Quantitative genetic-by-soil microbiome interactions in a perennial grass affect functional traits

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Abstract

Plant–microbe interactions can impact plant growth and performance, and contribute to local adaptation. However, few studies have explored the impact of microbial communities from distinct native locations on plant functional traits, and less is known about how host-microbe interactions affect the quantitative genetics of plant traits. We used a recombinant inbred line (RIL) mapping population derived from upland and lowland ecotypes of the diploid C4 perennial bunch grass *Panicum hallii* to explore quantitative genetic responses to soil microbiomes. We show that the growth and development of ecotypes and their trait divergence depends on soil microbiomes. Moreover, we find that broad-sense H^2 is modified by soil microbiomes, revealing important plant genotype-by-microbiome interactions for quantitative traits. We detected a number of quantitative trait loci (QTL) that interact with the soil microbiome. Our results highlight the importance of microbial interactions in ecotypic divergence and trait genetic architecture in C4 perennial grasses.

Introduction

Plants have evolved alongside microbes for millions of years and have formed intricate relationships with soil microbial communities via their root systems. Soil microbial community composition is shaped by soil abiotic conditions and varying soil types contain microbiomes with distinct taxonomic distributions (Fierer 2017; Hartman & Tringe 2019). Plant host genetics also drive the assembly of rhizosphere and endosphere microbial communities (Jones et al. 2019; Trivedi et al. 2020), and crop varieties or natural ecotypes (or genotypes) grown in a common environment can differ in root and rhizosphere community structure (Bowsher et al. 2020; Li et al. 2018; Perez-Jaramillò et al. 2017; Wagner et al. 2016). To some degree, the root microbiome can be thought of as an extended phenotype of the plant. Plant-soil-microbiome relationships can influence plant traits and there is strong evidence that microbes can yield positive effects on plant performance directly or indirectly by impacting plant functional traits (Egamberdieva et al. 2017; Lau & Lennon 2012; Wagner et al. 2014) or negatively as pathogens. Plant root associated microbiomes impact root traits, can increase nutrient acquisition, provide indirect impacts on shoot traits (such as increasing shoot biomass) and promote tolerance to abiotic and biotic stress (Friesen et al. 2011; Mendes et al.2013; Santhanam et al. 2015; Sukumar et al. 2013). In synthetic community research, growing the same genotype in the presence or absence of differing sets of selected microbiomes produces a wide range of plant trait modulation (De Souza et al. 2020; Vorholt et al. 2017). Given the growing evidence of microbial effects on plant growth and development, it's possible that plant microbial interactions also play a role in the process of local adaptation, where plant populations diverge and exhibit different niche characteristics and habitat preferences (Petipas et al. 2021).

Many plant species are composed of highly varied ecotypes across their range, each of which may show a high degree of trait divergence. Quantitative traits can be profoundly influenced by environmental factors

and the degree to which these factors influence plant traits can vary widely across genotypes. These types of interactions are termed genotype-by-environment interaction (GxE; Des Marais et al. 2013). Many studies focus on local adaptation and GxE in response to changing habitats or conditions (Leimu & Fischer 2008; Midolo & Wellstein 2020), however, the relative contribution of abiotic and biotic factors in driving GxE is often unclear (Runquist et al. 2020). Plants encounter diverse biotic factors including competition, herbivory, pathogens and an array of microbial communities (Bischoff et al. 2006; Järemo et al. 1999), but little is known about how specific interactions between plants and microbial communities contribute to adaptation. Numerous quantitative trait loci (QTL) mapping studies have explored the genetic architecture of GxE in natural and crop populations for a number of abiotic factors and this approach has become widely utilized to study plant responses to abiotic stress and to understand plant trait plasticity (Des Marais et al. 2016; Vij & Tyagi 2007). Far less is known about the influence of biotic factors, especially microbiomes. Additional experimental studies exploring the impact of microbial communities are critically needed to fully elucidate aspects of plant-microbe interactions and local adaptation.

Panicum hallii is a diploid, C4, self-fertilizing, North American native perennial bunch grass that occurs across a large geographical range with diverse habitats and climate. There are two naturally occurring ecotypes of *P. hallii* that are classified as separate varieties: an upland xeric ecotype, *P. hallii* var. *hallii*(hereafter referred to as *hallii*) and a lowland mesic ecotype,*P. hallii* var. *filipes* (hereafter referred to as*filipes*). These ecotypes display trait divergence in a similar direction and magnitude to other perennial grass species with upland and lowland ecotypes, a pattern which is thought to be driven by adaptive evolution along precipitation gradients across a species range (Gray et al. 2014; Khasanova et al. 2019; Lowry et al. 2014). Many observations have shown that both ecotypes of *P. hallii* display a large degree of plasticity in several shoot traits in response to changes in abiotic factors including light (Weng et al. 2019) and precipitation (Lovell et al. 2018), yet these differences are minor in comparison to the differences inherent between the ecotypes. Compared to abiotic factors, little is known about the importance or relative contribution of biotic environmental variation, especially soil microbiota, in shaping plant shoot and root traits in the *P. hallii* system and plants in general.

