Received: 4 November 2022 | Revised: 1 September 2023 | Accepted: 18 October 2023

DOI: 10.1111/pce.14755

ORIGINAL ARTICLE

Plant, Cell & Environment | PC&E | WILEY

Interactions between root hairs and the soil microbial community affect the growth of maize seedlings

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Funding information

National Science Foundation; University of Nebraska-Lincoln; The National Science Foundation EPSCoR Center, Grant/Award Number: #1557417

Abstract

Root hairs are considered important for rhizosphere formation, which affects root system functioning. Through interactions with soil microorganisms mediated by root exudation, root hairs may affect the phenotypes and growth of young plants. We tested this hypothesis by integrating results from two experiments: (1) a factorial greenhouse seedling experiment with *Zea mays B73-wt* and its root-hairless mutant, *B73-rth3*, grown in live and autoclaved soil, quantifying 15 phenotypic traits, seven growth rates, and soil microbiomes and (2) a semi-hydroponic system quantifying root exudation of maize genotypes. Possibly as compensation for lacking root hairs, *B73-rth3* seedlings allocated more biomass to roots and grew slower than *B73-wt* seedlings in live soil, whereas *B73-wt* seedlings grew slowest in autoclaved soil, suggesting root hairs can be costly and their benefits were realized with more complete soil microbial assemblages. There were substantial differences in root exudation between genotypes and in rhizosphere versus non-rhizosphere microbiomes. The microbial taxa enriched in the presence of root hairs generally enhanced growth compared to taxa enriched in their absence. Our findings suggest the root hairs' adaptive value extends to plant-microbe interactions mediated by root exudates, affecting plant phenotypes, and ultimately, growth.

KEYWORDS

exudates, functional traits, plant-microbe interactions, resource allocation trade-offs, rhizosphere, soil microbiome

1 | INTRODUCTION

Roughly 50% of the world's caloric intake depends on cereal crops (Singer et al., 2019). However, crop productivity is limited by the availability of water and nutrients and is being affected by declines in soil health and climate change (Wang et al., 2018). Plant root systems enable the acquisition of resources from soil that are necessary for photosynthesis and aboveground plant growth. Understanding the belowground determinants of cereal crop productivity is thus important for crop improvement efforts required to feed growing

human populations. While availability of the resources in the soil affects nutrient uptake, the acquisition of soil resources by root systems is also influenced by the functional properties of the root system, including root hairs, root exudation-defined as the release of chemical compounds from plant roots-, biomass allocation to roots versus shoots, and the phenotypic traits of roots (Canarini et al., 2019; Kohli et al., 2022; Lynch et al., 2021; Poorter & Nagel, 2000). Functional root traits have poorly understood consequences for interactions with soil microorganisms in the region of soil surrounding and in close contact with plant roots, known as the rhizosphere (Cardon and Whitbeck, 2011; Edwards et al., 2015; Marschner, 2011; Mathesius, 2015).

Root hairs are single-cell wide extensions of root epidermal cells that, due to their narrow width, length, and abundance, have high surface-area-to-volume ratio (Kohli et al., 2022; Lynch et al., 2021). By forming intimate connections with the soil and pore-space between soil particles, root hairs help anchor the growing root and are essential for the development of the distinct soil environment of the rhizosphere, which strongly affects the functioning of the root system and its ability to provide soil resources to shoots (Aslam et al., 2022; Bengough et al., 2016; Burak et al., 2021; Saengwilai et al., 2021). The large surface area of root hairs may also promote microbial colonization and growth in the rhizosphere (Burak et al., 2021; Canarini et al., 2019; Cotton et al., 2019; Dennis et al., 2010; Doan et al., 2017; Gebauer et al., 2021; Holz et al., 2018). Root hairs can thus enhance crop productivity by promoting nutrient and water acquisition (Aslam et al., 2022; Bates & Lynch, 2000; Bengough et al., 2016; Brown et al., 2013; Gilroy and Jones, 2000; Hochholdinger et al., 2008; Kohli et al., 2022; Marin et al., 2021; Saengwilai et al., 2021), but the costs and benefits of root hair production in relation to interactions with soil microorganisms are poorly understood.

Root structures influence the microbial composition of the rhizosphere (rhizobiome) by providing habitats for colonization and by altering the physicochemical environment, in part through exudation (Aslam et al., 2022; Bilyera et al., 2021; Edwards et al., 2015; Reinhold-Hurek et al., 2015; Tkacz et al., 2020; Williams et al., 2022). Root exudates are molecules, like amino acids, simple sugars, and plant hormones, that are released from roots and root hairs (Doan et al., 2017; Hochholdinger et al., 2008; Horn et al., 2016; Marin et al., 2021; Saengwilai et al., 2021). Exudates promote several functions, including nutrient uptake by facilitating cation exchange near the root, binding of soil near roots, and mediation of interactions between soil microorganisms and the plant (Aslam et al., 2022; Chiniquy et al., 2021; Galloway et al., 2022; Seitz et al., 2022; Vives-Peris et al., 2020; Wang et al., 2022). Certain exudates can promote microbial growth by providing resources to beneficial microbes, while others hinder the growth of pathogens in the rhizosphere (Tkacz et al., 2020; Vives-Peris et al., 2020). Crop genotypes differ in their exudate profiles and other root traits, which can affect rhizosphere microbial communities (Berg and Smalla, 2009; Bulgarelli et al., 2015; Lopez-Guerrero et al., 2022; Wang et al., 2020; Williams et al., 2022). Exudate profiles and amounts have been found to differ between a wild-type barley and its root-hairless mutant, indicating that root hairs can affect root exudation and, in consequence, the rhizobiomes (Galloway et al., 2022).

The root system, while essential for supplying nutrients and water necessary for photosynthesis, is costly to build and maintain and does not contribute directly to photosynthetic carbon fixation. In consequence, plants may only as much as is necessary to roots to maximize photosynthesis and ensure survival, given the long-term expected environmental variation (Ledder et al., 2020; Lerdau, 1992; Reynolds and Pacala, 1993; Sterck and Schieving, 2011). Otherwise, plants would experience lost opportunity costs from not investing in photosynthetically productive shoots (Bloom et al., 1985; Ledder et al., 2020; Westoby et al., 2000). Tradeoffs between investment in roots versus shoots have consequences for crop productivity (Eissenstat, 1997), particularly at the seedling stage.

Given their importance in belowground resource acquisition, root hair production may be involved in these cost–benefit trade-offs. To the extent that root hairs increase efficiency in root functioning, they may lower the threshold for optimal mass investment in roots. Specifically, the total investment in root mass may be reduced if root hairs increase nutrient and water absorption directly through their high surface area to volume ratio, or indirectly through interactions with rhizosphere microorganisms (Kumar et al., 2019; Zhu et al., 2010). How interactions between plants and soil microorganisms influence cost–benefit trade-offs of investment in root systems is not well understood (Bergmann et al., 2020; Lynch et al., 2005; Richardson et al., 2011). A study with *Zea mays* found that relative to wild-type plants, root-hairless plants exhibited compensatory changes in both root traits and investment in arbuscular mycorrhizal fungi, particularly in phosphorus-depleted soils, suggesting that root hairs provide benefits in the form of greater nutrient uptake, but the costs of root hair production were not examined (Kumar et al., 2019). Few studies have examined the role of root hairs in interacting with soil bacteria and archaea. Given the multifaceted functions of root hairs, there may be synergistic effects of root hairs with a wide variety of members of the soil microbial community, partly mediated by exudate production, that affects biomass allocation, and ultimately, plant productivity (Bilyera et al., 2021; Zhang et al., 2020). Therefore, an improved understanding of how to enhance the early growth of cereal crops, like maize, may be achieved through knowledge of how the interconnected mechanisms of root hairs, root exudates, and biomass allocation affect the biomass, diversity, and composition of rhizosphere microbial communities, and how their combined effects govern plant growth (Figure 1).

