

Article

Impacts of Fertilizers with Varying Nitrogen Contents on Millet Yield and Rhizosphere Soil Microbial Communities: Implications for Sustainable Agricultural Development

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Abstract: Nitrogen (N) is a vital macronutrient affecting crop productivity, yet the influence of different N contents in fertilizers on rhizosphere soil microbial diversity is not well understood. This study used high-throughput 16S rRNA and fungal ITS sequencing to examine the impact of varying N contents (low (LN, 90 kg/ha), medium (MN, 120 kg/ha), and high (HN, 150 kg/ha)) on root-associated microbial communities. The results revealed that Millet yield increased with N level: HN (7.14 Kg/ha) > MN (6.33 Kg/ha) > LN (5.62 Kg/ha), with HN yields significantly higher than LN ($p < 0.05$). No significant differences in microbial α -diversity were noted among three groups. *Gemmatimonadota*, *Acidobacteriota*, and *Ascomycota* were the dominant phyla, whereas *Sphingomonas*, *Vicinamibacteraceae*, and *Fusarium* were the predominant genera. LEfSe analysis showed that *Entotheonellaeota* were substantially enriched in the LN group at the phylum level. At the genus level, there was marked enrichment of *Rhodothermaceae*: *Fusicolla* were in the LN group and *PLTA13*, *Luteimonas*, and *Edaphobaculum* were in the MN group, whereas *Aridibacter* and *Parvoiterribacte* were enriched in the HN group. Fertilizers with varying N contents affected rhizosphere soil microbial community composition and millet yield. These findings provide valuable insights for developing scientifically-based fertilization strategies to promote sustainable agricultural ecosystems.



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Keywords: millet; fertilizer application; microbial diversity; microbial community; sustainable agricultural development

1. Introduction

Millet (*Setaria italica* (L.) Beauv.) is a traditional crop with a dominant position in China and a cultivation history of 8000 years [1]. China accounts for 80% of millet cultivation worldwide. Millet is the sixth most produced cereal crop worldwide [2]. Millet grains are rich in various nutrients, including proteins, starch, fats, polyphenols, and flavonoids [3]. In addition to its nutritional value, millet, a gluten-free grain, plays a significant role in diabetes management and blood sugar reduction [4–6]. Additionally, millet is highly drought-resistant, efficient in water use, and tolerant of poor soils, earning it the nickname “iron crop” [7,8]. It is a crucial crop in dryland farming, contributing significantly to dryland agriculture and maintaining dietary diversity [9,10]. Although these traits are excellent, the millet yield has not yet improved.

Soil microorganisms are critical components of ecosystems and drive essential processes, such as nutrient cycling, carbon sequestration, and nitrogen (N) fixation [11,12].

They are crucial for preserving soil health and preventing plant diseases. This includes enhancing nutrient recycling and the transformation of organic matter in soil, boosting plant productivity, and aiding in the management of soil-borne diseases [13,14]. Optimal nitrogen fertilizer application has been shown to increase thousand-grain weight, nitrogen use efficiency, and wheat yield [15]. Similarly, within certain limits, nitrogen fertilization significantly enhances maize yield [16]. Balanced nitrogen application improves the yield and biomass formation of pearl millet [17]. However, excessive application of N fertilizers can lead to suboptimal vegetative growth, impede reproductive development, cause nutrient imbalances, diminish yield and quality, and disrupt the absorption of other essential nutrients such as potassium and phosphorus [18–20]. Conversely, insufficient N supply adversely affects leaf size, plant height, photosynthesis, chlorophyll content, and the synthesis of proteins and nucleic acids [21,22].

Soil microorganisms are influenced by both the beneficial and detrimental effects of soil management practices or disturbances, resulting in alterations in their taxonomy and function [23]. Both the diversity and composition of microorganisms play a crucial role in shaping their ecological functions. Fertilizers, organic amendments, and rhizodeposits have been found to affect the structure and functionality of soil bacterial communities [24–26]. Research indicates that fertilization significantly reduces the diversity of soil microorganisms, including fungi [27,28]. The application of manure fertilizers, including animal waste, plant residues, and composted organic materials, can modify the composition and functionality of soil bacterial communities, thereby influencing the prevalence of N-cycling microorganisms [29,30]. A study reported an increase in the abundance of *Bacteroidetes* and *Gammaproteobacteria* in soils amended with manure [31]. Another study demonstrated that rice husk biochar improved soil bacterial proliferation and significantly increased soil phosphorus availability [32]. However, one piece of research reported that N fertilization did not exert a significant influence on the composition of the microbial community [33]. Additionally, there was no notable variation in the population of rhizosphere fungi when comparing organic and inorganic amendments in long-term fertilization trials [34]. The observed discrepancies could potentially result from variations in nitrogen levels applied across different experiments, along with differences in ecosystems, seasons, and plant species.

Limited research has focused on the structure and diversity of rhizosphere bacterial communities in millet fields under various fertilization rates. High-throughput DNA sequencing provides a swift and thorough method for investigating soil microbiota. In this research, field experiments were carried out on millet (Datong 29) using fertilizers with different nitrogen levels. The bacterial community structure in rhizosphere soil under these treatments was analyzed through 16S rRNA sequencing, while fungal communities were assessed using ITS sequencing. Our objective was to ascertain the predominant bacterial communities and establish an optimal fertilization rate to enhance millet growth and yield.

2. Materials and Methods

The millet variety Datong 29, developed by the Crop Research Institute of the Alpine Region, Shanxi Academy of Agricultural Sciences, was used in this study. In the summer of 2023, during the foxtail millet growing season, field trials substituting organic fertilizers were conducted at the Huaiyuan Maozao Base in Shanxi Province (elevation 1020 m; 113.29° E, 39.93° N). This region exhibits a warm temperate continental monsoon climate, with an average yearly temperature of 13.6 °C and an annual precipitation of 350 mm. Prior to fertilization, soil samples were taken from a depth of 20 cm utilizing the grid sampling technique. Soil properties were as follows: pH, 8.51; total phosphorus, 313 mg/kg; total N, 535 mg/kg; total potassium, 9.41×10^3 mg/kg; available potassium, 178 mg/kg;

available phosphorus, 178 mg/kg; alkali-hydrolyzable N, 145 mg/kg; organic matter content, 32.2 g/kg; moisture content, 15.4%. The soil type was sandy loam.

