RESEARCH ARTICLE

Effects of maize-potato intercropping and different fertilization methods on maize rhizosphere soil microorganisms and yield

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Continuous maize monoculture often leads to soil micro-ecological imbalance and yield decline. Rational intercropping combined with strategic nitrogen (N) fertilizer management represents a promising approach to mitigate these negative effects and promote sustainable agriculture. This research conducted a field experiment using maize monoculture as the control (CK). Within the maize-potato intercropping system, four N fertilization treatments were established including conventional intraspecific N application (T1), reduced intraspecific N application (T2), conventional interspecific N application (T3), and reduced interspecific N application (T4). The rhizosphere bacterial and fungal community structures were analyzed *via* 16S rRNA and ITS gene sequencing, respectively. These microbiological data were integrated with key agronomic traits and yielded indicators to comprehensively evaluate the effects of intercropping and reduced N fertilization on the rhizosphere micro-ecosystem and maize growth. The results showed that the T3 treatment demonstrated superior performance in maintaining both bacterial richness and fungal diversity and significantly enhanced maize plant height (308.60 cm), stem diameter (32.46 mm), and leaf area (1,214.57 cm²). Notably, the T4 treatment successfully maintained high fungal diversity under reduced N conditions and achieved a maize yield of 24,432.75 kg/ha, representing a 26.3% increase over the control. Analysis of the microbial community structure revealed that intercropping significantly increased the abundance of beneficial phyla such as *Actinobacteriota*. In contrast, the T2 treatment induced an abnormal proliferation of the pathogenic *Olpidium* (*Phylum Olpidiomycota*) to 12.7%, alongside an elevated proportion of unclassified bacteria, suggesting potential disruption to the community's stability. Maize-potato intercropping, particularly when combined with reduced interspecific N application, effectively optimized the rhizosphere microbial community structure, stabilized microbial diversity, and substantially improved maize yield. This study provided valuable theoretical support for developing efficient intercropping systems that reduced fertilizer inputs while enhancing productivity.

Keywords: intercropping; nitrogen reduction; interspecific; intraspecific; rhizosphere soil; microorganisms; yield.

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Introduction

Studies have shown that long-term maize monoculture and excessive application of compound fertilizers severely degrade soil structure [1], which leads to an imbalance in soil nutrient ratios, alterations in the microbial community structure, and a progressive increase in soil salinity [2]. These issues contribute to secondary problems such as soil salinization, acidification, and compaction alongside diminished water retention and aeration. Consequently, physical properties like soil bulk density increase while aggregate structure decreases, ultimately triggering severe replant disease symptoms including stunted growth, reduced yield, poor quality, and increased susceptibility to pests and diseases [3].

Maize-potato intercropping can optimize resource utilization. The practice of intercropping maize with potatoes has been widely validated by numerous studies as a primary strategy for mitigating potato-specific continuous cropping obstacles [4]. Intercropping systems generally enhance land use efficiency (LUE) with potato yields potentially increasing under favorable conditions. Although some studies indicate that potatoes exhibit competitive effects on maize, mutual benefits can be achieved through optimized planting density and intercropping row spacing [5]. Potatoes enhance soil nitrogen uptake, improve soil microbial activity, enzyme activity, and organic matter content, while corn provides mulching to prevent soil erosion. Long-term intercropping helps maintain favorable soil chemical properties [6]. Maize-potato intercropping when applied to continuously cropped soil can significantly increase organic matter content and facilitate a shift towards a more fertile, "bacteria-dominant" soil type, demonstrating significant efficacy in alleviating replant disorders [7]. Maize is one of the world's three major staple crops, and its high and stable yield is directly linked to national food security [8]. By optimizing cropping systems and nitrogen (N) fertilizer management, it can simultaneously reduce chemical fertilizer application, enhance land use efficiency, mitigate continuous cropping obstacles, and promote the development of resource-conserving agriculture [9]. Intercropping can foster mutual benefits between species through mechanisms such as complementary water use, nutrient mobilization, and root recognition cues, which guide root proliferation towards inter-species zones [10, 11]. Nitrogen is a critical component of plant defense-related enzyme systems, pathogenesis-related proteins, and other compounds essential for disease resistance. Therefore, rational N application is fundamental to enhancing plant resilience [12].

This study investigated the effects of maize-potato intercropping combined with differential N application strategies, specifically intraspecific (within-maize) versus interspecific (between-crop) placement along with moderate N reduction, on the rhizosphere soil microbiome of maize through a field experiment. The key parameters of maize rhizosphere soil microbial communities, key agronomic traits, and final grain yield were examined to elucidate the impact of these intercropping and fertilization strategies on maize performance. The results of this research would also provide crucial theoretical and technical support for the potato cultivation industry.

