Effects of Nitrogen Application Rate on Soil Microbial Abundance and Soil Enzyme Activity in a Maize/Soybean Intercropping System (Postprint)

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Abstract

To elucidate the regulatory mechanisms of soil nitrogen transformation and rhizosphere microecological effects in maize/soybean intercropping systems, a study was conducted with planting pattern as the main factor [maize monoculture (MM), soybean monoculture (SS), and maize/soybean intercropping (IMS) as three treatments] and total nitrogen application rate for maize and soybean (with a 3:1 nitrogen application ratio between maize and soybean) as the subfactor [no nitrogen (NN, 0 kg · hm ^{2}), reduced nitrogen (RN, 180 kg · hm ^{2}), and conventional nitrogen (CN, 240 kg · hm ^{2}) as three treatments] to investigate the effects of different nitrogen application rates on rhizosphere soil microbial populations and soil enzyme activities. The results demonstrated that, compared with corresponding monocultures, intercropping increased maize rhizosphere soil fungal and actinomycete populations by 25.37% and 8.79%, respectively; intercropping enhanced soybean rhizosphere soil fungal, actinomycete, and nitrogen-fixing bacterial populations compared with soybean monoculture; and intercropping significantly elevated protease and urease activities in maize rhizosphere soil and protease activity in soybean rhizosphere soil. Among different nitrogen application levels, reduced nitrogen application increased fungal populations in both maize and soybean rhizosphere soils compared with conventional nitrogen and no nitrogen applications; nitrogen application enhanced actinomycete populations in maize and soybean rhizosphere soils; soybean rhizosphere soil nitrogen-fixing bacterial populations were highest under reduced nitrogen application, being 17.78% and 5.67% higher than those under no nitrogen and conventional nitrogen applications, respectively; and protease activity in maize rhizosphere soil, urease activity in maize rhizosphere soil, and urease activity in soybean rhizosphere soil were all highest under reduced nitrogen application. Appropriate nitrogen application rates can not only increase populations of fungi, actinomycetes, and nitrogen-fixing bacteria in maize/soybean intercropping soils, but also enhance soil protease and urease activities, regulate soil nitrogen transformation, promote nitrogen uptake by maize and soybean from soil, and achieve energy conservation and efficiency enhancement.

Full Text

Effects of Nitrogen Application Rate on Soil Microbial Quantity and Soil Enzyme Activities in Maize/Soybean Intercropping Systems

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Abstract: To elucidate the regulatory mechanisms of soil nitrogen transformation and rhizosphere micro-ecological effects in maize/soybean relay strip intercropping systems, a two-factor experiment was conducted. The main factor was planting pattern [monoculture maize (MM), sole soybean (SS), and maize/soybean relay strip intercropping (IMS)], and the sub-factor was total nitrogen application rate for both crops (with a maize:soybean N ratio of 3:1) [no nitrogen (NN, 0 kg·hm ^{2}), reduced nitrogen (RN, 180 kg·hm ^{2}), and conventional nitrogen (CN, 240 kg·hm ^{2})]. The results showed that compared with corresponding monocultures, intercropping increased rhizosphere fungal and actinomycete counts in maize by 25.37% and 8.79%, respectively. Intercropped soybean exhibited higher rhizosphere counts of fungi, actinomycetes, and nitrogen-fixing bacteria than sole soybean. Protease and urease activities in intercropped maize rhizosphere soil, as well as protease activity in intercropped soybean rhizosphere soil, were significantly elevated. Across nitrogen treatments, reduced nitrogen application enhanced fungal counts in both maize and soybean rhizospheres compared with conventional and no nitrogen applications. Nitrogen application generally increased actinomycete counts in both crops. Soybean rhizosphere nitrogen-fixing bacteria reached maximum abundance under reduced nitrogen, being 17.78% and 5.67% higher than under no nitrogen and conventional nitrogen, respectively. Protease and urease activities in maize rhizosphere and urease activity in soybean rhizosphere were highest under reduced nitrogen. Appropriate nitrogen application rates not only increased fungal, actinomycete, and nitrogen-fixing bacterial populations in maize/soybean intercropping systems but also enhanced soil protease and urease activities, thereby regulating soil nitrogen transformation, promoting nitrogen uptake by both crops, and achieving energy savings with improved efficiency.

