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Genotype-specific rhizosphere microbiome assembly and its impact on soybean (*Glycine max* (L.) Merri.) yield traits

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Introduction: The rhizosphere microbiome plays a critical role in plant growth and productivity. However, the genotype-specific dynamics and functional influence of these microbial communities on soybean (*Glycine max* (L.) Merri.) yield traits remain underexplored. This study investigated the composition and functional implications of rhizosphere microbial communities in conventional and genetically modified (GM) soybean varieties under field conditions.

Methods: A comparative analysis was conducted on rhizosphere microbial communities associated with conventional H0269 and transgenic Z1510 and M579 soybean varieties at seedling and flowering stages. High-throughput sequencing of 16S rDNA and ITS regions was used to profile bacterial and fungal communities. Alpha and beta diversity metrics were assessed, and correlation analyses were performed to identify microbial taxa linked to yield traits.

Results: Distinct microbial community structures were observed across soybean genotypes and growth stages. *Proteobacteria*, *Acidobacteriota*, and *Actinobacteria* were dominant bacterial phyla, while *Ascomycota* predominated among fungi. Genotype-specific differences were evident, with M579 exhibiting the highest bacterial alpha diversity. Beta diversity analysis revealed significant shifts in microbial composition between growth stages, particularly for bacterial communities. Correlation analysis identified 15 bacterial and 13 fungal species significantly associated with yield traits such as plant density, grain weight, and theoretical yield.

Discussion: The results highlight genotype-dependent recruitment of rhizosphere microorganisms and their potential impact on soybean productivity. GM soybean varieties selectively enriched beneficial microbes that may enhance nutrient uptake, stress tolerance, and disease resistance. These findings provide valuable insights into plant-microbe interactions, paving the way for developing microbial inoculants and breeding strategies to optimize soybean yield in sustainable agriculture.

KEYWORDS

rhizosphere, soybean, microbiome, 16S rDNA and ITS sequencing, yield trait

1 Introduction

The rhizosphere microorganisms played a crucial role in plant growth, development, and health (Weng et al., 2024; Sharma et al., 2024). These beneficial bacteria, fungi, and archaea formed complex communities around plant roots, creating a dynamic environment. It has been reported that certain *Bacillus* strains have the potential to partially replace chemical fertilizers in rice production (Tripathi et al., 2022). The rhizosphere microorganisms can not only provide phosphorus and nitrogen for promoting plant growth, but also help plant tolerate abiotic stresses such as drought and salinity (Abrahão et al., 2018; Bandara and Kang, 2024). The interactions between plant and rhizosphere microorganisms were bidirectional and led to sustainable agricultural practices, including the use of plant growth-promoting rhizobacteria as biofertilizers and biopesticides (Olanrewaju et al., 2019; Ferreyra-Suarez et al., 2024; Vejan et al., 2016). Recent advances in multi-omics approaches have further revealed how plants actively shape their rhizobiome through root exudate profiles, creating genotype-specific microbial recruitment patterns (Levy et al., 2017; Zhalnina et al., 2018).

Soybean (*Glycine max* (L.) Merri.), a globally significant food and cash crop, serves as a primary source of high-quality protein and oil for human consumption. While traditional breeding has focused on improving aboveground agronomic traits, there is growing interest in the rhizosphere microbiome's potential to enhance crop productivity and sustainability (Kumar and Dubey, 2020). Soybean and legume microbiomes are shaped by plant genetics, environmental factors, and agricultural practices. Genetically related cowpea cultivars develop similar rhizobacterial communities (de Albuquerque et al., 2022), while soybean genotypes modulate microbial assembly via root exudates and nodulation (Liu et al., 2019; Zhong et al., 2019). Seasonal shifts and soil types alter peanut rhizosphere microbiota (Daraz et al., 2023), and organic farming enhances peanut microbiome diversity compared to conventional methods (Paudel et al., 2023). Soybean nodulation enriches nitrogen-cycling microbes, improving subsequent crop yields (Liu et al., 2024), while intercropping creates unique microbiome configurations (Lanzavecchia et al., 2024). Functional diversity in soybean rhizospheres supports stress resilience and nitrogen fixation (Ajiboye et al., 2022; Mayhood and Mirza, 2021).