Materials and methods

Research environment and plant resources

The field experiment was conducted in 2024 at the Practical Training Base of Kunming University (Anning, Yunnan, China) with the geographic coordinates of 102°32'22'' E and 24°56'14'' N, altitude 1,800 m. The site had an average annual temperature of 17.0°C, annual frost-free period of 232 days, and annul sunshine time of 1,933.2 hours. The average annual precipitation was 765.1 mm, which was concentrated in June (109.7 mm), July (873.4 mm), and August (484.0 mm) of 2024. The background values of the cultivation soil were 8.06 pH, 9.21 g/kg soil organic matter, 88.95 mg/kg alkali-hydrolyzable

nitrogen, 1.76 mg/kg available phosphorus, 103.93 mg/kg available potassium, 84.77 mg/kg total phosphorus, and 1,512.38 mg/kg total potassium. The planted potato was virus-free seed tubers “Fresh Tuber No. 6” obtained from Lijiang Hengtianran'an Ecological Agriculture Co., Ltd. (Lijiang, Yunnan, China), while the planted maize was the commercial variety “Jingdian 8” obtained from Yunnan Jingdian Seed Industry Co., Ltd. (Kunming, Yunnan, China). Controlled-Release Nitrogen (N) fertilizer composed of ≥ 28% N and High-Phosphorus (P), High-Potassium (K) Controlled-Release fertilizer with N:P:K ratio of 2:15:30 were obtained from Yunnan Yuntianhua Agricultural Technology Co., Ltd. (Kunming, Yunnan, China).

Plant planting and sampling

A total of five treatments were tested in this research, which included maize monoculture with conventional N fertilization at 150 kg/ha as control (CK), maize-potato intercropping with conventional intraspecific N application at 150 kg/ha as T1, maize-potato intercropping with reduced intraspecific N application at 75 kg/ha as T2, maize-potato intercropping with conventional interspecific N application at 150 kg/ha as T3, maize-potato intercropping with reduced interspecific N application at 75 kg/ha as T4. For both monoculture and intercropping systems, the row spacing within a crop's rows (intraspecific) and between different crops (interspecific) was standardized at 40 cm with a plant-to-plant spacing of 40 cm. The plot size was 5.5 m × 6.5 m, and the experiment was arranged in a randomized complete block design (RCBD) with three replicates. Controlled-release compound fertilizers were applied as a basal dressing, mixed into the soil in their designated positions immediately prior to sowing. Intraspecific fertilization referred to the application of fertilizer within the midpoint of a single crop row, while interspecific fertilization placed fertilizer in the space between the maize and potato rows. At the maize maturity stage, plants were selected by using a five-point sampling method. Rhizosphere soil was subsequently collected from each plant by using the root-shaking method [13]. After removing debris and fine roots, the soil samples were homogenized and then divided into three subsamples, sealed in self-sealing bags, and stored with one subsample being stored at -80°C for subsequent microbiological analysis.

Measurement of growth and yield indicators

Growth parameters including plant height, stem diameter, and leaf area were measured during the mid-to-late stages of maize growth. For each plot, ten plants were randomly selected, and measurements were repeated three times per treatment with data presented as the mean value. The plant height was measured from the base of the plant to the tip of the main stem (the apical meristem) using a tape measure in cm. The internode diameter at the third node was measured as stem diameter using a vernier caliper in cm. The leaf area was calculated using the length-width coefficient method with leaf length and width being measured by a tape. The area was determined by multiplying these values with a correction factor of 0.75. For biomass and yield determination, maize samples were oven dried at 105°C for one hour to stop enzymatic activity (killing green) followed by drying at 85°C until reaching a constant weight. The dry weights of both the whole maize plant (biomass) and its grain (yield) were recorded and combined with the planting density for each treatment to calculate the final yield (kg/ha) and total biomass as follows.

Leaf area (LA) = Leaf length (L) × Maximum leaf width (W) × Correction coefficient (0.75)

Theoretical yield (kg/ha) = Number of ears per unit area (ears/ha) × Number of grains per ear (grains) × 1,000-grain weight (g) ÷ 10^6

Biomass per plant = Sum of the oven-dried weights of all aboveground organs including leaves, stems, leaf sheaths, husks, ears, etc.).

Total biomass = Average biomass per plant × Total plant count per unit area

Soil total DNA extraction, polymerase chain reaction (PCR) amplification, and library construction

Total soil DNA was extracted by using the CTAB-SDS method [14]. The purity and concentration of the extracted DNA were assessed *via* agarose gel electrophoresis. The extracted DNA was dissolved in sterile water to a final concentration of 1 ng/µL as template. The barcoded primer pair 515F-806R for the V4 region of the 16S rRNA was employed for bacterial community PCR amplification. The reaction was conducted with Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs, Ipswich, MA, USA) following manufacturer's instructions. The resulting PCR products were purified by using magnetic beads. Following quantification, samples were pooled in equal amounts based on their concentration and subjected to 2% agarose gel electrophoresis followed by purification using Qiagen gel extraction kit (Qiagen, Germantown, MD, USA). TruSeq® DNA PCR-free sample preparation kit (Illumina, San Diego, CA, USA) was applied for library construction following manufacturer's instructions. The constructed libraries were quantified by using a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Sequencing was carried out by using an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

