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Response of Maize Root Endophytes to Carbon-Nitrogen Coupling in the Irrigated Area of the West Liaohe Plain, Inner Mongolia

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Abstract

Based on a five-year positioning experiment conducted in the irrigated area of the West Liaohe Plain, this study employed a two-factor split-plot design. Two factors were set: the straw stover return rate (no stover return; half stover return, 4500 kg/ha; full stover return, 9000 kg/ha) and the nitrogen application rate (conventional nitrogen, 450 kg/ha; reduced nitrogen, 300 kg/ha), resulting in six treatment combinations: NN (no stover return + conventional nitrogen), NR (no stover return + reduced nitrogen), HN (half stover return + conventional nitrogen), HR (half stover return + reduced nitrogen), TN (full stover return + conventional nitrogen), and TR (full stover return + reduced nitrogen). Root tissue samples were collected at the R1 stage (silking) of maize for analysis of bacterial and fungal community structures. High-throughput sequencing of the 16S rRNA gene V5-V7 region and 18S rRNA gene ITS2 region, combined with Accu 16S™ and Accu ITS™ absolute quantification technologies for bacteria and fungi, was used to systematically investigate the diversity, community structure, functional potential, and coupling relationships with the soil

nutrient factors of maize root endophytes. The results revealed that the dominant bacterial phylum was Pseudomonadota, and the dominant genus was *Pseudomonas*. Unlike the NN treatment, which maintained relatively high bacterial diversity and complex structure, the NR treatment led to increased dominance of *Pseudomonas* and extreme community simplification, with high total fungal abundance but a uniform structure. In the HN treatment, the bacterial communities were dominated by Pseudomonadota, enriching the organic matter-degrading groups, whereas the fungal communities included opportunistic pathogens such as *Fusarium*. The HR treatment significantly enriched Bacteroidota, inhibited *Pseudomonas*, had the highest number of unique bacterial OTUs but decreased diversity, and it presented the strongest fungal heterogeneity, with an increase in functional groups such as *Mortierella*. The TN treatment had a conserved bacterial structure enriched with *Enterobacterales*, which presented the lowest total fungal abundance but was enriched with yeast-like groups. The TR treatment significantly inhibited *Pseudomonas*; increased the abundance of *Rhizobium*, *Agrobacterium*, and Actinomycetota; maintained high functional differentiation potential; and enriched *Mortierella* and *Penicillium* with a stable fungal structure. The dominant fungal phylum was Ascomycota. Under reduced nitrogen, stover return amplified community differentiation and increased the contribution of *Mortierella*. A comprehensive correlation analysis revealed that alkali-hydrolyzable nitrogen and available potassium were the core environmental factors driving changes in the root endophytic microbial community structure. The TR treatment was more conducive to constructing a highly functionally diverse community with both nitrogen fixation and organic matter transformation capabilities, providing a microbiological basis for the synergistic management of maize stover utilization

and nitrogen fertilizer reduction in the irrigated area of the West Liaohe Plain.

Keywords: Maize root endophytes; Stover return; Nitrogen fertilizer; Silking stage; Relative quantification; Absolute quantification

1 Introduction

The sustainable development of agroecosystems relies on the coordination of efficient resource utilization and environmental carrying capacity (Anita Kumawat et al., 2023; Fuki Fujiwara et al., 2023). Stover return, as a key measure for enhancing soil organic matter (Yang et al., 2023), improving the soil structure (Zhang et al., 2024), and strengthening the soil carbon sequestration capacity (Lei et al., 2025), together with optimized nitrogen fertilizer management, constitute a core strategy for the green transformation of agricultural production (Zhang et al., 2023). Their combined application drives the structural reorganization and functional evolution of soil microbial communities (Liu et al., 2023) by altering the distribution pattern and stoichiometric characteristics of carbon and nitrogen resources in the rhizosphere microenvironment (Song et al., 2024), subsequently affecting soil nutrient transformation efficiency and crop productivity (Yang et al., 2024).

Maize (*Zea mays* L.) is a major cereal crop widely cultivated globally (Liu et al., 2020), and its root-associated microbiome plays a crucial role in regulating nutrient uptake, promoting crop growth, and enhancing stress resistance (Kour et al., 2023; Wasim Khan et al., 2024). Stover return can promote microbial community assembly, enhance nutrient cycling, and improve soil fertility and functional stability (Liu et al., 2023), whereas its absence adversely affects soil quality, crop yield, and the environment (Du et al., 2022). Therefore, stover return is considered an important measure for improving soil health (Li et al., 2024)

and agroecosystem function (Zhang et al., 2022). On the other hand, although increasing nitrogen fertilizer application is a common practice to increase maize yield (Coelho et al., 2020), excessive nitrogen application (S Sela et al., 2018) and its low utilization efficiency (Li et al., 2010) have led to issues such as soil acidification (Kazem Zamanian et al., 2024), environmental pollution (Zhou et al., 2017), and reduced microbial activity (Renpeng Sun et al., 2019; Lian et al., 2024). Scientifically reducing nitrogen fertilizer application and determining appropriate nitrogen levels are crucial for maintaining root-associated microbial diversity and soil health (Zou et al., 2024).

Research on the interactive effects of stover return and nitrogen fertilizer on maize root-associated microbial communities is insufficient. Many studies focus solely on the overall microbial community of the plough layer (Soheila Lahijanian et al., 2025), overlooking the key functional component of root-colonizing microorganisms that directly interact with plants (Muhammad Saleem et al., 2018), and lack in-depth analysis of their spatial heterogeneity. Furthermore, existing studies often fail to systematically quantify the interaction effects between stover return gradients and nitrogen fertilizer levels, making it difficult to clarify the precise regulatory thresholds of carbon and nitrogen resource inputs on microbiome assembly (Yuan et al., 2022; Zhang et al., 2023). Methodologically, traditional relative abundance-based analyses may mask real changes in the absolute abundance of key taxa due to fluctuations in the total microbial load across samples, thereby limiting accurate assessment of microbial functional potential (Catherine Preece et al., 2019; Azarbad Hamed et al., 2022). In recent years, the application of spike-in absolute quantification technology has provided an effective means to overcome this limitation, enabling a more accurate understanding of the true

response patterns of microbial communities to environmental drivers by simultaneously obtaining both relative and absolute abundance information.

Synergistically regulating the coupled carbon and nitrogen processes in farmlands through stover return and nitrogen fertilizer reduction has become an important pathway for enhancing soil health, stabilizing productivity, and reducing environmental pollution. Recent comprehensive multiscale studies have shown that stover input can reshape functional microbial communities and enhance soil multifunctionality by altering soil carbon pool components and enzyme activities (Zhang et al., 2024; Yan et al., 2026), but this effect is significantly constrained by stover return intensity and nutrient ratios. Global meta-analyses further indicate that stover return has directional effects on bacterial and fungal diversity and functional profiles, as stover return affects the duration of the process and the associated fertilization regimes (Bai et al., 2018; Zhou et al., 2016). Moreover, moderate nitrogen reduction combined with stover return has been repeatedly demonstrated to improve nitrogen use efficiency and reduce nitrogen surplus risk while maintaining or enhancing soil function (Fu Liu et al., 2025; Yao et al., 2017), suggesting that the carbon supply and inorganic nitrogen constraints jointly determine microbial nutritional strategies and niche differentiation, thereby influencing nutrient transformation pathways (Xu et al., 2021). However, current research still focuses primarily on bulk or rhizosphere soils, with insufficient attention given to plant root endophytes—a key functional pool directly involved in host nutrient acquisition and stress regulation. Bacteria and fungi, as the two core groups of root endophytes, exhibit distinct ecological strategies and functional roles. Bacterial communities typically respond more rapidly to resource fluctuations, whereas fungal

communities present greater compositional plasticity and structural stability; the synergistic response mechanisms of these two groups remain to be systematically elucidated.

On this basis, a field experiment with a factorial design of stover return gradients and nitrogen application levels was established in a typical irrigated maize system. Root samples were systematically collected during the silking stage (R1), a critical period for determining root physiological activity and plant–microbe interactions. By integrating 16S rRNA and ITS amplicon sequencing with spike-in absolute quantification technology, this study systematically analysed the interactive effects of stover return and nitrogen fertilizer reduction on the structure, diversity, and key species abundance of maize root endophytic bacterial and fungal communities in terms of both relative proportion and absolute quantity. This study also revealed potential functional differentiation driven by community changes and identified the core environmental factors driving these microbial changes, providing a microbiological theoretical basis for stover utilization and precise nitrogen management in ecologically intensive maize cultivation on the West Liaohe Plain and similar ecological regions.