With the increasing adoption of genetically modified (GM) soybeans in agricultural fields, field experiments have become crucial in assessing the interactions between GM soybeans and rhizosphere microbiomes. Global research reveals geographically distinct responses. North American studies in the U.S. Midwest show environmental factors outweigh GM traits in shaping bacterial diversity (Liu et al., 2019; Chang et al., 2021), while Argentine trials identify transient *Pseudomonadales* shifts post-glyphosate application mediated by soil organic content (Bento et al., 2019). Nigerian organic GM systems match conventional yields with rainfall-driven microbial shifts (Olowe et al., 2023), whereas Romanian field trials demonstrate microbiome stability patterns aligning with Central European observations (Ichim, 2019; Noack et al., 2024). Despite progress, critical gaps persist in understudied regions like Eastern European chernozems and Africa's Sahel, where unique genotype-environment-microbiome relationships emerge (Sarkar et al., 2022). Crops shape rhizosphere microbiomes via root exudates, selecting bacteria that aid stress resilience and nutrient cycling. While environmental factors and management practices drive community composition, functional microbial cores underpin plant health under local stressors (Cucio et al., 2016; Debenport et al., 2015). International comparisons further highlight system-specific dynamics. Brazilian glyphosate-resistant soybeans exhibit early *Bradyrhizobium* reductions (Zablotowicz and Reddy, 2004), Canadian systems show seasonal variability overriding genotypic effects, and Australian non-GM cultivars maintain stable variety-specific microbial signatures across soils (Jang et al., 2023). These disparities underscore the need for multinational consortia to disentangle genetic, environmental, and management factors shaping rhizosphere communities.

These findings highlight the need for continued research to fully understand long-term effects of GM soybeans on soil microbial communities in agricultural ecosystems (Ren et al., 2023). Our study addresses this through a comparative analysis of conventional and GM varieties in Inner Mongolia's agriculturally critical yet understudied chernozem (chestnut soil zone), integrating rhizosphere microbiome profiles with soybean phenomic data across growth stages. We reveal genotype-specific microbial recruitment dynamics while contributing foundational datasets from this climate-vulnerable region, elucidating how pedoclimatic conditions interact with soybean genetics to shape yield-linked microbial communities. This work bridges continental research divides, offering insights into how regional pedoclimatic conditions interact with plant genetics to structure rhizosphere communities that influence yield outcomes.

2 Materials and methods

2.1 Plant materials

Three distinct soybean varieties, Zhongliandou (Z1510), Hejiao (H0269), and Maiyu (M579) were used in this study. Z1510 and M579 are both genetically modified soybeans. The Z1510 transformed from ZhongHuang 6,106 with the *gat* and *eps*(g2) gene, conferring glyphosate tolerance. M579 derived from DBN9004, contains *epsps* and *pat* gene, providing glyphosate and glufosinate-ammonium tolerance. In contrast, H0269 is a conventional soybean variety that does not possess these modifications and relies on traditional weed control methods.

2.2 Experimental design and site description

Three distinct soybean varieties cultivated at the experimental base of the Xing'an League Agriculture and Animal Husbandry Science, Institute in Inner Mongolia, a region of significant importance for soybean production in China. The site (45°43′-46°18′N, 121°53′-122°52′E) has a temperate continental climate with chernozem and chestnut soils (pH 8.03, organic matter 34.76 g/kg, total N 3.437 g/kg, total K 2.22%, alkaline N 252.54 mg/kg, available P 20.78 mg/kg, available K 88.75 mg/kg). A randomized complete block design with three replications was employed. Planting utilized a double-row ridge system (60 cm ridge spacing, 0.6 cm plant spacing) at 280,000 plants/ha. Z1510 and M579 received 2,250 mL/ha of 41% glyphosate isopropylamine salt, while H0269 received conventional herbicides. Base fertilizer was applied at >450 kg/ha.

2.3 Rhizosphere soil sampling and microbial community analysis

Samples were collected at seedling and flowering stages using a five-point sampling method. Rhizosphere soil was obtained from roots at 10–30 cm depth, sieved (2 mm), and stored at −80°C. DNA was extracted using HiPure Universal DNA Kit. The V3 and V4 region of 16S rRNA and ITS1 region were amplified and sequenced on a Qsep-400 platform (Persoon et al., 2017). Sequences were processed using QIIME 2 (version 2021.4) and DADA2 to obtain amplicon sequence variants (ASVs; Kesim et al., 2023). Taxonomic annotation used the SILVA 138 database (Bars-Cortina et al., 2023). Alpha diversity was assessed in QIIME 2. Beta diversity was evaluated using principal coordinates analysis (PCoA) based on Bray-Curtis distances. Linear discriminant analysis effect size (LEfSe) identified differentially abundant taxa (LDA > 3.5). Functional classification used FUNGuild (version 1.0; Djemiel et al., 2017).

2.4 Yield trait measurements

At maturity on September 28, yield components were measured following national standards. Plants per m^2 were counted in 2 m^2 areas, while single plant grain weight (g) was calculated by dividing total grain weight by the number of plants in the same 2 m^2 area. The 100-grain weight (g) was determined as an average of 3 replicates, and moisture content (%) was measured using a moisture meter with 3 replicates. Finally, the theoretical yield (kg/ha) was calculated using the formula: plants/m^2 × 10,000 × single plant grain weight × 10^{-5} × 0.9. This comprehensive approach provides a standardized method for estimating crop yields based on key components measured in sample areas, with multiple replicates used for some measurements to enhance accuracy and reliability.