This study investigated the hypothesis that the benefits of root hairs to seedling growth are partly mediated by interactions between root hairs, soil microorganisms, and root exudation and their effects on phenotypic traits. We tested this hypothesis by integrating data from two experiments. First, we conducted a greenhouse pot experiment in which seedlings of *Zea mays* B73 wild-type (*B73-wt*) and its root hairless mutant (*B73-rth3*) were grown in a natural soil mixture with either the full complement of soil microorganisms (live soil) or experimentally altered soil microbiomes (autoclaved soil). We

quantified seven growth rates and 15 phenotypic traits of seedlings (Table 1), along with the structure of the soil microbial communities (defined here as bacteria and archaea) near (rhizosphere) and away from (bulk soil) seedling roots. Second, we used a semi-hydroponic system combined with targeted metabolomics to quantify variation in root exudate profiles between maize *B73-wt* and its root hairless mutant. We addressed the following research questions. Q1: How does the presence or absence of root hairs affect (Q1.1) community

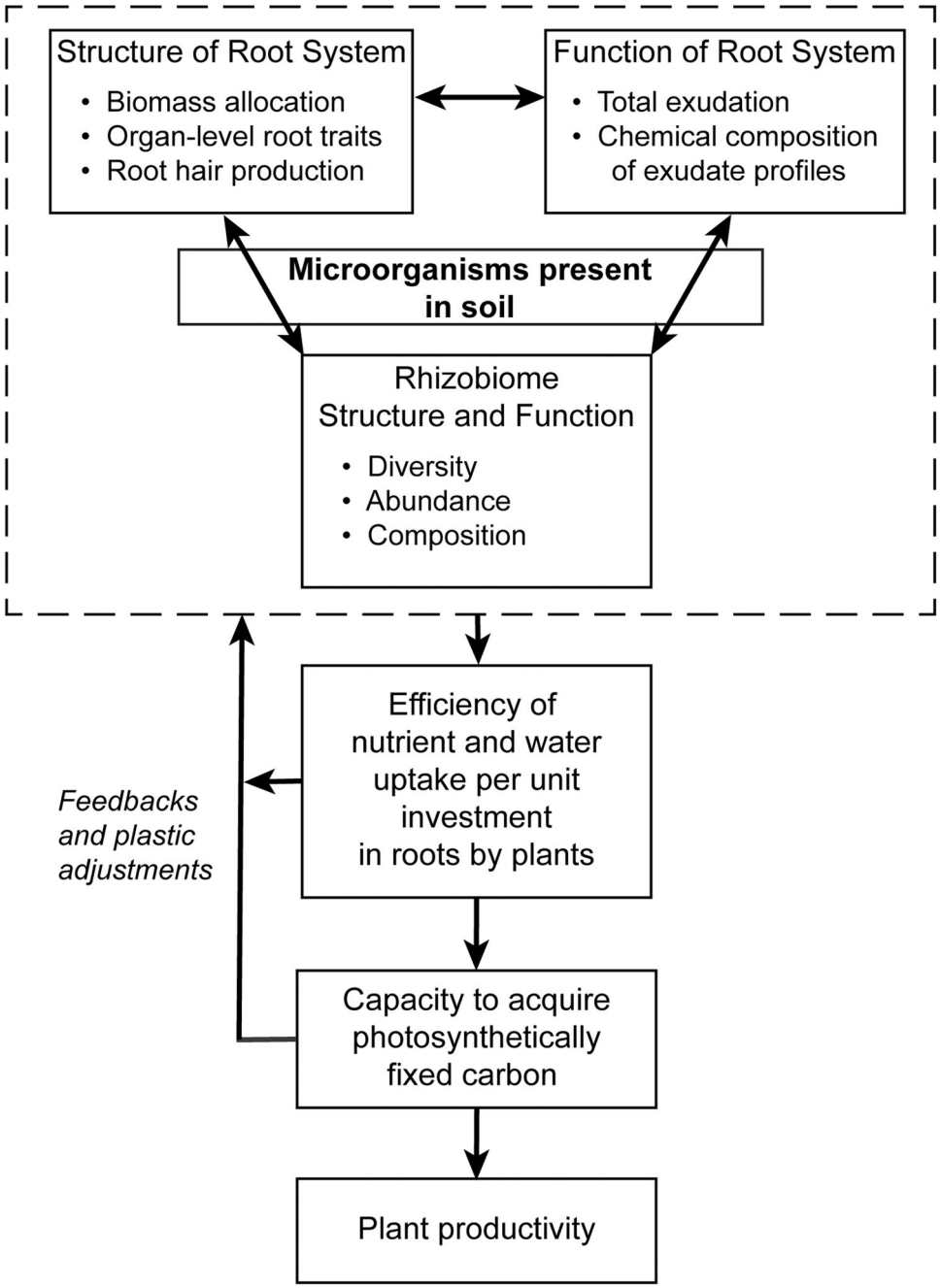


FIGURE 1 Interactions between soil microorganisms and root phenotypic variation and their consequences for plant productivity, emphasizing the subset of interactions, mechanisms, and microbial taxa (bacteria and archaea) that are the focus of this study. Root phenotypic variation, defined by the interdependence of the structure and function of the root system, interacts with soil microorganisms to produce the rhizosphere microbial community (rhizobiome). Rhizobiome structure and function are influenced by the soil environment near roots and are shaped by root phenotypes. The structure and function of the root system and the rhizobiome are interdependent and together influence a plant's access to soil resources that enhance the capacity for photosynthetic carbon fixation. Interactions among microbial taxa in the rhizosphere community can increase accessibility of soil resources to the plant, thereby reducing the need for investment of plant biomass in roots. Allocation of above and belowground resources is regulated by feedbacks and plastic adjustments, which, under a scenario of optimal allocation, would maximize plant productivity.

structure (abundance, alpha diversity, and composition of the microbial community) in the rhizosphere of maize seedlings compared to the bulk soil, (Q1.2) the structural traits, growth rates, and (Q1.3) exudate profiles of maize seedlings? Q2: Is seedling growth rate more strongly influenced by root traits or the structure of rhizosphere microbial communities? Q3: Which rhizosphere microbial taxa most promote or inhibit growth and are these taxa enriched in the rhizospheres of seedlings with root hairs?

We predicted that seedling growth rates and phenotypes, including root exudates, of maize B73 genotypes with (*B73-wt*) and without (*B73-rth3*) root hairs will differ with variation in the rhizobiome when grown in live soil, with more complex microbial assemblages, versus autoclaved soil with reduced microbial assemblages. Specifically, if plants experience cost–benefit trade-offs involving investment in root systems and interactions with soil microorganisms, then we expected overall greater investment in root systems in maize *B73-rth3* when compared with *B73-wt* genotype, which would be associated with slower whole-plant growth of *B73-wt* plants in autoclaved versus live soil and of maize *B73-rth3* compared to *B73-wt* in live soil. We expected root exudation profiles and rhizobiome composition to differ between genotypes and that rhizobiome composition would affect maize seedling phenotypes and

TABLE 1 Plant growth rates and phenotypic traits analyzed for seedlings of maize B73 wild type and B73 root hairless mutant (*rth3*) in a greenhouse experiment.

Abbreviation	Description	Units
Growth rates		
Total bm gr	total biomass growth rate per month	g plant biomass/month
Plant height gr	relative plant height growth rate per month	cm plant height/month
Ag bm gr	aboveground biomass growth rate per month	g aboveground/month
Leaf DWT gr	leaf dry weight growth rate per month	g leaf dry weight/month
LA g	total leaf area growth rate per month	cm^2 leaf/month
Root DWT gr	root dry weight growth rate per month	g root dry weight/month
RL gr	Root length growth rate per month	cm root/month
Phenotypic traits		
Prop L	proportional allocation of biomass to leaves	g leaf dry weight/g plant biomass
LAR	leaf area ratio	cm^2/g leaf dry weight
SLA	specific leaf area	cm^2 leaf area/g leaf dry weight
LDMC	leaf dry matter content	g leaf dry weight/g leaf fresh weight
LTD	leaf tissue density	g leaf dry weight/cm^3 leaf volume
Leaf thick	mean leaf thickness	mm
LA	mean area of a leaf	cm^2
Prop R	proportional allocation of biomass to roots	g root biomass/g plant biomass
RLR	root length ratio	cm root length/g plant biomass
SRL	specific root length	cm root/g root dry weight
RDMC	root dry matter content	g root dry weight/g root fresh weight
RTD	root tissue density	g root dry weight/cm^3 root volume
Total root length	total root length	cm
Total root SA	total root surface area	cm^2
Root diam	average root diameter	mm

Note: Trait abbreviations, a brief description, and the corresponding units for each phenotypic trait are provided.

growth, with rhizosphere microbial taxa varying in their effects on plant growth.

2 | MATERIALS AND METHODS

2.1 | Overview of the study design

We conducted a greenhouse experiment to investigate the effects of root hairs and their interactions with soil microorganisms on maize seedling growth and phenotypic traits using *Zea mays B73 wild type (B73-wt)* and *B73 root hairless mutant (B73-rth3). Z. mays B73-rth3* has gene regulatory elements that prevent the elongation of root hairs at the mature root zone, compared to the otherwise genotypically identical wild type (Wen & Schnable, 1994). Genotype was crossed with soil treatment, which was a factor manipulating the microbial community of the soils in which seedlings were sown into live or autoclaved soil (Wolf and Skipper, 1994). The experiment included a total of 32 seedlings, one in each pot of the following treatment combinations: *B73-wt* × live soil (eight pots), *B73-wt* × autoclaved soil (six pots), *B73-rth3* × live soil (nine pots), and *B73-rth3* × autoclaved soil (nine pots). To create the live and autoclaved soil treatment, soil was mixed in an 80:20 (volume/volume) ratio of a naturally sandy soil, which was autoclaved before mixing, and prairie soil, serving as the soil microbial inoculum. Soil microbial (bacterial and archaeal) communities of each pot were characterized from the rhizosphere (the soil adhering to the root surface at harvest, including the rhizoplane) and in the bulk soil (soil not associated with the root system). Seedling growth and phenotypic traits (Table 1) were quantified for each seedling at the V3 stage. Differences between genotypes in root exudation were quantified in a separate experiment in a growth chamber using a custom-built semi-hydroponic system (Lopez-Guerrero et al., 2022). For full details, refer to the Supporting Information S1: Appendix 1.