2 Results and Analysis

2.1 Relative Abundance and Distribution Characteristics of Maize Root-Endophytic Bacteria

The results of the relative quantitative analysis of the bacterial communities (Fig. 1a) revealed that the number of shared OTUs across the treatments was 268, while the number of unique OTUs followed the order HR > NN > TR > HN > TN > NR. Under conventional nitrogen application, the no stover return treatment (NN) resulted in the greatest number of unique OTUs and the most complex community structure; the stover return treatments (HN

and TN) tended toward community conservation. Under reduced nitrogen application, the full stover return treatment (HR) had the greatest number of unique OTUs and the greatest community uniqueness; the no stover return treatment (NR) was the most simplified. At the bacterial phylum level (Fig. 1b), Pseudomonadota was the dominant phylum (59.45–80.1%), with its relative abundance being greater in the NR, HN, and TN than in the HR. Bacteroidota and Bacillota were the second most dominant phyla. The abundance of Bacteroidota was enriched in HR and NN, whereas Bacillota had the highest relative abundance in NN (19.63%). The abundance of Actinomycetota was relatively high in HR and TR. HR specifically revealed the presence of Planctomycetota, Acidobacteriota, and Chloroflexota. Under conventional nitrogen application, NNs presented lower Pseudomonadota abundance but higher Bacillota abundance; both HNs and TNs presented high Pseudomonadota abundance, although Actinomycetota abundance was lower in TNs than in HNs. Under reduced nitrogen application, full stover application resulted in a smaller decrease in Pseudomonadota abundance, whereas no stover return led to an increase in its abundance; stover return increased Bacillota competition and increased Actinomycetota abundance; HR resulted in a significant increase in Bacteroidota and rare taxa, with Chloroflexota increasing significantly only in this treatment, suggesting potential functional substitution. At the bacterial genus level (Fig. 1c), *Pseudomonas* was the dominant genus (15.19–50.62%), with relatively high relative abundances in NR, TN, and TR and significantly low abundances in HR; *Priestia* had relatively high abundances in NN and TR; and *Streptomyces* was relatively enriched in HR, TR, and NR. Under conventional nitrogen application, NN had lower *Pseudomonas* but higher *Priestia* and *Rhizobium*; *Pseudomonas* dominated in HN. Under

reduced nitrogen application, *Pseudomonas* was absolutely dominant in NR, leading to
 community simplification; *Pseudomonas* was inhibited in HR, while the diversity of rare taxa
 such as *Streptomyces* increased; TR maintained a relatively high abundance of *Pseudomonas*
 and enriched *Streptomyces* and *Rhizobium*. Stover return promoted the enrichment of
Streptomyces and rhizobium-related genera, with the abundances of genera such as
Flavobacterium and *Chitinophagaceae* also increasing in the HR. Analysis of the diversity
 indices (Fig. 1d) revealed that the observed richness indices followed the order $HR > NN >$
 $TR > HN > NR > TN$, with the HR being significantly greater than that in the other treatments
 in most cases. The Shannon index followed the same order, whereas the Simpson index
 showed the opposite trend (highest in TN). The coverage indices were close to 1, indicating
 sufficient sequencing depth. PCoA based on weighted UniFrac distance (Fig. 1e) indicated
 that PC1 and PC2 explained 42.03% and 14.36% of the variation, respectively. HN and HR
 clustered on the positive side of PC1, showing similar community structures; NN, NR, and
 TN were distributed on the negative side of PC1, with TN showing a significant shift on PC2;
 TR partially clustered with the half stover returning treatments, indicating influence by
 common environmental factors.

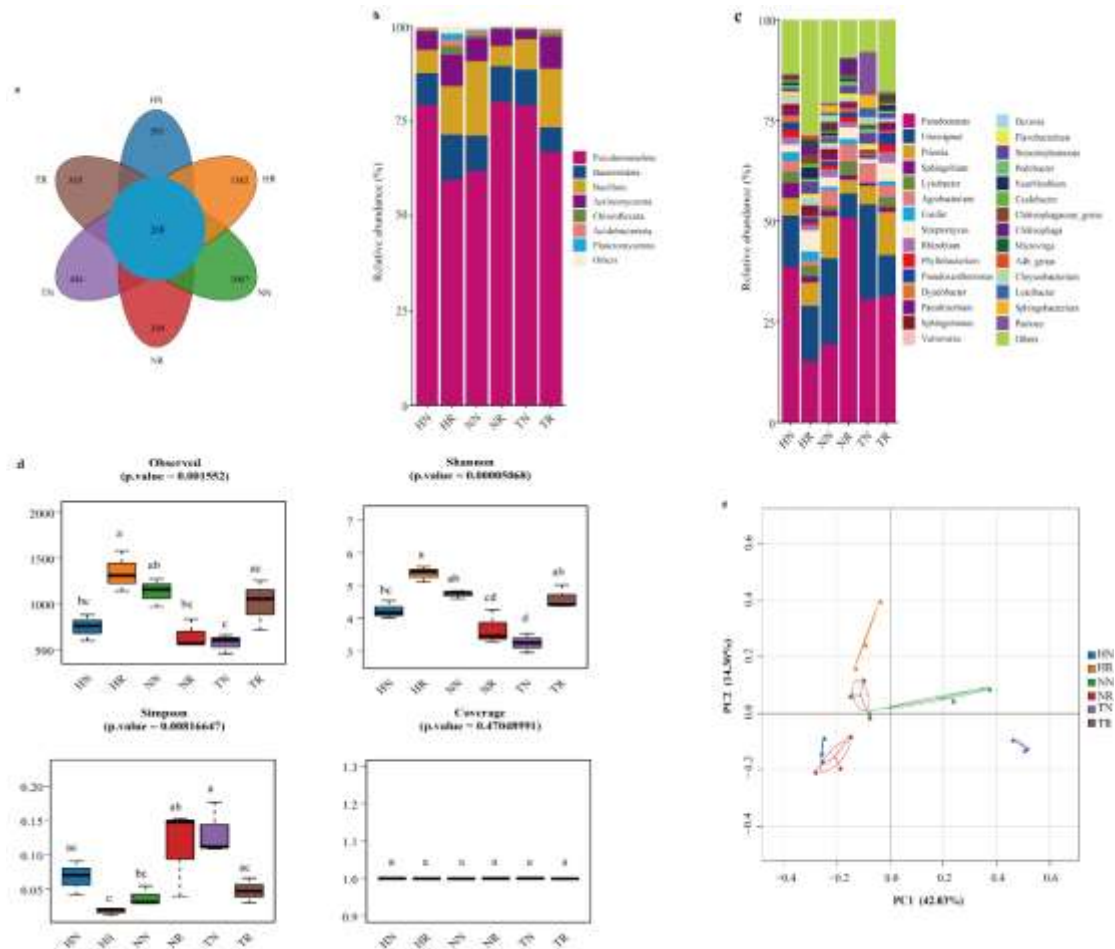


Figure 1 Community structure of maize root endophytic bacteria under different nitrogen application and stover return treatments

Note: a. OTU Venn diagram of bacterial communities based on relative quantification (relative quantification); b. Relative abundance of bacterial communities at the phylum level; c. Relative abundance of bacterial communities at the genus level; d. α diversity indices; e. PCoA based on weighted UniFrac distance (relative quantification). In panel a, each color represents a treatment group, overlapping areas indicate shared OTUs, and nonoverlapping areas represent unique OTUs for each treatment group. In Panel e, each point represents a sample, the colors correspond to the treatment groups, and the distances between points reflect community similarity.

2.2 Relative Abundance and Distribution Characteristics of Maize Root Endophytic Fungi

OTU-level Venn analysis (Fig. 2a) revealed that 74 OTUs were shared across treatments. The number of unique OTUs varied significantly among the treatments, following the order HR (312) > TR (240) > NN (188) > TN (172) > HN (157) > NR (152). The combination of reduced nitrogen and stover application significantly increased fungal community heterogeneity. At the fungal phylum level (Fig. 2b), the fungal community was mainly composed of Ascomycota, Mucoromycota, Mortierellomycota, and Basidiomycota, with Ascomycota being the dominant phylum across all the treatments. The relative abundance of Ascomycota varied among the treatments, remaining high in HN, HR, NR, and TR but significantly lower in TN. Mucoromycota was relatively enriched in NN and NR, Mortierellomycota maintained a relatively high abundance in TN and TR, and Basidiomycota increased relatively in HR and TR. Additionally, low-abundance phyla such as Chytridiomycota, Glomeromycota, and Rozellomycota were detected in HR and TR. At the fungal genus level (Fig. 2c), the community consisted of a few dominant genera and a large number of low-abundance genera, with *Fusarium*, *Actinomucor*, *Mucor*, *Rhizopus*, and *Mortierella* being the dominant genera. Under conventional nitrogen application, the NN treatment was dominated by genera such as *Actinomucor* and *Mucor*, with a relatively concentrated community structure; the HN treatment had a relatively high abundance of *Fusarium*. Under reduced nitrogen application, the dominant genera in the NR treatment were highly concentrated, with significant community simplification; in contrast, the HR treatment resulted in increased proportions of *Mortierella*, *Podospora*, *Didymella*, and other

nondominant and rare taxa, increasing community evenness. The TR treatment maintained a certain proportion of dominant genera while enriching with functional fungi such as *Mortierella* and *Penicillium*. α -Diversity analysis (Fig. 2d) revealed no significant differences in the observed indices among the treatments ($P = 0.2566$); the Shannon and Simpson indices also showed no significant differences ($P = 0.3277$ and 0.4370 , respectively). The coverage indices were close to 1, indicating sufficient sequencing depth to comprehensively reflect the composition characteristics of the fungal community. PCoA revealed significant differences in fungal community structure among the treatments (Fig. 2e). PC1 and PC2 explained 26.23% and 15.31% of the community variation, respectively. The HR and TR treatments deviated significantly from the other treatments in terms of ordination space and presented greater within-group dispersion. In contrast, the HN and NN treatments were relatively similar in terms of the PCoA space; the NR and TN treatments were more concentrated, with relatively stable community structures but limited diversity potential. Overall, the results indicate that reduced nitrogen is the primary factor driving differentiation in fungal community structure, whereas stover return significantly amplifies this ecological effect under reduced nitrogen conditions.

