

Effects of Fertilization on Rhizosphere Microbial Community Characteristics of Tiger Nut (Post-print)

Authors:

Date: 2021-12-12T00:00:00+00:00

Abstract

This study investigated the effects of different fertilization treatments on the composition and diversity of rhizosphere soil microbial communities of tiger nuts in Xinjiang sandy areas, which is of great significance for field nutrient management and soil fertility improvement of this crop in the region. The results showed that fertilization significantly increased the contents of organic matter (OM), available phosphorus (AP), alkaline hydrolyzable nitrogen (AN), and available potassium (AK) in the rhizosphere soil of tiger nuts ($P < 0.05$). The diversity indices (Chao, Ace, and Shannon indices) of bacteria and fungi in the rhizosphere soil of tiger nuts showed a decreasing trend with increasing fertilization rates. The dominant bacterial phyla in the rhizosphere soil were Actinobacteria, Proteobacteria, Firmicutes, and Chloroflexi, while the dominant fungal phyla were Ascomycota and Basidiomycota. Redundancy analysis indicated that soil pH and electrical conductivity (EC) were significantly correlated with the dominant bacterial phyla, and soil moisture content was significantly correlated with the dominant fungal communities. In summary, fertilization altered the structure and diversity of soil microbial communities in the rhizosphere of tiger nuts by affecting soil physicochemical properties, and the diversity of rhizosphere soil microorganisms decreased with increasing fertilization rates. Soil pH and EC were the driving factors influencing changes in soil bacterial communities, while the main factors affecting fungal community changes were soil organic matter and moisture content.

Full Text

Effect of Fertilization on the Characteristics of Rhizosphere Soil Microbial Communities in *Cyperus esculentus* in the Sandy Areas of Xinjiang

XU Jieliang¹, ZHANG Fenghua^{1,2}, LI Bianbian¹, WANG Jiaping^{1,2}, CHENG Zhibo^{1,2}

¹College of Agriculture, Shihezi University, Shihezi 832000, Xinjiang, China

²Key Laboratory of Oasis Ecological Agriculture, Shihezi University, Shihezi 832000, Xinjiang, China

Abstract

This study investigated the effects of different fertilization treatments on the composition and diversity of rhizosphere soil microbial communities in *Cyperus esculentus* cultivated in the sandy areas of Xinjiang, which is of great significance for field nutrient management and soil fertility improvement. The results demonstrated that fertilization significantly increased the contents of available potassium, available phosphorus, alkali-hydrolyzable nitrogen, and soil organic matter in the rhizosphere soil of *C. esculentus* ($P < 0.05$). The Shannon, Chao, and Ace diversity indices of both bacterial and fungal communities exhibited a decreasing trend with increasing fertilization rates. The dominant bacterial phyla in the rhizosphere soil were *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Chloroflexi*, while the dominant fungal phyla were *Ascomycota* and *Basidiomycota*. Redundancy analysis revealed that soil pH and electrical conductivity were significantly correlated with the major dominant bacterial phyla, whereas soil moisture content was significantly correlated with the dominant fungal communities. In conclusion, fertilization altered the structure and diversity of rhizosphere soil microbial communities in *C. esculentus* by influencing soil physicochemical properties, with microbial diversity decreasing as fertilization rates increased. Soil pH was identified as the primary driving factor affecting bacterial community changes, while soil organic matter and moisture content were the main factors influencing fungal community variation.

Keywords: fertilization; sandy area; *Cyperus esculentus*; rhizosphere soil; microbial community; Xinjiang

Introduction

Soil microorganisms represent the most active and important components of soil ecosystems, playing crucial roles in driving organic matter decomposition, nutrient cycling, and energy flow [?]. The rhizosphere serves as the primary gateway for nutrients and water entering plants, and its microbial diversity is closely associated with plant roots, making rhizosphere microbial community dynamics

an important biological indicator for evaluating soil quality [?]. *Cyperus esculentus* is an annual sedge plant and an oil-bearing herbaceous energy crop native to Africa [?]. This species exhibits strong stress resistance, tolerance to poor soil conditions, well-developed root systems, and strong tillering and regeneration capabilities, providing natural windbreak and sand fixation functions [?]. Loose sandy soil not only facilitates root and tuber growth in *C. esculentus* but also benefits subsequent harvesting operations [?]. However, sandy soils have poor capillary performance and are prone to water and fertilizer loss, resulting in low potential nutrient content. Therefore, cultivating crops on sandy soils requires rational water and fertilizer regulation measures based on crop requirements at different growth stages to achieve cost-effective and efficient production [?].

