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RESEARCH ARTICLE

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Freeze tolerance of poleward-spreading mangrove species weakened by soil properties of resident salt marsh competitor

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Abstract

1. Increasing temperatures associated with climate change are shifting plant species to higher latitudes. Soil communities could aid the plants' shift into novel areas by harbouring fewer soil-borne antagonists or more mutualists that influence the fitness and stress tolerance of the shifting species. Alternatively, they could contain novel antagonists or fewer mutualists. Thus, soil communities could positively or negatively affect plant range expansion, particularly if they influence plants' responses to climate, such as freeze tolerance, that feedback to affect expansion.
2. We used the northward range expansion of the black mangrove, *Avicennia germinans*, into a system dominated by marsh cordgrass, *Spartina alterniflora*, in northern Florida, USA to study how the novel soil environment (i.e. *S. alterniflora* soil) affects mangrove fitness, susceptibility to cold stress and the colonization of mutualist fungi. We quantified abundance of root mutualistic fungi in mixed marsh-mangrove habitat and conducted a laboratory experiment to test effects of steam-sterilized and live soils from *A. germinans* and *S. alterniflora* on the growth, condition, fungal colonization and freeze tolerance of *A. germinans* seedlings.
3. In the field, we found two times higher dark septate endophyte (DSE) colonization of *A. germinans* roots and three times higher fungal spore density in *A. germinans* soil compared to *S. alterniflora* roots and soil. In the laboratory experiment, seedlings in steamed *S. alterniflora* soil treatments had 50%–65% survival after freezing, compared to 0% survival in treatments with live *S. alterniflora* soil. *A. germinans* live soil mixed with *S. alterniflora* steamed soil yielded *A. germinans* roots with the highest DSE colonization and seedlings with greater shoot biomass and lower root:shoot ratios. *S. alterniflora* live soil lowered the freeze tolerance of *A. germinans*, decreased mangrove survival and depressed DSE colonization.
4. *Synthesis*. *S. alterniflora* soil could impede *A. germinans* establishment in salt marsh communities. As climate warming gradually allows *A. germinans* to displace *S. alterniflora*, the rhizosphere could become increasingly hospitable to *A. germinans*. Our work suggests the soil community associated with resident species mediates climatic stressors to affect expansion success.

KEYWORDS
allelopathy, biotic resistance, dark septate endophyte fungi, global change ecology, invasion ecology, plant–soil (below-ground) interactions, plant–soil feedbacks, salt marshes

1 | INTRODUCTION

Global warming redistributes species, which often causes range shifts to higher latitudes and elevations (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Pecl et al., 2017). Biotic interactions can further influence how climate change affects species distributions (Blois, Zarnetske, Fitzpatrick, & Finnegan, 2013; Gilman, Urban, Tewksbury, Gilchrist, & Holt, 2010; Wisz et al., 2013). As species expand with shifting climate envelopes, they will interact positively and negatively with resident species in the areas they colonize in ways that can affect both the rate and geographical extent of their expansion (HilleRisLambers, Harsch, Ettinger, Ford, & Theobald, 2013). In particular, plant interactions with soil mutualists and antagonists can affect plant expansion success into recipient systems (Bray, Kitajima, & Sylvia, 2003; Gribben et al., 2017; Klironomos, 2003; Reinhart & Callaway, 2006). For example, plant expansion could be enhanced if plants encounter fewer soil pathogens in recipient systems or if strong native mutualists facilitate plant invaders (Mitchell et al., 2006; Richardson, Allsopp, D'Antonio, Milton, & Rejmanek, 2000). Alternatively, resident soil pathogens or an absence of soil mutualists could inhibit plant expansion into new areas (Callaway, Montesinos, Williams, & Maron, 2013; Knevel, Lans, Menting, Hertling, & van der Putten, 2004). The relative importance of positive and negative plant–soil interactions influences plant expansion success, and it is important to consider these feedbacks in the context of climate change. Interactions with soil microbes can alter plant stress tolerances and susceptibility to environmental stressors, such as temperature or drought (Gehring, Sthultz, Flores-Renteria, Whipple, & Whitham, 2017; Xi, Chu, & Bloor, 2018). However, it is unclear how soil–plant interactions that alter plant stress tolerance influence expansion success during climate-driven range expansions.

Two broad categories of mutualistic fungi that are often associated with plant–soil interactions are arbuscular mycorrhizae (AM) and dark septate endophytes (DSE). These fungi often aid plants by facilitating nutrient uptake (Barrow & Osuna, 2002; da Silva, da Silva, de Souza, Oehl, & Maia, 2017; Karasawa, Hodge, & Fitter, 2012) and may be particularly valuable symbionts in stressful environments (Singh, Gill, & Tuteja, 2011). Like AM fungi, DSE fungi can promote nutrient uptake and mitigate heat and drought stress in extreme environments (Jumpponen, 2001; Newsham, 2011; Ramirez-Viga et al., 2018), although the beneficial functions of DSE fungi are relatively understudied relative to those of AM fungi (Mandyam & Jumpponen, 2015). AM fungi–plant symbioses can also competitively exclude root parasites or pathogens and modulate environmental stressors such as shade, drought, salinity and nutrient depletion (Garg & Chandel, 2010). Since nutrient cycling is typically slow in cold environments (Nadelhoffer, Shaver, Giblin, & Rastetter, 1997), plants may rely on symbiotic fungi to acquire adequate nitrogen and phosphorus. In fact, AM fungi and DSE fungi may both perform important functions for plant growth in cold environments, as they are both abundant in arctic and alpine systems (Schmidt, Sobieniak-Wiseman, Kageyama, Halloy, & Schadt, 2008; Väre, Vestberg, & Eurola, 1992). For example, AM has recently been directly linked to freeze tolerance in barley plants (Hajiboland, Joudmand, Aliasgharzad, Tolra, & Poschenrieder, 2019). AM symbiosis can improve nutrient acquisition, increase antioxidant enzyme activities of the plants and protect root hydraulic conductance from cold stress (Aroca, Porcel, & Ruiz-Lozano, 2007; Karasawa et al., 2012; Zhou, Ma, Liang, Huang, & Pinyopusarerk, 2012). However, DSE is found across a broader range of climatic conditions, including at extreme elevations in the Himalayas where AM is conspicuously absent (Kotilinek et al., 2017). High melanin concentrations in DSE have been cited as an adaptation to cold temperatures (Jumpponen & Trappe, 1998; Robinson, 2001). Thus, these two fungal groups have strong associations with plants, and often seemingly play important roles in plant–soil feedbacks including in cold-stressed environments.

Mangroves provide an opportunity to examine the relative importance of positive and negative plant–soil interactions in the context of a climate-driven range expansion. As temperatures warm, mangroves are expanding poleward and replacing salt marshes between subtropical and warm temperate climatic zones world-wide (Guo, Zhang, Lan, & Pennings, 2013; Saintilan, Wilson, Rogers, Rajkaran, & Krauss, 2014; Smith, Blaze, Osborne, & Byers, 2018). The northern distribution of mangroves in the southeastern United States is controlled primarily by the frequency of extreme cold events (Cavanaugh et al., 2014; Osland, Enwright, Day, & Doyle, 2013). In this region, the black mangrove, *Avicennia germinans*, is moving north into salt marshes that are dominated by smooth cordgrass, *Spartina alterniflora* (Simpson, Osborne, & Feller, 2017). Although the southeastern United States has increased in mean temperatures over the past 30 years, this area is still subject to the sharp cold snaps and heightened climate variability that affect northward-moving tropical and subtropical species along the southeastern US coastline (Canning-Clode, Fowler, Byers, Carlton, & Ruiz, 2011). Because fungal symbionts facilitate plant growth in stressful conditions (Rodriguez et al., 2008; Singh et al., 2011), losing mutualistic fungi or gaining antagonistic interference from the *S. alterniflora* soil could decrease stress tolerance of *A. germinans*. These interactions with soil symbionts could be particularly important for freeze tolerance, which influences mangrove expansion into cooler climates.

Saltmarsh and mangrove systems are characterized by different soil environments. These two foundation species fundamentally differ in their ability to sequester carbon (Coldren et al.,

2016; Doughty et al., 2016). They also have variable root physiology (Purnobasuki & Suzuki, 2004) and associated soil chemical properties, including salinity, soil ammonium and redox potential (Perry & Mendelssohn, 2009). All of these factors contribute to different sediment microbial communities between mangrove and marsh systems (Barreto, Morrissey, Wykoff, & Chapman, 2018). AM and DSE fungi both commonly occur in mangrove-dominated habitats (Kothamasi, Kothamasi, Bhattacharyya, Kuhad, & Babu, 2006; Wang & Li, 2003; Wang et al., 2010) where phosphorus is often a limiting element for plant growth (Koch & Snedaker, 1997; Lovelock, Ball, Choat, et al., 2006; Lovelock, Ball, Feller, Engelbrecht, & Ling Ewe, 2006). In contrast, *S. alterniflora* does not readily associate with AM fungi (Cooke & Lefor, 1990; Hoefnagels, Broome, & Shafer, 1993; Mandyam & Jumpponen, 2005), but the plant has been shown to form root associations with DSE in stressful abiotic conditions, and root-colonization is positively correlated with plant growth (Moore, 2016). In common garden experiments, *S. alterniflora* is poorly colonized by AM fungi; when inoculated with a commercially available AM inoculum mix, *S. alterniflora* developed fungal hyphae, but no arbuscules (McHugh & Dighton, 2004). In addition to differences in fungal root associates between these two plants, soil antagonists and allelopathic effects associated with *S. alterniflora* could also inhibit *A. germinans* expansion. For example, in China where *S. alterniflora* is invasive, its endophytic fungal pathogen, *Fusarium palustre*, is associated with dieback of the native plant *Phragmites communis* (Li et al., 2014). *S. alterniflora* can also depress the association between AM fungi and neighbouring plants (Liang et al., 2016), and the quantity of AM fungi phospholipid fatty acids in the soils gradually declines following *S. alterniflora* invasion (Liang et al., 2016; Yang, Jeelani, Leng, Cheng, & An, 2016). Thus, not only do soil properties differ based on plant communities but also soil fungi can influence plant interactions and community composition.