2.1.1 | Greenhouse experiment: Seedling growth, phenotyping, and collection of soil samples

Seeds of *Zea mays B73-wt* and *B73-rth3* were obtained from self-pollinated plants grown in a greenhouse. Seeds were surface sterilized by soaking in 2% TWEEN (polysorbate) solution. Seeds were then incubated in 1 mM $CaCl_2$ in a dark growth chamber at 25°C to stimulate germination (Mahboob et al., 2013). One seedling was transplanted into each pot and grown to the maize V3 growth stage (11 days). Greenhouse temperatures were set at 23.8°C during daylight and 21.1°C at night, with lamps supplementing natural light for 12 h each day. Seedlings were watered daily with 20 mL sterile ddH_2O, and supplemented weekly with 20 mL sterile 25% Hoagland's solution (Hoagland & Arnon, 1950).

At the V3 stage, 1–2 g of bulk soil (soil not visibly in contact with any root) and rhizosphere samples were collected from each biological replicate (pot) at harvest (Edwards et al., 2015; Marschner, 2011; Mathesius, 2015). The remaining soil was manually separated from roots using aseptic technique while keeping the seedling intact, leaving the soil adhering to the root system (rhizosphere), which was then sonicated (Branson 450D, 30% amplitude, 0.3 s duty cycle) in sterile 1x phosphate buffered saline solution and pelleted (6000 g for 10 min). Rhizosphere and bulk soil samples were flash-frozen in liquid N_2 and stored at −78°C until DNA extraction.

Following sonication, the aboveground portion of the seedling was severed from the root system, and measurements were collected to estimate growth rates and phenotypic traits (Table 1) for each seedling. Roots were cleaned and measured for total fresh weight. The root system was scanned using the WinRhizo Epson Perfection scanner (20 × 25 cm) at 300 dpi resolution and analyzed using WinRhizo image analysis software (Regent Instruments, version 2008a) to estimate the total root length, average root diameter, and total surface area of roots for each seedling. Leaves were cut below the collar adjacent to the stem. Three mature leaves were selected for leaf-level measurements of thickness, area, fresh mass, and dry mass. Leaf area was measured by scanning each leaf and analyzing images with ImageJ software (v1.51) (Schneider et al., 2012). Leaf-level trait values were averaged to obtain a single trait value for each seedling. The stems, roots, three leaves, and remaining leaves were separately dried at 60°C for 48 h to measure dry biomass. Calculations, abbreviations, and units for the growth rates and phenotypic traits used in statistical analyses derived from these measurements are in Table 1.

2.1.2 | Greenhouse experiment: soil DNA extraction, qPCR, amplicon sequencing, and bioinformatic analyses

DNA was extracted from soil samples by bead beating in 5% CTAB (Cetyltrimethylammonium bromide) followed by phenol:chloroform:i-soamyl alcohol (25:24:1) extraction (Zhou et al., 1996). The DNA was purified and precipitated using a 40% poly-ethylene glycol and 1.6 M NaCl solution with 1uL of glycogen (Griffiths et al., 2000). The qPCR copies were determined using the KAPA HiFidelity HotStart Polymerase of the 16 S V4 gene regions (515 F and 806 R primers) for approximately 10x sequencing coverage. Paired end amplicon sequencing (2 × 300 bp) with the Illumina Miseq was performed using the 515F- (5'-GTGCCAGCMGCCGCGGTAA) and 806 R (5'-GGACTACHVGGGTWTCTAAT) standard primer set for bacteria and archaea (Thompson et al., 2017). Across 145 bulk and rhizosphere soil samples, we obtained 4.85 million raw 16S rRNA v4 paired gene sequence reads, which were analyzed using DADA2 (v3.10; Callahan et al., 2016) in R (v3.6.0; R Core Team 2020) to produce an amplicon sequence variant (ASV) table for the bulk soil and rhizosphere samples from each seedling.

Using the 'dada2' and 'phyloseq' packages in R (Callahan et al., 2016; McMurdie & Holmes, 2013), low-quality sequences were filtered using a minimum average sequence quality score of 20.

Reads were then trimmed (250–300 bp position for forward reads; 200–300 bp, reverse reads) based on manual review of FastQC files for each sample. We produced an ASV table and removed chimeric sequences using the consensus method in DADA2 (Callahan et al., 2017). The ASV table used in statistical analyses had 459102 total reads across 5762 ASVs. Taxonomic classifications were assigned to the ASVs referencing the SILVA v 132 database to produce a taxonomy table (Quast et al., 2012; Yilmaz et al., 2014). Abundances of each ASV from sequencing duplicates, extract duplicates, and bulk soil sampling triplicates were averaged separately for bulk and rhizosphere samples within a biological replicate and rounded to the nearest whole number of raw read counts. Spurious ASV sequences comprising < 0.1% relative abundance across all samples were removed (Reitmeier et al., 2021), as were sequences identified as plant DNA, according to the SILVA taxonomic database.

2.1.3 | Semi-hydroponic experiment: Quantification of root exudate profiles

Exudate data was obtained using a semi-hydroponic exudate collection system that has been used to quantify differences in exudate production between maize genotypes (Lopez-Guerrero et al., 2022). Seeds of *B73-wt* and *B73-rth3* were surface-sterilized, germinated, and transferred to columns filled with 3 mm soda-lime beads. Seedlings were watered with a semiautomatic drip system of sterile nutrient solution using a 'flood and drain' method (Lopez-Guerrero et al., 2022). Exudates were collected at the V3 growth stage with sterile 1 mM $CaCl_2$ or sterile Milli-Q water and freeze-dried. Exudates were identified using liquid and gas chromatography-mass spectrometry (LC-MS/MS and GC-MS) from methods developed at the Proteomics and Metabolomics Facility of the University of Nebraska-Lincoln, USA. To analyze exudate composition from LC-MS/MS and GC-MS results, the concentration of each compound was standardized based on the fresh weight of roots harvested from each seedling. For further details, see Lopez-Guerrero et al. (2022).

2.2 | Statistical analyses

All analyses were performed using R Statistical software (v4.1.2; R Core Team, 2021). The 'microbiome', 'phyloseq', 'pairwise.t. test', 'vegan', 'ANCOMBC', 'stats', 'ecodist', 'car', 'Hmisc', and 'rrBLUP' packages were used for statistical analyses (Endelman, 2011; Fox, 2015; Goslee & Urban, 2007; Harrell, 2022; Lin & Peddada, 2020; McMurdie & Holmes, 2013; Oksanen et al., 2020; Shaffer, 1995; Shetty & Lahti, 2020; Warton et al., 2012). For all tests, statistical significance was assessed at $\alpha = 0.05$. In all models, nonsignificant interaction terms were dropped, and for interaction terms or multi-level main effect terms with statistically significant omnibus tests, we conducted *post hoc* pairwise comparisons of treatment combinations or levels using Student's *t*-tests adjusted for the false discovery rate using the Benjamini-Hochberg method.

2.2.1 | Q1.1 variation in microbial community structure

We assessed effects of sample type (bulk soil and rhizosphere), soil treatment (live and autoclaved), and genotype (*B73-wt*, *B73-rth3*) on microbial abundance, diversity, and composition based on the final ASV table, using both relative-abundance weighted and presence-absence metrics. Total microbial abundance was determined from qPCR copy number normalized to total soil wet mass extracted (copies/gram wet soil). We fit separate analysis of variance (ANOVA) models using Type III tests for each diversity metric with the main effects of sample type, soil treatment, and genotype, along with all two-way interactions, and the three-way interaction effect.

We assessed variation in microbial community composition due to sample type, soil treatment, and genotype using principal coordinate analysis (PCoA) in parallel with permutational ANOVA (perMANOVA). Relative abundance-weighted (Bray-Curtis) and presence-absence (Raup-Crick) distance metrics for ordination were plotted in separate PCoAs with 95% confidence ellipses estimated from the standard error of sample type × soil treatment combinations. We conducted the corresponding perMANOVAs with all main effects and interactions of sample type, soil treatment, and genotype. We used differential abundance analysis (Lin & Peddada, 2020) to identify microbial taxa at the ASV and genus (259 genera across all samples) levels that were differentially abundant in the rhizospheres of wild-type versus root-hairless seedlings in live soil.

2.2.2 | Q1.2 and 1.3 variation in seedling growth rates, phenotypic traits, and exudate profiles

To assess the effects of genotype and soil treatment on seedling growth and phenotypic traits in the greenhouse experiment, we fit separate ANOVAs for each growth rate and phenotypic trait with main effects of soil treatment, genotype, their interaction, and controlling for seedling age, using Type III tests.

To assess the effect of genotype on root exudation from data in the semi-hydroponic experiment, we first calculated the average contribution of each of the 47 exudates identified to the overall Bray-Curtis dissimilarity between maize genotypes. For the 14 exudates with an average individual contribution > 1% across samples, we used two-sided Student's *t*-tests with unequal variance to test for differences between genotypes in individual concentrations (weighted by fresh root mass) and total exudation (the sum of the concentrations of the 47 exudates). Differences in exudate profiles between genotypes were visualized in separate nonmetric multi-dimensional scaling (NMDS) plots with two kernels (k) using concentration-weighted (Bray-Curtis) and presence-absence (Raup-

Crick) distance metrics. Corresponding perMANOVAs statistically tested for multivariate differences between genotypes in exudation.

