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Microbial mediation of salinity stress response varies by plant genotype and provenance over time

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Abstract

Although it is becoming widely appreciated that microbes can enhance plant tolerance to environmental stress, the nature of microbial mediation of exposure responses is not well understood. We addressed this deficit by examining whether microbial mediation of plant responses to elevated salinity is contingent on the environment and factors intrinsic to the host. We evaluated the influence of contrasting environmental conditions relative to host genotype, provenance and evolution by conducting a common-garden experiment utilizing ancestral and descendant cohorts of *Schoenoplectus americanus* genotypes recovered from two 100+ year coastal marsh seed banks. We compared *S. americanus* productivity and trait variation as well as associated endophytic microbial communities according to plant genotype, provenance, and age cohort under high and low salinity stress with and without native soil inoculation. The magnitude and direction of microbial mediation of *S. americanus* responses to elevated salinity varied according to individual genotype, provenance, as well as temporal shifts in genotypic variation and G×E (gene by environment) interactions. Relationships differed between plant traits and the structure of endosphere communities. Our findings indicate that plant-microbe associations and microbial mediation of plant stress are not only context-dependent but also dynamic. Our results additionally suggest that evolution can shape the fate of marsh ecosystems by altering how microbes confer plant tolerance to pressures linked to global change.

KEYWORDS
coastal marsh, endophytes, plant-microbe interaction, resurrection ecology, *Schoenoplectus americanus*, soil microbes

1 | INTRODUCTION

Ongoing global environmental change circumscribes conditions such as a warming climate and sea level rise that are presenting new or more intense challenges to plants worldwide. There is a growing body of evidence suggesting that endophytic symbionts (i.e., microbes inhabiting plant tissues) and associations with soil microbes can confer greater capacity to cope with global change (e.g., Lau & Lennon, 2012; Porter et al., 2020). Colonization of fungal and bacterial endophytes can, for example, increase plant tolerance to salinity stress (Gupta et al., 2021; Rodriguez et al., 2008). Similarly, soil microbes can promote greater plant growth (Kearl et al., 2019) and accelerate flowering phenology (Wagner et al., 2014). Yet microbial mediation of stress appears to be contingent on a range of factors that influence the formation and persistence of associations with plants (Gehring et al., 2017; Rodriguez et al., 2019)

including environmental factors and heritable variation in plant hosts (Gonzalez Mateu et al., 2020; Lumibao et al., 2020). This contrast raises the possibility that outcomes of microbial associations might range from physiological acclimation to constitutive adaption of plants to abiotic stressors. Additional insight could be gained by conducting experiments designed to concurrently determine how plant performance varies according to relationships with microbial associates; and how different factors influence microbial associations and thus potential mediation of stress responses (Kellenberger et al., 2018; Suter & Widmer, 2013). Insight about the balance of underlying ecological and evolutionary mechanisms might also be gained by examining the nature of plant-microbial associations over space and time and potential mediation of stress response (hereafter, microbial mediation).

There is good reason to think that the formation and persistence of plant-microbe associations are subject to prevailing environmental conditions. For example, if favourable to a host under a particular regime, plant-microbe associations might arise and persist, even over successive generations (i.e., that span a period of relative environmental constancy) (Vannier et al., 2018; but see Rezki et al., 2018). Accordingly, associations might shift with environmental change. Associations might, for example, become beneficial or symbiotic under environmental stress (i.e., facilitation) compared to more benign conditions where competition is expected to prevail (sensu the stress gradient hypothesis), although this can depend on life-history-stage of the plant (David et al., 2020). Shifts might also arise because microbial communities and host plants differ in response to environmental change (Lau & Lennon, 2012; Whittle et al., 2021). While both scenarios are plausible, neither has been well tested, largely because of the challenges involved with tracking plant-microbe associations over time. As a consequence, most studies to date have relied on space-for-time substitutions (e.g., Lau & Suwa, 2016) that may not accurately convey the dynamics of plant-microbe associations such as the magnitude or rate of change over time.

The nature of plant-microbe associations can also be contingent on constitutive biotic factors like plant genotype (Bowen et al., 2017; Gehring et al., 2017) as well as heritable variation in functional traits that corresponds to plant genotype (Lumibao et al., 2020; Torres-Martínez et al., 2021). Both genotypic and trait variation can – but does not always (terHorst et al., 2014) – differ by provenance (Bernik et al., 2018, 2020), which can result from adaptation to local environmental conditions including in situ soil microbial communities (Schultz et al., 2001; Young et al., 2018). Microbial associations can also reflect genetic drift or historical contingency, where priority effects dictate local occurrence and composition of soil microbial communities that may colonize host plants. Priority effects may be dampened or exacerbated, however, depending on whether microbial communities are influenced by plant host (Lumibao et al., 2020). Thus consideration should be given to abiotic and constitutive biotic factors, including host provenance (i.e., population origin reflecting both "native" or in situ soil microbial communities and site variations) and evolution (i.e., shifts in the genetic composition of host populations over time), when evaluating whether and how plant-associated microbes confer greater tolerance to environmental stress (Rúa et al., 2018).

In this study, we examined the performance of the foundational sedge *Schoenoplectus americanus* according to variation in associations with root endophytes (microbes living inside root tissues). We focused on *S. americanus* in part because it dominates brackish marshes across the Atlantic and Gulf coasts of North America, where it can govern vital ecosystem processes like carbon cycling and accretion. Prior studies also have demonstrated that *S. americanus* can be "resurrected" from century-long soil-stored seed banks (Summers et al., 2018; Vahsen et al., 2021), and that plants originating from the early 20th century exhibit different heritable responses to salinity and atmospheric carbon dioxide (CO_2) relative to descendants (Blum et al., 2021; Gentile, 2015). Building on these findings, we evaluated the potential for microbial mediation of salinity stress by conducting a common-garden experiment using ancestral and descendant cohorts of *S. americanus* from two Chesapeake Bay marshes. We evaluated productivity and phenotypic trait variation within and among age cohorts of *S. americanus* genotypes from both marshes (i.e., source populations) under (1) high and low salinity exposure, and (2) with and without native soil microbial inoculant. We examined root endophytes because prior work indicates that the community is responsive to environmental factors (e.g., Kandalepas et al., 2015) and conditions intrinsic to plant hosts (Naylor et al., 2017). We elected to focus on salinity stress because brackish marsh ecosystems are becoming increasingly threatened by saltwater intrusion as sea level continues to rise with unfolding changes in global climate conditions.

The design (Figure 1) of our common garden experiment enabled us to test a series of related hypotheses about microbial mediation. First, we tested the hypothesis that (H1) soil microbiota alter phenotypic responses of *S. americanus* to salinity stress. We expected that the performance of plants grown with inoculation of soil microbes would consistently be greater than plants grown without soil microbial inoculation when exposed to salinity stress. Recognizing that sea level rise could be acting as a widely experienced selective pressure, we further hypothesized (H2) that descendant cohorts exhibit higher salinity tolerance than ancestral cohorts regardless of provenance, with the expectation that differences in performance would be greater in comparisons of ancestral and descendant cohorts grown without than with soil microbe inoculation. Building on this expectation, we further examined whether (H3) observable differences in performance are in part an expression of heritable variation in plasticity, reflecting genotype-by-environment (G×E) interactions. We tested these hypotheses with the additional aim of evaluating whether (H4) the composition of plant-associated microbial communities is contingent on salinity conditions (Whittle et al., 2021). This approach not only allowed us to gain insight about the influence of microbes on plant performance, but also offered detailed perspectives on how mediation might manifest according to variation in constitutive factors, environmental conditions, and combinations thereof.