Here, we conducted a quantitative genetic experiment to examine the impact of soil microbiomes and host genetics on root and shoot traits. A recombinant inbred population (RIL) derived from a cross between the upland and lowland ecotypes of *P. hallii* allowed us to identify plant genomic regions contributing to microbial-mediated traits. To overcome the limitations of synthetic community approaches and the complexity of natural soils, we took a hybrid approach of inoculating sterilized soils with naturally derived microbial communities in a greenhouse setting. Specifically, we sought to answer four questions: 1) Does the native soil microbiome drive plasticity in *P. hallii*above- and below-ground traits? 2) Are microbiome effects general, or specifically related to the location of origin of the microbiome? 3) Do*P. hallii* ecotypes exhibit GxE in response to variable soil microbiomes? And, 4) Can we map genetic effects and their interactions with the microbiome to the genome? Overall, our experiment demonstrates the impact of living soil microbiomes on the quantitative genetic architecture of both root and shoot traits in *P. hallii* and highlights the potential importance of microbiomes in local adaptation.

Materials and Methods

Plant Material

We used a population of recombinant inbred lines (RILs) derived from a cross between *P. hallii* var. *hallii* (HAL2 genotype) and*P. hallii* var. *filipes* (FIL2 genotype) to evaluate the genetic basis of plant-microbiome interactions. A single F_1 hybrid was used to generate a F_2population which was bred by single seed descent to the F_7 generation (Khasanova et al. 2019). Parents have full genome assemblies that are publicly available (https://phytozome-next.jgi.doe.gov/; Lovell *et al.* , 2018). The RIL population linkage map was constructed by shallow whole-genome resequencing and is congruent with the order of the physical genome. Seeds of 293 F_7RILs and parents were sterilized, germinated, transplanted into treatment pots in a greenhouse located at the University of Texas at Austin (16-h days at 500 μE m^{-2} s^{-1}, 28°C; 8-h nights at 24°C; full protocol in Appendix S1).

Microbial Inoculum Collection and Treatment Soil Preparation

RIL and parental replicates were grown in the presence and absence of native microbiome inoculations obtained from the locations where parents were collected (Lady Bird Johnson Wildflower Center (WFC) in Austin, TX for var. *hallii* and the Corpus Christi Botanical Garden (CCBG), in Corpus Christi, TX for var. *filipes* ; native soils properties in Appendix S1 and Table S1).

To create treatments, we mixed 1% by volume of native soil inoculum (to minimize the effect of nutritive and textural soil properties from the inoculum) with a twice-autoclaved horticultural soil mix of compost, decomposed granite and vermiculite (Thunder Dirt, Geo Growers, Austin, TX) and incubated it for two weeks in closed 400-liter plastic containers (as in Edwards et al. 2019). We selected this particular commercial soil mix because it homogenizes well and facilitates root extraction and cleaning. For soils utilized in the control treatments, the 1% soil inoculum was twice autoclaved over a 24-hour period before mixing and incubation. Given the nature of this large-scale greenhouse experiment under an open-air environment, true sterility of the control treatments is not possible and thus we refer to the treatments by their inoculum source: microbiome treatments as Austin Inoculated (AI) and Corpus Inoculated (CI), and control treatments as Mock Austin Inoculated (MAI) and Mock Corpus Inoculated (MCI). Nevertheless, we feel this system allows us to assess the holistic impact of soil microbes, above and beyond what could be obtained from studies of individual microbes or experiments under more artificial conditions.

Microbial DNA extraction and 16S rRNA gene sequencing

To characterize the microbial community composition of native locations, we collected samples of rhizosphere and root from eight haphazardly selected *hallii* individuals growing at Austin (WFC) and nine*filipes* individuals growing at Corpus Christi (CCBG) – we note that in this natural sampling scheme location and ecotype are confounded. Additionally, five bulk soil samples (all plant material removed) from each site were collected in areas adjacent to living*hallii* plants (44 samples total) and DNA was obtained with the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany). 16S ribosomal RNA gene regions were amplified using the 515F-806R primer pair, barcoded and sequenced on the Illumina Novaseq platform on the SP flowcell using 250x250 reads. To characterize treatments in the greenhouse experiment, this procedure was repeated on rhizosphere, root and soil samples taken at harvest from seven replicates of each parent in each treatment (four treatments x 14 parents x three compartments = 168 samples).

