat-adapted microbiomes in high-salinity environments, finding evidence that endophytes cultured from coastal sites can confer salt tolerance on rice, other crops, and beach grass (e.g., Rodriguez et al., 2008; Soares et al., 2016). Our work builds on and complements this progress, making several novel contributions to understanding the importance of salinity legacy effects on plant–microbiome interactions. For instance, we show for the first time that salinity legacy effects on microbiomes are important for a native foundation species that not only naturally dominates native habitats but also is fundamental to structuring these communities and maintaining ecosystem functions. In addition, our study considered the salinity legacy of the microbiome across a continuous gradient of the whole range of saline environments the host organism experiences (0–40 ppt salinity), in contrast to previous work (Soares et al., 2016) that collected endophytes from extreme habitats (and often without explicitly considering replication of high- and low-salinity sites, i.e., no replication of salinity legacy). By incorporating replicate microbiomes from 30 sites and characterizing the salinity variation at these sites, our study provides robust insight into how the relationship between the salinity legacy of the microbiome and its ability to confer benefits changes across the entire salinity gradient.

Several nonmutually exclusive explanations could underpin the role of salinity legacy in microbial conferral of resilience to salinity stress. One likely explanation is that freshwater legacy microbiomes lack key endophytes (i.e., microbes of large effect) (Afkhami et al., 2020) or interacting groups of endophytes (i.e., network modules of microbes) that underpin the benefits being conferred on the mangroves. Previous work has shown that environmental stress can cause substantial changes in microbial communities (Zimmerman & Vitousek, 2012), filtering out otherwise competitively superior microbes that are unable to persist in those conditions due to niche limitations. Future research into the community composition, diversity, and network structure of the microbiome, using approaches similar to those of Adair and Douglas (2017), Hernandez et al. (2021), and Wagg et al. (2019), across a salinity gradient would be a valuable next step for linking this ecological mechanism to the legacy effects our experiment revealed. Second, local adaptation within the microbiome to high-salinity stress environments could be crucial for maintaining benefits provided to host plants. Compared to freshwater legacy endophytes, endophytes from high-salinity environments may be better equipped to fully function under salinity stress, including the metabolic and physiological functions that directly or indirectly (i.e., mediated through intermicrobial interactions) affect plant performance in high-salinity environments. Previous researchers highlighted many examples of species interactions where partners experiencing extreme stress were unable to provide resources or services, including reduced nutrient trade (Vitousek & Walker, 1989), seed dispersal (Terborgh et al., 2008), pollination (Cox & Elmqvist, 2000), and defensive mutualisms (Palmer et al., 2008), illustrating the importance of a mutualist’s own stress tolerance in its ability to confer rewards. Moreover, recent studies have shown increased beneficial microbiome functions and functional groups in extreme stress environments (e.g., Hernandez et al., 2021; Regina et al., 2002; Rolli et al., 2015), and in one case that individual endophytes cultured from a coastal environment are themselves more salt tolerant (Soares et al., 2016). In addition to future research directly investigating metabolic changes in the microbiome under salinity stress, metatranscriptomic studies (e.g., Li, Long, et al., 2021; Hou et al., 2018) aimed at identifying changes in the expression of genes involved in microbial community (and plant) stress tolerance could prove valuable for assessing the importance of microbial local adaptation to stress in the salinity legacy effects on plant performance.

CONCLUSION

Salinity stress in the Anthropocene is becoming an issue of increasing importance that has enormous implications for primary producers and the communities they support (Ogurcak et al., 2019; Taillie et al., 2019). Our results support the conclusion that interacting with stress-experienced microbiomes is an important pathway for in situ adaptation to salinity stress for coastal plants faced with sea level rise and storm surges. In particular, our study illustrates the importance of microbiome salinity legacy, emphasizing that the overall performance of a key coastal foundation species in high-stress environments depends not only on having a microbiome but also on the microbiome's having experience with a given stressor. By identifying a factor (i.e., microbiome salinity legacy) that can be easily characterized using simple tools and that also explains when microbes can benefit a foundation species, this work contributes to the formation of a predictive framework for understanding when to expect the microbiome mitigation of salinity stress. Based on this insight, we also suggest that future work that examines microbiome composition, niche limitation, and functional responses in nature is needed, as are experimental manipulations to further distinguish the underlying mechanisms for the legacy effects described herein. By combining the critical knowledge obtained from our study—that microbiome salinity legacy effects matter at a crucial life history stage of a foundation species—with studies that elucidate the ecological mechanism for these effects, insight will be gained into how foliar microbiomes affect plant response to increasing stress, perhaps suggesting key roles for microbiomes in the management of coastal ecosystems. For instance, identifying which components of salinity legacy microbiomes are involved in conferring benefits on plants as well as their functional roles and how they are transmitted can lead to the development of careful and effective strategies for improving plant restoration with microbial inoculation. Overall, we hope that this study will inspire more research into microbial mitigation of salinity stress and, because of the important role mangroves play in protection from hurricanes, resistance of coastal erosion, and economically and ecologically important fisheries, will represent a step toward practical consideration of microbiomes in the management and restoration of coastal plant communities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Subedi et al., 2022) are available in Dryad: https://doi.org/10.5061/dryad.xsj3tx9gm.