The experimental millet field was organized into plots measuring 13.34 m² (2.0 m × 6.67 m), with each treatment conducted in triplicate using a randomized block design. Basal fertilization included 150 kg/ha² N, 90 kg/ha² P₂O₅, and 90 kg/ha² K₂O. This study included three nitrogen fertilization treatments, applied using urea (46% N), with each treatment replicated twice: (1) low-dose N (LN, 90 kg/ha N), (2) medium-dose N (MN, 120 kg/ha N), and (3) high-dose N (HN, 150 kg/ha N). Preliminary research identified 120 kg/ha as the standard nitrogen application rate for local production. In all fertilization strategies, P₂O₅ and K₂O were applied at 90 kg/ha. After sowing, the millet was regularly irrigated and sprayed with millet-specific herbicides. During the harvest season, the millet panicles were collected for yield assessment. The panicles were dried and threshed to determine the yield. At maturity, soil samples associated with roots were gathered from each plot. To achieve this, loosely attached soil was first shaken off the roots, followed by brushing off the tightly adhered soil, which was then collected as rhizosphere soil. The collected soil samples were subsequently stored at −80 °C for later high-throughput sequencing of 16S rRNA and fungal ITS regions. Five biological replicates were prepared to ensure reliable results.

2.1. DNA Isolation, Library Preparation, and Sequencing

DNA was isolated from each sample utilizing the OMEGA Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) following the protocol provided by the manufacturer. The DNA concentration was determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The 16S rRNA gene and the internal transcribed spacer (ITS) region were sequenced to investigate bacterial and fungal communities, respectively. For the ITS1 region, primers ITS-1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS-1R (5'-GCTGCGTTCTTCATCGATGC-3') were employed. The V3–V4 region of the 16S rRNA gene was amplified using primers 338F (5'-ACTCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Unique 7 bp barcodes were added to each sample for multiplex sequencing. The PCR protocol began with an initial denaturation at 98 °C for 5 min, followed by 25 cycles consisting of 30 s of denaturation at 98 °C, 30 s of annealing at 53 °C, and 45 s of extension at 72 °C. The procedure ended with a final extension at 72 °C for 5 min. PCR products intended for 16S rRNA and ITS sequencing were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified with a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). The purified amplicons were then pooled in equal concentrations and sequenced using paired-end 2 × 300 bp reads on the Illumina MiSeq platform with the MiSeq Reagent Kit v3 (Personal Biotechnology, Shanghai, China).

2.2. Bioinformatics Analysis

The initial sequencing data were processed using the QIIME software (v1.8.0) [35]. Reads matching the barcodes were assigned to their respective samples and considered valid. Low-quality sequences were removed, and paired-end reads were merged using the FLASH software (version 1.2.3) [36]. After detecting and removing chimeras, high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using UCLUST software [37]. Representative sequences for each OTU were selected using the default settings. These sequences were then aligned against UNITE (for ITS sequences) and SILVA 132 databases (for 16S rRNA sequences) [38] to determine their taxonomic classification. To examine the phylogenetic relationships among OTUs, multiple sequence alignments were conducted using Muscle (v3.8.31) [39]. QIIME2 (v2019.10) was used to calculate the Weighted Unifrac distances and create UPGMA clustering trees.

Alpha (α)-diversity, which includes both species richness and evenness, was assessed using QIIME2 (v2019.10). Chao1 and observed species indices were used to assess species richness, whereas Shannon and Simpson indices were used to evaluate species diversity. Pielou's evenness metric was used to assess species evenness, and Good's coverage was calculated to evaluate species coverage. Differences in α -diversity among groups were statistically analyzed using the Kruskal–Wallis test, with a p -adjusted cutoff value of 0.05, implemented via the agricolae package. Beta diversity analysis was performed to explore the structural variation in microbial communities across samples using Bray–Curtis metrics, which were visualized using principal coordinate analysis (PCoA). A Venn diagram was created to show the shared and unique OTUs among samples or groups based on the occurrence of OTUs, regardless of their relative abundance. Taxonomic abundances at various levels (phylum, class, order, family, and genus) were statistically compared among the samples using Metastats. Linear discriminant analysis effect size (LEfSe) was used to identify differentially abundant taxa across groups using default parameters. Additionally, we employed an R package to perform Pearson correlation analysis to determine the correlation between millet yield and the composition of bacterial and fungal communities.

2.3. Statistical Analysis

The differences in millet yield among the groups were analyzed using one-way analysis of variance (ANOVA) in GraphPad Prism 8. The results were presented as the mean $\pm$ standard deviation (SD), with statistical significance defined as a p -value less than 0.05.

3. Results

3.1. Effects of Different Fertilization Rates on Millet Yield

The millet yields were in the order of HN (7.14 Kg/ha) > MN (6.33 Kg/ha) > LN (5.62 Kg/ha). The HN group exhibited a significantly higher millet yield than the LN group ($p < 0.05$, Figure 1). However, no significant differences in millet yield were found between the LN and MN groups or between the MN and HN groups ($p > 0.05$, Figure 1).

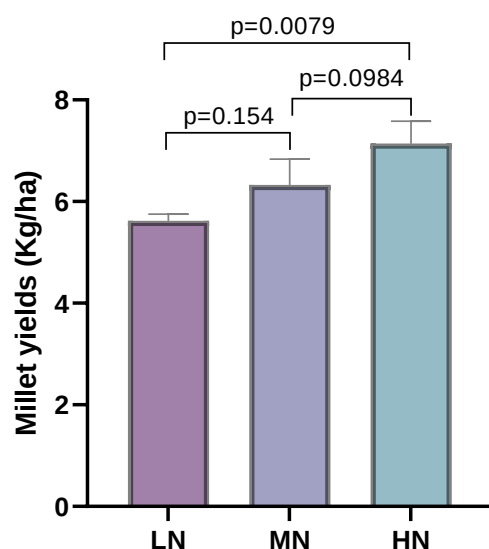


Figure 1. The yield of millet in the three fertilizer treatment groups ($n = 3$).

3.2. Bacterial Diversity

The values of the indices Chao1 and observed species index of species richness initially increased and then decreased with increasing fertilization rates. Similarly, the values of Shannon diversity index first increased and then decreased as fertilization levels increased,

whereas those of the Simpson index consistently decreased at higher fertilization rates. The values of the Pielou's index of species evenness decreased with increasing fertilization levels, whereas those of the Good's coverage index initially decreased and then increased as fertilization rates increased. No notable variations were detected among the three groups in terms of richness, diversity, evenness, or coverage ($p > 0.05$; Figure 2A).

