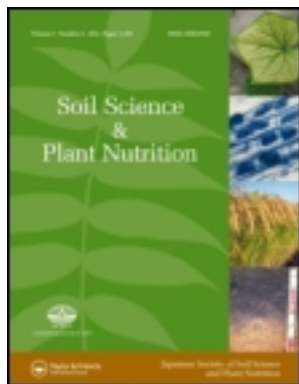


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A Study on the Improvement Iron Nutrition of Peanut Intercropping with Maize on Nitrogen Fixation at Early Stages of Growth of Peanut on a Calcareous Soil

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A glasshouse study employing a split-root technique was conducted to investigate the influence of intercropping with maize (*Zea mays* L.) in a calcareous soil on N₂ fixation by peanut (*Arachis hypogaea* L.) at early stages of growth. In this intercropping system, competitive interactions between maize and peanut for N and improvement of Fe uptake were likely to be important factors affecting N₂ fixation of peanut. The experiment was comprised of three treatments which included treatment I: peanut monocropping; treatment II: maize/peanut intercropping (the major and the minor compartments with low N, 50 mg kg⁻¹); treatment III: maize/peanut intercropping (the major compartment with low N, 50 mg kg⁻¹ and the minor compartment with high, N 200 mg kg⁻¹). The minor compartment of treatment III was fertilized with 200 mg kg⁻¹ N for reducing or eliminating the competition of N coming from intercropping maize. Intercropping with maize corrected Fe chlorosis of peanut by significantly increasing plant Fe concentration and uptake. Compared with the monocropping treatment, iron uptake increased from intercropping treatment II and III by 22 and 24% per plant, 30 and 29% shoots, 38 and 60% nodules. Iron uptake by the root nodules was especially enhanced in the intercropping system. In contrast, intercropping with maize had little effect on NO₃⁻-N concentrations in the soil rhizosphere of peanut or on N concentrations and uptake by peanut compared with plants in monoculture. The results indicate that the improvement in Fe nutrition was an important factor promoting N₂ fixation by peanut in the intercropping system at the flowering stage of peanut growth, and that competition for N by intercropped maize had little effect on N₂ fixation by peanut under the experimental conditions.

Key Words: intercropping, iron deficiency chlorosis, nutrient competition, split-root.

Iron is an essential nutrient required by both legume and root nodule bacteria for many metabolic functions at several key stages in the symbiotic N₂ fixation process and is critical for N₂ fixation due to its role in the activity of both leghemoglobin and nitrogenase (Dakora 1995; Kaiser et al. 2003). These induce N₂-fixing nodules on the roots of legumes and in this symbiotic state have a very high demand for Fe, since several polypeptides made specifically in the nodule (e.g. nitrogenase and leghemoglobin) are Fe-proteins (Johnston et al. 2001; Wexler et al. 2003; Benson et al. 2004). Some

legume species of soybean (*Glycine max* L. Merr.) and *Lupinus angustifolius* showed that induction of Fe—deficiency responses (proton release and Fe(III) reduction) is much stronger in the roots of plants that have actively fixing nodules than in the roots of uninoculated plants, and Fe is directly involved in nodule initiation and nodule development (Terry et al. 1991; Tang et al. 1992; Levier 1996). Peanut inoculated with *Bradyrhizobium* sp. has shown that Fe-deficient plants initiated nodules but had decreased nodule development, lowered amount of leghemoglobin in nodules, fewer bacteroids present in nodules, and lower N₂ fixation activity (O'Hara et al. 1988a, b). These studies demonstrate that Fe plays a key role in nitrogen-fixing symbioses.

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Iron deficiency chlorosis in peanut is one of the most common yield limiting nutrients and serious economic problems in peanut monocropping systems on calcareous soils in northern China, but is much less commonly observed in peanut/maize intercropping systems. The maize/peanut intercropping improved Fe nutrition, increased Fe uptake and corrected Fe deficiency chlorosis symptoms in peanut. Particularly there is considerable interest in devising practical agronomic approaches for the correction or avoidance of Fe deficiency in peanut crops and intercropping has become the most common cropping system for peanut production in northern China (Zuo 1997). Iron, an important component of peanut nitrogenase, participates directly in N_2 fixation. The improvement in Fe nutrition of peanut by intercropping with maize may therefore influence N_2 fixation by peanut.