2.2.3 | Q2 association of seedling growth rates with rhizobiome community structure and root traits

To evaluate whether seedling growth rates depended on rhizobiome community structure, we fit separate linear models for each seedling growth rate (dry leaf biomass growth rate, dry root biomass growth rate, total biomass growth rate, and leaf area growth rate) as a function of microbial abundance, richness, evenness, and relative proportion of rare taxa of the rhizobiome across genotypes and soil treatments using Type III ANOVA tests. In a multivariate context, we evaluated seedling growth rates as a function of root and leaf trait variation and rhizobiome community composition using multiple regression distance matrices that produced an approximation of the Student's *t* test statistic that is used for permutation-based significance testing (Anderson, 2001; Lichstein, 2007). Distances between all pairs of seedlings were estimated using the Gower metric for growth rates and leaf and root traits using the Bray-Curtis and Raup-Crick distance metrics for rhizobiome composition.

2.2.4 | Q3 identification of microbial ASVs influencing seedling growth

To identify rhizosphere microbial taxa with the strongest effects on the biomass growth rates of seedlings with and without root hairs, we used mixed models with soil treatment and genotype as fixed effects and the ASV relative abundances as random effects. We compared this mixed model to a fixed effects-only model and found that including the ASV relative abundances in the random effect model was supported based on Akaike Information Criterion, *pseudo* R^2, and prediction accuracy (Supporting Information S1: Table 1). Prediction accuracy of each model was assessed based on five k-fold cross-validation (Fushiki, 2011; Shao, 1993). *Pseudo-*R^2 values (Nakagawa et al., 2017) were calculated to compare the variance explained by the fixed effects alone (marginal R^2) versus the fixed effects conditioned on the random effects (conditional R^2). Random effect slope parameter estimates, reflecting the change in seedling growth rate per unit change in ASV relative abundance, were obtained using reduced maximum likelihood. We calculated a metric for effect size that accounts for variance in ASV abundance: the slope estimate squared multiplied by the variance in ASV relative abundance, applying the sign of the slope to the metric to indicate positive or negative effects on growth. We ranked the top 10% of all ASVs with the greatest variance-weighted effect sizes (5% positive and 5% negative) and obtained genus and family-level taxonomic assignments for them. To relate the microbial ASVs influencing growth to those differing significantly in relative abundance between maize genotypes, we cross-referenced these ASVs with the ASVs in live-rhizosphere samples that had shown significant differential abundance between seedlings with and without root hair genotypes.

3 | RESULTS

After dereplication in the DADA2 pipeline, filtering, and averaging technical replicates, we obtained 172565 16S rRNA reads for 3564 ASVs across maize genotypes, soil treatments, and sample types (Supporting Information S1: Table 2). ASVs were taxonomically classified into 153 families and 259 genera spanning across one archaeal phylum (Thaumarcheota) and 17 bacterial phyla (Supporting Information S1: Table 3). Soil treatments showed no dramatic differences in the number of reads, but autoclaved soils tended to have fewer unique ASVs (Supporting Information S1: Table 2).

3.1 | Q1.1 variation in microbial community structure

Soil microbial community structure varied most strongly with soil treatment (live vs. autoclaved) and sample type (bulk soil vs. rhizosphere), while maize genotype (presence-absence of root hairs) was only significant with the interaction of sample type for microbial abundance (Figure 2; Supporting Information S1: Table 4 and 5, Supporting Information S1: Figure 1 and 2). Microbial abundance was higher in the rhizosphere than bulk soil, but this effect depended on the maize genotype, with lower abundance in the rhizosphere of *B73-rth3* than *B73-wt* (Figure 2a). All measures of alpha diversity (richness, diversity, relative proportion of rare taxa) except evenness were higher in the bulk soil than the rhizosphere (Figure 2b–e).

Microbial community composition and its variability differed between soil treatments, bulk versus rhizosphere soil, and maize genotypes based on both abundance-weighted (Supporting Information S1: Figure 2A,C) and presence-absence (Supporting Information S1: Figure 2B,D) analyses of ASVs (Supporting Information S1: Figure 1 and Supporting Information S1: Table 5). Maize genotype had no effect on abundance-weighted composition, which was principally influenced by sample type and soil treatment. The presence-absence of ASVs, however, was affected by maize genotype, sample type, and soil treatment. Maize genotype and sample type also had significant interactions with soil treatment, suggesting that the effect of autoclaving differed between genotypes and sample types. Focusing on live rhizosphere soils, 12 of 78 (15%) genera were significantly differentially abundant between maize genotypes, indicating that these taxa were likely influenced by the presence-absence of root hairs (Supporting Information S1: Table 7 and Supporting Information S1: Figure 3). Although the differences between genotypes were small in magnitude (LFC = −0.005 to 0.006), the relative abundances of *Stenotrophomonas* in Proteobacteria and *Gaiella* in Actinobacteria, were significantly higher

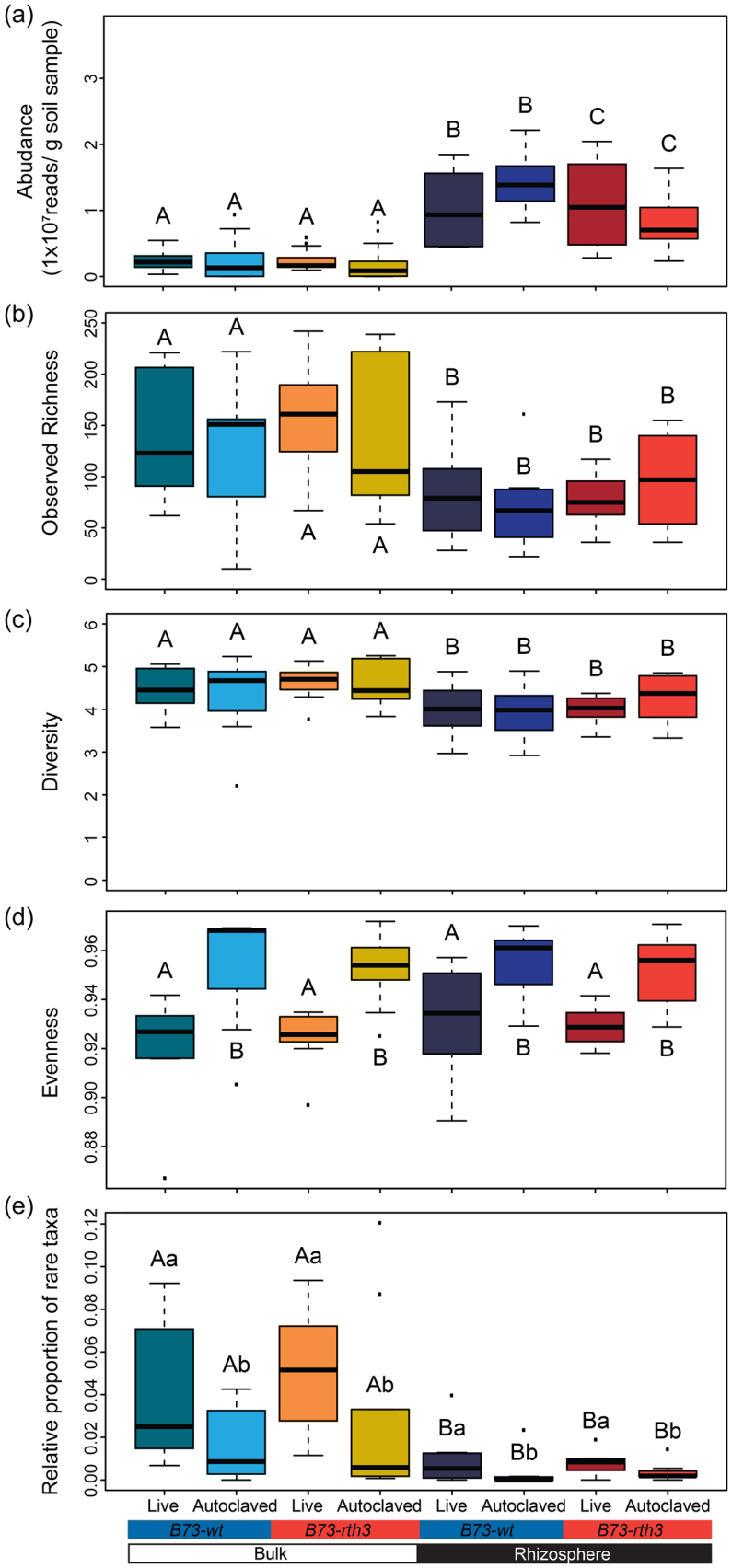


FIGURE 2 Variation in microbial abundance and alpha diversity metrics for bulk and rhizosphere soil samples collected from maize seedlings of B73 wild type and B73 root hairless mutant (*rth3*) in live and autoclaved soils. Variation in microbial community alpha diversity between maize *B73-wt* (blue hues) and *B73-rth3* (red to yellow hues) genotypes grown in live (darker hues) and autoclaved (lighter hues) soil treatments was quantified using microbial amplicon sequence variants (ASVs) and qPCR reads collected from bulk (left to middle) and rhizosphere (middle to right) soil samples in a greenhouse pot experiment. The boxplots (a–e) show a combination of genotype, soil treatment, and sample type differences for (a) microbial biomass estimates from qPCR reads in comparison to the initial soil sample mass, (b) the observed richness of unique ASVs, (c) Shannon's Diversity Index based on ASV abundances, (d) Pielou's evenness based on ASV abundances, and (e) the relative proportion of rare taxa, estimated by a cutoff of 0.2% in ASV relative abundance. The box represents the interquartile rate, the center represents the median, and the whiskers indicate ±1.5 times the interquartile range. Dots above or below the whiskers represent extrema in the data set. If an interaction effect was marginally significant ($p < 0.10$), then *post-hoc* tests corrected for the false discovery rate (FDR) using the Benjamini-Hochberg correction method. Letters indicate statistically significant differences between treatment combinations. When both upper and lowercase letters are present in a panel, then uppercase letters indicate significant ($p < 0.05$) differences between sample types, and lowercase letters indicate significant differences between soil treatments. If only uppercase letters are present in a panel, then they indicate significant differences among all treatment combinations. Summary statistics for the analysis of variance for all microbial community structure variables are presented in Supporting Information S1: Table 4. [Color figure can be viewed at wileyonlinelibrary.com]