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REFERENCES

Adair, K. L., and A. E. Douglas. 2017. "Making a Microbiome: The Many Determinants of Host-Associated Microbial Community Composition." *Current Opinion in Microbiology* 35: 23–9.

Afkhami, M. E., B. K. Almeida, D. J. Hernandez, K. N. Kiesewetter, and D. P. Revillini. 2020. "Tripartite Mutualisms as Models for Understanding Plant–Microbial Interactions." *Current Opinion in Plant Biology* 56: 28–36.

Afkhami, M. E., P. J. McIntyre, and Y. Strauss. 2014. "Mutualist-Mediated Effects on Species' Range Limits across Large Geographic Scales." *Ecology Letters* 17: 1265–73.

Ahmad, P., A. Hashem, E. F. Abd-Allah, A. A. Alqarawi, R. John, D. Egamberdieva, and S. Gucel. 2015. "Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system." *Frontiers in Plant Science* 6: 868.

Ahmed, S., T. Y. Heo, A. R. Choudhury, D. I. Walitang, J. Choi, and T. Sa. 2021. "Accumulation of Compatible Solutes in Rice (*Oryza sativa* L.) Cultivars by Inoculation of Endophytic Plant Growth Promoting Bacteria to Alleviate Salt Stress." *Applied Biological Chemistry* 64: 1–14.

Ali, A., R. Shahzad, A. L. Khan, B. A. Halo, R. Al-Yahyai, A. Al-Harrasi, A. Al-Rawahi, and I. J. Lee. 2017. "Endophytic Bacterial Diversity of *Avicennia marina* Helps to Confer Resistance against Salinity Stress in *Solanum lycopersicum*." *Journal of Plant Interactions* 12: 312–22.

Allen, C. D., D. D. Breshears, and N. G. McDowell. 2015. "On Underestimation of Global Vulnerability to Tree Mortality and Forest Die-Off from Hotter Drought in the Anthropocene." *Ecosphere* 6: 1–55.

Amarasinghe, M. D., and S. Balasubrananiam. 1992. "Net Primary Productivity of Two Mangrove Forests Stands on the Northwestern Coast of Sri Lanka." *Hydrobiologia* 247: 37–47.

Ananda, K., and K. R. Sridhar. 2002. "Diversity of Endophytic Fungi in the Roots of Mangrove Species on the West Coast of India." *Canadian Journal of Microbiology* 48: 871–8.

Arnold, A. E., Z. Maynard, G. S. Gilbert, P. D. Coley, and T. A. Kursar. 2000. "Are Tropical Fungal Endophytes Hyperdiverse?" *Ecology Letters* 3: 267–74.

Arnold, A. E., L. C. Mejía, D. Kyllo, E. I. Rojas, Z. Maynard, N. Robbins, and E. A. Herre. 2003. "Fungal Endophytes Limit Pathogen Damage in a Tropical Tree." *Proceedings of the National Academy of Sciences* 100: 15649–54.

Ball, M. C. 1988. "Salinity Tolerance in the Mangroves *Aegiceras corniculatum* and *Avicennia marina* I. Water Use in Relation to Growth, Carbon Partitioning, and Salt Balance." *Australian Journal of Plant Physiology* 15: 447–64.

Barbier, E. B., E. W. Koch, B. R. Silliman, S. D. Hacker, E. Wolanski, J. Primavera, E. F. Granek, et al. 2008. "Coastal Ecosystem-Based Management with Nonlinear Ecological Functions and Values." *Science* 319: 321–3.

Blumwald, E., G. S. Aharon, and M. P. Apse. 2000. "Sodium Transport in Plant Cells." *Biochimica et Biophysica Acta (BBA) - Biomembranes* 1465: 140–51.

Brader, G., S. Compant, B. Mitter, F. Trognitz, and A. Sessitsch. 2014. "Metabolic Potential of Endophytic Bacteria." *Current Opinion in Biotechnology* 27: 30–7.