A legume–non-legume intercrop provides excellent intercropping advantages which are mainly from the N efficiency of intercrop systems. The success of intercrop farming systems depends initially on effective N_2 fixation and subsequent transfer of N to the non-legume. The non-legume crop has a unique capacity to lower the mineral N concentration in the rhizosphere of legume to an extent that symbiotic N_2 fixation on an individual plant basis expressed as % Ndfa (N derived from atmosphere) can actually be increased over the sole-crop legume. The negative effect of residual N or fertilizer N on fixation of atmospheric N_2 can be reduced by intercropping through competition from the non-leguminous crop for available soil N (Jensen 1996; Ito et al. 1997; Dakora 1998; Karpenstein-Machan and Stuelpnagel 2000).

In our intercropping systems, the two factors that may affect N_2 fixation of peanut are: competition for N by the intercropped maize and the improvement of Fe nutrition of peanut. Effects of competition for N by intercropping maize on peanut N_2 fixation must therefore be eliminated (or at least decreased) to study the effects of improvement of Fe nutrition of intercropped peanut on N_2 fixation. The aim of the present study was to investigate whether competition for N by intercropped maize or the improvement of Fe nutrition of intercropped peanut had the greater effect on peanut N_2 fixation in maize/peanut intercropping systems at the flowering stage of peanut.

MATERIALS AND METHODS

Rhizobox design. Plants were grown in two kinds of PVC rhizoboxes as shown in Fig. 1. The design used for intercropping (Fig. 1A) was divided by a PVC plate into two compartments. The larger compartment

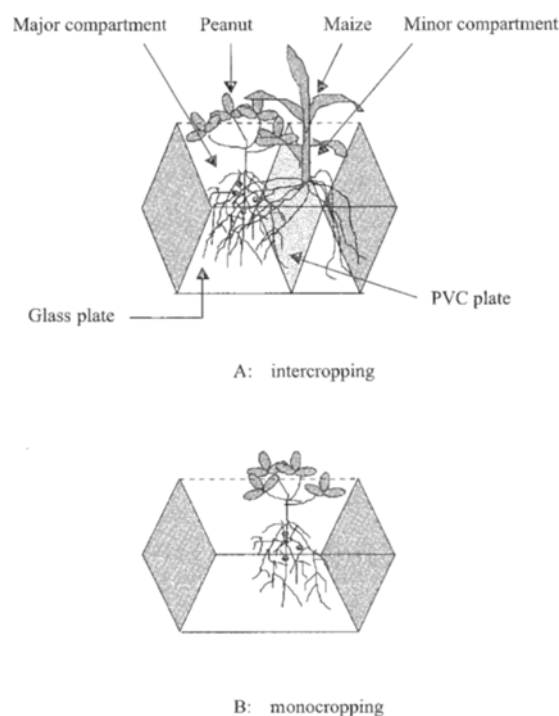


Fig. 1. Diagrammatic representation of the rhizobox design for intercropping (A) and monocropping (B).

occupied two-thirds of the pot volume and the smaller occupied one-third. All peanut roots and the majority of roots of maize grew in the major compartment in intercropping system (just a few roots of maize were in minor compartment). The other design, used for monocropping, had the same dimensions but was not separated into two compartments (Fig. 1B). One side of each rhizobox consisted of a glass plate to permit observation of plant root growth. The experiment was comprised of three treatments with four replicates per treatment, treatment I: peanut monocropping; treatment II: maize/peanut intercropping (the major and the minor compartments with low N 50 mg kg^{-1} as $\text{Ca}(\text{NO}_3)_2$); treatment III: maize/peanut intercropping (the major compartment with low N 50 mg kg^{-1} and the minor compartment with high N 200 mg kg^{-1} as $\text{Ca}(\text{NO}_3)_2$). The minor compartment of treatment III was fertilized 200 mg kg^{-1} N for reducing or eliminating the competition of N coming from intercropping maize.