Keywords: Maize/soybean relay strip intercropping; Nitrogen application rate; Rhizosphere soil; Fungi; Actinomycetes; Nitrogen-fixing bacteria; Soil enzyme activity

Introduction

Soil enzymes play crucial roles in biogeochemical cycling and the transformation of organic matter and minerals in soil, while soil microorganisms are the primary drivers of soil nutrient transformation. Numerous important biochemical reactions in living soil organisms, including oxidation-reduction reactions and compound hydrolysis, are catalyzed by enzymes. Both microbial populations and enzyme activities are highly sensitive to changes in the rhizosphere environment, with fertilization practices, tillage systems, root exudates, and soil moisture exerting significant influences. Appropriate cropping patterns can improve land use efficiency and increase grain output per unit area. The maize/soybean (*Zea mays* L./*Glycine max* L.) relay strip intercropping system efficiently utilizes soil nutrient resources, regulates soil nitrogen cycling, and significantly improves nitrogen use efficiency in both crops, delivering substantial economic, social, and ecological benefits since its promotion in southwestern China. Therefore, investigating changes in soil microbial populations and enzyme activities under different nitrogen levels in intercropping systems is essential for understanding soil nitrogen cycling and improving nitrogen use efficiency.

Previous studies have demonstrated that maize/soybean intercropping enhances crop nutrient uptake and yield while reducing fertilizer requirements, increases rhizosphere bacterial counts, modulates ammonia-oxidizing and denitrifying bacterial community structures and diversity, and improves soil nitrogen transformation processes. Additionally, intercropping promotes soybean roots to secrete more protons and organic acids, mobilizing sparingly soluble phosphorus into plant-available forms. These findings suggest that the high nutrient use efficiency and yield benefits of maize/soybean intercropping are associated with improved rhizosphere micro-environments, particularly changes in microbial populations and enzyme activities. While extensive research has been conducted on the effects of monoculture and long-term fertilization on crop ecology, microbial populations, and enzyme activities, few studies have reported on the relationship between rhizosphere microbial counts, enzyme activities, and efficient nitrogen utilization in maize/soybean intercropping systems. This study compares differences in rhizosphere soil microbial populations and enzyme activities between monoculture and intercropping systems under varying nitrogen rates to clarify the dynamic changes and temporal variations in these parameters, thereby revealing the regulatory mechanisms of soil nitrogen transformation and rhizosphere micro-ecological effects to provide theoretical foundations for scientific fertilization strategies in this system.

1.1 Experimental Time, Location, and Materials

The field experiment was conducted from March 2013 to October 2014 at the Sichuan Modern Grain Industry (Renshou) Demonstration Base (30°07 N, 104°18 E). The soil type was purple soil. The maize variety used was ‘Denghai 605’ provided by Shandong Denghai Seed Industry Co., Ltd., and the soybean variety was ‘Nandou 12’ provided by the Nanchong Agricultural Science Research Institute of Sichuan Province. Baseline soil fertility in 2013 was: pH 6.6, organic matter 29.8 g · kg ^{1}, total nitrogen 1.6 g · kg ^{1}, total phosphorus 1.28 g · kg ^{1}, total potassium 16.3 g · kg ^{1}, available nitrogen 317 mg · kg ^{1}, available phosphorus 42.2 mg · kg ^{1}, and available potassium 382 mg · kg ^{1}.

1.2 Experimental Design

The experiment employed a two-factor split-plot design with planting pattern as the main factor [monoculture maize (MM), sole soybean (SS), and maize/soybean relay strip intercropping (IMS)] and total nitrogen application rate for both crops (maize:soybean N ratio of 3:1) as the sub-factor [no nitrogen (NN, 0 kg · hm ^{2}), reduced nitrogen (RN, 180 kg · hm ^{2}, 75% of local maize N rate), and conventional nitrogen (CN, 240 kg · hm ^{2}, based on local total N rates for maize and soybean)]. Nine treatments were established, each planted in three continuous strips (6 m length × 2 m width, 36 m^{2} plot area) with three replications.

Planting configurations are illustrated in [Figure 1: see original paper]. Monoculture plots used equal row spacing: 100 cm for maize and 50 cm for soybean, with 17 cm between holes and one plant per hole. The intercropping system used wide-narrow row spacing: maize wide rows were 160 cm and narrow rows 40 cm. Two soybean rows were sown in the maize wide rows during the maize bell stage (V12). Soybean row spacing was 40 cm with 60 cm spacing between maize and soybean rows. Hole spacing was 17 cm for both crops, with one maize plant per hole (density 58,500 plants · hm ^{2}) and two soybean plants per hole (density 117,000 plants · hm ^{2}). Monoculture and intercropping systems maintained identical planting densities.