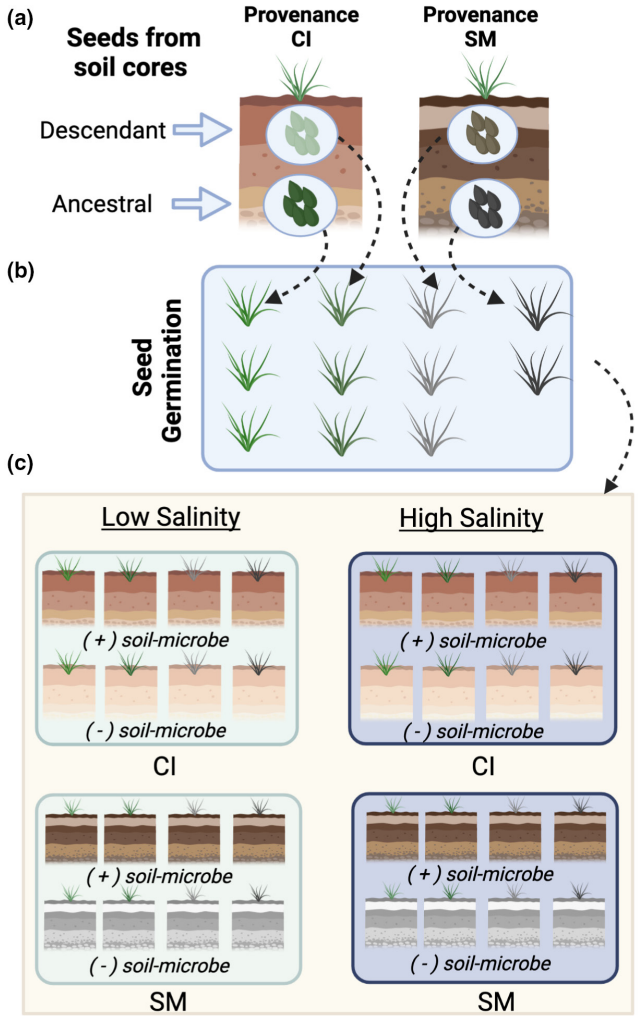

FIGURE 1 Common-garden experimental set-up. (a) Seeds were retrieved from soil cores at different depths – representing ancestral and descendant cohorts – obtained from two provenances (Corn Island [CI] and Sellman marsh [SM]). (b) Ancestral and descendant seeds of different genotypes were germinated in trays in growth chambers. Four sets of five replicate clones were created for each 11 genotype (3 ancestral and 3 descendant genotypes for CI; 2 ancestral and 3 descendant genotypes for SM provenance). (c) the replicate sets of clones for each genotype were grown under high (15 ppt) and low (0 ppt) salinity treatments crossed with (+) soil-microbe and (–) soil-microbe inoculation treatments in a walk-in environmental chamber. Figure created with BioRender.com [Colour figure can be viewed at wileyonlinelibrary.com]

2 | MATERIALS AND METHODS

2.1 | Experimental design

We conducted a common-garden experiment (Figure 1) using plants derived from seeds comprising an ancestral (c. 100 year-old seeds) and a descendant (c. 10 year-old) age cohort (Summers et al., 2018; Blum et al., 2021; see Methods S1). Seeds were retrieved from representative cores of highly persistent, time-stratified seed banks formed by *S. americanus* populations in two high marshes (~10 km apart) in the Rhode River estuary of Chesapeake Bay -- Sellman (SM) and Corn Island (CI). The stratigraphy of buried seeds was reconstructed from ^{210}Pb and ^{137}Cs dated soil cores, with all seeds retrieved and germinated in trays in environmental chambers at the University of Tennessee-Knoxville (UTK) following Summers et al. (2018) (see Methods S1).

SM and CI exhibit differences in soil biogeochemistry and plant community composition. For instance, SM soils are more organic and have less Fe (Weiss et al., 2004). Vegetation surveys of both sites conducted in 2018 (independent of the current study) showed that SM harbours more plant species (i.e., plant diversity, n = 16), with the community dominated primarily by *Distichlis spicata*, *Spartina patens* and *S. americanus*. In comparison, CI (n = 10) is dominated by *Distichlis spicata* (Whigham et al., 2020). Total biomass across all plant species, however, appears to be lower at SM than at CI (Whigham et al., 2020).

Native inoculum was obtained in 2018 for the common garden experiment by collecting soil from areas within the SM and CI coring sites where *S. americanus* was present. At each site, we sampled ~20 L from the top 30 cm of soil from three proximate locations. We then pooled all of the sampled soil from each site, respectively, and transported it to UTK in sterilized 50 L sealed containers kept at 4°C. The two pooled soil samples were sieved separately by hand to remove any plant matter and live *S. americanus* rhizomes. Each pooled sample was then divided into aliquots set aside for either "live" soil inoculum (i.e., no sterilization treatment) or "sterile" soil inoculum that was autoclaved at 121°C for 1.5 h, twice, allowing it to cool down before the second sterilization. Although the soils used for inoculation had different characteristics, a common soil matrix (i.e., sterile background soil) was used for all plants in the common-garden experiment.

The common-garden experiment was initially conducted with 220 plants. Ancestral and descendant plant cohorts were grown from seeds originating from SM and CI. Each ancestral and descendant cohort was composed of three genotypes except for ancestral SM cohorts, which were composed of two genotypes. Four sets of five replicate clones were created for each genotype. The replicate sets of clones for each genotype were grown under high (15 ppt) and low (0 ppt) salinity treatments crossed with live and sterile soil inoculation treatments (Figure 1). Each clone was grown in an individual sterile pot with a separate watering sleeve (see below).

The experimental treatments were established following a process designed to prevent potential contamination and to encourage the formation of plant–soil associations. For example, all materials- including pots, beakers and miscellaneous tools- were sterilized thoroughly prior to the experiment via autoclaving and/or being subjected to a bleach treatment followed by exposure to UV in a laminar flow hood for at least 1 h. All water used in the experiment was purified using the Milli-DI Water Purification System for Deionized Water (Millipore Sigma). We also prepared a sterile 1:1 ratio premix of topsoil (Baccto Premium Soil) and sand (All-purpose Premium Sand), with equal amounts (1.5 kg) of the premix poured into sterile pots (30.48 cm height, 36 cm^2 diameter) in sterile watering sleeves. The experiment also was conducted in a sterile walk-in

environmental chamber. Accordingly, we first established the soil inoculation treatments by treating individual plants with either live soil ([+] soil-microbe) inoculant or sterile soil inoculant from their site of origination (e.g., SM plants were treated only with SM soil inoculants).

For each soil inoculation treatment ([+] soil-microbe or [−] soil-microbe), soil inoculum (5% of the total soil mass) was mixed with the top layer of the sterilized 1:1 premix of topsoil and sand. Clones of *S. americanus* were then transplanted into individual pots, with the plants put in close contact with the inoculum to encourage new roots to potentially take up microbes from the soil inoculum. All plants were initially grown for 3 weeks to allow establishment of microbes before the start of the salinity treatment. Half of the plants were then introduced to high salinity (15 ppt). Saline water that was prepared with Instant Ocean salt and filtered distilled water was subsequently used for watering the plants under the high salinity treatment. The experiment was conducted for 4 months with the chamber set at 26°C (day)/25°C (night), 10,000 LUX light intensity, and a 12 h day: night cycle. In order to avoid a potential "greenhouse effect", we rotated pots every 2 weeks within each soil microbial inoculation treatment (i.e., rotated placement of plots within sterile and within inoculated) to avoid contamination. Twice-weekly upkeep was performed to maintain salinity and water levels (at 1 cm below the soil surface) for the duration of the experiment.

We acknowledge the potential limitations of using clones from plants grown from nonsterile seeds. Tissues almost certainly harboured an endophytic microbiome prior to the onset of the experiment. We thus took measures to minimize the potential influence of pre-existing endophytic microbiomes on estimates of plant performance. For example, all shoots used in the experiment originated from the same environmental and soil conditions. We also restricted sampling of belowground tissues to new roots to profile endophytes. Additionally, we included initial propagule (i.e., clone) weight as a covariate in statistical analyses (described below) with genotype as a random factor in most analyses. Notably, preliminary analysis of stem height 3 weeks after soil microbe inoculation but prior to salinity exposure revealed that plants inoculated with soil inoculum that contained no microbes (i.e., [−] soil-microbe inoculation) were significantly taller than plants inoculated with soil inoculum that contained microbes (i.e., [+] soil-microbe inoculation) (Figure S1) regardless of cohort or provenance (Table S1). We have accordingly interpreted and discussed the outcomes of the experiment in light of this finding.