In this study, we use *A. germinans* expansion into *S. alterniflora* marshes in north Florida, USA to examine how the survival, performance, freeze tolerance and fungal root colonization of *A. germinans* seedlings may change at the leading edge of the mangrove expansion as a function of soil associated with the two different dominant plants. First, we conducted a field survey in mixed salt marsh and mangrove habitats to quantify fungal colonization of *A. germinans* and *S. alterniflora* roots. We also measured fungal spore density in the soil surrounding both *A. germinans* and *S. alterniflora* plants. Second, we used a laboratory experiment to ensure uniform, controlled conditions to assess how combinations of live and steam-sterilized soils collected from the rhizosphere of *S. alterniflora* and *A. germinans* affect the density of AM and DSE mutualists associated with *A. germinans* seedlings, as well as seedling survival, performance and freeze tolerance. We expected that fungi would improve *A. germinans* seedling responses by improving nutrient acquisition, increasing antioxidant enzyme activities and protecting root hydraulic conductance. We hypothesized that root fungi colonization of *A. germinans* seedlings would be lower in *S. alterniflora* soil than in *A. germinans* soil because of relatively lower spore density in *S. alterniflora* soil. Lastly, we hypothesized that *S. alterniflora* soil would inhibit performance of *A. germinans* seedlings and decrease their freeze tolerance relative to *A. germinans* soil.

2 | MATERIALS AND METHODS

2.1 | Field survey

To assess mutualist fungi colonization in roots and soil of *S. alterniflora* and *A. germinans*, we haphazardly selected five mixed species plots (2 m^2) within a 0.5 km section of the salt marsh-mangrove transition zone in Marineland, FL (29.672633°N, 81.218414°W) in November 2017. In each plot, we collected roots from one *A. germinans* seedling and one *S. alterniflora* ramet and the associated soil from each plant. To quantify root fungal abundance, we removed the fine roots from each plant, cleaned the roots in 10% KOH and stained them with trypan blue (Phillips & Hayman, 1970). After staining, we determined the percentage of fungi colonization by microscopic visual examination using the gridline intersection method (Giovannetti & Mosse, 1980). For the soil, we extracted fungal spores by centrifuging soil samples in a 20% sugar solution followed by a 60% sugar solution adapted from the INVAM method (West Virginia University, Davis College of Agriculture, 2017). Fungal spores were concentrated in the supernatant and we decanted them into a Petri dish for visual examination. We counted fungi spore density under a stereoscopic microscope at 40× magnification.

We analysed the data with R (R Development Core Team, 2017). For both AM and DSE colonization, we used a paired *t* test to compare their values between *A. germinans* and *S. alterniflora* roots collected from the same plots. We also used a paired *t* test to examine the difference in fungal spore density for *A. germinans* and *S. alterniflora* soils collected from the same plots.

2.2 | Laboratory experiment

To examine how different soil compositions influence the survival, biomass, fungal colonization and freeze tolerance of *A. germinans* seedlings, we performed a two-factor laboratory experiment that manipulated seedling exposure to soil type and freezing temperatures (freeze, control).

2.2.1 | Soil collection and treatment preparation

We collected the soil from *S. alterniflora* and *A. germinans* single-species zones (10 × 50 m) in areas with continuous vegetation (*A. germinans* or *S. alterniflora*) in Crescent Beach, Florida (29.763889°N, 81.262694°W). We collected soil to a depth of 10 cm in the rhizosphere of the target plants. We homogenized the soil collected from each plant species to create separate pools of *A. germinans* and *S. alterniflora* soils. Although there is debate about the merits of soil

homogenization (see Rinella & Reinhart, 2018; Teste et al., 2019), we collected soil from a single site, and we wanted to quantify *A. germinans* response to a standardized amount of *S . alterniflora* soil and its associated biotic and abiotic components (e.g. chemical compounds and fungal spores). Next, we sieved the soils through a 2-mm mesh to remove large infauna and debris. Sodium hypochlorite is commonly used to disinfect seeds and isolated fungal spores from bacteria (Liang et al., 2015). Thus, to reduce the natural bacterial community present in the soil while leaving fungal spores intact, we saturated all soil with 10% sodium hypochlorite for 15 min. All field collected soils were conditioned the same. We then rinsed the soils repeatedly with fresh water (5×) to remove traces of sodium hypochlorite.

To create the soil treatments, we split each soil origin type into two batches of equal volume and steam-sterilized one batch of each soil origin type (*A. germinans*, *S. alterniflora*) for 45 min to kill all fungal spores (Sioux Steam-Flo Steam Generator). Soil steam-sterilization is commonly used as an alternative to chemical sterilization in agricultural and plant biology studies to inactivate fungal spores in soil (Baker & Chandler, 1957; Bollen, 1985; Runia & Molendijk, 2010; Warcup, 1951). Our soil treatments comprised four combinations that each included both soil origins (*A. germinans*, *S. alterniflora*) that varied in their fungal spore presence (steamed, live). We added an equal volume (50 ml) of *A. germinans* (*Ag*) and *S. alterniflora* (*Sa*) soil to each treatment to maintain consistent nutrient contributions from *A. germinans* and *S. alterniflora* soils. Thus, our treatments were as follows: (a) both steamed (*Sa*(*s*) + *Ag*(*s*)); (b) live *S. alterniflora* (*Sa* + *Ag*(*s*)); (c) live *A. germinans* (*Sa*(*s*) + *Ag*); (d) both live (*Sa* + *Ag*). Steamed soil is denoted by (*s*) after the soil type abbreviation.

2.2.2 | Propagule collection and experiment set-up

We collected *A. germinans* propagules from adult trees located at the same site as the soil collections. We floated the propagules in water for a week to approximate optimal flotation time prior to planting (Simpson et al., 2017). We then disinfected propagules (15 min, 5% bleach), rinsed them with water and individually planted each propagule into one of 192 square pots (10 × 10 × 15 cm depth) that we had three-quarters filled with a 1:5 mix of steam-sterilized playground sand and commercial topsoil (Figure 1: Step 1). We placed all the pots under grow lights (8 a.m.–8 p.m. each day) and housed them in tubs (60 × 40 × 20 cm) filled 10 cm deep with Instant Ocean saline water (20 ppt salinity). Holes in the bottom of the pots enabled water to wick up into the soil from below. We replaced any dead propagules during the first 2 weeks, and we changed the salt water in the tubs weekly to maintain salinity and minimize microalgal growth.

After 1 month, *A. germinans* seedlings in all 192 pots developed true leaves. We measured initial seedling height and randomly assigned the soil treatments to seedlings by adding 50 ml of each appropriate soil type (n = 48 per treatment, 100 ml per pot; Figure 1: Step 2). To minimize fungal contamination among pots in different treatments, we placed six pots of the same soil treatment in a smaller tub (35 × 25 × 20 cm) filled with saline water as described above. We randomly re-assigned the pots of the same soil treatment in the small tubs every week.

To assess baseline seedling responses to soil conditions prior to the freeze treatments, we recorded seedling heights and survival 3 months after we added the soil treatments, and destructively sampled 10 seedlings in each soil treatment to confirm presence of mycorrhizal fungi (pre-test group) (Figure 1: Step 3). We measured AM and DSE colonization in fresh roots using the protocol described above.

2.2.3 | Simulated freeze events

For each soil treatment, we froze 20 seedlings (freeze group) at −5°C ± 1°C in a darkened walk-in freezer for 6 hr (Freeze 1), and left the remaining seedlings (n = 12–17) as controls that did not receive freeze exposure (Figure 1: Step 4). We based the temperature and duration of the freeze treatment on previous work that demonstrated 100% mortality of mangroves exposed to −6.5°C for 24 hr and 50% mortality for mangroves exposed at the same temperature, but for shorter duration (Pickens, 2012). During the freeze event, we kept the control plants on a nearby benchtop at room temperature (19°C) in the dark. Immediately before the freeze event, we removed all seedlings from their salt-water bath. Temperature fluctuated ±1°C during the freezing period.

After the freeze event, we evaluated seedling cold tolerance with same-day measurements of chlorophyll fluorescence and by recording seedling survival 2 weeks post-freeze. Chlorophyll fluorescence is a non-invasive measure of the efficiency of Photosystem II, which we measured as the variable to maximum fluorescence ratio (Fv/Fm) of dark-adapted leaves. Fv/Fm is correlated with other estimators of frozen leaf damage and is widely used to estimate plant freeze tolerance and stress (Cook-Patton, Lehmann, & Parker, 2015; Huner et al., 1993; Sierra-Almeida, Cavieres, & Bravo, 2009). Low Fv/Fm values indicate a decrease in efficiency of Photosystem II. This change could be a temporary response to stress or an indication of more severe damage to the plant (Khanal, Bray, Grisnich, Moffatt, & Gray, 2017). We measured Fv/Fm with a FIRe Fluorometer System (Satlantic LP Company). We measured and averaged Fv/Fm for the two top fully expanded leaves of each seedling in both the freeze and control group before and after the freeze treatment. We took before and after freeze measurements at the same time of day to control for daily fluctuations in Fv/Fm. We took the after-measurements as soon as the seedlings returned to room temperature. We estimated the response of each plant as the photoinactivation ratio (PHI) PHI = 1 − Fa/Fb, where Fb is Fv/Fm tested before freezing and Fa is Fv/Fm tested after freezing (Perez, Hinojosa, Ossa, Campano, & Orrego, 2014). A high PHI indicates higher levels of photoinactivation corresponding to plant stress.

