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Maize grain concentrations and above-ground shoot acquisition of micronutrients as affected by intercropping with turnip, faba bean, chickpea, and soybean

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Most research on micronutrients in maize has focused on maize grown as a monocrop. The aim of this study was to determine the effects of intercropping on the concentrations of micronutrients in maize grain and their acquisition via the shoot. We conducted field experiments to investigate the effects of intercropping with turnip (*Brassica campestris* L.), faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), and soybean (*Glycine max* L.) on the iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) concentrations in the grain and their acquisition via the above-ground shoots of maize (*Zea mays* L.). Compared with monocropped maize grain, the grain of maize intercropped with legumes showed lower concentrations of Fe, Mn, Cu, and Zn and lower values of their corresponding harvest indexes. The micronutrient concentrations and harvest indexes in grain of maize intercropped with turnip were the same as those in monocropped maize grain. Intercropping stimulated the above-ground maize shoot acquisition of Fe, Mn, Cu and Zn, when averaged over different phosphorus (P) application rates. To our knowledge, this is the first report on the effects of intercropping on micronutrient concentrations in maize grain and on micronutrients acquisition via maize shoots (straw+grain). The maize grain Fe and Cu concentrations, but not Mn and Zn concentrations, were negatively correlated with maize grain yields. The concentrations of Fe, Mn, Cu, and Zn in maize grain were positively correlated with their corresponding harvest indexes. The decreased Fe, Mn, Cu, and Zn concentrations in grain of maize intercropped with legumes were attributed to reduced translocation of Fe, Mn, Cu, and Zn from vegetative tissues to grains. This may also be related to the delayed senescence of maize plants intercropped with legumes. We conclude that turnip/maize intercropping is beneficial to obtain high maize grain yield without decreased concentrations of Fe, Mn, Cu, and Zn in the grain. Further research is required to clarify the mechanisms underlying the changes in micronutrient concentrations in grain of intercropped maize.

intercropping, maize, micronutrient, harvest index, dilution

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Intercropping, which is the cultivation of two or more crops on a single piece of land, has many advantages over monocropping. It can increase yields, has fewer negative effects on the environment, and is more ecologically sustainable [1]. Maize-based intercropping, especially with legumes, is predominant among intercropping systems. This cultivation

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practice is widespread in India, Southeast Asia, Latin America, Africa, and China [2]. The most significant benefits of maize-based intercropping are increased yields of maize. This may result from direct or indirect growth promotion by legumes or other crops, or from interspecific temporal and spatial niche complementarities. For example, legumes can directly facilitate nitrogen (N) or phosphorus (P) uptake by maize [3,4]. Beneficial microorganisms can also mobilize nutrients and indirectly facilitate their uptake by maize [3]. Maize can also capture more solar radiation and water and nutrient resources through interspecific differences in above-ground plant height, belowground rooting depth, or seasonality (cool/warm) [4].

As a strategy-II plant, maize can secrete low-molecular-mass secondary amino acids (mugineic acids) known as 'phytosiderophores' from its roots. These substances chelate sparingly soluble iron (Fe) for absorption and use [5]. Consequently, intercropping of maize and legumes on neutral or alkaline soils could ease Fe uptake to correct Fe deficiencies and increase the concentration of Fe in legume seeds [6]. However, few studies have focused on the acquisition of micronutrients by above-ground plant parts, or on the concentrations of micronutrients in grain of intercropped maize. Very recently, there has been some research on maize intercropping for bioremediation of heavy metal-contaminated soils, since maize is a hyperaccumulator of such metals [7].

Maize is a staple crop in many parts of the world, and is often targeted for micronutrient 'biofortification'. The three micronutrients most often lacking from human diets and necessary for maize growth are Fe, zinc (Zn), and copper (Cu) [8]. Research on maize biofortification has included conventional breeding and genetic modification [9] or agronomy practices [10], but all of these strategies have focused on maize cultivated as a monoculture. Fe and Zn remain the most studied micronutrients in terms of biofortification, while only limited research has been conducted on Cu. Manganese (Mn) is one of the eight trace elements essential for higher plants, but only a few studies have focused on Mn biofortification of maize [8].

The three most important factors for a successful biofortification strategy are high concentrations of the micronutrient(s) in the edible part of the crop, high yield, and high profitability [11]. Water regimes (pre-anthesis drought vs. irrigation throughout the vegetation cycle) did not affect the mineral composition of tropical maize grain. Application of N fertilizer increased the concentration of Mn in the grain, but the higher grain yield resulting from N fertilization resulted in a grain Zn 'dilution' effect [10]. Increasing N supply significantly improved the yield but had little effect on the grain Zn concentration in maize [12]. In addition, modern maize varieties with higher yields tend to contain lower concentrations of micronutrients in the grain, compared with those in grain of the lower-yield conventional varieties [10].

It is, therefore, important to know whether intercropping could be an effective agronomy practice to enhance or maintain concentrations of Fe, Mn, Cu, and Zn in the maize grain, alongside the higher yields of intercropped maize, or whether there will be a 'dilution' effect as reported in studies on monocultured maize [10]. In addition, continuous P application over recent decades has led to a cumulative surplus of P in croplands. For example, the average Olsen-P has increased from 7.4 to 24.7 mg kg^{-1} during the last 30 years in China [13]. This situation threatens both the quality of the environment and food security because of over-fertilization and limited reserves of phosphate rock [14]. Complex interactions of P with Fe, Mn, Cu, and especially Zn have been studied since the 1960s, using various crops cultivated under different systems [15]. P application can negatively affect grain Zn concentration in cereal crops [16].

The objective of this research was to analyze the effects of intercropping on the micronutrient concentrations in maize tissues. We intercropped maize with faba bean, chickpea, soybean, and turnip and analyzed the concentrations of Fe, Mn, Cu, and Zn in above-ground maize grain and straw). Also, to determine the effects of P on these interactions, we applied P at different rates to the various monocropping and intercropping systems. The four maize-based intercropping systems tested in this study are widespread and produce high crop yields in irrigated areas of temperate zones in northwestern China. This intercropping-rich part of China generally has only one relatively short cropping season each year because of temperature limitation.

1 Materials and methods

1.1 Field location

The field experiment was conducted in 2010 at Baiyun Experimental Station, Institute of Soils, Fertilizers and Water-Saving Agriculture, Gansu Academy of Agricultural Sciences, Gansu Province, China. Baiyun Experimental Station (38°37′N, 102°40′E) is located 15 km north of Wuwei City, Gansu Province, at 1504 m above sea level. The annual mean temperature is 7.7°C. The accumulated temperatures above 0°C and 10°C are 3646°C and 3149°C, respectively. The frost-free period is 170–180 d. Total solar radiation is 5988 MJ m^{-2} a^{-1}, annual precipitation is 150 mm, and potential evaporation is 2021 mm. The area is classified as having a typical arid climate and the soil at the site is classified as Aridisol (serozem).

1.2 Experimental design

We used a split-plot design with three replicates. The main plot treatments comprised three P application rates (0, 40, or 80 kg P hm^{-2}, applied as triple superphosphate) and the split-plot treatments consisted of nine cropping systems:

maize (*Zea mays* L.) intercropping with turnip (*Brassica campestris* L.), faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), or soybean (*Glycine max* L.), and corresponding monocultures of each crop.

