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Maize/Peanut Intercropping Affects Legume Nodulation in Semi-Arid Conditions

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Abstract: Maize/peanut intercropping is practiced widely to increase land productivity and considered a sustainable way for using and saving resources through peanut's complementary N source via biological N_2 fixation. Our study aims to understand how maize/peanut intercropping affects the nodulation of peanuts under water-limiting conditions and different nitrogen inputs. A two-year micro-plot experiment in 2015–2016 and a two-year field experiment in 2017–2018 were conducted to quantify nodulation in maize/peanut intercropping and sole peanut cropping under four N fertilization rates (N-free, low, medium, and high N) in rain-fed water-limited conditions. In the micro-plot experiment, intercropped peanuts increased nodule biomass compared to sole peanuts. The nodule number of intercropped peanuts was 51.6% (p = 0.001) higher than that of sole cropped peanuts, while nodule weights did not differ at high N fertilization rates and were lower in the no-N fertilization control. However, the results were different in the field experiment. Both the nodule number and single weight of the sole cropped peanut were 48.7% (p = 0.020) and 58.9% (p = 0.014) higher than that of the intercropped peanut. The ratio of the nodule weight to aboveground dry matter at the beginning peg in the dry year of 2017 was lower in intercropping than sole cropping, especially at low N fertilization rates. The potential increase in nodulation found in a well-controlled micro-plot environment might be limited by strong water and light competitions in field conditions. The results could contribute to the understanding of interspecific interactions in cereal/legume intercropping.

Keywords: cereal/legume intercropping; dryland agriculture; interspecific interaction; nodule number; nodule weight

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1. Introduction

Intercropping grows multiple crop species in one field and has been practiced by farmers for many years in various ways and in most areas of the world [1–3]. Intercropping improves the total crop productivity per area and soil fertility; reduces soil erosion, weed abundance and disease, and insect damage; and increases the efficiency of resource use, such as of water, light, and nutrients [4–9]. In rain-fed agricultural systems, the cultivation of maize and peanuts as primary food and cash crops faces sustainability challenges. Maize cultivation tends to deplete soil moisture resources, whereas peanut yields are negatively impacted by plant–soil feedback and wind erosion, particularly in regions with water limitations. Intercropping maize with peanuts presents a promising strategy for enhancing resilience to drought conditions. This is due to the differing water acquisition patterns between maize and peanuts. Additionally, the presence of maize stubble in the intercropped system helps mitigate soil erosion caused by wind within the peanut strips. Thus, maize/peanut intercropping is an important cereal/legume intercropping system in China and semi-arid regions. Previous studies showed that maize/peanut intercropping can increase the yield and resource use efficiencies such as of light and water [10–14]. However, we barely know anything about the response of nodulation under rain-fed semi-arid conditions and differential N fertilization, which is of great significance for enhancing the biological nitrogen fixation ability of peanuts in intercropping and vulnerable conditions.

Cereal/legume intercropping is widely recognized within the agricultural research community as a sustainable production system. This designation is primarily attributed to the system's demonstrated capacity to enhance overall land productivity while concomitantly reducing the need for nitrogen fertilizer applications. The underlying mechanism driving these benefits is the symbiotic nitrogen fixation process facilitated by the leguminous component of the intercrop. Through this process, atmospheric nitrogen is effectively captured and converted into a plant-available form, thereby enriching the soil and promoting the robust growth of the associated cereal crop [10,14–16]. The nodule biomass increased by 4–73% under maize/faba bean intercropping and reduced the negative effects of N fertilization [17]. Semi-dwarf sorghum/soybean intercropping led to a 2.6-fold increase in N_2 fixation, while the nodule number increased by 62% [18]. Rice/mung bean intercropping increased N acquisition by 64% and nodulation by 54%, and the percentage of total N transferred from mung bean to rice leaves [19].

Cereal/legume intercropping increases N use via the stimulation of nodulation and N_2 fixation as soil N is depleted by the competing cereal [1,20] and/or flavonoid synthesis (signaling compounds for rhizobia) by root exudates of cereals, stimulating the nodulation of the companion legume [21]. However, legume nodulation in intercropping at the field scale are not consistently found in experiments. Soybean/maize intercropping decreased the nodule number of soybean in a humid subtropical climate [22]. Soybean nitrogen fixation was reduced in soybean/sorghum intercropping via a reduction in nodule numbers, weights per nodule, and specific nodule activity [18]. Groundnut/cereal intercropping reduced nodulation and N_2 fixation compared to sole cropping [23,24]. These negative effects of intercropping were ascribed to shading by the taller cereal reducing photosynthesis [23].

In maize/peanut intercropping, the taller maize plants compete for light, which may result in fewer and smaller nodules in intercropped peanuts due to an inadequate supply with photosynthates. However, the belowground interspecific interactions of other resources (e.g., water and nitrogen) would be more severe, especially under stress situations such as water-limited conditions in semi-arid areas. According to the "stress gradient hypothesis (SGH)", the strength of facilitation should increase with the severity of environmental stress [25,26], which suggests that nodulation may be enhanced in maize/peanut intercropping. Therefore, we hypothesized a higher nodulation, i.e., higher number of nodules and nodule weights in intercropping compared to sole cropping. Moreover, we considered whether the positive effects of nodulation in intercropping could be offset by the negative effects from interspecific competition for nitrogen. In this study, a two-year micro-plot experiment and a two-year field experiment with three and four N fertilization rates were conducted, and the hypotheses were tested under semi-controlled and little controlled field conditions.