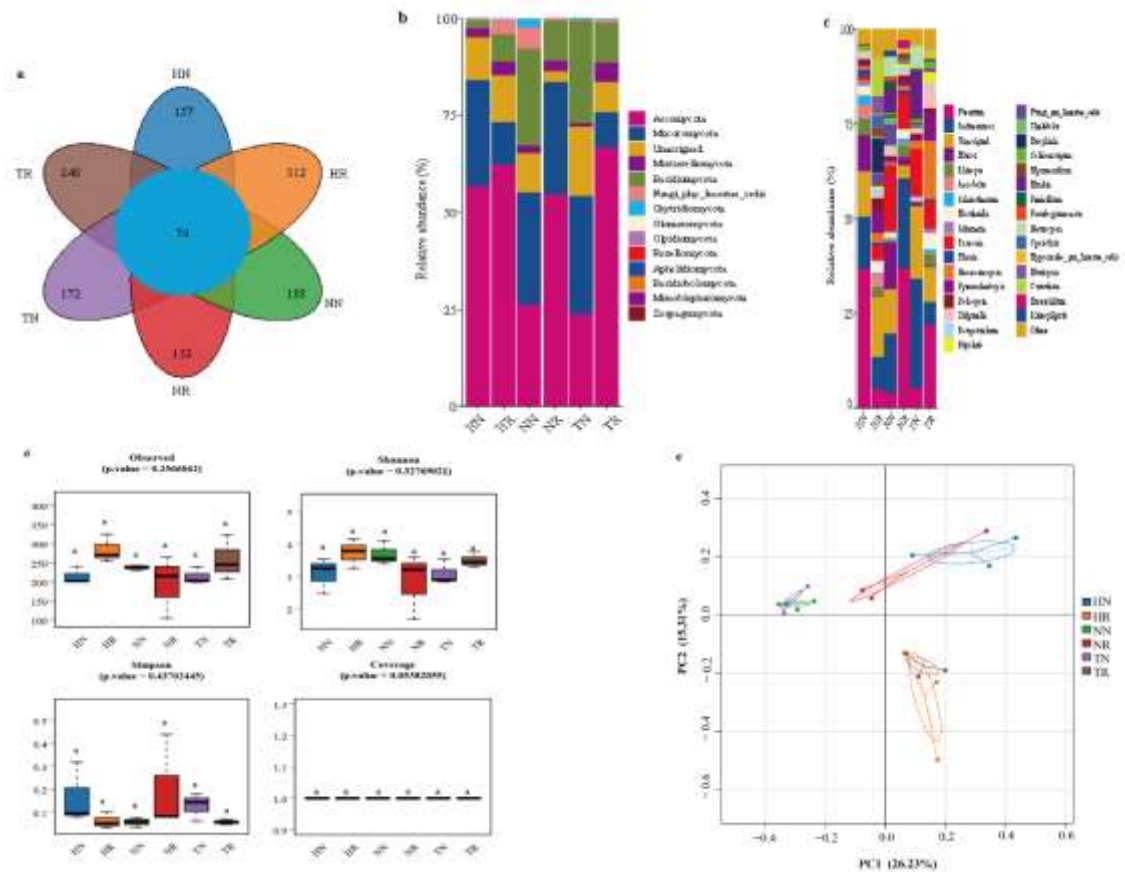


Figure 2 Community structure of maize root endophytic fungi under different nitrogen application and stover return treatments

a. OTU Venn diagram of fungal communities based on relative quantification; b.

Relative abundance of fungal communities at the phylum level; c. Relative abundance of

fungal communities at the genus level; d. α diversity indices (based on relative abundance); e.

PCoA based on weighted UniFrac distance (based on relative abundance).

2.3 Absolute Abundance and Distribution Characteristics of Maize

Root Endophytic Bacteria

The results of the absolute quantification analysis were highly consistent with the relative quantification results in terms of the number of shared OTUs (272), the ranking of unique OTUs, and the diversity indices (HR > NN > TR > HN > NR > TN) (Fig. 3a).

However, regarding subtle community composition, absolute quantification further revealed that the TR treatment maintained a relatively high total OTU count despite having a moderate number of unique OTUs and exhibited a pattern of balanced coexistence between Actinomycetota (7.7%) and Bacteroidota (7.78%). At the phylum level (Fig. 3b), Pseudomonadota remained the dominant phylum (63.96% – 80.5%), but the peak of Bacillota in NN was more pronounced (8.59%), and the specific enrichment of Planctomycetota (3.37%) and Chloroflexota (3.81%) in HR was more clearly defined. At the genus level (Fig. 3c), absolute quantification revealed 29 taxa, confirming that *Priestia* (5.01%) and *Agrobacterium* (5.62%) were relatively highly abundant in NN, whereas the absolute dominance of *Pseudomonas* (36.19%) in NR under reduced nitrogen highlighted community simplification. Moreover, the increased abundance of rare taxa such as *Ensifer* (1.97%) in the HR was more quantitatively characterized. α -Diversity analysis (Fig. 3d) revealed no significant differences among treatments for the observed, Shannon, and Simpson indices ($P > 0.05$), with trends consistent with the relative abundance. PCoA revealed that while the overall distribution trend among the treatments was similar to that of the relative quantification, the explained contributions of PC1 and PC2 were adjusted to 34.18% and 15.09%, respectively, with an increased distance between HN and HR on PC2, and TR exhibited greater independence in the ordination plot (Fig. 3e).

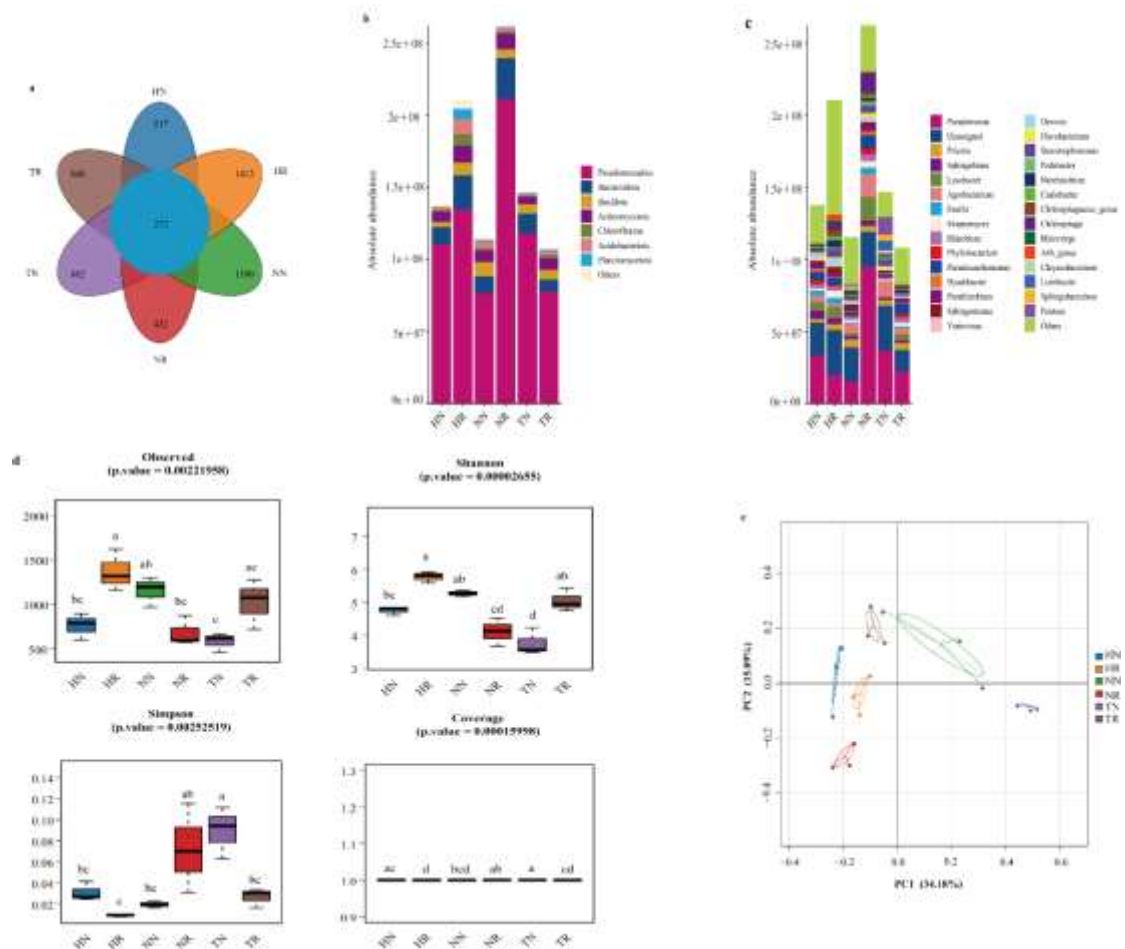


Figure 3 Community structure of maize root endophytic bacteria under different nitrogen application and stover return treatments (absolute quantification)

a. OTU Venn diagram of bacterial communities based on absolute quantification; b. absolute abundance of bacterial communities at the phylum level; c. absolute abundance of bacterial communities at the genus level; d. α -diversity indices (based on absolute abundance); e. PCoA based on weighted UniFrac distance (based on absolute abundance).

The legend details are the same as those in Figure 1.

2.4 Absolute Abundance and Distribution Characteristics of Maize Root Endophytic Fungi

OTU-level Venn analysis (Fig. 4a) revealed that the number of shared OTUs across

261 treatments was consistent with the relative abundance results (74). However, the ranking of
262 unique OTU numbers was adjusted to HR (294) > TR (223) > TN (171) > NN (166) > NR
263 (151) > HN (138). In terms of absolute abundance at the phylum level (Fig. 4b), Ascomycota
264 remained the dominant group driving changes in community abundance. The NR treatment
265 had the highest total absolute abundance, which was attributed mainly to the simultaneous
266 increase in Ascomycota and Mucoromycota, whereas the TN treatment had the lowest total
267 abundance, with a notable decrease in Ascomycota. Additionally, Mortierellomycota and
268 Basidiomycota maintained relatively high levels in the HR and TR. At the genus level (Fig.
269 4c), the total absolute abundance of fungi and the abundance of dominant groups such as
270 *Fusarium* and *Actinomucor* were highest in the NR treatment. Under conventional nitrogen
271 application, the distribution of the dominant genera in NN and HN was relatively balanced,
272 while the absolute abundance of most genera was low in TN. The HR and TR treatments,
273 while increasing the total abundance, also increased the absolute abundance of fungi such as
274 *Mortierella*, *Podospora*, *Didymella*, and *Penicillium*. α -Diversity analysis (Fig. 4d) revealed
275 no significant differences among treatments for the observed, Shannon, and Simpson indices
276 ($P > 0.05$), with trends consistent with the relative abundance. PCoA based on the Bray–
277 Curtis distance (Fig. 4e) revealed significant differences in the absolute abundance structure
278 among the treatments, with PC1 and PC2 explaining 20.47% and 13.43% of the variation,
279 respectively. Unlike the relative abundance results, the NR treatment resulted in a distinct
280 separation along the PC1 axis in the ordination plot, whereas the conventional nitrogen
281 application group was more concentrated.

