Accumulation, partitioning, and bioavailability of micronutrients in summer maize as affected by phosphorus supply

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ABSTRACT

Decreased micronutrient concentration in cereal grains caused by excessive application of phosphorus (P) fertilizer may contribute to reduce their nutritional quality. To help correct this problem in maize grain, a 3-year field experiment was conducted to determine how P application rate affects micronutrient partitioning in maize shoots and other plant organs and micronutrient bioavailability in grain. Phosphorus application significantly decreased shoot zinc (Zn) and copper (Cu) concentrations at all growth stages but had no effects on shoot iron (Fe) and manganese (Mn) concentrations. As the P application rate increased, shoot Zn and Cu contents decreased, and shoot Fe and Mn contents increased. The ratios of pre-anthesis to post-anthesis mineral contents were not affected by P application rate except Zn. P application increased the percentage of Zn that was allocated to grain and decreased the percentage that was allocated to other tissues, but had no effects on the allocation of other micronutrients among tissues. The bioavailability of Zn, Cu, Fe, and Mn in grain decreased as P application rate increased. Overall, taking account of grain yield and nutrients concentration, P fertilizer rates should range from 12.5 to 25.0 kg P ha⁻¹ under the local condition. It can be concluded that not only grain yields, but also nutritional quality, should be considered in assessing optimal P rates in maize.

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1. Introduction

Maize (*Zea mays* L.) is one of the world's leading cereal grains and is very popular because of its diverse functionality as a food for both humans and animals (Nuss and Tanumihardjo, 2010). Maize is also a dietary staple for more than 200 million people and it provides around 15% of the protein and 20% of the calories in the world food diet (Brown et al., 1988). As a result, nutrient-biofortification of maize is attracting increasing attention from researchers (Xue et al., 2014). According to recent publications, the target concentrations of zinc (Zn) and iron (Fe) in biofortified maize are 38 mg kg⁻¹ and 60 mg kg⁻¹, respectively (Bouis and Welch, 2010), but the concentrations of Zn and Fe in global maize grain are only about 22.0 mg kg⁻¹ and 21.0 mg kg⁻¹, respectively (Gibson, 2012; Welch, 2002).

In the intensive cultivation of high-yielding crop cultivars, at least 30–50% of the crop yield is attributable to the application of commercial fertilizers (Stewart et al., 2005). Although phosphorus (P) fertilizer can substantially increase maize grain yield (Banaj

et al., 2006), excessive P fertilizer typically does not result in additional yield increases (Bai et al., 2013) but can lead to environmental pollution (Guo et al., 2010; Le et al., 2010) and deficiencies in Zn, copper (Cu), and Fe of maize (Cakmak, 2002; Stein, 2010). In China, for example, approximately 40% of the soils are Zn and Fe deficient, and about 30% are manganese (Mn) and Cu deficient (Liu, 1991). P application can reduce micronutrient concentrations and especially Zn and Cu concentrations in wheat grain (Zhang et al., 2012). It is therefore important to clarify how the micronutrient status in maize grain and stover is affected by P application in the field.

Many studies have reported how the accumulation of minerals in maize shoots is related to maize yields (Bender et al., 2013; Karlen et al., 1988). For instance, maize that yielded 12.0 Mg of grain ha⁻¹ accumulated 49.8 kg of P, 0.1 kg of Cu, 1.4 kg of Fe, 0.5 kg of Mn, and 0.5 kg of Zn ha⁻¹ (Bender et al., 2013). If the yield increased to 19.3 Mg ha⁻¹, the accumulation of P, Cu, Fe, Mn, and Zn at physiological maturity increased to 69.9, 0.14, 1.9, 0.9, and 0.8 kg ha⁻¹, respectively (Karlen et al., 1988). Furthermore, Xue et al. (2014), who evaluated shoot micronutrient accumulation at different growth stages under different yield and nitrogen levels, found that the reciprocal internal efficiencies (g of micronutrient requirement in plant dry matter per Mg of grain) of Fe, Mn, and Cu were not greatly affected by the yield and N levels. Although researchers have

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Fig. 1. The precipitation and mean temperature of month in the three years: 2012 (A), 2013 (B), and 2014 (C). The bars indicate the precipitation and the lines indicate mean temperature of month.

reported that high rates of P application can decrease Zn and Cu accumulation in wheat (Kizilgoz and Sakin, 2010), little information is available about how the dynamics of micronutrient accumulation (such as Zn, Fe, Mn, and Cu) in maize are affected by P application rate. Researchers have also reported that the quantity of minerals accumulated before anthesis determined the quantity that is remobilized into grain of wheat (Stomph et al., 2009). Pearson and Rengel (1994) indicated that a high accumulation of available Zn pre-anthesis is the main source for the Zn that is retranslocated to grain. In maize, however, the effect of P application on Zn accumulation pre- and post- anthesis is unclear. Thus, the effect of P fertilizer supply on the accumulation of Zn and other micronutrients pre- and post- anthesis should be evaluated in maize.