2.2 | Plant trait measurements

Plant growth was monitored monthly by measuring the height of all stems. At the end of the four-month experiment, we harvested all plants and measured the following traits for each plant: number of stems (SN), stem density (SD, stem number cm^{-2}), average stem diameter (SDi), and final average stem height (SH). We calculated plant size (PS) as the average height x stem number. Aboveground (AG) wet biomass, belowground (BG) wet biomass, and biomass of green stems (GB) were measured at the end of the experiment, with GB used as an index of stress. For AG biomass, all stems including brown ones were measured. This allowed us to determine root-to-shoot biomass ratios (R:S) and total biomass.

2.3 | Microbial community assessment

We focused on assessing fungal and bacterial colonization into new roots (i.e., the root endosphere) at the end of the experiment. New root samples were taken, cut into 2–3 mm^2 pieces and surface-sterilized in a sequential immersion of 70% ethanol for 10 s, 3.125% sodium hypochlorite for two min, and two rounds of rinsing with sterile distilled water, then stored at −20°C prior to processing. Roots were then ground in liquid nitrogen and 10 mg of the resulting material was used for extraction of total genomic DNA with a DNeasy PowerPlant Pro DNA isolation Kit (Qiagen).

Microbial communities were profiled by amplifying and sequencing the 18S rDNA internal transcribed spacer (ITS1) and 16S rRNA V5–V6 regions for fungi and bacteria, respectively. Libraries were generated by a two-step amplicon polymerase chain reaction (PCR) approach using primers modified with the Illumina TruSeq adapter. For fungi, we used the standard ITS1 region primers ITS1F (Gardes & Bruns, 1993) and ITS2 (White et al., 1990) modified with the Illumina TruSeq adaptor (see Appendix S1). For bacteria, we used the modified primers 799F and 1115R primers (Hanshew et al., 2013; Kembel et al., 2014; Appendix S1). In order to normalize across all samples, 10 ng of DNA template per sample was used for the first PCR. PCR conditions for the first amplification reaction were as follows: initial denaturation 95°C 5 min, 30 cycles of 98°C 20 s, 52–56°C 15 s and 72°C for 30 s; final elongation at 52°C for 5 min. For each sample, PCR was done in triplicate at three different annealing temperatures (52, 54 and 56°C) to remove amplification bias towards certain fungal taxa. When necessary, purification was done to clean up the amplicon and to remove primer dimers before we indexed PCR products. Indexed libraries were purified, pooled and run separately for fungi and bacteria on the paired-end Illumina MiSeq platform at the UTK Genome Centre.

MiSeq sequences were filtered for quality, and adaptors/distal priming sites were removed, keeping a minimum sequence length of 50 bp using cutadapt version 1.7.1 (Martin, 2013). Mothur version 1.34.4 (Schloss et al., 2009) was used for further filtering of the sequences, which included removal of homopolymers ≤9 bp at both ends of sequences and removing short sequences (<125 bp) and those containing ambiguous base pair calls. Paired-end sequences for fungi were then merged using pear version 0.9.8 (Zhang et al., 2014). Only forward reads were used for the 16S region (bacteria), as the overlap between forward and reverse reads was too short to merge the two without significant sequence loss. Filtered sequences were then dereplicated and clustered into operational taxonomic units (OTUs) at a 97% threshold. Fungal OTUs were picked using a chain-picking method adapted from Nguyen et al. (2015). OTUs were first picked using USEARCH with chimera detection and removal using the uparse algorithm (Edgar, 2013), followed by additional reclustering using UCLUST (Edgar, 2010) implemented in Qiime (Caporaso

et al., 2010). Bacterial OTUs were picked using the open-reference method in Qiime following the uclust method, with chimera detection and removal. Singleton OTUs (OTUs with sequence count = 1) were excluded to minimize potential PCR and sequencing artefacts (Nguyen et al., 2015). Taxonomic identity was assigned using BLAST methods against UNITE (Nilsson et al., 2019) and SILVA version 138 (Quast et al., 2013) database for fungi and bacteria, respectively (see Appendix S1).

2.4 | Statistical analysis

To address H1 and H2, we examined the influence of soil microbes on the expression of plant traits according to salinity treatment by conducting restricted maximum likelihood linear mixed-effects models (LMMs) analyses. The full models included salinity, soil inoculation, provenance, cohort, and their interactions as fixed effects, individual genotype as random effect, and weight of initial propagule as a covariate in all models. We examined all individual traits using the lmer function from the lme4 package (Bates et al., 2015). Significance of main effects was determined using the lmerTest package (Kuznetsova et al., 2017), and where significant interactions were found, the estimated marginal means (EMMs) were used to explore treatment differences (emmeans; Lenth, 2016, multcomp; Hothorn et al., 2008). Furthermore, we calculated a "microbe effect" response variable (similar to Petipas et al., 2020) to determine whether soil microbe inoculation or its interaction with other fixed factors had a significant effect on a particular trait. To calculate the microbe effect, we examined data from (+) soil-microbe and (−) soil-microbe treated pairs of clones of the same genotype within specific treatments, subtracting the response trait value of the (+) soil-microbe inoculated plant from the trait value of the matching (−) soil-microbe inoculated plant. The resulting values were either positive (i.e., positive inoculation effect), around zero (i.e., no effect), or negative (i.e., negative effect). We then plotted values based on the microbe effect EMMs.

As described above, we conducted linear mixed modelling to analyse plant growth (i.e., height) prior to the start of the salinity treatment to determine initial effects of the soil inoculation treatment. Likelihood ratio tests were conducted to determine the significance of random effects by comparing models with and without genotype as a random factor. Variables were log-transformed to meet assumptions of normality.

We also conducted linear mixed modelling to address H3. LMMs were constructed as described above but without provenance as a factor and with a cohort x salinity x inoculation interaction term. We determined genotypic differences in response to salinity and soil inoculation for each provenance, where the slope and intercept were allowed to vary among genotypes for both treatments (Appendix S1). Separate analyses were carried out for each individual trait. If model was found to be significant for a particular trait, we visualized the reaction norm of that trait and estimated the mean trait value of each genotype by calculating the best linear unbiased predictors (BLUPs) from that fitted linear mixed model. Results were then plotted in ggplot2 (Wickham, 2016).

To test H4, we assessed the diversity and composition of root endosphere microbial communities. Endosphere data were rarefied to $n = 6000$ and $n = 12{,}000$ for fungi and bacteria, respectively. We determined microbial (alpha) diversity by calculating the effective number of species based on the probability of interspecific encounter (ENS_{PIE}), a scale-independent metric that is less sensitive to rare taxa compared to other diversity metrics (Seabloom et al., 2019). ENS_{PIE} was calculated as $1 / \sum_{i=1}^{S} p_i^2$ (Inverse of Simpson's Index) where S is the total number of species and p_i is the proportion of the community represented by species i (Chase & Knight, 2013). We investigated whether endosphere diversity detected in new roots of soil-inoculated plants differed across treatments by conducting a linear mixed-effects regression with salinity, provenance, cohort and their interactions as fixed factors, with genotype as random effect, and ENS_{PIE} as the response variable.

To determine differences in OTU-based microbial composition according to treatment, we partitioned variation in community composition across all plants using permutational multivariate analysis of variance (PERMANOVA) on abundance-weighted Bray-Curtis pairwise dissimilarity values. The model included the nested effects of provenance, cohort nested within provenance, genotype nested within cohort, and the experimental treatments and their interactions. The model was run using adonis in vegan (Oksanen et al., 2019) with 9999 permutations. To identify OTU(s) driving multivariate patterns and the OTU(s) characteristic of a specific treatment group combination (e.g., high salinity-inoculated-ancestral), we conducted a species indicator analysis using the multipatt function of the indicspecies package (De Cáceres & Legendre, 2009), with the association function "r.g." and max.order = 3 parameter settings and significance tested with 9999 permutations. Lastly, visual groupings of microbial communities were examined by db-RDA based on Bray-Curtis index values using the capscale function in vegan for all plants and for (+) soil-microbe treated plants only (Appendix S1).

We examined relationships between microbiota and plant phenotype to gain further perspective on how microbes can shape plant responses to stress. We did so by investigating the strength of associations between ENS_{PIE} diversity (response variable) and each plant trait (predictors) with partial least square regression (PLSR) using the pls package (Mevik & Wehrens, 2007). All traits were standardized to a mean of zero and variance of one. We then determined which component of microbial communities (based on Bray-Curtis index) influence observed phenotypic responses (similar to Wagner et al., 2014). For traits where soil microbe inoculation was significant in the full linear mixed model for plant traits, we regressed the residual from that model onto the mean principal coordinate (PCo) score ("site" score, representing Bray-Curtis index values) of microbial communities from the capscale analysis. The latter captures how differences in the composition of microbial communities correlate with phenotypic responses. Analyses were done separately for endosphere fungi and bacteria, to determine whether there were differences in the respective relationship(s) with plant phenotype. All analyses were conducted in R version 3.6 (R Core Team, 2020).

