

Effect of frequency of nitrogen fertilization on the rhizosphere soil bacterial community and yield of forage maize in arid areas

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ABSTRACT

Precise N fertilization is crucial to increasing crop yields and maintaining soil ecological balance. However, under drip irrigation conditions, the specific topdressing frequency and its impact on soil bacterial communities and forage maize (*Zea mays* L.) yields are still unknown. In this field experiment, the effects of different N application frequencies (four applications of N fertilizer at 37.5%:12.5%:12.5%:37.5% (NF1), three applications at 33.75%:32.5%:33.75% (NF2), and two applications at 62.5%:37.5% (NF3)) on the rhizosphere soil bacterial community diversity and yield of forage maize were analyzed, with no N fertilization as the control (CK). The results showed that NF3 treatment had the largest number of unique operational taxonomic units (OTUs) among the treatments (211). The Shannon index of NF3 treatment was significantly higher than that of NF1 and CK treatments. The relative abundance of Proteobacteria of NF3 treatment was 4.49, 5.33, and 3.94 percentage points higher than that of CK, NF1, and NF2 treatments, respectively, and the copiotroph Actinobacteria was significantly enriched in NF3 treatment. In addition, the abundance of N cycle functional genes (chemoheterotrophy, aerobic chemoheterotrophy, and aromatic compound degradation) of NF3 treatment was significantly higher than that of other treatments ($p < 0.05$). The yield of forage maize of NF3 treatment was as high as 73 925.83 kg ha⁻¹, which was 176% and 103% higher than that of CK and NF1 treatments ($p < 0.05$), respectively. These results provide a scientific basis for increasing forage maize yield and optimizing N fertilization, promoting soil microecological balance and sustainable production.

Key words: Crop yield, forage maize, nitrogen fertilizer, soil microorganisms, topdressing times, *Zea mays*.

INTRODUCTION

Forage maize (*Zea mays* L.) has always been an important source of feed for the livestock industry in Xinjiang, an important livestock production base in arid northwest China. However, water scarcity and unfertile soil in Xinjiang restrict the sustainable production of forage maize. Nitrogen is a key factor impacting forage maize growth. Traditional extensive agricultural management modes, characterized by excessive N application, are prone to N leaching and ammonia volatilization, significantly lowering N fertilizer utilization efficiency (Liu et al., 2023). Therefore, optimizing N fertilization modes is crucial for the sustainable forage maize production in arid areas (Baum et al., 2025). Effective N fertilization can not only improve the photosynthetic performance of crops but also increase crop yields (Cheng et al., 2025).

In recent years, drip fertigation has solved the problem of canopy closure induced difficulty in topdressing in the later stage of crop growth in Xinjiang, China. Drip fertigation can significantly increase crop yield by precisely regulating the spatial and temporal distribution of water and N (Cui et al., 2024; Yang et al., 2024). In field management, split application of fertilizers can accurately meet the nutrient needs of crops and minimize nutrient fixation in the soil (Ouoba et al., 2022). Studies have shown that split application of N fertilizer by drip fertigation can alleviate N loss caused by N leaching and ammonia volatilization (Kaur et al., 2023). At present,

research on N fertilizer management for forage maize is mainly focused on controlling the total N application rate (Hammad et al., 2018; Liimatainen et al., 2022). Although optimizing N fertilization at different growth stages, such as the jointing stage can promote yield formation of maize (Cheng et al., 2025), how to increase the yield of forage maize by adjusting the frequency of N fertilization under drip fertigation conditions is still unclear.

Furthermore, soil microorganisms play a crucial role in the soil N cycle (Yin et al., 2024b). Changes in N fertilization regime often affect soil bacterial community composition and diversity (Tong et al., 2024; Li et al., 2025). Functional groups such as *Nitrospira* and *Sphingomonas* that participate in the N cycle are often reported to be enriched in high N treatments (Zhao et al., 2025). However, high levels of N application significantly reduce the diversity of bacterial communities (Wang et al., 2018; Yin et al., 2025), and also cause soil acidification, increased N emissions, and soil ecological imbalance and unhealth (Wang et al., 2023b; Chi et al., 2025). Therefore, after determining the total N application rate throughout the growth period, more and more scholars have turned to optimizing N fertilization strategies such as combined application of N fertilizer and manure and increasing topdressing times to improve crop yield and maintain the diversity of soil bacterial communities (Qu et al., 2019; Wang et al., 2024). However, currently, in the forage maize planting, the impact of frequency of N fertigation on soil bacterial community diversity is still not clear.