We performed a second freeze treatment 2 weeks after Freeze 1 to simulate a more intense freeze event because no seedlings died

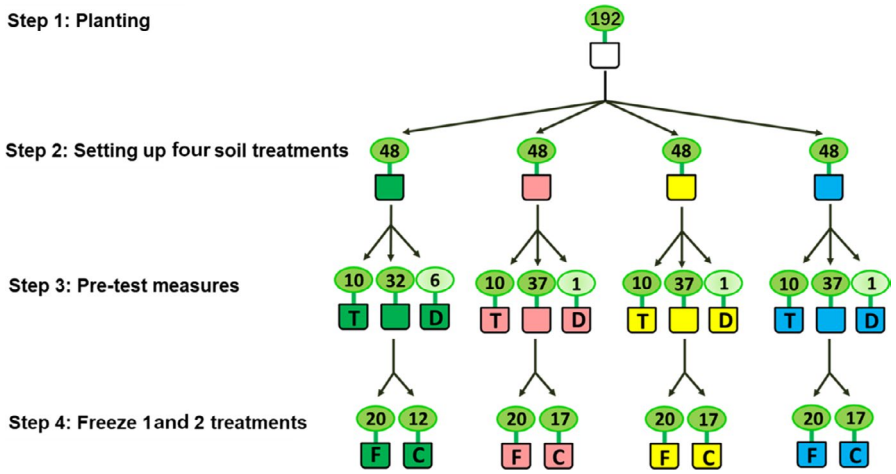

FIGURE 1 Laboratory experiment design and procedure. Numbers represent the number of seedlings in each step of the experiment. Colour of the square pots indicates the four soil treatments (Green: both steamed (*Sa*(*s*) + *Ag*(*s*)); Red: live *Spartina alterniflora* (*Sa* + *Ag*(*s*)); Yellow: live *Avicennia germinans* (*Sa*(*s*) + *Ag*); Blue: both live (*Sa* + *Ag*)). Letters on square pots denote T : pre-test group destructively sampled to quantify fungal colonization before freezing; D: dead seedlings before freeze treatment for Step 3, and F: freeze group (freeze exposed); C: control group (no-freeze exposure) in Step 4. *Ag*, *Avicennia germinans*; *Sa*, *Spartina alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

from Freeze 1. The second freeze was designed to test seedling performance through successive freeze events and determine whether our treatment groups varied in response to increased freeze severity. We froze the seedlings overnight at −5°C for two 12-hr intervals, with a 12-hr break between the intervals (Freeze 2). We used the same control seedlings from Freeze 1 as controls during Freeze 2. As in Freeze 1, we measured chlorophyll fluorescence before and after the freeze treatment. We recorded seedling survival 2 weeks after Freeze 2. After that, we harvested all the seedlings and measured their total fresh biomass, dry above-ground biomass (dried in an oven at 70°C for 72 hr), and root AM and DSE colonization as described above.

2.2.4 | Analyses

For the laboratory experiment, we analysed the effect of soil treatments ((*Sa*(*s*) + *Ag*(*s*)), (*Sa* + *Ag*(*s*)), (*Sa*(*s*) + *Ag*), (*Sa* + *Ag*)) on AM and DSE fungal colonization of mangrove seedlings before Freeze 1 (pre-test group) using a one-way ANOVA for each fungal group. After Freeze 2, we analysed fungal colonization as a function of soil treatments, freeze treatment (control, freeze) and their interaction with a two-way ANOVA for each fungal group. Because neither freeze treatment nor the interaction between soil and freeze treatments significantly affected AM or DSE colonization, we used Tukey's post hoc tests to examine for overall differences among soil treatments for each fungal group.

For photosynthetic analyses, we examined the effects of soil treatment, freeze treatment and their interaction on the Fv/Fm of each seedling (averaged over the two leaves measured) before and after Freeze 1 and Freeze 2 with four separate two-way ANOVAs. We also used two two-way ANOVAs to analyse the effects of the same factors on PHI for Freeze 1 and Freeze 2. We used Tukey's post hoc tests to determine significant soil and freeze treatment differences for PHI responses in each freeze trial.

For performance measurements (i.e. height, total fresh biomass, dry shoot biomass, fresh root–shoot ratio), we used separate ANOVAs to test each response as a function of soil treatment. We performed these tests on the heights of all the plants after 3 months (before Freeze 1), and the height, shoot biomass, total fresh biomass and root:shoot ratio on the control plants after 4 months (2 weeks after Freeze 2). For each response variable, we used Tukey's post hoc tests to examine differences in the seedling performance between soil treatments.

Finally, we used a logistic regression (generalized linear model fit with a binomial distribution) to examine all seedling survival before Freeze 1 as a function of soil treatment using the 'LME4' package (Bates, Machler, Bolker, & Walker, 2015). We also examined seedling survival in the freeze treatment 2 weeks after Freeze 2 as a function of soil treatment with logistic regression. In this case, because there was an issue of separation in the data (i.e. 100% mortality in two of our treatments), we applied Bayesian inference with non-informative prior assumptions to obtain stable logistic regression coefficients and account for quasi-complete separation using the 'ARM' package (Gelman, Jakulin, Pittau, & Su, 2008; Gelman & Su, 2018). We used post hoc tests for each model to examine differences in seedling survival among the soil treatments.

3 | RESULTS

3.1 | Field survey

DSE colonization of *A. germinans* roots was more than twofold greater than on *S. alterniflora* roots (paired $t = -2.86$, $df = 4$, $p = .046$, Table 1), but AM root colonization did not differ between *S. alterniflora* and *A. germinans* (paired $t = 1.71$, $df = 4$, $p = .162$, Table 1). Soil fungal

spore density in *A. germinans* patches was three times higher than in *S. alterniflora* patches (paired $t = -4.75$, $df = 4$, $p = .009$, Table 1).

3.2 | Laboratory experiment

3.2.1 | AM and DSE colonization

After 3 months, soil treatment significantly affected AM and DSE colonization of *A. germinans* seedlings (pre-test group; AM colonization: $F_{3,36} = 11.20$, $p < .001$; DSE colonization: $F_{3,36} = 79.58$, $p < .001$). AM and DSE fungi did not colonize seedlings in steamed soil (*Sa*(*s*) + *Ag*(*s*)), which confirms the efficacy of the steam sterilization treatment to inactivate fungal spores. AM colonization of seedlings was low (<5%) in all soil treatments. DSE colonization of seedlings in the *Sa*(*s*) + *Ag* soil was three times higher than the next closest soil treatment (*Sa* + *Ag*) (Table 2). DSE colonization was nearly equivalent to levels measured in the field surveys (Tables 1 and 2). After 4 months and two freeze events, AM and DSE colonization were significantly affected by soil treatments (AM colonization: $F_{3,134} = 18.08$, $p < .001$, DSE colonization: $F_{3,134} = 72.42$, $p < .001$; Figure 2), but not freeze treatments (AM colonization: $F_{1,134} = 1.89$, $p = .172$; DSE colonization: $F_{1,134} = 0.87$, $p = .353$), nor the interaction between soil and freeze treatments (AM colonization: $F_{3,134} = 2.08$, $p = .106$; DSE colonization: $F_{3,134} = 0.37$, $p = .778$). As before, AM colonization of seedlings was very low in all soil treatments (<5%), and DSE colonization ranged between 0% and 32% depending on the soil treatment. Average AM and DSE colonization of seedlings was greatest in the *Sa*(*s*) + *Ag* soil (3% and 18%, respectively; Figure 2; Table S1).

TABLE 1 AM and DSE colonization on roots of *Spartina alterniflora* (*Sa*) and *Avicennia germinans* (*Ag*) and soil fungal spore density in *S. alterniflora* and *A. germinans* soils in the field surveys

Root or soil type	AM colonization (%)	DSE colonization (%)	Soil fungal spores (per g)
S. alterniflora	2.34 ± 0.85^{a}	8.00 ± 2.26^{a}	2.52 ± 0.23^{a}
A. germinans	1.14 ± 0.83^{a}	20.00 ± 5.29^{b}	8.20 ± 1.37^{b}

Note: Significant differences for each response variable from a paired *t* test ($p < .05$) are indicated by dissimilar letters ($n = 5$, $M \pm SE$).

Abbreviations: AM, arbuscular mycorrhizae; DSE, dark septate endophyte.

TABLE 2 AM and DSE colonization on *Avicennia germinans* (*Ag*) seedlings in pre-test group in different soil treatments after 3 months

Soil treatments	AM colonization (%)	DSE colonization (%)
Sa(*s*) + *Ag*(*s*)	0^{a}	0^{a}
Sa + *Ag*(*s*)	0^{a}	3.5 ± 1.07^{ab}
Sa(*s*) + *Ag*	4.20 ± 1.19^{b}	19.5 ± 1.17^{c}
Sa + *Ag*	0.40 ± 0.27^{a}	6.5 ± 1.07^{b}

Note: Significant differences among treatments for each response variable from Tukey's post hoc tests ($p < .05$) are indicated by dissimilar letters ($n = 10$, $M \pm SE$).

Abbreviations: AM, arbuscular mycorrhizae; DSE, dark septate endophyte; *Sa*, *Spartina alterniflora*; *Ag*, *Avicennia germinans*.