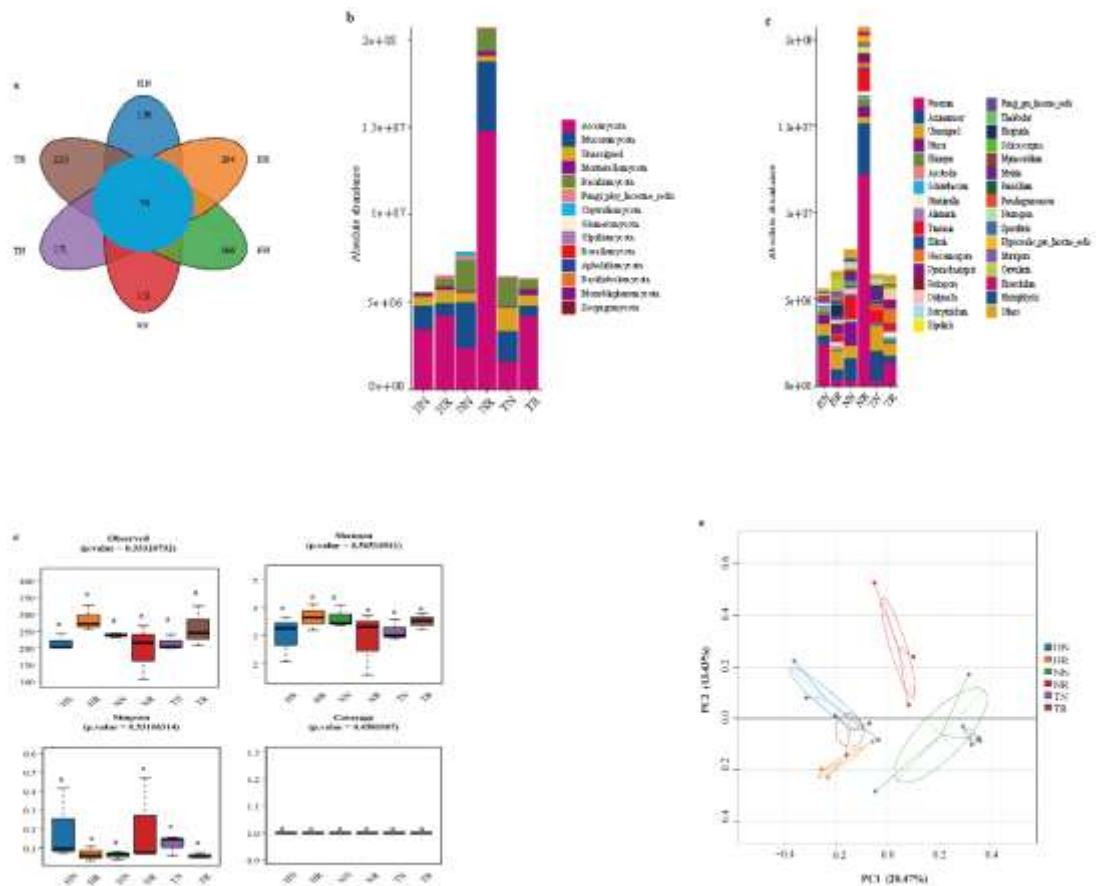


Figure 4 Community structure of maize root endophytic fungi under different nitrogen application and stover return treatments (absolute quantification)

a. OTU Venn diagram of fungal communities based on absolute quantification; b. absolute abundance of fungal communities at the phylum level; c. absolute abundance of fungal communities at the genus level; d. α -diversity indices (based on absolute abundance); e. PCoA based on Bray–Curtis distance (based on absolute abundance). The legend details are the same as those in Figure 1.

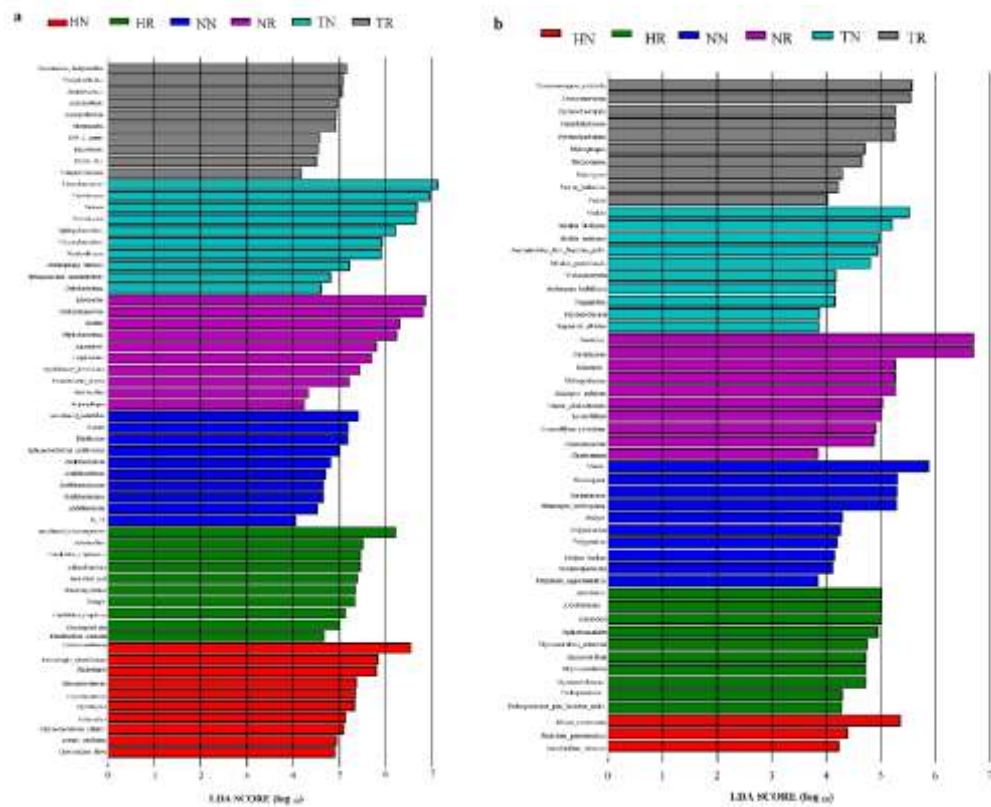
2.5 Differential Taxa and Functional Potential Analyses of Bacteria and Fungi

The LEfSe analysis results shown in Figure 5a indicate significant differences in the indicator taxa of the root endophytic bacterial communities among the treatments. The NN

treatment significantly enriched taxa such as *Abditibacteriota*, *Abditibacteriales*,
Abditibacteriaceae, and *Abditibacterium*. The HN treatment enriched *Comamonadaceae*,
Glycomycetaceae, and related taxa such as *Glycomycetales* and *Glycomyces*. The TR
 treatment significantly enriched taxa closely related to nitrogen cycling, such as
Azospirillales, *Azospirillaceae*, and *Rhizobiales*. In contrast, the HR treatment enriched
 mainly *Planctomycetales*, *Dongia*, and various low-abundance taxa. The TN treatment
 specifically recruited taxa such as *Enterobacterales*, *Yersiniaceae*, *Pantoea*, and
Sphingobacterium, with *Pantoea* having potential nitrogen-fixing capacity. The NR treatment
 enriched mainly *Lysobacter*, *Chitinophagaceae*, *Ensifer*, *Phyllobacterium*, and *Agromyces*,
 which are associated with antagonism or plant growth promotion. The LEfSe analysis results
 shown in Figure 5b revealed significant differences in the root endophytic fungal
 communities among the treatments. The TR treatment enriched mainly *Neocosmospora*,
Rubicola, *Pyrenochaetopsis*, and their affiliated family *Cucurbitariaceae*. The TN treatment
 significantly enriched yeast-like fungi such as *Mrakia* (including several species),
Vishniacozyma, *Naganishia*, and *Filobasidiaceae*. The NR treatment enriched mainly
Fusarium, *Nectriaceae*, *Rhizopus*, and *Chaetomiaceae*. The NN treatment significantly
 enriched taxa with strong decomposition capacity, such as *Neurospora*, *Sordariaceae*,
Polyporales, and *Picipes*. The HR treatment enriched mainly *Ascobolus*, *Ascobolaceae*,
Myrmecridium, and related taxa. The HN treatment specifically enriched *Mucor_racemosus*,
Fusarium_graminearum, and *Sarocladium_strictum*.

The fungal cladogram shows that differential features were distributed across multiple
 higher taxonomic levels, suggesting that community structure is driven by both environmental

filtering and habitat interactions. Differential nodes covered Ascomycota-related groups such as *Eurotiomycetes* and *Hypocreales* and, at the genus level, included *Fusarium*, *Neocosmospora*, *Albifimbria*, and *Melanospora*, indicating that saprotrophic and potentially plant-associated fungi occupy key ecological niches in different groups. The Basidiomycota-related lineages included *Agaricomycetes*, *Tremellomycetes*, *Polyporales*, and *Picipes*, along with *Mrakia*, *Tausonia*, *Naganishia*, and *Vishniacozyma* (yeast-like groups), suggesting that the enrichment of lignin-degrading and environmentally adaptive fungi is group specific.