Chaum, S., and C. Kirdmanee. 2009. "Effect of Salt Stress on Proline Accumulation, Photosynthetic Ability and Growth Characters in Two Maize Cultivars." *Pakistan Journal of Botany* 41: 87–98.

Cintron, G., A. E. Lugo, D. J. Pool, and G. Morris. 1978. "Mangroves of Arid Environments in Puerto Rico and Adjacent Islands." *Biotropica* 10: 110–21.

Costa, I. P., L. C. Maia, and M. A. Cavalcanti. 2012. "Diversity of Leaf Endophytic Fungi in Mangrove Plants of Northeast Brazil." *Brazilian Journal of Microbiology* 43: 1165–73.

Cox, P. A., and T. Elmqvist. 2000. "Pollinator Extinction in the Pacific Islands." *Conservation Biology* 14: 1237–9.

Cram, J. W., P. G. Torr, and D. A. Rose. 2002. "Salt Allocation during Leaf Development and Leaf Fall in Mangroves." *Trees* 16: 112–9.

David, A. S., P. Quintana-Ascencio, E. Menges, K. Thapa-Magar, M. E. Afkhami, and C. A. S. Searcy. 2019. "Soil Microbiomes Underlie Population Persistence of an Endangered Plant Species." *The American Naturalist* 194: 488–94.

David, A. S., K. B. Thapa Magar, and M. E. Afkhami. 2018. "Microbial Mitigation-Exacerbation Continuum: A Novel Framework for Microbiome Effects on Hosts in the Face of Stress." *Ecology* 99: 517–23.

Debbab, A., A. H. Aly, and P. Proksch. 2013. "Mangrove Derived Fungal Endophytes–A Chemical and Biological Perception." *Fungal Diversity* 61: 1–27.

DeLaune, R. D., S. R. Pezeshki, and W. H. Patrick, Jr. 1987. "Response of Coastal Plants to Increase in Submergence and Salinity." *Journal of Coastal Research* 3: 535–46.

Dissanayake, N. P., and K. M. C. Amarasena. 2009. "Assessment and Comparison of Salt Content in Mangrove Plants in Sri Lanka." *Ruhuna Journal of Science* 4: 21–7.

Duke, N. C., J. O. Meynecke, S. Dittmann, A. M. Ellison, K. Anger, U. Berger, S. Cannicci, et al. 2007. "A World Without Mangroves?" *Science* 317: 41–2.

Egamberdieva, D., S. J. Wirth, V. V. Shurigin, A. Hashem, and E. F. Abd-Allah. 2017. "Endophytic Bacteria Improve Plant Growth, Symbiotic Performance of Chickpea (*Cicer arietinum* L.) and Induce Suppression of Root Rot Caused by *Fusarium solani* under Salt Stress." *Frontiers in Microbiology* 8: 887.

Feeley, K. J., E. M. Rehm, and B. Machovina. 2012. "Perspective: The Responses of Tropical Forest Species to Global Climate Change: Acclimate, Adapt, Migrate, or Go Extinct?" *Frontiers of Biogeography* 4: 2.

Fromard, F., H. Puig, E. Mougin, G. Marty, J. L. Betoulle, and L. Cadamuro. 1998. "Structure, Above-Ground Biomass and Dynamics of Mangrove Ecosystems: New Data from French Guiana." *Oecologia* 115: 39–53.

Gechev, T. S., and J. Hille. 2005. "Hydrogen Peroxide as a Signal Controlling Plant Programmed Cell Death." *The Journal of Cell Biology* 168: 17–20.

Gernns, H., H. Alten, and H. M. Poehling. 2001. "Arbuscular Mycorrhiza Increased the Activity of a Biotrophic Leaf Pathogen–Is a Compensation Possible?" *Mycorrhiza* 11: 237–43.

Hameed, M., and M. Ashraf. 2008. "Physiological and Biochemical Adaptations of *Cynodon dactylon* (L.) Pers. from the Salt Range (Pakistan) to Salinity Stress." *Flora: Morphology, Distribution, Functional Ecology of Plants* 203: 683–94.

Hardoim, P. R., L. S. van Overbeek, and J. D. van Elsas. 2008. "Properties of Bacterial Endophytes and Their Proposed Role in Plant Growth." *Trends in Microbiology* 16: 463–71.

Harris, J. 2009. "Soil Microbial Communities and Restoration Ecology: Facilitators or Followers?" *Science* 325: 573–4.

Hayat, S., B. A. Mir, A. S. Wani, S. A. Hasan, M. Irfan, and A. Ahmad. 2011. "Screening of Salt-Tolerant Genotypes of *Brassica juncea* Based on Photosynthetic Attributes." *Journal of Plant Interactions* 6: 53–60.