Based on this, this study intended to optimize the frequency of N fertigation for forage maize under a certain total N application rate. It was hypothesized that the optimized N fertigation frequency might enhance (1) the diversity of rhizosphere soil bacterial communities, (2) the yield of forage maize, and (3) the expression of N cycle genes. To verify these hypotheses, this study used high throughput sequencing technology to analyze the effect of different frequencies of N fertigation on the characteristics of rhizosphere soil bacterial communities and the yield of forage maize. The study results will provide a scientific basis for increasing forage maize yield and optimizing N fertilization, promoting soil microecological balance and sustainable production.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in 2023 at the experimental field of Tianye Agricultural High Tech Co., Ltd. in Xinjiang (85°59'47" E, 44°19'26" N; 450 m a.s.l.), China. The annual accumulated temperature ($\geq 10^{\circ}\text{C}$) was 3608-3996 $^{\circ}\text{C}$. The average annual sunshine duration was 2500 h. The frost-free period lasted 140-185 d. The average annual rainfall was 150-200 mm, and the average annual evaporation was 300-400 mm. The soil organic matter content was 18.1 g kg⁻¹. The contents of soil total N, alkaline N, available P, and available K were 1.23 g kg⁻¹, 29.4 mg kg⁻¹, 38.4 mg kg⁻¹, and 242.6 mg kg⁻¹, respectively. A drip irrigation system was used. The inner diameter of the drip tape was 16 mm, the wall thickness was 0.18 mm, and the dripper spacing was 30 cm.

Experimental design

This experiment adopted a randomized complete block design. The forage maize (*Zea mays* L.) 'Xinsiyu 13' was used for this experiment. Before sowing, 150 kg ha⁻¹ KH₂PO₄ were applied. Forage maize seeds (provided by Xinjiang Academy of Agricultural and Reclamation Science, China) were sown on 15 July 2023. The row spacing was 60 cm. The plant spacing was 17 cm. There were a total of four treatments, including no N fertigation (CK), emergence to jointing stage 37.5%, jointing to tasseling stage 12.5%, tasseling to silking stage 12.5%, silking to dent stage 37.5% (NF1); emergence to jointing stage 33.75%, tasseling to silking stage 32.5%, silking to dent stage 33.75% (NF2); and emergence to jointing stage 62.5%, silking to dent stage 37.5% (NF3); with a total N application rate of 150 kg ha⁻¹. Each treatment had four replicates, with a total of 16 plots. The area of each plot was 15 m² (7.5 m × 2 m). Other field managements were consistent across the treatments. The frequency, timing, and quantity of N fertigation are detailed in Table 1. The total irrigation amount for each treatment during the entire growth period was 200 m³. The specific irrigation amount for each growth stage is shown in Table 2.

Table 1. Nitrogen fertilization strategies for drip-irrigated forage maize. CK: Control group without N fertilization; NF1, NF2, and NF3: four-, three-, and two-application N-fertilization regimes, respectively. Values within phenological intervals are presented as kg N ha⁻¹, with the percentage of the total 150 kg N ha⁻¹ in parentheses.

Treatments	N fertilizer application		Proportion of top-dressed N, kg ha ⁻¹ (% of total N)			
	rate (kg ha ⁻¹)	Topdressin g times	Emergence to jointing stage	Jointing to tasseling stage	Tasseling to silking stage	Silking to dent stage
CK	0	0	-	-	-	-
NF1	150	4	56.25 (37.5%)	18.75 (12.5%)	18.75 (12.5%)	56.25 (37.5%)
NF2	150	3	50.625 (33.75%)	-	48.75 (32.5%)	50.625 (33.75%)
NF3	150	2	93.75 (62.5%)	-	-	56.25 (37.5%)

Table 2. Irrigation amount at different growth stages of forage maize. All treatments received the same irrigation schedule. The total irrigation amount reported for each treatment was 200 m³, distributed among phenological stages according to the listed proportions.

Irrigation amount	Emergence to jointing stage	Jointing to tasseling stage	Tasseling to silking stage	Silking to dent stage
Proportion, %	25	20	20	35
Irrigation volume, m ³	50	40	40	70

Measurements

Rhizosphere soil sampling. The rhizosphere soil samples of forage maize were collected during the grouting stage. For each treatment, 18 maize plants were randomly selected and uprooted with a sterilized shovel. The rhizosphere soil attached to the root system (about 2 mm) was brushed with an alcohol sterilized brush. The collected rhizosphere soils were sieved (2 mm) to remove impurities. The soil samples of each treatment were mixed evenly and then transferred into a sterile centrifuge tube (5-10 g per tube). Each treatment had three centrifuge tubes. The tubes were stored in a refrigerator at -80 °C for measuring soil microbial diversity by sequencing.

Measurement of forage maize yield. At the late milk ripe stage (15 October 2023), 4 m² of maize plant shoots were collected from each plot, followed by the measurement of shoot fresh weight. Each sample was measured three times, and the average value was calculated.

DNA extraction and PCR amplification

Total microbial genomic DNA was extracted from rhizospheric soil samples using the E.Z.N.A. soil DNA kit (Omega Bio-tek, Norcross, Georgia, USA) according to the manufacturer's instructions. The quality and concentration of DNA were determined by 1% agarose gel electrophoresis and a NanoDrop2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). Then, the DNA was kept at -80 °C before further use. The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by T100 Thermal Cycler PCR thermocycler (BIO-RAD, Hercules, California, USA). The PCR reaction mixture (20 µL in total) was composed of 4 µL 5 × Fast Pfu buffer, 2 µL 2.5 mM dNTPs, 0.8 µL each primer (5 µM), 0.4 µL Fast Pfu polymerase, 10 ng template DNA, and ddH₂O. The PCR amplification cycling conditions were as follows: Denaturation at 95 °C for 3 min, followed by 27 cycles of three procedures (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s) and extension at 72 °C for 10 min, finally ending at 4 °C. The PCR product was extracted from 2% agarose gel, purified using the PCR Clean Up kit (YuHua, Shanghai, China) according to the manufacturer's instructions, and quantified using Qubit 4.0 fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Illumina sequencing

Purified amplicons were pooled in equimolar amounts and paired-end sequenced on an Illumina Nextseq2000 platform (Illumina, San Diego, California, USA) according to the standard protocols by Majorbio Bio Pharm Technology Co., Ltd. (Shanghai, China). The raw sequencing reads were deposited into the NCBI Sequence Read Archive (SRA) database (National Center for Biotechnology Information [NCBI], Bethesda, Maryland, USA).