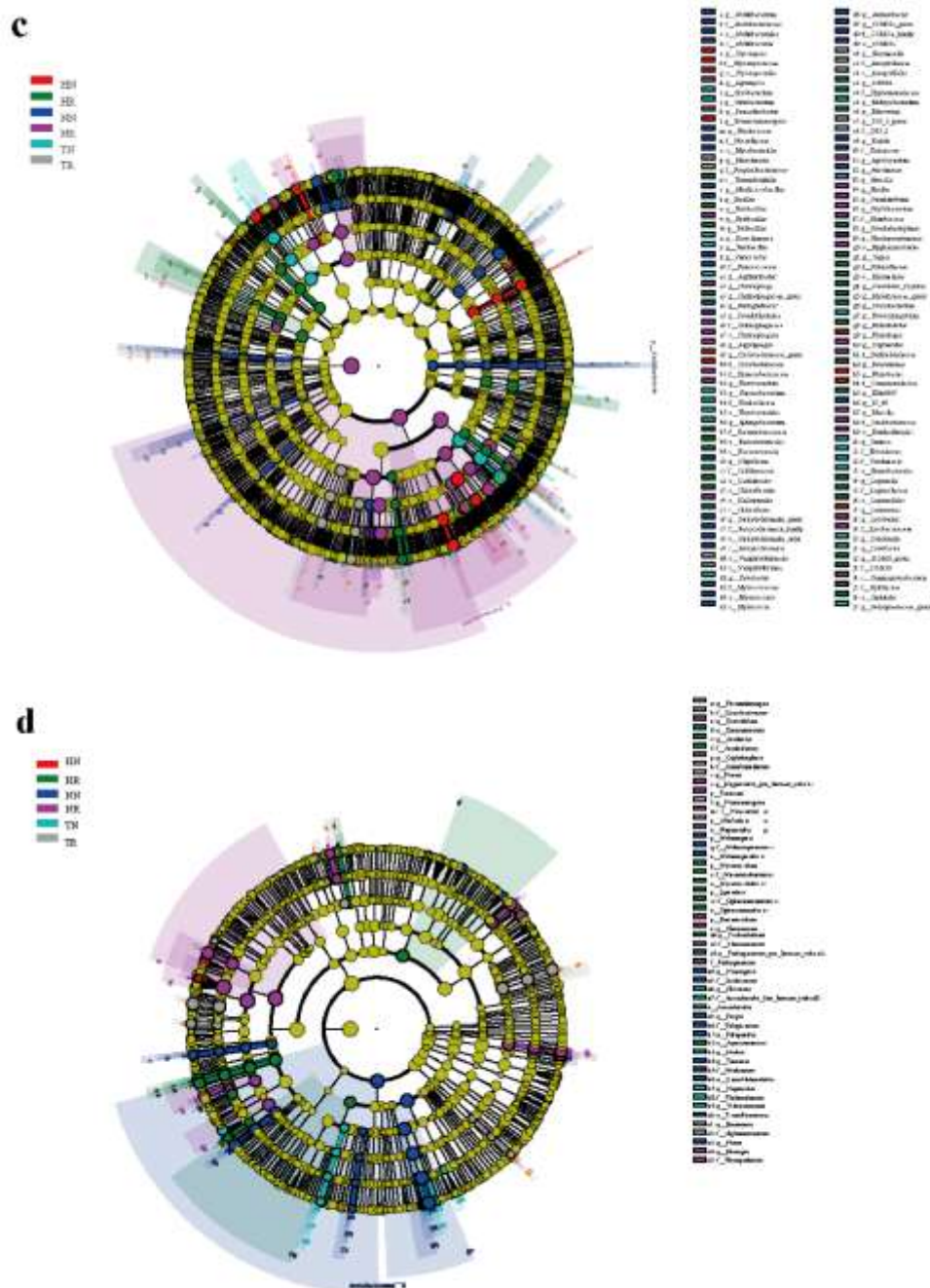


Figure 5 LEfSe analysis of maize root endophytic bacteria (a) and fungi (b) based on absolute quantification and corresponding cladograms for bacteria (c) and fungi (d)

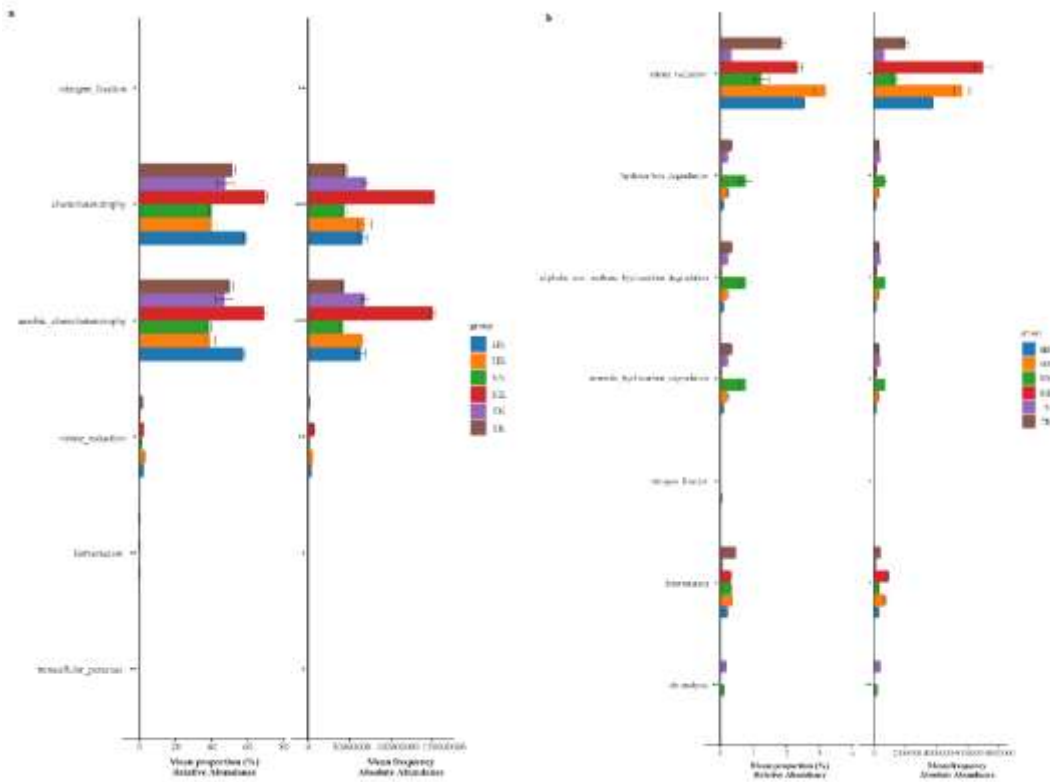
Note: Different colors represent different treatments. Red nodes in the cladogram indicate species with significantly greater abundance in the red group, green nodes indicate species with significantly greater abundance in the green group, and yellow nodes indicate

species with no significant differences in abundance between groups. The node diameter is proportional to the abundance. Nodes from the inner to outer layers represent phylum, class, order, family, genus, and species. Annotations for each layer from outer to inner represent phylum, class, order, family, genus, and species; phylum-level annotations are shown on the outermost ring, whereas annotations for other levels are shown in the legend on the right.

The ANOVA results (Fig. 6a) revealed that chemoheterotrophy and aerobic chemoheterotrophy were the core functional groups in the community. The relative and absolute abundances of these genes were highest in NR ($P < 0.05$). Additionally, functional categories such as nitrogen fixation ($P < 0.05$), fermentation ($P < 0.01$), and intracellular parasites ($P < 0.01$) also reached significant levels among the treatment groups. The results of the Kruskal–Wallis tests further confirmed (Fig. 6b) that nitrate reduction ($P < 0.05$) was significantly enriched in NR, whereas carbon metabolism-related functions such as hydrocarbon degradation, aliphatic nonmethane hydrocarbon degradation, and aromatic hydrocarbon degradation were relatively active in NN ($P < 0.05$). Chitinolysis highly significantly affected TN ($P < 0.01$). These results indicate that the NR treatment significantly enhances bacterial heterotrophic metabolism and nitrogen cycling potential, potentially playing an important role in soil organic matter dynamics.

Figure 6c shows that different treatments significantly regulated the relative abundance of major fungal functional groups. The soil saprotroph-undefined saprotroph was the dominant functional group in the community, particularly in TN, where its average relative abundance was significantly greater than that in the other treatments ($P < 0.01$). Additionally, the relative abundance of the plant pathogen wood saprotroph was significantly enriched in

the TRs, with a significant increasing trend ($P < 0.01$). In terms of absolute abundance, the results of the Kruskal–Wallis tests further confirmed the significant differences in community functions (Fig. 6d). The results indicated that functional groups such as plant pathogen–wood saprotrophs and animal pathogen–plant pathogens significantly differed among the treatments ($P < 0.05$). Specifically, in the TR treatment, the mean frequency of plant pathogen–wood saprotrophs peaked, which was significantly greater than that in HN and NN. Moreover, minor functional groups, such as fungal parasites, soil saprotrophs and wood saprotrophs, also presented sensitive responses to environmental disturbances in terms of absolute abundance.