2. Materials and Methods

2.1. Experimental Site

The experiments were carried out at the National Agricultural Experimental Station for Agricultural Environment in Fuxin (121°43′ E, 42°09′ N), Liaoning, Northeast China, in 2015–2018. The experiment site is situated in a region with rain-fed agriculture under typical semi-arid conditions. The average precipitation is about 470 mm (mean of last

30 years). The climate is temperate continental with a hot summer and a cold dry winter, which allows only one crop per year. The rainfall was 247 mm in 2015, 492 mm in 2016, 351 mm in 2017, and 327 mm in 2018 from May to September, which is the growing season. The measured temperature and rainfall during the growing season are shown in Figure 1.

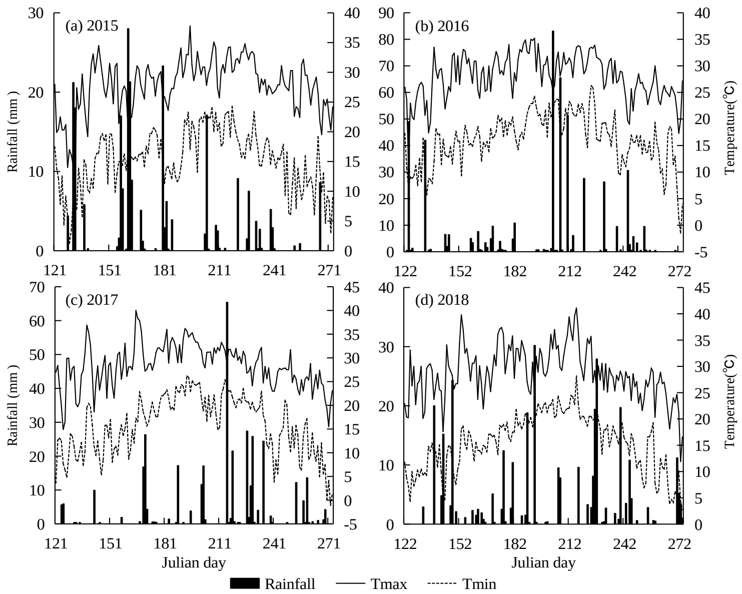

Figure 1. The maximum (Tmax) and minimum (Tmin) temperatures and rainfall in 2015 to 2018 during the crop growing season (from May to September) in Fuxin, Liaoning.

2.2. Experimental Design

2.2.1. Micro-Plot Experiment

The micro-plot experiments were conducted in 2015–2016 in a randomized complete block design with 3 replicates. The micro-plot was constructed out of frames of polyethylene plates. Each micro-plot had an area of 8 m^2 (4 m $\times$ 2 m) with a 1m depth to avoid the root interaction between treatments. The soil's physical and chemical properties are listed in Table 1. A total of 27 plots were used for three cropping systems: sole maize, sole peanut, and maize/peanut strip intercropping with 2 rows of maize and 2 rows of peanut. The 27 plots were also used under three N levels: N free (0 kg ha^{-1}), medium N (100 kg ha^{-1}), and high N (200 kg ha^{-1}). Furthermore, 90 kg ha^{-1} of P_2O_5, 58 kg ha^{-1} of K_2O were applied to all plots at sowing. Maize (*Zea mays* L.) cultivar Zhengdan 958 and for peanut (*Arachis hypogaea* L.) cultivar Baisha 1016 was chosen, respectively. The row spacing was 50 cm in both sole and intercropping for both crop plants (Figure 2). The interplant distance in the rows was the same for intercropping and sole cropping, 33.3 cm for maize and 14 cm for peanut, resulting in 6 plants m^{-2} in sole and 3 plants m^{-2} in intercropping for maize and 28 plants m^{-2} in sole and 14 plants m^{-2} in intercropping for peanuts.

All crops were sown on 20 May 2015 and 13 May 2016. The harvesting time for all crops was on 23 September 2015 and 28 September 2016. Weeds were removed by hand. Irrigation was only used after sowing to ensure the establishment of the crop seedlings.

Table 1. The soil physical and chemical properties of micro-plot experiment and field experiment.

Experiment	Bulk Density	Organic Matter	pH	Cation Exchange Capacity	Total N	Available N	Olsen-P	Available K
	g cm^{-3}	g kg^{-1}		mmol kg^{-1}	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Micro-plot	1.35	13.8	6.2	191	0.18	29.4	26.6	112
Field	1.48	14.1	5.3	311	0.08	87.5	6.9	69

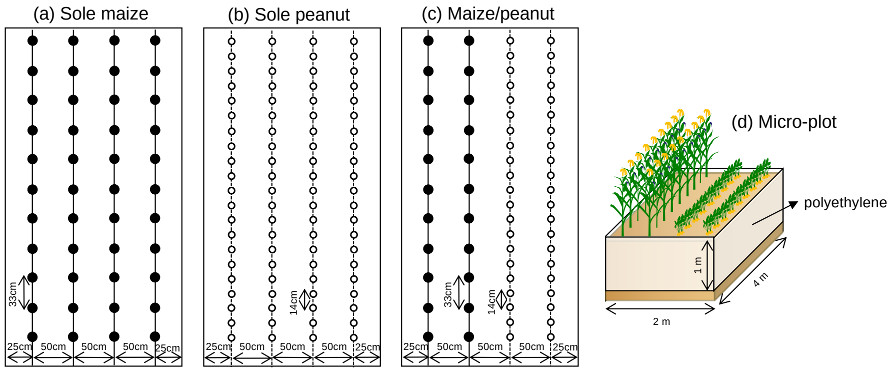

Figure 2. Layout of sole maize (**a**), sole peanut (**b**), and maize/peanut strip intercropping (**c**) in micro-plot experiment (**d**). Solid circles in solid lines represent maize plants, and open circles in dashed lines indicate peanut plants. The inter-row distance was 50 cm, while the interplant distance within rows was 33 cm for maize and 14 cm for peanuts.

