American Journal of Botany

RESEARCH ARTICLE

Plant response to fungal root endophytes varies by host genotype in the foundation species *Spartina alterniflora*

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Manuscript received 2 April 2020; revision accepted 3 August 2020.

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Citation: Hughes, A. R., A. F. P. Moore, and C. Gehring. 2020. Plant response to fungal root endophytes varies by host genotype in the foundation species *Spartina alterniflora*. *American Journal of Botany*. 107(12): 1645–1653.

doi:10.1002/ajb2.1573

PREMISE: Root-associated fungi provide a wide range of functions for their host plants, including nutrient provisioning, pathogen protection, and stress alleviation. In so doing, they can markedly influence host-plant structural and physiological traits, although the degree to which these effects vary within particular plant host species is not well understood.

METHODS: We conducted a 7-month common-garden inoculation experiment to test the potential effects of a marine fungus (*Lulwoana* sp.) on the phenotypic traits of different genotypes of the host, the salt marsh plant species *Spartina alterniflora*. *Lulwoana* belongs to the dark septate endophytes (DSE), a polyphyletic group of fungi that are commonly found colonizing healthy plant roots, though their ecological role remains unclear.

RESULTS: We documented significant impacts of *Lulwoana* on *S. alterniflora* morphology, biomass, and biomass allocation. For most traits in our study, these impacts varied significantly in direction and/or magnitude across *S. alterniflora* genotypes. Effects that were consistent across genotype were generally negative. Plant response was not predicted by the percentage of roots colonized, consistent with findings that dark septate endophytes do not necessarily influence plant growth responses through direct contact with roots.

CONCLUSIONS: The observed changes in stem height, biomass, and biomass allocation have important effects on plant competitive ability, growth, and fitness, suggesting that plant–fungal interactions have community and ecosystem level effects in salt marshes.

KEY WORDS cordgrass; dark septate endophyte; intraspecific variation; *Lulwoana*; Poaceae; root-associated fungi; salt marsh; *Spartina alterniflora*; symbiont.

Plants and their interactions with root-associated fungi have wide-reaching ecological effects, from influencing individual traits to modifying interactions with other species to affecting biodiversity and biogeochemical cycles (Johnson et al., 2012). While mycorrhizal fungi have long been of interest, there is growing evidence that other root-associated fungi markedly influence host plant structural and physiological traits, particularly when mycorrhizal fungi are absent or in low abundance (Porras-Alfaro and Bayman, 2011; Clemmensen et al., 2013; Kivlin et al., 2013; Berthelot et al., 2019). Plant trait differences can result from functions provided by fungal endophytes, including the production of secondary metabolites with antibacterial properties and hormones that influence plant growth and biomass allocation (Andrade-Linares and Franken, 2013; Berthelot et al., 2019).

Dark septate endophytes (DSE) are a polyphyletic fungal endophyte group with high colonization rates in the roots of a broad range of host plants in nearly all biomes (Mandyam and Jumpponen, 2008, 2014; Newsham, 2011) and with melanized hyphae that may allow them to withstand extreme conditions (Jumpponen and Trappe, 1998; Kivlin et al., 2013). Unlike mycorrhizal fungi, DSE do not have specialized structures for resource exchange with host plants, but their presence is associated with increased plant shoot nitrogen and phosphorus concentrations (Newsham, 2011). Recent genomic evidence suggests that DSE have a spectrum of enzymes capable of degrading organic matter (Knapp et al., 2018), consistent with an ability to increase nutrient availability to plants, particularly in nutrient-poor soils (Newsham, 2011; Knapp et al., 2018). Dark septate endophytes can also protect against plant pathogens (Andrade-Linares and

Franken, 2013) and increase plant resistance or tolerance to stresses such as drought and warming (Kivlin et al., 2013). However, the degree to which DSE are a critical dimension of plant performance remains an open question.

Although DSE are a common component of the microbial communities colonizing healthy plant roots, many aspects of their ecology remain unknown (Mandyam et al., 2012). Host plant responses to DSE colonization vary along a mutualism–parasitism continuum (Mandyam et al., 2012), with colonization resulting in increased shoot and root biomass in some plant species, particularly in systems with low inorganic nitrogen availability (Newsham, 2011), and others showing neutral to negative biomass responses (Alberton et al., 2010; Mayerhofer et al., 2013). Plant responses to DSE can also vary within species (Tellenbach et al., 2011), leading to unpredictable effects of DSE that depend on plant genotype (Mandyam and Jumpponen, 2015). A better understanding of intraspecific variation in plant–DSE relationships is needed (Johnson et al., 2012), particularly in nonmodel systems of high ecological importance where plant–fungal interactions may influence plant host responses to environmental stress (Kivlin et al., 2013; Berthelot et al., 2019).

Spartina alterniflora Loisel (Poaceae), or saltwater cordgrass, is a perennial grass and a dominant plant species in low-elevation salt marshes of the Gulf of Mexico and the Atlantic coast of the United States (Pennings et al., 2005). Although *S. alterniflora* is colonized by a diverse suite of root-associated fungi (Kandalepas et al., 2015), little is known regarding the roles of these endosymbionts in salt marshes. *S. alterniflora* is not mycorrhizal (McHugh and Dighton, 2004; Daleo et al., 2008), but it is commonly colonized by other root-associated fungi, including DSE (Kandalepas et al., 2015; Lumibao et al., 2018). These DSE are responsive to variation in environmental conditions (Kandalepas et al., 2015; Moore et al., in press), and they may influence *S. alterniflora* growth and biomass (A. R. Hughes and C. Gehring, unpublished data).