Raw FASTQ files were demultiplexed using an inhouse Perl script, quality filtered by fastp version 0.19.6 (HaploX Biotechnology, Shenzhen, China), and merged by FLASH version 1.2.7 (Johns Hopkins University School of Medicine, Baltimore, Maryland, USA). Then, the optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 (<http://drive5.com/uparse/>) with 97% sequence similarity level. The most abundant sequence for each OTU was selected as the representative sequence. To minimize the effects of sequencing depth on α - and β -diversity measurements, the number of 16S rRNA gene sequences from each sample was rarefied to 20000, which still yielded an average Good's coverage of 99.09%.

The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Center for Microbial Ecology, Michigan State University, East Lansing, Michigan, USA) against the 16S rRNA gene database using a confidence threshold of 0.7. The FAPROTAX (Functional Annotation of Prokaryotic Taxa) database has been widely used for predicting the metabolic functions of soil bacterial communities (Sansupa et al., 2021). In this study, the metagenomic function was predicted by FAPROTAX based on OTU representative sequences.

Data analysis

Bioinformatic analysis of the soil microbiota was carried out using the Majorbio Cloud platform (<https://cloud.majorbio.com>). Based on the OTUs information, rarefaction curves and α -diversity indices, including observed OTUs, Shannon and Simpson indices were calculated with Mothur v1.30.1. Column charts representing the bacterial community structure at different taxonomic levels were generated using R software (version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria). The similarity among the microbial communities in different samples was determined by principal component analysis (PCA) using the Vegan v2.5-3 package. Nonmetric multidimensional scaling (NMDS) analysis was conducted for verification. The linear discriminant analysis (LDA) effect size (LEfSe) was conducted to identify the significantly abundant taxa (phylum to genera) of bacteria among the treatments (LDA score > 3, $p < 0.05$) (<http://huttenhower.sph.harvard.edu/LEfSe>). Treatment differences in yield, α -diversity indices, and FAPROTAX results were analyzed by one-way ANOVA followed by Tukey's honestly significant difference (HSD) test, with significance set at $p < 0.05$.

RESULTS

Diversity of bacterial communities in the rhizosphere soil of forage maize

The OTU-based classification (Figure 1) showed that NF1, NF2, NF3, and CK treatments had 165 OTUs in total, accounting for 2.41% of the total OTUs. The NF3 treatment had the largest number of unique OTUs among all treatments (211), while NF1 treatment had the smallest number of unique OTUs (156). The NF2 and CK treatments had 186 and 177 unique OTUs, respectively. Significant changes were observed in the diversity indices of rhizosphere soil bacterial communities of NF1, NF2, and NF3 treatments compared with CK. Among them, the Shannon index of NF3 treatment was 7.07% and 7.20% higher than that of NF1 and CK treatment, respectively ($p < 0.05$; Figure 2a). The Simpson's index of NF3 treatment was the lowest among all treatments ($p < 0.05$; Figure 2b).

The structure of bacterial communities in the rhizosphere soil of forage maize also showed significant changes under NF1, NF2, and NF3 compared with CK (Figure 2c). The principal component analysis (PCA) showed that, at the species level, PC1 (23.67%) and PC2 (10.70%) explained a total of 34.37% of the variation in community structure. Along the PC2 axis, CK, NF1, and NF3 samples were distributed mainly on the positive side, whereas NF2 samples were distributed on the negative side. The CK and NF1 samples clustered together, whereas NF2 and NF3 were separated from CK ($R = 0.58$; $p = 0.002$). Nonmetric multidimensional scaling (NMDS) further supported treatment-related differences, with NF3 separated from CK (stress = 0.029; $R = 0.64$; $p = 0.001$; Figure 2d).