In recent years, the role of rhizosphere microorganisms in farmland soil ecosystems has received increasing attention [?]. The combined application of nitrogen, phosphorus, and potassium fertilizers can not only increase crop yield and soil nutrient content but also enhance soil microbial diversity, thereby contributing positively to the maintenance of soil ecological environments [?]. However, some studies have found that chemical fertilizer application can alter microbial community structure and reduce microbial community diversity [?]. Although these findings are not entirely consistent, they demonstrate that fertilization has widespread effects on farmland ecosystems. Consequently, in-depth research on the impacts of different fertilization treatments on rhizosphere soil microorganisms is essential for guiding agricultural production and sustainable farmland development.

Previous studies have shown that soil microbial biomass carbon and nitrogen, soil respiration, and microbial functional activity change with fertilization rates [?]. Long-term fertilization significantly increases rhizosphere soil microbial biomass carbon and nitrogen content [?], while the application of organic or compound fertilizers promotes microbial growth and metabolic activity, thereby increasing rhizosphere soil microbial diversity [?]. Building upon this foundation, the present study utilized high-throughput sequencing technology to analyze the characteristics of rhizosphere soil microbial communities in *C. esculentus* under different fertilization treatments at a long-term experimental site in the sandy areas of Xinjiang. The objective was to clarify the response of *C. esculentus* rhizosphere microorganisms to fertilization and provide a scientific basis for nutrient management strategies for this crop in Xinjiang's sandy regions.

1. Materials and Methods

1.1 Experimental Design The field experiment was conducted at the Shache Farm of the Third Division in Xinjiang (38°38' N, 77°6' E), which features a warm temperate continental climate with an average annual temperature of 12.3°C, average annual precipitation of 56.6 mm, and average annual evaporation of 192 mm. The test soil was classified as sandy soil, with basic physicochemical

properties shown in . The experiment began in 2017 and has involved continuous cropping of *C. esculentus* (variety Zhongyou 1) sown in early April each year. Under traditional local cultivation practices, the nitrogen-phosphorus-potassium fertilization rates for *C. esculentus* are 200–283–88 kg · hm⁻². Based on this baseline with $\pm 20\%$, F2(218–68 kg · hm⁻²), F3(200–283–88 kg · hm⁻²), and CK (no fertilization). Fertilizers included urea (CO(NH₂)₂), monoammonium phosphate (NH₄H₂PO₄), and *C. esculentus* at different growth stages. Each application was completed in three installments. Before each fertilization, 2% was applied, followed by fertigation. The experiment employed a randomized block design with each plot measuring 10 m × 10 m (100 m²). Each treatment was replicated three times. Planting was conducted using hole sowing at a density of 35 cm spacing, with 6,500 plants per hectare. Drip irrigation was implemented in a one-tube-two-row pattern, with a total irrigation volume of 4,500 m³ · hm⁻² across the growing season. Intertillage and weeding were performed during the growth period.

1.2 Sample Collection Soil samples were collected at the end of August during the peak tuber formation stage of *C. esculentus*. For each treatment, five plant holes were randomly selected, and rhizosphere soil was collected using the shaking method. The collected soil samples were thoroughly mixed, placed in sterile sealed bags, and transported in a cooler box with ice packs to the laboratory. Each sample was divided into two portions: one stored at -80°C for microbial sequencing analysis, and the other air-dried and passed through a sieve for determination of physicochemical indicators.