Plants in all intercropping and monocropping plots were grown in an east-west row orientation. The area of the individual plots was 4.0 m×5.5 m for monocropped maize, turnip, faba bean, chickpea, and soybean, and 5.6 m×5.5 m for the intercropping systems (Figure 1). Each intercropped plot consisted of four strips, each 1.4-m wide. Two rows of maize alternated with three rows of legumes or turnip were planted in each strip; the inter-row distance was 40 cm for monocropped and intercropped maize and 20 cm for monocropped and intercropped legumes or turnip. There was a 30-cm gap between maize rows and associated crop rows in the intercropping systems. Theoretically, the row design of the intercropping system resulted in two maize rows occupying 80 cm of the 140-cm wide strip, and the three rows of the companion crop occupying 60 cm. The inter-plant distance was 20 cm for maize and legume species. Turnip was planted by broadcast sowing in each row. The spacing was specifically designed to represent typical intercropping practices in the region. Maize rows occupied 80/140=57% of the intercropped area and the companion rows occupied 60/140=43%. To compare intercropped with monocropped plants, the density of maize or other crops in the intercropped plots was designed to be equal to that in the monoculture, based on the per unit of sown row area for maize or the companion crop. The experiment had a replacement design where the sum of relative sowing densities of the two species (sowing density in intercrop/sowing density in monocrop) was equal to one.

Nitrogen fertilizer was applied at an identical rate (112.5 kg hm^{-2} as urea) to all legume species and turnip. This amount was 50% of that applied to maize. No potassium (K) or organic manure was applied to any crop. All the P fertilizer and 112.5 kg hm^{-2} of the N fertilizer were evenly broadcasted and incorporated into the upper 20 cm of the soil prior to sowing. The other half of the N fertilizer for maize was divided into two portions and applied by top-dressing along with irrigation at the maize stem elongation stage and the pre-tasseling stage. All plots were irrigated adequately and all plants were weeded manually during the growing season. No fungicides were applied to either crop. At the peak flowering stage, omethoate (2-dimethoxyphosphinoylthio-N-methylacetamide; Dazhou Xinglong Chemical Co., Ltd., Dazhou, China) was used as a foliar spray to control aphids on faba bean.

The sowing dates were March 28 for faba bean, chickpea, and turnip and April 26 for maize and soybean. The harvest dates were June 28 for turnip when maize was at the elongation stage, July 28 for faba bean and chickpea when maize was at the tasseling stage, August 31 for soybean when maize was at the grain-filling stage, and October 5 for maize. The maize harvest date was almost 3 months after the harvest date for turnip, 2 months later than those for faba bean and chickpea, and 1 month later than that of soybean.

1.3 Sample preparation and micronutrient analysis

Grain and straw yields of intercropped and monocropped maize at maturity were measured by harvesting one intercropping strip from each intercropping plot and two adjacent rows of maize from each monoculture plot. After harvesting, maize straw and grain samples threshed by hand were dried at 65–70°C for 72 h after being washed rapidly with deionized water. Plant samples were ground with a stainless steel grinder (Model HY-04B, Beijing Xinhuanya, China) and subsamples were digested with HNO_3:H_2O_2 (6 mL:2 mL) in a microwave accelerated reaction system (CEM Corp., Matthews, NC, USA). The concentrations of Fe, Mn, Cu, and Zn in the digested solutions were determined by inductively coupled plasma atomic emission

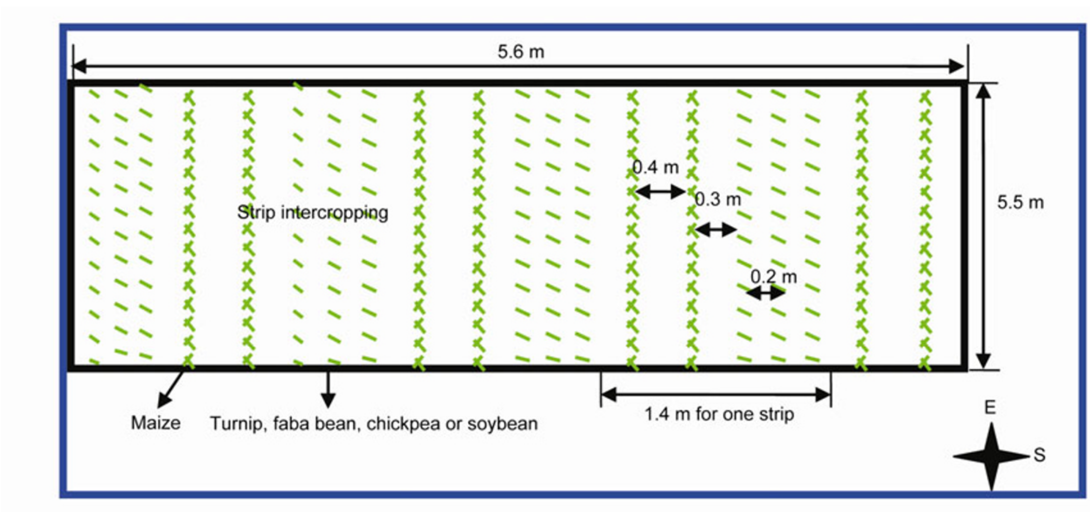

Figure 1 Diagram of strip intercropping field experiment.

spectroscopy (ICP-AES; OPTIMA 3300 DV, Perkin-Elmer, Norwalk, CT, USA). Blanks and international certified reference materials (IPE556 for grain and IPE883 for straw, Wageningen University, The Netherlands) were used in each batch of digestions to ensure analytical quality.

1.4 Statistical analysis

Data from the split-plot design experiment were subjected to analysis of variance (ANOVA) using SAS for Windows ver. 8 and mean values (n=3) were compared using the least significant difference (LSD) test at the 5% level. Linear regressions and Pearson correlations were used to analyze the relationships between maize grain Fe, Mn, Cu, Zn concentrations and grain yields or harvest indexes. All figures were drawn using SigmaPlot v. 10.0 (Systat Software Inc., San Jose, CA, USA).

2 Results

2.1 Maize grain Fe, Mn, Cu, and Zn concentrations

The maize grain Mn concentration was higher in the 80 kg P hm^{-2} treatment than in the 0 kg P hm^{-2} treatment, averaged over monoculture and intercropping systems (Figure 2). Compared with the Fe concentration in monocropped maize grain, Fe concentration in intercropped maize grain was not significantly lower, except for maize intercropped with faba bean or soybean in the 80 kg P hm^{-2} treatment (Figure 2). Under all P treatments, Mn and Cu concentrations were significantly lower in grain of maize intercropped with faba bean, chickpea, and soybean than in grain of monocropped maize, except for the Mn concentration in maize intercropped with chickpea in the 80 kg P hm^{-2} treatment (Figure 2). Compared with that in monocropped