2.2.2. Field Experiment

The experiments were carried out in 2017–2018 in a randomized complete block design with 3 replicates. The soil's physical and chemical properties in the field are listed in Table 1. The tested sole and intercropping systems were the same as in the micro-plot experiment, i.e., (i) sole maize, (ii) sole peanut, and (iii) maize/peanut strip intercropping (2 rows of maize and 2 rows of peanut). The N fertilization treatments comprised the fertilization rates: N-free (N0) and low N (N1) as 180, 40, and 110 kg ha^{-1} for sole maize, sole peanuts, and intercrops; medium N (N2) as 240, 80, and 160 kg ha^{-1}; and high N (N3) as 300, 120, and 210 kg ha^{-1}, respectively. Moreover, 120 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} were applied as a basal fertilizer to the plots when sowing. The maize cultivar was the locally commonly used pioneer 335 and peanut cultivar Baisha 1016. The row spacing for all crops was 50 cm in both sole and intercropping. The intra-row distances between adjacent maize plants were 20 cm and that between adjacent peanut plants were 10 cm (Figure 3). The plant densities of maize were 10 plants m^{-2} in sole and 5 plants m^{-2} in intercropping. The plant densities of peanut were 20 plants m^{-2} in sole and 10 plants m^{-2} in intercropping. The plot area was 42 m^2 (7 m in length $\times$ 6 m in width) for all treatments.

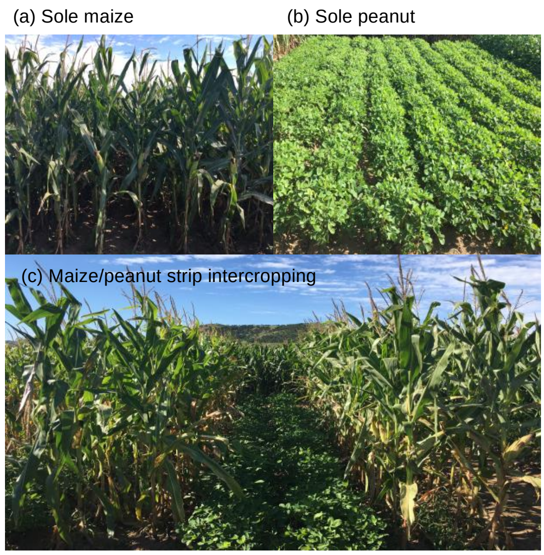

Figure 3. Layout of sole maize (**a**), sole peanut (**b**), and maize/peanut strip intercropping (**c**) in the field experiment in Fuxin, Liaoning, China. The inter-row distance was 50 cm, while the interplant distance within rows was 20 cm for maize and 10 cm for peanut.

All crops were sown on 21 May 2017 and 15 May 2018. Harvesting time for all crops was on 26 September 2017 and 18 September 2018. Weeds were removed manually, and no irrigation was used as in farmers' practice.

2.3. Measurements

To determine the aboveground dry matter of peanuts at different developmental stages in the field plot experiment, a sub-sampling area of 1 m^2 was chosen and three plants were randomly harvested. The plants were sampled at 49, 78, 107, and 127 DAS (days after sowing) in 2017 and 36, 63, 92, and 125 DAS in 2018. The samples were oven-dried at 80 °C for 2 consecutive days.

To determine the nodulation of peanuts under different treatments in the micro-plot experiments, 4 peanut plants were randomly selected from the strip adjacent to the maize rows in intercropping and from the two middle rows in sole cropped peanut. We sampled the nodules in different stages at the micro-plot experiment: the beginning peg stage (63 DAS in 2015 and 65 DAS in 2016), pod setting stage (91 DAS in 2015 and 101 DAS in 2016), and maturity stage (126 DAS in 2015 and 138 DAS in 2016). In the field plot experiment, nodulation was determined at the beginning peg stage (78 DAS in 2017 and 67 DAS in 2018). A spade was used to dig out the whole root system of peanuts. Soil that adhered to the roots was carefully shaken off, the roots rinsed, and the nodules picked from the roots as soon as possible. Nodule numbers were counted and nodule weights were measured after air-drying to an 8% water content.

2.4. Statistical Analysis

Analyses of variance (ANOVA) in the nodule number, nodule weight, weight per nodule, nodule weight per aboveground dry matter, and peanut aboveground dry matter were run using the statistical software package SPSS 20 (IBM, Endicott, NY, USA). The univariate general linear model was used to assess the effects of cropping system, N fertilization rate, year, and their interactions. The cropping system, N fertilization rate, and year were treated as fixed factors. The replicate was defined as the random factor. The factor replicate was nested in the factor year. Least significant differences (LSDs) were used to separate treatment means at the 5% significance level.

3. Results

3.1. Micro-Plot Experiment

3.1.1. Nodule Number

The nodule number per plant was significantly affected by the cropping system, N fertilization rate, and year at the needle and pod stages, while at the latter cropping system, N fertilization rate, and year, all interacted (Table 2). The nodule number of intercropped peanuts was 51.6% higher than that of sole cropping peanuts across both developmental stages and N fertilization rates in 2015 and 2016, except for N0 at the pod setting stage (133 and 87 nodules per plant in sole cropping and intercropping, respectively), N0 (118 and 106 nodules per plant in sole cropping and intercropping), and N100 (80 and 51 nodules per plant in sole cropping and intercropping) at maturity (Figure 4) in 2016. The nodule number was also affected by the N fertilization rate in both years. With increasing N inputs, nodule numbers decreased in inter- and sole cropping. Nodule numbers differed significantly between the years, with lower numbers in 2015 than 2016.

Table 2. Results of ANOVA for the effects of cropping system, N fertilization, and year on nodulation of peanut in sole and intercropping with maize at three developmental stages in the micro-plot experiments.