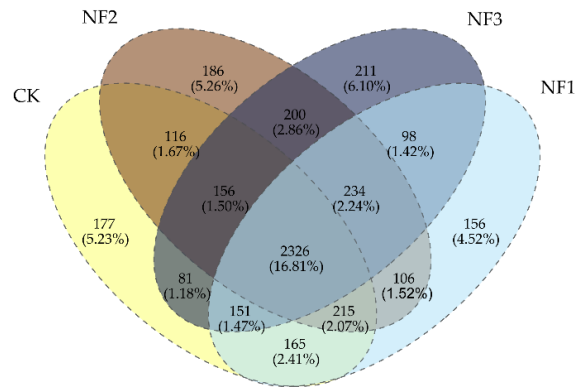


Figure 1. Number of unique and shared operational taxonomic units (OTUs) in soil bacterial communities under different N fertilization frequencies. CK: Control group without N fertilization; NF1: four applications totaling 150 kg N ha⁻¹, with 37.5%, 12.5%, 12.5%, and 37.5% applied from emergence to jointing, jointing to tasseling, tasseling to silking, and silking to dent, respectively; NF2: three applications totaling 150 kg N ha⁻¹, with 33.75%, 32.5%, and 33.75% applied from emergence to jointing, tasseling to silking, and silking to dent, respectively; NF3: two applications totaling 150 kg N ha⁻¹, with 62.5% and 37.5% applied from emergence to jointing and silking to dent, respectively.

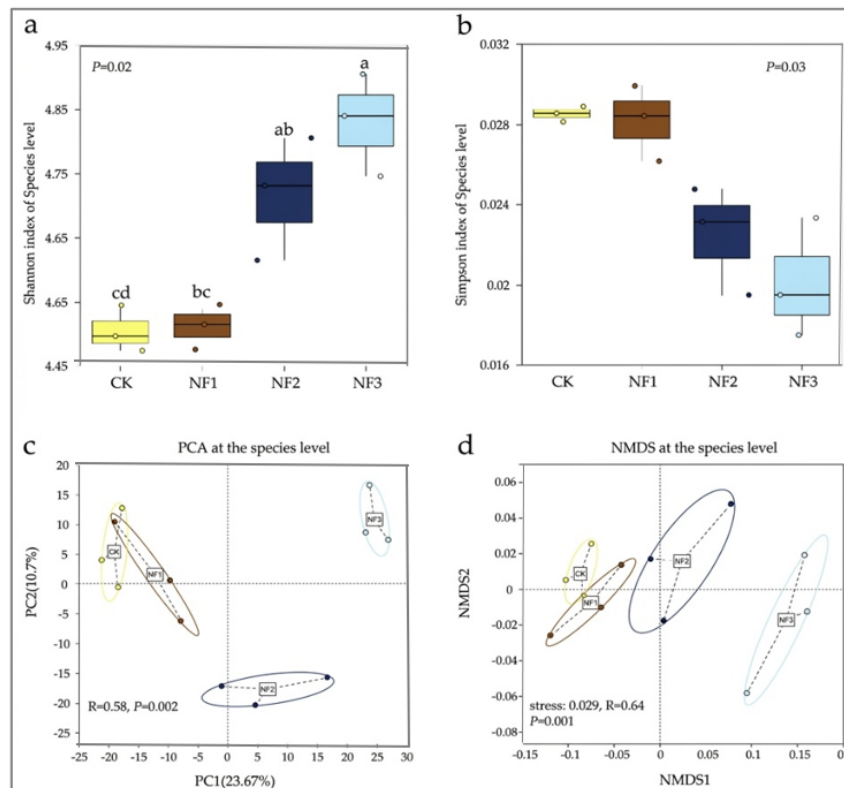


Figure 2. α -diversity (a: Shannon index; b: Simpson index) and β -diversity (c: principal component analysis (PCA); d: nonmetric multidimensional scaling (NMDS)) analysis of soil bacterial communities at the species level under different N fertilization frequencies. The PCA and NMDS show community dissimilarity among treatments. CK: Control group without N fertilization; NF1: four applications totaling 150 kg N ha⁻¹, with 37.5%, 12.5%, 12.5%, and 37.5% applied from emergence to jointing, jointing to tasseling, tasseling to silking, and silking to dent, respectively; NF2: three applications totaling 150 kg N ha⁻¹, with 33.75%, 32.5%, and 33.75% applied from emergence to jointing, tasseling to silking, and silking to dent, respectively; NF3: two applications totaling 150 kg N ha⁻¹, with 62.5% and 37.5% applied from emergence to jointing and silking to dent, respectively.

Composition and functional genes of bacterial communities in the rhizosphere soil of forage maize

At the phylum level (Figure 3a), Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes, and Gemmatimonadetes were the dominant bacterial phyla in the treatments. Among them, the relative abundance of Proteobacteria of NF3 treatment (28.36%) was numerically higher than that of CK, NF1, and NF2 treatments, respectively. The dominant bacterial classes in the treatments were Acidobacteria, Actinobacteria, Alphaproteobacteria, Gemmatimonadetes, Planctomycetia, and Gammaproteobacteria (Figure 3b). The relative abundance of Acidobacteria in NF3 treatment was numerically lower than that of CK, NF1, and NF2 treatments, respectively. At the order level, norank_c_Acidobacteria, Rhizobiales, Planctomycetales, and Gemmatimonadales were the dominant bacterial orders in the treatments, and Rhizobiales had the highest relative abundance in the NF3 treatment (9.07%) (Figure 3c).

