RESEARCH ARTICLE

Impact of different N, P, and K fertilization levels on the rhizosphere microbial community structure of strawberry

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Excessive application of chemical fertilizers in strawberry cultivation often leads to soil compaction and reduced soil fertility. Rhizosphere microorganisms play a crucial role in soil nutrient transformation and thus strongly influence strawberry growth and yield. The effects of varying nitrogen, phosphorus, and potassium levels on strawberry rhizosphere microbial communities remain unclear. This research used the strawberry cultivar "Hongyan" to test the rhizosphere soil under three fertilization treatments of biochar + organic fertilizer (C1), full-rate chemical fertilizer + biochar + organic fertilizer (C2), and half-rate chemical fertilizer + biochar + organic fertilizer (C3). Next-generation sequencing was employed to analyze microbial diversity and community composition based on the 16S rDNA V4–V5 region and the ITS1 region of rhizosphere soil. The results showed that bacterial operational taxonomic unit (OTU) abundance and Shannon index followed the order of C1 (1,106, 6.07) > C3 (1,105, 6.06) > C2 (1,099, 6.05), while fungal OTU abundance and Shannon index followed the order of C2 (311, 4.82) > C3 (295, 4.75) > C1 (286, 4.61), respectively. Proteobacteria was the dominant phylum with the relative abundances of C1 and C2 being 34.42% and 35.34%, respectively, and C2 being 0.92% more abundant than C1. The dominant phyla in fungi showed that *Ascomycota* in C3 (68.33%) was 12.46% higher than that in C1 (55.87%), while *Basidiomycota* in C1 (23.04%) was 6.22% higher than that in C2 (16.82%). This study elucidated the effects of different nitrogen, phosphorus, and potassium levels on strawberry rhizosphere microbial community structure, providing theoretical support for optimizing nutrient management and achieving high-yield cultivation of strawberries.

Keywords: chemical fertilizer; strawberry; rhizosphere soil; microorganisms; diversity.

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Introduction

Strawberry (*Fragaria ananassa* Duch.) is a perennial herbaceous plant belonging to the *Rosaceae* family. Its fruit is soft, juicy, and nutritionally rich, containing a wide range of vitamins [1]. In recent years, driven by increasing market demand and the development of protected agriculture, China's strawberry industry has experienced steady and rapid growth. According to the 2024 China Strawberry Industry Data Analysis Brief, China's strawberry cultivation area reached 147,563 hectares in 2022 with total production approaching 4 million

tons, which accounted for more than one-third of global output, establishing China as the world's largest strawberry producer [2]. Strawberry cultivation in Yunnan, China entered a period of rapid development in the early 21st century and experienced explosive growth after 2015 [3]. By 2022, Yunnan ranked eighth nationwide in both cultivation areas and production with approximately 90,267 acres under cultivation and a total yield of 165,000 tons. The region has become an important winter strawberry production base and the primary summer strawberry production area in China, contributing more than 80% of the country's summer fresh strawberry supply [4].

With the expansion of strawberry cultivation, fertilizer inputs have continued to increase. However, excessive fertilizer application can result in soil compaction, salinization, declining soil fertility, soil acidification, reduced soil organic matter content, and altered soil enzyme activities, all of which severely restrict improvements in strawberry yield and fruit quality [5]. The rhizosphere is the most biologically active zone of interaction among plant roots, soil, and microorganisms [6]. Rhizosphere microorganisms play a vital role in plant growth by participating in organic matter decomposition and nutrient transformation, converting nutrients into forms readily absorbed and utilized by plants [7]. Previous studies have demonstrated that different fertilization practices significantly influence soil microbial community structure and function. In agricultural production systems, rational fertilizer applications can improve soil physicochemical properties and enhance microbial functional diversity [8]. Phosphorus and potassium fertilizers have been shown to increase soil sucrase and cellulase activities, thereby accelerating organic matter decomposition and enhancing plant resistance [9]. Increased application of organic fertilizers promotes the abundance of bacteria, actinomycetes, and fungi, whereas sole application of chemical fertilizers tends to reduce microbial populations [10]. Different fertilizer types exert varying effects on microbial abundance, community structure, and functional diversity in soils [11]. Long-term nitrogen fertilization has also been reported to significantly reduce soil microbial activity [12]. Liu *et al*. investigated the effects of different fertilization regimes on microbial functional diversity in sorghum rhizosphere soil and found that changes in soil nutrient status altered rhizosphere microbial diversity, thereby influencing nutrient uptake processes [13]. Hao *et al*. examined the effects of compost and chemical fertilizers on rhizosphere microbial communities of Lanzhou lily and concluded that organic fertilizers effectively improved microbial community structure [14]. Wu *et al*. demonstrated that combined application of straw and chemical fertilizers helped maintain soil ecological health and fungal community diversity, whereas nitrogen fertilization increased fungal abundance but significantly reduced richness and diversity [15]. Jiang *et al*. reported that organic fertilizers enhanced the metabolic activity of tea rhizosphere microorganisms involved in nutrient utilization [16]. Studies also showed that dominant bacterial phyla in strawberry rhizosphere soils included *Proteobacteria*, *Actinobacteria*, *Bacillobacteria*, *Acidobacteria*, and *Chloroflexi*, while dominant fungal phyla included *Ascomycota*, *Zygomycota*, and *Basidiomycota* [17]. However, research on how different fertilizer application rates influence the composition and response mechanisms of strawberry rhizosphere microbial communities remains limited. This gap has constrained the development of precision fertilization strategies and soil ecological regulation technologies for strawberry production.

This study investigated strawberry rhizosphere soils under different fertilization levels to evaluate the effects of varying nitrogen, phosphorus, and potassium application rates on rhizosphere microbial community structure and to clarify microbial response characteristics to changes in fertilization intensity through high-throughput sequencing technology. The results of this study provided a theoretical basis for

nutrient management and scientific fertilization of strawberry rhizosphere soils and offered scientific support for the sustainable development of the strawberry industry.

Materials and methods

Plant, soil, and fertilizer resources

The strawberry (*Fragaria ananassa* Duch.) variety "Hongyan" was purchased from the Strawberry Seedling Propagation Base of the Institute of Alpine Economic Plants, Yunnan Academy of Agricultural Sciences (Kunming, Yunnan, China). The tested soil was a typical acidic, unmatured primary red soil from the protected strawberry cultivation area in Chenggong District, Kunming, Yunnan, China with the geographic coordinates of 24°53'N and 102°49'E. Soil samples were collected from the 0 – 20 cm cultivation layer with plant debris being removed. Samples were air-dried naturally and sieved through a 2 mm mesh. Organic fertilizer with organic matter larger than 45% and total nutrients more than 5% was supplied by Kunming Shenrui Agricultural Company (Kunming, Yunnan, China). Biochar was provided by Yunnan Weixin Agricultural Technology Co., Ltd. (Kunming, Yunnan, China).