Data processing and statistical analysis

Raw sequencing data was demultiplexed based on barcode sequences and PCR primers. The resulting reads for each sample were then assembled by using FLASH software (https://ccb.jhu.edu/software/FLASH/) and underwent a stringent quality filtering process to generate high-quality clean tags [15, 16]. Following the QIIME 1.9.1 quality control pipeline (https://qiime.org/) [20], chimeric sequences were identified and removed by aligning the tags against a reference database using UCHIME within the USEARCH suite (https://usearch.com/) [17, 18], ultimately yielding the final effective tags before clustering into operational taxonomic units (OTUs) at a 97% sequence identity threshold using the Uparse algorithm [19]. Representative sequences for each OTU defined as the most frequent sequence within that cluster were selected and then taxonomically annotated by alignment against the SILVA 138 SSU rRNA database (https://www.arb-silva.de/) using the Mothur method (https://mothur.org/) with a confidence threshold of 0.8 – 1.0 to generate kingdom, phylum, class, order, family, genus, and species [20, 21]. A multiple sequence alignment of all OTU representative sequences was conducted by using multiple sequence comparison by log-expectation (MUSCLE) (https://drive5.com/muscle/) to establish their phylogenetic relationships [22]. All samples were normalized by subsampling to the same sequencing depth (the lowest read count among all samples) to ensure comparability for subsequent diversity analyses. Within-sample (Alpha) diversity was assessed by using QIIME 1.9.1. Key metrics including observed OTUs, Chao1, Shannon, Simpson, ACE, Good's Coverage, and PD_whole_tree were calculated. Rarefaction curves, rank abundance curves, and species accumulation curves were generated by using R 2.15.3 software (https://www.r-project.org/). Differences in Alpha diversity indices between groups were statistically tested. For comparisons between two groups, a t-test or Wilcoxon rank-sum test was employed. For comparisons involving more than two groups, Tukey's HSD test and the Kruskal-Wallis H test *via* the 'agricolae' package were used. Between-sample (Beta) diversity was evaluated by calculating unweighted UniFrac distances using QIIME 1.9.1 to assess community dissimilarity. An unweighted pair-group method with arithmetic mean (UPGMA) clustering tree was then constructed. Principal component analysis (PCA) was performed in R 2.15.3 to visualize sample clustering based on community composition. Agronomic traits, yield, and other phenotypic data were organized by using Excel 2016 (Microsoft, Redmond, WA, USA). Statistical analysis was performed by using SPSS Statistics 26 (IBM, Armonk, NY, USA). Significant differences between treatments were determined by using Duncan's multiple range test (DMRT) with *P* value less than 0.05 as

Table 1. Sample operational taxonomic unit (OTU) abundance and alpha diversity index.

Variety	Samples	Alpha diversity				
		ACE	Chao1	Simpson	Shannon	Coverage
Bacteria	CK	3,573.08 ± 72.29^{a}	3,554.59 ± 42.15^{a}	1.00 ± 0.00^{a}	9.53 ± 0.04^{a}	0.96 ± 0.00^{a}
	T1	3,261.66 ± 14.06^{b}	3,361.22 ± 157.80^{a}	0.99 ± 0.01^{a}	9.24 ± 0.43^{a}	0.96 ± 0.00^{a}
	T2	3,488.81 ± 88.27^{a}	3,337.39 ± 98.89^{a}	0.99 ± 0.00^{a}	9.17 ± 0.15^{a}	0.97 ± 0.00^{a}
	T3	3,497.30 ± 45.01^{a}	3,442.57 ± 163.54^{a}	0.99 ± 0.01^{a}	9.46 ± 0.21^{a}	0.96 ± 0.00^{a}
	T4	3,461.40 ± 12.29^{a}	3,462.02 ± 4.54^{b}	0.97 ± 0.01^{b}	8.56 ± 0.19^{a}	0.96 ± 0.00^{a}
Fungus	CK	806.94 ± 9.38^{a}	828.60 ± 6.86^{a}	0.97 ± 0.00^{a}	6.76 ± 0.17^{a}	0.96 ± 0.00^{a}
	T1	698.26 ± 21.94^{c}	709.45 ± 27.92^{c}	0.97 ± 0.00^{a}	6.33 ± 0.06^{a}	0.96 ± 0.00^{a}
	T2	637.30 ± 5.33^{d}	644.46 ± 3.65^{d}	0.95 ± 0.05^{a}	6.00 ± 0.63^{a}	0.97 ± 0.00^{a}
	T3	763.17 ± 9.57^{b}	778.19 ± 7.55^{b}	0.97 ± 0.00^{a}	6.53 ± 0.17^{a}	0.96 ± 0.00^{a}
	T4	799.19 ± 13.38^{a}	819.58 ± 12.50^{a}	0.97 ± 0.00^{a}	6.75 ± 0.18^{a}	0.96 ± 0.00^{a}

Note: different lowercase letters indicated significant differences ($P < 0.05$).

statistically significant. Visualizing graphs were created by using Origin 2018 (OriginLab, Northampton, MA, USA).