Properties and treatment of the calcareous soil. The calcareous sandy soil used in the experiments was collected from the plough layer of a calcareous meadow on a farm near Beijing, North China ($39^\circ 40' \text{ N}$, $116^\circ 15' \text{ E}$). It had the following properties: $\text{pH}(\text{CaCl}_2)$ 8.3, CaCO_3 8.32%, organic matter 0.39%, DTPA-Fe 4.52 mg kg^{-1} , total N 0.027%, Olsen-P 3.9 mg kg^{-1} , $\text{NH}_4\text{OAc-K}$ 60.4 mg kg^{-1} . The soil was air-dried, sieved ($< 1 \text{ mm}$), and amended with basal nutrients at the following concentration (mg kg^{-1}): N 50

as $\text{Ca}(\text{NO}_3)_2$; P 150 and K 189 as KH_2PO_4 ; Mg 50 as MgSO_4 ; Zn 5 as ZnSO_4 , and Cu 5 as CuSO_4 .

Plant growth. Peanut (cv. Haihua) and maize (cv. Danyu) seeds of uniform size were surface sterilized in 5% (v/v) H_2O_2 for 30 min, washed with deionized water and germinated at 25°C in the dark in sterile coarse quartz sand (2-mm diameter) moistened with saturated CaSO_4 solution. After 2 d the germinated peanut seedlings were inoculated with *Bradyrhizobium* strain (*Arachis*) 3201 (originally isolated from peanut root nodules in calcareous soil) and were planted in the rhizoboxes. Maize seeds were planted 10 d later than peanut. In the intercropping treatments, peanut seeds were planted in the major compartments, and maize was planted on the PVC separated plate. Therefore, maize roots were divided into two parts, the majority grew in the major compartment and a few grew in the minor compartment. The plants were grown at 26°C / 20°C and 14 h / 10 h day / night cycles with a relative humidity of 70–75%. Soil water content was maintained at about 80% of field capacity (20%) by daily supply of water. The daily fluctuation of water content in the soil before and after the daily irrigation was 10–12% and 13–17%. The soil is a very sandy calcareous soil with good penetrability and poor water retention ability. There were six plants in each rhizobox of monocropping and intercropping system respectively. The ratio of peanut to maize was 1 : 1 in the intercropping treatments.

Plant analysis.

Plant harvest: After 60 d growth, the peanut plants were harvested and separated into shoots, roots, and nodules, and maize plants were separated into shoots and roots. Plants were oven dried at 80°C for 48 h and ground. The whole growth period of peanut is about

150 d, so 60 d growth would be an early stage of growth i.e. the flowering stage of peanut.

Chlorosis: The chlorophyll SPAD value of the young leaves of peanut in the monocropping and intercropping system was determined by using SPAD-202 chlorophyll apparatus.

Acetylene reduction activity: Roots samples washed with distilled water were placed in 650 mL mannitol bottles and sealed with a rubber septum. Acetylene (50 mL) was injected into the bottles which was then incubated without light at 25°C for 1 h. A 0.5 mL gas sample from each bottle was obtained by microinjector. The release capacity of ethylene was determined by GC-14B Shimadzu gas chromatograph (detector: FID, determined condition: column temperature 80°C, injector port temperature 100°C, detector temperature 150°C, N flow 50 mL min⁻¹, hydrogen flow 50 mL min⁻¹, air flow 500 mL min⁻¹).

Total N of plants: Plant shoots and roots of maize and peanut, and nodules of peanut were dried at 80°C for 48 h and ground. The N content of plant samples was analyzed by Kjeldahl digestion and colorimetric determination of ammonium in the digests by an automated indophenol blue method (Schuman et al. 1973).

Total Fe of plants: Plant shoots, roots, and nodules of peanut were dry ashed at 550°C for about 10 h and the ash dissolved in 1 : 30 (v/v) HNO_3 , the concentration of total Fe in shoots, roots, and nodules were determined by atomic absorption spectrophotometry.

The concentration of NO_3^- ion in the soil samples was determined within 24 h of plant harvest. Soil samples (12 g wet weight) were shaken for 1 h with 100 mL 0.01 mol L⁻¹ CaCl_2 . The extract was filtered over a 11-cm diameter ash-less filter paper. The amounts of NO_3^-