We conducted a 7-month common-garden inoculation experiment to assess whether inoculation of *S. alterniflora* with a root-associated marine DSE fungus influenced morphology and biomass and whether these responses differed among host genotypes. Specifically, we tested the independent and interactive effects of fungal treatment (control vs. inoculation with a single isolate of the DSE *Lulwoana* sp.) and host plant genotype (18 genotypes of *S. alterniflora*) on host plant morphology and biomass. *Lulwoana* belongs to the order Lulworthiales, a group of root-associated fungi restricted to marine environments (Kohlmeyer et al., 2000), whereas most prior functional studies of DSE have focused on different orders (Heliotiales, Pleosporales). Although the biology of *Lulwoana* sp. is poorly known, it is in the family *Lulworthiaceae*, which includes the dominant root endophytes of *S. alterniflora* at some sites in the northern Gulf Coast (Kandalepas et al., 2015; Lumibao et al., 2018). Prior studies have shown substantial variation across *S. alterniflora* genotypes in key plant traits (e.g., stem height, stem production) and both plant–plant and plant–consumer interactions (Proffit et al., 2003, 2005; Hughes, 2014; Zerebecki et al., 2017). Thus, we hypothesized that the response to *Lulwoana* would also differ by plant genotype. By focusing on a nonmodel plant and nonmycorrhizal fungus, this study aims not only to advance general understanding of host–fungal associations, but also to address a persistent deficit in our understanding of how endophytic associations influence host plant performance, focusing on the hypothesis that outcomes vary due to host genotypic differences.

MATERIALS AND METHODS

Colonization and isolation of DSE from *S. alterniflora*

We conducted a preliminary survey to determine the most common DSE species in St. Joseph Bay (SJB), Florida, United States, where the *S. alterniflora* genotypes for this study were collected. First, we analyzed the percentage root length colonized by DSE on *S. alterniflora* roots collected from 40 plots, each 0.25 m^2, within an area of approximately 200 m^2 in a natural marsh in SJB using the grid-line intersect method with 100 intersections per sample (McGonigle et al., 1990): 100% of plots sampled were colonized by DSE, with root colonization averaging 20.6% (4.31 SD). No colonization by mycorrhizal fungi was observed, consistent with previous studies (McHugh and Dighton, 2004; Daleo et al., 2008).

To isolate species of DSE from *S. alterniflora* growing in SJB, we collected additional roots from three sites that spanned an ~9-km^2 area of the bay (Appendix S1); sites were a minimum of 3.2 km from one another, and 2–8 plants were sampled per site. We surface-sterilized 1-cm root sections (200 root segments, 16–17 per sample) in 50% v/v bleach, then 70% v/v ethanol. We rinsed them in sterile water, plated them on malt extract agar (MEA), and incubated them at room temperature. We isolated fungal hyphae originating from roots and grouped them based on morphological characteristics and identified the isolates using the methods of Lamit et al. (2014) for DNA extraction, amplification, and sequencing of the internal transcribed spacer region of the ribosomal RNA gene of the fungal genome.

We found seven different culturable root-associated taxa in *S. alterniflora*, with a member of the genus *Lulwoana* the most common, as it was isolated from 36% of the 200 surface-sterilized root segments. Putative members of the genus *Lulwoana* were also observed in high abundance using next-generation sequencing (NGS) of DNA from roots collected at SJB (C. Gehring, unpublished data). Only one described species occurs in this genus [Campbell et al., 2005; *Lulwoana uniseptata* (Nakagiri) Kohlm., Volkm.-Kohlm., J.Campb., Spatafora & Gräfenhan], which was observed as an endophyte in the roots of *Posidonia oceanica*, a Mediterranean seagrass (Torta et al., 2015). The morphological description of *Lulwoana* by Torta et al. (2015) closely matched our observations, except for slight differences in culture morphology that could be due to differences in the media used. In March 2013, we used subcultures from one of these *Lulwoana* isolates (from site S8; Appendices S1, S2; GenBank accession MT271362.1) to inoculate plants in a greenhouse experiment (see below). This isolate showed ~91% (99% query coverage) similarity to the DNA sequences deposited by Torta et al. (2015). Using a single isolate allowed us to focus on the relationship between *Lulwoana* and plant genotype without the potentially confounding effects of differences among isolates (Berthelot et al., 2019).

Greenhouse experiment

For the greenhouse common garden experiment at the Florida State University Coastal and Marine Laboratory, 18 genotypes of *S. alterniflora* were haphazardly selected from a larger greenhouse stock. These genotypes were originally collected in 2009 in SJB

from multiple sites (Appendices S1, S3). We confirmed that genotypes were genetically distinct using eight DNA microsatellite loci designed for this species (Blum et al., 2004; Sloop et al., 2005; see Hughes, 2014 for additional details). In November 2012, we isolated 10 replicate single stems of each genotype with attached root and rhizome and planted them individually in a 50:50 mix of commercial potting soil and sand in 6.4-L pots. We randomly assigned pots to locations within three water tables in the greenhouse. We irrigated pots with freshwater daily, immersed them in flow-through seawater from the Gulf of Mexico weekly, and fertilized them monthly with 2.81 g L^{-1} Miracle-Gro (Marysville, OH, USA) All Purpose liquid fertilizer (NPK at 24:8:16 with trace elements B, Mo, Cu, Fe, Zn, and Mn).