1.3 Determination of Soil Physicochemical Properties Soil moisture content was determined using the oven-drying method [?]. Soil pH was measured using a pH meter (HQ411D, Hach, USA) with a soil-to-water ratio of 1:2.5. Soil electrical conductivity was determined using a conductivity meter (DDSJ-308F, INESA Scientific Instrument, Shanghai, China) with a soil-to-water ratio of 1:5. Soil alkali-hydrolyzable nitrogen was measured using the alkaline diffusion method [?]. Available phosphorus content was determined using the 0.5 mol · L⁻¹ NaHCO₃ extraction-spectrophotometry method [?]. Soil available potassium content was measured using the 1 mol · L⁻¹ NH₄OAc extraction-flame photometry method [?]. Soil organic matter content was determined using the potassium dichromate external heating method [?].

1.4 High-Throughput Sequencing of Soil Microorganisms Total DNA was extracted from soil samples using the FastPrep DNA extraction kit. For bacteria, the V4-V5 hypervariable region of the 16S rRNA gene was amplified using universal primers 5'-GTGCCAGCMGCCGCGG-3' and 5'-CCGTCAATTCMTTTRAGTTT-3'. For fungi, the ITS region was amplified using primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). The amplification products were sequenced by Shanghai Majorbio Bio-Pharm Technology Co., Ltd.

1.5 Data Processing Raw sequencing data were processed and analyzed using the cloud platform provided by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (<https://cloud.majorbio.com>). Excel 2016 and SPSS 20.0 software were used for data processing and graphing. One-way ANOVA was performed for significance testing, and Pearson correlation coefficient analysis was conducted for correlation assessment. CANOCO 4.5 software was used for redundancy analysis (RDA) and visualization of relationships between microbial communities and environmental factors.

2. Results

2.1 Analysis of Soil Physicochemical Properties The physicochemical properties of rhizosphere soil under different fertilization treatments are presented in . Soil electrical conductivity ranged from high to low as $F3 > F2 > F1 > CK$, though differences among treatments were not significant ($P > 0.05$). The contents of available potassium, available phosphorus, and alkali-hydrolyzable nitrogen in the F3 treatment were $47.54 \text{ mg} \cdot \text{kg}^{-1}$, $29.65 \text{ mg} \cdot \text{kg}^{-1}$, and $19.83 \text{ mg} \cdot \text{kg}^{-1}$, respectively, which were significantly higher than those in the CK treatment ($P < 0.05$). Soil organic matter content increased significantly under all fertilization treatments ($P < 0.05$), but no significant differences were observed among the different fertilization treatments ($P > 0.05$). Soil pH decreased significantly with increasing fertilization rates ($P < 0.05$), with the lowest value observed in the F3 treatment.

2.2 Effects of Different Fertilization Treatments on Rhizosphere Soil Microbial Community Diversity High-throughput sequencing analysis revealed that the numbers of effective bacterial sequences obtained from rhizosphere soil samples under different fertilization treatments were: CK (38,886), F1 (33,522), F2 (31,667), and F3 (29,663). The average coverage of all samples exceeded 99%, indicating that the sequencing results were valid. At the 97% similarity level, different fertilization treatments shared 1,087 bacterial OTUs, with the CK treatment having the highest number of unique OTUs (155) and the F3 treatment the lowest (111). For fungi, different treatments shared 35 OTUs, with the CK treatment showing the most unique OTUs (38) and the F3 treatment the fewest (29). No unique OTUs were detected in the F2 treatment.

As shown in , significant differences in microbial community diversity indices were observed among treatments ($P < 0.05$). The Shannon and Simpson indices for bacteria followed the order $CK > F1 > F2 > F3$, with significant differences between treatments ($P < 0.05$). For fungi, the Shannon index followed the order $CK > F1 > F2 > F3$, while the Simpson index showed the opposite trend ($F3 > F2 > F1 > CK$), though differences were not significant ($P > 0.05$). All fertilization treatments significantly reduced the Shannon, Chao, and Ace indices of both bacterial and fungal communities compared to the CK treatment ($P < 0.05$).

2.3 Effects of Different Fertilization Treatments on Relative Abundance of Rhizosphere Soil Bacteria and Fungi

The species composition of rhizosphere soil bacterial and fungal communities at different taxonomic levels under various fertilization treatments is illustrated in [Figure 2: see original paper]. The dominant bacterial phyla were *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Bacteroidetes*, and *Planctomycetes*. Although bacterial community composition was similar across fertilization treatments, the relative abundance of these phyla varied. Compared to the CK treatment, all fertilization treatments increased the relative abundance of *Firmicutes*, *Chloroflexi*, and *Planctomycetes* to varying degrees, while decreasing that of *Bacteroidetes*.