Results and discussion

Richness and diversity of rhizosphere soil microbial communities

The analysis of the alpha diversity index of bacteria demonstrated that the abundance-based coverage estimator (ACE) index of treatment T1 was 3,261.66 ± 14.06, significantly lower than both treatment CK's 3,573.08 ± 72.29 and other treatments ($P < 0.05$). The Chao1 index showed that treatment T4 was 3,462.02 ± 4.54, which was markedly lower than treatment CK's 3,554.59 ± 42.15 ($P < 0.05$), whereas there was no significant difference between treatment T1 (3,361.22 ± 157.80) and treatment CK. Simpson index showed that treatment T4 exhibited 0.97 ± 0.01, a notable decrease compared to all other treatments. Although variations existed in the Shannon index values among different treatments, there was no statistical significance observed (Table 1). The results suggested that, following maize-potato intercropping, the implementation of interspecific nitrogen reduction (T4) and intraspecific conventional fertilization (T1) led to a decline in the richness and evenness of the bacterial community in the maize rhizosphere soil. In contrast, the indices for treatments T2 and T3 showed no significant disparity with those of treatment CK, implying that intraspecific nitrogen reduction and interspecific conventional fertilization exerted no substantial influence on the diversity and abundance of the bacterial community in the maize rhizosphere soil. The analysis results of the alpha diversity index of fungi demonstrated that the ACE index of T1 and T2 treatments were 698.26 ± 21.94 and 637.30 ± 5.33, respectively, which were significantly lower than CK treatment's 806.94 ± 9.38 ($P < 0.05$) with T2 treatment exhibiting the lowest ACE index. T3 treatment showed 763.17 ± 9.57, which was notably lower than CK but higher than T1 and T2. There was no significant difference between T4 treatment (799.19 ± 13.38) and CK. The trend of the Chao1 index results was largely consistent with that of the ACE index with T1 and T2 treatments being significantly lower than CK, T3 treatment being significantly lower than CK yet higher than T1 and T2, and T4 treatment showing no significant difference from CK. No significant differences were observed in the Simpson index and Shannon index among various treatments (Table 1). The results suggested that, after maize-potato intercropping, intraspecific conventional fertilization (T1) and intraspecific nitrogen reduction (T2) treatments markedly decreased the richness of the fungal community in the rhizosphere soil of maize. Interspecific conventional fertilization (T3) treatment also

reduced richness, albeit with less impact compared to T1 and T2. In contrast, the indices of interspecific nitrogen reduction (T4) treatment showed no significant difference from those of CK treatment, implying that interspecific nitrogen reduction had no notable effect on the diversity and abundance of the fungal community in the rhizosphere soil of maize. Soil microorganisms are central drivers of soil ecosystem functions, and their community structure and diversity serve as key biological indicators for assessing soil health and predicting the stability of agricultural ecosystems [23]. The results demonstrated that maize-potato intercropping in combination with varying nitrogen fertilization strategies significantly reshaped the rhizosphere microbial community structure. The richness and evenness of the bacterial communities in the treatments involving interspecific nitrogen reduction (T4) and intraspecific conventional fertilization (T1) were reduced. Conversely, intraspecific nitrogen reduction (T2) and interspecific conventional fertilization (T3) maintained diversity levels comparable to those observed in monoculture (CK), suggesting that moderate nitrogen reduction or optimized planting configurations within intercropping systems could stabilize microbial diversity.

Analysis of the impact of different treatments on bacterial community distribution characteristics

The analysis of the ten major phyla in the sample information revealed that the dominant groups were *Proteobacteria* (18.5 - 25.6%), *Actinobacteriota* (11.4 - 25.5%), unclassified_Bacteria (7.6 - 31.3%), *Bacteroidota* (7 - 13.7%), *Firmicutes* (4.1 - 11.4%), *Acidobacteriota* (3.7 - 6.8%), *Myxococcota* (2.3 - 4.8%), *Verrucomicrobiota* (2.8 - 3.2%), *Chloroflexi* (1.9 - 3.2%), and *Planctomycetota* (1.5 - 3.3%). The relative abundances of *Bacteroidota*, *Firmicutes*, *Chloroflexi*, and *Planctomycetota* were lower in the conventional fertilization treatment for monoculture maize (CK) compared to other treatments. In CK, the relative abundances of *Actinobacteriota*, *Acidobacteriota*, *Verrucomicrobiota*, *Chloroflexi*, and *Planctomycetota* were all lower than those in T1 and T3 treatments under maize-potato intercropping. After T2 treatment, the relative abundances of *Proteobacteria*, *Actinobacteriota*, *Acidobacteriota*, *Myxococcota*, *Chloroflexi*, and *Planctomycetota* decreased compared to that in T1 treatment. After T4 treatment, the relative abundances of *Bacteroidota*, *Firmicutes*, and *Verrucomicrobiota* decreased compared to that in T3 treatment. Under conventional fertilization with maize-potato intercropping, except for *Bacteroidota* and *Firmicutes*, the relative abundances of the remaining phyla were higher in T1 treatment than in T3 treatment. Under reduced nitrogen applications, the relative abundances of *Bacteroidota*, *Firmicutes*, and *Verrucomicrobiota* were higher in T2 treatment than in T4 treatment (Figure 1A). The results indicated that maize-potato intercropping increased the relative abundances of *Actinobacteriota*, *Acidobacteriota*, *Verrucomicrobiota*, *Chloroflexi*, and *Planctomycetota*, while different fertilization methods within and between species easily affected the relative abundances of *Bacteroidota* and *Firmicutes*. At the bacterial genus level, the analysis of major bacterial genera in the samples showed that the dominant groups were unclassified_Bacteria (7.6 - 31.3%), *Terrimonas* (1.2 - 3.6%), unclassified_67_14 (1.4 - 3.3%), *Gaiella* (1.2 - 2.9%), *Solirubrobacter* (1.0 - 2.9%), unclassified_*Chitinophagaceae* (1.0 - 2.5%), *Bacillus* (1.0 - 2.0%), unclassified_ *Vicinamibacteraceae* (0.8 - 2.1%), unclassified_ *Vicinamibacterales* (0.8 - 1.9%), and *Candidatus*_ *Omnitrophus* (0.9 - 1.7%). The relative abundances of *Terrimonas*, unclassified_ *Chitinophagaceae*, and *Bacillus* were higher in CK than in other treatments. After maize-potato intercropping, the relative abundances of *Gaiella*, *Solirubrobacter*, unclassified_ *Vicinamibacteraceae*, and unclassified_ *Vicinamibacterales* significantly increased in T1 treatment. After T2 treatment, the relative abundances of most dominant genera including *Terrimonas*, *Solirubrobacter*, unclassified_ *Vicinamibacteraceae*, *Gaiella*, *etc*. decreased