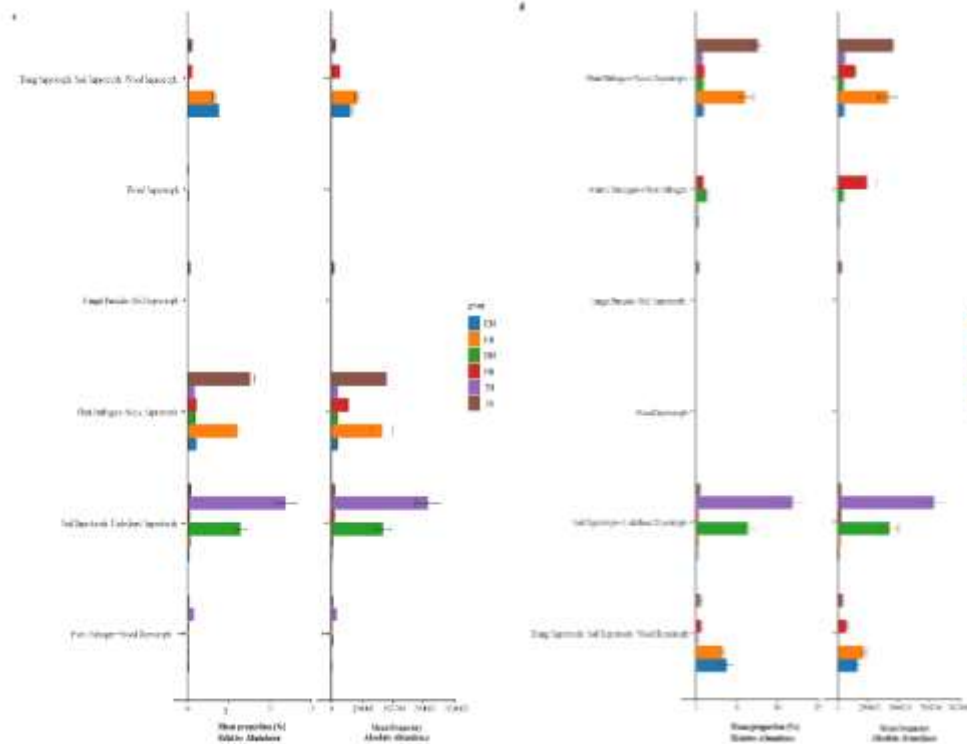


Figure 6 ANOVA and Kruskal–Wallis analysis of maize root-associated microbiota

Note: Panels "a", "b", "c", and "d" represent bacterial ANOVA, bacterial Kruskal–Wallis, fungal ANOVA, and fungal Kruskal–Wallis analyses, respectively. "*", "**", "***", and "****" indicate $P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$, respectively.

2.6 Relationships between Root Endophytic Bacterial Communities and Environmental Factors

The Mantel test results (Fig. 7a) revealed that available potassium (AK) and alkali-hydrolyzable nitrogen (AN) were the core environmental factors driving the assembly of maize root endophytic microbial communities. The bacterial relative and absolute diversity were significantly correlated with AN ($P < 0.01$, $0.25 \leq r < 0.5$), and the bacterial absolute community composition was significantly correlated with AN ($P < 0.05$, $0.25 \leq r < 0.5$). Fungal relative and absolute diversity were primarily significantly affected by AK ($P < 0.05$, r

374 ≥ 0.5), and the correlations of fungal relative and absolute community composition with AK
 375 were highly significant ($P < 0.01$, $0.25 \leq r < 0.5$). The soil pH, AP, SOC, and TN did not
 376 have significant direct regulatory effects on the diversity or composition of the root-associated
 377 microbial groups ($P > 0.05$).

378 As shown in Figure 7b, significant correlations existed between bacterial genera and soil
 379 nutrient factors (AP, TN, AK, AN). AP was highly significantly positively correlated with
 380 *Mucilaginibacter*, *Mycobacterium*, *Rhodopseudomonas*, *Curtobacterium*, *Chryseobacterium*,
 381 *Oligoflexus*, *Sphingopyxis*, *Novosphingobium*, *Oligoflexaceae*, *Terribacillus*, and *Legionella*
 382 and significantly positively correlated with *Bacteroidetes_VC2.1_Bac22*, *Agriterribacter*,
 383 *Pedosphaeraceae*, *Rhodococcus*, *Singulisphaera*, *Micavibrionales*, *01D2Z36*, *IS-44*,
 384 *Abditibacterium*, *Cryobacterium*, *Methylobacterium*, *Rosellomorea*, *Sphingosinicella*,
 385 *Unassigned_SJA-28*, *Kapabacteriales*, *NS11-12*, *Lysinibacillus*, *Methylophilus*, *Reyranelia*,
 386 *TM7a*, *Planctomycetales*, *Fodinicurvataceae*, *Mitochondria*, and *Babeliales*. AP was highly
 387 significantly negatively correlated with *Promicromonospora*, *Rhizobacter*, *Phyllobacterium*,
 388 and *Glycomyces* and significantly negatively correlated with *Cyclobacteriaceae*, *Lacibacter*,
 389 *Hydrogenophaga*, *Oscillochloris*, *Rhizobium*, *Pararhizobium*, *Cellvibrio*, *Massilia*,
 390 *Cupriavidus*, *Herpetosiphon*, and *Algoriphagus*. TN was highly significantly positively
 391 correlated with *Fluviicola*; significantly positively correlated with *ABI_genus*, *Nitrosomonas*,
 392 *Luteolibacter*, *Rhodothermaceae*, *Taibaiella*, *Escherichia-Shigella*, and *UCG-005*; highly
 393 significantly negatively correlated with *Aurantisolimonas* and *Pseudorhizobium*; and
 394 significantly negatively correlated with *Herpetosiphon*, *AKIW781*, *Sphingomonadaceae*,
 395 *Thermomonas*, *Gaiella*, *Pelosinus*, and *Algoriphagus*. AK was significantly positively

correlated with *Promicromonospora*, *Candidatus_Alysiosphaera*, *Terrabacter*, and *PAUC26f*;
 highly significantly negatively correlated with *Stenotrophomonas* and *Desertimonas*; and
 significantly negatively correlated with *OLB13*, *Achromobacter*, *Armatimonadaceae*,
Altericroceibacterium, *Pedosphaeraceae*, *Hydrogenedensaceae*, *Fictibacillus*,
Chthoniobacter, *BD7-2BR169*, *SJA-28*, *Kapabacteriales*, *Vicinamibacteraceae*,
Saprospiraceae, *Haliangiaceae*, *Planctomycetes*, *Bdellovibrio*, *NB1-j*, *Aquicella*, and *S085*.
 AN was highly significantly positively correlated with *Cystobacter*; significantly positively
 correlated with *Luteibacter*, *Xanthomonas*, *Paraburkholderia*, and *Patulibacter*; highly
 significantly negatively correlated with *Saccharimonadales*, *Ardenticales*,
Longimicrobiaceae, *Hyphomicrobium*, *Anaerolineaceae*, *Ilumatobacteraceae*,
Rokubacteriales, *Polaromonas*, *Litchfieldia*, *Metabacillus*, *Flavitalea*, *Adhaeribacter*,
Aridibacter, *KD4-96_genus*, and *Rhizorhabdus*; and significantly negatively correlated with
SH-PL14, *Candidatus_Ovatusbacter*, *Herminiimonas*, *Cohnella*, *Sphingoaurantiacus*,
CCD24_genus, *Roseiflexaceae*, *Puia*, *Latescibacterota*, *211ds20*, *Micavibrionales*,
Subgroup_17, *Arenimicrobium*, *Pedomicrobium*, *Hyphomicrobiales_Incertae_Sedis*, *Tagaea*,
Enterococcus, *A4b_genus*, *AKYH767*, *Acidibacter*, *PLTA13*, *Tautonia*, *Subgroup_5*, *MS-44*,
Alkaliphilus, *Turicibacter*, *Pseudolabrys*, *Nitros*

As shown in Figure 7c, significant correlations existed between fungal genera and soil
 nutrient factors (AP, TN, AK, AN). AP was significantly positively correlated with *Hannaella*;
 highly significantly negatively correlated with *Lecythophora*; and significantly negatively
 correlated with *Thanatephorus*, *Funneliformis*, *Dominikia*, *Rhizoctonia*, *Agrocybe*,
Lobulomycetales, and *Striaticonidium*. TN was significantly positively correlated with

418 *Kurtzmanomyces*; highly significantly negatively correlated with *Fusicolla*,
419 *Pseudogymnoascus*, *Solicoccozyma*, *Occultifur*, *Cladosporium*, and *Paraphoma*; and
420 significantly negatively correlated with *Aspergillus*, *Pyrenochaeta*, *Cephalotrichum*, and
421 *Hypocreales*. AK was highly significantly positively correlated with *GS05*, *Leptosphaeria*,
422 *Protocreopsis*, *Exophiala*, and *Vishniacozyma*; significantly positively correlated with
423 *Rhizophlyctis*, *Neurospora*, *Naganishia*, *Picipes*, *Pseudomicrodochium*, *Ustilago*,
424 *Myrmecridium*, *Talaromyces*, *Mrakia*, *Saitozyma*, and *Nectriopsis*; highly significantly
425 negatively correlated with *Rhizopus* and *Fusarium*; and significantly negatively correlated
426 with *Lecythophora*, *Malassezia*, *Murispora*, and *Albifimbria*. AN was highly significantly
427 positively correlated with *Cephalotrichum* and *Filobasidium*; significantly positively
428 correlated with *Dictyosporiaceae*, *Eucasphaeria*, *Conocybe*, *Trichoderma*, *Meyerozyma*,
429 *Oliveonia*, *Cladosporium*, *Niesslia*, *Lobulomycetales*, and *Striaticonidium*; highly
430 significantly negatively correlated with *Trichocladium*; and significantly negatively correlated
431 with *Hannaella*, *Plectosphaerella*, and *Pseudocyclothyriella*.

nonsignificant Mantel correlations; the green and blue lines indicate correlations at significance levels of $0.01 < P \leq 0.05$ and $0.001 < P \leq 0.01$, respectively.

Panels b and c show heatmaps of correlations between bacterial/fungal genera and soil nutrient factors (AP, TN, AK, AN). All correlation coefficients included in the analysis had absolute values ≥ 0.3 . "*" and "***" indicate significant correlations at $P < 0.05$ and $P < 0.01$, respectively.