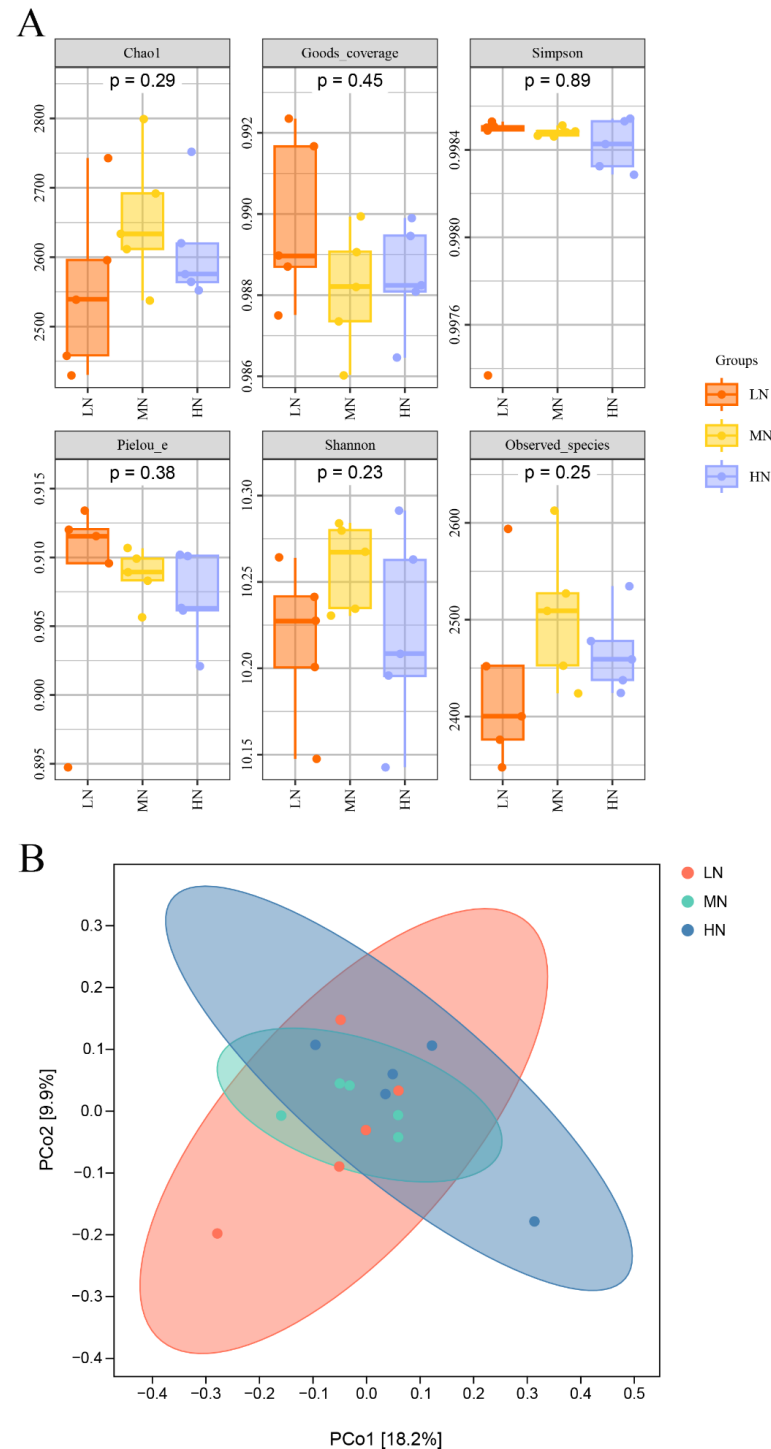


Figure 2. Bacterial diversity after chemical fertilizer treatment with different nitrogen content. **(A)** Alpha diversity; all the data of alpha diversity are presented based on the box line plots of Chao1, Goods_coverage, Simpson, Pielou_e, Shannon, and Observed_species of OTUs. **(B)** Principal coordinate analysis (PCoA) of bacterial community composition in chemical fertilizer treatment with different nitrogen content. The results indicate no significant differences among the three groups ($p > 0.05$). LN, low-dose N; MN, medium-dose N; HN, high-dose N.

Subsequently, we employed PCoA to assess the β -diversity of bacterial communities across the three groups. The PCoA plot indicated that the samples from the different groups did not exhibit significant separation or distinct clustering, suggesting no notable differences among the three groups (Figure 2B).

3.3. Bacterial Community Structure

The top ten bacterial phyla in each group were Proteobacteria, Gemmatimonadota, Acidobacteriota, Actinobacteriota, Chloroflexi, Bacteroidota, Myxococcota, Verrucomicrobiota, Planctomycetota, and Methyloirabilota. Among these, the dominant bacterial phyla in all rhizosphere soil samples were Proteobacteria, Gemmatimonadota, Acidobacteriota, and Actinobacteriota, each with relative abundance exceeding 10%, accounting for 21.92–22.34%, 19.34–21.03%, 18.39–19.94%, and 17.32–18.65%, respectively (Figure 3A).