Experimental Design

Each treatment contained 293 RILs and seven replicates of each parent for a total of 307 plants per treatment in the experiment (four treatments x 293 RILs + 56 parents =1,228 plants). Incubated soil for treatments and controls was transferred to 950 ml 3" x 8" Mini-Treepots (Stuewe and Sons, Tangent, OR), which were lined with sterile plastic bags perforated at the bottom to allow water drainage and facilitate easy root system removal. Pots for all treatments were randomized in a single block design and left in the greenhouse for acclimation in open air for two weeks before seedlings were transplanted. Plants were watered with UV sterilized tap water for the duration of the experiment.

Harvest and Phenotyping

Plants were harvested prior to panicle emergence after six weeks of growth over a five-day period. Individual plants were extracted from pots by pulling the plastic bag to prevent root damage and plants with attached roots were removed from the soil by shaking over wire mesh. Soil rhizosphere samples for parents were collected by dipping root systems into sterilized 50 ml tubes filled with 1X phosphate buffered saline. Plants were then hung by the shoot base on a clamping apparatus and soil particles were removed from the root system with a spray of UV-sterilized water and roots were separated from shoots and preserved in 90% ethanol for future phenotyping. Tiller number and flag leaf area of the main tiller was measured. Shoot and leaf tissue were dried at 55°C and weighed separately to obtain aboveground biomass and to calculate specific leaf area (SLA; fresh leaf area / dry mass of the leaf (cm^2 g^{-1})).

Root systems were phenotyped by scanning the entire intact root system and one representative nodal root

with attached lateral roots on an EPSON 12000XL flatbed scanner (Epson America, Inc., San Jose, CA, USA) calibrated for use with WinRhizo Pro 2019 root image analysis software (Regent Instruments Inc., Canada) (full description in Appendix S1). For parents, a small portion of the central root system was frozen for DNA extraction and PCR amplification to determine root endosphere microbial community composition. The remaining roots were collected and dried for 96 hours at 55°C, and weighed.

Sequence Analysis

Demultiplexed sequences were trimmed to remove adapter and primer binding sites using Cutadapt (Martin 2011). Amplicon sequence variants (ASVs) were inferred using DADA2 (Callahan et al. 2016). Errant ASVs due to chimerization were detected using the "consensus" method in DADA2 and discarded. Any ASV with a sequence length of greater than 256 bp or less than 250 bp were discarded. Taxonomic classifications were assigned to each ASV using DADA2's assignTaxonomy () function using the Silva reference database (version 132, Quast et al. 2013).

Microbiome data was analyzed in R software (R Core Team 2020). ASVs assigned to mitochondrial and chloroplast lineages were discarded prior to normalization. For principal coordinates analysis (PCoA) and phylum level abundance statistics, raw counts were normalized to account for differences in sequencing depth between samples by dividing each ASV count by sequencing depth of a particular sample and multiplying by 1000 to place the counts on a per mille scale. Principal coordinate analyses were conducted using the capscale () function in the package Vegan (Oksanen et al. 2020). Bray Curtis dissimilarity on log2 transformed abundances was used for all PCoAs unless otherwise noted. Alpha diversity was calculated using Shannon Entropy from the diversity () function in Vegan. Differential abundance of aggregated phylum abundances was performed using linear models on log2 transformed abundances. Differential abundance of ASVs between conditions was conducted using DESeq2 on raw counts (Love et al. 2014).

Plant trait data from all parental replicates was analyzed to test genotypic and microbial treatment effects on plant morphological traits. We fit factorial linear mixed models using PROC MIXED in SAS (Littell et al. 1996) consisting of Ecotype, Treatment, and Ecotype x Treatment interactions as fixed effects. Preliminary analysis did not show any significant differences (in all cases, $P > 0.113$ between MAI and MCI treatments for parents and RILs), thus the average between them was used for this and all subsequent analyses (hereafter referred to as the Mock Inoculated (MI) treatment).

To explore the impact of the microbiome on the quantitative genetic architecture of our measured traits, we fit linear mixed models testing for GxE using the sommer package (Covarrubias-Pazaran 2018) in R based on the additive and epistatic relationship matrix determined from the genotypic data of the RIL (full approach described in Appendix S1). We calculated broad-senseH^2 as $(V_a + V_{aa})/V_p$ and present variance components and model comparisons.