3 | RESULTS

3.1 | Do soil microbiota alter plant phenotype and always elevate plant performance? (H1)

We found mixed support for the hypothesis that soil microbiota alter phenotypic responses to *S. americanus* to salinity stress, and likewise, our findings did not always align with the expectation that the performance of plants grown with soil microbes would be greater than plants grown without soil microbes. Consistent with our hypothesis and expectation, we found that soil microbe inoculation increased plant size ($F_{1,160}$ = 8.10, p = .01; Table S2, Figure S2) and green biomass production ($F_{1,180}$ = 7.04, p = .01; Figure S3, Table S3) regardless of other factors. Less support was found in the other plant traits. For example, (+) soil-microbe inoculated plants had thinner stems (SDi) than (−) soil-microbe treated plants regardless of salinity treatment (Table S2, Figure S2). Comparisons following salinity exposure also revealed that differences in productivity-related and architectural traits between (+) soil-microbe plants and (−) soil-microbe plants sometimes depended on salinity treatment, provenance and cohort. For example, although soil microbe inoculation influenced overall stem height ($F_{1,186}$ = 5.40, p < .01, Table S2), this was more apparent for CI than SM plants (Figure 2a, Table S4). CI plants in the (+) soil-microbe treatment were shorter than CI plants in the (−) soil-microbe treatment under low salinity conditions (Figure 2a), whereas soil microbe inoculation only increased stem height of the ancestral cohort of CI plants under elevated salinity conditions based on microbe effect analysis (Figure 2b). Analyses of other traits further illustrated that outcomes of soil microbe inoculation differed according to provenance. This was particularly evident in measures of aboveground traits like stem number (SN, salinity: $F_{1,187}$ = 27.09, p < .01; soil inoculation x provenance: $F_{1,189}$ = 7.70, p = .01) (Figure 2c,d) and stem density (SD, $F_{1,187}$ = 25.11, p < .01; soil microbe inoculation x provenance: $F_{1,189}$ = 8.84, p = .01) (Figure 2e,f, Table S2). CI plants produced more stems when treated with the (+) soil-microbe inoculum (Figure 2c), reflecting a positive microbe effect regardless of salinity and cohort (Figure 2d). On the other hand, SM plants in the (+) soil-microbe treatment had fewer stems than SM plants in the (−) soil-microbe treatment, reflecting a negative microbe effect particularly under high salinity conditions (Figure 2c, d). A similar pattern was observed for stem density (SDi, Figure 2e,f).

3.2 | Do descendants exhibit greater salinity tolerance than ancestral plants? (H2)

We hypothesized that descendant cohorts would exhibit higher salinity tolerance, with the expectation that differences in performance would be greater in comparisons of cohorts grown without than with soil microbe inoculation (H2). Comparisons based on architectural traits did not provide clear support for our hypothesis or expectation. For example, ancestral plants (mean = 2.47 mm ± 0.011) exhibited thicker shoots than descendant plants (SDi mean = 2.14 mm ± 0.013) ($F_{1,6}$ = 5.41, p = .05), regardless of treatment (Table S2, Figure S2 inset). We also found that (+) soil-microbe soil inoculation enhanced growth of the CI ancestral cohort, which exhibited the largest positive microbe effect (emmean = 10.71, Figure 2b) under elevated salinity conditions. Soil microbe inoculation also enhanced plant size among ancestral CI plants compared to descendant CI plants regardless of salinity treatment (inoculation x cohort $F_{1,93}$ = 6.77, p = .01) (Table S4, Figure S4), which was not observed among SM plants.

Biomass-based measures of performance also did not provide clear support for our hypothesis, with differences in aboveground and belowground biomass reflecting salinity, provenance, and cohort. For instance, green biomass (GB) production was notably lower in all plants subjected to high salinity ($F_{1,180}$ = 28.44, p < .01, Table S3), with no difference found between ancestral and descendant cohorts. Similarly, we recovered a significant effect of salinity on R:S values across all plants independent of cohort, but the effect varied by provenance (salinity x provenance, $F_{1,186}$ = 5.23, p < .01, Table S3) (Figure S5). We did, however, detect provenance-specific differences in biomass production between ancestral and descendant cohorts in response to salinity and soil inoculation (Figure 3). For example, differences in total biomass were detected between ancestral and descendent cohorts in response to both treatments for CI plants ($F_{1,101}$ = 5.03, p = .03) but not SM plants ($F_{1,81}$ = 0.31, p = .58) (Table S5).

Notably, a significant interaction between salinity, soil microbe inoculation, and cohort was observed for aboveground biomass production in CI plants ($F_{1,101}$ = 5.64, p = .02, Table S5), with ancestral CI cohorts producing more aboveground biomass than descendant CI cohorts (Figure 3a). Soil microbe inoculation boosted aboveground biomass production in ancestral CI cohorts under high salinity conditions by as much as 37% ((+) soil-microbe plants mean = 0.59 g m^{-1} ± 0.00, (−) soil-microbe plants mean = 0.33 g m^{-1} ± 0.00) (Figure 3a). On the other hand, a significant interaction between salinity, inoculation, and cohort was observed for belowground biomass production in SM plants ($F_{1,77}$ = 3.80, p = .05, Table S5). Overall, descendant SM cohorts exhibited more belowground biomass production than ancestral SM cohorts (Figure 3b), whereas (+) soil-microbe inoculation reduced belowground biomass production of ancestral SM cohorts by 50% under low salinity conditions ((+) soil-microbe mean = 0.09 g m^{-1} ± 0.00, (−) soil-microbe mean = 0.18 g m^{-1} ± 0.00). Under high salinity conditions, (+) soil-microbe inoculation enhanced belowground biomass production of ancestral SM cohorts by ≤25% ((+) soil-microbe plants mean = 0.12 g m^{-1} ± 0.00, (−) soil-microbe plants mean = 0.07 g m^{-1} ± 0.00; Figure 3b).

3.3 | Do differences in plant performance reflect GxE interactions? (H3)

We found evidence of G × E interactions, where salinity tolerance and soil microbial mediation of *S. americanus* responses to