The dominant fungal phyla in rhizosphere soil samples were *Ascomycota* and *Basidiomycota*, with relative abundances of 42.72%–60.62% and 17.64%–51.46%, respectively, making them the dominant fungal groups at the phylum level. With increasing fertilization rates, the relative abundance of *Ascomycota* showed an increasing trend, while that of *Basidiomycota* decreased. The most abundant fungal genera included *Filobasidium*, *Alternaria*, *Podospora*, and *Acremonium*.

2.4 Relationship Between Rhizosphere Soil Microbial Community Diversity and Soil Physicochemical Properties

Correlation analysis revealed significant relationships between microbial community diversity indices and soil physicochemical properties (). The bacterial Shannon index was significantly negatively correlated with available potassium, available phosphorus, alkali-hydrolyzable nitrogen, and organic matter content, and significantly positively correlated with soil pH. The bacterial Simpson index showed significant negative correlations with available potassium, available phosphorus, alkali-hydrolyzable nitrogen, and organic matter content. The fungal Shannon index was significantly positively correlated with soil pH and significantly negatively correlated with alkali-hydrolyzable nitrogen. The fungal Simpson index was significantly positively correlated with soil available potassium.

2.5 Relationship Between Rhizosphere Soil Microbial Community Composition and Soil Physicochemical Properties

To investigate the influence of soil environmental factors on microbial community composition at the phylum level under different fertilization treatments, redundancy analysis (RDA) was performed to elucidate relationships between microbial communities and environmental factors. Soil physicochemical properties explained 61.64% of the variation in bacterial community structure, with the first and second axes accounting for 50.36% and 11.28% of the variation, respectively. The relative abundance of *Actinobacteria* was significantly positively correlated with pH and electrical conductivity, while that of *Proteobacteria* was significantly positively correlated with pH. The relative abundance of *Firmicutes* was significantly positively correlated with alkali-hydrolyzable nitrogen. The primary environmental factors driving bacterial community structure changes were pH and electrical conductivity.

Soil physicochemical properties explained 56.72% of the variation in fungal community structure, with the first and second axes accounting for 36.17% and 20.55% of the variation, respectively. The relative abundance of *Ascomycota* was significantly positively correlated with available potassium, available phosphorus, alkali-hydrolyzable nitrogen, and organic matter, while that of *Basidiomycota* was significantly positively correlated with organic matter and moisture content. The main environmental factors influencing fungal community structure were organic matter and moisture content.

3. Discussion

3.1 Effects of Different Fertilization Treatments on Soil Physicochemical Properties All fertilization treatments increased the contents of available nutrients to varying degrees compared to the CK treatment, while significantly decreasing soil pH. This acidification effect is consistent with previous research findings [?] and may be attributed to the accumulation of nitrates produced from urea decomposition in the soil [?]. The present study found no significant changes in soil organic matter content among different fertilization treatments, likely due to the inherently low organic matter content of the sandy test soil. With increasing fertilization rates, the contents of available potassium, available phosphorus, and alkali-hydrolyzable nitrogen showed significant increasing trends ($P < 0.05$), which aligns with the results of Tang et al. [?]. However, some studies have reported that available phosphorus does not change significantly with increasing fertilization rates [?], and these discrepancies may be caused by differences in fertilizer types and soil conditions among experimental sites.

3.3 Relationship Between Soil Physicochemical Properties and Rhizosphere Soil Microorganisms in *Cyperus esculentus* Under Different Fertilization Treatments Previous studies have demonstrated that heavy application of inorganic nitrogen, phosphorus, and potassium fertilizers reduces soil bacterial and fungal diversity [?, ?]. The present study similarly found that the diversity indices (Shannon) and evenness indices (Simpson) of all fertilization treatments were significantly lower than those of the CK treatment, indicating that nitrogen-phosphorus-potassium fertilization reduces the diversity of rhizosphere soil microorganisms in *C. esculentus*. This reduction may be due to changes in the rhizosphere environment after fertilization that prevent some microorganisms from surviving or lead to convergent evolution, resulting in decreased microbial community diversity compared to the unfertilized treatment. The decreasing trend in microbial diversity indices with increasing fertilization rates may be attributed to soil compaction caused by excessive chemical fertilizer application [?], which leads to insufficient oxygen supply and negatively affects the survival of aerobic microorganisms, thereby reducing diversity.