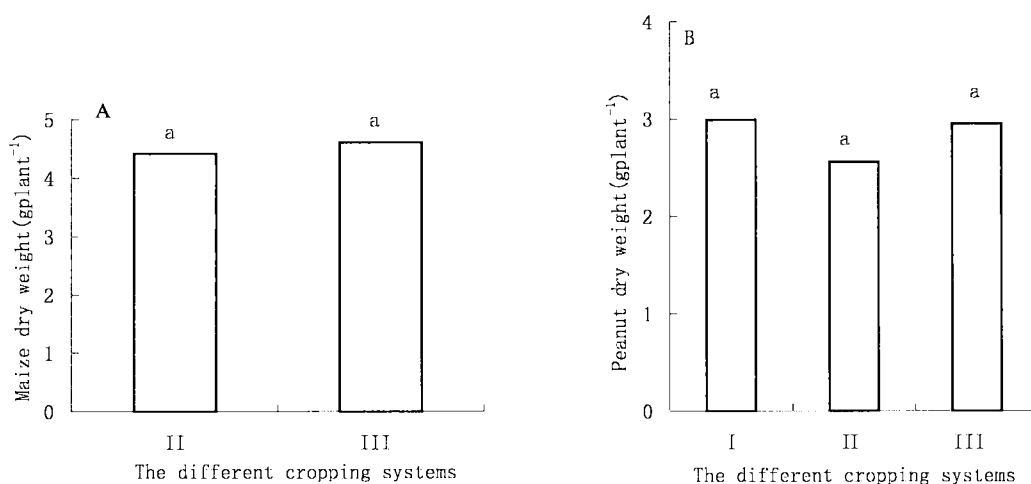


Fig. 2. Peanut and maize dry weight per plant in the different cropping systems. Treatments: I, peanut monocropping; II, maize / peanut intercropping (the major and the minor compartments with low N 50 mg kg⁻¹); III, maize / peanut intercropping (the major compartment with low N 50 mg kg⁻¹ and the minor compartment with high N 200 mg kg⁻¹).

in the extracts were determined colorimetrically using a continuous flow analyzer (TRAACS 2000).

Test of significance. Significant differences in the data between each treatment were estimated by Duncan's multiple-range test.

RESULTS

Effects of peanut intercropping with maize on the growth of plants dry weight

The dry weight of peanut and maize was not significantly different in different cropping systems (Fig. 2). That is to say, it was not serious for peanut plants to encounter nutritional stress from N competition of maize in the intercropping system at the early stages

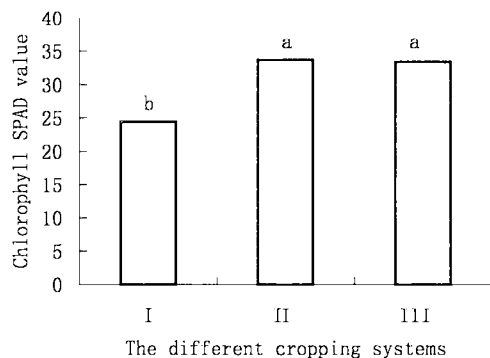


Fig. 3. The chlorophyll content in the young leaves of peanut in the different cropping systems. Treatments: I, peanut monocropping; II, maize/peanut intercropping (the major and the minor compartments with low N 50 mg kg⁻¹); III, maize/peanut intercropping (the major compartment with low N 50 mg kg⁻¹ and the minor compartment with high N 200 mg kg⁻¹).

of growth.

The effects of peanut intercropping with maize on Fe nutrition of peanut plants

The chlorophyll readings in the young leaves of peanut in the two treatments of the intercropping system were respectively higher 38 and 36% than that of peanut in the monocropping system. The chlorophyll content in the young leaves of peanut in the intercropping system (treatment II and III) was obviously enhanced (Fig. 3). Thus, maize in the intercropping systems markedly improved the Fe nutrition and corrected Fe chlorosis of peanut plants.

Iron uptake in the organs of peanut in the intercropping system (treatment II and III) was higher than that of the monocropping treatment (Table 1). Compared with the monocropping treatment, Fe uptake increased from intercropping treatment II and III by 22 and 24% per plant, 30 and 29% shoots, 38 and 60% nodules. Iron concentration of shoots of the intercropping treatment II and III were increased 52 and 33%, respectively. Iron uptake in roots was unaffected by treatments. Both Fe uptake of nodule and Fe concentration of peanut shoot of the intercropping treatment II and III were significantly higher than those of the monocropping treatment (Table 1). Thus, peanut intercropping with maize improved Fe nutrition of peanut plant and especially increased Fe uptake in nodule and peanut shoot.