Plant culture and fertilization treatments

Three fertilization treatments were applied including biochar (270.00 g/pot) plus organic fertilizer (500.00 g/pot) with no potassium nitrate or dipotassium phosphate as C1 group, chemical fertilizer (13.86 g/pot potassium nitrate and 6.93 g/pot dipotassium phosphate) combined with biochar (270.00 g/pot) and organic fertilizer (500.00 g/pot) as C2 group, half chemical fertilizer (4.20 g/pot potassium nitrate and 2.10 g/pot dipotassium phosphate) combined with biochar (270.00 g/pot) and organic fertilizer (500.00 g/pot) as C3 group. Each treatment group comprised nine pots with three strawberry plants per pot and was arranged in three replicates. The experiment employed a random arrangement with fixed-position pots and quantitative fertilization and was conducted in the smart agricultural greenhouse in the College of Agriculture and Life Sciences at Kunming University (Kunming, Yunnan, China) with the geographic coordinates of 25°01'N and 102°68'E and an elevation of approximately 1,890 m. The greenhouse soil was loamy with a neutral pH and exhibited good water- and nutrient-retention properties. A drip-irrigation system was employed to supply stable water and provide excellent drainage.

Sample collection

Uniform and healthy strawberry seedlings were selected and cultivated in pots containing 3 kg of red soil each. The plants were irrigated once every two days throughout the entire growing season. Soil samples were collected by using the shaking-root method during three key growth stages including the vigorous vegetative growth stage (July 20, 2024), the flowering and fruiting stage (October 24, 2024), and the peak fruiting stage (December 12, 2024). The top 1 – 2 cm of surface soil around the rhizosphere was carefully removed before sampling. The rhizosphere soil from a depth of 5 – 15 cm was collected by using a specialized soil sampler. Five soil samples were collected, placed in sterile plastic bags, transported to the laboratory immediately, and stored at 4 °C.

Extraction of soil metagenomic DNA

Genomic DNA from rhizosphere soil was extracted by using the TIANamp Soil DNA Kit (Tiangen Biotech, Beijing, China). The quality of the extracted DNA was assessed by 1% agarose gel electrophoresis, and its concentration was determined by using NanoDrop OneC micro-UV spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Polymerase chain reaction (PCR) amplification and recovery of the V4-V5 and ITS1 regions of 16S rDNA

The V4–V5 region of the bacterial 16S rDNA and the ITS1 region of the fungal rDNA were amplified by PCR using primers 515F (5'-GTG YCA GCM GCC GCG GTA A-3')/926R (5'-(CCG YCA ATT YMT TTR AGT TT-3') and ITS1F (5'-(CTT GGT CAT TTA GAG GAA GTA A-3')/ITS1R (5'-(GCT GCG TTC TTC ATC

GAT GC-3'), respectively [18]. The PCR amplification was performed in a 25 µL reaction mixture containing 12.5 µL of 2× Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µM of each forward and reverse primer, 1 µL of template DNA (approximately 10 ng), and nuclease-free water to a final volume of 25 µL. PCR amplification was carried out by using Bio-Rad C1000 Touch thermal cycler (Bio-Rad Laboratories, Fort Worth, TX, USA) under the conditions of 98°C for 30 s followed by 25 cycles of 98°C for 10 s, 55°C for 30 s, 72°C for 30 s, and a final 72°C for 5 min. PCR products were purified by using AMPure XP magnetic beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA) according to the manufacturer's instructions. The purified target amplicons were sent to Microbase Biotechnology Co., Ltd. (Shanghai, China) for high-throughput sequencing.

Data processing and analysis

After sequencing, paired-end reads were merged by using fast length adjustment of short reads (FLASH) (https://ccb.jhu.edu/software/FLASH/) [19]. Quality filtering of raw reads was performed by using quantitative insights into microbial ecology (QIIME) (version 1.9.1) (https://qiime.org) [20], where sequences with ambiguous bases, low-quality scores, or mismatched barcodes were removed. Filtered sequences were aligned against reference ChimeraSlayer Gold database (http://drive5.com/uchime/gold.fa) to improve alignment accuracy. Chimeric sequences were detected and removed by using UCHIME (https://drive5.com/uchime/) [21], resulting in high-quality effective sequences. Operational taxonomic units (OTUs) were clustered at 97% sequence similarity using UPARSE software (https://drive5.com/uparse/) [22]. Representative sequences from each OTU were taxonomically assigned using the ribosomal database project (RDP) classifier (https://rdp.cme.msu.edu/) [21] against the Greengenes reference database (http://greengenes.secondgenome.com/) [23]. Alpha diversity analyses were conducted by using Mothur software (version 1.30.2) (https://mothur.org/). Briefly, sequences were randomly subsampled to an equal sequencing depth across samples to minimize sampling bias before generating rarefaction (dilution) curves. Library coverage was calculated based on Good's coverage estimator, while Chao1 richness was used to estimate species richness, and the Shannon index was calculated to evaluate microbial diversity considering both richness and evenness [24]. Principal component analysis (PCA) was performed by using Canoco (version 5.0) (https://www.canoco5.com/) to visualize differences in microbial community structure among samples. Phylogenetic distance matrices were calculated by using Fast UniFrac (http://unifrac.colorado.edu/). Hierarchical clustering was subsequently conducted by using the unweighted pair group method with arithmetic mean (UPGMA) algorithm based on weighted UniFrac distances to evaluate relationships among microbial communities.