in *B73-rth3* rhizobiome, whereas *Luteibacter*, *Lysobacter Fulvimonas*, *Klebsiella*, and *Altererythrobacter* in Proteobacteria, *Terrimonas* and *Parafilimonas* in Bacteroidetes, *Fimbriimonas* in Armatimonadetes, *Edaphobacter* in Acidobacteria, and *Schlesneria* in the Planctomycetes phylum were present only in *B73-wt* rhizobiome (Supporting Information S1: Table 7, and Supporting Information S1 Figure 3B).

3.2 | Q1.2 variation in growth rates and phenotypic traits

Seedling growth rates and phenotypic traits varied significantly between genotypes and soil treatments (Figure 3, Supporting Information S1: Table 8). Compared to *B73-wt*, the root hairless mutant *B73-rth3* seedlings exhibited a compensatory increase in allocation to roots. Growth in root dry weight, growth in root length, proportional biomass allocation to roots, and total root length were significantly higher in *B73-rth3* than *B73-wt* seedlings (Figures 3b,e,g,h). This came at the expense of aboveground growth in terms of plant height, leaf area, and leaf dry weight, particularly in autoclaved soil (Figures 3a,c,f). In terms of total plant biomass, *B73-wt* seedlings in autoclaved soil grew the slowest (Figure 3d) and produced leaves that were the smallest in area (Figure 3i).

In multivariate analyses of all growth rates and phenotypic traits, there were statistically significant differences between genotypes and soil treatments. For roots, there were significant differences between genotypes (perMANOVA; $R^2 = 0.18$, $F_{1,\ 22} = 5.10$, $p = 0.001$). For leaves, both genotypes (perMANOVA; $R^2 = 0.10$, $F_{1,\ 22} = 2.85$, $p = 0.045$) and soil treatments (perMANOVA; $R^2 = 0.15$, $F_{1,\ 22} = 4.49$, $p = 0.008$) differed significantly.

3.3 | Q1.3 exudate composition variation between genotypes and soil treatments

Root exudation differed significantly between genotypes (Figure 4; Supporting Information S1: Table 9). The total mass of all exudates analyzed tended to be greater in *B73-wt* than *B73-rth3* seedlings, although this difference was not statistically significant due to the large variation among *B73-wt* seedlings (Figure 4a; unequal variance *t*-test; $t = -1.19$, $df = 8.04$, $p = 0.135$). Among exudates comprising ≥ 1% of the total variation in exudate profiles (Figure 4b–d), lysine was the only exudate that differed significantly between genotypes (Figure 4b), with lower concentrations produced by *B73-wt* versus *B73-rth3* roots (unequal variance t-test; $t = 3.44$, $df = 18.26$, $p = 0.014$). DIMBOA also exhibited marginally significant differences between maize genotypes (unequal variance *t*-test; $t = -1.89$, $df = 10.50$, $p = 0.087$). In a multivariate context, differences in the concentrations of individual exudates translated into large, significant overall differences in exudate profiles between maize genotypes (Figure 4c,d), using both concentration-weighted (perMANOVA; $R^2 = 0.14$, $F_{1,22} = 3.72$, $p = 0.002$) and presence-absence-based analyses (perMANOVA; $R^2 = 0.48$, $F_{1,22} = 20.11$, $p = 0.009$).

3.4 | Q2: Seedling growth rates associated with plant traits and microbial community structure

Across soil treatments and genotypes, seedling growth rates varied significantly with microbial abundance and with some components of alpha diversity in the rhizobiome, which explained a substantial amount of variation in seedling growth rates (23–44%) (Table 2). ASV richness and the proportion of rare taxa did not significantly affect any growth rate. However, faster growth rates in total biomass, leaf dry weight, and root dry weight were significantly associated with lower evenness and lower abundance, whereas faster leaf area growth rate was only associated with lower evenness. In a multivariate context, leaf and root traits and rhizobiome composition explained 12–15% of the variation in seedling growth rates (Supporting Information S1: Table 10). While root traits significantly affected seedling growth ($p = 0.050$), the strongest effect was the presence-absence of ASVs in the rhizobiome ($p = 0.005$).

3.5 | Q3 microbial ASVs strongly affecting biomass growth of maize seedlings

Microbial ASVs differed strongly in how variation in relative abundance affected seedling biomass growth rate, based on the 148 ASVs representing 8 bacterial phyla in the top 10% of variance-weighted effect sizes (Figure 5; Supporting Information S1: Table 11). ASVs had both positive and negative association with growth (Figure 5, Supporting Information S1: Table 11). ASVs which were taxonomically classified in *Moraxellaceae*, *Rhizobiaceae*, *Azospirillaceae*, and *Rhizobiales Incertae Sedis* families (8 ASVs) within the phylum Proteobacteria had predominantly positive associations with growth, whereas ASVs in *Weeksellaceae* and *Crocinitomicaceae* in Bacteroidetes, Streptomycetaceae in Actinobacteria, and Beijerinckiaceae and Xanthomonadaceae families in Proteobacteria (8 ASVs) had predominantly negative associations with growth. At the genus level, 30 of the 148 ASVs were unclassified (20.3%) and varied between positive (20 ASVs) and negative (10 ASVs) effects on growth. For the ASVs classified in the genera *Cupriavidus*, *Acinetobacter*, *Fulvimonas*, *Nordella*, *Phenylobacterium*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Neorhizobium*, and *Azospirillum* in the phylum Proteobacteria and *Sphingobacterium* in the Bacteroidetes phylum were positively associated with growth, whereas *Terrimonas*, *Chryseobacterium*, and *Fluviicola* in the Bacteroidetes phylum, *Pseudarthrobacter* and *Streptomyces* in Actinobacteria, *Edaphobacter* in Acidobacteria, and *Acinetobacter*, *Plot4-2H12* (Sphingomonadaceae), *Rhodopseudomonas*, *Caulobacter*, *Methylobacterium*, *Stenotrophomonas* in the Proteobacteria phylum were negatively associated with growth.

Of the 38 ASVs that were differentially abundant between maize genotypes in live rhizosphere soils (Figure 5; Supporting Information S1: Table 12), 13 ASVs (34%) also had strong effects on seedling biomass growth, based on variance-weighted effect