3.2.2 | Chlorophyll florescence

Before Freeze 1, Fv/Fm values were equivalent between freeze and control groups for all soil treatments (F1b in Table 3). After Freeze 1, there was a significant interactive effect of soil and freeze treatments on PHI ($F_{3,134} = 5.22$, $p = .002$, Table S2), but no differences in PHI among the control groups for all soil types (Figure 3A; Table S3); however, among the freeze groups, there was a significantly greater PHI for the mangrove seedlings in *Sa*(*s*) + *Ag*(*s*) soil (Figure 3A; Table S3). In this soil treatment, PHI was two times higher in the freeze group compared to the control. Those seedlings recovered by their next measurement (13 days later), and Fv/Fm ratios did not differ between freeze and control groups in all soil treatments before Freeze 2 (F2b in Table 3). After Freeze 2, there was a significant interactive effect of soil and freeze treatments on PHI ($F_{3,134} = 13.55$, $p < .001$, Table S2). The average PHI of seedlings in the freeze group was significantly higher in *Sa* + *Ag*(*s*)

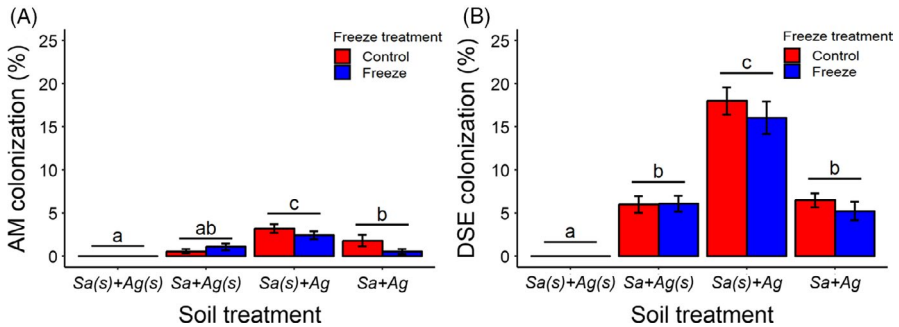

FIGURE 2 AM colonization (A) and DSE colonization (B) of *Avicennia germinans* seedlings in different soil treatments 2 weeks after Freeze 2 ($M \pm SE$). The four soil treatments are as follows: both steamed (*Sa*(*s*) + *Ag*(*s*)); live *Spartina alterniflora* (*Sa* + *Ag*(*s*)); live *A. germinans* (*Sa*(*s*) + *Ag*) and both live (*Sa* + *Ag*). Colour indicates two levels of the freeze treatment: Red: control group (no-freeze exposure); Blue: freeze group (freeze exposed). Dissimilar letters indicate significant differences among soil treatments from Tukey's honestly significant difference tests ($p < .05$). Tukey's tests are independent for each panel. *Ag*, *Avicennia germinans*; AM, arbuscular mycorrhizae; DSE, dark septate endophyte; *Sa*, *Spartina alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Result of two-way analysis of variance for the effects of soil and freeze treatments (control vs. freeze) on Fv/Fm of *Avicennia germinans* seedlings

		F1b		F1a		F2b		F2a	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Soil	3	0.41	.747	9.64	**<.001**	0.74	.528	3.04	**.031**
Freeze	1	0.99	.322	13.53	**<.001**	3.16	.078	42.44	**<.001**
Soil × freeze	3	1.08	.361	4.66	**<.001**	1.44	.235	7.86	**<.001**
Residuals	134								

Note: Boldface text indicates significance at the level of $p < .05$.

Abbreviations: Fv/Fm, variable to maximum fluorescence ratio; F1b, Fv/Fm measurement before Freeze 1; F1a, Fv/Fm measurement after Freeze 1; F2b, Fv/Fm measurement before Freeze 2; F2a, Fv/Fm measurement after Freeze 2.

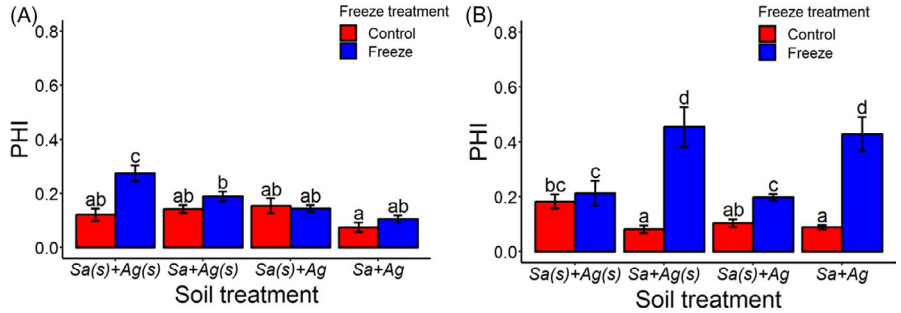

FIGURE 3 PHI of *Avicennia germinans* seedlings in different soil treatments across Freeze 1 (A) and Freeze 2 (B) ($M \pm SE$). The four soil treatments are as follows: both steamed (*Sa*(*s*) + *Ag*(*s*)); live *Spartina alterniflora* (*Sa* + *Ag*(*s*)); live *A. germinans* (*Sa*(*s*) + *Ag*) and both live (*Sa* + *Ag*). Colour indicates two levels of the freeze treatment: Red: control group (no-freeze exposure); Blue: freeze group (freeze exposed). Dissimilar letters within each panel indicate significant differences among soil and freeze treatment combinations from Tukey's honestly significant difference tests ($p < .05$). *Ag*, *Avicennia germinans*; PHI, photoinactivation; *Sa*, *Spartina alterniflora* [Colour figure can be viewed at wileyonlinelibrary.com]

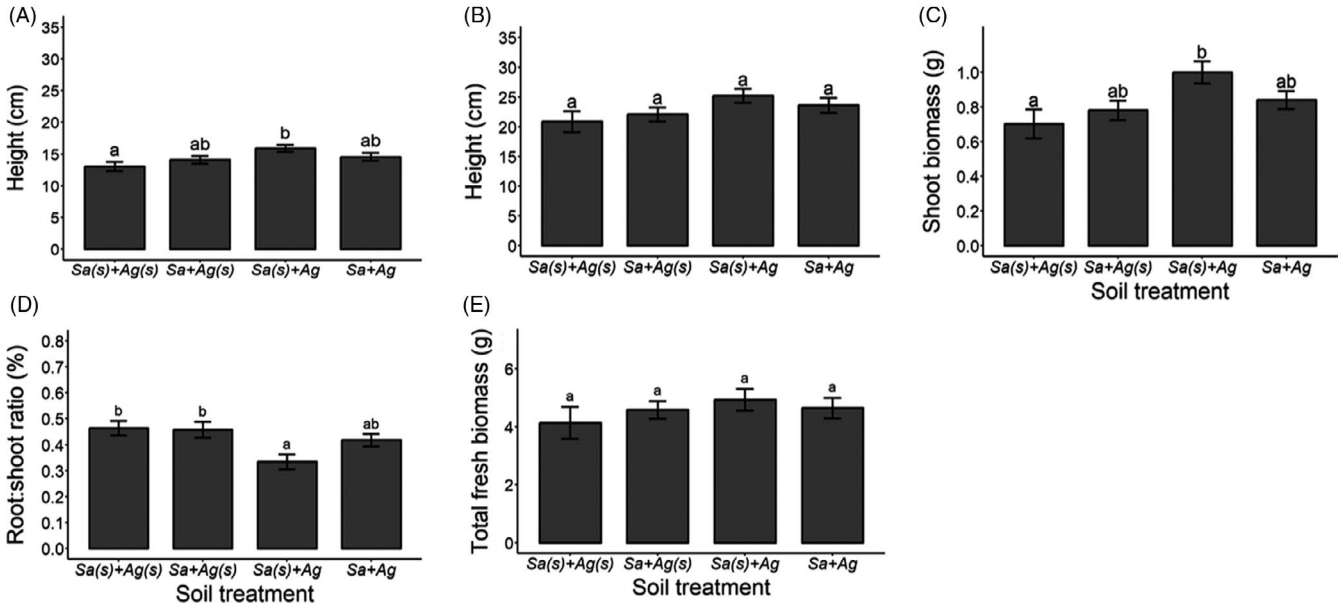

FIGURE 4 Height, biomass and root:shoot ratio of *Avicennia germinans* seedlings in different soil treatments ($M \pm SE$). Height (A) of *A. germinans* seedlings in different soil treatments after 3 months and before freeze treatment ($n = 183$), and height (B), dry shoot biomass (C), fresh root:shoot ratio (D) and total fresh biomass (E) of *A. germinans* seedlings in control group after 4 months (i.e. 2 weeks after Freeze 2) in different soil treatments ($n = 63$). The four soil treatments are as follows: both steamed (*Sa*(*s*) + *Ag*(*s*)); live *Spartina alterniflora* (*Sa* + *Ag*(*s*)); live *A. germinans* (*Sa*(*s*) + *Ag*) and both live (*Sa* + *Ag*). Dissimilar letters indicate significant differences among soil treatments from Tukey's honestly significant difference tests ($p < .05$) for each response variable. Tukey's tests are independent for each panel. *Ag*, *Avicennia germinans*; *Sa*, *Spartina alterniflora*

and *Sa* + *Ag* soils, and also showed the greatest increase in PHI between controls and freeze groups. There was no difference in PHI of seedlings between the control and freeze groups in *Sa*(*s*) + *Ag*(*s*) soil, and there was a significant but small increase in PHI between control and freeze groups in *Sa*(*s*) + *Ag* soil (Figure 3B; Table S3).

3.2.3 | Biomass and survival

Soil treatments significantly affected *A. germinans* seedling height after 3 months (before Freeze 1; $F_{3,179} = 3.57$, $p = .015$). Seedling height was highest in *Sa*(*s*) + *Ag* soil and lowest in *Sa*(*s*) + *Ag*(*s*) (Figure 4A; Table S4). After Freeze 2, we only measured the height and biomass in the control group because we had low survival in the freeze group. In the control group, soil treatments significantly affected dry shoot biomass ($F_{3,59} = 3.91$, $p = .013$; Figure 4C) and fresh root:shoot ratio ($F_{3,59} = 4.55$, $p = .006$; Figure 4D) of seedlings, but did not affect height ($F_{3,59} = 1.94$, $p = .133$; Figure 4B) or total fresh biomass ($F_{3,59} = 0.64$, $p = .593$; Figure 4E). Seedlings in *Sa*(*s*) + *Ag* soil had the highest dry shoot biomass and lowest fresh root to shoot ratio relative to the other treatments (Figure 4C,D; Table S4).

Before Freeze 1, there were no significant differences in seedling survival among soil treatments ($\chi^2 = 7.32$, $df = 3$, $p = .062$), with 87.5% of seedlings in *Sa*(*s*) + *Ag*(*s*) surviving, and 97.9% survival in *Sa* + *Ag*(*s*), *Sa*(s) + *Ag*, and *Sa* + *Ag* (Table S5). No seedlings died within 2 weeks after Freeze 1. After Freeze 2, mortality occurred only in the freeze treatment. In the freeze treatment, soil treatment significantly affected seedling survival ($\chi^2 = 40.91$, $df = 3$, $p < .001$). There was no difference in seedling survival between *Sa*(*s*) + *Ag*(*s*) (65%) and *Sa*(s) + *Ag* soil (50%), but survival in these two treatments was significantly greater than in *Sa* + *Ag*(*s*) and *Sa* + *Ag* soils where there was 0% survival (Table S5).