The effects of peanut intercropping with maize on N nutrition of peanut plants

Nitrogen uptake of peanut in the monocropping system was not higher than that of either intercropping treatments (Table 2). Thus, N transfer into various organs of peanut was not significantly different in two

Table 1. Fe concentration and uptake in the organs of peanut in different cropping systems.

Treatments	Fe concentration (mg kg ⁻¹)			Fe uptake (µg plant ⁻¹)			
	Shoot	Root	Nodule	Shoot	Root	Nodule	Whole plant
I	252.1 b	735.7 a	684.3 a	685.1 b	171.5 a	13.5 b	870.1 b
II	385.1 a	595.1 a	612.2 a	893.3 a	150.8 a	18.5 a	1,062.6 a
III	335.2 a	621.3 a	612.7 a	882.9 a	175.4 a	21.5 a	1,079.8 a

With each treatment, means in the same column and with different letters are significantly different ($p < 0.05$). Mean of four replications. Fe uptake (µg plant⁻¹) = Fe concentration (mg kg⁻¹) × dry weight (g plant⁻¹).

Table 2. N concentration and uptake in the organs of peanut in different cropping systems.

Treatments	N concentration (g kg ⁻¹)			N uptake (mg plant ⁻¹)		
	Shoot	Root	Whole plant	Shoot	Root	Whole plant
I	25.9 a	21.9 a	75.7 a	70.8 a	5.6 a	75.7 a
II	25.6 a	21.3 a	64.9 a	59.4 a	5.5 a	64.9 a
III	24.9 a	20.5 a	72.0 a	65.7 a	6.3 a	72.0 a

With each treatment, means in the same column and with different letters are significantly different ($p < 0.05$). Mean of four replications. N uptake (mg plant⁻¹) = N concentration (g kg⁻¹) × dry weight (g plant⁻¹).

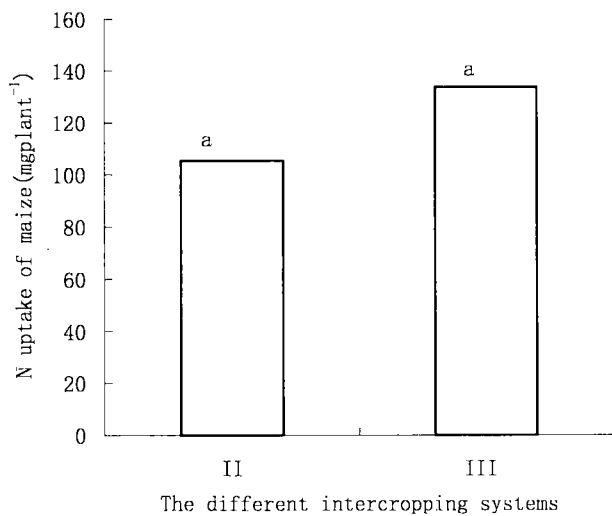


Fig. 4. Maize N uptake per plant in the different intercropping systems. Treatments: II, maize/peanut intercropping (the major and the minor compartments with low N 50 mg kg⁻¹); III, maize/peanut intercropping (the major compartment with low N 50 mg kg⁻¹ and the minor compartment with high N 200 mg kg⁻¹).

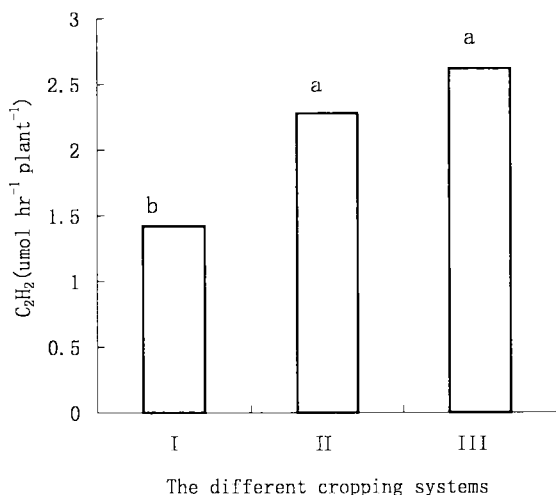


Fig. 5. Effects of intercropping on total acetylene reduction activity of peanut. Treatments: I, peanut monocropping; II, maize/peanut intercropping (the major and the minor compartments with low N 50 mg kg⁻¹); III, maize/peanut intercropping (the major compartment with low N 50 mg kg⁻¹ and the minor compartment with high N 200 mg kg⁻¹).

cropping systems studied. Ultimately, there was no serious effect of the competition of N from intercropping maize on N nutrition of peanut at the early stages of growth. In addition, N uptake of maize in the intercropping treatment III was not significantly different than that of treatment II (Fig. 4).