The observation of different QTL effects under different treatment conditions provides evidence for QTL x environment interactions. To detect QTL present in the AI, CI and MI treatments, we completed QTL mapping on RIL values in R using the R/qtl package (Broman & Sen 2009) in each environment separately (full description in Appendix S1). We further tested for QTL x environment interactions in a full linear model incorporating data from the three treatments using the PROC MIXED procedure of SAS. Marker x treatment interaction indicates QTL x environment interaction, marker x marker interaction represents epistasis averaged over the environments, and marker x marker x treatment interaction indicates environment specific epistasis. We performed this analysis to test GxE interaction effects by contrasting the AI, CI and MI microbiomes (e.g. potentially identifying different soil or residual microbiome impacts). To test the significance of individual marker alleles at each treatment, we used the slice function in SAS as tests of simple effects (Winer 1971) for all significant marker x treatment and marker x marker x treatment interactions (full approach described in Appendix S1).

Results

Treatment drives bacterial community composition

We used 16S rRNA gene amplicon sequencing classified into ASVs to characterize both the native microbial communities and communities generated by experimental inoculations as a representation for the overall biotic differences. For parental ecotypes growing under natural habitats, PCoA of bacterial ASV counts revealed strong location/ecotype and compartment effects (i.e. soil, rhizosphere, and root) across axes one and two, respectively (Fig. 1a). Permanova mirrored these results with location/ecotype explaining the most variance (R^2= 0.21, $P < 0.001$) and compartment explaining the second most ($R^2 = 0.15$, $P < 0.001$; Table 1). Microbiota varied significantly in alpha diversity between compartments, but not between location (Fig. 1b). Phylum level distributions were overall consistent between microbiota of plants growing at the two natural locations with *Proteobacteria* ,*Actinobacteria* , and *Acidobacteria* being dominant members (Fig. 1c, Fig. S1a), which is congruent with results from previous root-associated microbiome studies (Edwards et al. 2014; Lundberg et al. 2012; Singer et al. 2019; Wagner et al. 2016). Only three relatively low abundance phyla displayed significant differences between location-ecotype: WPS-2 and *Entotheonellaeota* in the rhizosphere and *Rokubacteria* in the root (Fig. S2). Conversely, microbiota from the two locations were more divergent at the ASV level and we identified 735 unique ASVs which were differentially abundant by compartment (440 in soil, 251 in rhizosphere, and 401 in roots; Fig. 1c).

We next analyzed microbiota acquired under experimental conditions in the greenhouse by sampling roots and rhizosphere from the RIL parents, along with soil from unplanted pots. PCoA revealed that inoculum and compartment significantly impacted microbiota composition (Fig. 1d). Alpha diversity was also impacted by compartment and inoculum: in general, plants inoculated with native soil slurries hosted microbiota with greater Shannon diversity compared to plants with heat-killed, mock microbiota (Fig. 1e). As expected, when comparing the effect of inoculum source within heat killed or native conditions, we found that microbial communities of plants and soil with heat-killed inocula were significantly more similar than if the inoculum was unsterilized and this effect was consistent independent of compartment (Fig. S3). Similar trends were observed at the phylum level where there were many more differentially abundant phyla by soil source with intact inoculum vs. heat treated (Fig. S1b, c). When identifying ASVs whose abundance was impacted by soil inoculation source, many more ASVs were differentially abundant in comparisons between native inoculum compared to heat killed (Fig. 1f). When analyzed together, we found that greenhouse and field microbiomes formed distinct communities, yet were still identifiable by soil source (Fig. S4a, more in Appendix S1). These results indicate that heat sterilization of inoculum dampens the effect of soil source on compositions of the resulting microbiome and that plants inoculated with native microbiota host significantly different communities in the rhizosphere and roots.