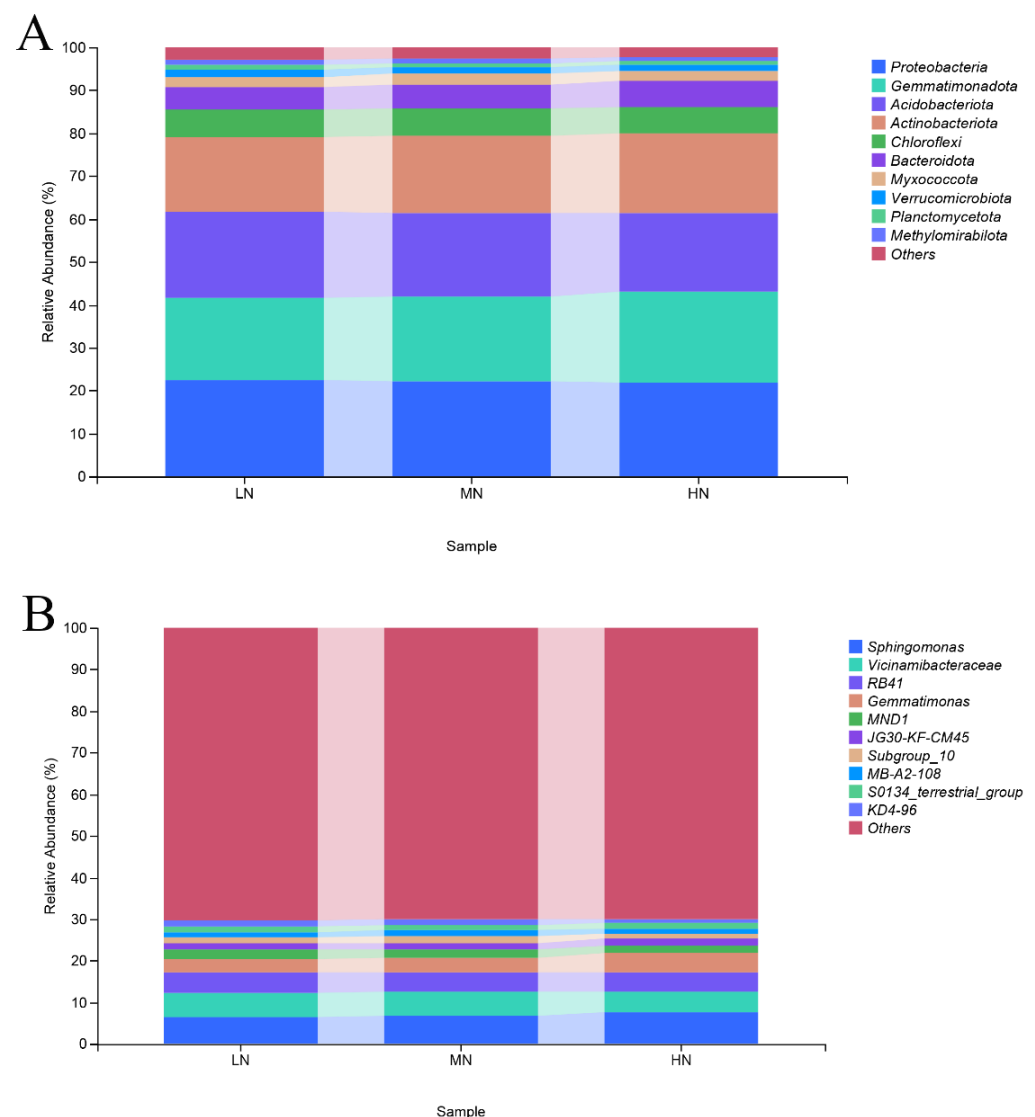


Figure 3. The 16S rRNA gene-based bacterial community compositions at the phylum (A) and genus (B) levels in three fertilizer treatment groups.

The top ten bacterial genera in each group were *Sphingomonas*, *Vicinamibacteraceae*, *RB41*, *Gemmatimonas*, *MND1*, *JG30-KF-CM45*, *Subgroup_10*, *MB-A2-108*, *S0134_terrestrial_group*, and *KD4-96*. Among these, the dominant bacterial genera in all rhizosphere soil samples were *Sphingomonas* and *Vicinamibacteraceae*, each with relative abundance exceeding 5%, accounting for 6.54–7.69% and 5.01–5.75%, respectively (Figure 3B).

3.4. Taxonomic Composition of Bacteria

The Venn diagram indicated that there were 2355 shared OTUs among the HN, MN, and LN groups. Additionally, the LN, MN, and HN groups had 3733, 3609, and 3741 unique OTUs, respectively (Figure 4A).

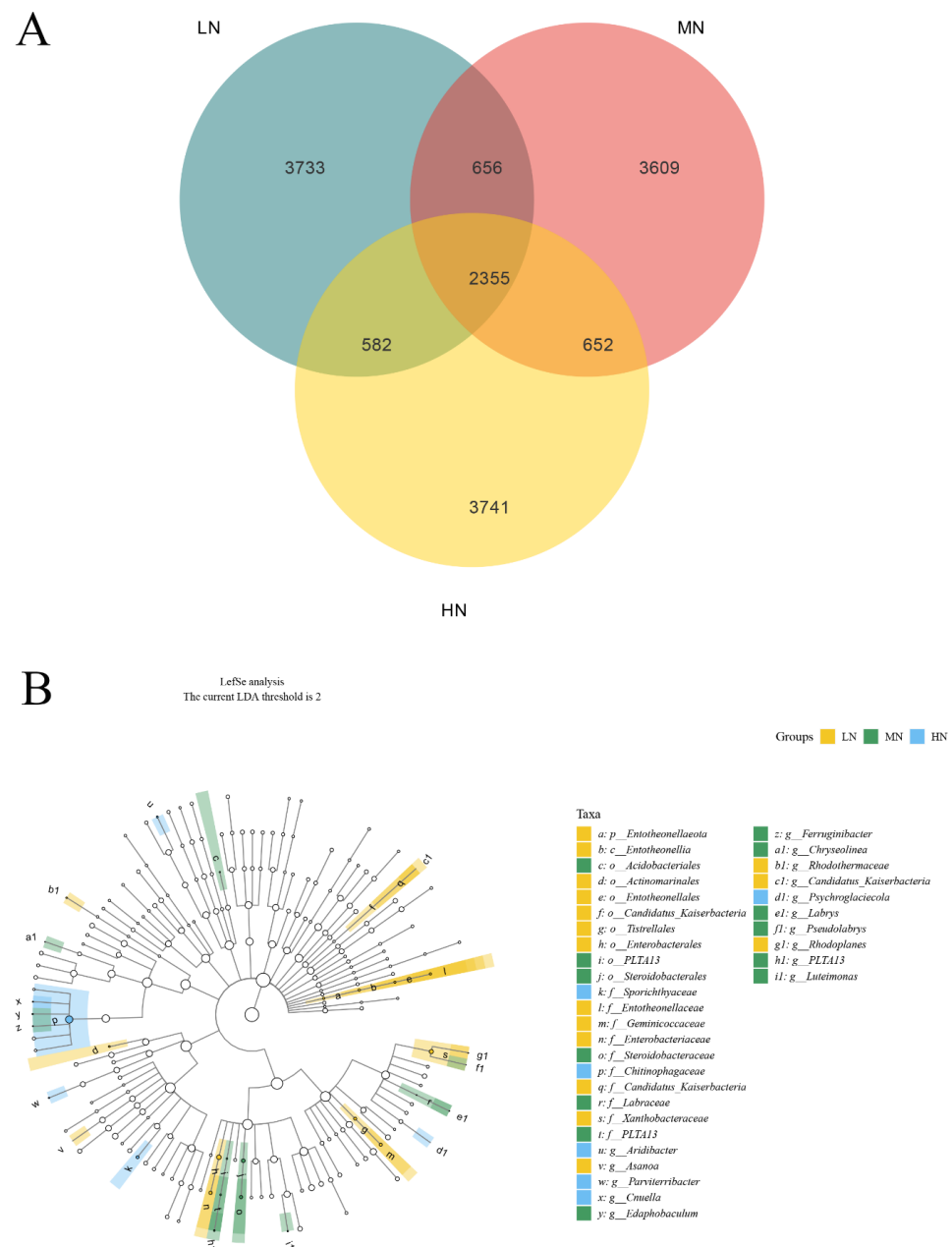


Figure 4. Classification and composition of microbial communities. (A) The Venn map of OTUs in three fertilizer treatment groups. (B) LEfSe cladograms showing taxa with different abundance values. Taxonomic cladogram obtained from LEfSe analysis of bacterial 16S rRNA sequences. Only taxa meeting an LDA significance threshold of 2 for bacterial communities are presented.

