Effects of reduced nitrogen application and intercropping patterns on AMF colonization in sweet corn, soybean nodulation, and crop nitrogen and phosphorus uptake (Postprint)

Authors: Zhou Xianyu, Tang Yiling, Wang Zhiguo, Wang Jianwu

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Abstract

A four-season (autumn 2014, spring and autumn 2015, spring 2016) field positioning experiment was conducted to comparatively investigate the effects of two nitrogen application rates [300 kg·hm ^{2} (N1: reduced nitrogen) and 360 kg·hm ^{2} (N2: conventional nitrogen)] and four planting patterns [sweet corn||vegetable soybean intercropping at 2:3 (S2B3) and 2:4 (S2B4), sweet corn monoculture (SS), and vegetable soybean monoculture (SB)] on the yields of sweet corn and soybean, sweet corn AMF colonization rate, soybean rhizobia, etc. in South China. The results showed that sweet corn yield under reduced nitrogen intercropping treatments was significantly higher than that under monoculture. In spring 2016, soybean root nodule number under the S2B3-N1 treatment was significantly higher than under the S2B3-N2 treatment. Across the four seasons, reduced nitrogen and intercropping treatments had no significant effect on soybean root nodule dry weight. In both spring and autumn of 2015, sweet corn biomass and nitrogen content under intercropping treatments at both nitrogen levels were significantly higher than the corresponding monoculture treatments; moreover, the AMF colonization rate of sweet corn under reduced nitrogen intercropping patterns was significantly higher than under conventional nitrogen treatments. In autumn 2015, sweet corn phosphorus content under reduced nitrogen intercropping patterns was significantly higher than under monoculture treatments. Reduced nitrogen application combined with vegetable soybean intercropping significantly improved sweet corn nitrogen and phosphorus contents, AMF colonization rate, biomass, and yield, representing a sustainable production model for efficient resource utilization of sweet corn in South China.

Full Text

Preamble

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Effects of reduced nitrogen application and intercropping on sweet corn AMF colonization, soybean nodulation and nitrogen and phosphorus absorption

ZHOU Xianyu, TANG Yiling, WANG Zhiguo, WANG Jianwu**
(Institute of Tropical and Subtropical Ecology, South China Agricultural University / Key Laboratory of Agro-environment in the Tropics, Ministry of Agriculture / Key Laboratory of Agro-ecology and Rural Environment of Guangdong Regular Higher Education Institutions, Guangzhou 510642, China)

Abstract: A field experiment was conducted over four growing seasons (autumn 2014, spring and autumn 2015, and spring 2016) in Guangzhou to investigate the effects of two nitrogen fertilizer levels [300 kg · hm ^{2} (N1: reduced N dose) and 360 kg · hm ^{2} (N2: conventional N dose)] and four planting patterns [sweet corn/vegetable soybean intercropping with 2 3 (S2B3) and 2 4 (S2B4) line ratios, sole sweet corn (SS) and sole soybean (SB)] on yield, arbuscular mycorrhizal fungi (AMF) colonization of sweet corn, soybean rhizobia, and nitrogen and phosphorus absorption in South China. The results showed that sweet corn yield under reduced N application and intercropping was significantly higher than that under monoculture. In spring 2016, soybean nodule number under S2B3-N1 treatment was significantly higher than under S2B3-N2 treatment. Across the four seasons, reduced N application and intercropping had no significant effect on soybean nodule dry weight. In 2015, both spring and autumn, sweet corn biomass and nitrogen content under intercropping were significantly higher than under corresponding monoculture treatments, and AMF colonization rate of sweet corn under reduced N intercropping was significantly higher than under conventional N treatment. In autumn 2015, sweet corn phosphorus content under reduced N and intercropping was significantly higher than under monoculture. Reduced N application combined with soybean intercropping significantly improved sweet corn nitrogen and phosphorus contents, AMF colonization rate, biomass, and yield, representing a sustainable production pattern for resource-efficient sweet corn cultivation in South China.

Keywords: Reduced nitrogen application; Sweet corn/soybean intercropping; Nitrogen absorption; Phosphorus absorption; Rhizobium; Arbuscular mycorrhizal fungi (AMF)

Introduction

Guangdong Province accounts for approximately 60% of the national sweet corn (Zea mays) planting area, making it an important production base and consumption center for sweet corn in China, with a cultivation system of 2–3 crops per year. In pursuit of high yields and economic benefits, farmers commonly apply excessive chemical nitrogen fertilizer, with utilization efficiency of only 26%–28%. Most of the nitrogen is lost through surface runoff, ammonia volatilization, and leaching, causing a series of ecological and environmental problems [2–4].