4 | DISCUSSION

Plant–soil interactions can facilitate or inhibit expanding species success (Andonian et al., 2011; Van Nuland, Bailey, & Schweitzer, 2017). During climate-driven range expansions, lost or novel interactions with mutualist or parasitic fungi can influence expanding species success (Dickie et al., 2017; Pringle et al., 2009), particularly when these interactions mediate plant stress responses to climatic variables (van der Putten, Bradford, Brinkman, van de Voorde, & Veen, 2016). We found that the expanding mangrove species, *A. germinans*, associates more with likely fungal mutualists relative to the resident salt marsh species, *S. alterniflora*. In our field survey, AM colonization was low for both *A. germinans* and *S. alterniflora*, but DSE colonization was more than two times greater on *A. germinans*, and fungal spore density in *A. germinans* soil was three times greater than that in *S. alterniflora* soil. Furthermore, *A. germinans*' association with soil mutualists declined in the presence of live *S. alterniflora* soil.

In our laboratory experiment, the steam sterilization process inactivated AM and DSE fungal spores, as indicated by the absence of fungal root colonization in *Sa*(*s*) + *Ag*(*s*). Across the other treatments, as in the field surveys, AM colonization was low, but was highest in live *A. germinans* soil combined with steamed *S. alterniflora* soil (*Sa*(*s*) + *Ag*). *A. germinans* seedlings also had the highest DSE levels in live *A. germinans* soil combined with steamed *S. alterniflora* soil (*Sa*(*s*) + *Ag*), and far lower levels of DSE in treatments with live *S. alterniflora* soil (Figure 2). The specific aspect of live *S. alterniflora* soil that depresses fungal colonization of *A. germinans* seedlings is not clear, but could likely be due to soil-associated pathogenic bacteria and fungi or allelopathic compounds. Nonetheless, our results suggest that steam sterilization negated the deleterious effects of *S. alterniflora* soil on AM and DSE colonization. Together, these findings indicate that live *S. alterniflora* soil may depress the association between *A. germinans* and AM, and especially, DSE fungi. Therefore, we suggest that as *A. germinans* expands into *S. alterniflora* communities, it has a weaker association with DSE fungi, which could initially limit *A. germinans* establishment. Previous studies showed that *S. alterniflora* has strong allelopathic effects on other plants (Wu, Chen, & Peng, 2006) and that fungal soil pathogens associated with *S. alterniflora* can depress its competitor, *Phragmites communis* (Li et al., 2014; Liang et al., 2016). In our study, depression of DSE root colonization by *S. alterniflora* seemingly provides some biotic resistance to mangrove expansion.

Mangrove ecosystems have productive and diverse microbial communities (Sahoo & Dhal, 2009), and mangrove-specific beneficial microbes can facilitate mangrove establishment and growth (Karthikeyan & Sivapriya, 2018). In the laboratory experiment, there was a positive correlation between DSE colonization and seedling performance. *A. germinans* seedlings growing in *Sa*(*s*) + *Ag* soil with the highest DSE colonization had the greatest shoot biomass relative to other soil treatments. DSE fungi can access sources of C, N, and P in detritus by producing arrays of hydrolytic enzymes (Mandyam & Jumpponen, 2005), which enable mangroves to obtain inorganic elements from organic matter and improves nutrient absorption efficiency. *A. germinans* seedlings with high DSE colonization also had low root:shoot ratios. Low root:shoot ratios suggest that DSE symbiosis reduces the burden of soil nutrient extraction from the root and that plants can invest more in above-ground material. In turn, increasing the distribution of materials and energy to above-ground growth facilitates sunlight acquisition and above-ground competition. We speculate that *A. germinans* that are initially expanding into salt marsh habitat will need to invest more in their root structures to obtain required nutrients because of low DSE colonization.

Freezing temperature is one of the primary limiting factors for mangrove poleward expansion (Saintilan et al., 2014; Stuart, Choat, Martin, Holbrook, & Ball, 2007), and plant–soil interactions could mediate plant responses to freezing stress. In our experiment, Freeze 1 did not induce mortality in any of the soil treatments. Seedlings exhibited relatively higher freeze tolerance overall compared to similar studies that measured *A. germinans* seedling

freeze tolerance in response to similar freeze regimes (McMillan & Sherrod, 1986; Pickens, 2011; Stevens, Fox, & Montague, 2006). One potential explanation for the differences could be variation in the location of studied mangrove populations. Specifically, the mangrove propagules that we collected from northern populations could be less susceptible to freezing conditions than southern populations due to selection or conditioning (Coldren & Proffitt, 2017; Hayes et al., 2020). Seedling age could also explain differences in survival. Previous work used 1-month-old seedlings to study the effects of freezing temperatures (Cook-Patton et al., 2015), which could be more sensitive than the 3- to 4-month-old seedlings we used. In our study, the unexpected tolerance of all seedlings to freezing temperatures necessitated the increased severity of Freeze 2.

PHI is a widely used indicator of plant stress responses that illuminated effects of the freeze and soil treatments on *A. germinans* (Ehlert & Hincha, 2008; Su, Dai, Li, & Xin, 2015; Yin et al., 2016). After Freeze 1, we observed the highest PHI levels in *Sa*(*s*) + *Ag*(*s*), which indicates that *A. germinans* seedlings were most stressed by freeze in the absence of any soil microbes (Figure 3). However, the stress from Freeze 1 was not strong enough to cause mortality. Interestingly, after Freeze 2, *A. germinans* seedlings in *Sa*(*s*) + *Ag*(*s*) soil shared the lowest PHI responses with *Sa*(*s*) + *Ag* and had the lowest seedling mortality. In contrast, seedlings in *Sa* + *Ag*(*s*) and *Sa* + *Ag* had high PHI values and then had complete mortality. The fact that seedlings in *Sa*(*s*) + *Ag*(*s*) had the highest relative stress response to Freeze 1, but the lowest stress response to Freeze 2 suggests that the sublethal exposure to freezing temperature during Freeze 1 could have had a positive acclimation effect. Many plants increase in freeze tolerance upon exposure to low temperature by activating the expression of certain cold-induced genes against freeze-induced injury (Hao et al., 2018; Kalapos et al., 2016; Thomashow, 1999; Xu, Zhang, Zhang, & Han, 2015). High PHI values in the *Sa*(*s*) + *Ag*(*s*) treatment during Freeze 1 could have triggered such protective responses.

Live *S. alterniflora* soil dramatically reduced freeze tolerance of *A. germinans* seedlings in Freeze 2. *Sa* + *Ag*(*s*) and *Sa* + *Ag* soil significantly increased PHI and reduced *A. germinans* seedling survivorship to zero (Figure 3B; Table S5). Low DSE colonization of *A. germinans* seedlings could have contributed to the poor freeze tolerance and low survivorship of these treatments. Although no study has directly examined the effects of DSE fungi on plant freeze tolerance, DSE are widespread in the roots of plants in cold-stressed environments (Treu, Laursen, Stephenson, Landolt, & Densmore, 1995; Väre et al., 1992), and they aid nutrient acquisition and stress modulation (Mandyam & Jumpponen, 2005). Although DSE colonization can help *A. germinans* survive, it is not necessary, since the steamed soil treatment (*Sa*(*s*) + *Ag*(*s*)) with no DSE had the best survivorship of all soil types. Perhaps in the absence of other *S. alterniflora* soil antagonists (i.e. when *S. alterniflora* is steamed), DSE is less essential. Poor survivorship in the soil treatments with live *S. alterniflora* and low DSE colonization suggest that other soil antagonists, such as allelochemicals or pathogenic bacteria or fungi, could be present and affecting freeze tolerance.

The negative effects of *S. alterniflora* soil could impede initial *A. germinans* colonization in *S. alterniflora* communities. Freeze events should be most severe on *A. germinans* at the northernmost, leading edge of the expansion (Stuart et al., 2007). In these areas, *A. germinans* seedlings are surrounded by *S. alterniflora* and its associated soil, which may have fewer beneficial mutualist microbes and more antagonistic properties due to their differences in soil microbial communities (Barreto et al., 2018). Mild winters give *A. germinans* a chance to survive with minimal mutualist assistance and less opportunity for antagonists to accentuate freeze stress. As *A. germinans* displaces *S. alterniflora*, the resulting switch in the plants' relative abundance should increasingly benefit *A. germinans* due to increased proximity to beneficial mutualists and dilution of the inhibitory factors associated with *S. alterniflora* soil that will thereby improve *A. germinans* growth and freeze tolerance. Several mild winters in a row could thus provide a 'window of opportunity' for *A. germinans* to accentuate such a positive plant–soil feedback (Balke, Herman, & Bouma, 2014), which could increase its population growth within an invaded site. Indeed, the direction and intensity of plant–soil feedbacks can shift over the course of a species invasion (Inderjit & Cahill, 2015). This scenario and the primacy of freeze resistance are consistent with studies that suggest that mangrove expansion along the southeastern coast of the United States is driven by decreased frequency of extreme cold events, not increased mean annual temperature (Cavanaugh et al., 2014; Osland et al., 2017).