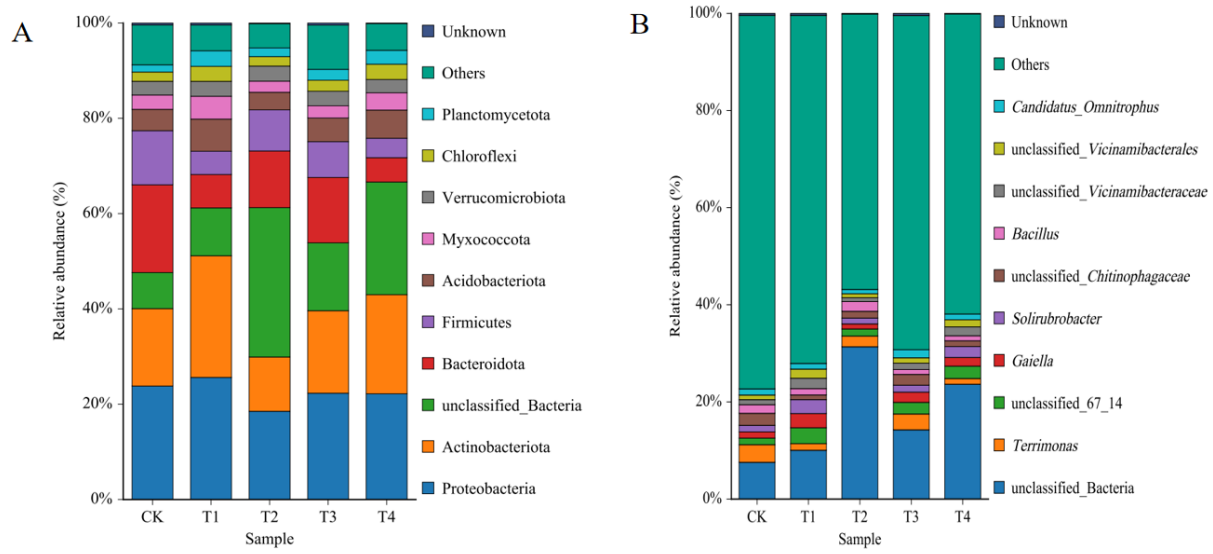

Figure 1. Relative abundance of bacterial species at the phylum (**A**) and genus (**B**) levels.

compared to that in T1 treatment, but the relative abundance of unclassified_Bacteria greatly increased. After T4 treatment, the relative abundances of *Gaiella*, *Solirubrobacter*, unclassified_*Vicinamibacteraceae*, and unclassified_*Vicinamibacterales* increased, while *Terrimonas*, unclassified_*Chitinophagaceae*, and *Bacillus* decreased compared to T3 treatment (Figure 1B). The results demonstrated that maize-potato intercropping combined with conventional fertilization (T1) increased the relative abundances of *Gaiella*, *Solirubrobacter*, and *Vicinamibacterales*-related taxa, while reduced nitrogen application, especially T2 treatment, led to an abnormal increase in the relative abundance of unclassified_Bacteria, which might indicate significant changes in the taxonomic composition of the bacterial community at the genus level or the emergence of a large number of poorly understood bacterial groups under reduced nitrogen conditions.