Results

OTU abundance and α diversity of bacteria and fungi

The numbers of high-quality sequences, OTU richness, Shannon diversity index, and coverage for the 16S rDNA V4–V5 and ITS1 regions in strawberry rhizosphere soil under different fertilizer treatments demonstrated that the effective sequence counts for bacteria were 36,133 (C1), 34,863 (C2), 32,875 (C3), while OTU abundances were 1,106 (C1), 1,099 (C2), 1,105 (C3) (Figure 1A), and Shannon diversity indices were 6.07 (C1), 6.05 (C2), 6.06 (C3), respectively, with the coverage ranged from 99.47% to 99.59%. The results indicated that bacterial OTU richness and Shannon diversity were the highest in C1, intermediate in C3, and the lowest in C2, i.e., chemical fertilizer reduced bacterial diversity in the rhizosphere soil with a larger reduction under the full-rate chemical fertilizer (C2) than the half-rate treatment (C3). The effective sequence counts for fungi were 46,868 (C1), 43,556 (C2), 42,898 (C3), while OTU abundances

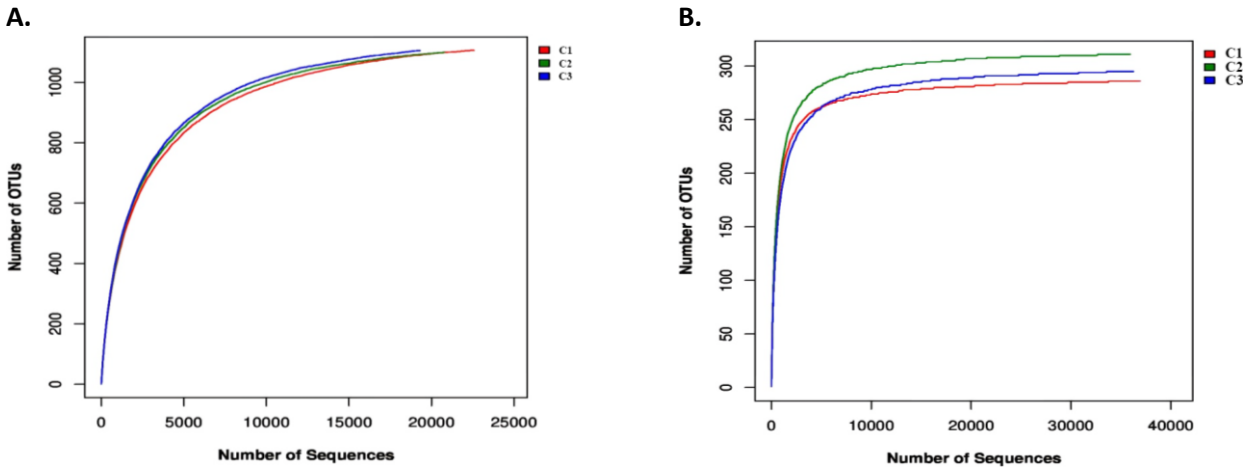

Figure 1. Dilution curves of OTUs abundance for bacteria (**A**) and fungi (**B**).

were 286 (C1), 311 (C2), 295 (C3) (Figure 1B), and Shannon indices were 4.61 (C1), 4.82 (C2), 4.75 (C3), respectively. The coverage was 99.98% for all fungal libraries. Fungal OTU richness and Shannon diversity were the highest in C2 followed by C3 and then C1, indicating that chemical fertilizer application increased fungal diversity in the rhizosphere soil with a stronger increase under the full-rate treatment than under the half-rate treatment.

Bacterial community species composition and abundance

In the rhizosphere soil without chemical fertilizer, bacterial phyla with a relative abundance greater than 10% were *Proteobacteria* (34.42%) and *Chloroflexi* (21.33%). In the treatment with chemical fertilizer, phyla with a relative abundance greater than 10% from high to low were *Proteobacteria* (35.34%), *Chloroflexi* (20.27%), and *Acidobacteria* (10.06%). In the half-rate chemical fertilizer treatment, phyla with a relative abundance greater than 10% were *Proteobacteria* (33.92%), *Chloroflexi* (22.14%), and *Actinobacteria* (11.13%) (Figure 2a). Thus, *Proteobacteria* and *Chloroflexi* were the dominant phyla shared by all three treatments. Compared with the treatment without fertilizer application, the relative abundance of *Proteobacteria* increased by 0.92% and 1.42%, whereas that of *Chloroflexi* decreased by 1.26% and 1.87%, respectively. At the taxonomic (class) level, bacteria with a relative abundance greater than 5% in rhizosphere soil without chemical fertilizer application were unclassified bacteria (18.46%), *Anaerolineae* (12.74%), *Alphaproteobacteria* (12.17%), *Gammaproteobacteria* (11.46%), and *Betaproteobacteria* (7.50%). With full chemical fertilizer application, groups with relative abundance greater than 5% included unclassified bacteria (18.22%), *Alphaproteobacteria* (13.07%), *Anaerolineae* (12.60%), *Gammaproteobacteria* (10.84%), and *Betaproteobacteria* (8.20%). In half-rate fertilizer application, groups with relative abundance greater than 5% included unclassified bacteria (16.57%), *Gammaproteobacteria* (13.09%), *Anaerolineae* (12.98%), *Alphaproteobacteria* (12.07%), and *Betaproteobacteria (*6.22%) (Figure 2b). The results indicated that unclassified bacteria, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Anaerolineae* were the common dominant groups across all three treatments. Compared with the full fertilizer treatment, the unfertilized treatment showed slightly higher abundances of unclassified bacteria (+0.24%), *Anaerolineae* (+0.14%), and *Gammaproteobacteria* (+0.62%), but lower abundances of *Alphaproteobacteria* (−0.90%) and *Betaproteobacteria* (−0.70%). Compared with the half-fertilizer treatment, the