Micro-Plot Experiment (2015–2016)		Pod Initiation Stage			Pod Setting Stage			Maturity Stage		
		Nodule Number plant^{-1}	Nodule Dry Weight mg plant^{-1}	Single Nodule Weight mg nodule^{-1}	Nodule Number plant^{-1}	Nodule Dry Weight mg plant^{-1}	Single Nodule Weight mg nodule^{-1}	Nodule Number plant^{-1}	Nodule Dry Weight mg plant^{-1}	Single Nodule Weight mg nodule^{-1}
Factor	System	0.002	0.039	0.152	0.000	0.041	0.006	0.265	0.223	0.290
	N	0.033	0.043	0.217	0.008	0.001	0.003	0.049	0.000	0.000
	Year	0.003	0.009	0.402	0.011	0.011	0.042	0.015	0.051	0.116
	System * N	0.301	0.803	0.278	0.000	0.013	0.032	0.308	0.973	0.055
	System * N * Year	0.519	0.603	0.292	0.000	0.009	0.005	0.309	0.201	0.001

Note: the system represents the effects of cropping system, N represents the effects of N fertilization, Year represents the effects of different years, System * N represents the interaction of cropping system and N fertilization, System * N * Year represents the interaction of cropping system, N fertilization and years. The same below.

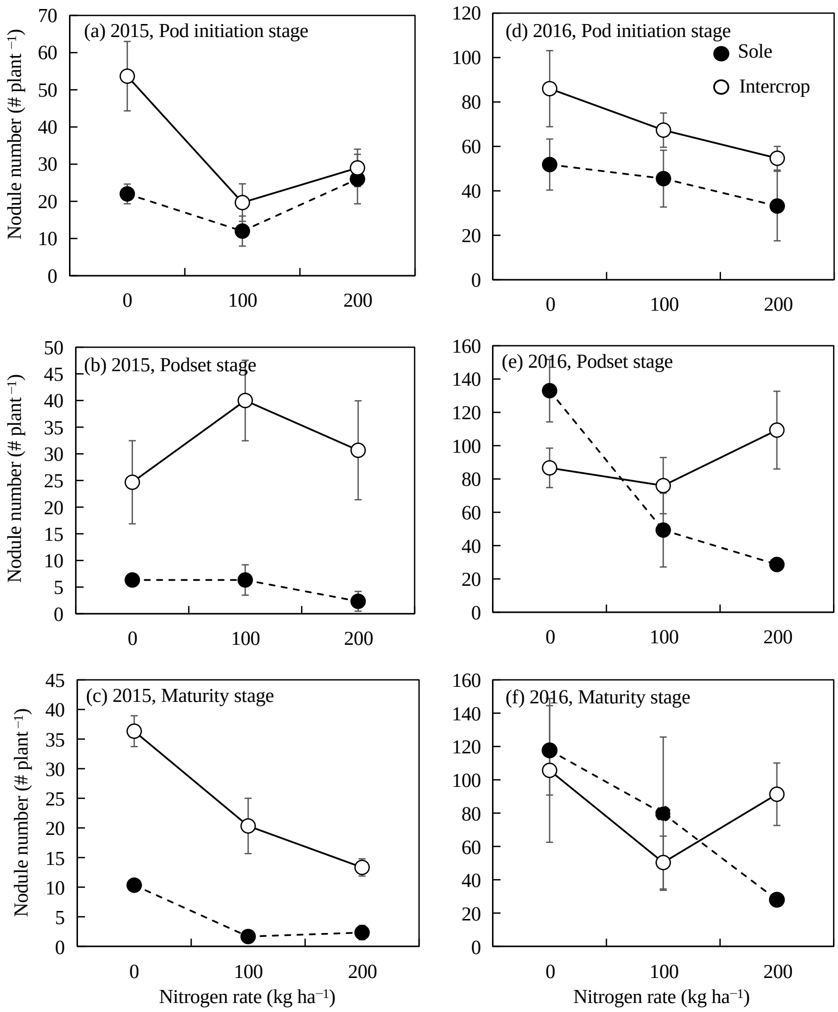

Figure 4. Nodule number of peanuts in sole and maize/peanut intercrop at pod initiation (60–70 days after sowing (DAS)), podset (90–100 DAS), and maturity (120–140 DAS) stages under different nitrogen rates at micro-plot experiment in 2015–2016.

3.1.2. Nodule Weight

The nodule dry weight per plant dropped with the N fertilization rate in both the intercropping and sole cropping of peanuts (Figure 5), and it was significantly affected by the cropping system, N fertilization, and year (Table 2). Intercropped peanuts (average 47 mg plant^{-1}) had a 1.4 times higher nodule dry weight than sole cropped peanut (average 34 mg plant^{-1}) at the pod initiation stage, across all years and N treatments. The nodule weight differed between years in both intercropping and sole cropping. The single nodule weight in intercropping was 42% (p = 0.020) lower than in sole cropping under no N addition, while there were no significant differences between the cropping systems at the other N fertilization rates (Figure 6).