Minerals in plants exhibit different degrees of mobility among tissues, especially during the grain formation stage (Hegelund et al., 2012). While P and Zn are highly mobile and begin to translocate to maize grain at the R2 growth stage, Mn, Cu, and Fe have limited mobility (Bender et al., 2013). A previous study found that large proportions of the N and P and a small proportion of K were translocated from other plant parts to developing grain (Hanway, 1962). In another study, about 60% of the Zn, 30% of the Cu, 20% of the Fe, and 15% of the Mn originally in maize shoots had accumulated in grain at maturity (Bender et al., 2013). Potential effects of P application levels on P and micronutrient partition among shoot tissues (i.e., among bract, rachis, grain, stem, and leaf) of maize under field conditions has not been studied. In addition, micronutrient bioavailability has been found to be negatively affected by P application. For instance, Zhang et al. (2012), who used the molar ratio of P to Zn to indicate Zn bioavailability, reported that the increase of P fertilizer rates increased the molar ratio of P to Zn and therefore decreased Zn bioavailability in wheat grain.

The aims of this study were to determine how P application rate affects (1) the temporal dynamics of micronutrient (Zn, Fe, Mn, and Cu) accumulation in maize, (2) the partitioning of P and micronutrients among different maize tissues pre- and post-anthesis, and (3) the micronutrient bioavailability in grain.

2. Materials and methods

2.1. Field location

A long-term field experiment with maize was conducted at the Quzhou Experiment Station (36.9°N, 115.0°E) in Hebei Province of China to determine the effect of P fertilizer application on grain yield and nutrients in grain and straw. The experiment was begun

in 2008, and the same plots, treatments, and crops were conducted from 2008 to 2014. In this report, all the data of biomass, yield, and nutrients in grain and straw were collected from 2012 to 2014 cropping years. At the beginning of the experiment, the initial soil Olsen-P concentration in 2008 was 7 mg kg^{-1} (0.5 M NaHCO_3 -extractable). The average soil DTPA (Diethylene Triamine Pentacetic Acid)-extractable Zn, Cu, Fe, and Mn concentrations before sowing were 0.40, 0.88, 8, and 13 mg kg^{-1} , respectively. The soil micronutrient concentrations were low mainly because the soil was a calcareous alluvial soil with a high pH (8.0, 1:2.5 w/v in water). The soil texture in the location was loam with 140 g kg^{-1} of sand (2–0.05 mm), 605 g kg^{-1} of silt (0.05–0.002 mm), and 255 g kg^{-1} of clay (<0.002 mm) on basis of the American soil particle-size classification standard. Meanwhile, the soil CaCO_3 and organic matter content were 65 g kg^{-1} and 10.3 g kg^{-1} , respectively. The monthly precipitation and mean temperature from a meteorological station installed the Experiment Station are presented in Fig. 1 among the three years.

2.2. Experimental design

Six P application rates, which were applied before sowing in each cropping year, were included in the experiment: 0, 12.5, 25, 50, 100, and 200 kg P ha^{-1} (recorded as P0, P12.5, P25, P50, P100, and P200, respectively). Each treatment was represented by four replicate plots, and each plot was 75 m^2 ($7.5 \text{ m} \times 10 \text{ m}$). The plots were arranged in a randomized block design. The summer maize (*Zea mays* L.) cultivar was 'Nongda 108' in 2012 and 2013 and 'Zhengdan 958' in 2014, and the maize density was $67,000 \text{ plants ha}^{-1}$. Before sowing, $75 \text{ kg nitrogen (N) ha}^{-1}$ as urea (46%N), $60 \text{ kg K}_2\text{O ha}^{-1}$ as K sulphate, and P fertilizer as calcium superphosphate (7% P content: 0, 179, 358, 716, 1432, and 2864 kg ha^{-1} , respectively) were applied. Another 150 kg N ha^{-1} as urea (46%N) was supplied at the 6-leaf (V6) stage. The $700 \text{ m}^3 \text{ ha}^{-1}$ irrigations were carried out after sowing and at V6 stage of maize for the three cropping years. Weeds and pests were controlled by standard practices including pre-emergence herbicide (sprays before emergence of seedlings) and pesticide (sprays at 12-leaf stage) to control weeds and aphids. No water, weed, or pest problems were observed during the experiment. In 2012, 2013, and 2014, the maize seeds were sown on June 12, June 15 and June 12, respectively and the grain was harvested on October 5, October 4, and October 2, respectively.