3 Materials and methods

3.1 Experimental Site and Natural Conditions

The experiment was conducted at the Agricultural High-tech Science and Technology Park in Horqin District, Tongliao city, Inner Mongolia Autonomous Region, China ($43^{\circ} 36'$ N, $122^{\circ} 25'$ E). This area is located in the irrigated area of the West Liaohe Plain and has a temperate continental monsoon climate. The annual average temperature is approximately 6.8 °C, the $\geq 10^{\circ}\text{C}$ growing degree days (GDDs) total approximately 3200 °C, the frost-free period lasts approximately 149 days, the annual average sunshine duration is approximately 3000 hours, and the multiyear average precipitation is approximately 390 mm.

The soil type at the experimental site is gray meadow soil. Before the experiment began, the organic matter content in the topsoil layer (0 – 20 cm) was 17.52 g kg^{-1} , the alkali-hydrolyzable nitrogen content was 45.20 mg kg^{-1} , the available phosphorus content was 28.32 mg kg^{-1} , and the available potassium content was $132.21 \text{ mg kg}^{-1}$.

3.2 Experimental Design and Field Management

This experiment employed a two-factor split-plot design. The main plot factor was the maize stover return rate, with three levels: no stover return, half stover return, and full stover

return. The corresponding stover input amounts for full and half stover return were 9000 and 4500 kg • ha⁻¹, respectively. The stover was chopped into 3 – 5 cm pieces after harvest in autumn and incorporated into the soil by deep ploughing. The subplot factor was the nitrogen application level, with conventional nitrogen application and reduced nitrogen application. Six treatments were established: no stover return + conventional nitrogen application (NN), no stover return + reduced nitrogen application (NR), half stover return + conventional nitrogen application (HN), half stover return + reduced nitrogen application (HR), full stover return + conventional nitrogen application (TN), and full stover return + reduced nitrogen application (TR). Each treatment had three replicates, and the plot area was 90 m².

The maize cultivar used was "Dika 159". The planting pattern employed wide and narrow rows (80 cm + 40 cm) with shallow buried drip irrigation, and the planting density was 7.5×10^4 plants ha⁻¹. All the treatments received uniform base fertilizer: diammonium phosphate (18--46--0) at 270 kg ha⁻¹ and potassium sulfate (0--0--50) at 90 kg ha⁻¹. The nitrogen source for topdressing was urea (46-0-0). The total urea amounts for the conventional and reduced nitrogen treatments were 450 and 300 kg • ha⁻¹, respectively, and were applied in three splits: 30% at V6, 60% at V12, and 10% at R1. Other field management practices were consistent with local conventional agronomic practices.

3.3 Sample collection, preservation, and measurement

The root samples were collected via a five-point sampling method. Representative plants with consistent growth status were randomly selected from each treatment, and 0–20 cm soil blocks were excavated along with the roots. After sampling, loosely adhered soil was gently shaken off (the soil was taken back to the laboratory and air-dried in a cool place for

determination of the soil nutrient content), and a sterile brush was used to remove rhizosphere soil tightly attached to the root surface, retaining only the root tissue. The samples were transported to the laboratory under dry ice conditions. Three biological replicates were used for each treatment. The soil nutrient indicators were determined according to the methods described in Bao's (2000) *"Soil and Agricultural Chemistry Analysis"* (Bao et al., 2000). The soil available phosphorus (AP) content was determined via the $0.5 \text{ mol} \cdot \text{L}^{-1} \text{ NaHCO}_3$ extraction-molybdenum blue colorimetric method; the available potassium (AK) content was determined via the $1 \text{ mol} \cdot \text{L}^{-1}$ neutral NH_4OAc extraction-flame photometric method; the total nitrogen (TN) content was determined via the Kjeldahl distillation method; the alkali-hydrolyzable nitrogen (AN) content was determined via the alkaline hydrolysis diffusion method; the soil pH was measured via a pH meter with a soil-to-water ratio of 5:1; the soil organic carbon (SOC) content was determined via the potassium dichromate oxidation-external heating method; and the soil carbon-to-nitrogen ratio (C/N ratio) = SOC/TN.

In the laboratory, fresh root samples were thoroughly rinsed with tap water to remove surface impurities, cut into approximately 1 cm segments, and the surface moisture was blotted dry. One gram of root tissue was weighed for surface sterilization, sequentially treated with 75% ethanol (30–60 s), 2–3% NaOCl (3–5 min), and 70% ethanol (30 s), followed by multiple rinses with sterile water to thoroughly remove disinfectant residues. The final rinse solution was plated on nutrient agar plates to verify the effectiveness of surface sterilization. The sterilized root samples were blotted dry with sterile filter paper, placed in sterile centrifuge tubes, and stored at -80°C .

Total DNA was extracted from roots via the Fast DNA[®] SPIN Kit for Soil (MP

Biomedicals, USA). Integrity was checked via 1% agarose gel electrophoresis, and precise quantification was performed via a Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA) to ensure the requirements for subsequent amplification and library construction.

Root endophytic bacterial community structure: The structure of the root endophytic bacterial communities was analysed via 16S rRNA gene high-throughput sequencing combined with Accu 16S™ absolute quantification technology. The V5-V7 region was amplified via specific primers (799F/1193R) to exclude host interference, and an internal spike-in sequence was introduced for absolute quantification of bacteria. After construction and quality control, the sequencing libraries were subjected to paired-end sequencing on the Illumina NovaSeq 6000 platform, and the obtained data were used for subsequent analysis.

Root endophytic fungal community structure: High-throughput sequencing and absolute quantification analysis were performed on the ITS2 region of root endophytic fungi. An artificially synthesized spike-in standard was added to the DNA samples as an internal reference. Amplification was performed via the primers ITS1F/ITS2R, followed by sequencing on the Illumina NovaSeq 6000 platform (2 × 250 bp).

3.4 Bioinformatics and Statistical Analysis

3.4.1 Sequencing Data Quality Control and ASV Construction

Raw paired-end sequencing data were demultiplexed via the Illumina platform and processed via QIIME2 software: adapters and primers were removed via Cutadapt, followed by quality control, denoising, merging, and chimera removal via DADA2 to obtain high-confidence amplicon sequence variants (ASVs). For the bacterial and fungal data, spike-in sequences were first removed. A standard curve was subsequently constructed on the basis of

these sequences, and the read counts of each ASV were subsequently converted to absolute copy numbers, ultimately yielding the absolute abundance of microorganisms per unit of DNA and per unit of sample mass.

3.4.2 Species Taxonomic Annotation Methods and Databases

Taxonomic annotation of ASVs was performed via the QIIME2 platform with the classify-sklearn (naive Bayes classifier) method. The SILVA rRNA database (v 138.2) was used for bacteria, with a confidence threshold of 0.8. The UNITE database (v 9.0) was used for fungi, with a confidence threshold of 0.6. In the bacterial absolute quantification workflow, the rdp classifier (v 2.14) was additionally used for reannotation to estimate and correct for 16S rRNA gene copy numbers at the genus level, yielding corrected absolute abundances. No copy number correction was performed for fungal analysis.

3.4.3 Community Diversity Analysis

On the basis of corrected absolute abundance data for bacteria and uncorrected absolute abundance data for fungi at the sample level, α diversity indices, including the observed, Chao1, Shannon, and Simpson indices, were calculated via R language (v 4.2.2). β diversity and its differences among groups were assessed via principal coordinate analysis (PCoA) on the basis of the Bray–Curtis distance and permutational multivariate analysis of variance (PERMANOVA, i.e., Adonis analysis).

3.4.4 Differential Taxa and Functional Prediction Analysis

The linear discriminant analysis effect size (LEfSe) method (LDA threshold > 2.0 , $P < 0.05$) was used to identify differential bacteria and fungi. For bacteria, functional prediction was performed via PICRUSt2 software, biogeochemical cycles were annotated via the

FAPROTAX (v 1.2.4) database, and phenotypes were predicted via the BUGBASE database (v 1.0). The Kruskal–Wallis test or ANOVA was used for intergroup difference analysis. For fungi, FUNGuild (v 1.1) and the FungalTraits database were used to annotate trophic modes and ecological functions.

3.4.5 Environmental Factors and Statistical Analysis

Redundancy analysis (RDA) was performed to analyse the relationships between soil environmental factors (e.g., organic matter, alkali-hydrolyzable nitrogen, available phosphorus, and available potassium) and the microbial community structure. One-way analysis of variance (ANOVA) with Duncan's multiple range test ($P < 0.05$) was performed via SPSS 25.0 software.

4 Discussion

4.1 Carbon and nitrogen reshaped the assembly of root endophytic bacteria and fungal communities

The coupling effect of stover return and nitrogen fertilizer reduction strongly selectively drives the directed assembly of maize root endophytic microbial communities by reshaping the availability and stoichiometric ratios of carbon and nitrogen resources in the rhizosphere microenvironment. In the bacterial community, full stover return with reduced nitrogen (TR) treatment, through an ample exogenous carbon supply and effective reduction in available nitrogen, creates a carbon-rich, nitrogen-limited microhabitat. This may inhibit the relative and absolute dominance of copiotrophic groups with rapid nitrogen utilization strategies, such as *Pseudomonas*, while simultaneously enriching groups associated with symbiosis, associative nitrogen fixation (e.g., *Rhizobium*, *Agrobacterium*), and complex organic matter

decomposition (e.g., Actinomycetota, represented by *Streptomyces*). This is corroborated by the increased copy numbers of nitrogen fixation genes in functional predictions (Meng et al., 2024), indicating that synergistic optimization of carbon and nitrogen resources can reshape the functional structure of bacterial communities, enhancing the system's nitrogen self-sufficiency and organic matter turnover potential (Zhao et al., 2024; D.L. Jones et al., 2018). Conversely, under the no stover return with reduced nitrogen (NR) treatment, owing to the lack of exogenous carbon input and highly constrained resources, oligotrophic groups with rapid nitrogen utilization capacity, such as *Pseudomonas* (with a relative abundance reaching 80.5%), occupy a nearly monopolistic position, leading to extreme simplification of community structure and functional convergence. This confirms the weakening of niche differentiation under single resource limitation (Hou, 2025). The half stover returned with reduced nitrogen (HR) treatment exhibited an assembly pattern oriented towards functional specialization: it significantly enriched efficient degrading groups represented by Bacteroidota (with a notable increase in absolute abundance), which play key roles in the degradation of cellulose, hemicellulose, and proteins (Taillefer et al., 2018; Deng et al., 2021), accompanied by some rare groups (e.g., Planctomycetota). This suggests that under moderate carbon input, the community tends to form a "function-enhanced" structure centered on specific decomposition functions, which may be related to the accumulation of intermediate products during stover decomposition (Duan et al., 2024).