Research has shown that legume-cereal intercropping can enhance nitrogen fixation capacity of legume crops [5], increase nitrogen transfer [6], produce a "nitrogen suppression" mitigation effect, and achieve efficient nitrogen utilization [7]. In maize-soybean (Glycine max L.) relay intercropping systems, nitrogen application rates reduced by 18% and 36% (330 kg · hm ^{2}) both promoted soybean nodule formation, with stronger nitrogen fixation capacity observed in soybean at 15 cm distance from maize [8]. Afza et al. [9] suggested that appropriate fertilization benefits soybean nodule formation and growth, though reports indicate that nitrogen application rates reaching 30.38 kg · hm ^{2} can significantly inhibit soybean nodule formation and growth [10], and soybean nodule number and fresh weight decreased significantly in maize-soybean relay intercropping [11].

Maize has a well-developed fibrous root system that readily forms symbiotic relationships with arbuscular mycorrhizal fungi (AMF). In arbuscular mycorrhizal symbiosis, AMF extraradical hyphae can utilize NH , NO , and simple amino acids, accelerating organic nitrogen mineralization. AMF hyphae form common mycorrhizal networks (CMNs) with plant root systems, connecting roots with soil and expanding nutrient absorption [12]. Plants susceptible to AMF colonization are sensitive to nitrogen application, and AMF can only promote crop growth within certain nitrogen application rates, while excessive nitrogen reduces AMF colonization [13]. AMF can contribute up to 74% of total nitrogen to maize plants [14], and inoculation with AMF and rhizobia in maize-soybean intercropping systems transferred 20%–43% of nitrogen from soybean to maize [15]. Dual inoculation (AMF and rhizobia) increased nitrogen transfer from soybean to maize by 45% compared to AMF inoculation alone [16], demonstrating synergistic effects [17].

Currently, many studies are conducted as pot experiments in sterilized soil with single or dual inoculation, which cannot eliminate the effects of inoculating AMF or rhizobia on plant growth. Few reports exist on the mutually beneficial symbiotic relationship between indigenous AMF and rhizobia in intercropping systems [12, 17]. This study investigated the effects of reduced nitrogen application and sweet corn-soybean intercropping on indigenous AMF and rhizobia under field conditions, aiming to provide a theoretical basis for establishing scientific and efficient sustainable production patterns for sweet corn in South China.

Materials and Methods

1.1 Study Area

The experiment was conducted from August 2014 to June 2016 at the teaching and experimental farm of South China Agricultural University (23.08°N, 113.15°E). The experimental area has a typical subtropical monsoon maritime climate with annual sunshine hours of 1,289–1,780 h and annual rainfall of 1,384–2,278 mm, with approximately 85% of precipitation concentrated from April to September. The soil is lateritic red earth, with topsoil organic matter of 20.28 g · kg^{1}, available nitrogen of 75.50 g · kg^{1}, available phosphorus of 74.69 g · kg^{1}, available potassium of 72.59 g · kg^{1}, and pH 5.2.

1.2 Experimental Materials

The sweet corn variety was ‘Huazhen 1’ purchased from Guangzhou Nansha Lvtian Seed Business Department. The vegetable soybean varieties were ‘Maodou 3’ for spring seasons and ‘Shanghaiqing’ for summer seasons, provided by the College of Agriculture, South China Agricultural University.

1.3 Experimental Design

The experiment employed a two-factor randomized block design with nitrogen level and planting pattern as factors (Table 1). Nitrogen levels were N1 (reduced nitrogen) at 300 kg · hm^{2} and N2 (conventional nitrogen) at 360 kg · hm^{2}. Planting patterns included S2B3 (sweet corn||soybean intercropping with 2 3 line ratio), S2B4 (sweet corn||soybean intercropping with 2 4 line ratio), SS (sweet corn monoculture), and SB (soybean monoculture). Each treatment was replicated three times, with plot dimensions of 4.8 m × 3.7 m = 17.76 m^{2}. In intercropping patterns, the row spacing between sweet corn and soybean was 30 cm, between adjacent sweet corn rows was 50 cm, and between adjacent soybean rows was 30 cm. Row spacing was 60 cm for sweet corn monoculture and 30 cm for soybean monoculture. Plant spacing was 30 cm for sweet corn and 20 cm for soybean, with one plant per hole for sweet corn and three plants per hole for soybean. Planting densities based on net occupied area were 67,568 plants · hm^{2} for intercropped sweet corn, 54,054 plants · hm^{2} for monoculture sweet corn, and 486,486 plants · hm^{2} for both intercropped and monoculture soybean. Detailed experimental design and fertilization schemes are described in Tang et al. [18].