LEfSe analysis was conducted to detect bacterial taxa that exhibited significantly different abundances across the groups, with an LDA score of >2. At the phylum level, *Entothaeonellaeota* were significantly enriched in the LN group. At the genus level, there was significant enrichment of *Rhodothermaceae*, *Asanoa*, *Candidatus Kaiserbacteria*, and *Rhodoplanes* in the LN group; *PLTA13*, *Luteimonas*, *Edaphobaculum*, *Ferruginibacter*, *Pseudolabrys*, *Labrys*, and *Chryseolinea* in the MN group; and *Aridibacter*, *Parviterribacter*, *Cnuella*, and *Psychroglaciecola* in the HN group.

3.5. Fungal Diversity

There were no notable differences in the fungal richness, diversity, evenness, or coverage among the three groups (Figure 5A). PCoA based on Bray–Curtis distances was employed to further elucidate the effects of different fertilization rates on the soil fungal community structure. As shown in Figure 5B, the first principal coordinate (PCo1) accounted for 23.7% of the total variation, whereas the second principal coordinate (PCo2) accounted for 16.6%. PCoA revealed that samples from different groups did not significantly separate or cluster, indicating minimal differences among the groups.

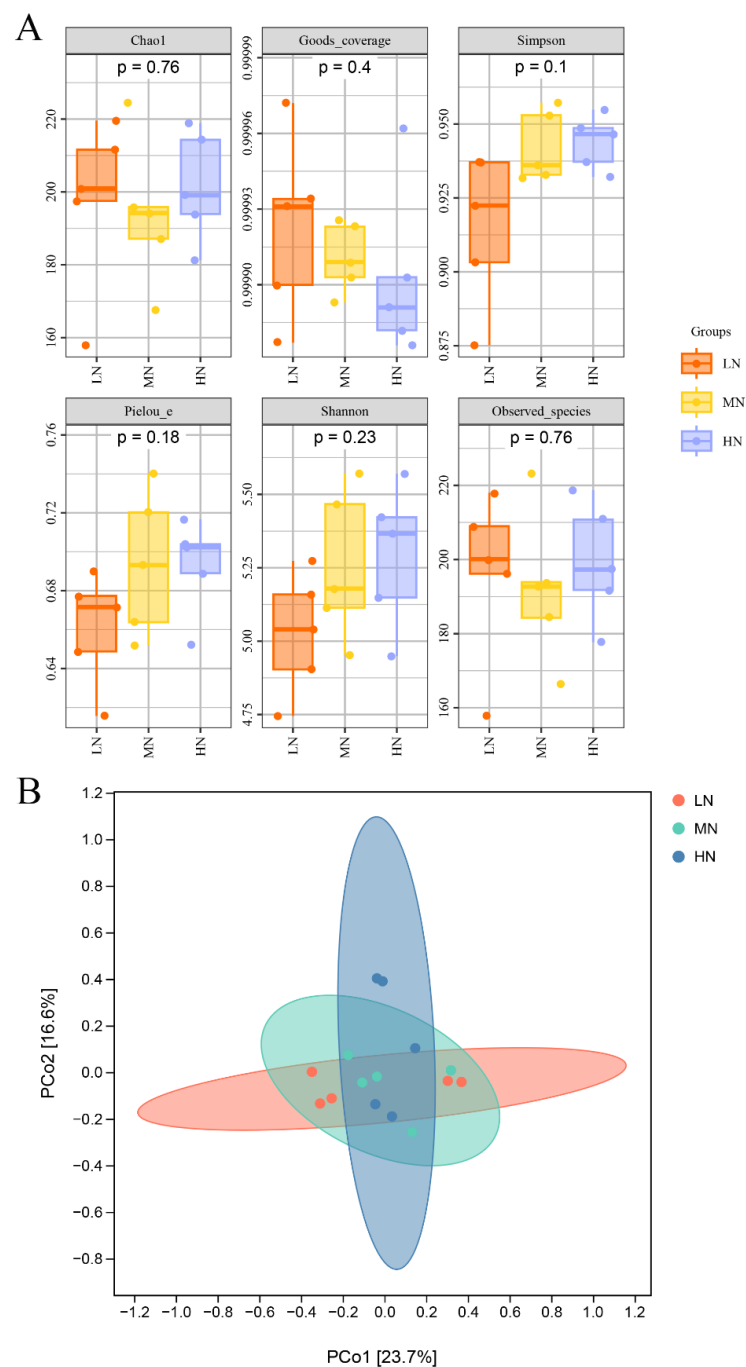


Figure 5. Fungal diversity after chemical fertilizer treatment with different nitrogen contents. (A) Alpha diversity; all the data of alpha diversity are presented based on the box line plots of Chao1, Goods_coverage, Simpson, Pielou_e, Shannon, and Observed_species of OTUs. (B) Principal coordinate analysis (PCoA) of fungal community composition in chemical fertilizer treatment with different nitrogen content. The results indicate no significant differences among the three groups ($p > 0.05$).

3.6. Fungal Community Structure

The predominant fungal phyla identified across all the groups were Ascomycota, Mortierellomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Aphelidiomycota, Mucoromycota, Kickxellomycota, Rozellomycota, and Olpidiomyota. Among these, Ascomycota emerged as the dominant phylum in all soil samples, exhibiting relative abundance exceeding 10% and accounting for 81.45–90.53% of the total fungal community (Figure 6A). Moreover, the LN and HN groups exhibited significantly higher abundances of Ascomycota than the MN group.