Effect of microbiome inoculation on parental traits

Trait differences among parents were driven by plant genotype, environment and genotype by environment interactions (GxE). Parents differed in shoot and root traits across all treatments. For example, FIL2 produced 1.64-fold more shoot biomass (P <0.0001), 1.98-fold more root biomass (P <0.0001), 1.80-fold lower specific root length (SRL) (P <0.0001) and 1.46-fold higher RTD (P <0.0001) relative to HAL2 (Fig. 2a, d, e; Table S2; Table S3). These results mirrored earlier descriptive studies of *P. hallii* ecotypes (Palacio-Meija *et al.* , 2021; Lowry*et al.* , 2014), including studies of the shoot and root traits studied in current work. (Khasanova et al. 2019). Treatment also had a significant effect on plant traits (Fig. 2a-c, e, f; Table S2, Table S3). For example, inoculated plants had greater shoot biomass (1.35-fold more biomass in CI and 1.17-fold more in AI treatments relative to the MI treatment (P =0.027)), lower lateral root length (1.2-fold less in CI and 1.53-fold less in AI relative to MI (P =0.046)), and showed changes in specific leaf area (SLA) dependent upon treatment (1.05-fold increase in AI and 1.06-decrease in CI relative to MI (P =0.039)). Importantly, we also identified several ecotype x microbiome interactions (Fig. 2d-f; Table S2, Table S3). For example, SRL of FIL2 decreased 1.17-fold under AI and 1.33-fold under CI relative to MI soil, while HAL2 showed 1.1-fold increase in SRL under AI and no change under CI relative to MI (P =0.039; Fig. 2d; Table S2, Table S3). Root tissue density (RTD) of FIL2 increased 1.1-fold under AI and 1.36-fold under CI relative to MI, while HAL2 showed 1.1-fold decrease under AI and 1.1-fold increase under CI relative to MI (P=0.046; Fig. 2e; Table S2, Table S3). In total, seven traits showed ecotype differences between parents, five traits were affected by microbial treatment and three traits had significant ecotype x microbiome interaction

(Fig. 2; Table S2, Table S3).

The impact of the microbiome on the quantitative genetic architecture of our measured traits was evaluated by comparing "base" and "GxE" linear mixed models. In 11 out of 12 cases, the GxE models were favored by AIC and log likelihood ratio tests (Table S4). Broad-sense heritability was low for most traits (ranging from 0.01 to 0.18; Table S2). Overall, we document considerable evidence that the microbiome modifies the expression of quantitative genetic variation in *P. hallii* .

QTL across and between microbial treatments

A total of 32 QTL were identified for 12 traits across all environments (Fig. 3; Table S5, Table S6). The additive effects of each QTL explained from 2.9 - 22% of trait variation (Table S5). Of these 32 QTL, six QTL occupied unique positions in the genome. The confidence intervals of all other QTL overlapped or colocalized with at least one other QTL. Eight traits (shoot biomass, tiller number, SRL, lateral root length, root diameter, root number, root biomass and total root length) had 16 QTL with overlapping confidence intervals grouped into two hotspots on chromosome three (Fig. 3; Table S5). The hotspot located on 3@4.3 (chromosome number @ centimorgan) showed an additive effect in the direction of parental ecotype divergence, while the other hotspot located on 3@58 showed an additive effect opposite the direction of parental divergence. Pleiotropic genes or linked genes with correlated effects may drive these genomic hotspots of correlated traits. We also found significant epistatic interaction between these two hotspots. Individuals possessing the *hallii* allele for the QTL on 3@58, masked the effects of their interactive QTL on 3@4.3 (Table S5).

We detected 11 genotype x microbiome QTL for 10 traits that show location specific QTL (Fig. 4a-k; Table S5, Table S6). Each of these QTL were analyzed to directly test in which treatment they were present, and to estimate the direction and magnitude of their effects (Fig. 4a-k; Table S7, Table S8). In the MI treatment, QTL for shoot biomass, root biomass and root number were detected with the *hallii* allele contributing to a higher trait value (Fig. 4a, i, j). In the CI treatment, QTL for RMR and SRL were detected with the *filipes*allele contributing to a higher trait value (Fig. 4b, c). In the AI treatment, QTL for tiller number, root number, root diameter, lateral root length, shoot biomass, root biomass, root length and first order root length had allelic effects with the *hallii* allele contributing to a higher value for all traits except root diameter (Fig. 4d-k). Of these QTL, four have overlapping confidence intervals and are grouped into a hotspot on chromosome seven and three are grouped together on chromosome nine (Fig. 4e-k; Table S7, Table S8). Two of the three QTL present on chromosome nine were also detected in the MI treatment (Fig. 4i, j; Table S7, Table S8). Epistatic interactions between two QTL for root diameter (chr 1@68 and 2@78.8) were present only in AI and CI (Fig. 5a-c, Table S7, Table S8) and individuals possessing genotypes at the two loci from the same parent (recovering the ecotypic configuration) produced traits with the smaller magnitude (Fig. 5b, c).