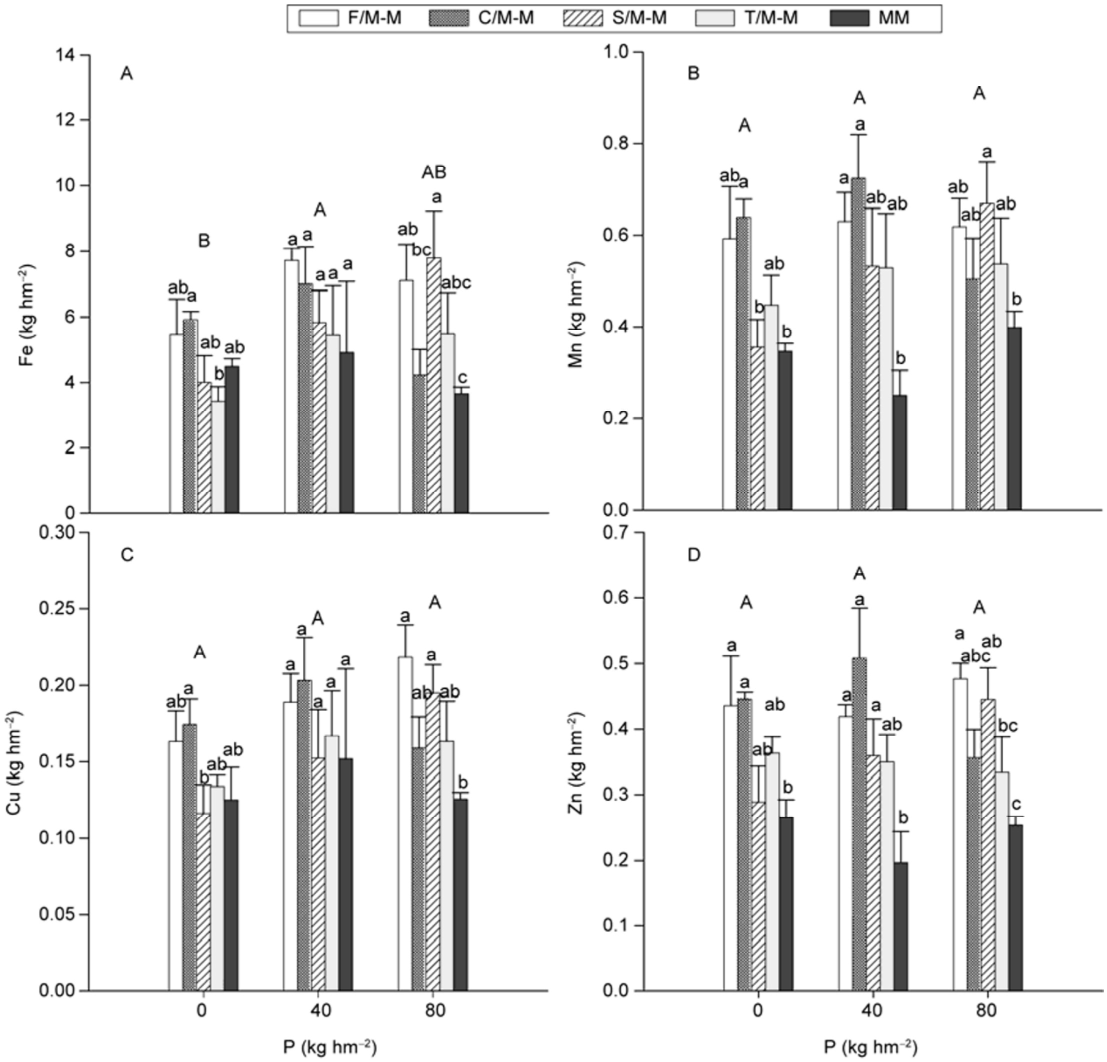

Figure 2 Concentrations of Fe, Mn, Cu, and Zn in grain of monocropped and intercropped maize under different P application rates. Different capital letters indicate significant differences (at $LSD_{0.05}$) among different P application rates. Different lowercase letters indicate significant differences (at $LSD_{0.05}$) among different cropping systems under the same P application rate. F/M-M, C/M-M, S/M-M, T/M-M, and MM indicate maize intercropped with faba bean, chickpea, soybean, and turnip, and monocropped maize, respectively.

maize grain, the Zn concentrations were lower in grain of maize intercropped with faba bean in all P treatments, with chickpea in the 0 and 40 kg P hm^{-2} treatments, and with soybean with no P application (Figure 2). Therefore, P application alleviated the decrease in Zn concentration of maize intercropped with chickpea and soybean, compared with that in monocropped maize grain (Figure 2). Intercropping with turnip did not significantly affect the Fe, Mn, Cu, and Zn concentrations in maize grain, compared with those in monocropped maize grain (Figure 2). Averaged over the three P application rates, intercropping with faba bean, chickpea, and soybean significantly decreased the concentrations of Fe, Mn, Cu and Zn in maize grain, while intercropping with turnip did not (Figure 2).

2.2 Maize grain Fe, Mn, Cu, and Zn acquisition

There were no significant differences in maize grain Fe, Mn, Cu, and Zn acquisition among the different P treatments, averaged over maize monocropping and intercropping systems (Figure 3). Irrespective of P application rates, the Fe contents in grain of maize intercropped with faba bean, chickpea, soybean, or turnip were less affected by intercropping than by monocropping (Figure 3). The contents of both Mn and Zn in maize grain were increased by interspecific interactions between maize and the three legumes in the 40 kg P hm^{-2} treatment (Figure 3). However, interspecific interactions did not significantly affect the maize grain Mn and Zn contents in the 0 and 80 kg P hm^{-2} treatments,

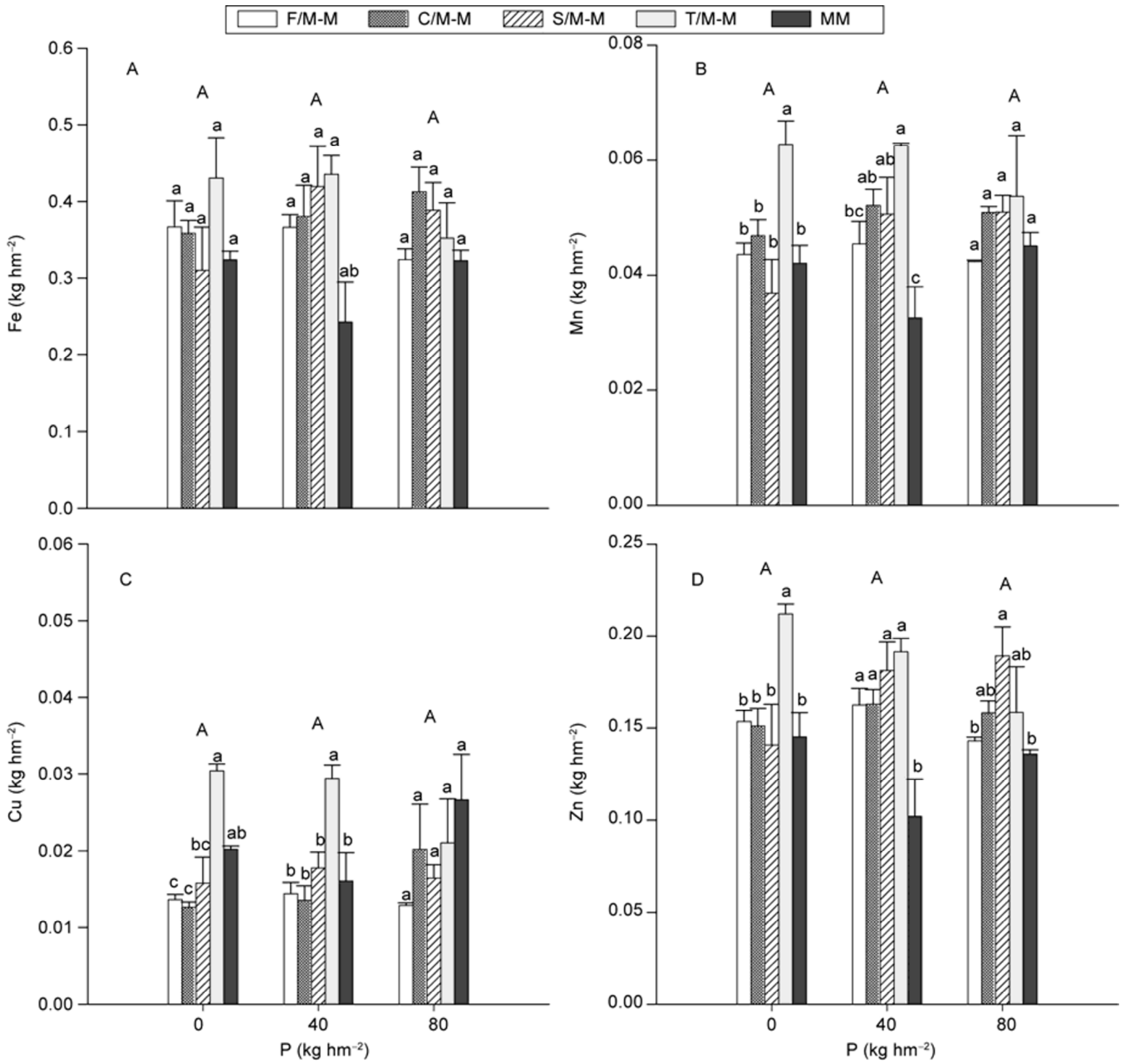

Figure 3 Grain Fe, Mn, Cu, and Zn acquisition of monocropped and intercropped maize under different P application rates. Different capital letters indicate significant differences (at $LSD_{0.05}$) among different P application rates. Different lowercase letters indicate significant differences (at $LSD_{0.05}$) among different cropping systems under the same P application rate. F/M-M, C/M-M, S/M-M, T/M-M, and MM indicate maize intercropped with faba bean, chickpea, soybean, and turnip, and monocropped maize, respectively.