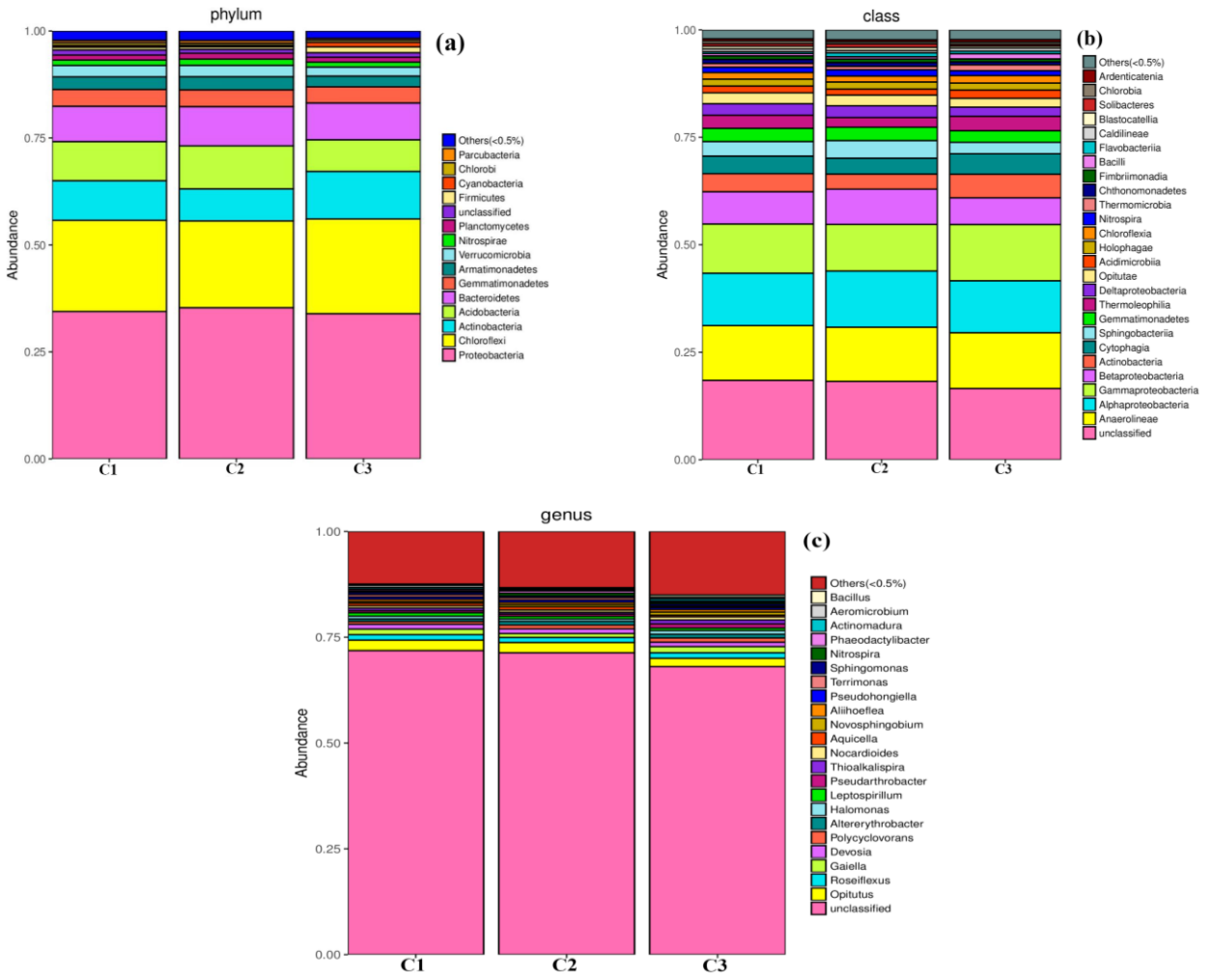

Figure 2. Relative abundance of bacteria at the phylum, class, and genus levels.

unfertilized treatment showed higher abundances of unclassified bacteria (+1.89%), *Alphaproteobacteria* (+0.10%), and *Betaproteobacteria* (+1.28%), and lower abundances of *Anaerolineae* (−0.24%) and *Gammaproteobacteria* (−1.63%). In the rhizosphere soil samples without fertilizer application, the genera with relative abundance greater than 1% were unclassified taxa (71.86%), *Opitutus* (2.45%), *Gaiella* (1.32%), *Roseiflexus* (1.30%), and *Devosia* (1.15%). In the full chemical fertilizer treatment, genera with relative abundance greater than 1% were unclassified taxa (71.34%), *Opitutus* (2.42%), *Roseiflexus* (1.20%), *Devosia* (1.10%), and *Gaiella* (1.00%), while in the half-rate fertilizer treatment, genera with relative abundance greater than 1% were unclassified taxa (68.07%), *Opitutus* (1.10%), *Gaiella* (1.45%), *Roseiflexus* (1.31%), and *Devosia* (1.10%). Thus, unclassified taxa and the genera *Opitutus*, *Gaiella*, *Roseiflexus*, and *Devosia* constituted the dominant genera shared among the three treatments. Compared with the fertilizer treatment, the unfertilized treatment increased the abundance of unclassified taxa by 0.52%, *Opitutus* by 0.03%, *Roseiflexus* by 0.10%, *Devosia* by 0.05%, and *Gaiella* by 0.32%. Compared with the half-rate fertilizer treatment, the unfertilized treatment increased the abundance of unclassified taxa by 3.79% and *Opitutus* by 1.35%, while *Roseiflexus* decreased slightly by 0.01%, *Devosia* increased by 0.05%,

and *Gaiella* decreased by 0.13%. In addition, *Derxia* (0.04%) was detected only in the unfertilized treatment, *Idomarina* (0.60%) and *Rubritepida* (0.03%) were unique to the full fertilizer treatment, and *Hymenobacter* (0.04%) was unique to the half-rate fertilizer treatment (Figure 2c).