Discussion

There is growing appreciation for the important and often complex interactions that exist between plants and their associated microbial communities. Exploring the genetic architecture of plant trait-microbiome interactions is an important step in determining if these interactions play a role in local adaptation and evolution. Here, we conducted a QTL study with a *P. hallii* RIL mapping population in soils inoculated with microbiomes from native *P. hallii*habitats to observe the impact of microbiomes on plant traits and genetic architecture. We found that the microbiota in the natural habitat of the RIL parents are distinct and served as suitable experimental treatments to quantify the effects of microbiota on host-plant traits. In this study, soils inoculated with native microbiomes drive trait plasticity in both above and below ground traits, and these effects were both general and location specific with respect to the origin of the microbial inoculum. We found QTL that displayed GxE for ten of twelve measured traits, suggesting widespread genetic variation in trait responses to plant-microbiome interaction. We also identified epistatically interacting QTL for root diameter present only in microbiomes from native locations, indicating that hybridization of ecotypes may disrupt genes and their interaction with microbes through root characteristics. Overall, our study suggests

that the genetic architecture of host functional traits is significantly impacted by microbial associations.

It is clear that host traits are impacted by microbial communities. Although soil microbes interact directly and indirectly with the root system, they can induce changes that affect the entire plant. The presence of microbiomes from native soil inocula induced trait plasticity in above and belowground traits for the parents that was general and location specific (Fig. 2). For example, traits linked to resource acquisition such as specific leaf area (SLA) and specific root length (SRL) were altered in response to the presence of microbiomes. High SLA correlates with high nitrogen content and low structural investment in leaves, which yields high rates of photosynthesis to promote rapid growth (Cornelissen et al. 2003; Reich et al. 1997), a trait necessary in xeric environments with short seasons terminated by drought. This is consistent with high SRL, where plants produce longer and thinner roots with less structural input to search for water (Balachowski et al. 2016). SLA showed a plastic response to location specific native microbiomes: SLA was increased for plants with the AI microbiome and decreased for plants with the CI microbiome. This observed pattern in SLA is consistent with the directionality of ecotypic divergence. Moreover, SRL showed GxE in response to microbiomes that was also concordant with the direction of parental trait divergence: with xeric adapted *hallii* showing higher SRL in the presence of native microbiomes while mesic adapted *filipes* showed lower SRL.

We detected two groups of QTL interacting with native microbiomes. The first responded to native soil inocula regardless of their origin and the second interacted with native soil inoculum from only one site. For example, QTL for root number 8@33.1 was present only in the MI treatment and not detected in native treatments, suggesting that native microbiomes reduce genetic divergence for this trait (Fig. 4a). This could be explained by microbial taxa which flourished under MI treatment given that the niche competition was relaxed. QTL for SRL (4@19.1) and RMR (3@74.9) showed location specific GxE (Fig. 4b, c); plants with the*filipes* allele in the CI treatment resulted in a higher trait value. This is opposite to the direction of SRL trait divergence in parental ecotypes and to their response to the CI treatment. Eight QTLs showed location specific GxE to the AI treatment (Fig. 4d-k). Our previous study conducted at the panicle emergence stage suggested that xeric *hallii* employs a fast-acquisitive strategy for drought escape by acquiring nutrients rapidly and flowering quickly to enter dormancy before the onset of summer drought (Khasanova et al. 2019). This is consistent with current study conducted at the tillering stage where plants with the *hallii* allele in interaction with the AI microbiome produced more root and shoot biomass. This is accomplished by the increased production of tillers with roots to support them. Root systems of plants with these *hallii* QTL hotspots produced longer and thinner roots, putatively allowing increased foraging and resource acquisition. Four of these QTL present in interaction with AI clustered in the genomic "hotspot" on chromosome seven and three QTL clustered on chromosome nine. This common genetic control of ecotype differentiating traits involving above and below ground traits suggests that these factors interact with the AI microbiome in tandem, potentially contributing to ecotype divergence and local adaptation.

We also identified epistatically interacting QTL for root diameter present only in treatments with microbiomes from native locations (Fig. 5). When lines are homozygous for either *hallii* or *filipes*alleles at both of the interacting QTL, individuals produce smaller diameter roots. In contrast, individuals with mismatched genotypes (HH/FF) at the pair of interacting loci develop larger diameter roots. However, the observed epistatic QTL effects did not translate to decreases in aboveground biomass. This hybrid mismatched that have unusual phenotypes could represent either hybrid vigor or breakdown – but selection studies looking at how these phenotypes impact performance will be needed to further evaluate links between epistasis-microbiome interactions and root developmental responses.