except for maize intercropped with soybean, which showed a significant increase in Zn content of the grain in the 80 kg P hm^{-2} treatment (Figure 3). Compared with that in grain of monocropped maize, the Cu contents in grain of maize intercropped with three legumes were decreased when no P fertilizer was applied, but were less affected by interspecific interactions with other crops in the 40 and 80 kg P hm^{-2} treatments (Figure 3). In contrast, the contents of Mn, Cu, and Zn in grain of maize intercropped with turnip were increased in the 0 and 40 kg P hm^{-2} P treatments, but not in the 80 kg P hm^{-2} treatment (Figure 3).

2.3 Maize above-ground shoot (grain+straw) Fe, Mn, Cu, and Zn acquisition

There were no significant differences in Mn, Cu, and Zn contents in above-ground shoots among the different P application rates (Figure 4). Only the Fe content was significantly increased in the 40 kg P hm^{-2} treatment, compared with that in the 0 kg P hm^{-2} treatment, averaged over monocropped and intercropped systems (Figure 4). Averaged over all P application rates, the Fe, Mn, Cu, and Zn contents in above-ground shoots of monocropped maize were generally lower than those of maize intercropped with turnip, which were also generally lower than those of maize intercropped with legume crops (Figure 4). The Fe and Cu contents in above-ground maize shoots were significantly enhanced by interspecific interactions with faba bean or soybean in the 80 kg P hm^{-2} treatment, but their contents were not affected by interspecific interactions in the 0 and 40 kg P hm^{-2} treatments (Figure 4). The Mn contents in above-ground shoots of maize intercropped with chickpea were significantly higher than those in above-ground shoots of monocropped maize in the 80 kg P hm^{-2} treatment (Fig-

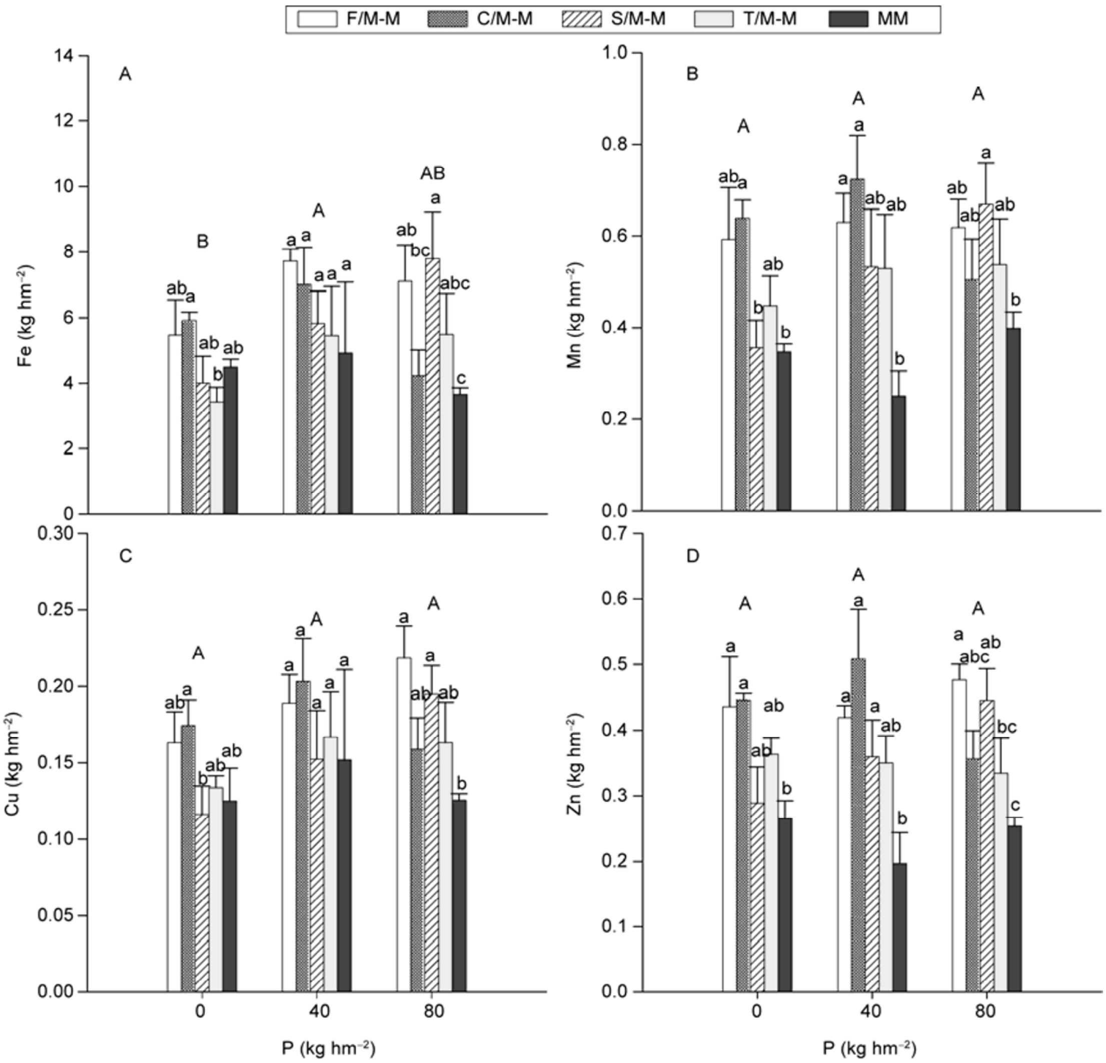

Figure 4 Fe, Mn, Cu and Zn acquisition via above-ground maize shoots (straw+grain) under different P application rates. Different capital letters indicate significant differences (at $LSD_{0.05}$) among different P application rates. Different lowercase letters indicate significant differences (at $LSD_{0.05}$) among different cropping systems under the same P application rate. F/M-M, C/M-M, S/M-M, T/M-M and MM indicate maize intercropped with faba bean, chickpea, soybean, and turnip, and monocropped maize, respectively.

ure 4). The Mn contents in above-ground shoots of maize intercropped with faba bean and soybean were greater than those in monocropped maize in the 40 and 80 kg P hm^{-2} treatments, respectively (Figure 4). The Zn contents in above-ground parts of maize intercropped with faba bean and chickpea were higher than those in above-ground shoots of monocropped maize in the 0 and 40 kg P hm^{-2} treatments (Figure 4). There was a significant difference in Zn content in above-ground shoots between monocropped maize and maize intercropped with faba bean in the 80 kg P hm^{-2} treatment (Figure 4). Compared with that in above-ground shoots of monocropped maize, the Zn contents in above-ground shoots of maize intercropped with soybean were enhanced in the 40 and 80 kg P hm^{-2} treatments (Figure 4).