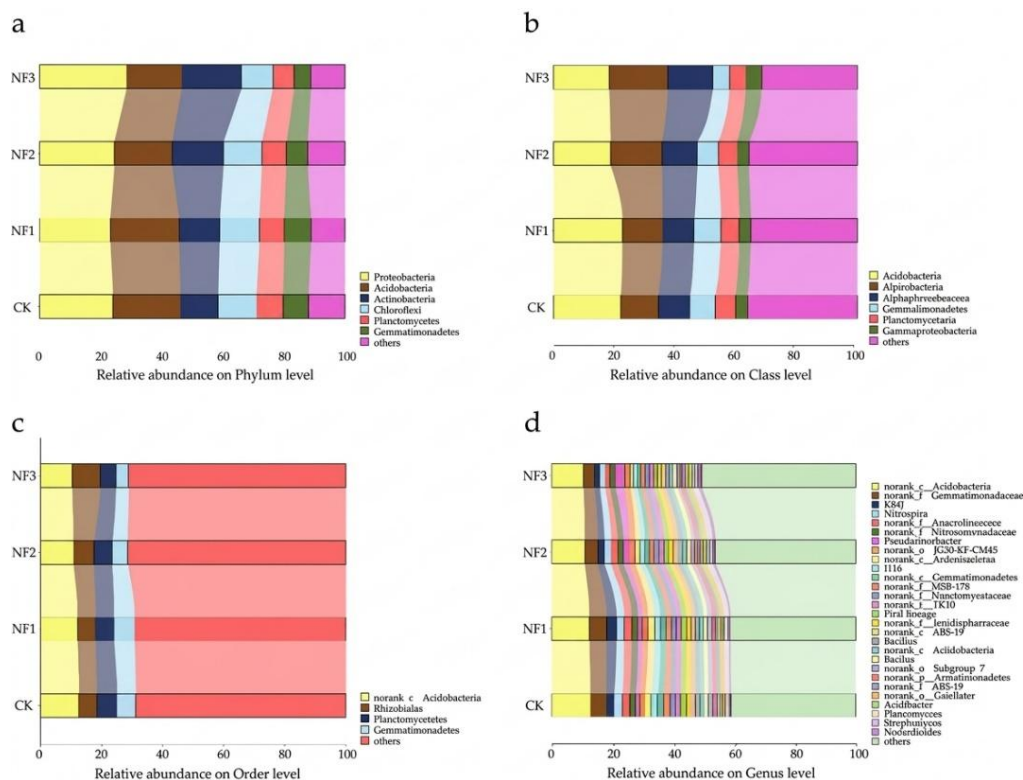


Figure 3. Soil bacterial community composition at the phylum (a), class (b), order (c), and genus (d) levels under different N fertilization frequencies. CK: Control group without N fertilization; NF1: four applications totaling 150 kg N ha⁻¹, with 37.5%, 12.5%, 12.5%, and 37.5% applied from emergence to jointing, jointing to tasseling, tasseling to silking, and silking to dent, respectively; NF2: three applications totaling 150 kg N ha⁻¹, with 33.75%, 32.5%, and 33.75% applied from emergence to jointing, tasseling to silking, and silking to dent, respectively; NF3: two applications totaling 150 kg N ha⁻¹, with 62.5% and 37.5% applied from emergence to jointing and silking to dent, respectively.

The LDA results showed that Actinobacteria was significantly enriched in NF3 treatment at the phylum and class levels (LDA threshold: 3; Figures 4a, 4b). In addition, the bacterial orders Micrococcales and Propionibacteriales, as well as the genera *Pseudarthrobacter* and *Nocardioides*, were also significantly enriched in NF3 treatment. The bacterial classes Subsection III and order Longimicrobiales, as well as the genera *Marmoricola* and *Adhaeribacter*, were significantly enriched in NF2 treatment. The bacterial phyla Acidobacteria and Gemmatimonadetes, as well as the class Acidobacteria, were significantly enriched in NF1 treatment.

