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RESEARCH ARTICL

BRIEF COMMUNICATION

Rhizosphere microbial communities reflect genotypic and trait variation in a salt marsh ecosystem engineer

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PREMISE: There is growing recognition that intraspecific genetic variation in plants can influence associated soil microbial communities, but the functional bridges linking plant genotype with microbial community structure are not well understood. This deficit is due in part to a prevailing focus on characterizing relationships between microbial communities and functional trait variation among plant species or across plant communities, rather than within a single species.

METHODS: We examined whether and how spatiotemporal variation in salt marsh rhizosphere microbial communities reflect plant provenance (genotypic variation) and associated trait variation within an ecosystem engineer, *Spartina alterniflora*. We planted *S. alterniflora* from four genetically distinct source populations in replicate sets of experimental plots across a shoreline in southeastern Louisiana, USA. After 2 years, we measured functional plant traits and profiled microbial communities.

RESULTS: Bacterial and fungal α-diversity and richness were significantly higher in winter than in summer and corresponded to plant trait variation associated with provenance. Notably, 20% of the variation in fungal community composition was explained by trait differences while bacterial community structure did not reflect plant provenance or trait variation. However, evidence was found suggesting that bacterial communities are indirectly shaped by the influence of plant provenance on soil physicochemical properties.

CONCLUSIONS: This study illustrates that intraspecific genetic and corresponding trait variation in an ecosystem engineer can shape rhizosphere microbial communities, with fungal communities being more responsive than bacteria to the influence of plant provenance and associated trait variation. Our results highlight the potential relevance of plant intraspecific variation in plant–microbe–soil feedbacks shaping naturally depauperate ecosystems like salt marshes.

KEY WORDS bacteria; fungi; plant functional traits; plant genetic variation; rhizosphere microbial diversity; *Spartina alterniflora*; salt marsh.

Soil microbial communities are often subject to the influence of plants (Bardgett and Van Der Putten, 2014). Microbial communities from the soil surrounding the roots (rhizosphere), for example, can reflect a host of conditions shaped by plants including temperature, pH, salinity, nutrient availability, and organic matter (Lozupone and Knight, 2007; Sharp et al., 2014). These and similar relationships raise the possibility of functional linkages between rhizosphere microbial community composition and trait variation

in associated plants. Though recent trait-based assessments indicate that plant functional variation can give rise to ecologically meaningful plant–microbe interactions (Berg and Smalla, 2009; de Vries et al., 2012; Li et al., 2018), understanding of the causal mechanisms underlying observed relationships remains elusive. Some elementary insights (e.g., the distribution of soil microbial communities) have been gained from studies of associations with broad functional variation among plant species or across plant communities (e.g., Wardle et al., 2004; Delgado-Baquerizo et al., 2018). A growing number of studies suggests, however, that greater insight can be obtained by examining associations with intraspecific trait variation (e.g., Benedek et al., 2019; Pérez-Izquierdo et al., 2019).

The diversity and composition of rhizosphere microbial communities can be shaped by intraspecific variation in plants (Pérez-Izquierdo et al., 2019). Work on agricultural crops, for example, has demonstrated that soil microbial communities vary with plant genotype (e.g., di Giovanni et al., 1999; İnceoğlu et al., 2012). Fewer studies have investigated plant genotype–soil microbiome relationships in a natural setting, but there are provocative indications that natural microbial communities can differ as a result of genetically based variation in soil-modifying traits like nutritional strategy, tissue chemistry, as well as leaf and root production (e.g., Seliskar et al., 2002; Schweitzer et al., 2008). Accordingly, further exploration is warranted to gain more detailed understanding about the nature of observed relationships (Delgado-Baquerizo et al., 2018), such as whether particular microbial groups or taxa might be favored by particular plant functional traits. Similarly, detailed assessment of intraspecific plant trait variation could strengthen understanding of microbial community stability, including seasonal dynamics and responses to climatic disturbances (Barboza et al., 2018).

In this study, we examined whether the structure of salt marsh rhizosphere microbial communities corresponds to intraspecific variation in the foundational grass *Spartina alterniflora* Loisel, an ecosystem engineer that is naturally distributed across the Atlantic and Gulf coasts of North America (Blum et al., 2007; Strong and Ayres, 2013). Taking advantage of replicated sets of common garden plots (Wagner et al., 2014; Sayer et al., 2017) established for a field-scale shoreline restoration experiment utilizing plants from genetically distinct source populations (Blum et al., 2014; Bernik et al., 2018b), we tested the hypothesis that rhizosphere microbial communities exhibit environmentally driven seasonal variation but nonetheless differ according to plant provenance and associated phenotypic trait variation.