Analysis of the impact of different treatments on fungal community distribution characteristics

The analysis of major fungal phyla in the sample information revealed that the dominant groups were *Ascomycota* (63.4 - 78.1%), *Basidiomycota* (9.8 - 16.5%), *Mortierellomycota* (4.7 - 9.5%), *Rozellomycota* (1.4 - 6.0%), *Olpidiomycota* (0.1 - 12.7%), *Chytridiomycota* (1.7 - 2.4%), and *Glomeromycota* (0.8 - 2.2%). The relative abundance of *Ascomycota* was the highest in the CK treatment at 78.1%. After intercropping maize with potato, the relative abundance of *Ascomycota* in both conventional fertilization and reduced nitrogen application treatments decreased, but *Basidiomycota* and *Mortierellomycota* were increased. Notably, in T2 treatment, the relative abundance of *Olpidiomycota* abnormally increased to 12.7%, significantly higher than all other treatments. Under maize-potato intercropping, T3 treatment had the highest relative abundance of *Mortierellomycota*, while T4 treatment had the highest relative abundances of *Basidiomycota* and *Glomeromycota* (Figure 2A). The results indicated that maize-potato intercropping reduced the relative dominance of *Ascomycota* and altered the phylum-level structure of the fungal community. Reduced nitrogen application, particularly in T2 treatment, might lead to a significant increase in specific pathogenic or parasitic phyla such as *Olpidiomycota*. The analysis of major fungal genera in the sample information showed that the dominant groups were unclassified_Fungi (0.7 - 3.1%), *Gibberella* (0.8 - 2.9%), *Solicoccozyma* (0.7 - 2.2%),

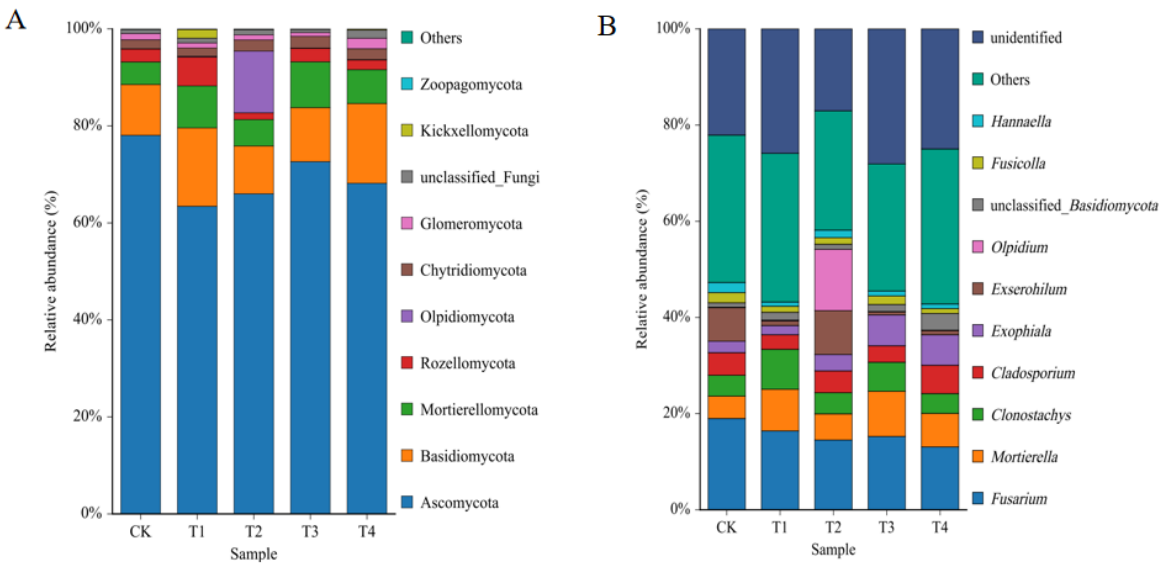

Figure 2. Relative abundance of fungus species at the phylum (**A**) / genus (**B**) levels.

unclassified_*Sordariomycetes* (0.6 - 2.0%), *Fusarium* (0.9 - 1.9%), unclassified_ *Didymellaceae* (0.5 - 1.8%), and unclassified_ *Pleosporaceae* (0.4 - 1.7%). The relative abundances of *Fusarium* and unclassified_*Pleosporaceae* were higher in CK treatment compared to other treatments. After intercropping maize with potato, T1 treatment significantly increased the relative abundances of *Gibberella* and unclassified_*Sordariomycetes*. In contrast, T2 treatment led to a substantial increase in the relative abundance of unclassified_Fungi and the lowest relative abundance of *Mortierella*. T3 treatment had higher relative abundances of *Solicoccozyma* and unclassified_*Didymellaceae*, while T4 treatment maintained high levels of *Gibberella* and unclassified_*Sordariomycetes* (Figure 2B). The results demonstrated that maize-potato intercropping and different fertilization methods significantly affected the genus-level composition of the fungal community. Intraspecific conventional fertilization (T1) tended to promote the growth of taxa such as *Gibberella*, while reduced nitrogen application, especially in the T2 treatment, might result in a large number of fungi being unclassifiable at the genus level, suggesting profound changes in the community structure. At the phylum level, intercropping treatments notably increased the abundance of beneficial phyla such as *Actinobacteriota* and *Acidobacteriota* with particularly pronounced effects in T1 and T3 treatments. At the genus level, T1 treatment significantly elevated the abundance of beneficial genera like *Gaiella* and *Solirubrobacter*, whereas T2 treatment led to an abnormal increase in unclassified_Bacteria to 31.3%, indicating potential disruptions in community structure due to nitrogen reduction. Fungal communities exhibited heightened sensitivity to nitrogen management. In T2 treatment, the relative abundance of *Olpidiomycota* surged to 12.7% accompanied by a substantial number of unclassifiable fungal genera, suggesting that intraspecific nitrogen reduction might elevate the risk of pathogenic bacteria. In contrast, T4 treatment preserved fungal diversity without significant deviation from CK, underscoring the mitigating effect of interspecific interactions on the adverse impacts of nitrogen reduction, which could be attributed to the distinct chemical compositions and release rhythms of root exudates from maize and potato. When intermingled, it created a mixed, diversified carbon source environment conducive to supporting a broader range of microbial taxa compared to monoculture systems [24]. Nitrogen