Fungal community species composition and abundance

The fungal taxa at the phylum level with relative abundances greater than 10% in the rhizosphere soil without chemical fertilizer application were *Ascomycota* (55.87%) and *Basidiomycota* (23.04%). In the full chemical fertilizer treatment, fungal phyla with relative abundances greater than 10% were *Ascomycota* (65.13%) and *Basidiomycota* (16.82%), while in the half chemical fertilizer treatment, fungal phyla with relative abundances greater than 10% were *Ascomycota* (68.33%) and *Basidiomycota* (17.07%). *Ascomycota* and *Basidiomycota* were the two dominant phyla shared among the three treatments. After halving the fertilizer rate, the relative abundance of *Ascomycota* increased by 3.20% and 12.46% compared with the full and zero chemical fertilizer treatments, respectively. The abundance of *Basidiomycota* in the treatment without fertilizer application was 6.22% higher than that in the full fertilizer treatment and 5.97% higher than that in the half-fertilizer treatment (Figure 3a). The dominant fungal taxa with a relative abundance greater than 5% at the class level in rhizosphere soil without fertilizer application were *Sordariomycetes* (31.36%), unclassified fungi (20.17%), *Agaricomycetes* (19.71%), *Glomeromycetes* (7.04%), *Dothideomycetes* (6.59%), and *Mortierellomycotina* (5.11%). In the full chemical fertilizers treatment, classes with a relative abundance above 5% included *Sordariomycetes* (35.24%), *Dothideomycetes* (12.34%), unclassified fungi (19.52%), *Glomeromycetes* (7.12%), and *Agaricomycetes* (11.26%). In samples receiving half the amount of chemical fertilizer, the dominant classes were *Sordariomycetes* (39.67%), unclassified fungi (17.87%), *Agaricomycetes* (13.57%), and *Dothideomycetes* (13.12%) (Figure 3b). Overall, *Sordariomycetes*, *Dothideomycetes*, unclassified fungi, and *Agaricomycetes* were the major fungal groups across all treatments. When the chemical fertilizer rate was reduced by half, the relative abundance of *Sordariomycetes* increased by 8.31% and 4.43% compared with the no-fertilizer and full-fertilizer treatments, respectively. The abundance of *Dothideomycetes* increased by 6.53% and 0.78% relative to the no-fertilizer and full-fertilizer treatments. Unclassified fungi increased by 0.65% and 2.30% compared with the full-fertilizer and half-fertilizer treatments, respectively. The abundance of *Agaricomycetes* increased by 8.45% and 6.14% compared with the full-fertilizer and half-fertilizer treatments. In addition, several taxa appeared to be treatment specific. *Cystobasidiomycetes* (0.21%) and *Orbiliomycetes* (0.08%) were detected only in the unfertilized soil. *Wallemiomycetes* (0.80%) and unclassified *Chytridiomycota* (0.13%) were unique to the full-fertilizer treatment, whereas *Taphrinomycetes* (0.10%) was detected exclusively in the half-fertilizer treatment. In the rhizosphere soil samples without fertilizer application, the fungal genera with relative abundances greater than 1% were unclassified taxa (33.53%), *Conocybe* (8.00%), *Coprinellus* (5.71%), *Mortierella* (5.11%), *Chaetomium* (4.60%), *Psathyrella* (3.04%), *Fusarium* (3.00%), *Nigrospora* (2.46%), *Dominikia* (2.17%), *Clonostachys* (2.10%), *Cryptococcus* (2.00%), *Myrothecium* (1.72%), *Cylindrocarpon* (1.42%), *Ilyonectria* (1.21%), *Efibulobasidium* (1.12%), *Septoglomus* (1.08%), and *Setophaeosphaeria* (1.05%). In the rhizosphere soil treated with full chemical fertilizer, genera with relative abundances greater than 1% included unclassified taxa (37.83%), *Conocybe* (8.61%), *Cladosporium* (4.73%), *Chaetomium* (4.68%), *Cylindrocarpon* (2.71%), *Fusarium* (2.71%), *Cryptococcus* (2.66%), *Alternaria* (2.47%), *Mortierella* (1.94%), *Clonostachys* (1.91%), *Dominikia* (1.82%), *Fusicolla* (1.76%), *Acremonium* (1.56%), and *Solicoccozyma* (1.25%). In the rhizosphere soil treated with half-reduced chemical fertilizer, the dominant genera were unclassified taxa (33.80%), *Chaetomium*

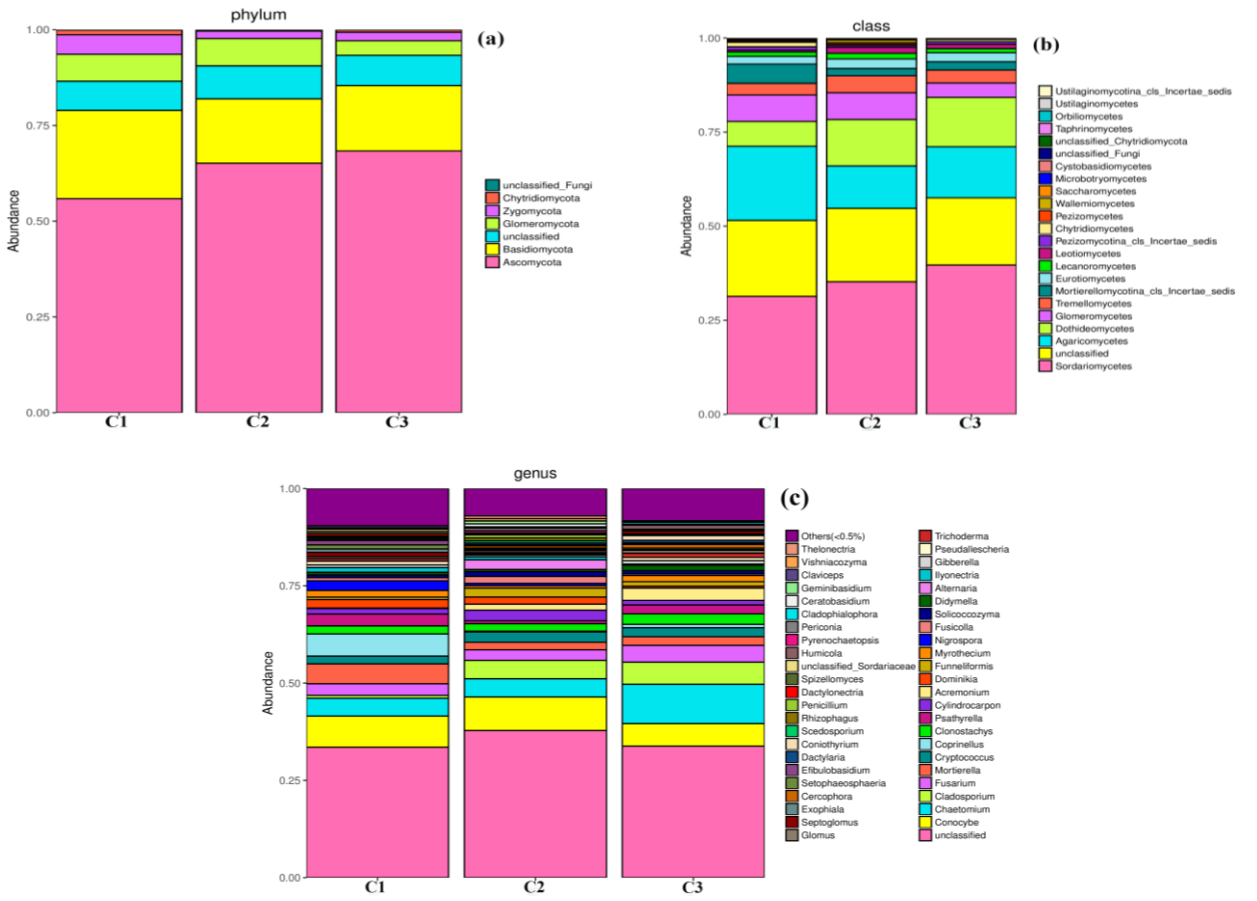

Figure 4. Relative abundance of fungi at the phylum, class, and genus level.