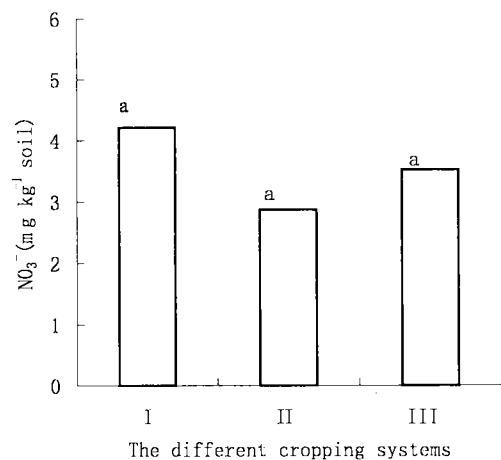


Fig. 6. NO₃⁻ concentration in the rhizosphere soil of peanut in the different treatments. Treatments: I, peanut monocropping; II, maize/peanut intercropping (the major and the minor compartments with low N 50 mg kg⁻¹); III, maize/peanut intercropping (the major compartment with low N 50 mg kg⁻¹ and the minor compartment with high N 200 mg kg⁻¹).

The effects of peanut intercropping with maize on acetylene reduction activity (ARA) of peanut plants

The acetylene reduction activity (ARA) of single peanut plants of both the intercropping treatment II and III were significantly higher than that of the monocropping treatment (Fig. 5), averaging 1.60 and 1.84 times higher than the monocropping treatment respectively. However, the two intercropping treatments were statistically similar.

The effects of peanut intercropping with maize on NO₃⁻ concentration of peanut soil rhizosphere

Nitrate concentration in the rhizosphere soil of monocropped peanut was not significantly different than that in the intercropping treatments (Fig. 6).

DISCUSSION

Improvement in peanut Fe nutrition in intercropping systems. Our results from rhizobox experiment made clear that peanut intercropping with maize could obviously correct Fe chlorosis of peanut (Fig. 3), increase Fe uptake of peanut, and especially enhance Fe uptake of nodule (Table 1). Zuo et al. (2000) reported that in the intercropping system, maize not only acquired adequate Fe to meet its own demand, but also improved the Fe status of peanut. The results strongly suggest that the improvement of Fe nutritional status of peanut intercropped with maize was mainly attributable to the rhizosphere effect of maize. The rhizosphere effect of maize in intercropping system could keep a

higher ferric reduction capacity for a longer time period and the relatively fine root morphology of peanut roots which may have assisted in the mobilization of sparingly soluble Fe(III) compounds from the apoplasmic Fe pool or the growth medium so that the peanut plants remained green. Another possibility is that maize released phytosiderophores into the rhizosphere of peanut and helped to make more root apoplasmic Fe available to peanut in mixture than in monoculture. Thus, it is possible that peanut plants grown as mixed crops may have higher apoplasmic Fe concentration and be able to make more efficient use of the apoplasmic Fe pool than in monocropping (Bienfait et al. 1985; Zuo et al. 2003). Our results showed that maize / peanut intercropping improved significantly DTPA-Fe concentration of the soil rhizosphere of peanut (data not shown). Zuo (1997) also found that DTPA-Fe concentration of rhizosphere soil under intercropping was significantly higher than that of monocropped peanut. Roots of peanut and maize shared a common rhizosphere in the intercropping system, so DTPA-Fe concentration of rhizosphere soil of the intercropping peanut was increased. This might explain the improvement of peanut Fe nutrition in maize/peanut intercropping system. Some have suggested shading can also have a great effect in improvement of Fe nutrition in plants (Pushnik and Miller 1982; Terry 1983; Bughio et al. 1997), but during our experiment, rhizoboxes were often moved to allow adequate sunlight. Thus, shading from maize was negligible and not a contributing factor.