In March 2013 (4 months after planting in the common garden), we measured the number of stems in all pots. We then gently removed plants from the pot by hand, shook off the loose soil, rinsed the roots in a freshwater bath, and transferred each plant to a new pot filled with a 50:50 mix of commercial potting soil and sand that had been sterilized by autoclaving at 121.1°C for 45 min. Pots were randomly assigned to 10 plastic bins (39 L; 90 × 42.5 × 15 cm) with 18 pots per bin (1 per genotype) across three water tables in the greenhouse. The plastic bins were randomly assigned to one of two experimental treatments: fungal inoculation or control. In each pot, we extracted a plug of soil to a depth of 5 cm using a 10-mL syringe corer, placed a 1-cm^2 piece of agar in the hole created by the core, and replaced the soil. Control treatments consisted of a piece of sterile potato dextrose agar, and fungal treatments consisted of agar with *Lulwoana*. Following the inoculation, plants were irrigated with freshwater daily and immersed in sterile seawater made with Instant Ocean (Blacksburg, VA, USA) for 8 h once per week for the duration of the experiment. This frequency is consistent with the intermittent flooding regime in St. Joseph Bay where the plants were collected. We did not fertilize the plants post inoculation. The bins were elevated above the bottom of the water table with bricks to prevent the mixing of water draining from the bins following irrigation.

In October 2013 (7 months after inoculation), we measured morphological, biomass, and biomass allocation traits important for plant fitness and species interactions (Aerts, 1999; Shipley and Meziane, 2002), including stem production, stem height, stem rigidity, and the number of leaves per stem. Stem production was calculated as the difference between the number of live stems at the end of the experiment and the number counted just before inoculation (March 2013). Stem height and number of leaves per stem were calculated as the mean of all stems in a pot. Stem rigidity was calculated for three stems per pot by attaching two paper clips to each stem at a height of 30 cm above the sediment surface and measuring the angle the leaf bent from its natural position (see Zerebecki and Hughes, 2013). We also measured flower production as the number of flowering stems produced per pot during the inoculation period. We then collected a segment of leaf tissue from the innermost leaf for nutritional content analysis and ~6 cm of root tissue per root from at least three roots for analysis of root fungal colonization. Root samples were kept cool (4°C) and shipped overnight directly to Northern Arizona University. Plants were subsequently harvested, rinsed of sediment, and shipped overnight on ice to Northeastern University. We then separated the plants into aboveground tissue, root tissue, and rhizome tissue and dried them at 60°C for at least 48 h to determine dry biomass. We quantified leaf tissue nutritional content (%C, %N, and C:N) by drying leaf samples for 24 h at 60°C and grinding them to a fine powder with a mixer mill (RETSCH MM 400, Haan, Germany). We analyzed a ground sample of known mass (3–5 mg) on an elemental analyzer (Thermo Fisher Scientific FlashEA 1112 NC Analyzer, Tampa, FL, USA) using aspartic acid (36.09% carbon and 10.52% nitrogen) as a reference standard.

We analyzed percentage colonization of a subset of the roots from the control (N = 31) and *Lulwoana* (N = 35) treatments to confirm that our inoculations were effective. We included at least one control and one *Lulwoana* treatment for each *Spartina* genotype. Roots from multiple locations from each sample were placed in a tissue cassette, cleared for 20 min in boiling 10% w/v potassium hydroxide, rinsed in distilled water, mounted on glass slides, and observed using a compound microscope at 400× magnification. The presence of melanized, septate hyphae and microsclerotia were used as indicators of DSE and quantified using the grid-line intersect method with 100 intersections per sample (McGonigle et al., 1990).

Statistical analyses

We used contingency table analyses to examine the presence/absence of DSE on the sampled roots at the end of the experiment by inoculation treatment (control vs. *Lulwoana*). We then used an effect size approach to test the effects of genotype and fungal inoculation on *S. alterniflora* morphological traits. An advantage of this approach is that it normalizes the responses to the inoculation treatments and allows a test of the null hypothesis that there is no response to inoculation (Mandyam et al., 2012; Kivlin et al., 2013; Mandyam and Jumpponen, 2015). We calculated the responsiveness of *S. alterniflora* to inoculation by genotype for each response variable as the difference between an individual pot of the inoculated treatment and the mean of the control pots for that genotype, divided by the mean of the control pots for that genotype. To examine whether genotypes differed in response to inoculation across all responses, we ran a PERMANOVA on the responsiveness metric for each trait with genotype as the predictor variable (Anderson, 2017). We then visualized these differences among genotypes in their response to inoculation across trait space by conducting a principal components analysis (PCA) in R v3.4.4 (R Core Team, 2018) using the prcomp function in the vegan package (Oksanen, 2013). We used genotype averages of the responsiveness metric for each trait as the input into the PCA.

The PERMANOVA indicated that genotypes differed across all traits. To examine which traits contributed to this variation, we ran independent mixed effects models on individual trait responsiveness metrics using the lme4 package in R v3.4.4 (R Core Team, 2018), including a fixed effect of genotype and a random effect of bin (to account for fungal treatments being assigned at the bin level). We visually inspected the residual vs. fitted values for deviations from normality. Flower production was the only variable that had clear deviations, so we log-transformed it for analysis. When there was a significant effect of genotype, we calculated 95% confidence intervals to examine which genotypes responded positively or negatively to inoculation. When there was no effect of genotype, we conducted a t-test to determine whether there was a significant response to inoculation across all genotypes. For traits that had significant positive or negative responses to inoculation, we ran linear models with independent effects of genotype and percentage colonization to test whether the percentage colonization of roots

at the end of the experiment predicted the absolute magnitude of this response.