2.3. Sample collection and nutrient analysis

Shoot samples of summer maize were collected at the 12-leaf (V12) stage, tasseling stage (VT), and physiological maturity stage (R6). At each of these stages, four plants were sampled. To reduce sampling variation, the four maize plants were collected from two adjacent rows with uniform growth height and growth stages which can be the representative of the whole plot. At the R6 stage, the shoot was divided into stem, leaf, bract, rachis, and grain. To evaluate the maize grain yield, all maize ears in a 7.2-m² (3 m × 2.4 m) area in the center of each plot were harvested. In the study, the grain yield was expressed on a 14.5% humidity basis. In this report, the term “shoot” refers to all aboveground parts of maize plants including the stover and grain. All samples were rapidly washed with tap water and then with deionized water before they were dried at 60–65 °C in the oven around 72 h to constant weight. Then all four plants samples were weighed after the grain was threshed and the harvest index was calculated as grain dry weight divided by total shoot dry weight. All plant samples were ground with a stainless steel grinder for nutrient analysis.

At maturity stage, soil samples were collected to measure the soil Olsen-P concentration and DTPA-Fe, Mn, Cu, and Zn concentrations in the three years. In each plot, six subsamples of soil were obtained from a 20 cm depth using a stainless steel auger then composited into a single sample. To reduce the soil sample variation, the sampling site was following an ‘S’ route. Sampling points homogeneously distributed in the plot, while, the soil from field ridge was avoided. All the soils from each plot were air-dried and crushed to pass a 1 mm sieve prior to analysis. The soil Olsen-P concentration was measured by the molybdovanado phosphate method with 0.5 mol L⁻¹ NaHCO₃ at pH 8.5 (180 rpm, 25 °C). The DTPA-Fe, Mn, Cu, and Zn concentrations were measured (0.005 mol L⁻¹ DTPA-extractable) according to the method described by Lindsay and Norvell (1978). Then nutrients in the extracted solution were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, OPTIMA 3300 DV, Perkin-Elmer, USA).

The plant samples were digested with HNO₃–H₂O₂ in a microwave-accelerated reaction system (CEM, Matthews, NC, USA). The P, Zn, Cu, Fe, and Mn concentrations in the digested solutions were determined by ICP-OES.

2.4. Statistical analysis

Two-way repeated measures analysis of variance (ANOVA) *F*-test by SPSS 18.0 was used to assess the effect of P application rate, year, and their interactions on the dependent variables. Before analysis, data normality and variance homogeneity were checked by SPSS ($P > 0.05$). When an ANOVA was significant, treatments were compared by Duncan's multiple comparisons test at the 0.05 probability level ($P < 0.05$). The linear-plateau program of SAS 8.0 was used to quantitatively evaluate the effect of P application on grain yield ($P < 0.05$).

The molar ratios of P to micronutrients were used to estimate the effect of P application on micronutrient bioavailability. As before, repeated measures ANOVAs were used to assess the effect of P application rate, year, and interactions on these molar ratios, and means were compared with Duncan's multiple comparisons test. Molar ratios in the grain and stover were calculated as molar mass of P divided by molar mass of micronutrients.

3. Results

3.1. Grain yield, harvest index (HI), and shoot biomass

The linear-plateau model showed that 17, 22, and 19 kg P ha⁻¹ (estimated values but not real fertilizer rates tested) in 2012, 2013, and 2014 were the threshold fertilizer rates for grain yield. High P application resulted in no increases of grain yield (Fig. 2A). Phosphorus application did not significantly affect the harvest index (HI) (Fig. 2B). Below a P fertilizer rate, shoot biomass at all growth stages increased with increasing P rates ($P < 0.05$) (Fig. 3A–C). For instance, in 2012 shoot biomass significantly increased with P application from 0 to 12.5 kg ha⁻¹ at V12 and R6 stages then plateaued, whereas the plateau at VT stage appeared at 25 kg ha⁻¹ P level. The plateau for shoot biomass also appeared at 25 kg ha⁻¹ P rate in 2013 and 2014 at all growth stages, while it was at 50 kg ha⁻¹ P rate at VT and R6 stages in 2013.