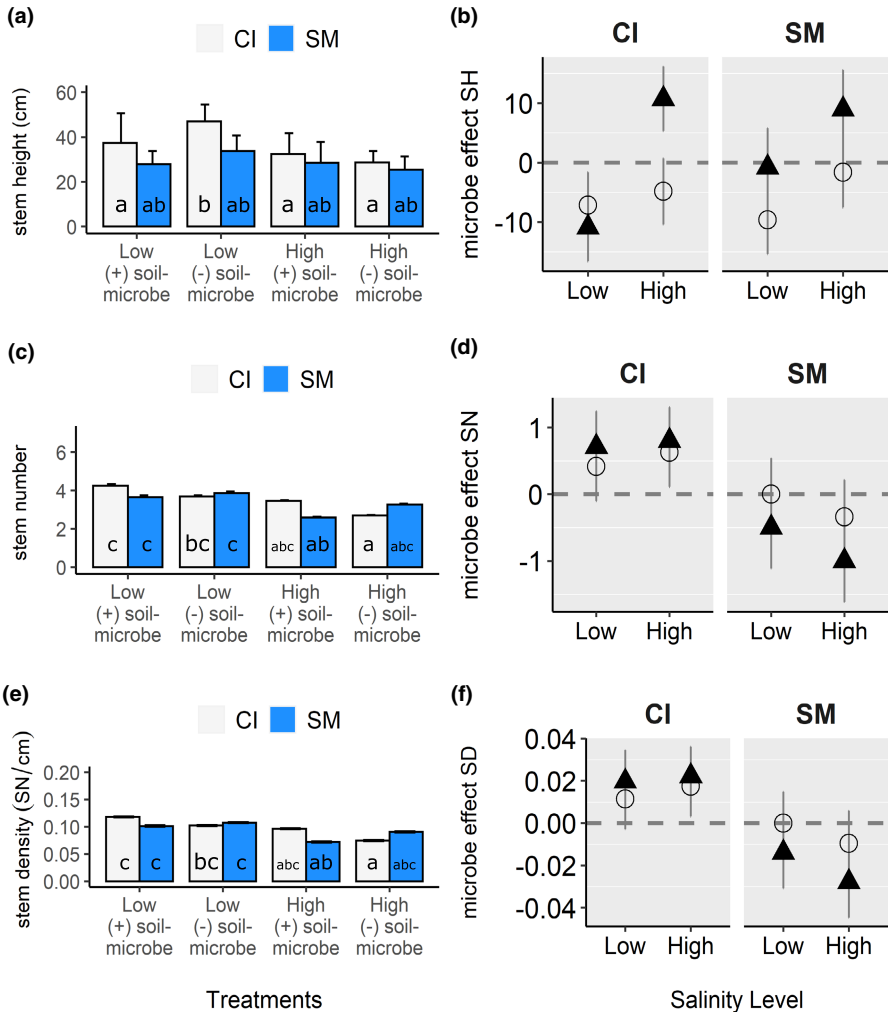

FIGURE 2 *Schoenoplectus americanus* responses to salinity and soil inoculation. Mean raw stem height, SH (a), stem number, SN (c) and stem density, SD (e) values according to salinity and soil-microbe inoculation for *S. americanus* from Corn Island (CI) and Sellman marsh (SM). Microbe effect on SH (b), SN (d) and SD (f) within each provenance. Points represent the estimated marginal mean values – Above zero means a positive inoculation effect, zero indicates a neutral effect and below zero corresponds to a negative effect. Filled triangles are ancestral plants and open circles are descendant plants. Low and high indicate low and high salinity treatments, respectively. Note that letters in bars indicate results from Tukey's honest significant pairwise comparisons of estimated marginal means (emmeans) among treatments from the full linear model whereas bars represent raw means [Colour figure can be viewed at wileyonlinelibrary.com]

salinity stress differed according to genotype. Notably, genotypic variation in exposure responses was evident in different subsets of traits reflecting provenance, independent of cohort. SM plants exhibited greater genotypic variation in productivity-related measures of response, whereas CI plants exhibited greater genotypic variation in measures of architectural traits (Figure 4). For example, without soil microbe inoculation, SM plants exhibited genotypic differences in AG, BG and total biomass under both low and high salinity conditions (Figure 4a–d right panels). Descendant genotypes M1 and M2, for instance, showed opposite patterns in their estimated mean belowground biomass trait value when comparing low to high salinity treatments without soil microbe inoculation. (M1: low salinity = 0.131; high salinity = − 0.123; M2: low salinity = − 0.295, high salinity = 0.04) (Figure 4b). Soil microbe inoculation dampened genotypic variation in biomass production, regardless of salinity condition (Figure 4a–c, left panels). Trait values also changed in different salinity treatments for a subset of genotypes (e.g., BG in genotype MSR1). It is also notable that CI plants exhibited a striking range of genotypic variation in stem height, diameter, and plant size (Figure 4d–f, respectively) regardless of the treatment.

3.4 | Does salinity determine the structure of endosphere microbial communities? (H4)

We found evidence indicating that colonization of fungi into *S. americanus* roots is not predominantly determined by salinity conditions. PERMANOVA revealed that the composition of root endosphere fungal communities across all plants differed according to provenance and soil inoculation but not salinity (Table S6, Figure S6). Analysis of Bray-Curtis index values also illustrated that endosphere fungal communities clustered more by provenance than salinity (Figure S6). However, other measures indicated that salinity can still exert appreciable influence on endosphere fungal communities. For example, among plants inoculated with soil microbes, root endosphere fungal ENS_{PIE} diversity (equivalent to alpha diversity) was notably lower under elevated salinity conditions (high salinity mean = 1.87 ± 0.84, low salinity mean = 2.18 ± 0.51) ($F_{1,86}$ = 3.68, p = .058; Figure 5a, Table S7). Fungal community composition also differed more though only slightly by salinity than by cohort and provenance (Figure 5b; for reference, comparisons to sterile plants are available in Appendix S1).

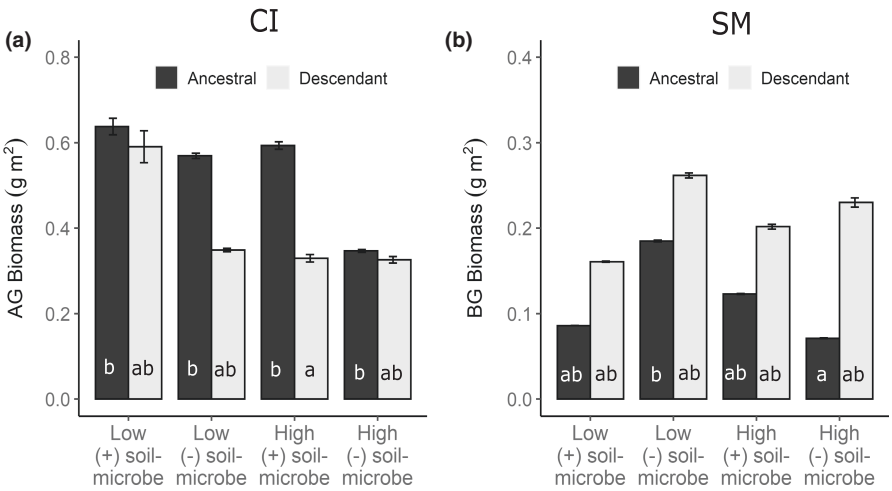

FIGURE 3 *Schoenoplectus americanus* responses to salinity and soil inoculation. (a) Aboveground (AG) mean biomass production for each Corn Island (CI) cohort (ancestral, black bars; descendant, grey bars) according to salinity and soil inoculation ([+] soil-microbe, [−] soil-microbe) treatments. (b) Below ground (BG) mean biomass production for each Sellman Marsh (SM) cohort in response to salinity and inoculation. BG and AG values are based on raw means, with all error bars representing standard errors. Low and high indicate low and high salinity treatments, respectively. Note that letters in bars indicate results from Tukey's honest significant pairwise comparisons of estimated marginal means (emmeans) among treatments from the full linear models whereas bars represent raw means.

The majority of identifiable endosphere fungal taxa across all plants were saprotrophs (e.g., *Zopfiella* sp.), mycorrhizae or endophytes (e.g., *Serendipita indica*). The most dominant family was Chaetomiaceae, which was strongly associated with descendant cohorts. Indicator species analysis identified six fungal endosphere OTUs that were strongly associated with ancestral plants inoculated with soil microbes under high salinity conditions, with the strongest association recovered for *Wongia garrettii* (Table S8). Only one fungal OTU (*Lulworthia* sp.) was associated with descendant cohorts under high salinity conditions (Table S8).

The influence of salinity on endosphere bacterial communities was also not predictable. Across all plants, PERMANOVA revealed that the influence of salinity on endosphere bacterial community composition depended on the soil inoculation treatment (i.e., salinity x inoculation; $F_{3,60} = 2.45$, $R^2 = 0.01$, $p < .01$; Table S6, Figure S6). Bacterial endosphere colonization of (+) soil-microbe plants also reflected cohort and provenance more so than salinity (Table S7). Likewise, bacterial ENS_{PIE} diversity did not differ between low (mean = 16.29 ± 6.21) and high salinity treatments (mean = 14.01 ± 2.97) ($F_1 = 0.60$, $p = .44$) (Figure 5c), but it did vary by provenance ($F_{1,6} = 13.36$, $p = .01$) (Table S7). However, the composition of bacterial communities in (+) soil-microbe plants differed by salinity as well as by provenance as illustrated in the visual clustering based on Bray-Curtis values (Figure 5d; results for (−) soil-microbe inoculated plants are reported in the Appendix S1).

The majority of identifiable endosphere bacterial OTUs across all plants were Alphaproteobacteria and Gammaproteobacteria (49 and 25% of total sequences, respectively). Indicator species analysis revealed that several bacteria OTUs were strongly associated with ancestral and descendant cohorts under different conditions (Table S9). For instance, otu_4406139 (*Burkholderiales*) was most strongly associated with (+) soil-microbe ancestral cohorts under high salinity conditions (Table S9).