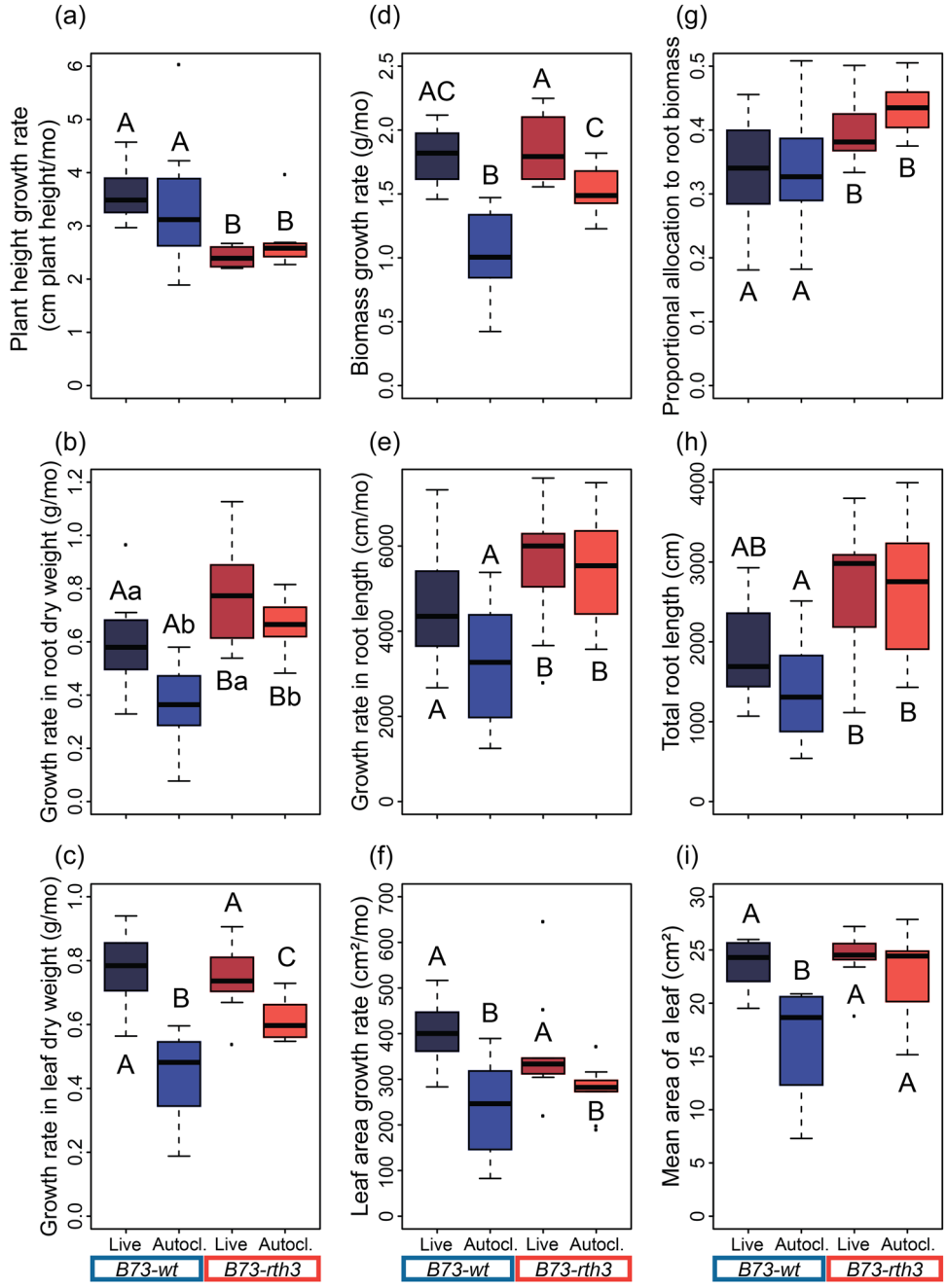
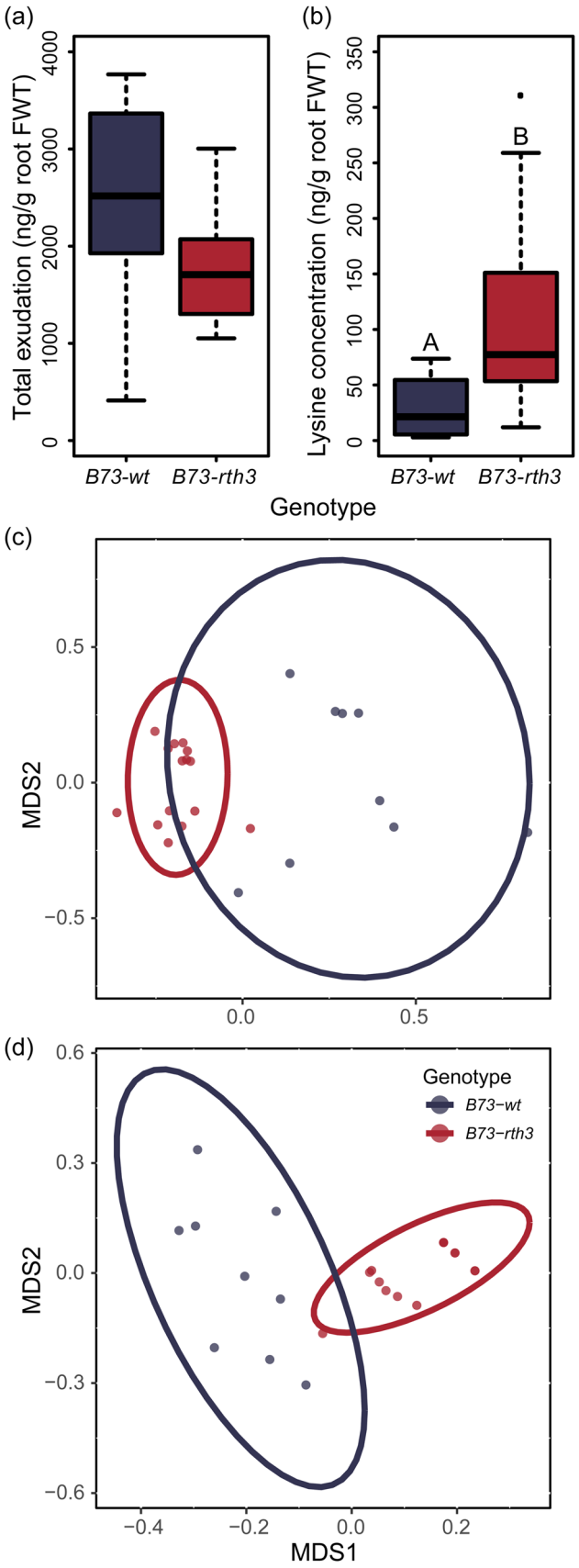


FIGURE 3 Variation in seedling growth rates and phenotypic traits of maize B73 wild type (*B73-wt*) and B73 root hairless mutant (*B73-rth3*) in live and autoclaved soils. Variation in growth rates and phenotypic traits between maize *B73-wt* (blue hues) and *B73-rth3* (red hues) grown in live (darker hues), and autoclaved (lighter hues) soils was quantified in a greenhouse experiment. Boxplots (a–i) show a combination of genotype and soil treatment differences for growth rates (a–f), including (a) seedling height growth rates, (b) root biomass growth rates, (c) leaf biomass growth rates, (d) seedling biomass growth rates, (e) root length growth rates, and (f) leaf area growth rates, projected for a month of growth, along with phenotypic traits including (g) allocation of total seedling biomass to the roots, (h) the total root length at harvest, and (i) average leaf area of one leaf. The box represents the interquartile rate, the center line represents the median, and the whiskers indicate ±1.5 times the interquartile range. Dots above or below the whiskers represent extrema in the data set. If an interaction effect was marginally significant ($p < 0.10$), then *post hoc* tests corrected for the false discovery rate (FDR) using the Benjamini-Hochberg correction method. Letters indicate statistically significant differences between treatment combinations. When both upper and lowercase letters are present in a panel, then uppercase letters indicate significant ($p < 0.05$) differences between genotypes, and lowercase letters indicate significant differences between soil treatments. If only uppercase letters are present in a panel, then they indicate significant differences among all treatment combinations. Summary statistics for the analysis of variance for all growth rates and phenotypic trait variables are presented in Supporting Information S1: Table S8. [Color figure can be viewed at wileyonlinelibrary.com]



sizes (Supporting Information S1: Table 12). The ASVs strongly affecting growth and were also significantly enriched in maize *B73-wt* rhizospheres had mostly (88%) positive effects on growth, whereas those that were significantly enriched in maize *B73-rth3* pots all had negative effects on growth (Figure 5, Supporting Information S1: Table 12).

TABLE 2 Effects of rhizobiome microbial community structure on growth rates of seedlings of maize *B73-wt* and the root hairless mutant *B73-rth3* genotypes in live and autoclaved soils.

Plant growth rate	Richness	Evenness	Abundance	Relative proportion of rare taxa
Total biomass $R^2_{adj} = 0.48$	$p = 0.218$	$p < 0.001$	$p = 0.002$	$p = 0.396$
	0.131	−0.301	−0.188	−0.077
Leaf dry weight $R^2_{adj} = 0.46$	$p = 0.489$	$p = 0.003$	$p = 0.002$	$p = 0.905$
	0.032	−0.104	−0.083	0.005
Root dry weight $R^2_{adj} = 0.27$	$p = 0.151$	$p = 0.015$	$p = 0.013$	$p = 0.237$
	0.090	−0.109	−0.088	−0.064
Leaf area $R^2_{adj} = 0.37$	$p = 0.476$	$p = 0.016$	$p = 0.195$	$p = 0.208$
	23.59	−57.77	−23.39	36.69

Note: Linear models were fitted separately for each seedling growth rate variable (Table 1) with the main effects of richness, Pielou's evenness, abundance, and relative proportion of rare taxa in the rhizosphere microbial community based on amplicon sequence variants (ASVs). See Methods Section 2.2.3 for details. The probabilities (p) in the first row refer to the significance of the marginal effect, and the second row reports the slope estimate. The probability that the slope is different from zero is the same as those in the first row. Predictor variables were scaled before analysis, so that slope estimates are comparable with each model. For all models, the variance inflation factors for all predictors were <3.8.