Hernandez, D. J., A. S. David, E. S. Menges, C. A. Searcy, and M. E. Afkhami. 2021. "Environmental stress destabilizes microbial networks." *The ISME Journal* 15(6): 1722–34.

Himabindu, Y., T. Chakradhar, M. C. Reddy, A. Kanygin, K. E. Redding, and T. Chandrasekhar. 2016. "Salt-Tolerant Genes from Halophytes Are Potential Key Players of Salt Tolerance in Glycophytes." *Environmental and Experimental Botany* 124: 39–63.

Ho, H. H., S. Y. Hsieh, and H. S. Chang. 1990. "*Halophytophthora epistomium* from Mangrove Habitats in Taiwan." *Mycologia* 82: 659–62.

Hou, J., T. Xu, D. Su, Y. Wu, L. Cheng, J. Wang, Z. Zhou, and Y. Wang. 2018. "RNA-Seq Reveals Extensive Transcriptional Response to Heat Stress in the Stony Coral *Galaxea fascicularis*." *Frontiers in Genetics* 9: 37.

Iannone, L. J., M. V. Vignale, A. D. Pinget, A. Re, P. D. Mc Cargo, and M. V. Novas. 2017. "Seed-Transmitted *Epichloë* sp. Endophyte Alleviates the Negative Effects of Head Smut of Grasses (*Ustilago bullata*) on *Bromus auleticus*." *Fungal Ecology* 29: 45–51.

Jiang, Z. K., L. Tuo, D. L. Huang, I. A. Osterman, A. P. Tyurin, S. W. Liu, D. A. Lukyanov, et al. 2018. "Diversity, Novelty, and Antimicrobial Activity of Endophytic Actinobacteria from Mangrove Plants in Beilun Estuary National Nature Reserve of Guangxi, China." *Frontiers in Microbiology* 9: 868.

Jogawat, A., S. Saha, M. Bakshi, V. Dayaman, M. Kumar, M. Dua, A. Varma, R. Oelmüller, N. Tuteja, and A. K. Johri. 2013. "*Piriformospora indica* Rescues Growth Diminution of Rice Seedlings during High Salt Stress." *Plant Signaling and Behavior* 8: e26891.

Khan, A. L., M. Hamayun, M. Waqas, S. M. Kang, Y. H. Kim, D. H. Kim, and I. J. Lee. 2012. "Exophiala sp. LHL08 Association Gives Heat Stress Tolerance by Avoiding Oxidative Damage to Cucumber Plants." *Biology and Fertility of Soils* 48(5): 519–29.

Kogel, K. H., P. Franken, and R. Hückelhoven. 2006. "Endophyte or Parasite–What Decides?" *Current Opinion in Plant Biology* 9: 358–63.

Krauss, K. W., C. E. Lovelock, K. L. McKee, L. López-Hoffman, S. M. Ewe, and W. P. Sousa. 2008. "Environmental Drivers in Mangrove Establishment and Early Development: A Review." *Aquatic Botany* 89: 105–27.

Kuldau, G., and C. Bacon. 2008. "Clavicipitaceous Endophytes: Their Ability to Enhance Resistance of Grasses to Multiple Stresses." *Biological Control* 46: 57–71.

Lata, R., S. Chowdhury, S. K. Gond, and J. F. White, Jr. 2018. "Induction of Abiotic Stress Tolerance in Plants by Endophytic Microbes." *Letters in Applied Microbiology* 66: 268–76.

Latef, A. A. H. A., and H. Chaoxing. 2014. "Does Inoculation with *Glomus mosseae* Improve Salt Tolerance in Pepper Plants?" *Journal of Plant Growth Regulation* 33: 644–53.

Li, X., X. Geng, R. Xie, L. Fu, J. Jiang, L. Gao, and J. Sun. 2016. "The Endophytic Bacteria Isolated from Elephant Grass (*Pennisetum purpureum* Schumach) Promote Plant Growth and Enhance Salt Tolerance of Hybrid Pennisetum." *Biotechnology for Biofuels* 9: 1–12.

Li, H., S. La, X. Zhang, L. Gao, and Y. Tian. 2021. "Salt-Induced Recruitment of Specific Root-Associated Bacterial Consortium Capable of Enhancing Plant Adaptability to Salt Stress." *The ISME Journal* 15: 2865–82.

Li, J., L. Long, Y. Zou, and S. Zhang. 2021. "Microbial Community and Transcriptional Responses to Increased Temperatures in Coral *Pocillopora damicornis* Holobiont." *Environmental Microbiology* 23: 826–43. https://doi.org/10.1111/1462-2920.15168.