A strength of our inoculation approach was prioritizing community effects, as opposed to the effect of single bacterial inoculants. However, given the exciting experimental advances of isolated bacterial strains in synthetic communities, targeted communities using locally adapted bacterial strains or combinatorics (Paredes 2018) could be used to address how the presence / absence of particular microbes impact plant phenotypes. In addition, it is intriguing to speculate on the genes and molecular mechanisms underlying the host x microbiome QTL detected in our study. It could be that these QTL harbor genes that interact only indirectly with

the host microbiome, perhaps through abundance of soil nutrients as modified by microbes. For example, certain soil microbes in our inoculates may alter the abundance or availability of soil nutrients with subsequent consequences for genetic variation in root or shoot growth. It may be that QTL are related to root exudates or metabolites released that may recruit or amplify key beneficial microbes with subsequent impacts on available nutrients. There are many examples of soil resource abundances of key nutrients impacting plant growth, including genes that demonstrated plastic responses to nutrient availability (Brumbarova & Ivanov 2019). Alternatively, it may be that the genes within QTL intervals are involved in more direct interactions with microbes. For example, recent studies show that phytohormones, microRNAs and secreted peptides are known to recruit and foster the establishment of symbiotic arbuscular mycorrhizal fungi (Müller & Harrison 2019). Moreover, Finkel et al. 2020 recently discovered an important role of the bacterial genus *Variovorax* in attenuating the negative effects on root growth imposed by other bacterial isolates via modification of auxin concentration gradients in the rhizosphere. Plants also deploy extensive immune repertoires to ward off pathogens and control access of microbes to endophytic compartments (Chen et al. 2020) and some of our interactions may be related to ecotypic specific resistance or susceptibility. Our observation of an epistatic interaction is especially interesting as they may represent sensing and signaling pathways that are triggered or directed by microbes. In our case, epistatic interactions may also represent hybrid incompatibilities between ecotypes that are driven by the microbial community. Given the broad confidence intervals of our genome wide scans, we resist the temptation to consider and discuss specific candidate genes. Nevertheless, we emphasize that our approach leads to a direct pathway of fine-mapping and the identification and cloning of new genes involved in plant-microbiome interactions.

Our results show that microbiomes impact the influence of genetic architecture on plant traits in two ecotypes of *Panicum hallii* . These effects were broadly divided into two categories, effects dependent upon the presence of inoculated microbiomes in general and effects dependent upon microbiomes originating from a specific location of origin. This pattern sheds light on the role biotic factors may play in ecotype divergence and raises questions about how microbes impact the genetic architecture of plant quantitative traits, potentially leading to local adaptation and ultimately speciation. Further work in this system has several pathways forward. Broad characterization of microbial communities can be used to determine how genetic variation shapes microbial communities as well as individual microbes. For example, this approach may allow for differentiating the effects that are mediated by plant-fungal interactions vs. plant-bacterial interactions. Once more is known about specific members of the microbial community that play large roles in impacting plant traits, reductionist approaches including targeted inoculations of bacterial / fungal strains and reverse genetic approaches could be used to identify specific mechanisms underlying plant-microbe interactions.

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Figure Legends

Fig. 1 Parental genotypes grown under natural conditions host distinct microbiota. Panels: (a), principal coordinate graph based on Bray-Curtis dissimilarities; (b), Shannon diversity of samples from native parental habitats; (d), number of ASVs with differential abundance between parental habitats broken down by phylum where bars to the left indicate number of ASVs enriched in the Austin habitat, while bars to the right indicate number of ASVs enriched in the Corpus habitat. Color legends in b and d are consistent with panel a. Microbial treatments differ significantly in community composition for plants growing in the greenhouse. Panels: (d), principal coordinate graph based on Bray-Curtis dissimilarities; (e), Shannon diversity of greenhouse samples; (f), number of differentially abundant ASVs when comparing AI vs. CI soil inoculum for both native and mock treatments where bars to the left indicate the comparison for the mock treatments

while bars to the right indicate native treatments. The color scheme in panels e and f are consistent with panel d.

Fig. 2 Effect of plant ecotype (E) and microbial treatment (TRT) and their interaction (E*TRT) on plant functional traits. Traits: (a), shoot biomass; (b), specific leaf area; (c), lateral root length; (d), specific root length; (e), root tissue density; (f), first order root length (data are means +S.E.).

Fig. 3 Genetic map of the *Panicum hallii* RIL population with locations of significant trait QTL by microbial treatment.