MATERIALS AND METHODS

Study site and experimental design

Following Seliskar et al. (2002), experimental plots of transplanted *S. alterniflora* were established along 400 m of shoreline during the summer of 2011 and monitored over 2 years as part of a marsh restoration experiment in Bay Jimmy, Louisiana (Appendix S1) (Bernik, 2015). The study evaluated alternative methods for oil removal and shoreline remediation in response to the 2010 *Deepwater Horizon* oil spill (Michel et al., 2013; Blum et al., 2014; Zengel et al., 2015). To test for the effects of intraspecific variation on aboveground (AG) and belowground (BG) conditions, replicate plots were planted with *S. alterniflora* from four genetically distinct populations (Appendices S2 and S3) comprising two natural and two cultivated sources (Bernik, 2015; Bernik et al., 2018b). Plants were obtained from a nearby area of Bay Jimmy (BJ) that was not affected by the oil spill and from Catfish Lake (CL), which is a habitat similar to Bay Jimmy located 40 km west of the study site. Source material (i.e., plugs with rhizomes and roots) was gathered from BJ and CL so that genotypic diversity within each plot (i.e., per unit area) would approximate that found within a comparable area at the source location (Richards et al., 2004; Bernik, 2015; Appendices S2 and S3). We also elected to work with Vermilion (V), which is a cultivar used almost exclusively in restoration projects on the Gulf coast since 1989 (USDA NRCS, 2018). Likewise, we worked with the CP9 (CP) cultivar, which is a derivation of six accessions of *S. alternifora* selected for seed production (Utomo et al., 2010). We obtained V and CP cultivars from nurseries at Nicholls State University and the LSU AgCenter, respectively. All source material was initially brought to a greenhouse, thoroughly rinsed clean, and separated into similarly sized rhizomes with a single stem node for subsequent planting. A subsample of the starter plants from each source were genotyped at a suite of species-specific microsatellite loci (Blum et al., 2004; Sloop et al., 2005) to characterize genotypic variation and differentiation according to provenance (Appendices S2 and S3).

Following a random block design to control for physiographic gradients along the shoreline (Bernick et al., 2018a), we established five replicate 25-m^2 plots with *S. alterniflora* from each source, except for the CP cultivar, which was only planted in two plots due to the scarcity of starting material. Within each plot, 55 bare root starter stocks were hand-planted along measured rows, giving a planting density of 2–3 individuals m^{-2}. Plants were allowed to establish for a full year before the onset of data collection so that measurements would reflect mature vegetation grown under common garden conditions. To enable comparisons to unrestored conditions, a set of control plots (NC) were kept unvegetated for the first year, after which natural colonization was allowed to proceed (Appendix S1).

Plant functional traits

Previous work has shown that *S. alterniflora* exhibits considerable heritable phenotypic variation (e.g., Seliskar et al., 2002; Bernick et al., 2018b). To assess relationships between phenotypic traits and soil microbial community structure, we quantified phenotypic variation by provenance following methods adapted from Seliskar et al. (2002). Vegetation was sampled at the end of the second growing season in November 2013. All plots (n = 17) other than the control plots were sampled by harvesting three 10 cm diameter cores that included all AG (aboveground) and BG (belowground) plant tissues from which we measured total shoot height and density, tiller height and density, and the length, density, and mass of seed heads as described by Bernick (2015) and Bernick et al. (2018b). We also measured shoot diameter, leaf count, and leaf length for three mature shoots per core. Dried mass was obtained for AG and BG tissue, with BG samples divided into depth intervals of 10 cm. Concentrations of carbon (C) and nitrogen (N) were measured for soils and for homogenized tissues using a CN analyzer (Bernick, 2015).

Rhizosphere microbial community variation

In January and June 2013, 2 cm diameter cores of rhizosphere soil (i.e., soil proximate to plant roots) were taken in all planted and control plots using butyrate tubes. The top 1–2 cm of material from each sample was analyzed to characterize aerobic soil. All

samples were immediately put on ice and frozen at −20°C within 24 h. Genomic DNA was extracted from all samples using a MoBio Powersoil DNA Isolation Kit (Qiagen, Hilden, Germany), and then sent to ACGT, Inc. (Wheeling, IL, USA) for amplification of the ITS1 (fungi) and 16 rRNA V4 (bacteria) regions and next-generation sequencing (Appendix S3). Resulting sequences were quality-filtered, demultiplexed, and processed. Operational taxonomic units (OTUs) were picked in QIIME 1.9.1 (Caporaso et al., 2010) and used to delineate among microbes for characterization of microbial diversity to facilitate comparisons with previous findings and studies. Taxonomic identity was assigned with UCLUST (Edgar 2010) using combined Greengenes 13_8 (McDonald et al., 2012) and UNITE databases (Nilsson et al., 2018) (Appendix S3).