Lin, G. H., and L. D. S. Sternberg. 1992. "Effect of Growth Form, Salinity, Nutrient and Sulfide on Photosynthesis, Carbon Isotope Discrimination and Growth of Red Mangrove (*Rhizophora mangle* L.)." *Functional Plant Biology* 19: 509–17.

Lin, G., and L. D. S. Sternberg. 1993. "Effects of Salinity Fluctuation on Photosynthetic Gas Exchange and Plant Growth of the Red Mangrove (*Rhizophora mangle* L.)." *Journal of Experimental Botany* 44: 9–16.

Malinowski, C. P., and D. P. Beleskey. 2000. "Adaptations of Endophyte-Infected Cool-Season Grasses to Environmental Stresses: Mechanisms of Drought and Mineral Stress Tolerance." *Crop Science* 40: 923–40.

Maschinski, J., M. S. Ross, H. Liu, J. O'Brien, E. J. von Wettberg, and K. E. Haskins. 2011. "Sinking Ships: Conservation Options for Endemic Taxa Threatened by Sea Level Rise." *Climatic Change* 107: 147–67.

Mateu, M. G., A. H. Baldwin, J. E. Maul, and S. A. Yarwood. 2020. "Dark Septate Endophyte Improves Salt Tolerance of Native and Invasive Lineages of *Phragmites australis*." *The ISME Journal* 14: 1943–54.

McFadden, L., T. Spencer, and R. J. Nicholls. 2007. "Broad-Scale Modelling of Coastal Wetlands: What Is Required?" *Hydrobiologia* 577: 5–15.

Moore, F. P., T. Barac, B. Borremans, L. Oeyen, J. Vangronsveld, D. Van der Lelie, C. D. Campbell, and E. R. Moore. 2006. "Endophytic Bacterial Diversity in Poplar Trees Growing on a BTEX-Contaminated Site: The Characterisation of Isolates with Potential to Enhance Phytoremediation." *Systematic and Applied Microbiology* 29: 539–56.

Ngumbi, E., and J. Kloepper. 2016. "Bacterial-Mediated Drought Tolerance: Current and Future Prospects." *Applied Soil Ecology* 105: 109–25.

Nicholls, R. J., and A. Cazenave. 2010. "Sea-Level Rise and Its Impact on Coastal Zones." *Science* 328: 1517–20.

Nicoletti, R., M. M. Salvatore, and A. Andolfi. 2018. "Secondary Metabolites of Mangrove-Associated Strains of Talaromyces." *Marine Drugs* 16: 12.

Oberhofer, M. & A. Leuchtmann 2013. Hordelymus europaeus Benefits from Natural Hybrid and Non-hybrid Endophytes under Drought Stress. *International Symposium on Fungal Endophytes of Grasses* 20(11850): 57752. https://doi.org/20.500.11850/57752.

Obeysekera, J., J. Barnes, and M. Nungesser. 2015. "Climate Sensitivity Runs and Regional Hydrologic Modeling for Predicting the Response of the Greater Florida Everglades Ecosystem to Climate Change." *Environmental Management* 55: 749–62.

Ogurcak, D. E., J. P. Sah, R. M. Price, and M. S. Ross. 2019. "Shifting Baselines in Coastal Forests: Rising Seas Transform Plant Communities from the Ground Up." *Forest Ecology and Management* 453: 117581.

Oh D. H., K. P. Kowalski, Q. N. Quach, C. Wijesinghege, P. Tanford, M. Dassanayake, and K. Clay. 2021. Novel Genome Characteristics Contribute to the Invasiveness of *Phragmites australis* (Common Reed). bioRxiv. https://doi.org/10.1101/2021.04.19.440155.

Palmer, T. M., M. L. Stanton, T. P. Young, J. R. Goheen, R. M. Pringle, and R. Karban. 2008. "Breakdown of an Ant-Plant Mutualism Follows the Loss of Large Herbivores from an African Savanna." *Science* 319: 192–5.

Proffitt, C. E., and S. E. Travis. 2010. "Red Mangrove Seedling Survival, Growth, and Reproduction: Effects of Environment and Maternal Genotype." *Estuaries and Coasts* 33: 890–901.

Putnam, H. M., K. L. Barott, T. D. Ainsworth, and R. D. Gates. 2017. "The Vulnerability and Resilience of Reef-Building Corals." *Current Biology* 27: R528–40.

van der Putten, W. H., M. A. Bradford, E. Pernilla Brinkman, T. F. van de Voorde, and G. F. Veen. 2016. "Where, When and How Plant–Soil Feedback Matters in a Changing World." *Functional Ecology* 30: 1109–21.