2.5 Statistical analysis

The 16S rRNA gene and ITS amplicon sequencing data were processed using the SILVA 138/16S rRNA database and Unite 8.0 database for taxonomic assignment. Quality control measures were implemented to ensure data integrity, including the detection and removal of chimeric sequences using the UCHIME (v1.9.1) software. Alpha diversity indices were calculated using the MOTHUR (v1.30.2) software to assess the richness and diversity of microbial communities across samples. To investigate the overall community composition and assess the similarities or dissimilarities between samples, beta diversity analysis was performed using principal coordinate analysis (PCoA) implemented in the QIIME (v1.9.1) software package (Caporaso et al., 2010). The resulting PCoA plots were generated using the R packages "vegan," "ecodist," and "ggplot2" in R (v4.1.3), enabling the visual representation and interpretation of beta diversity patterns (Schloss et al., 2009; Cole et al., 2014; Chen and Boutros, 2011). Correlations between microbial community composition (top 20 dominant bacterial taxa) and yield traits were examined using Pearson's correlation coefficient. Data were subjected to a one-way analysis of variance with factors of treatments and expressed as means +SD. Comparisons between any two groups were performed by unpaired Student's t-tests.

3 Results

3.1 Overall analysis of 16S rDNA

The 16S rDNA and ITS sequencing analyses revealed significant microbial diversity in rhizosphere soil samples. Using the 341F-806R primer pair, 404,470 denoised and non-chimeric ASVs were obtained from rhizosphere soil samples, averaging 67,411 ASVs per replicate (Supplementary Table S1). The ITSIF-ITSIR primer pair yielded 807,404 ASVs from surrounding soil samples, with an average of 134,567 ASVs per replicate (Supplementary Table S2). The higher ASV count in rhizosphere soil samples suggested potentially greater microbial diversity.

3.2 Alpha diversity of microbial community in rhizosphere soils

The alpha diversity of rhizosphere microbial communities in M579, Z1510, and H0269 soybean varieties revealed distinct patterns

TABLE 1 Analysis of alpha diversity of three soybean rhizosphere soil microbial communities.

Sample	ACE	Chao1	Shannon
Z1510-Seeding Stage	1144.68 ± 87.1[b]	1144.36 ± 87.02[b]	8.81 ± 0.39[ab]
H0269-Seeding Stage	1089.51 ± 143.97[b]	1088.74 ± 143.52[b]	8.8 ± 0.34[ab]
M579-Seeding Stage	1395.86 ± 51.66[a]	1394.94 ± 51.27[a]	8.96 ± 0.12[a]
Z1510-Flowering Stage	1202.24 ± 250.32[ab]	1201.49 ± 249.98[ab]	8.8 ± 0.31[ab]
H0269-Flowering Stage	1085.39 ± 43.8[b]	1084.45 ± 43.58[b]	8.43 ± 0.09[b]
M579-Flowering Stage	1313.95 ± 47.44[a]	1312.74 ± 47.39[a]	8.81 ± 0.23[ab]
Z1510-Seeding Stage	362.98 ± 15.49[ab]	362.99 ± 15.95[a]	4.88 ± 0.26[a]
H0269-Seeding Stage	367.01 ± 33.57[ab]	366.82 ± 33.68[a]	4.77 ± 0.48[a]
M579-Seeding Stage	452.89 ± 57.84[a]	452.97 ± 58.79[a]	5.21 ± 0.57[a]
Z1510-Flowering Stage	281.12 ± 135.3[ab]	280.81 ± 135.82[a]	3.86 ± 2.22[a]
H0269-Flowering Stage	350.33 ± 24.99[b]	350.6 ± 25.47[a]	5.26 ± 0.05[a]
M579-Flowering Stage	239.29 ± 20.63[ab]	239.13 ± 20.62[a]	3.68 ± 2.47[a]

Different letters in the superscripts of the column indicate significant differences (p < 0.05).

for bacterial and fungal populations (Table 1). At both seeding and flowering stages, bacterial abundance consistently followed the order M579 > Z1510 > H0269, with M579 exhibiting significantly higher Chao1 and ACE indices compared to H0269. M579 maintained 5.8% higher Shannon diversity than H0269 across growth stages. Notably, bacterial diversity indices remained stable within each soybean variety, suggesting that genetic factors have a stronger impact on bacterial community composition than developmental changes. In contrast, fungal communities showed more conserved traits, with 123 shared ASVs across all samples and no significant diversity differences among varieties or growth stages. However, fungal ASV abundance showed diverged between seedling and flowering stages. M579 and Z1510 decreased significantly, while H0269 remained stable. Shannon indices showed no significant differences among varieties. These findings revealed the complex dynamics of soybean rhizosphere microbiomes, showcasing distinct bacterial and fungal responses to plant genotype and developmental stage.