To examine relatedness among genotypes, we conducted an individual-based PCA on the eight DNA microsatellite loci. We ran the PCA using the adegenet and ade4 packages (Jombart, 2008, 2016) in R v2.4.4 (R Core Team, 2018) on a matrix of allele frequencies, with each row representing an individual and each column representing an allele. The strength of this approach is that it does not assume Hardy–Weinberg equilibrium (Jombart, 2008, 2016). To test whether genetic differences (the matrix of microsatellite allele frequencies) and trait responses to inoculation (the matrix of response ratios) were correlated, we conducted a Mantel test with 999 permutations using the mantel function in the vegan package (Oksanen, 2013).

RESULTS

Inoculation resulted in differential colonization by DSE between the inoculated and control treatments ($\chi^2 = 45.43$, $P < 0.0001$), suggesting our inoculation was effective. One hundred percent (35/35) of the sampled roots in the inoculation treatment were colonized by DSE, indicating that every genotype was capable of being colonized. Colonization ranged from 2 to 64%, with a mean [SE] of 19.5 [2.1]%, similar to average rates of colonization observed in the field collections described above. In the control treatment, 81% of samples (25/31) were not colonized by DSE, and mean [SE] colonization of the remaining samples was 2.0 [1.0]%. This small amount of colonization in noninoculated controls may be the result of airborne or potting soil contamination or from DSE occurring naturally within the roots of the initial transplants. Our genotype replicates came from field-collected plants that likely harbored endophytes at the time of propagation, yet the strong differences in DSE colonization that we observed between inoculated and control plants in all *Spartina* genotypes suggest that these endophytes did not contribute significantly to the patterns described below. Furthermore, low levels of colonization in inoculated controls are commonly observed in studies of root-colonizing fungi (e.g., Berruti et al., 2015; Mueller et al., 2019).

Genotypes varied in their response to fungal inoculation across all traits (PERMANOVA: genotype $F_{17, 64} = 3.32$, $P < 0.001$; Fig. 1). In our principal component analysis of trait responses, PC1 (31%) and PC2 (21%) accounted for a combined 52% of the responsiveness to fungal inoculation across *Spartina* genotypes. PC1 was primarily a function of plant biomass: stem mass (0.47), leaf number (0.39), rhizome mass (0.38), average height (0.37), and root mass (0.34). Principal component 2 was mainly a function of leaf nutrient content: leaf C:N (0.62) and leaf %N (−0.61).

When we examined traits individually, inoculation alone had a significant effect on several key *S. alterniflora* traits (Fig. 2). These effects were consistent across genotypes, and negative overall, for average stem height (genotype $F_{17, 64} = 1.43$, $P = 0.15$; R t-test $t = -2.36$, df = 85, $P = 0.02$), number of leaves (genotype $F_{17, 64} = 1.36$, $P = 0.18$; R t-test $t = -1.97$, df = 85, $P = 0.05$), and final root mass (genotype $F_{17, 64} = 1.09$, $P = 0.38$; R t-test $t = -2.05$, df = 85, $P = 0.04$). There was also a marginally significant negative effect of inoculation on final rhizome mass (genotype $F_{17, 64} = 1.34$, $P = 0.19$; R t-test $t = -1.84$, df = 85, $P = 0.07$). Percentage colonization was not correlated with the responsiveness of any trait.

Consistent with the multivariate analysis, the response to inoculation differed significantly by genotype for most individual traits, including stem production (genotype $F_{17, 64} = 1.89$, $P = 0.03$;