FIGURE 4 Genotypic variation in root exudation of maize seedlings. Variation in root exudation between *B73-wt* (dark blue) and *B73-rth3* (red) was quantified in a sterile lab hydroponics system. Boxplots (a, b) show genotypic differences for (a) total exudation of all analyzed exudates and lysine concentrations (b). Ordinations (c, d) show variation in all exudates based on concentration-weighted (c) and presence-absence (d) analyses using Bray-Curtis and Raup-Crick dissimilarity indices, respectively. Boxes represent the interquartile range, the center line represents the median, and the whiskers indicate the first and third quartiles ± 1.5 times the interquartile range. Dots above or below the whiskers represent extrema in the data set. Letters above each boxplot indicate statistically significant differences ($p < 0.05$) based on Welch's two-sample t-tests assuming unequal variances. Points in the ordination plots represent individual exudate profiles from each sample in the semi-hydroponic system, color-coded according to the legend in panel d. Ellipses are 95% confidence ellipses. [Color figure can be viewed at wileyonlinelibrary.com]

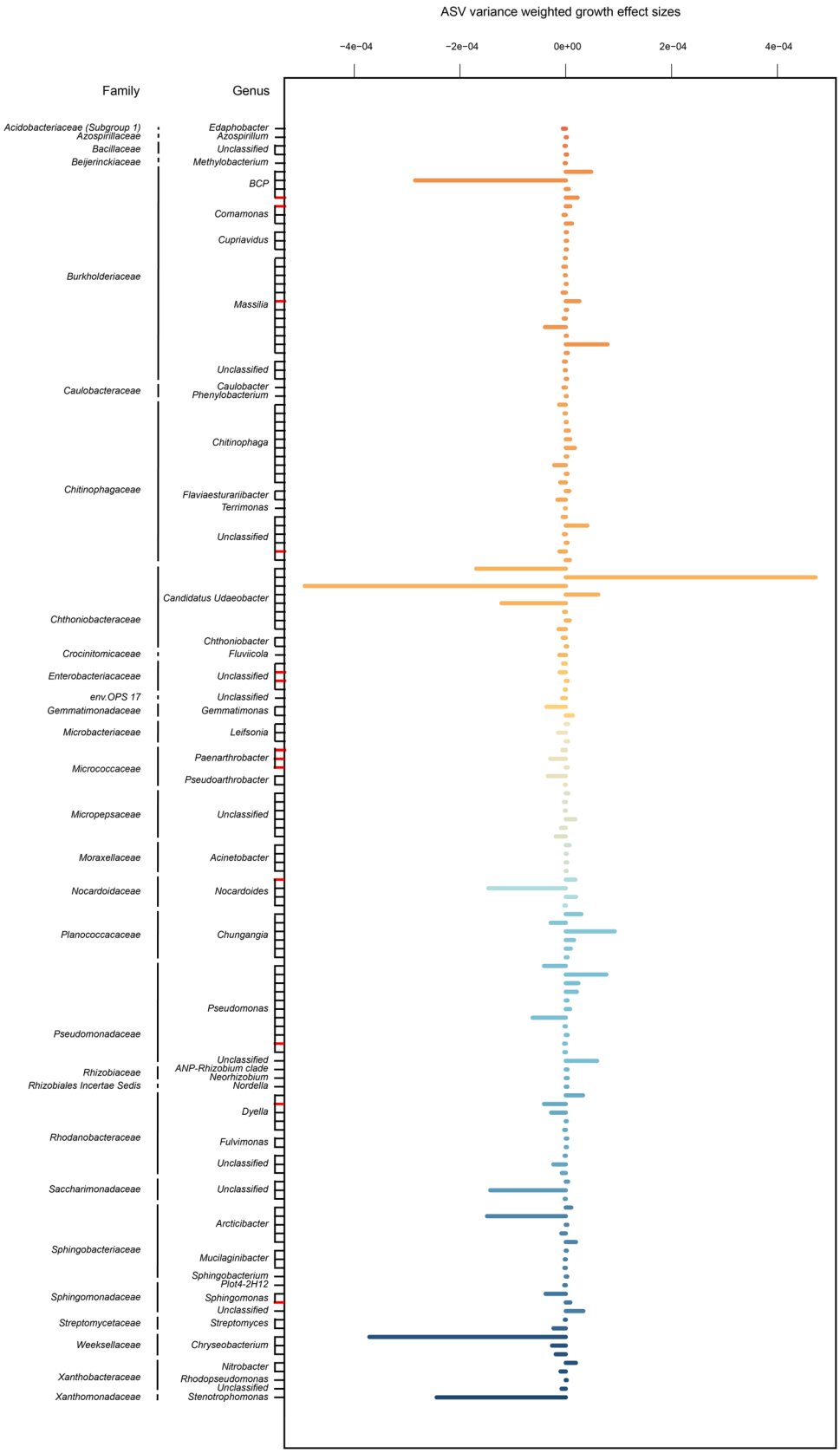


FIGURE 5 (See caption on next page).

4 | DISCUSSION

There is a growing appreciation for the interactive roles of soil microorganisms and the structure and functioning of the root system in determining plant productivity (Bergmann et al., 2020; de la Riva et al., 2021; Lynch et al., 2021; Roley et al., 2021). Biomass allocation to roots versus shoots is part of a zero-sum game, in that resources allocated to making more roots come at the cost of allocating mass to shoots that could increase photosynthetic capacity, ultimately slowing plant growth (Iwasa and Roughgarden, 1984; Sterck and Schieving, 2011; Thornley, 1998). Our results suggest that the maize root hairless mutant genotype (*B73-rth3*) suffers these costs. *B73-rth3* seedlings allocated more biomass to roots and had altered root phenotypic traits compared to *B73-wt*, which were ultimately associated with slower plant height growth rates compared to *B73-wt* seedlings. These patterns of variation match those that would be expected if changes to the *B73-rth3* root system functioned to compensate for the lack of root hairs. The role of the soil microbial community in modulating these effects was also suggested by our findings. In soils with experimentally altered microbiomes, the root-hairless mutant grew slower than in live soils. While the absence of root hairs reduced growth rates, the production of root hairs was not without cost. The biomass growth rate of *B73-wt* seedlings, which produce root hairs, was the slowest in the autoclaved soil with an altered microbiome, not only in comparison with live soil, but also compared to *B73-rth3* in both live and autoclaved soil. These results suggest that root hair production can be costly and that the benefits of root hairs were only fully realized in the presence of an appropriate soil microbial assemblage in these maize seedlings. Our results, elaborated below, support the less appreciated function of root hairs in interacting with soil bacteria, and indicate that these interactions affect plant phenotypes and growth rates. As these are novel interpretations, we suggest that they should be further evaluated as hypotheses in experiments with other plant species for which root hairless genotypes are available (e.g., barley).

In addition to the presence or absence of root hairs, our findings indicate that these belowground plant-microbe interactions may also be mediated in part by other root phenotypic traits, including chemical exudation from roots. As it is extremely difficult to isolate plant-derived root exudates from microbial exudates in soil, we employed a semi-hydroponic system that was specifically developed to quantify genotypic variation in root exudation in maize (Lopez-Guerrero et al., 2022). Using the semi-hydroponic system enabled us to identify substantial differences in exudation profiles between *B73-wt* and *B73-rth3* seedlings, which has not yet been demonstrated in maize. Although plant growth can differ in soil versus semi-hydroponic media (Lin et al., 2016; Oburger and Jones, 2018; Williams et al., 2021), none of our data analyses involved comparisons across the plants in the semi-hydroponic and greenhouse experiments. Semi-hydroponic systems are presently the only way to ensure that inferences about root exudation are not strongly confounded with microbial exudation. Integrating the interpretations of the complementary findings from these two experiments, our study yielded novel insights on the functioning of the plant-root-microbe system as a whole (Figure 1). We cannot directly test the hypothesis that differences in root exudation contributed to rhizobiome variation, but the semi-hydroponic experiment showed that root exudation tended to be lower in total amount in the root-hairless mutant seedlings and differed strongly in composition from seedlings expressing root hairs. This, combined with genotypic variation in the relative abundance of microbial ASVs, supports the hypothesis that exudation is likely an important mechanism by which root hairs affect the rhizobiome.

The effects of seedling roots on microorganisms were clearly shown by the differences in microbial communities between bulk and rhizosphere soil, which varied among genotypes. These rhizobiome differences had consequences for seedling growth. The microbial ASVs that were more abundant in the rhizosphere of maize seedlings with root hairs generally had positive effects on seedling growth, whereas the reverse was true for the root-hairless seedlings, suggesting that root hairs may promote beneficial bacteria in the rhizobiome that can have positive feedbacks on plant growth. By integrating data on above and belowground seedling growth and phenotypic traits, root exudation, and soil microbial communities, our study helps synthesize this mosaic of complex interactions involving root systems and soil microorganisms, leading to a better understanding of how they influence belowground functioning and aboveground productivity in maize seedlings.