3.3 Beta diversity of microbial community in rhizosphere soils

Beta diversity analysis using PCoA revealed significant variations in rhizosphere microbial community composition across soybean growth stages and genotypes. The PCoA1 axis accounted for 18.55 and 33.78% of the total variance in bacterial and fungal community composition, respectively. At seedling stage, Z1510 and H0269 showed similar bacterial and fungal communities, distinct from M579. At flowering stage, bacterial communities of Z1510 and M579 converged, while H0269 diverged (Figure 1A). However, Fungal communities at flowering stage showed less distinct clustering among varieties, but M579 displayed high within-group variability (Figure 1B). These findings underscored the dynamic nature of rhizosphere microbiomes, influenced by both plant genotype and developmental stage.

3.4 Alterations in bacterial and fungal community composition

Analysis of soybean microbiomes across growth stages revealed significant variations in bacterial community structure. At the bacterial phylum level, the dominant groups identified were Proteobacteria, Acidobacteriota, and Actinobacteria. During the seedling stage, Proteobacteria exhibited the highest abundance in the H0269 variety, while M579 showed the lowest levels. Conversely, Acidobacteriota was most abundant in M579, with H0269 and Z1510 displaying similar abundances. Notably, the abundance of Actinobacteria remained relatively consistent across all three soybean varieties. The flowering period saw increased Proteobacteria and Acidobacteriota across all varieties, while Actinobacteria declined (Figure 2A). At the genus level, the dominant genera during both seedling and flowering periods were *Sphingomonas*, *Gemmatimonas*, *RB41*, *Vicinamibacteraceae*, and *WD2101_soil_group* (Figure 2B).

Fungal communities were dominated by Ascomycota, Basidiomycota, and Zygomycota phyla. At the seedling stage, Ascomycota was most abundant in H0269, followed by Z1510 and M579, while Basidiomycota peaked in M579. These abundance patterns persisted into the flowering stage, with Ascomycota increased in H0269 and Z1510 but decreased in M579 (Figure 2C). At the genus level, *Fusarium* and *Guehomyces* dominated, with *Guehomyces* showing higher abundance in M579 during the seedling stage. Conversely, *Fusarium* became more prevalent in H0269 during flowering (Figure 2D).

3.5 LEfSe analysis of bacterial and fungal communities

The LEfSe analysis of rhizosphere bacteria across three soybean varieties revealed significant differences in functional bacteria recruitment between seedling and flowering stages. At seedling stage, a total of 89 bacterial species were recruited, with M579 recruiting the highest number
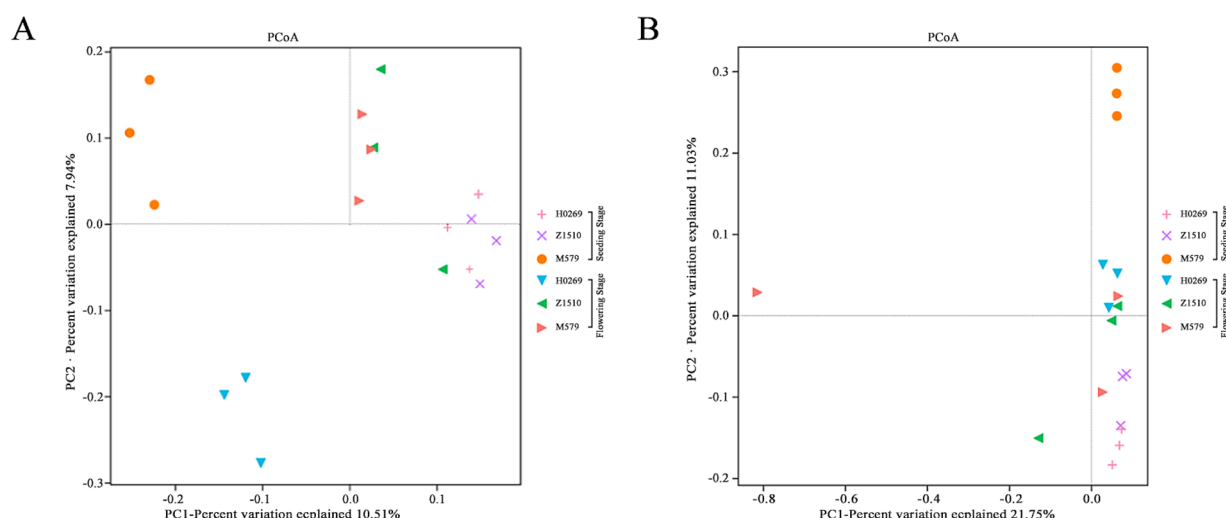