Table 2. Comparison of agronomic traits of Maize under different planting patterns.

Treatment	Indicator		
	Plant height (cm)	Stem thickness (cm)	Leaf area (cm ²)
CK	293.88 ± 7.32^{d}	24.62 ± 2.03^{c}	1,110.03 ± 50.95^{c}
T1	306.87 ± 3.59^{ab}	31.91 ± 1.84^{a}	1,164.13 ± 57.75^{b}
T2	297.50 ± 3.98^{c}	30.50 ± 1.93^{b}	1,132.72 ± 83.33^{bc}
T3	308.60 ± 5.01^{a}	32.46 ± 1.51^{a}	1,214.57 ± 74.61^{a}
T4	304.31 ± 2.12^{b}	31.31 ± 1.47^{ab}	1,150.66 ± 74.72^{bc}

Note: different lowercase letters indicated significant differences ($P < 0.05$).

levels exerted a strong influence on the "quality" and "quantity" of root exudates with variations in nitrogen application altering the structural composition of rhizosphere carbon sources [25]. Furthermore, the rate and placement of nitrogen fertilization directly determined the concentration and spatiotemporal distribution of ammonium and nitrate nitrogen in the rhizosphere, thereby generating unique microhabitats [26].

Variations in biological traits and yield across different treatments

1. Agronomic trait indicators

Notable disparities were observed in the impact of various treatments on maize agronomic traits. All treatment groups exhibited significantly greater heights than the CK with T3 demonstrating the most pronounced increase at 5.01% above CK followed by T1 and T4, while T2 showed the least enhancement. In terms of stem diameter, all treatments surpassed CK significantly following the order of T3 > T1 > T4 > T2, wherein T3 recorded the highest improvement of 31.84%. T3 demonstrated markedly leaf area superior to other treatments followed by T1 treatment, while T2 and T4 elicited relatively minor increases (Table 2). The results collectively indicated that all applied treatments could substantially bolster maize growth performance. However, nitrogen-reducing fertilization strategies appeared to offer limited promotional effects, particularly under intraspecific fertilization regimes (T2), which yielded the weakest outcomes. Conversely, T3 emerged as the most efficacious treatment, potentially owing to interspecific complementarity effects coupled with adequate nitrogen supply.

2. Effects of different cultivation patterns on maize quality traits

The determination of maize ear traits revealed significant differences between intercropping treatments and CK. Based on the analysis of percentage changes, the ear lengths of T1, T3, T4 were 193.82 mm, 189.89 mm, and 186.28 mm and increased by 18.4%, 15.9%, and 13.8%, respectively, compared to CK (163.77 mm), which all were significantly higher than CK with only T2 (165.76 mm) showing no significant difference from CK. The number of rows per ear showed 34.29 for T1, 34.80 for T3, and 34.18 for T4, which rose by 16.8%, 18.5%, and 16.4%, respectively, compared to CK (29.36) and all demonstrated significantly surpassing to CK with only T2 (30.6) having no significant difference from CK. Kernels per row showed that T3 was 16.27, slightly increased by 1.7% compared to CK (16) and was significantly higher than the others. T1 (16.18) and T2 (16.13) had no significant difference from CK, while T4 (15.82) decreased by 1.1% compared to CK. As for ear diameter, T1 (53.36 mm) and T3 (52.99 mm) increased by 14.2% and 13.4%, respectively, compared to CK (46.74 mm) with significant difference, while T4 (52.13 mm) increased by 11.5% and T2 (48.8 mm) increased by 4.4% compared to CK with no significant difference (Table 3). The maize-potato intercropping model, especially the conventional nitrogen application within/between species treatments could significantly enhance ear traits such as ear length, number of rows per ear, and ear diameter. The interspecific nitrogen

Table 3. Effects of different cultivation patterns on the quality traits of corn.

Treatment	Ear length (mm)	Row count	Grain count	Ear thickness (mm)
CK	163.77 ± 13.06^{b}	29.36 ± 3.08^{b}	16.00 ± 0.27^{ab}	46.74 ± 2.29^{c}
T1	193.82 ± 7.84^{a}	34.29 ± 1.66^{a}	16.18 ± 0.16^{ab}	53.36 ± 1.38^{a}
T2	165.76 ± 12.43^{b}	30.6 ± 3.15^{ab}	16.13 ± 0.14^{ab}	48.80 ± 2.36^{bc}
T3	189.89 ± 9.95^{a}	34.80 ± 1.42^{a}	16.27 ± 0.27^{a}	52.99 ± 2.51^{a}
T4	186.28 ± 5.04^{a}	34.18 ± 1.79^{a}	15.82 ± 0.08^{b}	52.13 ± 0.85^{ab}

Note: different lowercase letters indicated significant differences ($P < 0.05$).