Statistical analyses

We conducted a linear discriminant analysis (LDA) to assess whether and how plant traits varied by provenance. We first clustered plant trait variables using Ward's *D* minimum variance to determine collinearity (Murtagh and Legendre, 2014), which enabled us to identify a subset of representative traits within each cluster to circumscribe plant productivity and trait-based functional variation for further statistical analyses (Appendix S4). We conducted the LDA on the subset of traits using lda in MASS with the "moment" method (Venables and Ripley, 2002) in R v 3.6 (R Core Team, 2016), followed by Pearson correlation tests between the LDA discriminant values and the original trait variables. Variables were scaled to standard *z*-scores with a mean of 0 and standard deviation of 1. We also conducted linear regressions to determine whether soil C and N (i.e., soil properties) varied according to plant provenance and LDA discriminant values.

We identified predictors of rhizosphere microbial diversity according to estimates of α-diversity (within-sample diversity) and seasonal change in α-diversity (i.e., temporal diversity) of bacteria and fungi. Bacterial and fungal samples were rarefied to a depth of 15,000 and 1000 sequences, respectively, which were then used to calculate Shannon diversity in vegan (Oksanen et al., 2013) and Chao1 (Chao, 1984) as an estimate of species richness. A nonparametric two-sample *t*-test was run using 999 Monte Carlo permutations to test for seasonal differences in α-diversity metrics. We then conducted partial least squares regression (PLSR) analyses to assess whether α-diversity metrics (Shannon or Chao1) were responsive to plant provenance, individual plant traits, soil properties, season, and the presence of residual oil (total polycyclic aromatic hydrocarbons [PAHs], Appendix S2) given the history of oiling at our study site (Michel et al., 2013; Kandalepas et al., 2015). Plot location was not included in the model because we did not detect evidence of spatial autocorrelation among samples based on pairwise similarity and Mantel tests (Appendix S5) and because spatial location was collinear with the presence of residual oil. We also performed PLSR analyses to assess whether seasonal change in α-diversity (i.e., summer-to-winter change in a given plot) reflected provenance, individual traits, soil properties, and residual oil. PLSR analyses were conducted using the pls package (Mevik and Wehrens, 2007), first with CV as a validation method to determine the optimum number of components based on adjusted CV, and then with the ascribed number of components. We tested for significance with the jackknife method and visualized the results by plotting the correlations between response and explanatory factors.

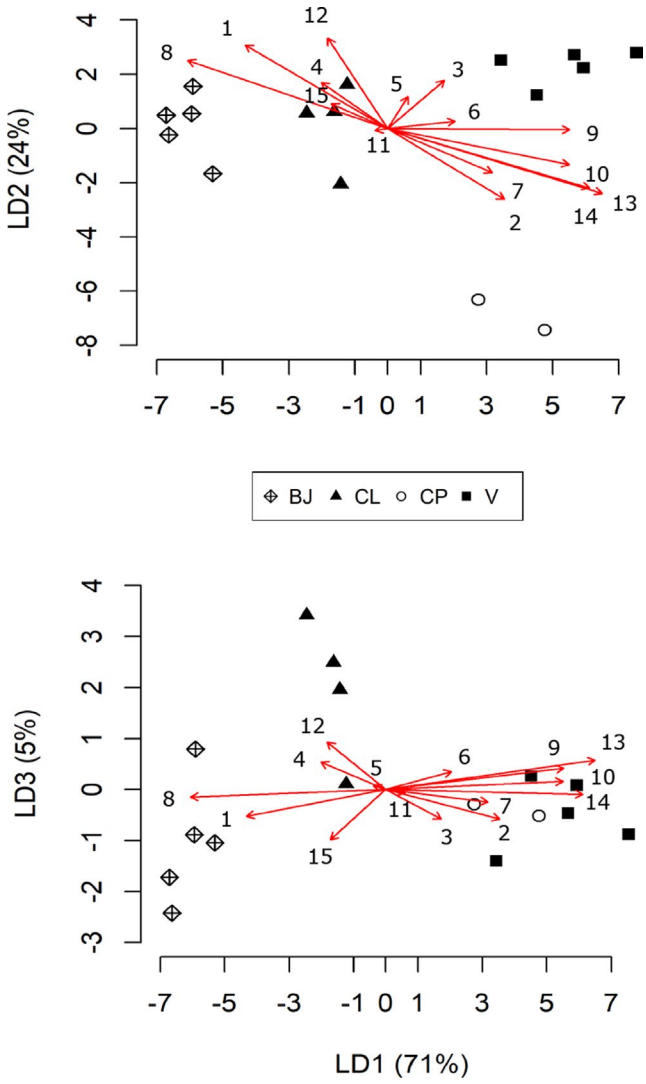

FIGURE 1. Functional trait variation according to plant provenance based on discriminant function analysis. Discriminant functions LD1, LD2, and LD3 explain 71%, 24%, and 5% of trait variation between provenances, respectively. Plant trait vectors depict coefficients of variation relative to the discriminant axes. See Appendix S4 for coefficient values. 1, BG biomass; 2, AG biomass; 3, shoot height; 4, shoot density; 5, tiller density; 6, tiller height; 7, leaf height; 8, leaf number; 9, R:S; 10, stem diameter; 11, seed mass; 12, $C:N_{[AG]}$; 13, $C:N_{[BG]}$; 14, $N_{[BG:AG]}$; 15, $C_{[BG:AG]}$.