2.4 Maize grain harvest indexes

We analyzed the maize grain harvest indexes (HIs) of Fe (FeHI), Mn (MnHI), Cu (CuHI) and Zn (ZnHI). There were no significant differences in maize grain FeHI, MnHI, CuHI and ZnHI among the different P treatments, averaged over monocropped and intercropped systems (Figure 5). Averaged over all of the P treatments, grain MnHI, CuHI, and ZnHI of monocropped maize and grain FeHI, MnHI, CuHI and ZnHI of maize intercropped with turnip were higher than those of maize intercropped with legume crops (Figure

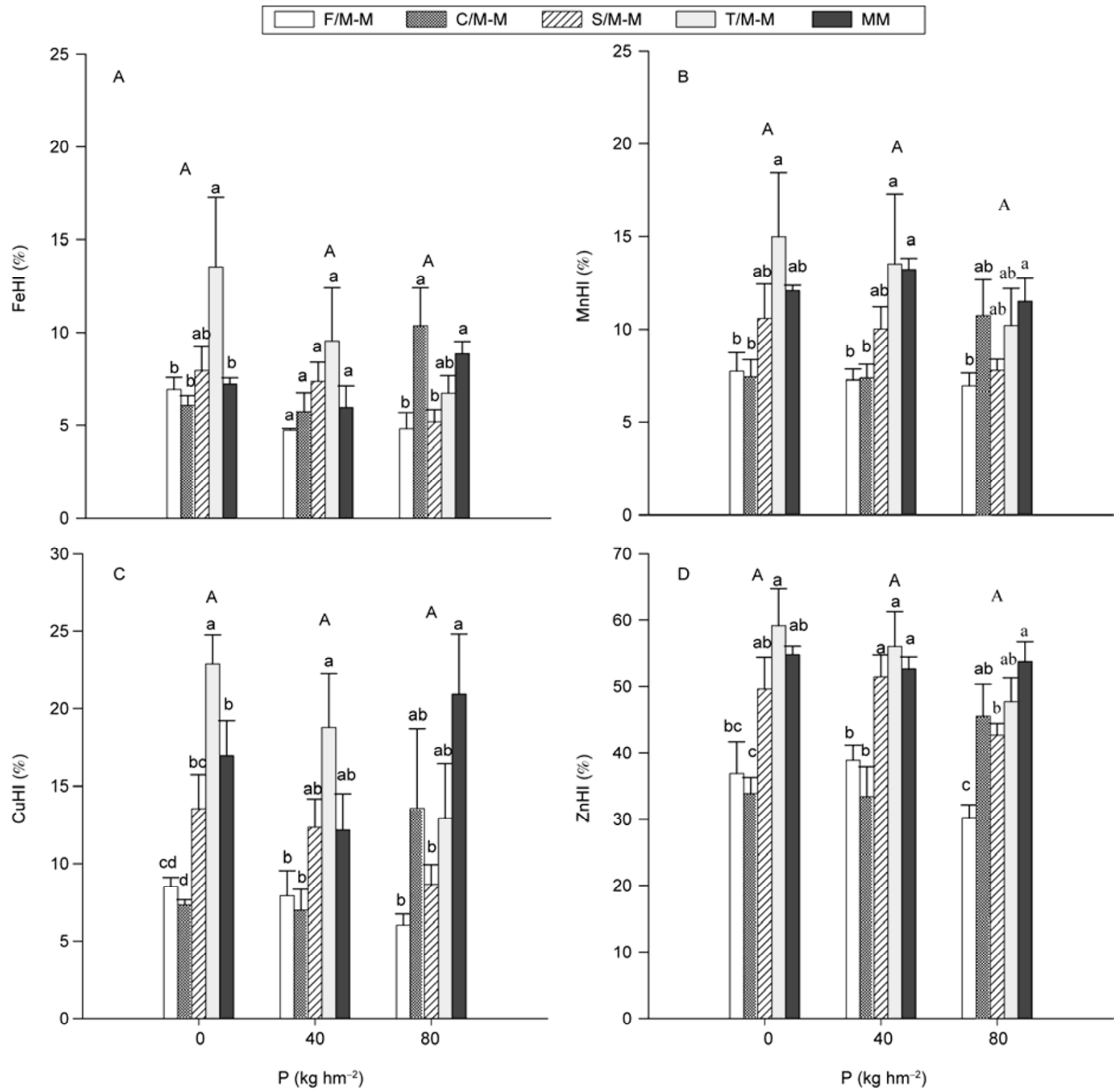

Figure 5 Harvest indexes (HIs) of grain Fe, Mn, Cu, and Zn of monocropped and intercropped maize under different P application rates. Different capital letters indicate significant differences (at $LSD_{0.05}$) among different P application rates. Different lowercase letters indicate significant differences (at $LSD_{0.05}$) among different cropping systems under the same P application rate. F/M-M, C/M-M, S/M-M, T/M-M, and MM indicate maize intercropped with faba bean, chickpea, soybean, and turnip, and monocropped maize, respectively.

5). The FeHIs of maize intercropped with faba bean and soybean were significantly lower than that of monocropped maize in the 80 kg P hm^{-2} treatment (Figure 5). The MnHIs of maize intercropped with faba bean in the 40 and 80 kg P hm^{-2} treatments and chickpea in the 40 kg P hm^{-2} treatment were significantly lower than that of monocropped maize (Figure 5). The CuHIs of maize intercropped with faba bean in the 0 and 80 kg P hm^{-2} treatments, chickpea in the 0 kg P hm^{-2} treatment, and soybean in the 80 kg P hm^{-2} treatment were significantly decreased by interspecific interactions, compared with the CuHI of monocropped maize (Figure 5). The ZnHIs of maize intercropped with faba bean in the 40 and 80 kg P hm^{-2} treatments, chickpea in the 0 kg P hm^{-2} treatment, and soybean in the 80 kg P hm^{-2} treatment were also significantly decreased by interspecific interactions, compared with the ZnHI of monocropped maize (Figure 5). There were no significant differences in FeHI, MnHI, CuHI, and ZnHI between monocropped maize and maize intercropped with turnip, except that the FeHI and CuHI of maize intercropped with turnip was significantly higher than those of monocropped maize in the 0 kg P hm^{-2} treatment (Figure 5).

2.5 Relationships between maize grain Fe, Mn, Cu, and Zn concentrations and grain yields

The grain yields of intercropped maize were greater than that of monocropped maize, but the concentrations of Fe, Mn, Cu, and Zn were lower in intercropped maize grain than in monocropped maize grain (Figures 2 and 6). Maize grain Fe and Cu concentrations showed significant negative linear correlations with grain yields, irrespective of P application rates and cropping systems (Figure 6).

2.6 Relationships between maize grain Fe, Mn, Cu, and Zn concentrations and harvest indexes

There were significant positive linear correlations between maize grain Fe, Mn, Cu, Zn concentrations and their respective HIs (Figure 7).

3 Discussion

3.1 Effects of phosphorus application rate

The results of this study show that the effects of P application rates on the concentrations of Fe, Mn, Cu, and Zn in maize grain, on the acquisition of Fe, Mn, Cu, and Zn via maize grain and total above-ground shoot, and on their corresponding harvest indexes, were generally not significant (Figures 2–5). The different P application rates did not affect maize grain yields significantly (data not shown). This may be because of a higher cumulative surplus of P in soils from previous over-fertilization by local farmers.