PLSR analyses revealed that most plant traits correlated positively with fungal ENS_{PIE} diversity (Figure 6a), although residual linear regression showed that plant traits were only weakly influenced by compositional differences (PCo1) among fungal communities (Figure 6b). In contrast, bacterial ENS_{PIE} diversity was negatively correlated with almost all plant traits (Figure 5c). Plant traits also appear to be more strongly influenced by compositional differences in bacterial communities (PCo2) (Figure 6d, Appendix S1).

4 | DISCUSSION

Our findings add to the growing body of evidence that microbial symbionts can enhance plant performance by conferring greater tolerance to stress (Acuña-Rodríguez et al., 2020; Porter et al., 2020), but that plants do not universally benefit from interactions with microbes (Petipas et al., 2020). We found that microbial mediation of *S. americanus* responses to salinity exposure was contingent on several factors. While we cannot assess the full extent of microbial mediation in the present study given the use of nonsterile seeds, our results show that outcomes of microbial associations (i.e., following soil microbial inoculation) reflected plant genotype and provenance, as well as temporal shifts in genotypic variation (i.e., age cohorts) and G × E interactions. Microbial profiling revealed that the composition of endosphere fungal and bacterial communities also reflected plant genotype, provenance, and age cohort – though this inference should be viewed with some caution given the possibility of priority effects during the assembly of endogenous microbiota in the seeds used in this study. Nonetheless, it appears that outcomes

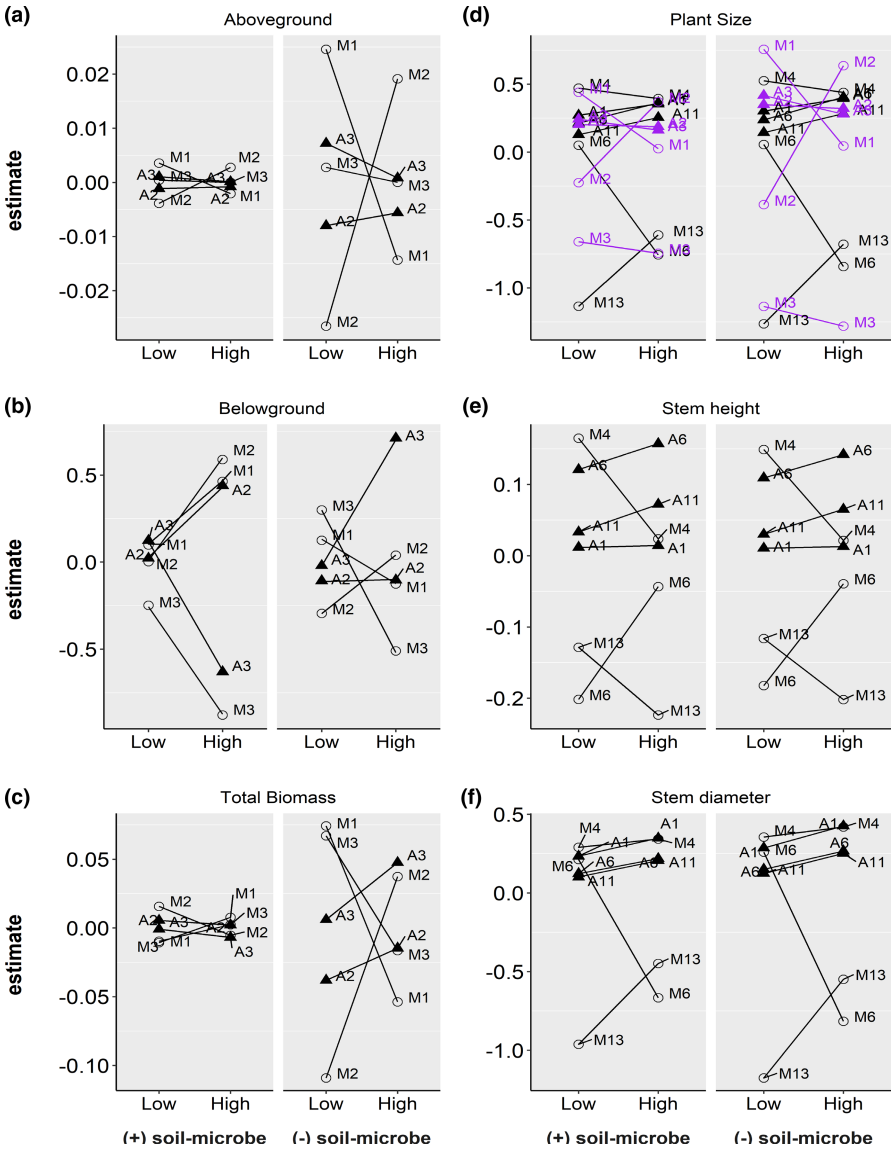

FIGURE 4 Genotypic differences in salinity and inoculation response. Best linear unbiased predictor (BLUPs) estimation (similar to a reaction norm) of productivity-related and architectural traits for each genotype from (a–d) Sellman marsh (SM) and (d–f) Corn Island (CI). Among-genotype variation in response to salinity and soil microbe inoculation was observed in (a) aboveground, (b) belowground, (c) total biomass and (d) plant size (black colour) of SM plants, and (d) plant size (purple) of CI plants, (e) stem height and (f) stem diameter of CI plants. Estimates were extracted from linear models that revealed a significant salinity (low, high) by soil inoculation ([+] soil-microbe, [−] soil-microbe) interaction for each ecotype. Filled triangles denote ancestral cohort; open circles denote descendant cohort. Letters on the graph indicate genotype. Different line colours in panel d indicate the two provenances (CI, purple; SM, black) [Colour figure can be viewed at wileyonlinelibrary.com]

of plant-microbe associations are not only context-dependent but also dynamic, where associations differ among populations and over time.

4.1 | Microbiota mediate responses of *S. americanus* to salinity stress

As has been previously shown, our study indicates that microbial associates can mediate the response of plants to stress by altering the expression of functional traits (Acuña-Rodríguez et al., 2020; Petipas et al., 2020). Consistent with our first hypothesis (H1), we found that microbiota can alter phenotypic responses of *S. americanus* to elevated salinity. Overall, microbial influence was more apparent in architectural traits than those related to productivity. Notably, without soil microbe inoculation, elevated salinity constrained *S. americanus* productivity and diminished a range of associated traits like stem height, diameter and number, suggesting that microbiota exert broad influence on plant phenotype.

4.2 | Microbial mediation of plant stress response differs by provenance

Our study also demonstrated that microbial mediation of *S. americanus* responses to salinity stress reflects plant provenance. Like the observed differences in salinity tolerance, microbial inoculation elicited responses in CI and SM plants that were not found in the other. For instance, CI plants exhibited shifts in aboveground traits related to light capture (e.g., height) whereas soil microbe inoculation elicited shifts in belowground traits in SM plants, such as greater belowground growth in plants subjected to elevated salinity. It is possible that the observed disparities in response to soil microbial inoculation reflect local adaption to differences in nutrient availability -- especially nitrogen (N) availability -- between the two study sites, with more limited availability favouring greater belowground allocation to foster nutrient capture (Lu et al., 2019). Prior work at the Global Change Research Wetland, which is part of the same Kirkpatrick marsh complex as our CI site, demonstrated that biomass allocation in *S. americanus* strongly responds to the balance of plant N demand