We examined the relative influence of plant provenance and associated traits on microbial community composition according to pairwise community distances. We calculated weighted UniFrac dissimilarity values for bacterial communities and Bray–Curtis dissimilarity values for fungal communities. We first characterized variation in rhizosphere communities according to plant provenance by graphically examining phylum- and class-level changes in the abundance of microbial taxa using a heatmap. For bacteria, we presented phylum-level abundance changes as it provided the best resolution for emergent patterns of diversity and composition. We then used a variation-partitioning approach to identify predictors of microbial community composition (Borcard et al., 1992). This approach enabled us to decompose the total variation of microbial community composition into the amount of variance explained

by (1) provenance, (2) plant traits, (3) season, (4) residual oil, (5) all possible combinations of aforementioned factors (e.g. both plant traits and provenance, etc.), and (6) unexplained variation. We used the varpart function in vegan, with significance of the fractions attributed to the variable (e.g., provenance) tested with db-rda as implemented in vegan and with adjusted-R^2 values used to visually plot partitioned variances.

We also used nonmetric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities to assess shifts in microbial communities (metaMDS function). Stable solutions with stress scores <0.200 and $R^2 > 0.950$ were used for subsequent analyses, resulting in a two-dimensional solution for both bacteria and fungi. We further assessed which individual plant traits were correlated with shifts in microbial communities for each season by using a vector-fitting approach to the NMDS ordinations with the envfit function in vegan. Soil N, soil C, and PAHs were also included in these analyses. Significance values were generated with 9999 random permutations.

All data from control plots (NC) were excluded from trait-based analyses of microbial diversity and community composition because data on plant traits was not available. Unless otherwise indicated, all statistical analyses were carried out in R.

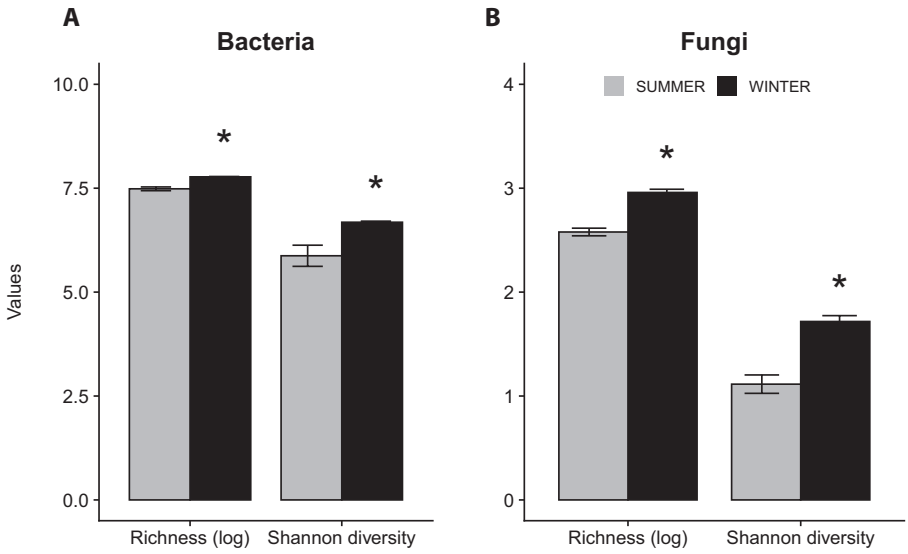

FIGURE 2. (A) Bacterial and (B) fungal diversity based on Shannon diversity and Chao1 log richness. Asterisks (*) represent significant differences between seasons.

RESULTS

Plant trait variation

Canonical functions LD1 (linear discriminant 1), LD2, and LD3 explained 71%, 24%, and 5% of trait variation according to plant provenance, respectively. The first canonical function reflects shifts in architectural traits (e.g., shoot height and tiller density) according to the canonical coefficients and how strongly each canonical function correlated with trait variation among samples (Fig. 1; Appendix S4). The second function, LD2, reflects shifts in overall productivity (e.g., increased BG and AG biomass) and carbon in root tissues. The third function, LD3, reflects the recalcitrance of AG plant matter, with increased C:N in leaf tissue, and shorter leaves on thinner stems that results in a higher proportion of structural tissue per cross-sectional area. Source populations were strongly distinguished according to the LD1 function, with local BJ and V plants bracketing overall variation (Fig. 1). The CP cultivar was strongly distinguished according to the LD2 function, reflecting greater investment in belowground or low-lying architecture versus aboveground architecture (Fig. 1). Plants of different provenance exhibited the greatest overlap across the LD3 function, with the exception of CL plants, which exhibited slightly greater recalcitrance in aboveground tissue (Fig. 1). Both soil C ($F_{6, 24} = 4.15$, $P = 0.005$) and soil N ($F_{6, 24} = 3.24$, $P = 0.02$) varied according to plant provenance; both were negatively associated with plant architectural traits (LD1) and positively associated with measures of productivity (LD2) (Appendix S6).