Putz, F. E., and H. T. Chan. 1986. "Tree Growth, Dynamics and Productivity in a Mature Mangrove Forest in Malaysia." *Forest Ecology and Management* 17: 211–30.

Qin, S., W. W. Feng, Y. J. Zhang, T. T. Wang, Y. W. Xiong, and K. Xing. 2018. "Diversity of Bacterial Microbiota of Coastal Halophyte *Limonium sinense* and Amelioration of Salinity Stress

Damage by Symbiotic Plant Growth-Promoting Actinobacterium *Glutamicibacter halophytocola* KLBMP 5180." *Applied and Environmental Microbiology* 84: e01533–18.

R Core Team. 2019. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/.

Rasool, S., A. Hameed, M. M. Azooz, T. O. Siddiqi, and P. Ahmad. 2013. "Salt Stress: Causes, Types and Responses of Plants." In *Ecophysiology and Responses of Plants under Salt Stress*, edited by P. Ahmad, M. M. Azooz, and M. N. V. Prasad, 1–24. New York: Springer.

Read, D. J. 1999. "Mycorrhiza—The State of the Art." In *Mycorrhiza*, edited by A. Varma and B. Hock, 3–34. Germany: Berlin Springer-Verlag.

Regina, R. S., K. B. Sheehan, R. G. Stout, R. J. Rodriguez, and J. M. Henson. 2002. "Thermotolerance Conferred to Plant Host and Fungal Endophyte during Mutualistic Symbiosis." *Science* 298: 1581.

Rho, H., M. Hsieh, S. L. Kandel, J. Cantillo, S. L. Doty, and S. H. Kim. 2018. "Do Endophytes Promote Growth of Host Plants under Stress? A Meta-Analysis on Plant Stress Mitigation by Endophytes." *Microbial Ecology* 75: 407–18.

Rodriguez, R. J., J. Henson, E. van Volkenburgh, M. Hoy, L. Wright, F. Beckwith, Y. O. Kim, and R. S. Redman. 2008. "Stress Tolerance in Plants Via. Habitat-Adapted Symbiosis." *The ISME Journal* 2: 404–16.

Rolli, E., R. Marasco, G. Vigani, B. Ettoumi, F. Mapelli, M. L. Deangelis, C. Gandolfi, et al. 2015. "Improved Plant Resistance to Drought Is Promoted by the Root-Associated Microbiome as a Water Stress-Dependent Trait." *Environmental Microbiology* 17: 316–31.

Ross, M. S., J. J. O'Brien, R. G. Ford, K. Zhang, and A. Morkill. 2009. "Disturbance and the Rising Tide: the Challenge of Biodiversity Management on Low-island Ecosystems." *Frontiers in Ecology and the Environment* 7(9): 471–8.

Ross, M. S., J. F. Meeder, L. Scinto, J. P. Sah, S. Stoffella, H. Biswas, and R. Vidales. 2019. *2018–2019 Annual Report: Ecosystem Dynamics in the White Zone: History, Drivers, and Restoration Implications*. Homestead, FL: South Florida Natural Resource Center, Everglades National Park.

Ross, M. S., P. L. Ruiz, G. J. Telesnicki, and J. F. Meeder. 2001. "Estimating Above-Ground Biomass and Production in Mangrove Communities of Biscayne National Park, Florida (USA)." *Wetlands Ecology and Management* 9: 27–37.

Ross, M. S., J. P. Sah, P. L. Ruiz, A. A. Spitzig, and S. C. Subedi. 2016. "Inferring Implications of Climate Change in South Florida Hardwood Hammocks through Analysis of Metacommunity Structure." *Diversity and Distributions* 22: 783–96.

Ross, M. S., S. L. Stoffella, R. Vidales, J. F. Meeder, D. C. Kadko, L. J. Scinto, S. C. Subedi, and J. R. Redwine. 2021. "Sea-Level Rise and the Persistence of Tree Islands in Coastal Landscapes." *Ecosystems*. 1–17. https://doi.org/10.1007/s10021-021-00673-1.

Saha, A. K., S. Saha, J. Sadle, J. Jiang, M. S. Ross, R. M. Price, L. S. Sternberg, and K. S. Wendelberger. 2011. "Sea Level Rise and South Florida Coastal Forests." *Climatic Change* 107: 81–108.

Sampangi-Ramaiah, M. H., P. Dey, S. Jambagi, M. V. Kumari, R. Oelmüller, K. N. Nataraja, K. V. Ravishankar, G. Ravikanth, and R. U. Shaanker. 2020. "An Endophyte from Salt-Adapted Pokkali Rice Confers Salt-Tolerance to a Salt-Sensitive Rice Variety and Targets a Unique Pattern of Genes in its New Host." *Scientific Reports* 10: 1–14.