Effects of the improvement of peanut Fe nutrition and maize N competition on N_2 fixation of peanut. Iron is an important nutrient in N_2 -fixing legume root nodules. Iron supplied to the nodule is used by the plant for the synthesis of leghemoglobin, while in the bacteroid fraction it is used as an essential cofactor for the bacterial N_2 -fixing enzyme, nitrogenase, and Fe-containing proteins of the electron transport chain (Kaiser et al. 2003). Iron appears to be required in greater amounts for nodule formation and function than for host legume growth (Tang et al. 1990). Nodule initiation and development are strongly dependent on Fe supply, but the stage of nodule initiation or development most sensitive to Fe deficiency appears likely to vary with legume species (Tang et al. 1992; Pike et al. 1997). Iron deficiency resulted in decreased nodule number and nodule mass in chickpea, lentil (Rai et al. 1982, 1984), French bean (Hemantaranjan 1988), and peanut (O'Hara et al. 1988a). Iron deficiency generally decreases nodule formation, leghemoglobin production, and nitrogenase activity, leading to low N concentrations in the shoots in some legumes. More Fe is required for plants reliant on N_2 fixation than for plants supplied with mineral nitrogen (Tang et al. 1992). It has also been shown that Fe deficiency in peanut induced N deficiency due to inhibi-

tion of the development of nodules (O'Hara et al. 1988b). Our results showed that peanut mixed cropping with maize remarkably improved Fe nutrition of peanut plants, and particularly increased total Fe uptake of peanut root nodule (Table 1). Therefore, the improvement in Fe nutrition of peanut intercropped with maize improved N_2 -fixation compared to peanut with Fe deficiency in monocropping system (Fig. 5).

A legume–non-legume intercropping can be defined as any form of cropping in which there is direct competition between two different crop species. In addition, where soil (one being a legume) N fertility is high, legumes in the field thrive without fixing atmospheric N_2 , so high levels of soil nitrate can be a potent inhibitor of N_2 fixation (Streeter 1988). In intercropping systems, competition for soil mineral N between legumes and cereals can result in stimulation of biological N_2 fixation and increased harvest of total interplanted crops (Rerkasem et al. 1988; Chandel et al. 1989; Peoples et al. 1995; Elabbadi et al. 1996). Both the competition of N from intercropping maize and the improvement of Fe nutrition of peanut could promote N_2 fixation of peanut in this intercropping system. Our experimental results showed that the improvement of peanut Fe nutrition in the intercropping system at the early stages of growth (only 60 d) was an important factor, which led to enhancement of peanut N_2 fixation. There were three reasons to support this idea. First, despite the intercropping system having potential competition for N, there was no significant difference in N concentration and uptake of peanut in different treatments at harvest, nor difference in NO_3^- -N concentration of peanut rhizosphere soil in different treatments (Table 2, Fig. 6). On the other hand, maize / peanut intercropping could correct Fe chlorosis of peanut, increase Fe transport into the organs of peanut—Fe uptake of nodule and Fe concentration of peanut shoots in different treatments were especially affected (Table 1, Fig. 3). These results indicate that improvement of peanut Fe nutrition was a more important effect on peanut than N competition from maize in the intercropping system at the early stages of growth. Second, the competition of N from the intercropping maize hardly affected N_2 fixation of peanut when the ratio of maize / peanut was 1:1 in the intercropping treatment. If there had been a difference in acetylene reduction activity between treatment I and II attributed to the competition of N, the nitrogenase activity of peanut should be significantly different between treatment II and treatment III which was eliminated the competition of N (compared to treatment II), but the fact was not so (Fig. 5). The effects of the competition of N from the intercropping maize on N_2 fixation of peanut under this planting mode at the early stages of growth was not obvious. Third, NO_3^- -N markedly affected the

number of nodule initials of monocropped peanut, while maize / peanut intercropping did not affect it (data not shown).

In conclusion, the results of our experiments strongly support that the improvement in N₂ fixation of peanut was mainly attributed to the improvement of Fe nutrition in the intercropping peanut. The competition of N from intercropping maize had little effect on N₂ fixation in peanut in this experiment.

The related mechanisms of improvement of Fe nutrition and competition for N nutrition on N₂ fixation in peanut in this intercropping system are much less understood. Further research is required to elucidate fully the respective contribution of Fe and N nutrition of peanut to N₂ fixation in intercropping system. Further characterization of effects of the improvement Fe nutrition of peanut intercropped with maize on nodule formation and development, N₂ fixation functions of peanut is currently in progress.

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