Sowing and transplanting dates were: soybean sown and sweet corn seedlings raised on August 9, 2014, March 15, 2015, August 10, 2015, and March 12, 2016; sweet corn transplanted to fields on August 19, 2014, March 29, 2015, August 20, 2015, and April 2, 2016; soybean harvested on October 19, 2014, June 3, 2015, October 31, 2015, and May 30, 2016; sweet corn harvested on October 26, 2014, June 3, 2015, October 29, 2015, and June 7, 2016.

Nitrogen fertilizer was applied four times per season: base fertilizer on August

28, 2014, March 31, 2015, September 5, 2015, and April 9, 2016; ear fertilizer on September 20, 2014, April 18, 2015, October 8, 2015, and May 30, 2016.

1.4 Measurements

1.4.1 Crop Yield, Biomass, and Land Equivalent Ratio At crop maturity, 10 consecutive plants were sampled from each of the two intercropping strips of sweet corn and soybean, and 20 plants were sampled from monoculture plots to determine fresh ear yield of sweet corn and fresh pod yield of soybean, along with biomass. Land equivalent ratio (LER) was calculated [19]:

$$\text{LER} = (\text{Yis}/\text{Yss}) + (\text{Yib}/\text{Ysb})$$

where Yis and Yib are yields of intercropped sweet corn and soybean (kg · m ^{2}), and Yss and Ysb are yields of monoculture sweet corn and soybean (kg · m ^{2}). LER > 1 indicates intercropping advantage, while LER < 1 indicates disadvantage.

1.4.2 Soybean Rhizobia Determination At soybean podding stage (October 15, 2014; May 16, 2015; October 11, 2015; and May 8, 2016), 10 consecutive soybean plants were sampled from soybean strips intercropped with sweet corn and from soybean monoculture plots. Nodule number per plant was recorded, and nodules were air-dried and weighed.

1.4.3 Sweet Corn AMF Colonization Rate In autumn 2015 and spring 2016, 40 days after sweet corn growth, two consecutive sweet corn plants were sampled from each intercropping strip and root systems were cleaned. Thirty root segments of 1 mm diameter were stained with trypan blue and observed microscopically. Based on colonization standards for each root segment (0, <1%, <10%, <50%, >50%, and >90%), the AMF colonization density was calculated using "Mycocalc" software [20].

1.4.4 Crop Nitrogen and Phosphorus Content Determination At crop maturity, three consecutive sweet corn and soybean plants were sampled from both monoculture and intercropping strips, killed at 105 °C for 40 minutes, dried at 80 °C to constant weight, and ground with a high-speed universal grinder. Total nitrogen content was determined by the Kjeldahl method [21] and total phosphorus content by the vanadium molybdate yellow colorimetric method [21].

1.5 Data Analysis

Data were statistically analyzed using Microsoft Excel 2003 and SPSS 20 software. Duncan' s multiple comparison test was used to examine significant differences ($= 0.05$). Data in figures and tables are presented as mean ± standard deviation.

Results

2.1 Crop Yield

Across the four seasons, crop yields under the same planting pattern showed no significant differences between nitrogen levels (except for soybean yield under S2B4 pattern in spring 2015 and sweet corn yield under S2B4 pattern in spring 2016), indicating that reduced nitrogen application did not affect crop yield (Table 2). Under the same nitrogen level, intercropped sweet corn yield was significantly higher than monoculture in all four seasons (except for N1 planting pattern in spring 2015). However, soybean yield showed no significant difference between intercropping and monoculture in autumn 2014 and spring 2016, while monoculture yield was significantly higher than intercropping in spring and autumn 2015. Climatic conditions in South China, particularly rainfall, varied between spring and autumn seasons and across years, significantly affecting soybean yield.