Fungal communities exhibited different ecological logics in response to the same management practices. Reduced nitrogen was the primary factor driving β diversity differentiation in fungal communities, and stover return significantly amplified this effect

under reduced nitrogen conditions. Unlike the drastic fluctuations in bacterial community abundance, the α diversity indices (Observed, Shannon) of fungal communities did not change significantly across treatments, indicating that management practices primarily induced species compositional turnover (i.e., community restructuring) in fungi rather than significant changes in species richness (Abrahão Anna et al., 2022). This confirms that fungal communities possess greater compositional plasticity and structural stability than bacteria do (Zhang et al., 2026). The full stover return with reduced nitrogen (TR) effectively mitigated the trend toward single-species dominance in fungi, enriching functional groups such as *Mortierella* and *Penicillium*, and significantly increasing the community's functional diversity potential. The addition of half stover in the reduced nitrogen (HR) treatment also increased fungal community heterogeneity and the number of unique OTUs. Although the no stover return with reduced nitrogen (NR) treatment had the highest total absolute abundance of fungi, its dominant genus (e.g., *Fusarium*) was highly concentrated, resulting in a uniform community structure and greater functional risk. These results collectively reveal that in carbon–nitrogen coupled management, bacterial communities tend to respond to resource changes through rapid adjustments in abundance and dominance (Wen et al., 2024), whereas fungal communities adapt functionally more through compositional turnover (Hao et al., 2024), forming complementary ecological response strategies.

4.2 Interconnections between Microbial Diversity, Functional Potential, and Community Assembly

Microbial diversity is the foundation of ecosystem functional stability and resistance to disturbance (Manuel Delgado-Baquerizo et al., 2016). This study revealed a complex trade-

off between the maintenance of diversity and functional enhancement in microbial communities under different carbon and nitrogen management strategies. In the TR treatment, full stover carbon input effectively mitigated the decline in bacterial Shannon diversity caused by reduced nitrogen, indicating that sufficient carbon sources create more ecological niches by increasing resource heterogeneity, buffering the negative impact of nitrogen limitation on community evenness (Jiao et al., 2020). This was accompanied by the synergistic enhancement of multifunctional potential, including nitrogen fixation and organic matter transformation, resulting in an ideal community that is both diverse and multifunctional (Delgado-Baquerizo Manuel et al., 2016). In stark contrast, the HR treatment exhibited a "function-strengthened, structure-simplified" trade-off pattern (Niu et al., 2021). Under this treatment, the degradation function of hydrocarbons associated with Bacteroidota was significantly enhanced, indicating that community resource allocation was tilted towards the rapid utilization of stover-derived carbon. However, this metabolic specialization may intensify interspecific competition, leading to a significant decrease in bacterial species richness and Shannon diversity (the observed index was the lowest among all the treatments) (Xun et al., 2021). This suggests that under the dual pressures of moderate carbon supply and nitrogen limitation, the microbial community ensures the maintenance of core decomposition functions at the expense of some species diversity. While this strategy may be beneficial for rapid stover decomposition in the short term (Lu et al., 2023), its long-term implementation could weaken community functional redundancy and ecological stability (Luo et al., 2023), increasing the risk of functional decline when facing environmental disturbances.

The functional response of fungal communities was primarily reflected in the reshaping

of trophic structure. In the HR and TR treatments, the enrichment of Basidiomycota, associated with lignin degradation, and saprotrophic fungal groups (A. Arun et al., 2022; An et al., 2023) indicates that stover return under reduced nitrogen conditions strengthens the functional module of fungi involved in the transformation of recalcitrant organic matter. In contrast, the no stover return treatments (NN and NR) presented increased proportions of saprotrophic and pathogenic fungi (e.g., *Fusarium*), suggesting that in the absence of stover carbon input, the fungal community may shift towards the utilization of root exudates or live plant tissues, increasing the potential risk of soil-borne diseases (Chen et al., 2022). These findings reveal that maize root endophytic microbial communities form a highly functionally differentiated and dynamically balanced system and that carbon and nitrogen management must be precisely regulated to find the optimal balance between functional enhancement and diversity maintenance.

4.3 Alkali-Hydrolyzable Nitrogen and Available Potassium are Potential Core Environmental Variables Affecting Root Endophytic Microbial Community Assembly

Through integrated analysis of redundancy analysis and correlation networks, this study identified AN and AK as core environmental factors driving structural changes in maize root endophytic bacterial and fungal communities, with the greatest number and strongest correlations with microbial taxa. AN, as a core indicator reflecting the instantaneous nitrogen supply capacity of soil, exhibited a strong deterministic screening effect on regulating the evolution of maize root endophytic bacterial communities. During the AN-driven community assembly process, correlations of moderate strength ($0.25 \leq r < 0.5$) suggested that AN

induces directional turnover of copiotrophic and oligotrophic bacterial groups by altering the nutrient flux in the root endophytic environment, forming a specific microbial co-occurrence pattern oriented by nitrogen availability. In contrast, the response of fungal communities to AN was significantly weaker than that of bacteria. This difference among microbial groups reveals niche differentiation in resource acquisition strategies within root endophytes: bacterial communities constitute an important link in maintaining root endophytic homeostasis in response to nitrogen fluctuations, whereas fungi are dominated by screening for mineral elements such as available potassium. The differential selection effect dominated by AN provides a basis for understanding the assembly logic of maize root endophytic bacteria and the synergistic nutrient interaction mechanism between host plants and endophytic microbiomes. Potassium, the most important cation in plants, is involved in osmotic regulation, enzyme activation, and signal transduction (Johnson et al., 2022). Changes in nutrient availability may indirectly select for and regulate microorganisms that colonize roots by directly affecting the composition and flux of root exudates. Taxa significantly positively correlated with AK, such as the bacteria *Promicromonospora* and *Terrabacter* and the fungi *Neurospora* and *Talaromyces*, may have functions such as growth promotion, stress resistance, or degradation of complex organic matter, suggesting that available potassium may act as an "ecological filter factor" (Tao et al., 2019; Song et al., 2026; Liu et al., 2025; Naike Kruhler et al., 2026), enriching beneficial microbes closely related to plant health. Conversely, the enrichment of potential pathogens, such as *Fusarium* and *Rhizopus*, was significantly negatively correlated with AK, further confirming that an insufficient potassium supply may worsen the microecological health of the rhizosphere

(Yang et al., 2023).

Stover return has been shown to promote the release of soil mineral potassium and the rapid cycling of stover potassium (Xiong et al., 2023), thereby effectively increasing the soil available potassium content (Fan et al., 2021). In this study, changes in soil AK levels under stover return treatments such as TR and HR were highly coupled with changes in microbial community structure, indicating that the synergistic effect of stover return and nitrogen reduction is partly achieved by optimizing the potassium supply (Li et al., 2025). This finding provides a new perspective for understanding how carbon and nitrogen management indirectly regulates the rhizosphere microbiome by affecting potassium cycling and highlights the necessity of considering the core role of potassium in maintaining microbial diversity and functional stability in integrated nutrient management, especially in potassium-deficient or potentially deficient farmland ecosystems in northern China.

5 Conclusions

This study demonstrated that the coupled effect of stover return and nitrogen fertilizer reduction significantly reshaped the structure and function of maize root endophytic microbial communities at the silking stage (R1). In bacterial communities, full stover return combined with reduced nitrogen treatment significantly enriched groups such as *Rhizobium*, *Agrobacterium*, and Actinomycetota by inhibiting the relative dominance of *Pseudomonas*, thereby synergistically enhancing the system's potential for biological nitrogen fixation and complex organic matter transformation. The addition of half stover in the reduced nitrogen treatment specifically enriched Bacteroidota and strengthened hydrocarbon degradation functions but led to a decline in community diversity. In contrast, treatments without stover

return, especially under high nitrogen, allowed Pseudomonadota to achieve absolute dominance, leading to simplified community structure and functional convergence. Fungal communities also respond significantly to management practices. Under reduced nitrogen, stover return increased the abundance of lignin degradation-related groups, such as Basidiomycota, and increased community heterogeneity. Environmental factor correlation analysis confirmed that soil alkali-hydrolyzable nitrogen and available potassium were the core abiotic factors driving the differentiation of root endophytic bacterial and fungal community structures. In summary, from the perspective of microbial community assembly and functional regulation, full stover return combined with nitrogen fertilizer reduction is identified as the most promising agronomic practice in the irrigated area of the West Liaohe Plain. By optimizing the input pattern of carbon and nitrogen resources, beneficial functional microbial groups can be recruited, providing a reliable microbiological basis for constructing a multifunctional and stable root microecosystem and achieving sustainable intensification of agricultural production.

Declarations

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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Author Contribution

Wu Chengcheng performed the experimental operation, conducted the data analysis, and wrote the manuscript. Sa Rula conceived the study, designed the scheme, managed the project, provided experimental guidance, revised the manuscript, reviewed the work, and acquired the funding. Liu Naijia collected the samples and supervised the study. Chen Xingbao performed the translation and polished the language. Chu Junqi refined the figures. Tai Jicheng designed the scheme, provided the materials, and analyzed the data.

Ethical Statement

Not applicable.

Consent for Publication

Not applicable.

Consent to Participate

Not applicable.

Clinical Trial Number

Not applicable.

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