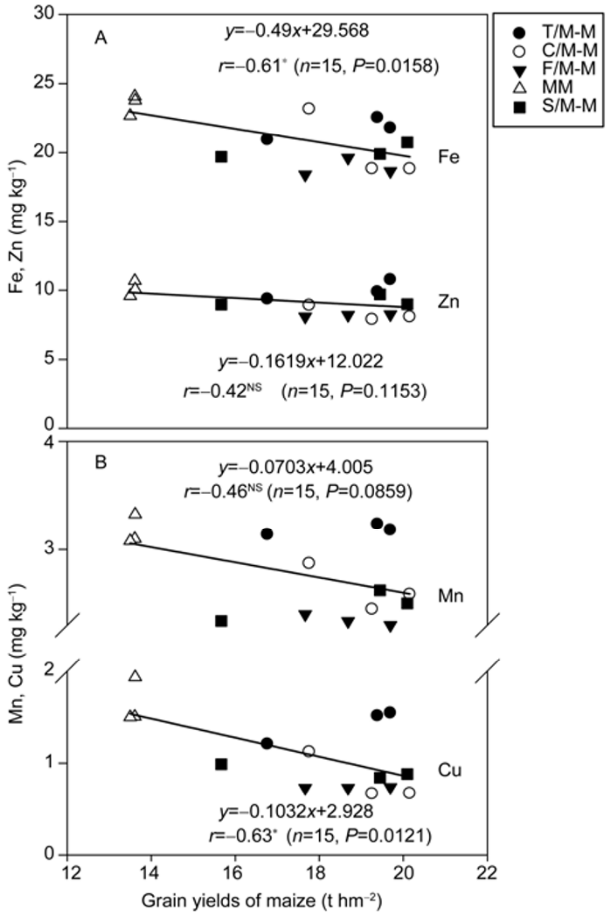

Figure 6 Relationships between maize grain Fe, Mn, Cu, Zn concentrations and maize grain yields. NS indicates not statistically significant; *, significant at P<0.05.

3.2 Grain micronutrient 'dilution' may not be caused by higher grain yields

Previous studies showed that intercropping of maize, especially with legumes, led to higher grain yields of maize compared with that of monocropped maize [4]. To our knowledge, this is the first report that grain of maize intercropped with faba bean, chickpea, and soybean shows dramatically decreased Fe, Mn, Cu, and Zn concentrations (Figure 2). One reason for this may be that the higher yields of maize led to a 'dilution' effect on micronutrients in the grain. In a previous study, N fertilization led to higher maize grain yield, which had a dilution effect on grain Zn concentration [10]. In another study, multiple regression analyses showed that both increasing yield and harvest index were significant factors explaining the downward trend in wheat grain mineral concentrations [17]. Similarly, yield stimulation caused by rising CO_2 led to a decrease in Zn concentration in wheat grain [18].

In the present study, the maize grain harvest index was not affected by intercropping (data not shown). Pearson

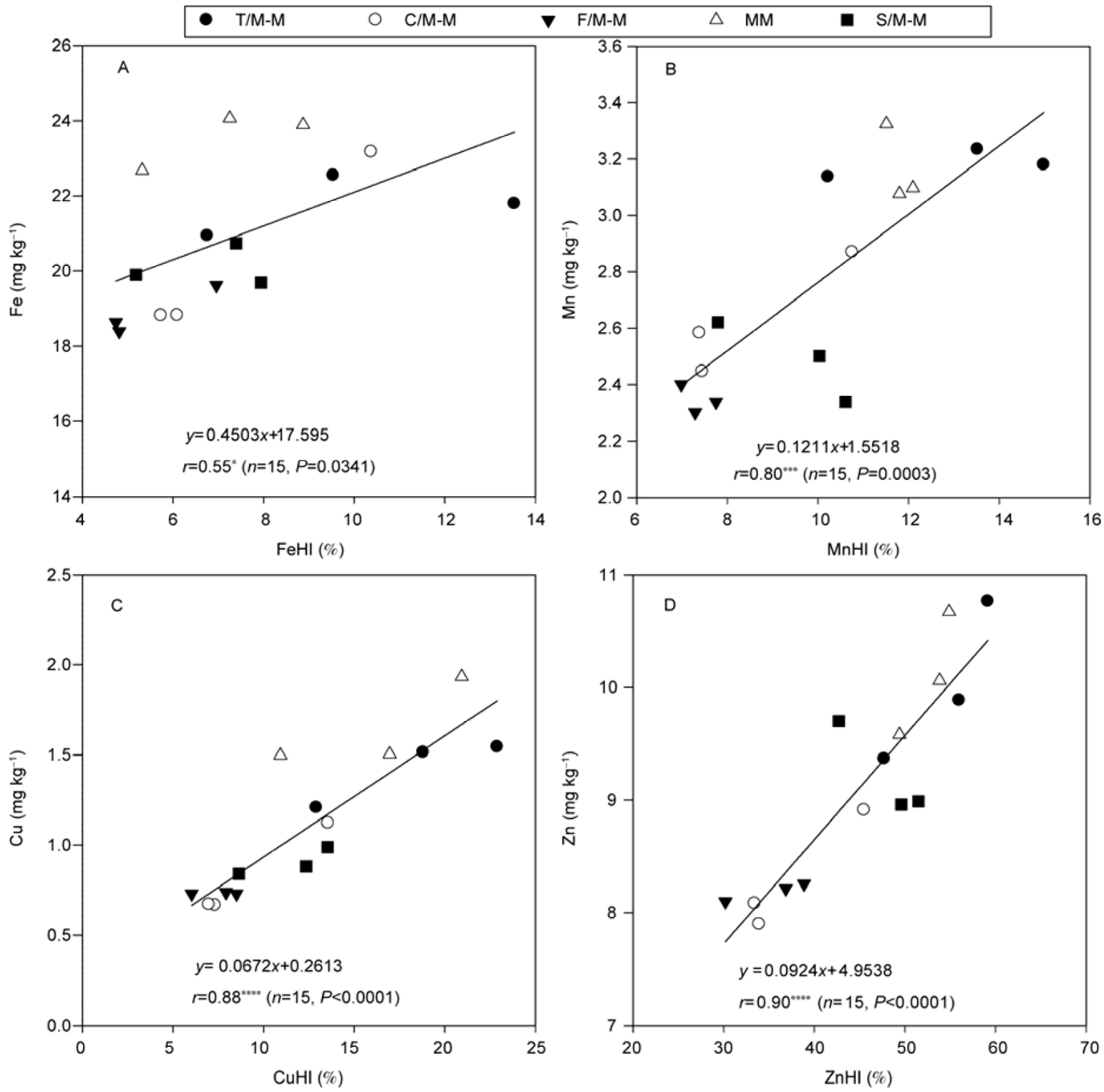

Figure 7 Relationships between maize grain Fe, Mn, Cu, Zn concentrations and their corresponding HIs. NS indicates not statistically significant; *, significant at $P<0.05$; ***, significant at $P<0.001$; ****, significant at $P<0.0001$.

correlation analyses showed there were no significant correlations between harvest indexes of grain yields and grain Fe, Mn, Cu, Zn concentrations (Table 1). Higher grain yields of maize were also not sufficient to explain the dilution of grain Fe, Mn, Cu, and Zn. There were significant negative linear correlations between maize grain yields and grain Fe or Cu concentrations, but the negative correlations between maize grain yields and Mn and Zn concentrations were not significant (Figure 5, Table 1). Therefore, the 'dilution' effect caused by higher grain yields did not appear to be responsible for the reduction in maize grain Mn and Zn concentrations. In a previous study, N application increased the maize grain Mn concentration [10]. Another study showed that increasing the N supply significantly improved yield, but it had little effect on maize grain Zn concentration [12].