Microbial community variation

Bacteria—Bacterial α-diversity was much higher in winter than summer. Seasonal differences were significant for Shannon diversity (winter = 6.68, summer = 5.87, $t = -3.23$, $P = 0.003$) and for Chao1 (winter = 3552.32, summer = 2820.53; $t = -3.34$, $P = 0.002$) (Fig. 2A). The PLSR analyses revealed that season (estimated regression coefficient, r, = 0.38, $P = 0.003$) and shoot density ($r = 0.20$, $P = 0.04$) were predictors of bacterial Shannon diversity (Appendix S7). Meanwhile, we found that season ($r = 0.13$, df = 9, $P = 0.01$) was the only significant predictor of bacterial richness (Chao1, Appendix S7). The strongest predictors of seasonal change in α-diversity of bacteria were PAH, $(C:N)_{AG}$, and soil N, but the observed relationships were not statistically significant (Appendix S8).

Variation in bacterial community composition reflected prevailing environmental conditions more so than plant provenance and associated differences in traits. According to variance partitioning, 4% and <0.1% of total bacterial community variation was attributable to plant traits ($adjR^2 = 0.04$, df = 12, $P = 0.25$) and provenance, respectively. Season and residual oil accounted for 10% ($adjR^2 = 0.10$, df = 1, $P = 0.01$) and 5% ($adjR^2 = 0.05$, df = 1, $P = 0.08$) of variation in bacterial community composition, whereas the remaining amount of variation (81%) was unexplained. The NMDS visualizations showed that bacterial communities were similar, especially the BJ, CP, and V plots during the winter, but that they diverged from one another by summer (Fig. 3A). And while we recovered a diverse range of bacterial phyla, only a few phyla consistently dominated bacterial communities across all provenances: Proteobacteria was the most dominant phylum, followed by Acidobacteria and Chloroflexi (Fig. 3B). Vector fitting revealed that differences in bacterial communities did not correspond to any plant or soil trait in winter or in summer (Fig. 4). These findings do not support our hypotheses that rhizosphere microbial communities exhibit clear signatures of plant provenance, associated trait variation, and seasonal change.

Fungi—Similar to bacteria, fungal α-diversity was significantly higher during winter (Shannon = 1.72; Chao1 = 22.27) compared

to summer (Shannon = 1.12, t = −3.31, P = 0.002; Chao1 = 15.04, t = −3.41, P = 0.001; Fig. 2B). The PLSR analyses revealed that season and $C_{[BG:AG]}$ were predictors of fungal Shannon diversity (r = 0.44, df = 9, P < 0.05; r = 0.17, df = 9, P = 0.03; Appendix S7) and richness (Chao1; r = 5.59, df = 9, P < 0.05; r = 1.60, df = 9, P = 0.03; Appendix S7). In addition, $(C:N)_{BG}$ was correlated with fungal richness (r = 1.45, df = 9, P = 0.04). The strongest predictors of seasonal change in α-diversity of fungi were provenance, tiller height, $N_{[BG:AG]}$, and $(C:N)_{AG}$, (Appendix S8), but the observed relationships were not statistically significant.

In contrast to bacterial communities, rhizosphere fungal community composition reflected plant trait variation as much as prevailing environmental conditions. According to variance partitioning, 20% of observed variation in fungal community composition (adjR^2 = 0.20, df = 10, P = 0.009) was attributable to plant traits, while season and residual oil accounted for 23% (adjR^2 = 0.23, df = 1, P = 0.001) and 5% (adjR^2 = 0.05, df = 1, P = 0.03) of compositional variation, respectively. Provenance accounted for a negligible (<0.1%) amount of variation. Notably, fungal communities were more dissimilar in summer than in winter (Fig. 3C). Differences in fungal communities reflected shifts in several classes, though Dothideomycetes and Eurotiomycetes remained the dominant classes across all plots (Fig. 3D). Vector fitting of the NMDS ordination revealed that variation in fungal communities corresponded to differences in BG biomass and shoot density (Fig. 4), which were significantly correlated with fungal community composition in winter (R^2 = 0.62, P = 0.006) and summer (R^2 = 0.51, P = 0.009), respectively (Fig. 4). Though not fully supportive of our hypotheses, these findings illustrate that plant traits associated with provenance influence the diversity and structure of rhizosphere fungal communities.