Table 4. Effects of different cultivation patterns on the Yield of corn.

Treatment	Dry matter (g)	Thousand-grain weight (g)	Yield (kg/hm²)
CK	303.78 ± 18.98^{c}	329.00 ± 14.74^{c}	19,354.56 ± 2,704.02^{c}
T1	425.94 ± 4.26^{a}	403.30 ± 15.29^{a}	27,992.17 ± 2,299.14^{a}
T2	323.85 ± 9.36^{bc}	343.82 ± 17.92^{bc}	21,186.45 ± 2,082.17^{bc}
T3	412.40 ± 18.80^{a}	369.74 ± 27.92^{ab}	26,244.66 ± 3,429.65^{a}
T4	346.86 ± 9.83^{b}	361.02 ± 14.16^{bc}	24,432.75 ± 2,191.77^{ab}

Note: different lowercase letters indicated significant differences ($P < 0.05$).

reduction treatment (T4) could also improve most ear traits, while the intraspecific nitrogen reduction treatment (T2) had an insignificant promoting effect on ear traits. The results suggested that intercropping combined with conventional nitrogen application was more conducive to optimizing maize ear development, playing a positive role in improving yield components.

3. Effects of different cultivation patterns on maize yield

The impact of different cultivation modes on maize yield in the first year revealed that intercropping treatments exhibited varying degrees of enhancement compared to CK. The dry matter in T1, T3, T4, and T2 were 425.94 g, 412.40 g, 346.86 g, and 323.85 g, respectively, demonstrating increases of 40.2%, 35.8%, 14.2%, and 6.6%, respectively, over CK (303.78 g) with T1 and T3 being significantly higher than CK. The thousand-grain weight results showed that T1, T3, T4, and T2 reached 403.30 g, 369.74 g, 361.02 g, and 343.82 g with the improvements of 22.6%, 12.4%, 9.7%, and 4.5%, respectively, compared to CK (329 g) with T1 being notably superior to CK. Concerning the yields, T1 (27,992.17 kg/hm²), T3 (26,244.66 kg/hm²), T4 (24,432.75 kg/hm²), and T2 (21,186.45 kg/hm²) surpassed CK (19,354.56 kg/hm²) by 44.6%, 35.6%, 26.3%, and 9.5%, respectively, with T1 and T3 exhibiting significant advantages over CK (Table 4). Overall, the maize-potato intercropping system positively influenced maize dry matter, thousand-grain weight, and yield. Notably, conventional nitrogen fertilization within species (T1) and between species (T3) yielded the most pronounced enhancements. Interspecific nitrogen reduction (T4) also effectively augmented yield-related traits, albeit to a lesser extent. Conversely, intraspecific nitrogen reduction (T2) exerted a relatively modest promotional effect. The results underscored that intercropping coupled with conventional nitrogen fertilization was more conducive to enhancing maize's material accumulation and yield levels. Moreover, interspecific nitrogen reduction could achieve nitrogen savings while ensuring certain production benefits. Intercropping treatments universally enhanced maize agronomic traits with T3 treatment yielding the most favorable outcomes in plant height, stem diameter, and leaf area. Analysis of ear characteristics revealed that T1 and T3 treatments significantly improved ear length, rows per ear, and ear diameter. In terms of yield, T1 and T3 treatments achieved

respective increases, while T4 treatment still managed an increase despite nitrogen reduction, highlighting the synergistic effect of intercropping combined with optimized fertilization in achieving both reduced fertilizer uses and increased productivity. These results aligned with the reports of Nie *et al*. [27], further corroborating the enhanced potential for fertilizer reduction within intercropping systems. However, the microbial community disturbances and limited yield improvements associated with intraspecific nitrogen reduction treatment (T2) underscored the necessity for tailored adjustments in fertilizer reduction rates and application methods based on specific production requirements, cautioning against indiscriminate fertilizer cuts.

Conclusion

The intercropping of maize and potato could enhance the abundance of beneficial bacterial phyla such as *Actinobacteria* by optimizing the rhizosphere microenvironment. However, nitrogen fertilizer management strategies significantly regulated the structure of microbial communities. Conventional fertilization between species demonstrated superior performance in maintaining microbial diversity and promoting maize growth, whereas reduced nitrogen application between species emerged as a feasible measure to balance ecological benefits with yield outcomes. Solely reducing nitrogen within the same species might induce disturbances in fungal communities such as proliferation of *Olpidium*, thereby constraining yield improvements. Thus, caution against arbitrary reductions in fertilizer usage was warranted in agricultural practice. Promoting the "maize-potato intercropping + interspecific nitrogen reduction" model offered a promising approach to ensure crop yields while mitigating environmental risks, aligning with the demands for sustainable agricultural development.

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