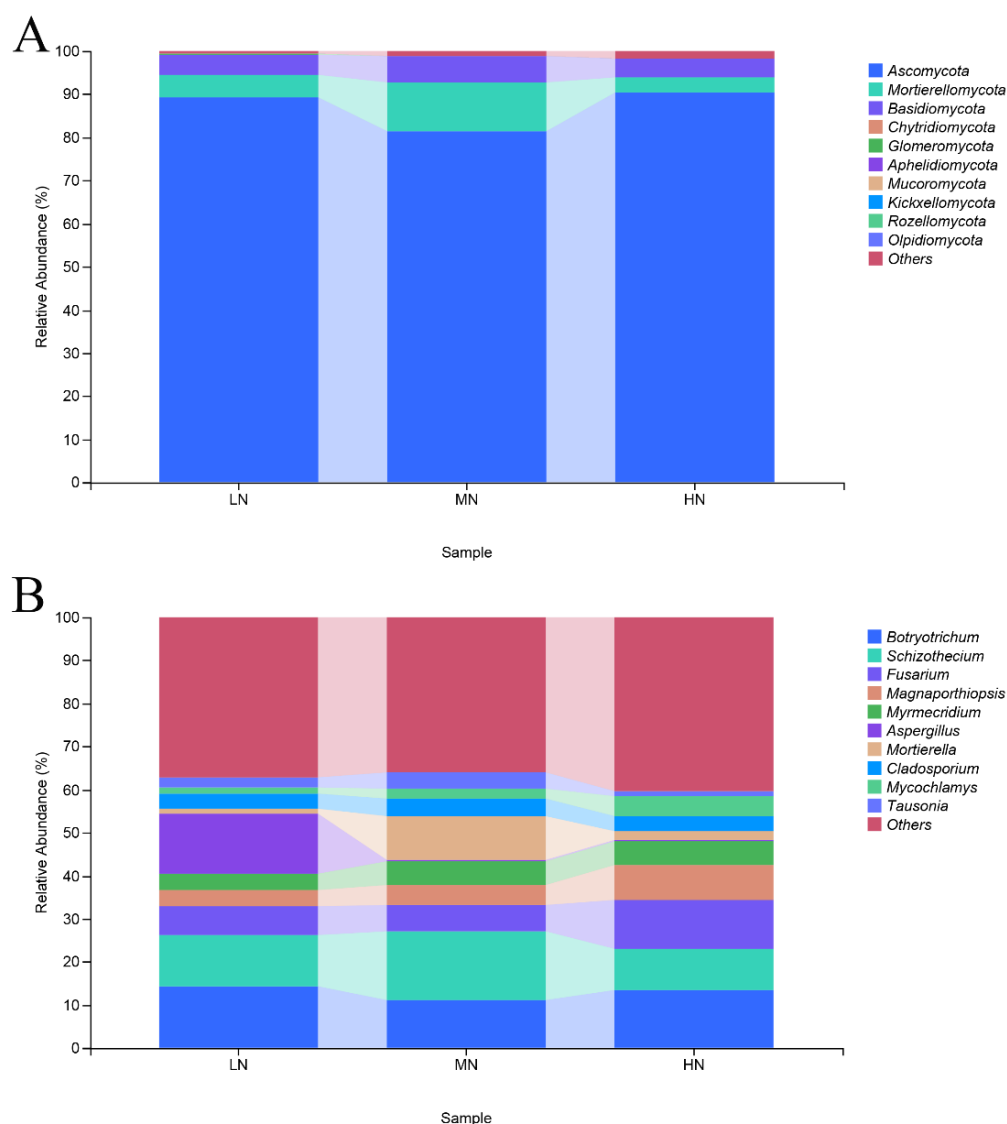


Figure 6. The ITS gene-based fungal community compositions at the phylum (A) and genus (B) levels in three fertilizer treatment groups.

The most prevalent fungal genera identified in each group were Botryotrichum, Schizothecium, Fusarium, Magnaporthiopsis, Myrmecridium, Aspergillus, Mortierella, Cladosporium, Mycochlamys, and Tausonia. Among these, Botryotrichum, Schizothecium, and Fusarium were the dominant genera across all soil samples, with relative abundances greater than 5%, accounting for 11.26–14.38%, 9.48–15.85%, and 6.20–11.37% of the total fungal population, respectively (Figure 6B). Furthermore, the LN and HN groups showed an increased abundance of Botryotrichum and Fusarium and a decreased abundance of Schizothecium, compared with the MN group.

3.7. Taxonomic Composition of Fungal

The Venn diagram illustrates 155 shared OTUs among the HN, MN, and LN groups (Figure 7A). Additionally, the LN, MN, and HN groups had 357, 336, and 411 unique OTUs, respectively.

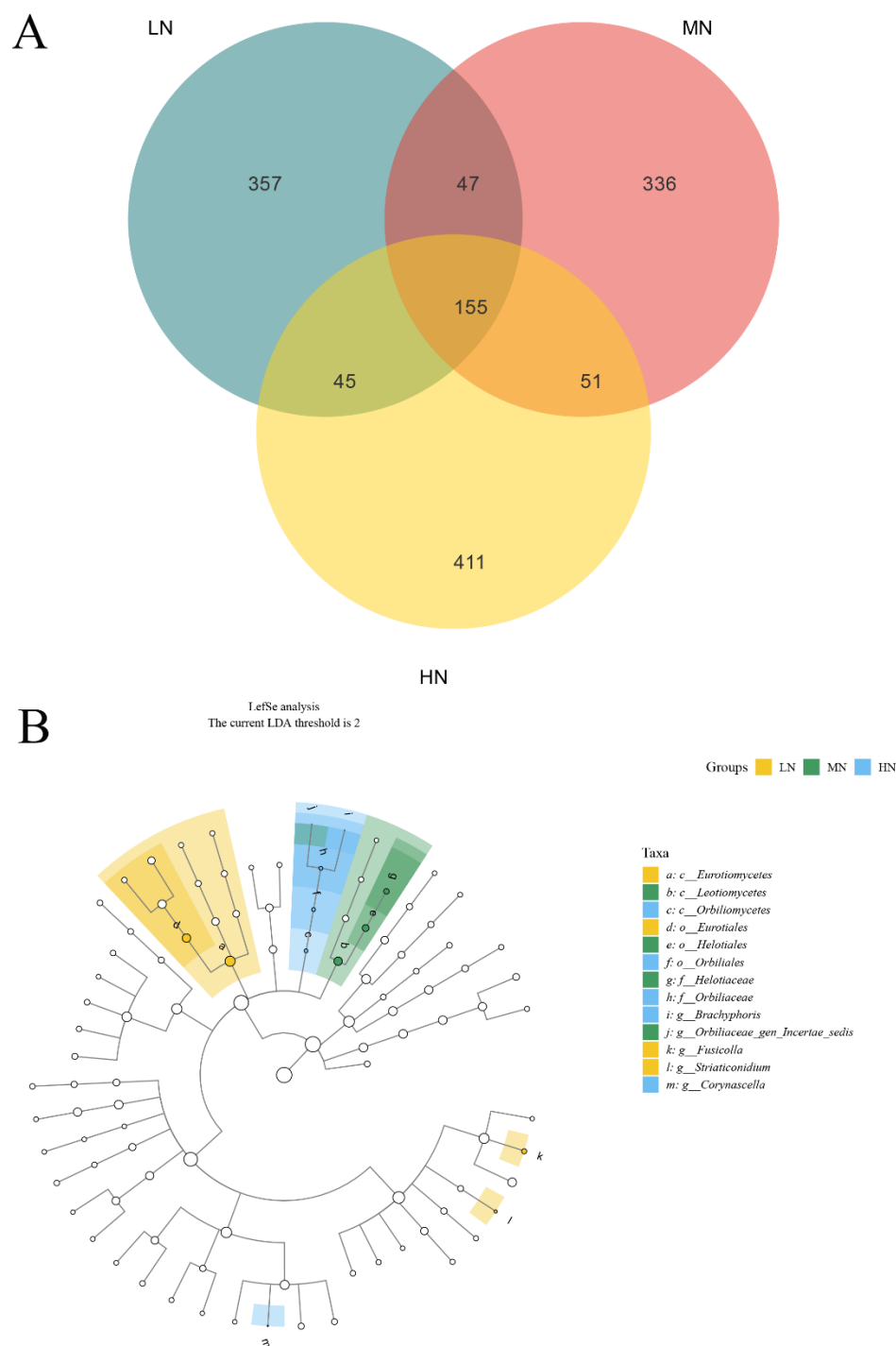


Figure 7. Classification and composition of fungal communities. **(A)** The Venn map of OTUs in three fertilizer treatment groups. **(B)** LEfSe cladograms showing taxa with different abundance values. Taxonomic cladogram obtained from LEfSe analysis of fungal ITS rRNA sequences. Only taxa meeting an LDA significance threshold of 2 for fungal communities are presented.