FIGURE 1
Principal component analysis based on the distance matrix calculated using the Bray−Curtis distance matrix for rhizosphere soil. The rhizosphere soil compartments cause the largest source of variance amongst bacterial **(A)** and fungal **(B)** communities of conventional soybean variety H0269 and genetically modified soybeans Z1510 and M579 at seeding stage and flowering stage.
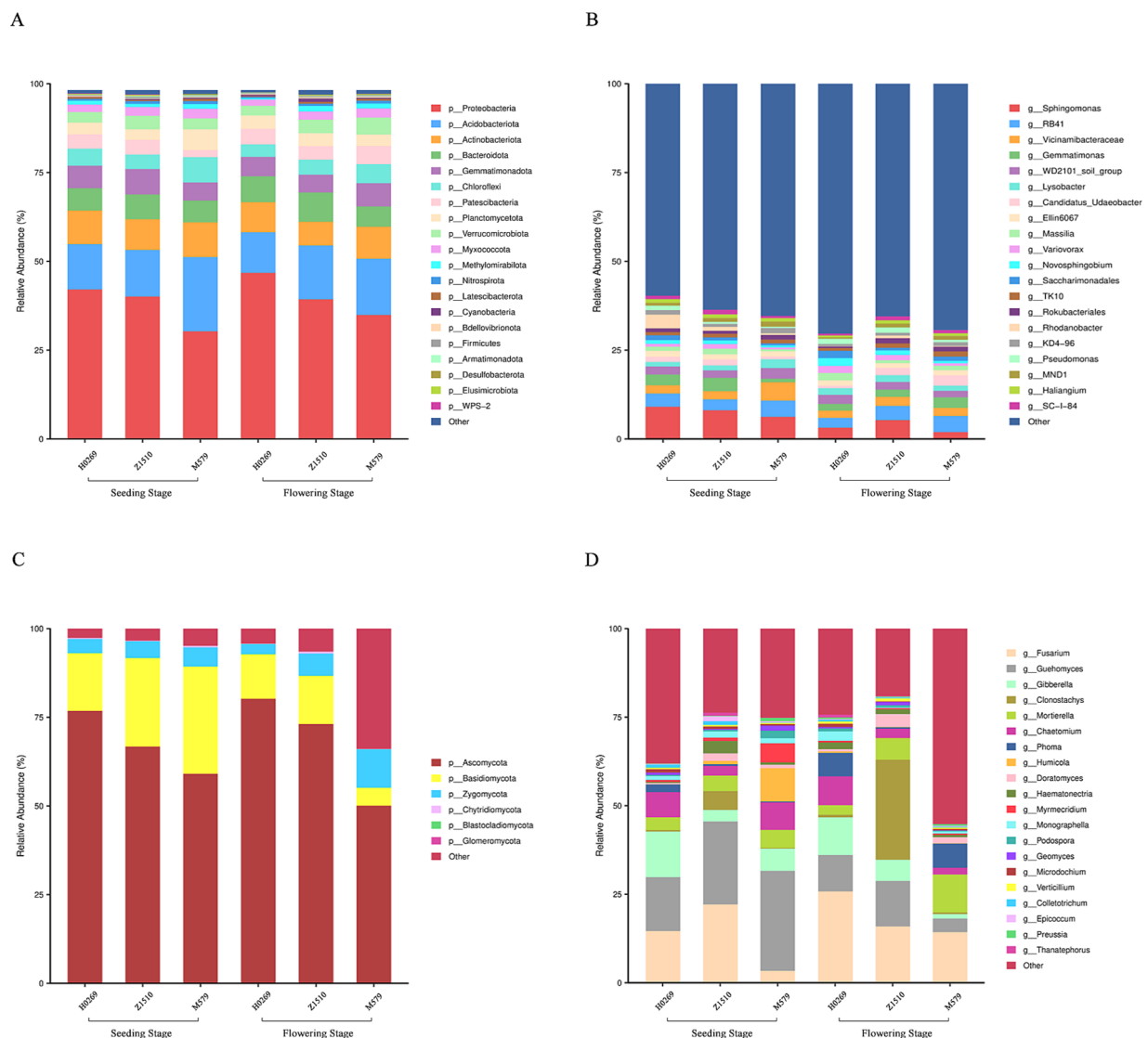
FIGURE 2
Composition and structure of bacterial and fungal communities of conventional soybean variety H0269 and genetically modified soybeans Z1510 and M579 at seeding stage and flowering stage. **(A,B)** Represent phylum and genus level of bacteria, respectively. **(C,D)** Represent phylum and genus level of fungi, respectively. The top 20 relative abundances are shown, and the remaining abundances are indicated as 'Others'.

(48 species), followed by Z1510 (21 species), and H0269 (20 species). M579 was enriched with taxa such as *P_Acidobacteria*, *c_Vicinamibacteria*, and *P_Chloroflexi*. H0269 had *o-Xanthomonaadales*, *f_Rhodanobaceae*, and *d_Rhodanobacte*; and Z1510 had *o-Gemmatimonadales* and related taxa. During flowering stage, the total number of bacterial species decreased to 51, with H0269 recruiting 25 species, and both Z1510 and M579 recruiting 13 species (Figures 3A,B). H0269 showed enrichment in *c_Alphasproteobacteria* and *c_Actinobacteria*. Z1510 in *o_Burkholderiales* and *f_Comamonadaceae*, and M579 in *f_Oxalobacteraceae* and related taxa. From seedling to flowering stage, M579 experienced a substantial decrease of 33 bacterial species, Z1510 decreased by 8, while H0269 increased by 5.