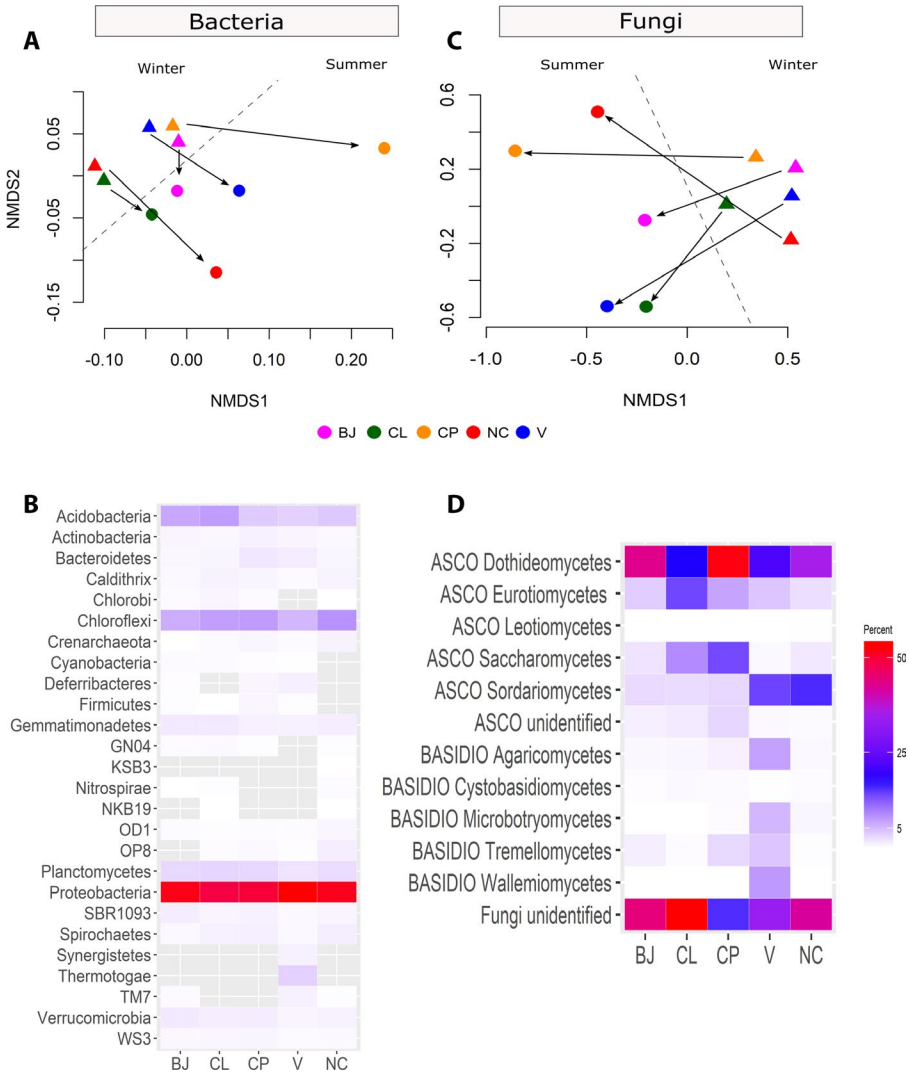

FIGURE 3. (A) NMDS ordination of bacterial community composition in summer and winter, based on mean pairwise UniFrac distances by plant provenance. (B) The relative abundances of the top 20 bacteria and archaea phyla according to plant provenance. (C) NMDS ordination of fungal community composition in summer and winter based on mean pairwise Bray–Curtis distances by plant provenance. (D) Relative percentage abundances of fungal taxa at the class level, with phyla shown in all caps for emphasis. Abundances are shown as percentage of sequence reads of each class within each plant provenance. ASCO = Ascomycota, and BASIDIO = Basidiomycota.

DISCUSSION

It is becoming increasingly apparent that intraspecific genetic variation in plants can influence aboveground macrocommunity structure and ecosystem attributes (e.g., Crutsinger et al., 2009; Des Roche et al., 2018) as well as belowground conditions, including the diversity and composition of rhizosphere microbial communities (Pérez-Izquierdo et al., 2019). Our study offers further evidence affirming prior findings that intraspecific genetic variation in ecosystem engineers can influence salt marsh rhizosphere microbial communities (Bowen et al., 2017; Zogg et al., 2018). Our findings also illustrate that bacterial and fungal communities differ in their association with *S. alterniflora*. Fungal communities appear to be more responsive to heritable trait variation that reflects provenance, whereas shifts in bacterial communities more strongly reflected seasonal dynamics and abiotic conditions such as residual oiling from the *Deepwater Horizon* oil spill.