LEfSe analysis was performed to detect fungal taxa with significantly different abundances among the groups, using a p -value threshold of 0.05 and LDA score of 2 (Figure 7B). At the fungal genus level, there was significant enrichment of *Fusicolla* and *Striaticonidium*

in the LN group, *Orbiliaceae_gen_Incertae_sedis* in the MN group, and *Corynascella* and *Brachyphoris* in the HN group.

3.8. The Correlation Between Millet Yield and the Composition of Bacterial and Fungal Communities

Pearson correlation analysis demonstrated a relationship between millet yield and the composition of bacterial and fungal communities. Millet yield was positively correlated with the abundance of soil bacterial communities belonging to Actinobacteriota ($p_{\text{Actinobacteriota}}$) and negatively correlated with those belonging to Chloroflexi ($p_{\text{Chloroflexi}}$) ($p < 0.05$, Figure 8). However, no significant correlation was observed between millet yield and fungal community abundance ($p > 0.05$, Figure 8).

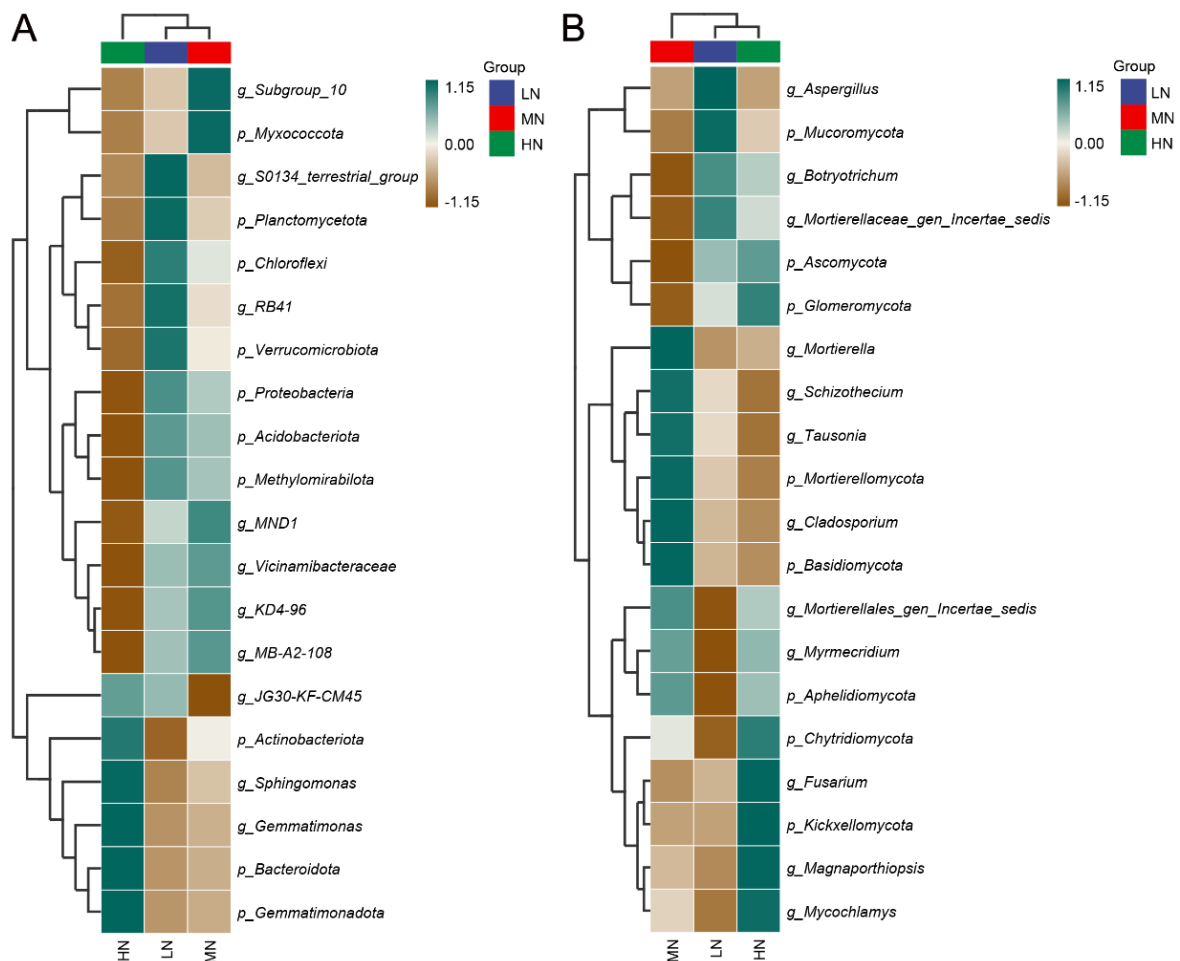


Figure 8. A heatmap depicting the correlation between millet yield and the composition of bacterial (A) and fungal (B) communities.

4. Discussion

Millet, which belongs to the family Poaceae, is among the world's oldest domesticated crops [40]. Recently, it has attracted considerable interest because of its exceptional drought resistance, adaptability, and efficient water use [41]. Despite its extensive history of cultivation and consumption, much remains to be understood regarding the best cultivation practices, particularly in terms of fertilization. Crops absorb beneficial substances, including minerals and micronutrients, from the soil and fertilizers to promote the growth and development of the aboveground parts [42]. Fertilizer application is a common agricultural practice aimed at enhancing crop productivity [43]. In this study, the effects of fertilizers with different N contents on millet yield and rhizosphere microbial diversity were eval-

uated. Our findings indicate that, compared to LN fertilizer, HN fertilizer significantly increased millet yield and altered the rhizosphere microbial community.

Previous studies have demonstrated that fertilization increases nutrient concentrations in agricultural soils [44,45]. Nitrogen fertilizers are particularly effective in enhancing crop yields, especially for cereals such as maize and wheat [46]. Research has shown that the combined application of N and phosphorus promotes the formation of soil aggregates, accelerates fertilizer utilization, enhances the ability of the soil to supply nutrients, and ultimately boosts crop yield [47]. Another study found that integrating N fertilizers with other types of fertilizer could significantly boost crop yields [48]. Consistent with previous findings, we observed that millet yields followed the order HN > MN > LN. The HN group demonstrated a markedly greater millet yield than the LN group.