Santoyo, G., G. Moreno-Hagelsieb, M. del Carmen Orozco-Mosqueda, and B. R. Glick. 2016. "Plant Growth-Promoting Bacterial Endophytes." *Microbiology Research* 183: 92–9.

Sarma, V. V., and K. D. Hyde. 2001. "A Review on Frequently Occurring Fungi in Mangroves." *Fungal Diversity* 8: 1–34.

Schulz, B., and C. Boyle. 2005. "The Endophytic Continuum." *Mycological Research* 109: 661–86.

Shabala, S., and A. Mackay. 2011. "Ion Transport in Halophytes." *Advances in Botanical Research* 57: 151–87.

Shahzad, R., A. L. Khan, S. Bilal, M. Waqas, S. M. Kang, and I. J. Lee. 2017. "Inoculation of Abscisic Acid-Producing Endophytic Bacteria Enhances Salinity Stress Tolerance in *Oryza sativa*." *Environmental and Experimental Botany* 136: 68–77.

Soares, M. A., H. Y. Li, K. P. Kowalski, M. Bergen, M. S. Torres, and J. F. White. 2016. "Evaluation of the Functional Roles of Fungal Endophytes of *Phragmites australis* from High Saline and Low Saline Habitats." *Biological Invasions* 18: 2689–702.

Soldan, R., F. Mapelli, E. Crotti, S. Schnell, D. Daffonchio, R. Marasco, M. Fusi, S. Borin, and M. Cardinale. 2019. "Bacterial Endophytes of Mangrove Propagules Elicit Early Establishment of the Natural Host and Promote Growth of Cereal Crops under Salt Stress." *Microbiological Research* 223: 33–43.

Song, M., X. Li, K. Saikkonen, C. Li, and Z. Nan. 2015. "An Asexual Epichloë Endophyte Enhances Waterlogging Tolerance of *Hordeum brevisubulatum*." *Fungal Ecology* 13: 44–52.

de Souza Sebastianes, F. L., A. S. Romao-Dumaresq, P. T. Lacava, R. Harakava, J. L. Azevedo, I. S. de Melo, and A. A. Pizzirani-Kleiner. 2013. "Species Diversity of Culturable Endophytic Fungi from Brazilian Mangrove Forests." *Current Genetics* 59: 153–66.

Subedi, S., P. Allen, R. Vidales, L. Sternberg, M. Ross, and M. Arkhami. 2022. "Salinity Legacy: Foliar Microbiome's History Affects Mutualist-Conferred Salinity Tolerance." Dryad, dataset. https://doi.org/10.5061/dryad.xsj3tx9gm.

Subedi, S. C., L. Sternberg, D. L. DeAngelis, D. E. Ogurcak, and M. S. Ross. 2020. "Using Carbon Isotope Ratios to Verify Predictions of a Model Simulating the Interaction between Coastal Plant Communities and their Effect on Ground Water Salinity." *Ecosystems* 23: 570–85.

Sun, L., P. Lei, Q. Wang, J. Ma, Y. Zhan, K. Jiang, Z. Xu, and H. Xu. 2020. "The Endophyte *Pantoea Alhagi* NX-11 Alleviates Salt Stress Damage to Rice Seedlings by Secreting Exopolysaccharides." *Frontiers in Microbiology* 10: 3112.

Szabados, L., and A. Savoure. 2010. "Proline: A Multifunctional Amino Acid." *Trends in Plant Science* 15: 89–97.

Taillie, P. J., C. E. Moorman, B. Poulter, M. Ardon, and R. E. Emanuel. 2019. "Decadal-Scale Vegetation Change Driven by Salinity at Leading Edge of Rising Sea Level." *Ecosystems* 22: 1918–30.

Terborgh, J., G. Nuñez-Iturri, N. C. A. Pitman, F. H. Cornejo Valverde, P. Alvarez, V. Swamy, E. G. Pringle, and C. E. T. Paine. 2008. "Tree Recruitment in an Empty Forest." *Ecology* 89: 1757–68.

Tomlinson, P. B. 2016. *The Botany of Mangroves*. Cambridge, UK: Cambridge University Press.

Turner, T. R., E. K. James, and P. S. Poole. 2013. "The Plant Microbiome." *Genome Biology* 14: 209.

Valiela, I., J. L. Bowen, and J. K. York. 2001. “Mangrove Forests: One of the World’s Threatened Major Tropical Environments.” *BioScience* 51: 807–15.