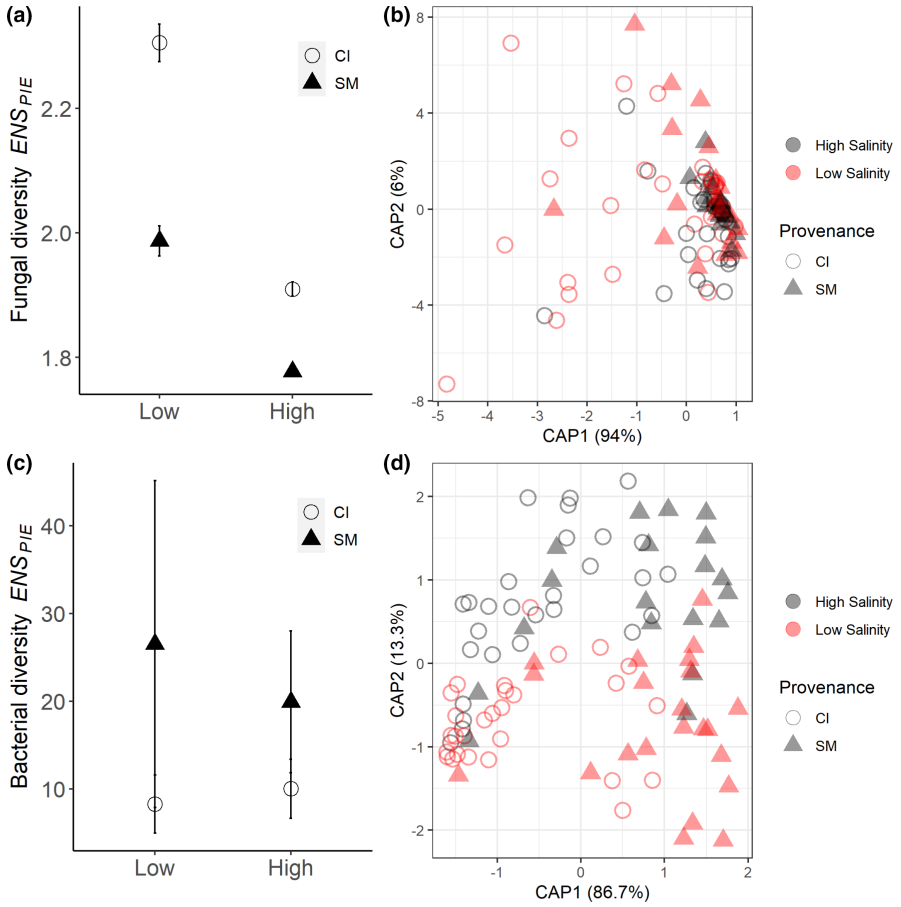

FIGURE 5 Variation in endosphere diversity and composition by provenance and salinity of (+) soil-microbe inoculated plants. Endosphere diversity (effective number of species based on the probability of interspecific encounter [ENS_{PIE}] left panel) and bray–Curtis dissimilarity in community composition (right panel) in fungi (a–b) and bacteria (c–d), respectively, that colonized new roots of soil- microbe-inoculated *S. americanus* ([+] soil-microbe plants only) from Corn Island (CI) and Sellman marsh (SM) under high and low salinity conditions. Error bars in a and c are standard errors [Colour figure can be viewed at wileyonlinelibrary.com]

and microbial N supply (Noyce et al., 2019). High salinity can interfere with plant N root uptake via higher production of hydrogen sulphide (H_2S) by sulphate-reducing bacteria or by direct H_2S toxicity (Koch et al., 1990; Lamers et al., 2013). Thus, the nature of microbial mediation might differ by provenance because metals (especially oxidized Fe) in the mineral-rich soils at SM can rapidly remove H_2S from solution, buffering the impact of H_2S production on plants at high salinity. In contrast, soils at CI lack minerals and accumulate H_2S to concentrations of up to 4 mM (Keller et al., 2009). While the logic of this hypothetical scenario has a certain appeal, it may not hold up to scrutiny. It could be tested by undertaking a two-factor (i.e., N availability and salinity) common garden study or reciprocal transplant study to determine whether variation in growth strategies is attributable to biogeochemical interactions or differences in biogeochemical regimes at the CI and SM study sites.

The observed differences in response might also be due to compositional variation in native soil microbial communities, and by extension, the pool of microbes that can potentially associate with *S. americanus*. Even though all plants were grown in the same soil (and thus the mineral content of the soils in our experiment was standardized across provenance), initial differences in microbial community composition of the soil inoculum might have nonetheless influenced microbial processes, including mediation of *S. americanus* responses to salinity. Despite exhibiting similar levels of diversity, the CI and SM inoculant communities displayed notable differences in composition (Figure S7). For example, the most abundant bacterial taxa in the SM (+) soil-microbe inoculant were *Flavobacterium* sp. and *Gallionella* sp., while CI (+) soil-microbe inoculant was dominated by *Sideroxydans* sp. and *Gallionella* sp. Both *Sideroxydans* sp. and *Gallionella* sp. are lithotrophs that use ferrous iron as a source of electrons (i.e., energy) and CO_2 as a carbon source (Emerson et al., 1999; Hallbeck & Pedersen, 2014). Iron oxidation often occurs in the rhizosphere of wetland plants via iron-oxidizing bacteria (Emerson et al., 1999; Weiss et al., 2004), thus the presence of different abundances of *Gallionella* sp. between soil inoculant communities could be indicative of local variation in soil biogeochemistry.

Differences attributable to provenance might additionally reflect modification of rhizosphere and endosphere microbiota by locally adapted plant ecotypes (Bowsher et al., 2020; Lumibao et al., 2020). Alteration of microbial communities, and associations thereof, might be a strategy employed by plants to optimize resource capture through biomass allocation (White et al., 2012), which can differ due to heritable trait variation and plasticity among local populations (Bernik et al., 2018). Support for this possibility comes from prior work showing that plant-microbe associations can be highly context dependent (Petipas et al., 2020) and that microbial mediation of plant fitness can be habitat-specific, where the population origin of both plants and associated microbes are important in determining outcomes of interactions (Hoeksema et al., 2010; Rúa et al., 2018; Young et al., 2018). Conducting a time-series analysis of microbial communities in a reciprocal transplant experiment, or a common garden study using a common inoculant or perhaps reciprocal "home

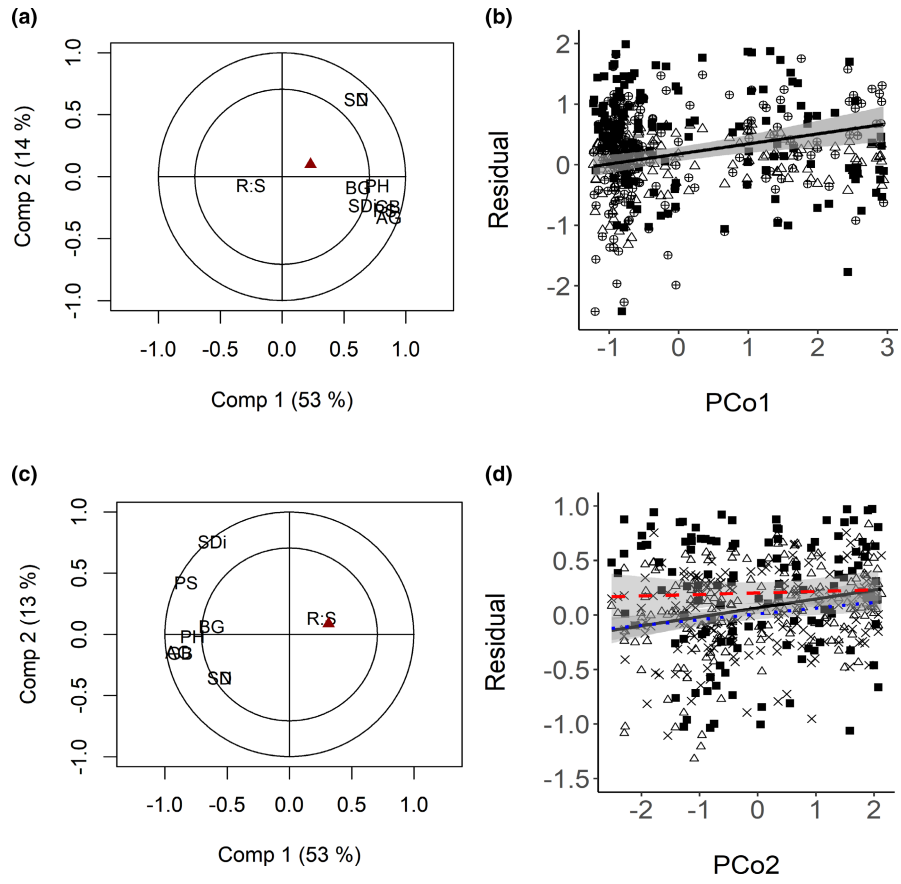

FIGURE 6 Associations between plant traits and endosphere microbes. PLSR-based correlations between effective number of species based on the probability of interspecific encounter (ENS_{PIE}) diversity (red triangles) and plant traits in endosphere (a) fungi and (c) bacteria. Fungi ENS_{PIE} diversity was positively correlated with most plant traits except for root-to-shoot ratio (R:S) while bacteria diversity exhibited negative correlations with plants traits. (b) Residuals of stem height (SH, open triangle), plant size (PS, filled square) and green biomass (GB, circle) responses (after controlling for genotype differences across all samples) from the full linear mixed models plotted against a gradient of endosphere fungal microbiota PCo1 based on Bray-Curtis index values, with a significant relationship depicted by the black regression line (grey indicates standard error) for GB. (d) Residuals of SH (open triangle), PS (filled square) and SN (cross) responses against bacterial endosphere microbiota PCo2. Significant relationships in (d): SH, black solid line; PS, dashed red regression line; SN, blue dotted line [Colour figure can be viewed at wileyonlinelibrary.com]

and away" inoculant treatments, could shed additional light on the possible importance of locally adapted plants acting on microbiota.