Soil microbial diversity is essential for the stability of soil ecosystems [49]. The diversity index is an important measure of soil microbial community diversity. A higher diversity index indicates greater richness and evenness in the distribution of microbial communities [35]. Our results demonstrate that varying the N content of fertilizers does not affect the richness and diversity of fungi and bacteria in the rhizosphere of foxtail millet. This finding is consistent with those of previous studies. Wang et al. reported that different fertilization methods significantly influenced soil bacterial community structure but did not significantly affect overall soil bacterial α -diversity [50]. Similarly, Chen et al. found no significant correlation between the application methods of slow-release fertilizers and the α -diversity index [51]. However, Sun et al. demonstrated that prolonged N fertilization reduces diversity and modifies the composition of soil archaeal and bacterial communities [52]. This discrepancy may be attributed to the insufficient duration of fertilization in our study, as changes in soil microbial diversity owing to fertilization are likely long-term processes.

Microbial communities, including bacteria and fungi, are extensively found in agricultural soils and are essential for ecosystem processes and nutrient cycling within the soil [53–55]. Microbial communities have been studied extensively across various ecosystems, including forests, grasslands, tundras, and deserts [56]. Numerous studies have identified *Gemmatimonadota*, *Acidobacteriota*, *Actinobacteriota*, and *Ascomycota* as the dominant phyla within these bacterial and fungal communities, which is consistent with our findings. In this study, *Gemmatimonadota*, *Acidobacteriota*, *Actinobacteriota*, and *Ascomycota* were found to be the predominant phyla. *Gemmatimonadota* are the eighth most abundant bacterial phylum in the soil, comprising approximately 1–2% of global soil bacteria [57]. *Gemmatimonadota* are recognized for their pivotal influence on plant diversity and nutrient cycling (including carbon, N, phosphorus, and sulfur) within plant ecosystems [58]. *Acidobacteriota* are regarded as ubiquitous and abundant soil bacteria. The phylum *Acidobacteriota* was significantly correlated with soil pH and N availability [59]. Li et al. also found that *Acidobacteriota* were significantly positively associated with total N content [60]. Ji et al. observed that, compared with the control, NPK fertilizer, organic fertilizer, and their combination significantly increased the abundance of *Acidobacteriota* [61]. Among beneficial microorganisms, *Actinobacteria* are one of the most abundant bacterial groups and play a vital role in plant growth and yield performance [62,63]. The abundance of *Actinobacteria* was positively correlated with the rate of N application [64]. The increase in the relative abundance of *Actinobacteria* is linked to prolonged N fertilizer application, as this phylum thrives in nutrient-rich environments and is essential for regulating plant nutrient absorption and biological N fixation [65]. Wolna-Maruwka et al. found that the application of organic fertilizer led to an increased abundance of *Actinobacteria* [66], which partially aligns with our results. *Ascomycota* are essential for the decomposition of organic material in rhizosphere soil, and a decline in their population could negatively affect soil

fertility [67]. The dominant genera identified in this study were *Sphingomonas*, *Vicinamibacteraceae*, *Botryotrichum*, *Schizothecium*, and *Fusarium*. *Sphingomonas* are known to play a crucial role in fostering positive interactions within microbial communities in contaminated soils [68,69]. *Vicinamibacteraceae*, the first family within sd6 *Acidobacteria* [70], contain genes related to butyrogenic degradation and prefer to utilize complex organic compounds [71]. Additionally, a positive correlation between *Vicinamibacteraceae* and available soil N content has been documented [72]. Furthermore, *Botryotrichum* showed a positive correlation with soil pH, total N, and organic carbon [73]. These findings suggest that fertilizers with varying N contents lead to changes in the microbial community of millet root zone soils.

To further explore the effects of biofertilizers on rhizosphere communities, LEfSe analysis was used to identify microbial biomarkers among various groups. The LEfSe results demonstrated significant enrichment of *Entothionellaeota* at the phylum level in the LN group. This finding is consistent with that of Zhang et al., who reported a strong correlation between *Entothionellaeota* and soil nutrient content [74]. At the genus level, there was marked enrichment of *Rhodothermaceae*, *Rhodoplanes*, and *Fusicolla* in the LN group, *PLTA13*, *Luteimonas*, and *Edaphobaculum* in the MN group, and *Aridibacter*, *Parviterribacter*, *Corynascella*, and *Brachyphoris* in the HN group. These findings align with previous results. For example, Li et al. found a positive correlation between N uptake rate and *Parviterribacter* [75]. *Edaphobaculum* is also an important indicator of soil quality [76]. However, excessive N input appears to promote soil-borne pathogens such as *Luteimonas*, while inhibiting beneficial microorganisms such as nitrifiers [77]. These findings suggest an association between different N levels in fertilizers and competition among microbial species, which may coincide with alterations in the soil microbial community structure.

Additionally, Pearson correlation analysis indicated a positive relationship between millet yield and the abundance of soil bacterial communities associated with *Actinobacteriota* (*p_Actinobacteriota*), while a negative correlation was found with those related to *Chloroflexi* (*p_Chloroflexi*). There was no significant correlation between millet yield and the abundance of fungal communities. *Actinobacteriota* are known to enhance plant health and soil quality by decomposing organic matter and producing antibiotics. They convert organic matter into forms easily absorbed by plants, inhibit harmful microorganisms, and improve crop growth and disease resistance, while also enhancing soil structure and fertility [78]. Previous studies have demonstrated that *Actinobacteriota* produce active enzymes that efficiently degrade organic carbon, thus accelerating the decomposition of straw and other organic materials [79,80]. These findings imply that *p_Actinobacteriota* might be a contributing factor to the increased millet yield observed in the HN treatment.

This study has several limitations. Firstly, it only measured the microbial yield in the soil related to production efficiency, without assessing the nitrogen content in the plant stems. Secondly, the study did not investigate the long-term effects of fertilizers with varying nitrogen content on microbial communities and millet yield. Consequently, further research is needed to explore these issues.

5. Conclusions

In conclusion, fertilizers with different N contents significantly impact soil microbial communities and millet yield, with higher N levels notably increasing yield. Fertilizers with different N contents greatly influenced the species abundance and community composition of soil fungi and bacteria. This study provides evidence that soil biodiversity and key microbial taxa are crucial for improving millet yield and soil function under fertilizers with varying N contents. Future research should investigate the long-term effects of fertilizers with varying N contents on microbial communities and millet yield.

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Conflicts of Interest: The authors declare that they have no competing interests.

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