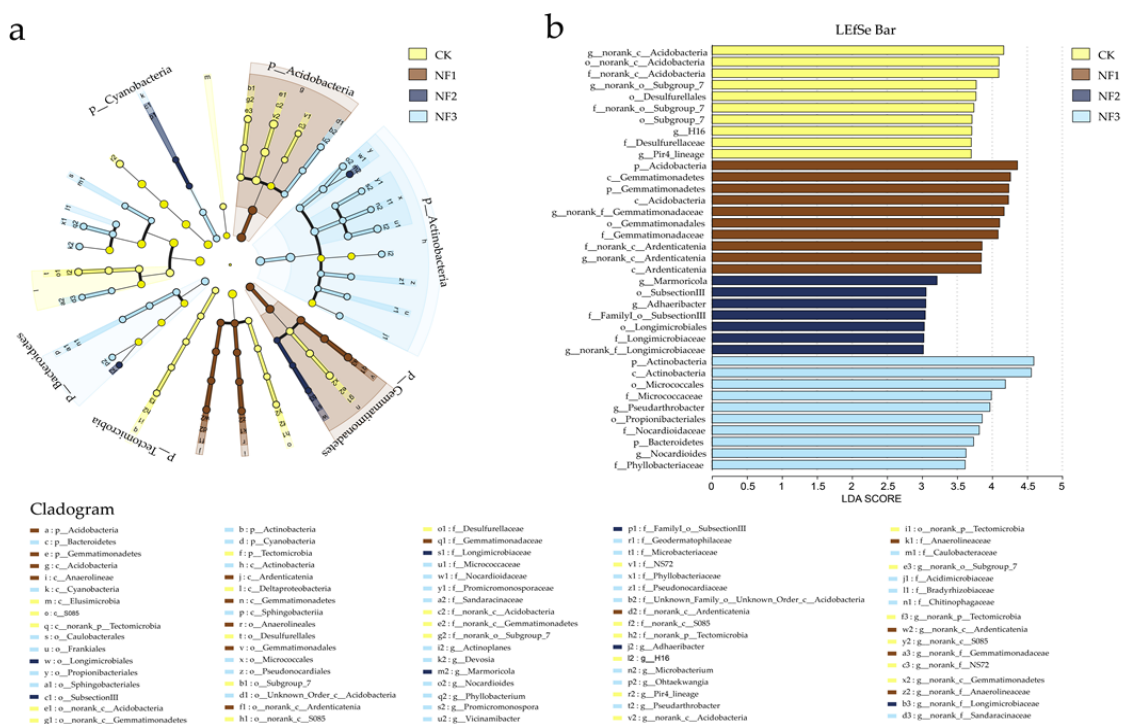


Figure 4. Differentially abundant bacterial taxa across N treatments. a: Cladogram illustrating the taxonomic hierarchy (phylum to genus) of differentially abundant bacterial taxa; b: Bar plot showing linear discriminant analysis (LDA) scores generated by linear discriminant analysis effect size (LEfSe) for taxa with significant differences in relative abundance (LDA threshold > 3). In taxon labels, p, c, o, f, and g denote phylum, class, order, family, and genus, respectively. CK: Control group without N fertilization; NF1: four applications totaling 150 kg N ha⁻¹, with 37.5%, 12.5%, 12.5%, and 37.5% applied from emergence to jointing, jointing to tasseling, tasseling to silking, and silking to dent, respectively; NF2: three applications totaling 150 kg N ha⁻¹, with 33.75%, 32.5%, and 33.75% applied from emergence to jointing, tasseling to silking, and silking to dent, respectively; NF3: two applications totaling 150 kg N ha⁻¹, with 62.5% and 37.5% applied from emergence to jointing and silking to dent, respectively.

The FAPROTAX predicted metabolic functional genes of the rhizosphere soil bacterial community under different treatments mainly included aerobic ammonia oxidation, aerobic nitrite oxidation, nitrate reduction, nitrate respiration, nitrification, nitrite respiration, N fixation, N respiration, and ureolysis (Figure 5a). The abundance of chemoheterotrophy, aerobic_chemoheterotrophy, aromatic compound degradation, and phototrophy of NF3 treatment was significantly higher than that of other treatments ($p < 0.05$, Figure 5b). However, the abundance of nitrification, aerobic nitrite oxidation, aerobic ammonia oxidation, respiration of S compounds, and S respiration of NF3 treatment was lower than that of CK, NF1, and NF2 treatments ($p < 0.05$, Figure 5b).

Effect of different N fertilization frequencies on the yield of forage maize

The yield of forage maize of NF3 treatment (73 925.83 kg ha⁻¹) was 176% and 103% higher than that of CK and NF1 treatments ($p < 0.05$, Table 3). There was no difference in the yield between NF2 (64 754.58 kg ha⁻¹) and NF3 treatments.

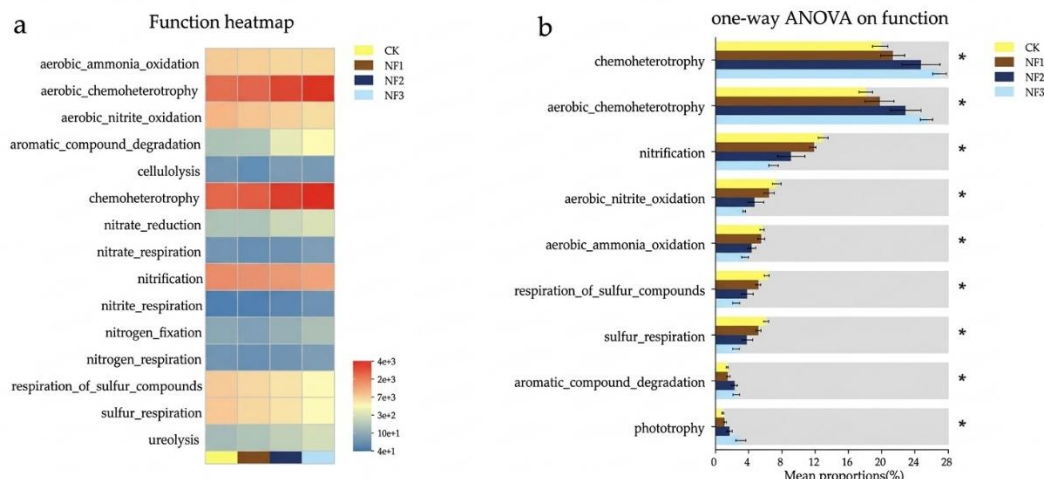


Figure 5. Functional prediction of soil bacterial communities under different N fertilization frequencies using Functional Annotation of Prokaryotic Taxa (FAPROTAX). a: Predicted metabolic functions, including N cycle-related functions; b: Significantly different functions. * $p < 0.05$; ** $p < 0.01$. CK: Control group without N fertilization; NF1: four applications totaling 150 kg N ha⁻¹, with 37.5%, 12.5%, 12.5%, and 37.5% applied from emergence to jointing, jointing to tasseling, tasseling to silking, and silking to dent, respectively; NF2: three applications totaling 150 kg N ha⁻¹, with 33.75%, 32.5%, and 33.75% applied from emergence to jointing, tasseling to silking, and silking to dent, respectively; NF3: two applications totaling 150 kg N ha⁻¹, with 62.5% and 37.5% applied from emergence to jointing and silking to dent, respectively.