Similarly, LEfSe analysis of rhizosphere fungi indicated significant differences in functional fungi recruitment between growth stages. In the seedling stage, a total of 67 fungal species were recruited: M579 recruited 36 species, Z1510 recruited 18 species, and H0269 recruited

13 species. During the flowering stage, this number dropped to 25 fungal species overall, with H0269 recruiting 12 species, M579 recruiting 8 species, and Z1510 recruiting 5 species. From seedling to flowering stages, M579 saw a decrease of 28 fungal species, Z1510 decreased by 13, and H0269 decreased by 6. Fungal taxa with LDA scores greater than 5 included *f_Nutriaceae* in H0269, *o_Hypocrales* and *s_Clonostachys_rasea_f_catenulata* in Z1510, and *d_Ratobasidium* and *s_Ratobasidium-ramicola* in M579 (Figures 3C,D).

3.6 Comparative analysis of soybean yield traits and rhizosphere microbial communities

Yield trait analysis across three soybean varieties revealed a consistent pattern, with Z1510 > M579 > H0269 for theoretical
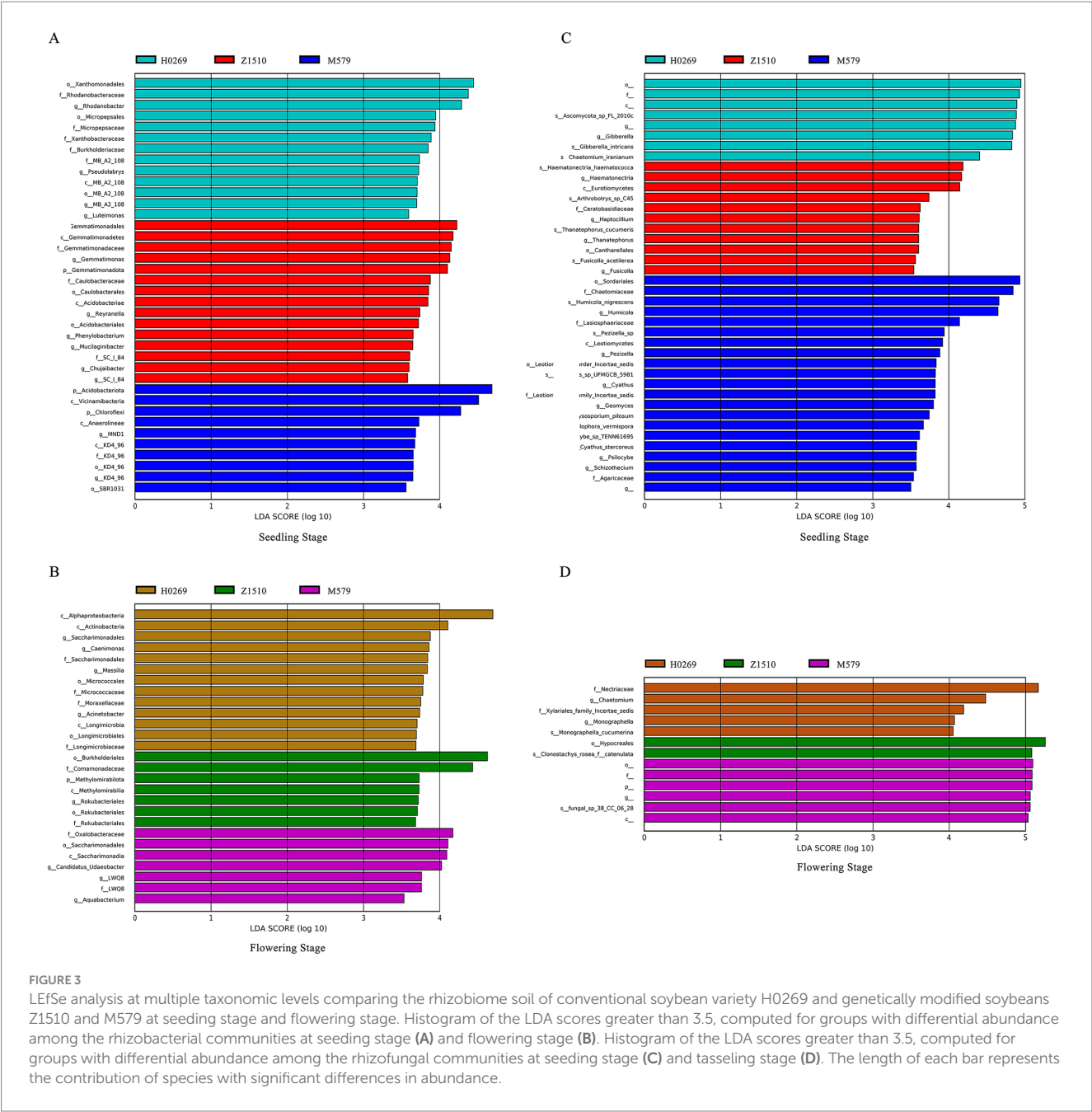
FIGURE 3
LEfSe analysis at multiple taxonomic levels comparing the rhizobiome soil of conventional soybean variety H0269 and genetically modified soybeans Z1510 and M579 at seeding stage and flowering stage. Histogram of the LDA scores greater than 3.5, computed for groups with differential abundance among the rhizobacterial communities at seeding stage **(A)** and flowering stage **(B)**. Histogram of the LDA scores greater than 3.5, computed for groups with differential abundance among the rhizofungal communities at seeding stage **(C)** and tasseling stage **(D)**. The length of each bar represents the contribution of species with significant differences in abundance.