(10.11%), *Cladosporium* (5.68%), *Conocybe* (5.60%), *Fusarium* (4.32%), *Acremonium* (3.17%), *Clonostachys* (2.69%), *Cryptococcus* (2.31%), *Psathyrella* (2.28%), *Mortierella* (2.21%), *Myrothecium* (1.61%), *Didymella* (1.37%), *Coniothyrium* (1.23%), *Cylindrocarpon* (1.17%), *Funneliformis* (1.15%), *Trichoderma* (1.10%), and *Humicola* (1.06%). Across all three treatments, unclassified taxa, *Chaetomium*, *Mortierella*, *Cryptococcus*, *Fusarium*, *Conocybe*, *Cylindrocarpon*, and *Clonostachys* were the dominant genera. Following chemical fertilizer application, the relative abundance of unclassified taxa increased by 4.30% and 4.03% compared with the unfertilized and half-reduced fertilizer treatments, respectively. *Cryptococcus* abundance increased by 0.66% and 0.35%, and *Conocybe* by 0.61% and 3.01%, respectively. The abundance of *Cylindrocarpon* increased by 1.29% compared with the unfertilized treatment and by 1.54% compared with the half-reduced fertilizer treatment. Under the half-reduced fertilizer treatment, *Fusarium* abundance increased by 1.32% relative to unfertilized treatment and by 1.61% relative to the full fertilizer treatment. *Clonostachys* increased by 0.59% and 0.78%, and *Chaetomium* increased substantially by 5.51% and 5.43% compared with the unfertilized and full fertilizer treatments, respectively. Without chemical fertilizer, *Mortierella* abundance was 3.17% and 2.90% higher than that in the full and half-reduced fertilizer treatments, respectively (Figure 3c).

Discussion

Compared with traditional culture and isolation methods, high-throughput sequencing offers advantages such as high sequencing depth, low cost, and accurate quantification [25]. This study applied high-throughput sequencing technology to investigate the effects of different nitrogen, phosphorus, and potassium levels on the rhizosphere microbial community of strawberries. The results showed that the application of chemical fertilizers increased the richness and Shannon index of fungal OTUs, while it reduced the richness and Shannon index of bacterial OTUs. *Proteobacteria* and *Chloroflexi* were the two dominant bacterial phyla shared among the three treatments, which was consistent with the findings of previous studies in strawberries [26], apples [27], grapes [28], peanuts [29], sesame fields [30], maize [31], and black soil of Northeast China after biochar application [32]. *Proteobacteria* and *Chloroflexi* played important roles in soil microbial communities. The reduction in bacterial OTU richness and Shannon index under chemical fertilizer application might be attributed to the inherently acidic soil conditions and the higher nitrogen content in fertilized soil compared with the unfertilized treatment, which could disrupt soil physical structure and reduce microbial activity, which was similar to the findings of previous research in maize [33]. The relative abundance of *Proteobacteria* in the full chemical fertilizer treatment increased compared with both the unfertilized and half-rate chemical fertilizer treatments, whereas the relative abundance of *Chloroflexi* decreased, which was in agreement with previous report [34], and might be because *Proteobacteria* were among the most abundant bacterial groups in soil, and the increased nutrient availability following chemical fertilizer application favored their growth. Previous studies have shown that *Proteobacteria* preferentially inhabit nutrient-rich soils and are significantly correlated with soil organic matter and total nitrogen content [35]. An increase in *Proteobacteria* can improve plant yield and quality, thereby promote plant growth and modify yield and quality attributes [36]. In contrast, *Chloroflexi* are Gram-negative, autotrophic bacteria that adapt to nutrient-poor environments through photosynthesis. Their abundance was the lowest in the half-rate chemical fertilizer treatment, possibly because *Chloroflexi* possessed a survival advantage that was less dependent on nutrient supply. In addition, the abundance of *Chloroflexi* has been reported to increase significantly during the rapid growth period of new shoots [37], suggesting that excessive chemical fertilizer application may inhibit new shoot growth. Overall, bacterial abundance in treatments without chemical fertilizer and with half-rate chemical fertilizer was higher than that in the full-rate chemical fertilizer treatment. An appropriate combination of chemical and organic fertilizers could increase bacterial abundance, which was consistent with the results of Zeng *et al*. [38]. At the class and genus levels, unclassified taxa were the most abundant. Their abundance decreased in the full and half-rate chemical fertilizer treatments compared with that in the unfertilized treatment, which might be due to limitations of current sequencing and annotation technology as many novel taxa remain poorly characterized, or it might indicate the emergence of new species in soil following fertilization. In rhizosphere soil samples treated with chemical fertilizer, the abundances of α-*Proteobacteria* and β-*Proteobacteria* were the highest. Previous studies have shown that these two classes contain many nitrogen-fixing bacteria, which can significantly enhance soil nitrogen fixation and play an important role in maintaining soil fertility [39]. This study found that, among the three different treatments, *Ascomycota* and *Basidiomycetes* were the common dominant fungal phyla with *Ascomycota* accounting for the largest proportion. The results were consistent with the findings of previous studies on strawberries [40], cantaloupe [41], and banana [42]. Fertilizer application did not alter the composition of the dominant fungal phyla but only changed their relative abundances. Soil itself was a natural medium containing large numbers of microorganisms. Under the half-fertilizer treatment, the abundance of *Ascomycota* increased compared with both the no-fertilizer

and full-fertilizer treatments. The abundance of *Basidiomycetes* decreased by 5.97% compared with the no-fertilizer treatment and increased by 0.25% compared with the full-fertilizer treatment. *Ascomycota* can occur in high abundance in acidic soils, and most members are saprophytic fungi capable of decomposing many recalcitrant organic compounds in soil [43]. Compared with *Basidiomycetes*, they also exhibit a faster evolutionary rate [44]. A plausible explanation was that the soil used for crop cultivation in this study was a typical acidic, uncultivated red soil, resulting in a higher abundance of *Ascomycota* than *Basidiomycetes*. In addition, high soil nitrogen content could restrict the utilization of lignin by *Basidiomycetes*, thereby inhibiting their growth [45]. Thus, the increase in soil nitrogen content after fertilization might account for the observed decrease in *Basidiomycetes* abundance. Under the half-fertilizer treatment, the abundances of *Agaricales* and *Sordariales* were the highest followed by the full-fertilizer treatment. After full fertilizer application, the abundance of *Fusarium* was the lowest at 2.71%. *Fusarium* can infect many plants including food crops, cash crops, oil crops, medicinal plants, and ornamentals, causing various rot diseases such as root rot, ear rot, and wilt, which lead to severe yield losses and substantial economic damage [46]. It was speculated that, after fertilizer application, the abundant nutrient supply reduced infection by pathogenic fungi and decreased the accumulation of pathogens in the rhizosphere soil, which was of great significance for increasing yield and improving crop quality. Because the characteristics of soil microbial communities are shaped by the combined effects of microorganisms and their environment, the impact of chemical fertilizers on rhizosphere microbial communities cannot be explained solely by changes in a single population or a few populations. In this study, only the effects of different nitrogen, phosphorus, and potassium levels on the abundance of microorganisms in strawberry rhizosphere soil were analyzed. However, different plant species harbor distinct bacterial and fungal communities, which in turn exert different effects on the plants. Therefore, further research is needed.