FIGURE 5 Effects of microbial genera and families in the live rhizosphere with the largest positive and negative effects on plant biomass growth. The top 10% of all rhizosphere ASVs (148/1474) with the largest positive (5%) and negative (5%) effects on growth were identified based on the variance-weighted effect sizes for the random effects (Supporting Information S1: Table 1; see Methods Section 2.2.4 for details). These variance-weighted effect sizes for each ASV were aggregated at (A) the genus level and (B) the family level and are ordered by the magnitude of the effect size. Classifications were based on the SILVA taxonomic database. All ASVs could be classified at the family level. All ASVs that could not be classified to the genus level are grouped under "Unclassified" for the relevant families. Red tick marks on the y-axis indicate ASVs which also were significantly differentially abundant between genotypes in the live rhizosphere soils (Supporting Information S1: Table 12). The acronym "BCP" at the genus level (A), refers to the *Burkholderia-Caballeronia-Paraburkholderia* genera, while "ANP-Rhizobium" refers to the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* clade of taxonomic classification. Refer to Supporting Information S1: Table 3 for phylum and class level taxonomic classification for each family. [Color figure can be viewed at wileyonlinelibrary.com]

4.1 | Root structural and functional traits influence soil microbial community structure

Microbial communities varied strongly due to the interactive effects of proximity to roots and the presence-absence of root hairs although the magnitude of their effects differed depending on the measure of the microbial community structure in question. Similar to previous studies (Reinhold-Hurek et al., 2015; Starr et al., 2018; Tkacz et al., 2020), it was evident that seedling roots had significant effects on soil microbial communities. Compared to the bulk soil, microorganisms were more abundant in the rhizosphere, but with lower taxonomic richness and diversity, along with strikingly different community composition, suggesting that plant roots mediate ecological filtering (*sensu* Kraft et al., 2015) of the soil microbiome which has been observed in other studies (Miller et al., 2018; Russo et al., 2012; Trivedi et al., 2021; Wang et al., 2013).

Our results suggest that these ecological filtering processes are mediated in part by environmental variation in the rhizosphere produced by root functional phenotypes, including root hairs and root exudation. Relative to the effects of proximity to the root and autoclaving, the main effect of the presence-absence of root hairs on soil microbiomes was not strong. To the extent that exudate profiles from the semi-hydroponic system are indicative of those in soil (Wang et al., 2022), the dramatic exudate variation between maize genotypes suggests that root hairs may strongly influence the rhizobiome through exudation. Root exudation is known to be an important mediator of interactions between soil microbes and plant roots (Canarini et al., 2019; Cotton et al., 2019; Dennis et al., 2010; Hu et al., 2018; Seitz et al., 2022; Vives-Peris et al., 2020; Wang et al., 2021, 2022) that can facilitate recruitment of bacteria into the rhizosphere through processes such as chemotaxis (Badri and Vivanco, 2009; Feng et al., 2021; Zhalnina et al., 2018), and has been shown to influence plant growth in the field (Lugtenberg and Kamilova, 2009). While plastic changes in root phenotypes have been observed in maize *B73-wt* in response to AMF colonization (Kumar et al., 2019), our findings suggest that there may also be a plastic response of root phenotypes to the bacteria in the rhizobiome, producing a feedback response between the microbial community and root functional traits.

4.2 | Costs and benefits of root hairs

A cost of root hair production was observed in that seedlings of *B73-wt* grew more slowly in autoclaved than live soils and more slowly than the root hairless mutant (*B73-rth3*) in both soil treatments for most dimensions of growth that we measured, particularly for biomass growth rate. These findings suggest that the full benefit of root hairs could not be realized without an appropriate soil microbial assemblage, thereby revealing the cost in terms of reduced growth rates of seedlings producing root hairs in autoclaved soil. It has long been appreciated that root hairs can be beneficial because they increase the surface area for absorption of water and nutrients (Bates and Lynch, 2000; Brown et al., 2013; Zhu et al., 2010) and facilitate penetration of the soil by the growing root (Bengough et al., 2016; Bibikova and Gilroy, 2002; Grierson et al., 2014), but only recently has the possibility that root hairs could facilitate the development of the rhizobiome been proposed in cereal crops (Bilyera et al., 2021; Koebernick et al., 2017; Pausch et al., 2016).

Root hairs have been found to be costly in terms of high rates of cellular respiration across an experimental phosphorus availability gradient, and only to be beneficial in low soil phosphorus conditions (Bates and Lynch, 2000). In parallel to these findings for phosphorus, a novel contribution of our study is the demonstration that at least some of the benefits of root hair production in maize seedlings depend on interactions with the soil microbial community. Conversely, root-hairless seedlings allocated a greater proportion of plant biomass to roots and had greater total root length, especially in autoclaved soils, suggesting a compensatory response to the lack of root hairs. The increased belowground investment was associated with slower aboveground growth rates in *B73-rth3* seedlings, while differences in the *B73-rth3* seedlings biomass investment could be considered a compensatory increase as the total biomass growth rate is similar to *B73-wt* seedlings in live soils. Our cost–benefit analysis supports the hypothesis that an important function of root hairs is to interact with the soil microbial community and facilitate the development of the rhizobiome as the root grows through the soil, potentially reducing the need for investment in the root system as a whole. Analogous results to ours were reported by Kumar et al. (2019), which found plastic changes in root phenotypes between root hairless and wild type plants in soils differing in phosphorus (P) availability. Root hairless plants built wider roots and had higher mycorrhizal colonization than wild type plants, particularly in low-P soil, perhaps as compensation for the lack of root hairs, suggesting that maize alters its root morphology and mycorrhizal interactions to maximize nutrient acquisition and hence growth (Kumar et al., 2019).

4.3 | Growth rates correlated with the rhizobiome

If interactions with microorganisms in the rhizosphere are important for plant productivity, then plant growth rates should correlate with variation in community structure of the rhizobiome. In support of this hypothesis, seedling growth rates were significantly negatively related to microbial abundance, evenness, and composition, but unrelated to richness, diversity, or the proportion of rare taxa in the rhizosphere. Our findings suggest that composition in the rhizosphere affects seedling growth. In this respect, our findings are inconsistent with the idea that diversity promotes productivity (Tilman et al., 1997, 2001), although our system involves interactions between plants and soil microorganisms, not among plants as was originally described for the diversity-productivity relationship. Considering the differences in microbial community structure between the bulk soil and rhizosphere, our results are more consistent with the selection effect, in which a few very productive plant species are responsible for enhanced productivity (Loreau and Hector, 2001). In

our case, it may be that the rhizobiome enhances plant productivity when it is restricted to microbial taxa that are beneficial to plants, which may comprise a filtered, less diverse, microbial community, relative to that in the bulk soil.

Ecological filtering processes can produce similar patterns in microbial communities based on environmental context and plant species, which are often referred to as core microbiomes (Grady et al., 2019; Hamonts et al., 2018). Previous work comparing microbial composition in the rhizosphere versus bulk soil across maize genotypes has identified a core maize microbiome (Walters et al., 2018). In live rhizosphere soils, 83% (10 of 12 genera; Supporting Information S1: Table 7) of genera significantly differentially abundant for a maize genotype were identified as members of the core maize microbiome (after reconciling taxonomic revisions). Microorganisms associated with the core microbiome and root hairs are likely to affect root growth. For example, *Methylobacterium spp*. and pathogenic *Pseudomonas spp*. have been shown to stimulate root hair growth while inhibiting primary root growth (Klikno and Kutschera, 2017; Pečenková et al., 2017). We found that ASVs in *Methylobacterium* were also associated with slower growth of maize seedlings, whereas some ASVs in *Pseudomonas* were associated with positive, and others negative, effects on growth. Our results suggest that root hairs may be involved in recruiting members of the core maize microbiome that can affect the growth of young maize plants.

4.4 | Conclusions

By integrating data on plant structural and functional traits, root exudation, and soil microbial communities, our study suggests that part of the adaptive value of root hairs is mediated through exudate-mediated plant-microbe interactions in the rhizosphere and through cost–benefit trade-offs related to root hair production, which, to our knowledge, has not been previously demonstrated for bacterial and archaeal communities. The seedling stage is a vulnerable period for plants, and our findings show that several functions of root hairs are likely to be important for seedlings as they develop organ systems and associations with microorganisms, ultimately influencing maize productivity. Improving understanding of root hair interactions and plastic adjustments in plant phenotypes promotes sustainable agriculture efforts for economically important crops in a changing agricultural landscape (Brown et al., 2013; Kohli et al., 2022).

ACKNOWLEDGMENTS

This project was funded by the National Science Foundation EPSCoR Center for Root and Rhizobiome Innovation Award #1557417. For the exudate data collection, we thank Sophie Alvarez and her team, Peng Wang, and other Daniel P. Schachtman lab group members. For the consultation for statistical methods, we thank Zhikai Yang and Stephen Kachman. We also thank Porshe Miller, part of the Nebraska EPSCoR Young Nebraska Scientist program, who helped with this project as a high school student, along with several others who helped with harvesting plants (A. Stengel, K. Stanke, B. Tripp, S. Johnson, H. Ngo, M. Allison, A.E. Beyene, F. Cordova).

DATA AVAILABILITY STATEMENT

Raw 16S rRNA gene amplicon sequences can be found in NCBI SRA BioProject (SUB12121156). The R code is available upon request to the corresponding author. Plant phenotypic traits, qPCR copy numbers, and the microbial metadata file used for analysis can be found in the Dryad data repository (https://doi.org/10.5061/dryad.0k6djhb3k).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Quattrone, A., Lopez-Guerrero, M., Yadav, P., Meier, M. A., Russo, S. E. & Weber, K. A. (2023) Interactions between root hairs and the soil microbial community affect the growth of maize seedlings. *Plant, Cell & Environment*, 1–18. https://doi.org/10.1111/pce.14755