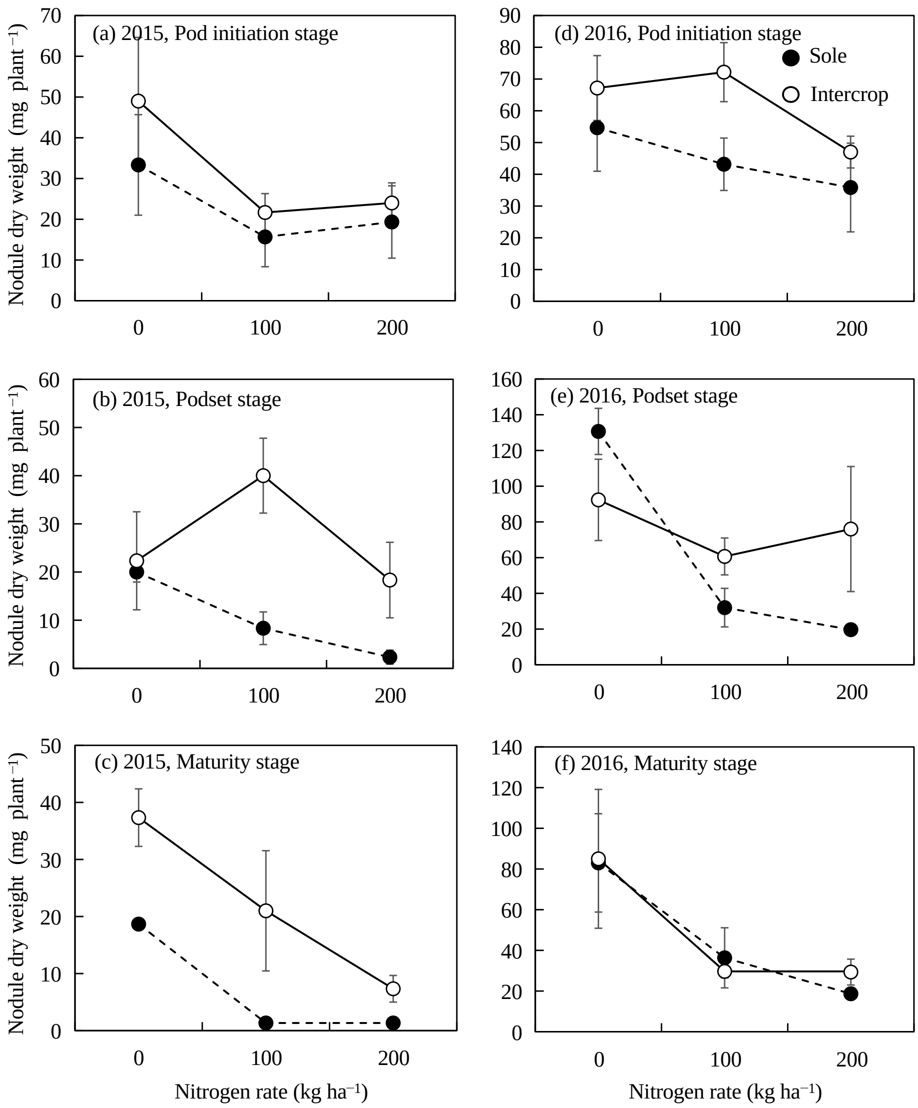

Figure 5. Nodule dry weight of peanut in sole and maize/peanut intercrop at pod initiation (60–70 days after sowing (DAS)), podset (90–100 DAS), and maturity (120–140 DAS) stages under increasing nitrogen fertilization rates of the micro-plot experiment in 2015–2016.