Conclusion

There were significant differences in rhizosphere soil microbial communities of the strawberry among different fertilizer treatments. The results showed that fertilizer application increased the richness and Shannon index of fungal OTUs but decreased the richness and Shannon index of bacterial OTUs. Under full fertilizer applications, the relative abundances of bacterial communities in the rhizosphere soil at the phylum, class, and genus levels increased significantly for *Proteobacteria*, α-*Proteobacteria*, and β-*Proteobacteria*, whereas the abundances of *Chloroflexi*, *Anaerobacteria*, γ-*Proteobacteria*, the genus *Gaiella*, and the genus *Roseobacter* decreased significantly. The abundance of fungal communities increased significantly for unclassified genera, the genus *Cryptococcus*, and the genera *Coniothyrium* and *Stachybotrys*, while the abundances of the phylum *Basidiomycetes*, the class *Agaricales*, the genus *Mortierella*, and the genus *Fusarium* decreased significantly. In the half-fertilizer treatment, the abundances of bacterial communities in the rhizosphere soil at the phylum, class, and genus levels increased significantly for *Chloroflexi*, γ-*Proteobacteria*, *Proteobacteria*, *Gaiella*, and *Roseobacter*, while the abundances of fungal communities increased significantly for *Ascomycota*, *Agaricales*, *Thecomycetes*, the genus *Chaetomium*, and the genus *Clonostachys*. In the treatment without chemical fertilizer application, the abundances of bacterial communities in the rhizosphere soil at the phylum, class, and genus levels increased significantly for unclassified taxa, the genus *Opitotus*, and the genus *Devosia*, while the abundance of fungal communities increased significantly for unclassified taxa at the class level.

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References

1. Liu C, Wang X, Li XS, Wang JQ. 2023. Status of strawberry production in China and comparative analysis with other countries. China Fruits. 2023(7):136–140.
2. Peng YQ, Lu WJ, Liu S, Tian XY, Ji MX, Ji GB. 2025. Impact of e-commerce sales on farmers' adoption of new green production technologies: Evidence from a survey of strawberry growers in Jiangsu Province. Cent South Agric Sci Technol. 46(2):198–204.
3. Ruan JW. Yunnan Strawberry. In Yunnan Strawberry. Kunming: Yunnan Science and Technology Press; 2023:235.
4. Li YM. 2024. Investigation of virus infection to strawberry plants in Yunnan and construction of regeneration system of virus-free nursery. Yunnan University.
5. Zhang XH, Fan ZW, Zhu JF, Lin L, Zhang R, Xu SG, *et al*. 2025. Effects of reduced chemical fertilizer application combined with methyl Jasmonate on NO synthesis and the AsA–GSH cycle in strawberry under continuous cropping. Guihaia. 45(8):1543–1552.
6. Zou ZY, Gao LH, Yang JC, Zhang ZR, Qiu Q, He Q, *et al*. 2025. Forest soil nutrient characteristics and fertility evaluation in the hilly red soil region of South China. J Southwest For Univ (Nat Sci). 45(6):61–69.
7. Ding HS, Guo JT, Pu XG, Li JF, Yang ZJ, Xu SG, *et al*. 2025. Research progress on biochar in alleviating crop continuous cropping obstacles. Jiangsu Agric Sci. 53(17):10–20.
8. Liu J, Gao Y, Ma YS, Shang Y. 2025. Progress and challenges of synthetic biology in agriculture. Synth Biol. 6(5):998–1024.
9. Wang XY, Wang GH, Xue ZL, Zhang B, Sui WJ, Jia HY, *et al*. 2024. Research and application progress of lignin in soil improvement and remediation. China Pulp Pap. 39(2):140–153.
10. Yu YJ, Xu QS, Zhang JH, Zhu CQ, Zhu LF, Tian WH, *et al*. 2024. Pathways and mechanisms of the effects of soil fertility improvement technologies on soil health. Soils Fert China. 2024(2):220–227.
11. Hu W, Zhang YP, Rong XM, Zhou X, Fei JC, Peng JW, *et al*. 2024. Biochar and organic fertilizer applications enhance soil functional microbial abundance and agroecosystem multifunctionality. Biochar. 6(1):3.
12. Zhang LQ, Zhao ZH, Jiang BL, Baoyin B, Cui ZG, Wang HY, *et al*. 2024. Effects of long-term application of nitrogen fertilizer on soil acidification and biological properties in China: A meta-analysis. Microorganisms. 12(8):1683.
13. Liu W, Deng GH, Zhang JC, Yang QP. 2011. Effects of different fertilization treatments on rhizosphere microorganisms of Moso bamboo and their PCR-DGGE analysis. J Northeast For Univ. 39(5):52–55.
14. Hao HT, Wang RY, Zhao X, Wang J, Guo ZH, Zhang YB, *et al*. 2017. Effect of compost on rhizosphere microbial diversity of Lanzhou lily based on high-throughput sequencing technology. Acta Agric Boreali-Occident Sin. 26(3):437–447.
15. Wu JN, Liu YX, Zhou X, Wang TY, Gao Q, Gao YH, *et al*. 2018. Analysis of the effects of long-term fertilization regimes on fungal communities in black soil using the Illumina MiSeq sequencing platform. Acta Microbiol Sin. 58(9):1658–1671.
16. Jiang YH, Lin S, Lin WW, Chen T, Yasir A, Wei XY, *et al*. 2017. Effects of different fertilizers on metabolic activity and community structure of rhizosphere soil microorganisms in degraded tea gardens. Chin J Ecol. 36(10):2894–2902.
17. Zhang SN, Gao HY, Yan DR, Li JW, Yang ZG, Huang HG, *et al*. 2025. Effects of vegetation restoration strategies on sandy soil bacterial community structure and potential functions. J Desert Res. 45(5):1–12.
18. Li Q, Wang H, Luan JW, Gao B, Wang Y, Liu SR. 2023. Effects of tree species diversity and soil microbial diversity on plantation productivity. Acta Ecol Sin. 43(12):4984–4994.
19. Wang YY, Xie ZJ, Kutschera E, Adams JI, Kadash-Edmondson KE, Xing Y. 2024. rMATS-turbo: An efficient and flexible computational tool for alternative splicing analysis of large-scale RNA-seq data. Nat Protoc. 19(4):1083–1104.
20. Lin LT, Li GX, Yu HY, Ma KM. 2024. pH nonlinearly dominates soil bacterial community assembly along an altitudinal gradient in oak-dominant forests. Microorganisms. 12(9):1877.
21. Srivastava A, Akhter Y, Verma D. 2024. A step-by-step procedure for analysing the 16S rRNA-based microbiome diversity using QIIME 2 and comprehensive PICRUSt2 illustration for functional prediction. Arch Microbiol. 206(12):467.
22. Fares M, Tharwat EK, Cleenwerck I, Monsieurs P, Van Houdt R, Vandamme P, *et al*. 2025. The unresolved struggle of 16S rRNA amplicon sequencing: A benchmarking analysis of clustering and denoising methods. Environ Microbiome. 20(1):51.
23. Spatola G, Giusti A, Gasperetti L, Nuvoloni R, Dalmasso A, Chiesa F, *et al*. 2025. 16S rRNA metabarcoding applied to the microbiome of insect products (novel food): A comparative analysis of three reference databases. Ital J Food Saf. 14(1):13171.
24. Xu TY, Li RL, Zhang YQ, Shen XX. 2025. Effects of temperature–flooding combined stress on rhizosphere bacterial community structure and diversity of the mangrove plant *Aegiceras corniculatum*. J Peking Univ (Nat Sci Ed). 61(4):781–790.