Although the grain yield of maize intercropped with turnip was greater than that of monocropped maize, it did not lead to a grain Fe, Mn, Cu, Zn 'dilution' effect; that is, the Fe, Mn, Cu, and Zn concentrations in grain of maize intercropped with turnip were the same as those in monocropped maize grain (Figure 2). This phenomenon differed markedly from that observed in maize intercropped with legume crops. As shown in Figure 6, the data of maize intercropped with turnip deviated widely from data of maize intercropped with legumes; this resulted in insignificant negative linear correlations between maize grain yields and grain Mn or Zn concentrations. If the data from maize intercropped with

Table 1 Pearson correlations between maize grain Fe, Mn, Cu, and Zn concentrations and grain yields or harvest indexes (HIs)[a)]

Maize grain micronutrients	Grain yields	Grain yields excluding T/M-M	HIs of grain yields	HIs of corresponding Fe, Mn, Cu, or Zn
Fe	–0.610*	–0.750**	0.195NS	0.549*
Mn	–0.458NS	–0.751**	0.233NS	0.802***
Cu	–0.629*	–0.883***	0.229NS	0.881****
Zn	–0.424NS	–0.722*	0.412NS	0.896****

a) T/M-M, maize intercropped with turnip; NS, not statistically significant; *, significant at $P<0.05$; **, significant at $P<0.01$; ***, significant at $P<0.001$; ****, significant at $P<0.0001$.

turnip were excluded, the negative linear correlations became significant (Table 1).

3.3 Lower concentrations of micronutrients in intercropped maize were due to lower micronutrient harvest indexes and delayed maize senescence

There were similar trends in maize grain Fe, Mn, Cu, and Zn concentrations (Figure 2) and their corresponding harvest indexes (Figure 5). This indicated that grain Mn, Cu, and Zn concentrations and their corresponding HIs of maize intercropped with faba bean, chickpea, and soybean were generally lower than those of monocropped maize and maize intercropped with turnip, irrespective of the P application rate. Compared with the correlations between grain Fe, Mn, Cu, and Zn concentrations and grain yields, grain Fe, Mn, Cu, and Zn concentrations were more significantly and positively correlated with their corresponding harvest indexes (Table 1).

There were no significant differences in HIs of grain yields between monocropped and intercropped maize. However, the Mn, Cu, and Zn HIs of maize intercropped with faba bean, chickpea, and soybean were lower than those of monocropped maize and maize intercropped with turnip (Figure 5). Thus, it is likely that the distribution or mobilization of Mn, Cu, and Zn from the vegetative tissue to the grain did not keep up with the distribution or translocation of photosynthates in maize grown with faba bean, chickpea, and soybean. Research has shown that translocation or remobilization of micronutrients from vegetative tissues to the grain may play an important role in determining the concentrations of micronutrients in maize grain. For example, under high N and Zn application rates, about 60% of Zn and 40% of Fe initially stored in vegetative parts were remobilized to wheat grain. A high N application rate contributed to uptake and remobilization of both Zn and Fe from wheat vegetative tissues under greenhouse conditions with sufficient irrigation and Zn supply [19]. Also, enhanced N supply increased grain Zn and Fe concentrations under field conditions [20]. There are several mechanisms that underlie the micronutrient 'dilution' effect. Because N and Zn are mainly located in the outer layers of maize kernels, a higher proportion of endosperm to total grain weight explains the yield 'dilution' effect of grain weight [21]. In maize, the number of grains also contributes to yield 'dilution' effects. This is likely because a larger number of grains depend on the development of a larger proportion of distal grains, which typically have lower levels of minerals such as Zn [22]. In a previous study, grain weight and grain number showed significant negative linear correlations with grain Zn concentration [18]. Our results suggest that the decreased concentrations of Mn, Cu, Zn, and Fe in maize grain resulted from reduced translocation or remobilization of these micronutrients from straw to grain. This explanation is more consistent with our results than is the 'dilution' effect caused by higher grain yield.

Senescence induced Fe mobilization in source leaves of barley (*Hordeum vulgare*) [23]. The *NAC* gene product, which regulates senescence, was shown to increase grain protein, zinc, and iron concentrations in wheat, while a reduction in the transcript levels of multiple NAM homologs by RNA interference delayed senescence by more than three weeks and decreased concentrations of protein, Zn, and Fe in wheat grain by more than 30% [24]. Similarly, in our research, maize grain Fe, Mn, Cu, and Zn concentrations and their corresponding harvest indexes may be related to the initiation of senescence. At harvest, maize grown with faba bean, chickpea, and soybean had a larger portion of green leaves compared with that of monocropped and maize intercropped with turnip. The latter two maize crops reached senescence about one week earlier (Figure 8), and consequently showed higher grain Fe, Mn, Cu and Zn HIs than those of maize intercropped with legumes. This may explain the higher concentrations of Fe, Mn, Cu, and Zn in monocropped maize and maize intercropped with turnip, compared with those in maize intercropped with legumes. Consistent with our results, other studies also suggested that differences in micronutrient concentrations/bioavailability can be due to the environmental conditions from the flowering to maturity stages, differences in the duration of the post-physiological maturity period during which the grain remains on the plant in the field, differences in genotypes, and/or differences in the harvest time [25,26]. Although appropriate N application may delay senescence [27], it can increase the maize grain Fe concentration [20]. The results of our research should be further verified under different conditions; for example, under different harvest times and different cropping seasons. There were higher contents of

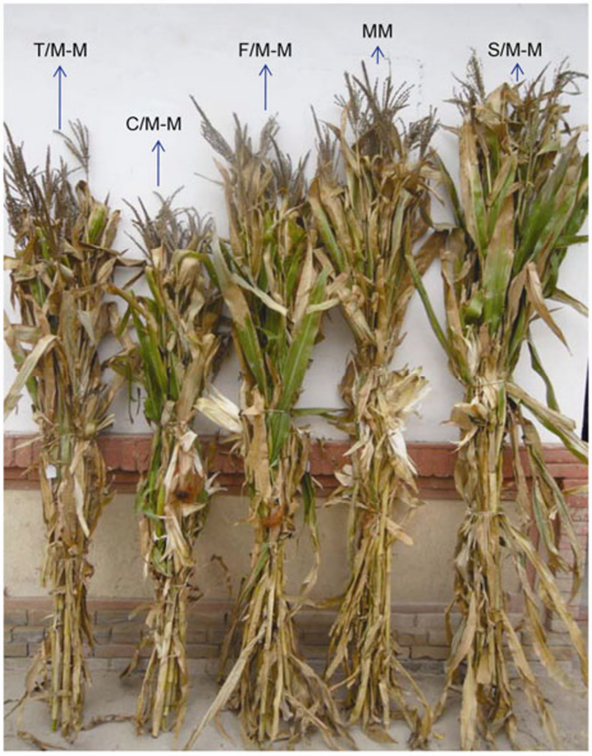

Figure 8 Harvested above-ground shoots of maize grown under field conditions. Compared with maize intercropped with turnip and monocropped maize, maize grown with faba bean, chickpea, and soybean matured later (visible as larger amounts of green leaves). MM, T/M-M, C/M-M, F/M-M, and S/M-M indicate monocropped maize and maize intercropped with turnip, chickpea, faba bean, and soybean, respectively.

Mn, Cu, Zn and Fe in the straw and lower translocation or remobilization ratios to grain in maize grown with legumes, compared with those in monocropped maize or maize intercropped with turnip. The mechanisms underlying these physiological differences in nutrient accumulation and translocation should be explored in future research.

3.4 Enhanced micronutrient acquisition by intercropped maize and its possible mechanisms

The concentrations of Fe, Mn, Cu, and Zn in grain of maize intercropped with faba bean, chickpea, and soybean were lower than those in grain of monocropped maize (Figure 2). However, the corresponding Fe, Mn, and Zn uptake by grain of intercropped maize with the legume species were not decreased, and even increased to some extent, when averaged over all P application rates (Figure 3). Maize intercropped with legumes showed higher contents of Fe, Mn, Cu, and Zn in above-ground shoots, compared with those in above-ground shoots of monocropped maize, when averaged over all P application rates (Figure 4). The Fe, Mn, Cu, and Zn contents in maize grain (Figure 3) and in the whole above-ground shoots of maize intercropped with turnip (Figure 4) were also higher than those in the respective tissues of monocropped maize, when averaged over all P application rates.