4.3 | Variation in responses to salinity stress over time

We found that microbial mediation of stress responses also differed among *S. americanus* age cohorts, with some of the observed differences reflecting provenance. This finding is partly attributable to differences in salinity tolerance among age cohorts. Consistent with our second hypothesis (H2), some measures indicate that descendants are more tolerant to salinity, though this is more apparent in CI than SM plants. We also found evidence that microbial mediation of stress response is dynamic, with some measures indicating that soil microbe inoculation elicited greater performance of ancestral cohorts than descendant cohorts. This shift could reflect evolutionary responses of *S. americanus* to changing environmental pressures (e.g., sea level rise). It might also in part reflect responses of endosphere microbial communities and in particular microbial associates to changing environmental pressures (Whittle et al., 2021). Thus, depending on the pace and concordance of responses, microbial mediation of plant stress tolerance might be a dynamic outcome of local adaptation of plant-microbe associations, akin to what has been suggested for ectomycorrhizal relationships (Rúa et al., 2018).

4.4 | Genotypic variation in responses to soil inoculation and salinity stress

Consistent with our third hypothesis (H3), we recovered evidence that *S. americanus* responses to salinity and inoculation reflect trait variation, including variation in plasticity (i.e., G×E interactions) among genotypes. Akin to the results of prior studies (Blum et al., 2021; Gentile, 2015), we detected evidence of variation in trait-based measures of salinity tolerance. We also detected evidence of variable reaction norms (Figure 4), which is consistent with findings from prior work showing that associations with soil

microbiota and endophyte recruits (Vannier et al., 2015) can mediate different plant responses to environmental pressures, including salinity tolerance. Notably, signatures of G×E interactions differed according to provenance. Heritable variation in CI plants was more evident in architectural traits whereas in SM plants, it was more evident in productivity-related traits. This finding, which aligns with other evidence indicating that responses of plants to stress can vary according to provenance (Bowsher et al., 2020; Diedhiou et al., 2016), further illustrates that population origin can be as important as individual genotype in determining the nature and range of plant stress responses.

Contrary to some theoretical predictions, phenotypic variation among genotypes was not consistently lower under elevated salinity conditions. Under the stress gradient hypothesis, mutually beneficial interactions should come to dominate as antagonistic interactions diminish with increasing levels of stress (Bertness & Callaway, 1994), suggesting that *S. americanus* should have exhibited less phenotypic variation under elevated salinity conditions. Inoculation with soil microbiota muted the effects of salinity on genotypic variation in some traits (Figure 4), contingent on provenance. This finding further illustrates the context-dependency of microbial mediation of plant stress response, with phenotypic variation reflecting plasticity and G×E interactions.

4.5 | Endosphere microbial associates of *S. americanus*

We hypothesized (H4) that colonization and association of microbial communities with plants would predominantly reflect salinity stress, with some differences contingent on provenance or genotype. Some of our results are consistent with this hypothesis, which aligns with recent findings that low salinity transitions can alter soil microbial communities in coastal ecosystems (Whittle et al., 2021). For example, we detected lower diversity of fungal endophytes under high salinity conditions, though we did not find a corresponding shift in composition between high and low salinity conditions (Figure S6). Lower endosphere fungal diversity without shifts in community composition under elevated salinity conditions could have resulted from the loss of rare OTUs or perhaps functionally-redundant OTUs. We also found that differences in the composition of endosphere bacterial communities relating to salinity corresponded to provenance, perhaps in part reflecting the different inoculants sourced at CI and SM, respectively.

Our experiment revealed that fungal and bacterial communities influence phenotypic variation in plants through possibly different mechanisms. For example, *S. americanus* traits were more strongly influenced by root endosphere fungal diversity than community composition, where greater diversity was positively correlated to plant trait variation. On the other hand, both root endosphere bacterial diversity and community composition appear to strongly influence *S. americanus* trait variation. These results likely reflect a strong influence of host filtering on microbial colonization into their root tissues. These results also suggest that bacteria play a more prominent role in mediating *S. americanus* responses to salinity stress than fungi, perhaps because wetland soils present anoxic and reducing conditions that are biogeochemically hostile to fungal communities (Onufrak et al., 2020). Culture-based studies have, however, shown that some fungi (e.g., dark septate endophytes) can improve salinity tolerance in other marsh plants like *Phragmites australis* (e.g., Gonzalez Mateu et al., 2020). These inferences should also be viewed with some caution because differences in interactions between microbiota in the soil inoculants versus constituents of the endogenous microbiome (i.e., within the seeds of the plants used in the study) might have affected the strength of associations with *S. americanus* traits. Further work (e.g., experiments using sterilized seeds) is thus warranted to identify and determine the basis of possible differences.

5 | CONCLUSIONS

Our findings highlight the potential importance of ecological interactions and evolution in determining the fate of ecosystems experiencing pressures linked to climate change. Natural surveys (Blum et al., 2010) and experiments (Blum et al., 2021; Erickson et al., 2007; Gentile, 2015) have shown that salinity strongly influences the distribution, growth, and phenotype of *S. americanus*. Our results indicate that microbial associations – particularly with salt-tolerant microbiota such as the plant-growth promoting Rhizobacteria – might dampen the impacts of sea level rise on marshes dominated by *S. americanus* by conferring greater tolerance to salinity, thus promoting both organic and inorganic contributions to marsh elevation gain and soil integrity (Lu et al., 2019; Mueller et al., 2016). Evidence that microbial mediation of salinity tolerance is context-dependent and dynamic, with variation attributable to plant genotype, provenance, and cohort, raises the possibility that functional outcomes of evolution can similarly influence vital ecosystem attributes (Blum et al., 2021). Although the amount of variation attributable to differences among cohorts was relatively small, even marginal changes in salinity tolerance over time could have pronounced aggregate impacts on marsh ecosystems (Baustian et al., 2012). By clarifying the mechanisms governing the structure and persistence of coastal marshes, further study of plant-microbial interactions could offer a stronger basis for identifying conditions that result in (mal)adaptive feedbacks among human actions, ecosystem integrity, and the availability of valued services. Better understanding of possible feedbacks could in turn improve societal capacity to anticipate and manage the socioeconomic consequences of climate change.

BENEFITS GENERATED

Benefits from this research accrue from the sharing of our data and results on public databases as described above.

AUTHOR CONTRIBUTIONS

Candice Y. Lumibao, Sunshine A. Van Bael, J. Patrick Megonigal and Michael J. Blum conceived the idea; Candice Y. Lumibao, Sunshine A. Van Bael and Michael J. Blum designed the experiment, Candice Y. Lumibao and J. Patrick Megonigal collected soil samples, Candice Y. Lumibao conducted greenhouse experiment, molecular work, and performed bioinformatics and statistical analyses, Lorena Torres Martínez performed statistical analyses, Candice Y. Lumibao and Michael J. Blum wrote the initial draft of the manuscript. All authors contributed significantly to the improvement and revisions of manuscript.

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CONFLICT OF INTEREST

The authors declare no competing interest.

DATA AVAILABILITY STATEMENT

Raw sequence data and associated metadata of root endosphere fungal and bacterial communities associated with Schoenoplectus americanus have been submitted to NCBI Sequence Read Archive; BioProjects PRJNA854091 (fungi) and PRJNA854092 (bacteria). Customs R scripts and input files used in the analyses are publicly available in https://github.com/LumibaoLab

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SUPPORTING INFORMATION

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