TABLE 2 Production characteristics across three soybean varieties.

Sample	Plants per square meter	Number of grains per plant	Hundred grain weight (g)	Moisture Content (%)	Theoretical yield (kg/hm²)
Z1510	24.80 ± 0.91	118.33 ± 4.78[A]	13.38 ± 0.47[B]	10.42 ± 0.92[b]	4322.7 ± 83.85[A]
M579	23.20 ± 2.20	85.95 ± 4.90[B]	20.19 ± 0.43[A]	11.56 ± 0.47[a]	3995.55 ± 117.6[B]
H0269	23.8 ± 2.49	42.10 ± 5.09[C]	18.23 ± 2.02[A]	11.64 ± 0.98[a]	1800.9 ± 63.75[C]

A, B, and C denote highly significant differences at the $p = 0.01$ level; a, b, and c indicate significant differences at the $p = 0.05$ level; no label indicates no significant difference.

yield and grain number per plant. Z1510 showed superior yield indicators but lower 100-kernel weight and moisture content compared to M579 and H0269 (Table 2), suggesting a compensatory mechanism of increased grain number offsetting reduced grain weight.

The study examined relationships between rhizosphere microbes and five yield indicators: plant density, grains per plant, 100-grain weight, moisture content, and theoretical yield. The results revealed 15 bacterial and 13 fungal species exhibited significant associations with yield components. Among the bacteria, taxa such as *s_wastewater* metagenome
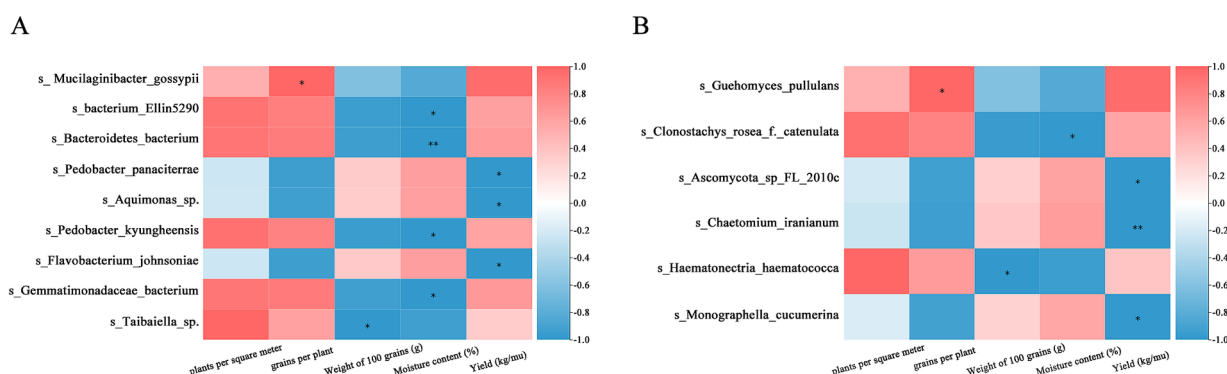
FIGURE 4
Correlation analysis between soybean rhizosphere microbial community and yield traits. **(A)** The rhizobacterial communities closely related to yield traits. **(B)** The rhizofungal communities closely related to yield traits.

and s_*Taibaiella* sp. demonstrated positive correlations with plant density but negative associations with grain weight. Conversely, species like *S_ Pedobacter panaciterrae* showed positive correlations with theoretical yield, grain weight, and water content (Figure 4A). Fungal species, including s_*Ascomycota* sp. *FL 2010c*, negatively correlated with grain number and yield per plant while positively associating with water content. Conversely, *S_Guehomyces pullulans* exhibited positive correlations with yield components but negative associations with water content (Figure 4B).