There are two main reasons that may explain the increased Fe, Mn, Cu, and Zn accumulation ability of intercropped maize. One is that intercropping can enhance secretion of acid phosphatase and organic acids from maize roots, and this may facilitate its absorption of soil micronutrients [4]. The other reason is that the longer life span and the larger space occupied by the roots of intercropped maize compared with those of monocropped maize may help intercropped maize acquire adequate micronutrients [28]. The concentrations of heavy metals, especially Cr, were lower in intercropped maize grain than in monocropped maize grain [7]. This was consistent with our results for Fe, Mn, Cu, and Zn (Figure 2). For phytoremediation of heavy metal-contaminated soils, maize intercropping is a practical strategy to obtain a crop that is safe for consumption [7]. For Fe, Mn, Cu, and Zn, intercropping of maize with faba bean, chickpea, and soybean may not be beneficial for 'biofortification' of maize grain. In future research, it would be useful to evaluate which parts of the maize grain contain decreased concentrations of Fe, Mn, Cu, and Zn, and whether the bioavailability and/or speciation of Fe, Mn, Cu, and Zn in maize grain is affected by intercropping.

In the present study, different dates for sowing and harvesting were specified for the different crops, monocropped maize and maize intercropped with turnip, faba bean, chickpea, and soybean were sown on the same date and also harvested on the same date. Different types and varieties of companion crops with different sowing and harvest dates, may affect the micronutrient nutrition of maize. For example, compared with the grain of monocropped maize, the grain of maize intercropped with legumes showed lower Fe, Mn, Cu, and Zn concentrations and lower corresponding harvest indexes, while those of maize intercropped with turnip were the same as those of monocropped maize.

It was reported that growing maize alongside legumes on neutral and alkaline soils could facilitate Fe uptake to correct Fe deficiencies and increase the concentration of Fe in legume seeds [6]. The aim of this study was to investigate the effects of intercropping with different companion crops on maize grain Fe, Mn, Cu and Zn concentrations and corresponding above-ground shoot acquisition; therefore, we did not analyze the micronutrient concentrations in turnip, faba bean, chickpea, and soybean plants. In future research, it would be useful to analyze micronutrients in the companion crops as well as in the main crop. This would give a better understanding of the effects of intercropping on micronutrient nutrition of the whole cropping system.

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1 Vandermeer J. The Ecology of Intercropping. New York: Cambridge University Press, 1989

2 Knoerzer H, Graeff-Hoenninger S, Guo B, et al. The rediscovery of intercropping in China: a traditional cropping system for future Chinese agriculture—a review. In: Lichtfouse E, ed. Climate Change, Intercropping, Pest Control and Beneficial Microorganisms. New York: Springer, 2009. 13–44

3 Hauggaard-Nielsen H, Jensen E S. Facilitative root interactions in intercrops. Plant Soil, 2005, 274: 237–250

4 Li L, Li S M, Sun J H, et al. Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc Natl Acad Sci USA, 2007, 104: 11192–11196

5 Römheld V, Marschner H. Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant Physiol, 1986, 80: 175–180

6 Zuo Y M, Zhang F S, Li X L, et al. Studies on the improvement in iron nutrition of peanut by intercropping with maize on a calcareous soil. Plant Soil, 2000, 220: 13–25

7 An L Y, Pan Y H, Wang Z B, et al. Heavy metal absorption status of five plant species in monoculture and intercropping. Plant Soil, 2011, 345: 237–245

8 White P J, Broadley M R. Biofortifying crops with essential mineral elements. Trends Plant Sci, 2005, 10: 586–593

9 Pixley K V, Palacios-Rojas N, Glahn R P. The usefulness of iron bioavailability as a target trait for breeding maize (*Zea mays* L.) with enhanced nutritional value. Field Crop Res, 2011, 123: 153–160

10 Feil B, Moser S B, Jampatong S, et al. Mineral composition of the grains of tropical maize varieties as affected by pre-anthesis drought and rate of nitrogen fertilization. Crop Sci, 2005, 45: 516–523

11 Bouis H E, Hotz C, McClafferty B, et al. Biofortification: a new tool to reduce micronutrient malnutrition. Food Nutr Bull, 2011, 32: S31–S40

12 Bruns H A, Ebelhar M W. Nutrient uptake of maize affected by nitrogen and potassium fertility in a humid subtropical environment. Commun Soil Sci Plant Anal, 2006, 37: 275–293

13 Li H, Huang G, Meng Q, et al. Integrated soil and plant phosphorus management for crop and environment in China. Plant Soil, 2011, 349: 157–167

14 Cordell D, Drangert J O, White S. The story of phosphorus: global food security and food for thought. Glob Environ Change, 2009, 19: 292–305

15 Stukenholtz D D, Olsen R J, Gogan G, et al. On the mechanism of phosphorus-zinc interaction in corn nutrition. Soil Sci Soc Am Proc, 1966, 30: 759–763

16 Ryan M H, McInerney J K, Record I R, et al. Zinc bioavailability in wheat grain in relation to phosphorus fertiliser, crop sequence and mycorrhizal fungi. J Sci Food Agric, 2008, 88: 1208–1216

17 Fan M S, Zhao F J, Fairweather-Tait S J, et al. Evidence of decreasing mineral density in wheat grain over the last 160 years. J Trace Elem Med Bio, 2008, 22: 315–324

18 Pleijel H, Danielsson H. Yield dilution of grain Zn in wheat grown in open-top chamber experiments with elevated CO_2 and O_3 exposure. J Cereal Sci, 2009, 50: 278–282

19 Kutman U, Yildiz B, Cakmak I. Effect of nitrogen on uptake, remobilization and partitioning of zinc and iron throughout the development of durum wheat. Plant Soil, 2011, 342: 149–164

20 Cakmak I, Kalayci M, Kaya Y, et al. Biofortification and localization of zinc in wheat grain. J Agric Food Chem, 2010, 58: 9092–9102

21 Peterson C J, Johnson V A, Mattern P J. Evaluation of variation in mineral element concentrations in wheat-flour and bran of different cultivars. Cereal Chem, 1983, 60: 450–455

22 Calderini D F, Ortiz-Monasterio I. Grain position affects grain macronutrient and micronutrient concentrations in wheat. Crop Sci, 2003, 43: 141–151

23 Shi R L, Weber G, Koster J, et al. Senescence-induced iron mobilization in source leaves of barley (Hordeum vulgare) plants. New Phytol, 2012, 195: 372–383

24 Uauy C, Distelfeld A, Fahima T, et al. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. Science, 2006, 314: 1298–1300

25 Oikeh S O, Menkir A, Maziya-Dixon B, et al. Genotypic differences in concentration and bioavailability of kernel-iron in tropical maize varieties grown under field conditions. J Plant Nutr, 2003, 26: 2307–2319

26 Oikeh S O, Menkir A, Maziya-Dixon B, et al. Environmental stability of iron and zinc concentrations in grain of elite early-maturing tropical maize genotypes grown under field conditions. J Agric Sci, 2004, 142: 543–551

27 He P, Jin J, Lin B. Effect of nitrogen application rates on leaf senescence and its mechanism in spring maize (*Zea mays* L.) (in Chinese). Sci Agric Sin, 1998, 31: 66–71

28 Li L, Sun J H, Zhang F S. Intercropping with wheat leads to greater root weight density and larger below-ground space of irrigated maize at late growth stages. Soil Sci Plant Nutr, 2011, 57: 61–67