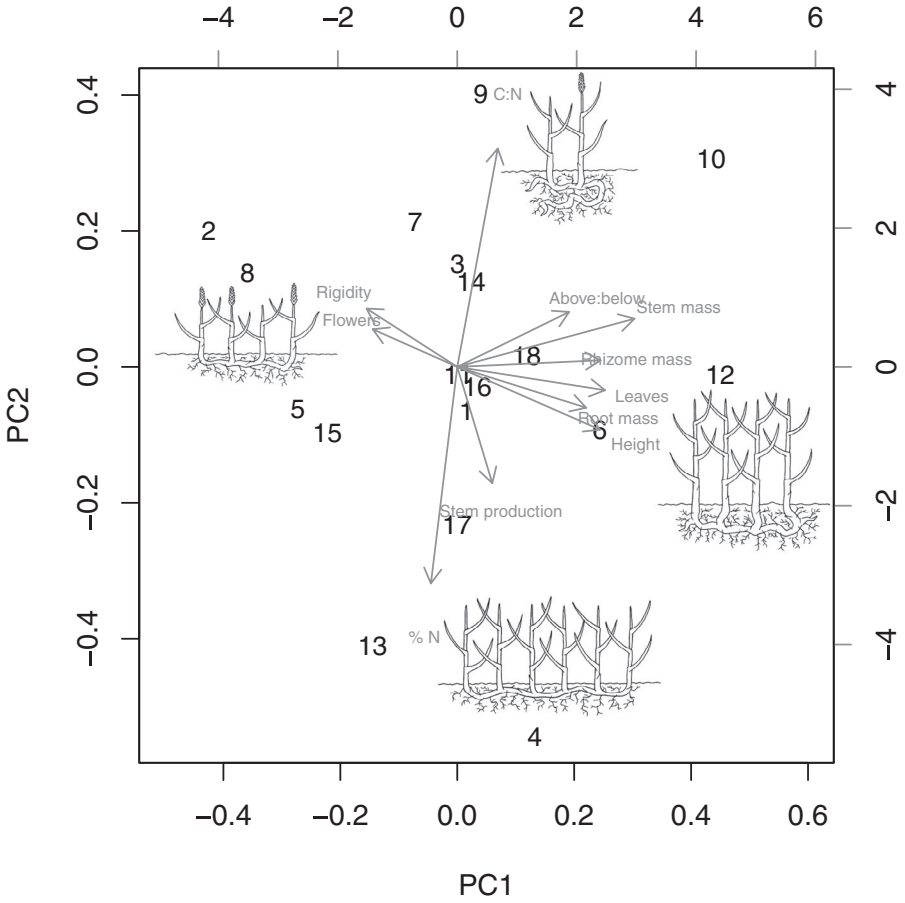

FIGURE 1. Principal component analysis (PCA) of *Spartina alterniflora* responsiveness to inoculation with *Lulwoana* across multiple traits: stem production, flower production, stem biomass, root biomass, rhizome biomass, ratio of above- to belowground biomass (Above:below), number of leaves, stem height, stem rigidity, leaf C:N, leaf %N. Black numbers represent the mean responsiveness to inoculation of each *S. alterniflora* genotype, reflecting how traits changed relative to the inoculated control of a given genotype. Trait loadings on the PCA axes are indicated in gray. Line drawings depict representative trait and allocation strategies in different regions of the PCA. Drawings by M. Freedman.

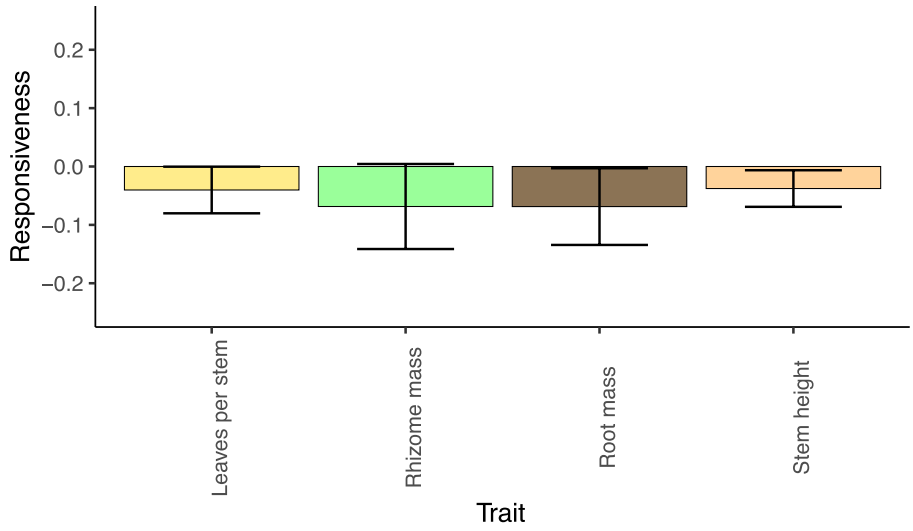

FIGURE 2. Responsiveness of *Spartina alterniflora* to *Lulwoana* inoculation was consistently negative across genotypes for number of leaves per stem, rhizome biomass, root biomass, and stem height. Responsiveness was calculated as the difference between an individual pot of the inoculated treatment for a given genotype and the average of the control treatment pots for that genotype, divided by the average of the control treatment pots for that genotype. Error bars represent 95% confidence intervals, which only overlap zero for rhizome biomass.

Fig. 3A), flower production (log-transformed; genotype $F_{17, 64} = 5.15$, $P < 0.001$; Fig. 3B), aboveground mass (genotype $F_{17, 64} = 2.42$, $P = 0.006$; Fig. 3C), above to belowground biomass ratio (genotype $F_{17, 64} = 3.36$, $P < 0.001$; Fig. 3D), C:N in leaf tissue (genotype $F_{17, 64} = 1.98$, $P = 0.02$; Fig. 3E), leaf %N (genotype $F_{17, 64} = 1.95$, $P = 0.03$), and stem rigidity (genotype $F_{17, 68} = 2.96$, $P < 0.001$; Fig. 3F). Inoculation significantly reduced stem production for two genotypes (7, 8) and significantly increased it for four genotypes (4, 11, 12, 15), relative to controls (Fig. 3A). Eleven genotypes altered their flower production in response to inoculation: genotypes 2, 5, and 18 produced more flowers, whereas 4, 9, 11, 12, 13, 14, 16, and 17 produced fewer flowers (Fig. 3B). Changes in aboveground mass and the ratio of above to belowground mass were similar, with five genotypes (5, 8, 13, 15, 17) producing less aboveground biomass relative to noninoculated plants (Fig. 3C, D). Inoculation increased C:N in leaf tissue for one genotype (9) and decreased it for three others (13, 17, 18; Fig. 3E); these differences were due to changes in %N, not %C. Stem rigidity significantly increased with inoculation for two genotypes (7, 8) and decreased for six (4, 10, 12, 14, 16, 18; Fig. 3F).