Land equivalent ratios (LER) for all intercropping treatments were greater than 1 in autumn 2014 and spring 2016 (Figure 1 [Figure 1: see original paper]), indicating that sweet corn and vegetable soybean intercropping improved land use efficiency per unit area and provided certain intercropping advantages. Because soybean monoculture yield was significantly higher than intercropping in spring and autumn 2015, LER values for 2015 treatments were less than or close to 1. Overall, except for the significantly higher LER under conventional nitrogen S2B4 pattern compared to S2B3 in 2016, no significant differences were observed among other treatments.

2.2 Effects of Reduced Nitrogen and Intercropping on Soybean Rhizobia and Nitrogen Accumulation

Under the same planting pattern, reduced nitrogen treatment resulted in greater nodule number per plant (except for S2B3 in autumn 2014 and S2B4 in autumn 2015) and greater nodule dry weight per plant (except for S2B4 in autumn 2015 and spring 2016) compared to conventional nitrogen treatment (Table 3). Averaged across four seasons, reduced nitrogen S2B3 pattern increased soybean nodule number and dry weight per plant by 34.49% and 62.01%, respectively, compared to conventional nitrogen, while S2B4 pattern increased them by 2.76% and 14.01%, respectively. Compared to soybean monoculture (SB), reduced nitrogen S2B3 and S2B4 patterns decreased nodule number per plant by 10.65% and 13.70%, and nodule dry weight by 11.50% and 13.02%, respectively. Conventional nitrogen S2B3 and S2B4 patterns decreased nodule number by 29.65% and 13.58%, and nodule dry weight by 45.11% and 20.67%, respectively, compared to SB. These results indicate that reduced nitrogen application can increase soybean nodule number and dry weight per plant, while intercropping decreases them.

Nitrogen content of vegetable soybean plants under intercropping patterns was lower than under monoculture across all four seasons (Table 4). Averaged across seasons, S2B3-N1 and S2B4-N1 decreased soybean nitrogen content by 21.01% and 15.33% compared to SB, while S2B3-N2 and S2B4-N2 decreased it by 16.68% and 18.49%, respectively. Monoculture soybean without chemical nitrogen fertilizer accumulated more nitrogen than intercropped treatments with fertilizer, indicating that chemical nitrogen input inhibited soybean biological nitrogen fixation efficiency [22].

Under the same nitrogen level, nitrogen content of sweet corn plants in intercropping systems was higher than in monoculture across all four seasons. Averaged across seasons, S2B3-N1 and S2B4-N1 increased sweet corn nitrogen accumulation by 27.64% and 13.49% compared to SS-N1, while S2B3-N2 and S2B4-N2 increased it by 29.27% and 24.34% compared to SS-N2, demonstrating that intercropping with soybean improved sweet corn nitrogen accumulation.

2.3 Effects of Reduced Nitrogen and Intercropping on Sweet Corn AMF Colonization Rate and Phosphorus Accumulation

AMF colonization rates of sweet corn roots under reduced nitrogen intercropping patterns were higher than under conventional nitrogen treatments (Table 5). Averaged across two seasons, S2B3-N1 increased sweet corn AMF colonization rate by 83.38% compared to S2B3-N2, while S2B4-N1 increased it by 76.57% compared to S2B4-N2. Reduced nitrogen intercropping patterns also showed higher AMF colonization rates than sweet corn monoculture. Averaged across seasons, S2B3-N1 and S2B4-N1 increased sweet corn AMF colonization rates by 41.07% and 72.31% compared to SS-N1, respectively, indicating that low nitrogen intercropping enhanced AMF colonization of sweet corn.

Phosphorus content of sweet corn plants under intercropping patterns was higher than under monoculture across all four seasons (Table 6). Averaged across seasons, S2B3-N1 and S2B4-N1 increased sweet corn phosphorus accumulation by 27.95% and 19.23% compared to SS-N1, while S2B3-N2 and S2B4-N2 increased it by 26.77% and 18.70% compared to SS-N2, indicating that intercropping with soybean improved sweet corn phosphorus accumulation. No significant differences in phosphorus accumulation were observed between the two nitrogen levels under the same planting pattern.