4 Discussion

This study analyzed rhizosphere microbial dynamics in Z1510, M579, and H0269 soybean varieties across growth stages. Bacterial communities were dominated by *Proteobacteria* (40–60%) showing positive correlation with plant development, consistent with global patterns observed in soybean rhizospheres from North American chernozem soils (Wang et al., 2024). The 18–25% reduction in nitrogen-fixing *Sphingomonas* abundance in Z1510 and M579 compared to H0269 aligns with genotype-driven microbiome variations reported in Brazilian GM soybean cultivars, where *Bradyrhizobium* dominance persisted despite genetic modifications (Shen et al., 2025). These findings underscore that genotype effects on microbial recruitment are conserved across continents, though regional soil properties (pH, salinity) may modulate their magnitude, as demonstrated in comparative U.S.–China trials (Wang et al., 2023; Wei et al., 2021).

Fungal communities comprised 50–80% *Ascomycota*, with *Guehomyces* increasing 30–40% during reproductive stages. Z1510 and M579 showed 35% greater microbial fluctuations across growth phases than the H0269 soybean variety. This genotype-driven bacterial diversity patterns (M579 > Z1510 > H0269) contrast with Liu et al.'s identification of stronger genotype-specific recruitment in wild-type cultivars (Liu et al., 2019), but support Yang et al.'s conclusion that environmental factors outweigh GM traits in shaping soybean microbiomes (Yang et al., 2022). However, the impact of GM soybeans on rhizosphere microbiomes appears subtle and context-dependent, influenced by factors such as growth stage and environmental conditions (Wang et al., 2023; Yang et al., 2022). Another study on glyphosate-tolerant soybeans observed transient reductions in

Bradyrhizobium abundance at the seedling stage but no long-term effects on microbial structure (Wei et al., 2021).

Fifteen bacterial and 13 fungal taxa correlated with yield traits, including *Mucilaginibacter gossypii*, *Bacterium Ellin5290*, *Guehomyces pullulans*, and *Doratomyces* sp. NG p07. Results suggest microbes influence yield through nitrogen cycling, water regulation, and plant density optimization. Soybean genetics impacted bacterial diversity twice as strongly as fungal diversity, aligning with Chang et al.'s demonstration of single-gene microbiome alterations, yet revealing broader genotype-level effects here (Chang et al., 2021). Simialy, high-yielding varieties showed rhizosphere microorganisms with higher activity and different carbon source utilization, suggesting superior recruitment and utilization of beneficial microbes, potentially enhancing nutrient absorption and yields (Popescu et al., 2022). Zheng et al. analyzed rhizosphere microbial community structures in three soybean genotypes (high oil, high protein, and high oil and high protein) further confirmed genotype-specific microbial communities and associated root characteristics (Zheng et al., 2019; Song et al., 2020).

While this field experiment provided valuable insights, further research was necessary to refine and expand upon these findings. These include addressing environmental variability, potential unaccounted microbial influences, and expanding the sample size to enhance statistical robustness. The single-site design (chernozem/ chestnut soils, pH 8.03) limited extrapolation to other agroecosystems, as environmental factors like soil type and regional climate influenced microbial recruitment. Future research should prioritize multi-location trials across soil types to assess microbiome stability, complemented by gnotobiotic systems validating causal relationships of key taxa (*Mucilaginibacter gossypii*). While identifying 15 bacterial and 13 fungal yield-associated taxa, taxonomic profiling through 16S/ITS sequencing captured microbial presence but not functional activity. The three-variety comparison with biological triplicates provides foundational insights, but expanded germplasm screening would better resolve subtle plant-microbiome interactions. Integration of metatranscriptomics could bridge taxonomic and functional analyses, while expanded sampling across growth stages would clarify temporal microbial dynamics.

In conclusion, this study illuminated the intricate interplay between soybean genotypes, developmental stages, and rhizosphere microbial communities. The findings advanced our understanding of plant-microbe interactions while revealing potential strategies for

enhancing soybean yield and promoting sustainable agriculture. As this field progresses, it holds promise for significantly improving global food security and agricultural sustainability, paving the way for more efficient and eco-friendly soybean cultivation practices.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

XH: Conceptualization, Investigation, Writing – original draft, Formal analysis, Supervision, Validation. XZ: Investigation, Software, Validation, Visualization, Writing – original draft. MS: Investigation, Validation, Visualization, Writing – original draft. SY: Data curation, Formal Analysis, Investigation, Writing – original draft. SF: Investigation, Visualization, Writing – original draft. TT: Investigation, Writing – original draft, Resources. NC: Investigation, Writing – original draft, Resources. GZ: Conceptualization, Data curation, Project administration, Supervision, Writing – original draft. LH: Project administration, Supervision, Investigation, Writing – original draft. GW: Data curation, Funding acquisition, Resources, Supervision, Writing – review & editing, Investigation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2025.1518597/full#supplementary-material

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