Fig. 4 Tests of effect slices for significant Treatment x Marker interactions for traits in the full model analysis of QTL x treatment interactions using PROC mixed in SAS with QTL modeled on the marker nearest the QTL peak. Panels: (a), root number at 8@33.1; (b), root mass ratio at 3@74.9; (c), specific root length at 4@19.1; (d), first order root length at 9@25.6; (e), lateral root length at 7@3.4; (f), tiller number at 7@17.0; (g), root diameter at 7@2.7; (h), root number at 7@0.3; (i), shoot biomass at 9@3.9; (j), root biomass at 9@3.9; (k), root length at 9@3.9.

Fig. 5 Pairwise epistatic QTL in the *P. hallii* RIL population detected only under Austin Inoculated and Corpus Inoculated treatments, with plotted points indicating two-locus genotype means ± 1SE for the two loci impacting root diameter. Root diameter QTL interactions for treatments: (a) Mock Inoculated (MI); (b), Austin Inoculated (AI); (c), Corpus Inoculated (CI).

Supportive Information Legends

Fig. S1 Panels: (a), phylum level distribution of microbiota from natural habitats; (b), phylum level distribution for microbiota from greenhouse samples; (c), differentially abundant phyla between soil treatments for mock and native microbiota where black boxes around tiles indicate a significant difference (adjusted P < 0.05) between soil treatments and the red color indicates a log fold change favoring the AI microbiota while blue favors CI microbiota;

Fig. S2 Phylum level differences with plants growing in their native habitats. Black boxes around tiles indicate a significant different between Austin Inoculated (AI) and Corpus Inoculated (CI) environments (adjusted P value < 0.05). A red color indicates a higher abundance in the AI environment, while a blue indicates higher abundance in the CI environment.

Fig. S3 Mock treatment microbiota are more similar than native treatment microbiota. The graph displays Bray-Curtis dissimilarities comparing inoculation within mock (Mock Austin Inoculated (MAI) vs. Mock Corpus Inoculated (MCI)) and native (Austin Inoculated (AI) vs. Corpus Inoculated (CI)) treatments levels.

Fig. S4 Field and greenhouse derived samples host non-identical microbiota. Panels: (a), PCoA graph displaying all samples collected in the study; (b), phylum level differences between greenhouse and field grown samples.

Table S1 Nutrient and mineral composition of native soils used for microbial inoculum.

Table S2 Means + SE for the parental ecotypes, RILs, RIL range and broad-sense heritability (H^2 + SE) for Mock Inoculated (MI), Austin Inoculated (AI), and Corpus Inoculated (CI) microbial treatments.

Table S3 P -values for genetic and microbial treatment effects of root and shoot traits for the *Panicum hallii* parental ecotypes across three microbial treatments (Mock Inoculates, Austin Inoculated and Corpus Inoculated).

Table S4 Comparison of "base" and "GxE" linear mixed models to evaluate the impact of the microbiome on the quantitative genetic architecture of our measured traits (The Diagonal model allows V_a or V_{aa} to vary by treatment).

Table S5 QTL effects (main and epistatic) of for the*Panicum hallii* RIL population.

Table S6 Full model analysis of QTL–treatment interactions using PROC mixed in SAS with QTL modeled on the marker nearest the QTL peak.

Table S7 Main and epistatic effects of GxE QTL for the*Panicum hallii* RIL population.

Table S8 Tests of effect slices of significant TRT x Marker interaction in the full model analysis of QTL x treatment interactions using PROC mixed in SAS with QTL modeled on the marker nearest the QTL peak.

Tables

Table 1 PERMANOVA Partitioning and Analysis of 16S community composition of native populations of *P. hallii* ecotypes and experimental plants grown in greenhouse.

Collected	Effect	Df	SS	MS	F.Model	R^2	P-Value
native environment	Location/Ecotype	1	1.893573	1.893573	14.06662	0.216195	**0.001****
	Compartment	2	1.35417	0.677085	5.0298	0.15461	**0.001****
	Location/Ecotype x Compartment	2	0.39553	0.197765	1.469119	0.045159	0.079
greenhouse	Ecotype	1	0.105545	0.105545	1.655188	0.004984	0.085
	Treatment	3	7.835636	2.611879	40.9604	0.370048	**0.001****
	Compartment	2	4.313753	2.156877	33.82489	0.203722	**0.001****
	Ecotype x Treatment	3	0.241243	0.080414	1.261085	0.011393	0.151
	Ecotype x Compartment	2	0.095659	0.047829	0.750079	0.004518	0.804
	Ecotype x Compartment	6	1.209483	0.20158	3.161255	0.057119	**0.001****
	Ecotype x Treatment x Compartment	6	0.231549	0.038592	0.605206	0.010935	0.999