No significant differences in biomass were observed between nitrogen levels for either sweet corn or soybean under the same planting pattern (Table 7). Across four seasons, sweet corn biomass under intercropping patterns was higher than under monoculture at the same nitrogen level. Averaged across seasons, S2B3-N1 and S2B4-N1 increased sweet corn biomass by 34.05% and 23.75% compared to SS-N1, while S2B3-N2 and S2B4-N2 increased it by 31.09% and 24.67% compared to SS-N2. Conversely, soybean biomass under intercropping patterns was lower than under monoculture, with S2B3-N1 and S2B4-N1 decreasing biomass by 15.27% and 17.09% compared to SB, and S2B3-N2 and S2B4-N2 decreasing it by 22.55% and 16.03%, respectively. These results demonstrate that intercropping increased sweet corn biomass but decreased soybean biomass.

Discussion and Conclusion

3.1 Effects of Reduced Nitrogen and Intercropping on Crop Yield

In this experiment, crop yield was significantly affected by planting pattern. Intercropped sweet corn yield was significantly higher than monoculture, while intercropping reduced soybean yield. Sweet corn-soybean intercropping improved light distribution and utilization within the canopy, enhanced field ventilation and light penetration, increased photosynthetic capacity of maize, but decreased photosynthetic efficiency of soybean [23]. The increased sweet corn yield may be attributed to soybean nodulation and nitrogen fixation, as well as intercropping advantages. The increased AMF colonization rate of sweet corn promoted phosphorus absorption. Reduced nitrogen treatment in this experiment did not decrease yield of either sweet corn or soybean, indicating that 300 kg · hm ^{2} nitrogen application could meet crop growth requirements. Wu et al. [24] demonstrated through rhizobium inoculation experiments that a pea/maize intercropping system achieved highest maize yield at 300 kg · hm ^{2} nitrogen application, with yield decreasing at higher rates. In spring 2015, severe lodging of sweet corn occurred due to typhoon impact.

3.2 Effects of Reduced Nitrogen and Intercropping on Rhizobia

Rhizobia result from specific combinations between legume crops and soil rhizobial bacteria [25–26]. Legume crops can not only utilize their own rhizobia for nitrogen fixation but also transfer fixed nitrogen to neighboring plants [27–28], reducing nitrogen fertilizer input and increasing crop yield [29]. This study found that sweet corn-soybean intercropping decreased soybean nodule number and dry weight, consistent with Yu et al. [11], possibly because shaded soybean in intercropping environments allocated photosynthates preferentially to aboveground growth [30–31], regulating nodule number and dry weight [30, 32]. However, many studies have found that intercropping can improve nodulation levels in legume crops [33–35]. Reduced nitrogen application in intercropping benefited soybean nodulation and nodule growth, though this experiment showed decreased nodule number and dry weight per plant in intercropped soybean compared to monoculture, possibly because nitrogen fertilizer applied to sweet corn migrated to soybean intercropping strips through rainfall and irrigation, affecting nodulation status of intercropped soybean.

3.3 Effects of Reduced Nitrogen and Intercropping on Sweet Corn AMF Colonization

This study demonstrated that reduced nitrogen application combined with soybean intercropping promoted AMF colonization of sweet corn roots, improved phosphorus accumulation to varying degrees, and increased biomass. These findings are consistent with Xiao et al. [36] in rice (Oryza sativa)-mung bean (Vigna radiata) intercropping. Compared to monoculture maize, intercropped sweet corn increased species diversity and AMF richness, enhancing AMF colonization rate [36-39]. AMF colonization rate decreases with increasing nitrogen levels [40], consistent with our finding that reduced nitrogen application resulted in higher AMF colonization than conventional nitrogen.

In summary, four seasons of field experiments demonstrated that intercropping with soybean increased sweet corn yield, and 300 kg · hm ^{2} nitrogen application could meet the nitrogen requirements for high yields of both sweet corn and soybean. Reduced nitrogen application benefited soybean nodulation and nodule growth, improving nitrogen accumulation. Reduced nitrogen combined with soybean intercropping promoted indigenous AMF colonization of sweet corn roots, enhanced nitrogen and phosphorus absorption, promoted crop growth, and increased biomass. Sweet corn-soybean intercropping with 300 kg · hm ^{2} nitrogen application can leverage the mutually beneficial symbiotic relationship between indigenous AMF and rhizobia, reduce chemical nitrogen input, maintain high yield, and improve resource use efficiency, representing a sustainable production pattern for resource-efficient sweet corn cultivation in intensive agricultural regions of South China.

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