Our findings are consistent with evidence from prior work indicating that rhizosphere microbial communities of *S. alterniflora* and other foundational grasses are associated with abiotic and biotic components of salt marsh ecosystems (Selisker et al., 2002; Bowen et al., 2017). For example, recent observational surveys have shown that salt marsh rhizosphere microbial communities are responsive to soil type, salinity, and oiling (e.g., Borruso et al., 2014; Wang et al., 2017; Lumibao et al., 2018, but see Angermeyer et al., 2018). Prior

studies have also found that salt marsh rhizosphere microbial communities reflect host lineage and other forms of intraspecific genetic variation (e.g., Nie et al., 2010; Zogg et al., 2018). Our results affirm that abiotic and biotic pressures shape rhizosphere microbial communities in salt marshes. Notably, we also found evidence indicating that trait variation in plants serves as a mechanistic bridge between plant genotypic variation and microbial community composition.

We recovered stronger relationships between intraspecific trait variation in *S. alterniflora* and fungal communities as opposed to bacterial communities. This finding contrasts with those of other studies showing that both rhizosphere bacterial and fungal communities are responsive to interspecific differences in plant traits (Sayer et al., 2017; Boeddinghaus et al., 2019; Chai et al., 2019). It also is inconsistent with evidence that rhizosphere bacterial communities reflect genetic variation in *S. alterniflora* (Zogg et al., 2018) and the marsh grass *Phragmites australis* (Bowen et al., 2017). While these discrepancies might be a consequence of our study design—we might have captured a stronger bacterial response had we examined a wider range of genotypic and associated trait variation (e.g., via comparisons among more geographically distant source populations; Blum et al., 2007; Bernik et al., 2018b)—there is good reason to think that fungi are more sensitive to intraspecific variation in plants. In general, fungi rely more on macromolecular polymers and more labile, less recalcitrant plant fractions (Newell, 2001; Boer et al., 2005; Paterson et al., 2008), and thus may be more readily influenced by resource availability or accessibility governed by intraspecific trait variation in associated plants. Greater sensitivity might also be a consequence of closer associations between fungi and plants, considering that ≥80% of extant plant species are symbiotic with fungi (Wang and Qiu, 2006).

Evidence that fungal community structure is shaped by BG biomass production might reflect the sensitivity of fungi to root attributes and exudates. BG biomass production by *S. alterniflora* differs according to provenance (Fig. 1; Bernik et al., 2018b), which might translate to differences in plant–fungal interactions due to concomitant shifts in morphology, ecophysiology (e.g., root tensile strength), or architecture (e.g., the ratio of rhizomes to roots) (Seliskar et al., 2002; Bernik et al., 2018b). It is also possible that plant–fungal interactions differ due to shifts in the quantity or quality of root exudates. For example, *S. alterniflora* that produce more BG biomass could also release more root exudates, thereby increasing the availability of resources supporting rhizosphere microbial communities (Philippot et al., 2013). Some work suggests that rhizosphere fungi are more responsive to root exudates than bacteria. For example, several studies have shown that increases in root biomass, exudates, and root-derived organic inputs result in greater increases in fungal biomass relative to bacterial biomass (Bardgett et al., 2014; Legay et al., 2014; Eisenhauer et al., 2017). Accordingly, differences in responsiveness might explain discrepancies between our findings and those of prior studies showing that bacterial communities reflect genetic variation in *S. alterniflora* (e.g., Zogg et al., 2018). It is also consistent with our finding that fungal communities varied according to BG biomass during winter, when the availability of resources is likely more constrained and thus shifts in prevailing conditions might elicit greater responses.

Though intraspecific variation in *S. alterniflora* does not seem to exert much influence on bacterial communities, our findings suggest that rhizosphere bacterial communities may be indirectly shaped by the influence of plant provenance on soil physicochemical properties. Intraspecific variation in *S. alterniflora* can give rise to differences in physicochemical properties like BG organic content (Seliskar et al., 2002) and nutrient availability (Bernik et al., 2018b), which in turn can give rise to variation in soil bacterial communities (Mavrodi et al., 2018). Thus, the influence of intraspecific variation in plants may be exerted not only by traits, but also by trait-driven differences in soil conditions (i.e., plant–soil relationships; Schweitzer et al., 2008). This is hinted at by the nonsignificant association between seasonal β-diversity of bacteria and soil N, which differs according to provenance and trait variation in *S. alterniflora*. Notably, we did not detect relationships between fungal communities and soil C or N, suggesting that fungal communities may be less responsive to indirect forms of influence.