Vitousek, P. M., and L. R. Walker. 1989. “Biological Invasion by *Myrica faya* in Hawai’i: Plant Demography, Nitrogen Fixation, Ecosystem Effects.” *Ecological Monographs* 59: 247–65.

Vo, Q. T., C. Kuenzer, Q. M. Vo, F. Moder, and N. Oppelt. 2012. “Review of Valuation Methods for Mangrove Ecosystem Services.” *Ecological Indicators* 23: 431–46.

Volkmar, K. M., Y. Hu, and H. Steppuhn. 1998. “Physiological Responses of Plants to Salinity: A Review.” *Canadian Journal of Plant Science* 78: 19–27.

Wagg, C., K. Schlaeppi, S. Banerjee, E. E. Kuramae, and M. G. van der Heijden. 2019. “Fungal-Bacterial Diversity and Microbiome Complexity Predict Ecosystem Functioning.” *Nature Communications* 10: 1–10.

Wake, D. B., and V. T. Vredenburg. 2008. “Are We in the Midst of the Sixth Mass Extinction? A View from the World of Amphibians.” *Proceedings of the National Academy of Sciences* 105: 11466–73.

Waqas, M., A. L. Khan, M. Kamran, M. Hamayun, S. M. Kang, Y. H. Kim, and I. J. Lee. 2012. “Endophytic Fungi Produce Gibberellins and Indoleacetic Acid and Promotes Host-Plant Growth during Stress.” *Molecules* 17: 10754–73.

Watson, C. S., N. J. White, J. A. Church, M. A. King, R. J. Burgette, and B. Legresy. 2015. “Unabated Global Mean Sea-Level Rise over the Satellite Altimeter Era.” *Nature Climate Change* 5: 565.

Werner, A., and R. Stelzer. 1990. “Physiological Responses of the Mangrove *Rhizophora mangle* Grown in the Absence and Presence of NaCl.” *Plant, Cell and Environment* 13: 243–55.

Wilkie, M. L., S. Fortuna, and O. Souksavat. 2003. “FAO’s Database on Mangrove Area Estimates.” Forest Resources Assessment Working Paper no. 62. Rome: Food and Agriculture Organization of the United Nations (FAO).

Woodruff, J. D., J. L. Irish, and S. J. Camargo. 2013. “Coastal Flooding by Tropical Cyclones and Sea-Level Rise.” *Nature* 504: 44–52.

Xia, L., X. Geng, R. Xie, F. Lei, J. Jiang, and G. Lu. 2016. “The Endophytic Bacteria Isolated from Elephant Grass (*Pennisetum purpureum* Schumach) Promote Plant Growth and Enhance Salt Tolerance of Hybrid Pennisetum.” *Biotechnology for Biofuels* 9: 190–201.

Xing, X., and S. Guo. 2010. “Fungal Endophyte Communities in Four Rhizophoraceae Mangrove Species on the South Coast of China.” *Ecological Research* 26: 403–9.

Xu, J. R., M. Urban, J. A. Sweigard, and J. E. Hamer. 1997. “The CPKA Gene of *Magnaporthe grisea* Is Essential for Appressorial Penetration.” *Molecular Plant-Microbe Interactions* 10: 187–94.

Yu, X., W. Zhang, D. Lang, X. Zhang, G. Cui, and X. Zhang. 2019. “Interactions between Endophytes and Plants: Beneficial Effect of Endophytes to Ameliorate Biotic and Abiotic Stresses in Plants.” *Journal of Plant Biology* 62: 1–13.

Zahn, G., and A. S. Amend. 2017. “Foliar Microbiome Transplants Confer Disease Resistance in a Critically-Endangered Plant.” *PeerJ* 5: e4020.

Zhang, X., X. Fan, C. Li, and Z. Nan. 2010. “Effects of Cadmium Stress on Seed Germination, Seedling Growth and Antioxidative Enzymes in *Achnatherum inebrians* Plants Infected with a *Neotyphodium* Endophyte.” *Plant Growth Regulation* 60: 91–7.

Zhou, J., X. Diao, T. Wang, G. Chen, Q. Lin, X. Yang, and J. Xu. 2018. “Phylogenetic Diversity and Antioxidant Activities of Culturable Fungal Endophytes Associated with the Mangrove Species *Rhizophora stylosa* and *R. mucronata* in the South China Sea.” *PLoS One* 13: e0197359.

Zimmerman, N. B., and P. M. Vitousek. 2012. “Fungal Endophyte Communities Reflect Environmental Structuring across a Hawaiian Landscape.” *Proceedings of the National Academy of Sciences* 109: 13022–7.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

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