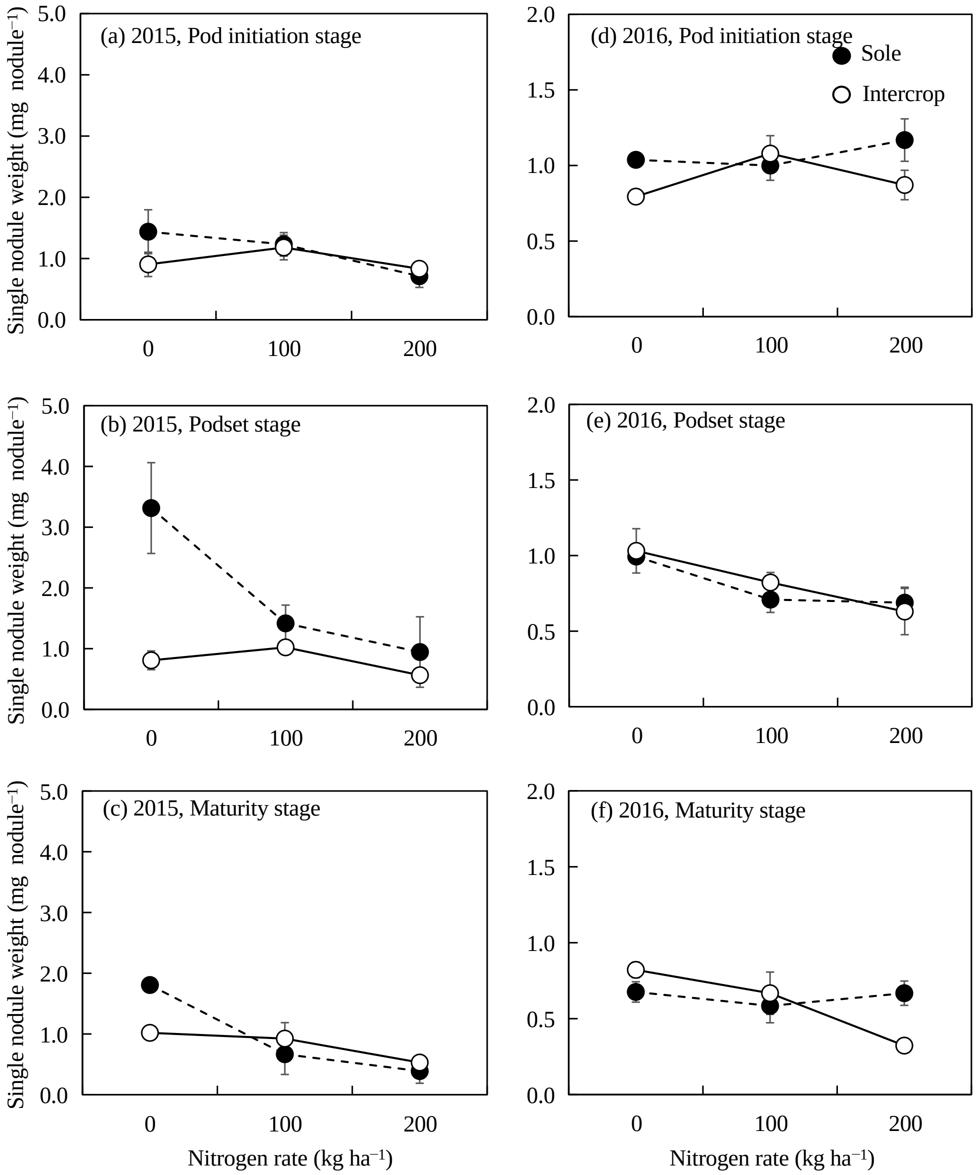

Figure 6. The single nodule weight of peanut in sole and maize/peanut intercrop at pod initiation (60–70 days after sowing (DAS)), podset (90–100 DAS), and maturity (120–140 DAS) stages under increasing nitrogen fertilization rates of the micro-plot experiment in 2015–2016.

3.2. *Field Experiment*

3.2.1. Aboveground Dry Matter

Peanut dry matter in both intercropping and sole cropping differed between the years. It was 38.4% (p = 0.0002) lower in intercropping (22.7 g plant^{-1}) than sole cropping (36.9 g plant^{-1}) in 2018 (Figure 7), a year with late-season (90–110 DAS) drought, while this difference was 12.7% in 2017 (75.5 g plant^{-1} for sole cropping and 65.9 g plant^{-1} for intercropping), a year of generally low yields. Over 2 years, the aboveground dry matter of peanut was 26% lower in intercropping than sole cropping. The aboveground dry matter did not differ with the N fertilization rate (p = 0.620).

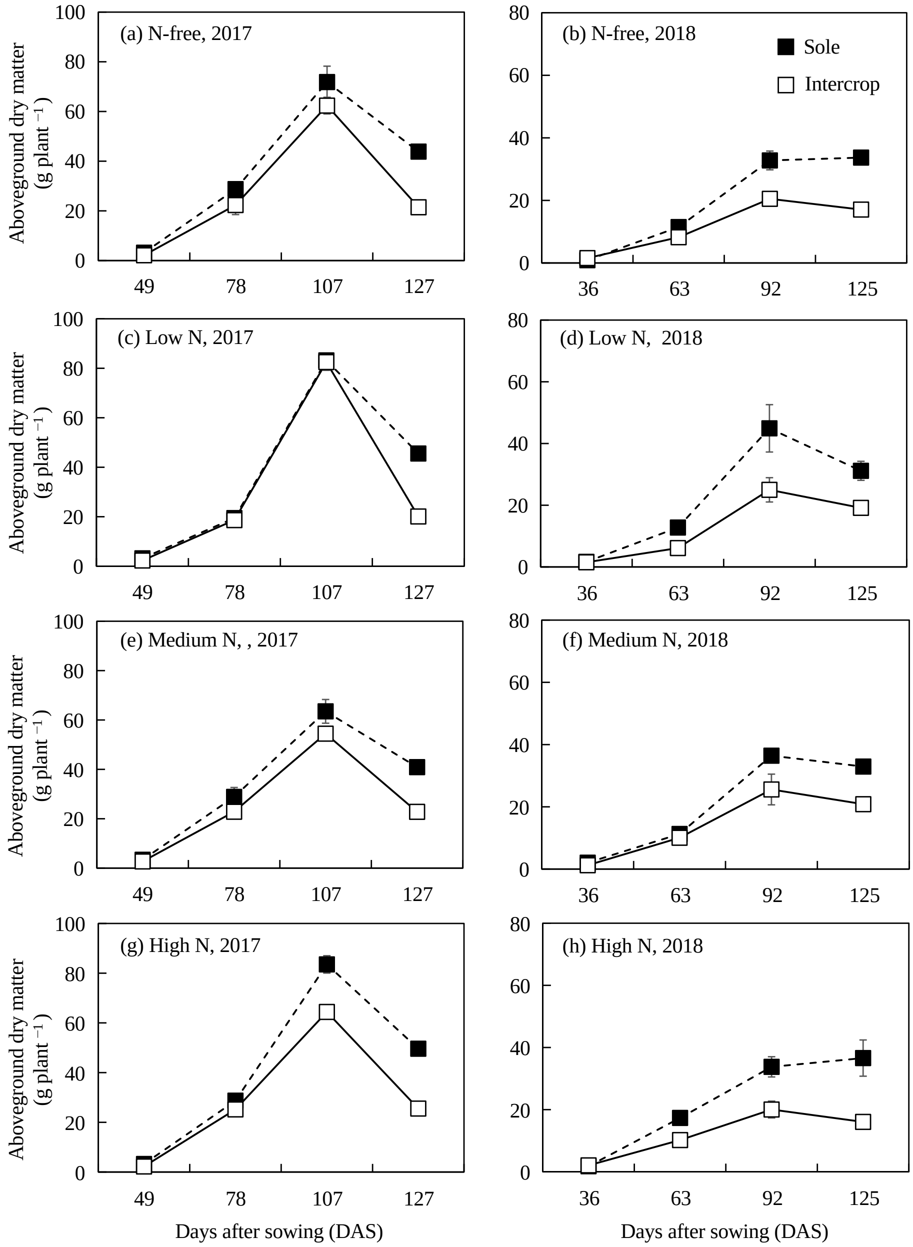

Figure 7. Aboveground dry matter dynamics of peanut in sole and maize/peanut intercropping systems under different nitrogen rates in the field experiment in 2017–2018.