In summary, we used the climate-driven range expansion of mangroves into salt marshes along the Atlantic coast of the United States to examine changes in plant–soil interactions and freeze tolerance. Our results suggest that the plant–soil interactions that affect plant stress responses can facilitate or inhibit climate-mediated range expansions. In particular, we found that resident *S. alterniflora* can inhibit *A. germinans* expansion by decreasing *A. germinans* seedling freeze tolerance and depressing DSE colonization. However, as the climate warms and *A. germinans* increasingly displaces *S. alterniflora* a positive plant soil feedback could accelerate *A. germinans* expansion into marshes. Previous work suggests that the balance between mutualists and pathogenic soil microorganisms can determine plant invasion trajectories (Callaway, Thelen, Rodriguez, & Holben, 2004; Inderjit & Cahill, 2015; Knevel et al., 2004). Our work demonstrates that it is also essential to consider plant–soil interactions in the context of climate variables that mediate expansion. Soil constituents can inhibit or facilitate climate-driven plant expansions by altering plant stress tolerance to freezing.

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AUTHORS' CONTRIBUTIONS

E.C., J.E.B., R.S.S. and S.P. conceived the ideas and designed the study. E.C., J.A.B. and R.S.S. performed the research and collected the data. E.C. and R.S.S. analysed the output data. E.C. and J.E.B. led the writing of the manuscript. All authors contributed substantially to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.vmcvdncp3 (Chen et al. 2020).

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REFERENCES

Andonian, K., Hierro, J. L., Khetsuriani, L., Becerra, P., Janoyan, G., Villarreal, D., … Callaway, R. M. (2011). Range-expanding populations of a globally introduced weed experience negative plant-soil feedbacks. *PLoS ONE*, *6*, 8. https://doi.org/10.1371/journal.pone.0020117

Aroca, R., Porcel, R., & Ruiz-Lozano, J. M. (2007). How does arbuscular mycorrhizal symbiosis regulate root hydraulic properties and plasma membrane aquaporins in *Phaseolus vulgaris* under drought, cold or salinity stresses? *New Phytologist*, *173*, 808–816. https://doi.org/10.1111/j.1469-8137.2006.01961.x

Baker, K. F., & Chandler, P. A. (1957). *UC system for producing healthy container-grown plants*. Oakland, CA: University of California, Division of Agricultural Sciences.

Balke, T., Herman, P. M. J., & Bouma, T. J. (2014). Critical transitions in disturbance-driven ecosystems: Identifying Windows of opportunity for recovery. *Journal of Ecology*, *102*, 700–708. https://doi.org/10.1111/1365-2745.12241

Barreto, C. R., Morrissey, E., Wykoff, D., & Chapman, S. (2018). Co-occurring mangroves and salt marshes differ in microbial community composition. *Wetlands*, *38*, 497–508. https://doi.org/10.1007/s13157-018-0994-9

Barrow, J., & Osuna, P. (2002). Phosphorus solubilization and uptake by dark septate fungi in fourwing saltbush, *Atriplex canescens* (Pursh) Nutt. *Journal of Arid Environments*, *51*, 449–460. https://doi.org/10.1006/jare.2001.0925

Bates, D., Machler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Blois, J. L., Zarnetske, P. L., Fitzpatrick, M. C., & Finnegan, S. (2013). Climate change and the past, present, and future of biotic interactions. *Science*, *341*, 499–504. https://doi.org/10.1126/science.1237184

Bollen, G. (1985). *Lethal temperatures of soil fungi. Ecology and management of soil-borne plant pathogens*. St. Paul, MN: The American Phytopathological Society.

Bray, S. R., Kitajima, K., & Sylvia, D. M. (2003). Mycorrhizae differentially alter growth, physiology, and competitive ability of an invasive shrub. *Ecological Applications*, *13*, 565–574. https://doi.org/10.1890/1051-0761(2003)013[0565:Mdagpa]2.0.Co;2

Callaway, R. M., Montesinos, D., Williams, K., & Maron, J. L. (2013). Native congeners provide biotic resistance to invasive *Potentilla* through soil biota. *Ecology*, *94*, 1223–1229. https://doi.org/10.1890/12-1875.1

Callaway, R. M., Thelen, G. C., Rodriguez, A., & Holben, W. E. (2004). Soil biota and exotic plant invasion. *Nature*, *427*, 731–733. https://doi.org/10.1038/nature02322

Canning-Clode, J., Fowler, A. E., Byers, J. E., Carlton, J. T., & Ruiz, G. M. (2011). 'Caribbean Creep' chills out: Climate change and marine invasive species. *PLoS ONE*, *6*, e29657. https://doi.org/10.1371/journal.pone.0029657

Cavanaugh, K. C., Kellner, J. R., Forde, A. J., Gruner, D. S., Parker, J. D., Rodriguez, W., & Feller, I. C. (2014). Poleward expansion of mangroves is a threshold response to decreased frequency of extreme cold events. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 723–727. https://doi.org/10.1073/pnas.1315800111

Chen, E., Blaze, J. A., Smith, R. S., Peng, S., & Byers, J. E. (2020). Data from: Freeze-tolerance of poleward-spreading mangrove species weakened by soil properties of resident salt marsh competitor. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.vmcvdncp3

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*, 1024–1026. https://doi.org/10.1126/science.1206432

Coldren, G. A., Barreto, C. R., Wykoff, D. D., Morrissey, E. M., Langley, J. A., Feller, I. C., & Chapman, S. K. (2016). Chronic warming stimulates growth of marsh grasses more than mangroves in a coastal wetland ecotone. *Ecology*, *97*, 3167–3175. https://doi.org/10.1002/ecy.1539

Coldren, G. A., & Proffitt, C. E. (2017). Mangrove seedling freeze tolerance depends on salt marsh presence, species, salinity, and age. *Hydrobiologia*, *803*, 159–171. https://doi.org/10.1007/s10750-017-3175-6

Cooke, J. C., & Lefor, M. W. (1990). Comparison of vesicular-arbuscular mycorrhizae in plants from disturbed and adjacent undisturbed regions of a coastal salt marsh in Clinton, Connecticut, USA. *Environmental Management*, *14*, 131–137. https://doi.org/10.1007/BF02394027

Cook-Patton, S. C., Lehmann, M., & Parker, J. D. (2015). Convergence of three mangrove species towards freeze-tolerant phenotypes at an expanding range edge. *Functional Ecology*, *29*, 1332–1340. https://doi.org/10.1111/1365-2435.12443

da Silva, I. R., da Silva, D. K. A., de Souza, F. A., Oehl, F., & Maia, L. C. (2017). Changes in arbuscular mycorrhizal fungal communities along a river delta island in northeastern Brazil. *Acta Oecologica-International Journal of Ecology*, *79*, 8–17. https://doi.org/10.1016/j.actao.2016.12.011

Dickie, I. A., Bufford, J. L., Cobb, R. C., Desprez-Loustau, M.-L., Grelet, G., Hulme, P. E., … Williams, N. M. (2017). The emerging science of linked plant–fungal invasions. *New Phytologist*, *215*, 1314–1332. https://doi.org/10.1111/nph.14657

Doughty, C. L., Langley, J. A., Walker, W. S., Feller, I. C., Schaub, R., & Chapman, S. K. (2016). Mangrove range expansion rapidly increases coastal wetland carbon storage. *Estuaries and Coasts*, *39*, 385–396. https://doi.org/10.1007/s12237-015-9993-8

Ehlert, B., & Hincha, D. K. (2008). Chlorophyll fluorescence imaging accurately quantifies freezing damage and cold acclimation responses in *Arabidopsis* leaves. *Plant Methods*, *4*, 12. https://doi.org/10.1186/1746-4811-4-12

Garg, N., & Chandel, S. (2010). Arbuscular mycorrhizal networks: Process and functions. A review. *Agronomy for Sustainable Development*, *30*, 581–599. https://doi.org/10.1051/agro/2009054

Gehring, C. A., Sthultz, C. M., Flores-Renteria, L., Whipple, A. V., & Whitham, T. G. (2017). Tree genetics defines fungal partner communities that may confer drought tolerance. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, 11169–11174. https://doi.org/10.1073/pnas.1704022114

Gelman, A., Jakulin, A., Pittau, M. G., & Su, Y. S. (2008). A weakly informative default prior distribution for logistic and other regression models. *Annals of Applied Statistics*, *2*, 1360–1383. https://doi.org/10.1214/08-aoas191

Gelman, A., & Su, Y. S. (2018). *arm: Data analysis using regression and multilevel/hierarchical models*. *R package version 1.10-1*. Retrieved from https://CRAN.R-project.org/package=arm

Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community interactions under climate change. *Trends in Ecology & Evolution*, *25*, 325–331. https://doi.org/10.1016/j.tree.2010.03.002

Giovannetti, M., & Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, *84*, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

Gribben, P. E., Nielsen, S., Seymour, J. R., Bradley, D. J., West, M. N., & Thomas, T. (2017). Microbial communities in marine sediments modify success of an invasive macrophyte. *Scientific Reports*, *7*, 8. https://doi.org/10.1038/s41598-017-10231-2

Guo, H., Zhang, Y., Lan, Z., & Pennings, S. C. (2013). Biotic interactions mediate the expansion of black mangrove (*Avicennia germinans*) into salt marshes under climate change. *Global Change Biology*, *19*, 2765–2774. https://doi.org/10.1111/gcb.12221

Hajiboland, R., Joudmand, A., Aliasgharzad, N., Tolra, R., & Poschenrieder, C. (2019). Arbuscular mycorrhizal fungi alleviate low-temperature stress and increase freezing resistance as a substitute for acclimation treatment in barley. *Crop & Pasture Science*, *70*, 218–233. https://doi.org/10.1071/cp18385

Hao, X., Wang, B., Wang, L., Zeng, J., Yang, Y., & Wang, X. (2018). Comprehensive transcriptome analysis reveals common and specific genes and pathways involved in cold acclimation and cold stress in tea plant leaves. *Scientia Horticulturae*, *240*, 354–368. https://doi.org/10.1016/j.scienta.2018.06.008

Hayes, M. A., Shor, A. C., Jesse, A., Miller, C., Kennedy, J. P., & Feller, I. (2020). The role of glycine betaine in range expansions; protecting mangroves against extreme freeze events. *Journal of Ecology*, *108*, 61–69. https://doi.org/10.1111/1365-2745.13243

HilleRisLambers, J., Harsch, M. A., Ettinger, A. K., Ford, K. R., & Theobald, E. J. (2013). How will biotic interactions influence climate change-induced range shifts? In A. L. Angert, S. L. LaDeau, & R. S. Ostfeld (Eds.), *Climate change and species interactions: Ways forward* (pp. 112–125). Oxford, UK: Blackwell Science Publishing.