In our PCA of the relatedness of genotypes across DNA microsatellite loci, PC1 (16.6%) and PC2 (14.4%) accounted for a combined 31% of the observed variation (Fig. 4A). Principal component 3 (12.6%) and PC4 (10.6%) explained similar amounts of variation in genetic relatedness (Appendix S4). There was no obvious pattern in relatedness among genotypes based on spatial proximity of their collection site (Appendix S3): although some genotypes from a single site clustered together (e.g., 9, 10, 11), others were quite distinct (e.g., 16, 17). In addition, there was no significant correlation between genetic relatedness among genotypes and their multivariate trait responsiveness to fungal inoculation (Mantel test, $P = 0.67$; Fig. 4B, Appendix S5).

DISCUSSION

We documented significant effects of a broadly distributed marine dark septate endophyte (Kandalepas et al., 2015; Lumibao et al., 2018) on the morphology, biomass, and biomass allocation of the dominant marsh plant species, *S. alterniflora*. For some above- and belowground traits, the effects of *Lulwoana* on *S. alterniflora* were consistently negative, in contrast to prior results that grasses tend to benefit from DSE (Mandyam et al., 2012; Mayerhofer et al., 2013). Further, plant response was not predicted by the percentage of roots colonized, consistent with previous studies (Berthelot et al., 2017) and with findings that DSE do not necessarily influence plant growth responses through direct contact with roots (Newsham, 2011). Rather, DSE may increase mineralization of organic compounds or solubilization of insoluble minerals in the rhizosphere (Mandyam and Jumpponen, 2015; Newsham, 2011). They also produce bioactive substances such as hormones and volatile organic compounds that diffuse in the soil, thereby promoting plant growth (Berthelot et al., 2019).

Previous studies of the effects of root-associated fungi on *S. alterniflora* have demonstrated the potential for widespread negative effects. For instance, *Fusarium* spp., a putative fungal pathogen, was consistently found at sites where *S. alterniflora* was experiencing dieback, yet it did not cause mortality of healthy plants in the greenhouse (Elmer et al., 2012). Subsequent experiments demonstrated that *Fusarium* had negative effects on *S. alterniflora* when in combination with drought stress and that herbivory was higher on drought-stressed, inoculated plants than healthy plants (Elmer, 2014). We also documented consistent negative effects of *Lulwoana* on important plant traits, but the plants in our study did not show signs of stress (e.g., yellowing leaves) pre-inoculation; thus, our results suggest that factors other than stress can contribute to negative effects of root-associated fungi in the marsh system.

Consistent with past findings (Newsham, 2011; Knapp et al., 2018), inoculation with DSE increased the percentage nitrogen in *S. alterniflora* leaf tissue for at least some genotypes. However, this increase did not translate to a general benefit on growth-related traits such as stem height or leaves per stem. Because the magnitude of DSE effects on plant nitrogen content can decrease with experimental duration (Newsham, 2011), it is possible that we missed positive nutrient responses that occurred before the end of our 7-month experiment. Alternatively, nutrients may not have been limiting in our greenhouse experiment, thereby minimizing positive effects of DSE, which are more common at low nutrient availability (Newsham, 2011). Given that plants were not supplemented with nutrients during the 7-month period post inoculation, we think it unlikely that nutrients were not limiting, but additional manipulations using field soil and a range of nutrient conditions would help refine our understanding of these relationships.

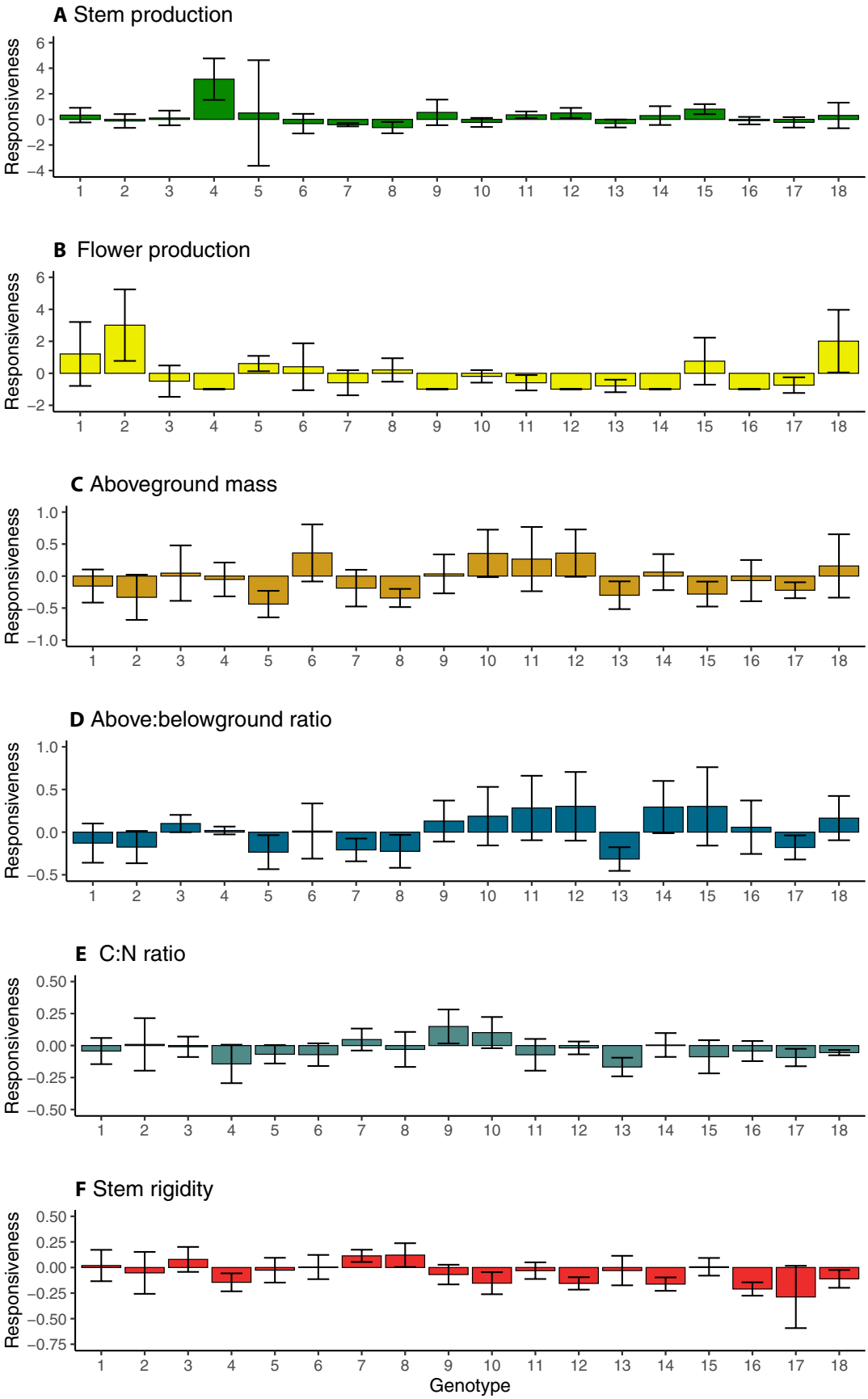
A Stem production
B Flower production
C Aboveground mass
D Above:belowground ratio
E C:N ratio
F Stem rigidity
Responsiveness
Genotype