3.2.2. Nodule Number

At the field experiment, the nodule number per plant differed between the cropping systems (p = 0.044), years and was affected by the N fertilization rate (p = 0.001), which had different effects between years (Table 3). Unlike in the micro-plot experiment, the nodule number in the sole cropped peanut was 48.7% (p = 0.02) higher than in the intercropped peanut (average 28 nodule per plant) and higher in year 2018 than 2017 (p = 0.029), except at the highest N fertilization rate of 120 kg ha^{-1} in 2018 when it was lower (23 nodules plant^{-1}, Figure 8).

Table 3. Results of ANOVA for the effects of cropping system, N fertilization, and year on nodulation of peanut in sole and intercropping with maize at three developmental stages in field experiments.

Field Experiment (2017–2018)		Pod Initiation Stage			
		Nodule Number plant^{-1}	Nodule Dry Weight mg plant^{-1}	Single Nodule Weight mg nodule^{-1}	Nodule Weight Per Aboveground Dry Matter mg g^{-1}
Factor	System	0.044	0.004	0.011	0.228
	N	0.001	0.000	0.178	0.000
	Year	0.029	0.757	0.013	0.070
	System * N	0.020	0.048	0.558	0.257
	System * N * Year	0.308	0.388	0.107	0.328

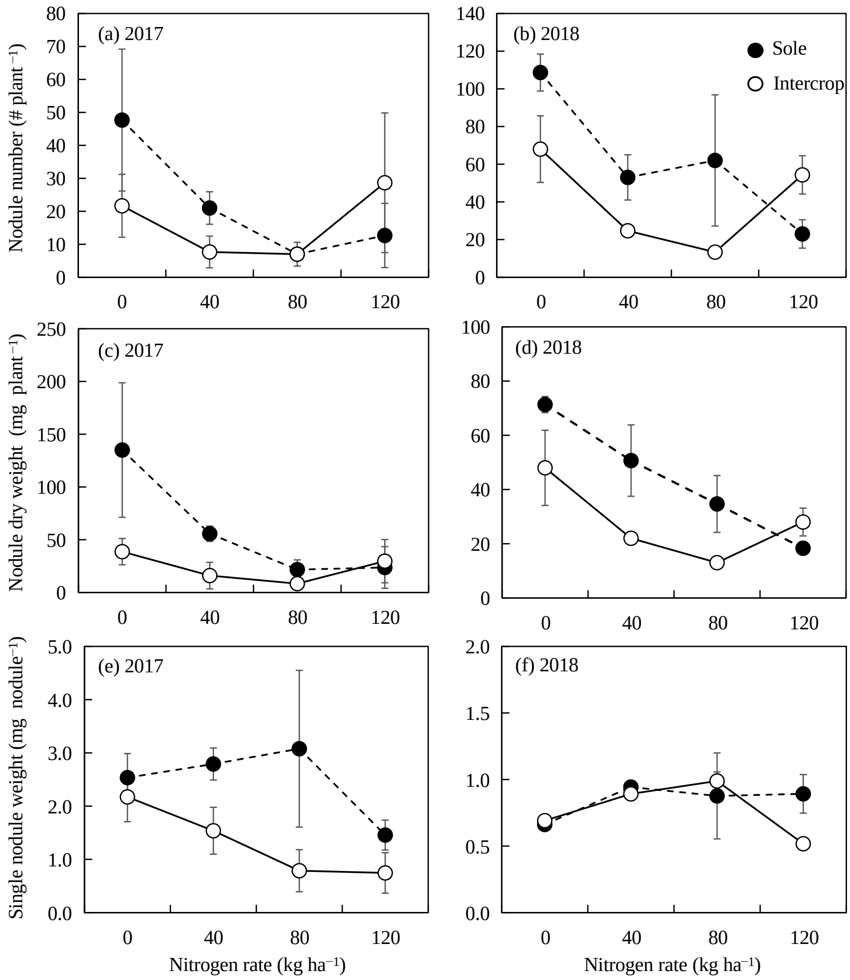

Figure 8. Nodule number, nodule dry weight, and single nodule weight of peanut in sole and maize/peanut intercrop at pod initiation (60–80 days after sowing (DAS)) stage under different nitrogen rates in the field experiment in 2017–2018.

3.2.3. Nodule Weight

The nodule weight per plant differed depending on the N fertilization rate and the cropping system ($p < 0.01$) over two years. At pod initiation, sole cropped peanuts showed a higher nodule weight per plant than intercropped peanuts, except at the highest N fertilization rate (Figure 8). The nodule weight tended to drop with increasing N fertilization, but under intercropping, it increased from 80 to 120 kg ha^{-1} of N, reflected in a significant interaction between the cropping system and N fertilization rate ($p = 0.048$) (Table 3). Single nodule weight was 58.9% higher ($p = 0.014$) in sole (1.65 mg nodule^{-1}) than intercropped peanut (1.04 mg nodule^{-1}); the advantage was significant in 2017, but not 2018 (Figure 8).

The nodule weight per aboveground dry matter tended to be higher in sole than intercropping in 2017 at a low N fertilization rate of 0–40 kg ha^{-1} of N (Figure 9). The differences in the single nodule weight between cropping systems and years were mainly due to the changes in the ratio of nodule weight over aboveground dry weight, indicating a strong influence by assimilate supply.