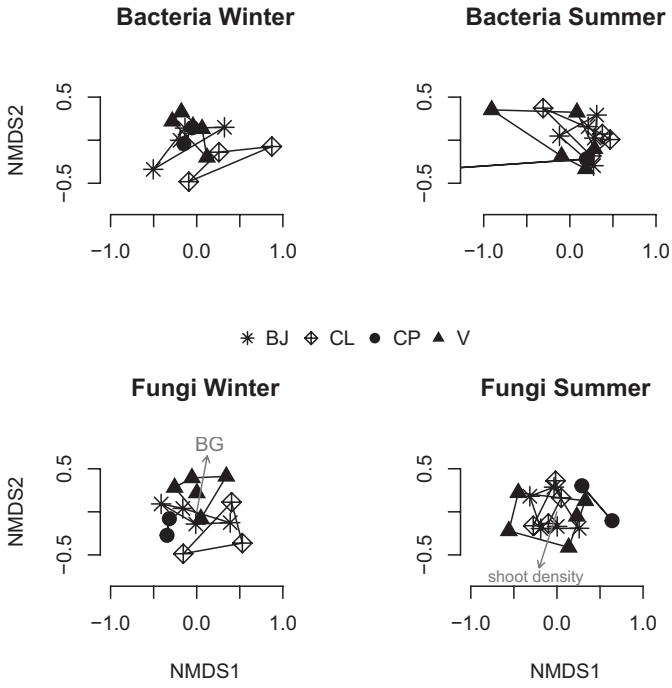

FIGURE 4. NMDS ordination of bacterial (top panel) and fungal communities (bottom panel) in winter and summer. Ordinations were based on Bray–Curtis dissimilarities and significant correlations of plant functional traits with ordination axes shown as arrows. Symbols represent different plant provenances, and ellipses are based on clustering by provenance.

It is important to note that the influence of large environmental disturbances like oiling from the *Deepwater Horizon* oil spill can potentially subsume, alter, or mask the influence of plant genotypic and associated trait variation on soil microbial communities (Beazley et al., 2012). It is thus not surprising that bacterial and fungal communities reflected residual oiling as much as or more so than other factors, including plant traits. Nonetheless, the majority of the observed variation in microbial communities was not explained by the variables we examined, suggesting that the communities are more strongly shaped by unmeasured abiotic factors (e.g., pH, salinity) or unmeasured plant traits.

CONCLUSIONS

Our study highlights the relevance of plant intraspecific variation in determining plant–microbe interactions that can potentially

shape ecosystem function and structure (Schweitzer et al., 2008; de Vries et al., 2012; Des Roche et al., 2018), particularly in naturally depauperate ecosystems like salt marshes dominated by a landform engineer. It would thus be a worthy endeavor to examine whether soil microbiome "engineering" by plants is a key constituent of plant–soil–microbe feedbacks that can govern vital biogeochemical or biogeophysical processes like shoreline erosion (de Vries et al., 2012; Des Roche et al., 2018). Investigating whether coastal marsh ecosystem responses to abiotic stress are moderated by intraspecific variation in plant–microbe interactions would be particularly worthwhile because it could offer valuable perspectives on resilience and fate under changing climate conditions.

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AUTHOR CONTRIBUTIONS

M.B. and B.B. conceived and designed the field experiment. S.V.B., M.B., D.K., and K.M. designed the microbiome assays. B.B. conducted plant genotyping, and D.K. performed DNA soil extraction. B.B. collected field data, J.P. conducted the PAH analyses, S.F. conducted bioinformatics analysis, and B.B. and C.L. conducted statistical analyses. C.L., B.B., and M.B. prepared the initial draft of the manuscript, with all authors contributing to subsequent drafts.

DATA AVAILABILITY

Sequence data are deposited in the NCBI SRA (BioProject PRJNA603629). Data are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) at https://data.gulfresearchinitiative.org (https://doi.org/10.7266/A59VQEW2; https://doi.org/10.7266/BQVFTBFY).

SUPPORTING INFORMATION

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

APPENDIX S1. Location of experimental plots in Bay Jimmy, Louisiana, USA.

APPENDIX S2. Additional information on plant microsatellite genotyping and genetic variation, microbial community profiling, bioinformatics analyses, and chemical analysis of residual oil.

APPENDIX S3. Discriminant analysis of principal components (DAPC) of genetic differentiation among *Spartina alterniflora* plants.

APPENDIX S4. Plant trait coefficients of variation for linear discriminants LD1, LD2, and LD3 and the correlation between plant traits and linear discriminants.

APPENDIX S5. Microbial community dissimilarity vs. pairwise geographic distance.

APPENDIX S6. Results from linear regression models showing how soil C and soil N varied by plant provenance and the linear discriminant analysis (LDA) values.

APPENDIX S7. Partial least square regression (PLSR) analyses of microbial richness based on Chao 1 estimate and Shannon diversity.

APPENDIX S8. Partial least square regression (PLSR) analyses of observed changes in microbial alpha diversity (seasonal dynamics—difference in diversity from summer to winter) and plant provenance, traits, residual oil, and soil properties in bacteria and fungi.

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