FIGURE 3. Responsiveness of *Spartina alterniflora* to *Lulwoana* inoculation varied in direction and magnitude across *S. alterniflora* genotypes for (A) stem production, (B) flower production, (C) aboveground biomass, (D) the ratio of above- to belowground biomass, (E) the ratio of carbon to nitrogen in leaf tissue, and (F) stem rigidity. Responsiveness was calculated as the difference between an individual pot of the inoculated treatment and the average of the control treatment pots for that genotype, divided by the average of the control treatment pots for that genotype. Flower production was log-transformed for analysis to improve normality; the untransformed data are presented here. Error bars represent 95% confidence intervals.

While meta-analyses of plant responses to fungal endophytes have reached contrasting conclusions about whether their effects are generally positive or negative (Newsham, 2011; Mandyam et al., 2012; Mayerhofer et al., 2013), our results highlight that plant intraspecific differences (i.e., genotypic variation) can affect the direction and magnitude of the relationship with a single fungal endophyte. For most of the traits studied, inoculation with *Lulwoana* had effects that varied in direction and/or magnitude across *S. alterniflora* genotypes. For example, while *Lulwoana* had no effect on stem production and increased flower production in genotype 2, it had opposing effects in genotype 4. Widespread differential responses of *S. alterniflora* genotypes to inoculation may influence competitive relationships among genotypes and the maintenance of genotypic diversity in the salt marsh. Further, these results indicate that genotypic diversity may bolster plant resistance to negative effects of DSE in natural marshes and increase marsh productivity. We used only a single DSE isolate, and other isolates may have yielded different results (Mandyam et al., 2013). Future studies that factorially manipulate both multiple host genotypes and multiple DSE isolates are needed to determine the full range of interaction signs and strengths, and the potential for local adaptation, in this system.

The variable plant–fungal interactions observed in our study have important management implications. Salt marshes are one of the primary targets of coastal habitat restoration (Grabowski et al., 2012), and *S. alterniflora* is one of the dominant species planted. There is growing interest in the role of soil microbes in plant restoration, with suggestions that nursery inoculation of plants with beneficial fungi could enhance restoration success in the marsh (McHugh and Dighton, 2004) and more broadly across terrestrial ecosystems (Neuenkamp et al., 2018). Our results suggest that identifying growth-promoting strains of DSE will be challenging given the negative effects of *Lulwoana* on several key metrics, as well as variation in the direction and magnitude of fungal effects across *S. alterniflora* genotypes on other traits. Our experiment was conducted in pots in the greenhouse; field experiments will be critical for testing the generality of these results as well as the effectiveness of inoculation in restoration, which can be highly context dependent (Neuenkamp et al., 2018). For instance, in a field setting, host genotype effects on DSE may influence interactions with other host-associated microbes as observed in loblolly pine associations