Nitrogen fertilizer was applied in two splits for maize (basal and bell stage topdressing) and once as basal fertilizer for soybean (Table 1). Monoculture plots received fertilizer in holes between plants, while intercropping plots used integrated fertilization: uniform basal application of 72 kg(N) · hm ^{2} for maize, with topdressing applied as a mixture of maize and soybean NPK fertilizers in a furrow 25 cm from maize rows between the two crops. Phosphorus and potassium fertilizers were applied with basal dressing: maize received P O 105 kg · hm ^{2} and K O 112.5 kg · hm ^{2}, while soybean received P O 63 kg · hm ^{2} and K O 52.5 kg · hm ^{2}. In 2013, maize was sown on April 3 and harvested on August 1; soybean was sown on June 11 and harvested on October 29. In 2014, maize was sown on March 29 and harvested on July 26; soybean was sown on June 9 and harvested on October 28.

1.3.1 Soil Sample Collection

Rhizosphere soil samples were collected at maize bell stage (V12), tasseling stage (VT), and maturity (R6), and at soybean fifth trifoliolate stage (V5), full bloom stage (R2), beginning seed stage (R5), and full maturity (R8). The excavation method was used: two representative plant sections per plot were selected, with two consecutive holes from two symmetric rows excavated (40 cm depth). After shaking off loosely attached soil, a flame-sterilized forceps was used to scrape the thin soil layer (<10 mm) adhering to roots as rhizosphere soil. Samples from the two sections were crushed, mixed, and quartered. Samples were immediately placed in ice boxes, transported to the laboratory, passed through a 2 mm sieve to remove visible roots, residues, and stones, then stored at 4°C for microbial counting and -20°C for enzyme activity analysis.

1.3.2 Soil Sample Analysis

Plate counting methods were used to determine fungal, actinomycete, and nitrogen-fixing bacterial populations under three nitrogen levels in both monoculture and intercropping systems. Fungi were cultured on Martin' s rose bengal medium, actinomycetes on modified Gause No. 1 medium, and free-living nitrogen-fixing bacteria on Ashby' s medium. Soil protease activity was measured using a commercial assay kit from Chengdu Huayang Biona Biotechnology Co., Ltd., and soil urease activity using a kit from Nanjing Jiancheng Bioengineering Institute.

1.4 Data Processing and Analysis

Data were organized and graphed using Microsoft Excel 2010. Analysis of variance (LSD test) was performed using DPS 7.05 software with significance level set at P=0.05.

Results

2.1.1 Soil Fungal Counts

Rhizosphere fungal populations in both maize and soybean reached 10 cfu · g 1 (Table 2 , Table 3). Planting pattern significantly affected maize rhizosphere fungal counts. Before crop co-growth at maize V12 stage, counts were MM>IMS, but after co-growth, IMS increased maize rhizosphere fungal counts by 25.37% compared with MM across both years. Nitrogen application enhanced maize rhizosphere fungal counts, with RN>CN>NN in both planting patterns. Soybean rhizosphere fungal counts increased progressively with growth stages, peaking at R8 stage (Table 3). At R2 and R8 stages, IMS>SS, with IMS exceeding SS by 3.79% at R8 stage averaged across years. Among nitrogen treatments, RN produced the highest soybean rhizosphere fungal counts (except at R2 stage). At R8 stage, RN increased fungal counts by 10.89% and 17.22% compared with NN and CN, respectively, under SS, and by 11.50% and 6.02% under IMS.

2.1.2 Soil Actinomycete Counts

Rhizosphere actinomycete populations reached 10 cfu·g ^{1} in both crops (Table 4 , Table 5). Planting pattern influenced maize rhizosphere actinomycete counts, which increased with growth stages with consistent interannual patterns. IMS consistently exceeded MM, with significant differences at R6 stage where IMS increased counts by 11.8% compared with MM. Among nitrogen treatments, IMS maize rhizosphere actinomycete counts increased with nitrogen rate, with CN exceeding NN and RN by 15.69% and 7.6%, respectively. Planting pattern also affected soybean rhizosphere actinomycete counts, with IMS>SS across all treatments. Under SS, RN and CN increased counts by 11.49% and 16.62% compared with NN, while under IMS, increases were 15.11% and 27.13%, respectively.