25. Zhang G, Ao Q, Zhou XY, Ding GT, Wang ZF, Teow YH, *et al*. 2025. Research progress on virus enrichment-related materials and devices. Mater China. 44(7):673–683.
26. Tong LH, Gao J, Jin YS. 2019. Dynamic changes of rhizosphere microorganisms of strawberry at different growth stages. J Beijing Univ Agric. 34(4):10–15.
27. Song XJ. 2017. Analysis of apple rhizosphere microbial community structure and screening of plant growth-promoting rhizobacteria. Shandong Agric Univ.
28. Song XJ. 2017. The study on bacterial diversity of vineyard soil. Qilu Univ Technol.
29. Liu XH. 2018. Effect of biochar and biochar-based fertilizer on bacterial diversity and peanut yield. Shenyang Agric Univ.
30. Luo Y. 2016. Effects of different flax cropping modes on soil enzymes activities and soil microbial community structure. Gansu Agric Univ.
31. Xu YR, Fang ZJ, Lu XP, Hao LJ. 2017. Effects of starane on maize soil bacterial diversity analyzed by high-throughput sequencing technology. Acta Microbiol Sin. 57(7):985-993.
32. Yao Q. 2017. Effect of biochar addition on soil physicochemical properties and microbial diversity in a black soil of northeast China. Univ Chinese Acad Sci (Northeast Inst Geography and Agroecology, CAS).
33. Wu JN. 2018. Effects of long-term different fertilization on microbial diversity and functional microflora in corn rhizosphere. Jilin Agric Univ.
34. Wang C, Chen LJ, Tian W, Huang SJ, Wang L, Xi YG. 2019. High-throughput sequencing analysis of the effects of microbial fertilizer on soil microbial communities in red soil organic farmland. Jiangsu Agric Sci. 47(2):272-277.
35. Chaturvedi S, Kumar A, Min L, Mohan K, Singh N, Kumar R. 2025. Insights into earthworms and fungi: pioneering roles in mitigating global food scarcity and combatting land degradation. Land Degrad Dev. 36(5):1405-1429.
36. Liu ZW, Liu N. 2025. Effects of rhythmic music stimulation on plant growth rhythm and developmental responses. Mol Plant Breed. 23(23):7981-7986.
37. Wang XD, Ye B, Dong Y, Wang XY, Zhao ZJ, Liu DN, *et al*. 2025. Effects of *Clostridium butyricum* and photosynthetic bacteria on the immune function of *Apostichopus japonicus*. Fish Sci. 44(5):685–694.
38. Zeng XB, Wang YN, Wang YZ, Lin ZL, Li LF, Bai LY, *et al*. 2013. Effects of different fertilization regimes on abundance and composition of the bacterial community in greenhouse vegetable soils. Sci Agric Sin. 46(1):69–79.
39. Liu DH, Liang SW, Zhang Z, Xiao ZX, Qiao Y, Ren J, *et al*. 2025. Effects of straw incorporation combined with nitrogen fertilizer on rhizosphere soil microbial community of winter rapeseed. Jiangsu J Agric Sci. 41(10):1962–1972.
40. Zhao F, Zhao MZ, Wang Y, Pang FH. 2017. Biodiversity of bacteria and fungi in the rhizosphere of strawberry under different years of continuous cropping. Microbiol China. 44(6):1377-1386.
41. Yang Y, Zhang XJ, Li MH, Wang DM, Wang GZ, Zhang YB. 2018. Effects of microbial fertilizer on rhizosphere soil fungal community structure in long-term continuously cropped Hami melon under protected cultivation. J Appl Environ Biol. 24(1):68-74.
42. Shen ZZ, Ruan YZ, Chao X, Zhang J, Li R, Shen Q. 2015. Rhizosphere microbial community manipulated by 2 years of consecutive biofertilizer application associated with banana Fusarium wilt disease suppression. Biol Fertil Soils. 51(5):553-562.
43. Sun K, Xia JY, Ye XY, Fang Z, Li AB, Zhou BZ, *et al*. 2025. Research progress on continuous cropping obstacles in medicinal and edible fungi. World For Res. 38(3):49-57.
44. Guo XW, Du SY, Wang FX, Ye Y, Yang MQ, Min W. 2022. Effects of long-term saline water irrigation on soil bacteria and fungi community structure in cotton field. Xinjiang Agric Sci. 59(12):2909-2923.
45. Tang W, Li ZG, Zhao QT, Sun J. 2025. Effects of oat (*Avena sativa*) planting density on the growth and rhizosphere fungal community structure of *Digitaria sanguinalis* and *Echinochloa crusgalli*. Acta Pratacult Sin. 34(8):149-164.
46. Wang ZY, Zhong YJ, Wang YF, Xie NH, Zhang Y, Jiang ZY, *et al*. 2025. Ecological functions of plant-beneficial microbiomes and their application prospects in sustainable agriculture. Chin J Appl Ecol. 36(5):1553-1566.