Table 3. Shoot fresh weight of forage maize under different N fertilization frequencies. SE: standard error. Values are means $\pm$ SE ($n = 4$). Different lowercase letters indicate significant differences among treatments at $p < 0.05$. CK: Control group without N fertilization; NF1: four applications totaling 150 kg N ha⁻¹, with 37.5%, 12.5%, 12.5%, and 37.5% applied from emergence to jointing, jointing to tasseling, tasseling to silking, and silking to dent, respectively; NF2: three applications totaling 150 kg N ha⁻¹, with 33.75%, 32.5%, and 33.75% applied from emergence to jointing, tasseling to silking, and silking to dent, respectively; NF3: two applications totaling 150 kg N ha⁻¹, with 62.5% and 37.5% applied from emergence to jointing and silking to dent, respectively.

Treatment	Fresh weight kg ha ⁻¹
CK	26 763.38 $\pm$ 76.41 ^d
NF1	36 379.29 $\pm$ 612.43 ^{cd}
NF2	64 754.58 $\pm$ 1544.71 ^{ab}
NF3	73 925.83 $\pm$ 1002.56 ^a

DISCUSSION

Optimized N fertilization frequency increased the diversity of bacterial communities in the rhizosphere soil of forage maize

There are currently different opinions on the impact of N fertilization strategies on soil bacterial community diversity, given the complexity of the ecosystems and the diversity of influencing factors. Previous studies have shown that conventional N fertilization, combined application of organic fertilizer and N fertilizer, and combined application of biochar and N fertilizer significantly impact soil microbial community diversity (Duan et al., 2025; Zhang et al., 2025). In this study, the number of operational taxonomic units (OTUs) unique to N fertilizer treatment emergence to jointing stage 62.5%, silking to dent stage 37.5% (NF3) was the largest among all treatments. The Shannon index of NF3 treatment was significantly higher than that of emergence to jointing stage 37.5%, jointing to tasseling stage 12.5%, tasseling to silking stage 12.5%, silking to dent stage 37.5% (NF1)

and control (CK) treatments, and the Simpson index of NF3 treatment was the lowest. It is generally believed that the higher the Shannon index and the lower the Simpson index, the higher the microbial community diversity (Yin et al., 2024a). Therefore, NF3 treatment has the highest diversity of rhizosphere soil bacterial communities in this study. Related studies have shown that high N levels cause significant enrichment of N cycle related bacteria, such as *Nitrospira* and *Sphingomonas*, reducing the diversity of soil bacteria (Zhao et al., 2025). However, the appropriate N levels enhance the diversity of soil bacterial communities (Zeng et al., 2025). In this study, the total N application rate of the three N fertilization strategies (NF1, NF2, NF3) was consistent (150 kg ha⁻¹). However, the diversity of bacterial communities in the rhizosphere soil of forage maize of NF1, emergence to jointing stage 33.75%, tasseling to silking stage 32.5%, silking to dent stage 33.75% (NF2), and NF3 treatments was all higher than that of CK. Particularly, the diversity of bacterial communities of NF3 treatment was significantly higher than that of CK and NF1 treatments. These findings indicate that under a certain total N application rate, the diversity of rhizosphere soil bacterial communities of forage maize can be improved by adjusting the frequency of N fertilization.

In this study, different N fertilization frequencies also significantly affected the structure of bacterial communities in the rhizosphere soil of forage maize. The nonmetric multidimensional scaling (NMDS) analysis detected a significant separation between NF3 and CK. This may be due to the fact that the bacterial community in the rhizosphere soil of forage maize has changed its structure to adapt to N level changes at different growth stages under different N treatments. Related studies have shown that N fertilization promotes interspecies competition among soil microorganisms, and soil microorganisms also make adaptive adjustments to N fertilization, leading to changes in the community structure (Lin et al., 2023; Wang et al., 2023a).