2.1.3 Soil Nitrogen-fixing Bacteria Counts

As microorganisms with specialized physiological functions, nitrogen-fixing bacteria play important roles in nitrogen cycling. Planting pattern significantly affected soybean rhizosphere nitrogen-fixing bacterial counts (Table 6), which increased initially then decreased with growth stages, peaking at R2 stage. IMS consistently exceeded SS, with a 12.31% advantage at R2 stage. Among nitrogen treatments, RN produced the highest counts: under SS, RN exceeded NN and CN by 17.78% and 5.67%, respectively, while under IMS, RN exceeded NN and CN by 10.46% and 2.85%, respectively.

2.2.1 Soil Protease Activity

Soil protease, a key enzyme in nitrogen transformation, converts protein components into amino acids. Planting pattern affected maize rhizosphere protease activity (Table 7), with IMS>MM and significant differences at tasseling stage. Nitrogen rate also influenced activity, with RN producing the highest values: at maize maturity, RN increased protease activity by 8.33% and 11.43% compared with NN and CN, respectively, under IMS. Intercropping enhanced soybean soil protease activity (Table 8), with the greatest increase at V5 stage (2.38% higher than SS). Among nitrogen treatments, RN increased R2 stage activity by 2.35% and 16% compared with CN and NN, respectively, and R8 stage activity by 3.57% and 17.57%, respectively.

2.2.2 Soil Urease Activity

Planting pattern affected maize soil urease activity, with IMS>MM and significant differences at R6 stage (Table 9). IMS increased urease activity by 16.72% at VT stage and 20.56% at R6 stage compared with MM. Among nitrogen treatments, RN produced the highest maize soil urease activity (RN>CN>NN). Soybean soil urease activity increased then decreased with growth stages, peaking at R5 stage (Table 10), likely due to reduced nitrogen absorption as biological nitrogen fixation intensified, leaving higher urea concentrations that maintained elevated urease activity. At R2 and R5 stages, IMS>SS for soybean rhizosphere urease activity, with RN producing the highest values across nitrogen treatments.

2.3 Correlations Among Soil Microbial Counts, Enzyme Activities, Soil Nutrients, and Plant Nitrogen Uptake

Significant correlations ($P<0.05$ or $P<0.01$) were observed between maize rhizosphere fungal and actinomycete counts, urease activity, soybean rhizosphere protease activity, and soil nutrient content and plant nitrogen uptake (Table 11). Maize rhizosphere fungal counts showed the strongest influence on soil total nitrogen, while maize rhizosphere urease and protease activities and soybean protease activity most strongly affected soil total phosphorus. Soybean protease activity was negatively correlated with soil total potassium. Maize rhizosphere bacterial and fungal counts were extremely significantly positively correlated with plant nitrogen uptake. These results indicate that soil microbial populations and enzyme activities are critical factors influencing soil nutrients and plant nitrogen absorption, and that scientific nitrogen application can alter microbial communities, enhance enzyme activities, and improve soil environments.

Discussion

3.1 Effects of Intercropping and Nitrogen Application on Soil Microbial Counts

Soil microorganisms drive soil nutrient transformation and cycling. Previous studies have shown that maize/soybean intercropping increases rhizosphere bacterial counts compared with monocultures, with the highest counts under reduced nitrogen application. This study demonstrates that intercropping increased maize rhizosphere fungal and actinomycete counts and soybean rhizosphere fungal, actinomycete, and nitrogen-fixing bacterial counts compared with corresponding monocultures. Zhang et al. reported that maize/peanut and maize/soybean intercropping significantly increased bacterial, fungal, actinomycete, and nitrogen-fixing bacterial counts compared with maize monoculture. You et al. found that wheat-tobacco relay intercropping increased bacterial, fungal, and actinomycete counts compared with continuous tobacco monoculture. These effects likely result from root system interactions after crop co-growth, increased root exudates, and crop residues providing humus for microbial growth, thereby improving the rhizosphere microenvironment. This study further reveals different patterns during co-growth versus non-co-growth periods, with stronger promotional effects on microbial counts after co-growth, indicating enhanced rhizosphere microbial populations in maize/soybean intercropping systems. Ma et al. reported that wheat soil actinomycete and nitrogen-fixing bacterial counts showed a unimodal response to nitrogen rate. This study found that nitrogen application increased fungal, actinomycete, and nitrogen-fixing bacterial counts compared with no nitrogen, with maize rhizosphere fungal counts following RN>CN>NN and soybean rhizosphere fungal counts (except at R2 stage) highest under RN. Excessive nitrogen application may inhibit enzyme activity and accumulate toxic compounds, particularly suppressing mycorrhizal fungi, while long-term nitrogen deficiency cannot sustain normal crop growth, and excessive nitrogen acidifies soil, inhibiting microbial proliferation.