Hoefnagels, M. H., Broome, S. W., & Shafer, S. R. (1993). Vesicular-arbuscular mycorrhizae in salt marshes in North Carolina. *Estuaries*, *16*, 851–858. https://doi.org/10.2307/1352444

Huner, N. P., Öquist, G., Hurry, V. M., Krol, M., Falk, S., & Griffith, M. (1993). Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosynthesis Research*, *37*, 19–39. https://doi.org/10.1007/BF02185436

Inderjit, & Cahill, J. F. (2015). Linkages of plant-soil feedbacks and underlying invasion mechanisms. *AoB Plants*, *7*, plv022. https://doi.org/10.1093/aobpla/plv022

Jumpponen, A. (2001). Dark septate endophytes—Are they mycorrhizal? *Mycorrhiza*, *11*, 207–211. https://doi.org/10.1007/s005720100112

Jumpponen, A., & Trappe, J. M. (1998). Dark septate endophytes: A review of facultative biotrophic root-colonizing fungi. *New Phytologist*, *140*, 295–310. https://doi.org/10.1046/j.1469-8137.1998.00265.x

Kalapos, B., Dobrev, P., Nagy, T., Vítámvás, P., Györgyey, J., Kocsy, G., … Galiba, G. (2016). Transcript and hormone analyses reveal the involvement of ABA-signalling, hormone crosstalk and genotype-specific biological processes in cold-shock response in wheat. *Plant Science*, *253*, 86–97. https://doi.org/10.1016/j.plantsci.2016.09.017

Karasawa, T., Hodge, A., & Fitter, A. H. (2012). Growth, respiration and nutrient acquisition by the arbuscular mycorrhizal fungus Glomus mosseae and its host plant *Plantago lanceolata* in cooled soil. *Plant Cell and Environment*, *35*, 819–828. https://doi.org/10.1111/j.1365-3040.2011.02455.x

Karthikeyan, A., & Sivapriya, N. B. (2018). Responses of *Bruguiera sexangula* propagules to beneficial microbes in the nursery. *Journal of Forestry Research*, *29*, 1093–1098. https://doi.org/10.1007/s11676-017-0502-8

Khanal, N., Bray, G. E., Grisnich, A., Moffatt, B. A., & Gray, G. R. (2017). Differential mechanisms of photosynthetic acclimation to light and low temperature in *Arabidopsis* and the extremophile *Eutrema salsugineum*. *Plants-Basel*, *6*, 29. https://doi.org/10.3390/plants6030032

Klironomos, J. N. (2003). Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, *84*, 2292–2301. https://doi.org/10.1890/02-0413

Knevel, I. C., Lans, T., Menting, F. B. J., Hertling, U. M., & van der Putten, W. H. (2004). Release from native root herbivores and biotic resistance by soil pathogens in a new habitat both affect the alien *Ammophila arenaria* in South Africa. *Oecologia*, *141*, 502–510. https://doi.org/10.1007/s00442-004-1662-8

Koch, M. S., & Snedaker, S. C. (1997). Factors influencing *Rhizophora mangle* L. seedling development in Everglades carbonate soils. *Aquatic Botany*, *59*, 87–98. https://doi.org/10.1016/S0304-3770(97)00027-2

Kothamasi, D., Kothamasi, S., Bhattacharyya, A., Kuhad, R. C., & Babu, C. (2006). Arbuscular mycorrhizae and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar island, India. *Biology and Fertility of Soils*, *42*, 358–361. https://doi.org/10.1007/s00374-005-0035-8

Kotilínek, M., Hiiesalu, I., Košnar, J., Šmilauerová, M., Šmilauer, P., Altman, J., … Doležal, J. (2017). Fungal root symbionts of high-altitude vascular plants in the Himalayas. *Scientific Reports*, *7*, 14. https://doi.org/10.1038/s41598-017-06938-x

Li, H., Zhang, X. M., Zheng, R. S., Li, X., Elmer, W. H., Wolfe, L. M., & Li, B. (2014). Indirect effects of non-native *Spartina alterniflora* and its fungal pathogen (*Fusarium palustre*) on native saltmarsh plants in China. *Journal of Ecology*, *102*, 1112–1119. https://doi.org/10.1111/1365-2745.12285

Liang, M., Liu, X., Etienne, R. S., Huang, F., Wang, Y., & Yu, S. (2015). Arbuscular mycorrhizal fungi counteract the Janzen-Connell effect of soil pathogens. *Ecology*, *96*, 562–574. https://doi.org/10.1890/14-0871.1.sm

Liang, X., He, C., Zhu, Xi'e, Chen, X., Lei, Y., Zhang, H., … Qi, X. (2016). Effect of exotic *Spartina alterniflora* on fungal symbiosis with native plants *Phragmites australis* and *Scirpus mariqueter*, and model plants *Lolium perenne* L. and *Trifolium repens*. *Aquatic Botany*, *130*, 50–58. https://doi.org/10.1016/j.aquabot.2015.10.003

Lovelock, C. E., Ball, M. C., Choat, B., Engelbrecht, B. M., Holbrook, N. M., & Feller, I. C. (2006). Linking physiological processes with mangrove forest structure: Phosphorus deficiency limits canopy development, hydraulic conductivity and photosynthetic carbon gain in dwarf *Rhizophora mangle*. *Plant, Cell & Environment*, *29*, 793–802. https://doi.org/10.1111/j.1365-3040.2005.01446.x

Lovelock, C. E., Ball, M. C., Feller, I. C., Engelbrecht, B. M., & Ling Ewe, M. (2006). Variation in hydraulic conductivity of mangroves: Influence of species, salinity, and nitrogen and phosphorus availability. *Physiologia Plantarum*, *127*, 457–464. https://doi.org/10.1111/j.1399-3054.2006.00723.x

Mandyam, K., & Jumpponen, A. (2005). Seeking the elusive function of the root-colonising dark septate endophytic fungi. *Studies in Mycology*, *53*, 173–189. https://doi.org/10.3114/sim.53.1.173

Mandyam, K. G., & Jumpponen, A. (2015). Mutualism-parasitism paradigm synthesized from results of root-endophyte models. *Frontiers in Microbiology*, *5*. https://doi.org/10.3389/fmicb.2014.00776

McHugh, J. M., & Dighton, J. (2004). Influence of mycorrhizal inoculation, inundation period, salinity, and phosphorus availability on

the growth of two salt marsh grasses, *Spartina alterniflora* Lois. and *Spartina cynosuroides* (L.) Roth., in nursery systems. *Restoration Ecology*, *12*, 533–545. https://doi.org/10.1111/j.1061-2971.2004.03109.x

McMillan, C., & Sherrod, C. L. (1986). The chilling tolerance of black mangrove, *Avicennia germinans*, from the Gulf of Mexico coast of Texas, Louisiana and Florida. *Contributions in Marine Science*, *29*, 9–16.

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., … Vazquez, D. P. (2006). Biotic interactions and plant invasions. *Ecology Letters*, *9*, 726–740. https://doi.org/10.1111/j.1461-0248.2006.00908.x

Moore, A. D. (2016). *Abiotic and biotic context influence species interactions in coastal vegetated ecosystems*. Doctoral dissertation, Northeastern University, Boston, MA.

Nadelhoffer, K. J., Shaver, G. R., Giblin, A., & Rastetter, E. B. (1997). Potential impacts of climate change on nutrient cycling, decomposition, and productivity in Arctic ecosystems. In W. C. Oechel, T. V. Callaghan, T. G. Gilmanov, J. I. Holten, B. Maxwell, & U. Molau (Eds.), *Ecological studies; global change and Arctic terrestrial ecosystems* (pp. 349–364). New York, NY: Springer-Verlag.

Newsham, K. K. (2011). A meta-analysis of plant responses to dark septate root endophytes. *New Phytologist*, *190*, 783–793. https://doi.org/10.1111/j.1469-8137.2010.03611.x

Osland, M. J., Enwright, N., Day, R. H., & Doyle, T. W. (2013). Winter climate change and coastal wetland foundation species: Salt marshes vs. mangrove forests in the southeastern United States. *Global Change Biology*, *19*, 1482–1494. https://doi.org/10.1111/gcb.12126

Osland, M. J., Feher, L. C., Griffith, K. T., Cavanaugh, K. C., Enwright, N. M., Day, R. H., … Rogers, K. (2017). Climatic controls on the global distribution, abundance, and species richness of mangrove forests. *Ecological Monographs*, *87*, 341–359. https://doi.org/10.1002/ecm.1248

Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I.-C., … Williams, S. E. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*, *355*, eaai9214. https://doi.org/10.1126/science.aai9214

Perez, F., Hinojosa, L. F., Ossa, C. G., Campano, F., & Orrego, F. (2014). Decoupled evolution of foliar freezing resistance, temperature niche and morphological leaf traits in *Chilean Myrceugenia*. *Journal of Ecology*, *102*, 972–980. https://doi.org/10.1111/1365-2745.12261

Perry, C. L., & Mendelssohn, I. A. (2009). Ecosystem effects of expanding populations of *Avicennia germinans* in a Louisiana salt marsh. *Wetlands*, *29*, 396–406. https://doi.org/10.1672/08-100.1

Phillips, J. M., & Hayman, D. (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-IN18. https://doi.org/10.1016/S0007-1536(70)80110-3

Pickens, C. N. (2012). *Influence of climate change on the ecophysiology and restoration ecology of black mangrove* (Avicennia germinans *(L.) L.)*. Lafayette, LA: University of Louisiana at Lafayette.