Optimized N fertilization frequency altered the composition and functional genes of bacterial communities in the rhizosphere soil of forage maize

Proteobacteria, Acidobacteria, Actinobacteria, and Gemmatimonadetes were the main dominant bacteria under different N fertilization frequencies in this study. These bacteria were also observed in other N treatment experiments (Yin et al., 2025). Related study (Dai et al., 2018) has shown that N fertilization increases the relative abundance of Proteobacteria in soil compared with the control. In this study, the relative abundance of Proteobacteria in NF1, NF2, and NF3 treatments was higher than that of CK, and the NF3 treatment had the highest abundance of Proteobacteria among the treatments. This may be due to the fact that Proteobacteria are copiotrophs that are involved in the soil N cycle, and most of the nitrification related bacteria are Betaproteobacteria and Gammaproteobacteria (Liu et al., 2014). The relative abundance of the oligotrophic phylum Acidobacteria was lower under NF2 and NF3 than under CK, suggesting that N availability may constrain taxa adapted to low-nutrient conditions. Actinobacteria was significantly enriched under NF3 at both the phylum and class levels. The orders Micrococcales and Propionibacteriales and the genera *Pseudarthrobacter* and *Nocardioides* were also enriched under NF3, indicating a coordinated response of Actinobacteria-affiliated taxa to the N-fertilization regime. This sensitivity to N management is consistent with Xu et al. (2025), who reported that excessive N application altered the rhizosphere microbial community of Tartary buckwheat and inhibited beneficial *Nocardioides*. Overall, adjusting N fertilization frequency at a fixed total N rate can alter rhizosphere bacterial composition across multiple taxonomic levels and may favor copiotrophic taxa.

Previous FAPROTAX analysis results revealed that N application promoted the functions of aerobic nitrite oxidation, nitrate reduction, and aerobic ammonia oxidation (Wang et al., 2021). In this study, the abundance of functional genes nitrification, aerobic nitrite oxidation, and aerobic ammonia oxidation was significantly lower in NF3 treatment than in NF1 and NF2 treatments. This may be due to the fact that the continuous N fertilization at different growth stages of forage maize in NF1 and NF2 treatments strengthens the N cycle function of soil bacterial communities and increases the abundance of related functional genes compared with the control. Notably, the abundance of bacterial functional genes chemoheterotrophy, aerobic chemoheterotrophy, and aromatic compound degradation of NF3 treatment was significantly higher than that of CK, NF1, and NF2 treatments. Related studies have shown that the bacterial community of chemoheterotrophy can obtain energy and C sources by decomposing N containing organic matter (such as proteins and humus), converting organic N into NH₄⁺, and promoting N mineralization (Phale et al., 2020). Bacterial community of aerobic chemoheterotrophy can participate in nitrification under conditions of sufficient oxygen, such as oxidizing NH₃ to NO₂⁻ and NO₃⁻. The bacterial community of aromatic compound degradation can release bound N by decomposing complex aromatic organic compounds such as lignin derivatives.

The bacterial taxa having these functional genes in this study play an important role in the soil N cycle, promoting crop N absorption and the shoot growth of forage maize of NF3 treatment, resulting in higher yields. In addition, the higher the diversity of bacterial communities, the higher the diversity of their metabolic functions (Guo et al., 2024). In this study, unlike NF1 and NF2 treatments, discontinuous N fertilization at different growth stages in NF3 treatment provides conditions for the construction of niches of non-N cycle functional microorganisms. This may also be the reason for the higher abundance of functional genes chemoheterotrophy, aerobic chemoheterotrophy, aromatic compound degradation, and phototrophy in NF3 treatment.

Optimized N fertilization frequency increased the yield of forage maize

Nitrogen fertilization has always been regarded as an important measure to increase maize yield (Meng et al., 2024). Different N application rates have different impacts on maize yield (Li et al., 2022). Related study (Hammad et al., 2018) has shown that N fertilization can increase the rate of photosynthesis during the vegetative development of maize, and the timing of N fertilization significantly affects maize yield. In this study, under a certain total N application rate, the yield of forage maize of NF3 treatment was significantly higher than that of CK and NF1 treatments. This may be due to the fact that the NF3 treatment obtains a higher N application rate from the seedling to jointing stage, which increases the photosynthesis rate of forage maize and promotes the shoot growth. In addition, in this study, the higher N application rate from the seedling to jointing stage in NF3 treatment may affect N absorption and shoot fresh weight by promoting root system growth. Li et al. (2022) reported that the optimized N application rate (180 kg ha⁻¹) affected maize N absorption and yield by increasing maize root length and root surface area.

CONCLUSIONS

This study analyzed the characteristics of rhizosphere soil bacterial communities and forage maize yield under different N fertilization frequencies. Overall, continuous N fertilization at four growth stages (NF1 treatment) increased the abundance of N cycle functional genes in the rhizosphere soil bacterial community, but did not necessarily improve bacterial community diversity or yield. The two-application N fertilization regime (NF3 treatment), with 62.5% applied from emergence to jointing and 37.5% from silking to dent, increased soil bacterial community diversity and the abundance of functional genes associated with chemoheterotrophy, aerobic chemoheterotrophy, and aromatic compound degradation, and produced the highest forage maize yield. This study provides new insights into crop water and N management under drip irrigation, and also provides a reference for N fertilization for forage maize.

Author contribution

Conceptualization: F.G., F.Y. Methodology: F.Y., M.Z. Software: F.G., M.Z. Validation: F.G., Z.B. Formal analysis: F.G., M.Z. Investigation: F.G., Z.B. Resources: F.Y., M.Z. Data curation: F.Y., M.Z. Writing-original draft: F.G., F.Y. Writing-review & editing: F.G., M.Z., Z.B. All co-authors reviewed the final version and approved the manuscript before submission.

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