3.2 Effects of Intercropping and Nitrogen Application on Soil Enzyme Activities

Soil enzymes, as proteins catalyzing numerous biochemical reactions, include proteases that decompose proteins and peptides into amino acids, playing crucial roles in nitrogen metabolism and cycling. Proteases convert organic nitrogen to inorganic nitrogen, which undergoes ammonification under urease action to generate ammonium ions, altering soil available nitrogen content. Xu et al. reported that intercropped pepper showed higher protease and urease activities in rhizosphere and non-rhizosphere soils than monoculture. Yong et al. found that wheat/maize/soybean relay intercropping produced the highest soil urease and protease activities among different planting patterns. Meng et al. demonstrated that wheat-cotton intercropping maintained significantly higher rhizosphere and non-rhizosphere enzyme activities than monoculture cotton throughout the growing season. This study shows that intercropping increased both protease and urease activities in maize and soybean rhizospheres compared with monocultures, likely due to root exudates regulating soil pH, temperature, and moisture, optimizing the rhizosphere environment and promoting microbial activity. Soybean nodulation increases total soil nitrogen in the intercropping system, potentially altering nitrogen balance and crop nitrogen absorption, thereby affecting enzyme activities. Deng et al. suggested that fertilization influences enzyme activities by improving soil hydrothermal conditions and microbial communities. Both measured enzymes responded positively to nitrogen application, with activities generally higher under nitrogen treatments than without nitrogen. Maize rhizosphere protease and urease activities and soybean rhizosphere protease and urease activities peaked under reduced nitrogen, highlighting the importance of moderate nitrogen reduction for enhancing enzyme activities. Guo et al. reported that 0–180 kg(N) · hm ^{2} application improved wheat soil total nitrogen and enzyme activities, with urease activity increasing with nitrogen rate. Appropriate nitrogen application improves soil nutrient status, prompting crops to secrete more related enzymes for nitrogen absorption, promoting microbial proliferation and ultimately enhancing enzyme activities.

3.3 Nitrogen Regulation Mechanism in Maize/Soybean Relay Strip Intercropping

Soil microorganisms participate in material cycling and energy transformation, while soil enzymes catalyze crucial biochemical reactions; both are important indicators for evaluating soil fertility. Correlation analysis revealed significant or extremely significant positive relationships between microbial counts, enzyme activities, soil nutrients, and plant nitrogen uptake. Changes in rhizosphere microbial counts and enzyme activities during maize and soybean growth reflect crop influences on microbial proliferation and enzyme activity, while microbial activity and enzyme levels conversely affect crop growth. Nitrogen application rate, soil enzymes, and microorganisms are inseparably linked: nitrogen application promotes root growth and exudation, enhances enzyme activities, and stimulates microbial proliferation. Intercropping improves the rhizosphere microenvironment, reduces ammonification intensity, ensures crop NO absorption, decreases NH volatilization, and enhances nitrogen fixation. In maize/soybean intercropping, root system interactions and exudates regulate soil temperature and moisture, increasing bacterial, fungal, actinomycete, and nitrogen-fixing bacterial populations, thereby facilitating nitrogen transformation into plant-available forms and improving nitrogen use efficiency. However, nitrogen application generates the major greenhouse gas N O, warranting further investigation into how nitrogen rate changes affect greenhouse gas emissions in this intercropping system.

Conclusion

Planting pattern and nitrogen application rate significantly affected rhizosphere microbial counts and enzyme activities in the maize/soybean relay strip intercropping system. Compared with monoculture, intercropping increased rhizosphere fungal, actinomycete, and nitrogen-fixing bacterial counts in both crops, and significantly elevated protease and urease activities in maize rhizosphere and protease activity in soybean rhizosphere. Reduced nitrogen application increased fungal counts in both crop rhizospheres and soybean rhizosphere nitrogen-fixing bacterial counts, while enhancing maize rhizosphere protease and urease activities and soybean rhizosphere urease activity compared with conventional nitrogen. Compared with no nitrogen, reduced nitrogen increased actinomycete counts in both crop rhizospheres. Microorganisms and enzymes are highly sensitive to soil environmental changes; appropriate planting patterns optimize the rhizosphere microenvironment, while nitrogen application regulates this environment, promotes soil nitrogen cycling, increases nutrient availability, and provides theoretical foundations for scientific fertilization in intercropping systems.

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Note: Figure translations are in progress. See original paper for figures.