Pickens, C. N., & Hester, M. W. (2011). Temperature tolerance of early life history stages of black mangrove *Avicennia germinans*: Implications for range expansion. *Estuaries & Coasts*, *34*, 824–830. https://doi.org/10.1007/s12237-010-9358-2

Pringle, A., Bever, J. D., Gardes, M., Parrent, J. L., Rillig, M. C., & Klironomos, J. N. (2009). Mycorrhizal symbioses and plant invasions. *Annual Review of Ecology Evolution and Systematics*, *40*, 699–715. https://doi.org/10.1146/annurev.ecolsys.39.110707.173454

Purnobasuki, H., & Suzuki, M. (2004). Aerenchyma formation and porosity in root of a mangrove plant, *Sonneratia alba* (Lythraceae). *Journal of Plant Research*, *117*, 465–472. https://doi.org/10.1007/s10265-004-0181-3

R Development Core Team. (2017) *R 3.3.4. R project for statistical computing*. Retrieved from http://www.r-project.org

Ramirez-Viga, T. K., Aguilar, R., Castillo-Arguero, S., Chiappa-Carrara, X., Guadarrama, P., & Ramos-Zapata, J. (2018). Wetland plant species improve performance when inoculated with arbuscular mycorrhizal fungi: A meta-analysis of experimental pot studies. *Mycorrhiza*, *28*, 477–493. https://doi.org/10.1007/s00572-018-0839-7

Reinhart, K. O., & Callaway, R. M. (2006). Soil biota and invasive plants. *New Phytologist*, *170*, 445–457. https://doi.org/10.1111/j.1469-8137.2006.01715.x

Richardson, D. M., Allsopp, N., D'Antonio, C. M., Milton, S. J., & Rejmanek, M. (2000). Plant invasions—The role of mutualisms. *Biological Reviews*, *75*, 65–93. https://doi.org/10.1017/s0006323199005435

Rinella, M. J., & Reinhart, K. O. (2018). Toward more robust plant-soil feedback research. *Ecology*, *99*, 550–556. https://doi.org/10.1002/ecy.2146

Robinson, C. H. (2001). Cold adaptation in Arctic and Antarctic fungi. *New Phytologist*, *151*, 341–353. https://doi.org/10.1046/j.1469-8137.2001.00177.x

Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., … Redman, R. S. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *The ISME Journal*, *2*, 404. https://doi.org/10.1038/ismej.2007.106

Runia, W., & Molendijk, L. (2010). Physical methods for soil disinfestation in intensive agriculture: Old methods and new approaches. *Acta Horticulture*, *883*, 249–258. https://doi.org/10.17660/ActaHortic.2010.883.31

Sahoo, K., & Dhal, N. (2009). Potential microbial diversity in mangrove ecosystems: A review. *Indian Journal of Marine Sciences*, *38*, 249–256.

Saintilan, N., Wilson, N. C., Rogers, K., Rajkaran, A., & Krauss, K. W. (2014). Mangrove expansion and salt marsh decline at mangrove poleward limits. *Global Change Biology*, *20*, 147–157. https://doi.org/10.1111/gcb.12341

Schmidt, S. K., Sobieniak-Wiseman, L. C., Kageyama, S. A., Halloy, S. R. P., & Schadt, C. W. (2008). Mycorrhizal and dark-septate fungi in plant roots above 4270 meters elevation in the Andes and Rocky Mountains. *Arctic Antarctic and Alpine Research*, *40*, 576–583. https://doi.org/10.1657/1523-0430(07-068)[schmidt]2.0.Co;2

Sierra-Almeida, A., Cavieres, L. A., & Bravo, L. A. (2009). Freezing resistance varies within the growing season and with elevation in high-Andean species of central Chile. *New Phytologist*, *182*, 461–469. https://doi.org/10.1111/j.1469-8137.2008.02756.x

Simpson, L. T., Osborne, T. Z., & Feller, I. C. (2017). Establishment and biomass allocation of black and red mangroves: Response to propagule flotation duration and seedling light availability. *Journal of Coastal Research*, *33*, 1126–1134. https://doi.org/10.2112/jcoastres-d-16-00108.1

Singh, L. P., Gill, S. S., & Tuteja, N. (2011). Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signaling & Behavior*, *6*, 175–191. https://doi.org/10.4161/psb.6.2.14146

Smith, R. S., Blaze, J. A., Osborne, T. Z., & Byers, J. E. (2018). Facilitating your replacement? Ecosystem engineer legacy affects establishment success of an expanding competitor. *Oecologia*, *188*, 251–262. https://doi.org/10.1007/s00442-018-4184-5

Stevens, P. W., Fox, S. L., & Montague, C. L. (2006). The interplay between mangroves and saltmarshes at the transition between temperate and subtropical climate in Florida. *Wetlands Ecology and Management*, *14*, 435–444. https://doi.org/10.1007/s11273-006-0006-3

Stuart, S. A., Choat, B., Martin, K. C., Holbrook, N. M., & Ball, M. C. (2007). The role of freezing in setting the latitudinal limits of mangrove forests. *New Phytologist*, *173*, 576–583. https://doi.org/10.1111/j.1469-8137.2006.01938.x

Su, L., Dai, Z., Li, S., & Xin, H. (2015). A novel system for evaluating drought–cold tolerance of grapevines using chlorophyll fluorescence. *BMC Plant Biology*, *15*, 82. https://doi.org/10.1186/s12870-015-0459-8

Teste, F. P., Kardol, P., Turner, B. L., Wardle, D. A., Zemunik, G., Renton, M., & Laliberte, E. (2019). Toward more robust plant-soil feedback research: Comment. *Ecology*, *100*, e02590. https://doi.org/10.1002/ecy.2590

Thomashow, M. F. (1999). Plant cold acclimation: Freezing tolerance genes and regulatory mechanisms. *Annual Review of Plant Biology*, *50*, 571–599. https://doi.org/10.1146/annurev.arplant.50.1.571

Treu, R., Laursen, G., Stephenson, S., Landolt, J., & Densmore, R. (1995). Mycorrhizae from Denali national park and preserve, Alaska. *Mycorrhiza*, *6*, 21–29. https://doi.org/10.1007/s005720050101

van der Putten, W. H., Bradford, M. A., Brinkman, E. P., van de Voorde, T. F. J., & Veen, G. F. (2016). Where, when and how plant-soil feedback matters in a changing world. *Functional Ecology*, *30*, 1109–1121. https://doi.org/10.1111/1365-2435.12657

Van Nuland, M. E., Bailey, J. K., & Schweitzer, J. A. (2017). Divergent plant-soil feedbacks could alter future elevation ranges and ecosystem dynamics. *Nature Ecology & Evolution*, *1*, 10. https://doi.org/10.1038/s41559-017-0150

Väre, H., Vestberg, M., & Eurola, S. (1992). Mycorrhiza and root-associated fungi in Spitsbergen. *Mycorrhiza*, *1*, 93–104. https://doi.org/10.1007/BF00203256

Wang, G.-W., & Li, H.-Y. (2003). Primary investigation on radicular endophytes of mangrove at Qinzhou Bay in Guangxi. *Guangxi Forestry Science*, *3*, 2.

Wang, Y. T., Qiu, Q., Yang, Z. Y., Hu, Z. J., Tam, N. F. Y., & Xin, G. R. (2010). Arbuscular mycorrhizal fungi in two mangroves in South China. *Plant and Soil*, *331*, 181–191. https://doi.org/10.1007/s11104-009-0244-2

Warcup, J. (1951). Effect of partial sterilization by steam or formalin on the fungus flora of an old forest nursery soil. *Transactions of the British Mycological Society*, *34*, 519–532. https://doi.org/10.1016/S0007-1536(51)80036-6

West Virginia University, Davis College of Agriculture. (2017). *Extraction of spores*. Retrieved from http://invam.wvu.edu/methods/spores/spore-extraction

Wisz, M. S., Pottier, J., Kissling, W. D., Pellissier, L., Lenoir, J., Damgaard, C. F., … Svenning, J.-C. (2013). The role of biotic interactions in shaping distributions and realised assemblages of species: Implications for species distribution modelling. *Biological Reviews*, *88*, 15–30. https://doi.org/10.1111/j.1469-185X.2012.00235.x

Wu, J. R., Chen, Z. Q., & Peng, S. L. (2006). Allelopathic potential of invasive weeds: *Alternanthera philoxeroide*, *Ipomoea cairica* and *Spartina alterniflora*. *Allelopathy Journal*, *17*, 279–285.

Xi, N. X., Chu, C. J., & Bloor, J. M. G. (2018). Plant drought resistance is mediated by soil microbial community structure and soil-plant feedbacks in a savanna tree species. *Environmental and Experimental Botany*, *155*, 695–701. https://doi.org/10.1016/j.envexpbot.2018.08.013

Xu, L. X., Zhang, M. L., Zhang, X. Z., & Han, L. B. (2015). Cold acclimation treatment-induced changes in abscisic acid, cytokinin, and antioxidant metabolism in zoysiagrass (*Zoysia japonica*). *HortScience*, *50*, 1075–1080. https://doi.org/10.21273/HORTSCI.50.7.1075

Yang, W., Jeelani, N., Leng, X., Cheng, X., & An, S. (2016). *Spartina alterniflora* invasion alters soil microbial community composition and microbial respiration following invasion chronosequence in a coastal wetland of China. *Scientific Reports*, *6*, 26880. https://doi.org/10.1038/srep26880

Yin, Y., Ma, Q.-P., Zhu, Z.-X., Cui, Q.-Y., Chen, C.-S., Chen, X., … Li, X.-H. (2016). Functional analysis of CsCBF3 transcription factor in tea plant (*Camellia sinensis*) under cold stress. *Plant Growth Regulation*, *80*, 335–343. https://doi.org/10.1007/s10725-016-0172-0

Zhou, Z. Z., Ma, H. M., Liang, K. N., Huang, G. H., & Pinyopusarerk, K. (2012). Improved tolerance of teak (*Tectona grandis* L.f.) seedlings to low-temperature stress by the combined effect of arbuscular mycorrhiza and paclobutrazol. *Journal of Plant Growth Regulation*, *31*, 427–435. https://doi.org/10.1007/s00344-011-9252-6

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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