The present study identified *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Chlo-*

roflexi, *Bacteroidetes*, and *Planctomycetes* as the dominant bacterial phyla in the rhizosphere of *C. esculentus*. Similar findings have been reported in rhizosphere soils of rice and tea plantations [?], indicating that even in sandy soils, the dominant bacterial phyla are not unique, though their abundances vary among fertilization treatments. The relative abundances of *Firmicutes*, *Chloroflexi*, and *Planctomycetes* were higher in all fertilization treatments compared to the CK treatment. Previous research has shown that nitrogen and phosphorus application can increase the relative abundance of *Chloroflexi* [?], which has facultative anaerobic characteristics and is widely distributed in nutrient-poor soils [?]. The increase in *Chloroflexi* abundance in this study may be partly attributed to the low fertility of the sandy soil and potential oxygen deficiency caused by fertilization-induced compaction [?]. *Planctomycetes* are important nitrogen-transforming microorganisms that convert ammonia nitrogen and nitrite nitrogen to nitrogen gas [?], and their increased abundance under nitrogen fertilization conditions has been confirmed by previous studies [?].

The dominant fungal phyla identified in this study were *Ascomycota* and *Basidiomycota*, which is consistent with findings from cucumber fields [?], yellow mud paddy soils [?], and *Camellia oleifera* forests [?], suggesting that these two phyla hold important positions in soil microbial communities. The relative abundance of *Ascomycota* increased after fertilization, possibly because nitrogen input from chemical fertilizers improved soil conditions favorable for *Ascomycota* survival. In contrast, the relative abundance of *Basidiomycota* was higher in fertilized treatments than in the CK treatment. *Basidiomycota* are important decomposers of soil litter [?], and the improved growth of *C. esculentus* after nitrogen-phosphorus-potassium fertilization likely increased litter input, thereby benefiting *Basidiomycota*.

Both bacterial and fungal diversity showed significant negative correlations with soil available nutrients and organic matter, indicating that environmental factors are the primary determinants influencing microbial community diversity. While some studies have reported positive correlations between soil organic matter and microbial diversity [?], this study found a significant negative correlation, possibly because the low background organic matter content in the sandy soil promoted the rapid proliferation of dominant microbial groups through fertilization and increased organic matter input. This competitive advantage of dominant groups may have suppressed less competitive taxa, ultimately reducing overall microbial diversity. The primary environmental factors affecting bacterial community structure in this study were soil pH and electrical conductivity, which differs from some reports showing that soil moisture, total nitrogen, and total phosphorus are significantly correlated with bacterial communities [?]. These inconsistencies may be attributed to differences in soil conditions and crop varieties, as different soils exhibit distinct physicochemical properties [?] and different crop root systems influence soil properties differently [?], leading to varied responses of bacterial community structure to soil physicochemical properties.

4. Conclusion

This study employed high-throughput sequencing technology to investigate the characteristics of microbial communities in the rhizosphere soil of *Cyperus esculentus* under different fertilization rates. The results indicated that fertilization significantly increased the contents of available potassium, available phosphorus, alkali-hydrolyzable nitrogen, and organic matter, but significantly decreased soil pH, causing soil acidification. Fertilization also reduced the diversity of both fungal and bacterial communities in the rhizosphere soil of *C. esculentus*. The dominant bacterial phyla were *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Bacteroidetes*, and *Planctomycetes*, with the relative abundance of *Bacteroidetes* decreasing after fertilization. The dominant fungal phyla were *Ascomycota* and *Basidiomycota*, with fertilization increasing the relative abundance of *Ascomycota* while decreasing that of *Basidiomycota*. Both bacterial and fungal diversity decreased significantly with increasing fertilization rates. Soil pH and electrical conductivity were identified as the main factors driving changes in the bacterial community, while organic matter and moisture content were the primary factors influencing the fungal community.

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