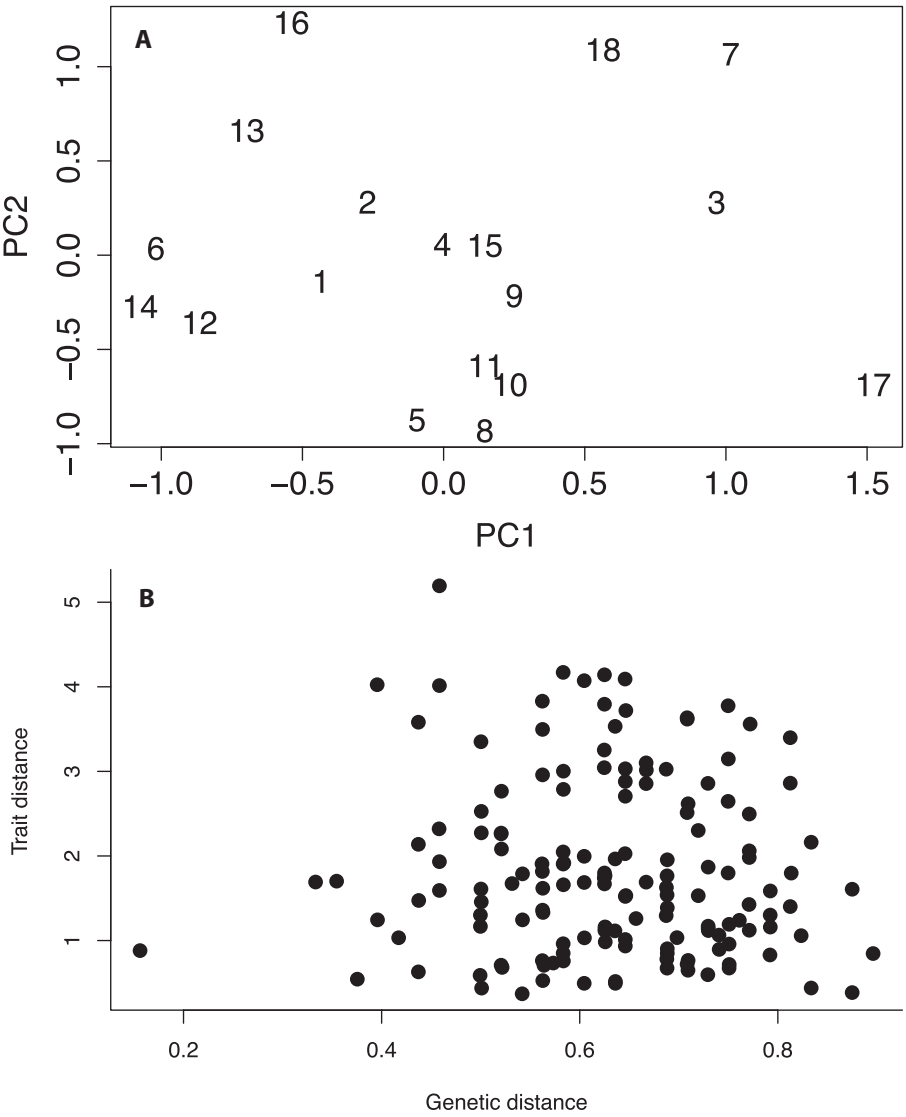

FIGURE 4. (A) Principal component analysis (PCA) indicating degree of genetic similarity among *Spartina alterniflora* genotypes based on eight DNA microsatellite markers. Black numbers indicate each *S. alterniflora* genotype. (B) Tests for correlations between trait responses and genetic distance. There was no significant correlation between genetic relatedness among genotypes and their multivariate trait responsiveness to fungal inoculation (Mantel test, $P = 0.67$; see Appendix S5 for an alternative analysis with consistent results).

with fungal pathogens and ectomycorrhizal fungi (Piculell et al., 2018).

Salt marshes span dramatic shifts in a variety of abiotic stresses over a tidal elevational gradient (Bertness and Ellison, 1987; Pennings et al., 2005). *Spartina alterniflora* exhibits extensive trait variation along this dynamic gradient, both across and within clearly differentiated tall- and short-form zones (Bertness and Ellison, 1987; Richards et al., 2005; Zerebecki et al., 2017). Our results suggest that trait variation that has previously been attributed to environmental conditions in natural marshes may also be influenced by genotype-specific responses to fungal associates. The observed genotypic variation in response to *Lulwoana* was not due to differential responses by one or a few genotypes in a particular trait, but instead due to multivariate differences across traits both above- and belowground that suggest potential trade-offs among traits (Fig. 1). Such changes in stem height, biomass, and biomass allocation have important effects on plant competitive ability, growth, and fitness (Aerts, 1999; Shipley and Meziane, 2002). For instance, increased allocation belowground has been linked with increased competitive ability among marsh plant species (Brewer et al., 1998; Emery et al., 2001). Because intraspecific variation in plant–fungal interactions may be common (Johnson et al., 2012), the determinants of genotype-specific changes in allocation in response to inoculation, as well as their implications for intra- and interspecific interactions, warrant further investigation.

ACKNOWLEDGMENTS

We thank B. Brady, R. Coker, T. Hanley, M. Murdock, F. Schenck, and R. Zerebecki for help maintaining the greenhouse experiment and processing samples. M. Freedman created the drawings for Fig. 3. We appreciate constructive comments from two anonymous reviewers. This project was supported by grants from the National Science Foundation (DEB-0928279 to ARH, IOS-1556738 to ARH, and IOS-1556087 to C.A.G.). This is contribution 408 from the Northeastern University Marine Science Center.

AUTHOR CONTRIBUTIONS

A.R.H., A.F.P.M., and C.A.G. conceived, designed, and executed the study. A.R.H. conducted the statistical analyses and wrote the first draft of the manuscript. All authors contributed to subsequent drafts.

DATA AVAILABILITY

Data are available from the Northeastern University Digital Repository Service (http://hdl.handle.net/2047/D20330489).

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Map of the sites in St. Joseph Bay, Florida, where genotypes of *Spartina alterniflora* and cultures of *Lulwoana* were collected for this study.

APPENDIX S2. Image of a subculture of the *Lulwoana* isolate used in our inoculation experiment. Photo credit: A. Moore.

APPENDIX S3. Information on *S. alterniflora* genotypes used in the inoculation experiment.

APPENDIX S4. Eigenvalues of the genotype principal component analysis.

APPENDIX S5. Tests for correlations between trait responses and genetic distance.

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