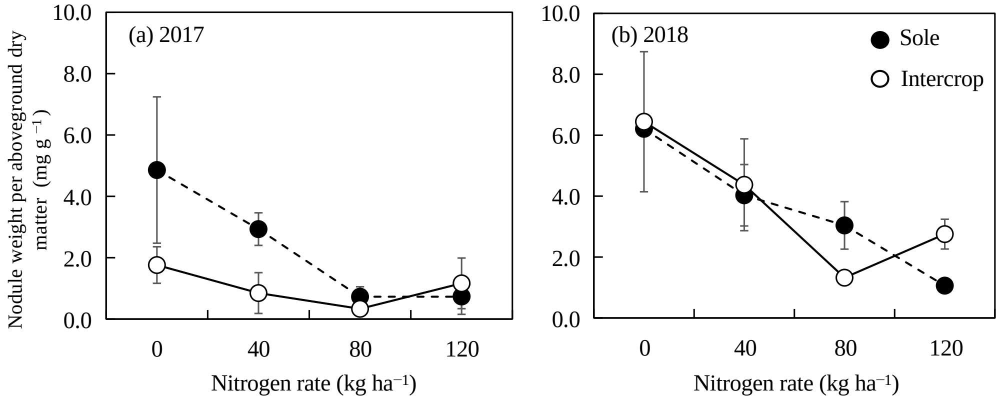

Figure 9. Nodule weight per aboveground dry matter of peanut in sole and maize/peanut intercrop at pod initiation (60–80 days after sowing (DAS)) stage under different nitrogen rates at field experiment in 2017–2018.

4. Discussion

This study revealed contradicting effects of sole and intercropping on the nodulation of peanuts under micro-plot and field plot conditions and a semi-arid climate. The cropping system and N fertilization rate significantly affected the nodule number and nodule weight in both the micro- and field-plot experiments. The nodule numbers and weights of the intercropped peanut were significantly higher than those of sole cropped peanuts in the well-controlled micro-plot experiment. However, they were significantly higher in sole than intercropping under normal field conditions, except at the highest N fertilization rate.

Similar to the results of our micro-plot experiments, previous pot experiment indicated that intercropping enhances nodulation and N_2 fixation in peanuts [27]. Intercropping stimulated nitrogen fixation in peanuts and increased nitrogen fixation efficiency in rice/peanut intercropping, as determined by the ^{15}N-isotope dilution method [28]. Nodule numbers and nodule nitrogenase activity were reported to be higher in maize/peanut mixed than sole cropping because of nitrate depletion from soil and iron mobilization by maize [26]. However, pot conditions differ largely from those in the field in above- and belowground conditions, affecting plant nutrition and nodulation. These differences might affect the competition and complementarity of the mixing crops and thus influence the nodulation formation.

Competition between intercropped species differs depending on the environmental conditions [29]. We hence set up our experiment with micro-plots on a soil with more moderate bulk density and pH, and sufficient available P and K than the field-plot experiment. Irrigation was only used in the micro-plot experiment, which mostly benefited peanuts in terms of their competitiveness against maize. The better development of peanut may thus have magnified the differences in nodulation between intercropped and sole cropped peanuts in the micro-plot experiments. Soil less affected by long-term cultivation used for the micro-plot experiment may explain the greater interspecific facilitation via nodulation and N_2 fixation [15,30–33].

Under field conditions, the N fertilization rate and application method affect the nodulation of legumes in intercropping [17,34]. Cereals are more competitive in inorganic soil N acquisition than legumes in intercropping because of faster and deeper root penetration and higher N demands due to greater dry matter formation. Increased soil N availability improves the N nutrition of cereals and their competitiveness for light, increasing cereal growth and decreasing legume growth [35]. In the present study, high N input and split applications during crop development in the field experiment must have improved N uptake and stimulated the growth of the maize plants, causing shading to the peanut plants. The lower allocation of photosynthates to nodulation under intercropping must thus explain the lower ratio of nodule weights over aboveground dry matter in intercropping compared to sole cropping in the field.

The nodulation of intercropped peanuts can also be affected by maize plant density [36–38]. Light interception and use efficiency differ with maize plant density in intercropping [13]. The leaf area index of maize increases but decreases in peanuts when maize plant density increases, leading to lower light interception by peanuts in maize/peanut intercropping. This reduces the assimilate supply for nodulation, even though light use efficiency increases in shaded peanuts [39]. Increasing the plant density of shaded crops increased both light interception and use efficiency in jujube/cotton intercropping [40]. The lower maize plant and higher peanut plant density in the micro-plot experiment provided a better light environment and benefit to contribute to the nodulation of intercropped peanut. Differences in nodulation depending on year must be explained by the sensitivity of photosynthesis and plant growth to drought, as well as mineral nutrient availability from soils and fertilizers [41].

There are consistent results with the micro-plot experiment, which showed that intercropping enhances the nodulation of peanut, as well as N_2 fixation and nodule nitrogenase activity compared to sole cropping [26–28]. In addition, previous studies also found similar results with the field experiment, which showed that there was negative effect of intercropping with maize, which inhibit the nodulation and N_2 fixation of peanuts compared to the sole cropping system [23,24]. Different results were obtained from the two-year micro-plot experiment and two-year field experiment. Our hypothesis was confirmed only under micro-plot and not field-scale conditions. The results of this preliminary study quantified the nodulation, including the nodule number, nodule biomass, and single nodule weight in maize/peanut intercropping and sole peanut cropping under different N fertilization rates. However, considering the interannual meteorological variability and complexity, longer term experiments need to be conducted to show the characteristics of nodulation in maize/peanut intercropping under different precipitation situation in semi-arid regions. Using statistical theory and applying ideas and methods such as hypothesis testing and linear regression may also help to reveal or explore the nodulation advantages of intercropped peanut from the perspective of probability space.

5. Conclusions

Our hypothesis predicts that intercropping increases nodulation was only confirmed under micro-plot and not field-scale conditions and differed among N fertilization treatments, which suggested a complex mechanism for nodulation in intercropping. Intercropping has the potential to increase nodulation in peanuts under rain-fed conditions but requires moderate N fertilization and possibly sufficient light and thus lower plant densities. The results will contribute to understanding the mechanism of nodulation in intercropping in relation to interspecific interactions, environments, and nitrogen managements. This study may also be helpful to guide farmers to choose a suitable amount of applied N fertilizer in cereal/legume intercropping and possibly optimize the seed density of tall cereals.

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