3.2. Shoot P, Zn, Cu, Fe, and Mn concentrations and contents at different growth stages as affected by P application rate

P application significantly increased the P concentration in shoots at all three growth stages (Fig. 4A–C). At the V12 stage in 2012, the P concentration in shoots increased with P application from 0 to 25 kg ha⁻¹ and higher P rates did not further enhance shoot P concentration; at the V12 stage in 2013 and 2014, the P concentration in shoots increased as the P application rate increased from 0 to 50 kg ha⁻¹. At the VT stage in the three cropping seasons, the P concentration in shoots increased as the P application rate increased from 0 to 50 kg ha⁻¹. At maturity, the P concentration in shoots continued to increase in response to increasing P application rates (Fig. 4A–C).

Shoot Zn, Cu, Fe, and Mn concentrations were higher at the V12 stage than at the VT and R6 stages irrespective of P application rate (Fig. 4D–O). Shoot Zn and Cu concentrations substantially decreased as the P application rate increased from 0 to 200 kg ha⁻¹ at all growth stages (Fig. 4D–I). P application had no significant effects on shoot Fe and Mn concentrations at all growth stages (Fig. 4J–O). Shoot P, Zn, Cu, Fe, and Mn contents were greater in the R6 stage than in other stages irrespective of P rate (Fig. 5A–O). The P content at all growth stages increased greatly as the P application rate increased from 0 to 50 kg ha⁻¹ (the inflection point appeared at 100 kg ha⁻¹ at maturity in 2014) but was no more increased by higher application rates (Fig. 5A–C). Shoot Zn and Cu contents decreased with increasing P rates (Fig. 5D–I), while shoot Fe and Mn contents increased with increasing P rates (Fig. 5J–O).

3.3. Concentrations and partitioning of Zn, Cu, Fe, and Mn in different tissues of maize at maturity as affected by P application rate

The concentrations of Zn, Cu, Fe, and Mn in maize tissues varied depending on the P application rates, years and their interaction (Table 1). Zinc and Cu concentrations in all tissues significantly decreased with P application rates. The year also had significantly effect on Zn and Cu concentrations in most tissues, while the effect of interaction of P application × year was not significant. The response of Fe concentration in rachis to P application varied with year (*P* value of interaction = 0.000). Iron concentrations in stem and leaf increased with P application, meanwhile it was not affected in bracts and grain. The Mn concentrations in all tissues were affected by years, while only in bract and grain were significantly increased by P application rates (Table 1).

The partitioning of Zn, Cu, and Mn was affected by P application rate and year, while the P application × year interaction had effect on Fe partitioning (Table 2). Phosphorus application significantly

Table 1
Concentrations of Zn, Cu, Fe, and Mn in tissues of summer maize as affected by P application rate on calcareous soil.

Cropping years	P rates (kg P/ha)	Micronutrient concentration (mg kg ⁻¹)																			
		Zn					Cu					Fe					Mn				
		Stem	Leaf	Bract	Rachis	Grain	Stem	Leaf	Bract	Rachis	Grain	Stem	Leaf	Bract	Rachis	Grain	Stem	Leaf	Bract	Rachis	Grain
2012	0	7.4 a	26.9 a	5.7 a	4.8 a	19.2 a	6.2 a	14.6 a	4.3 a	1.2 a	1.8 a	22.8 b	164 b	56 a	17 c	15.0 ab	6.7 ab	75 a	14.1 c	3.5 a	4.2 ab
	12.5	5.6 ab	18.8 b	3.6 b	4.8 a	16.4 b	5.8 ab	13.5 a	4.0 ab	1.0 ab	1.5 b	23.5 b	184 ab	58 a	17 c	15.0 ab	6.4 b	67 a	17.1 a	3.3 a	4.0 b
	25	5.2 b	15.9 c	4.3 b	3.8 a	14.2 c	5.3 b	11.5 b	3.8 ab	0.8 bc	1.5 b	23.6 b	185 ab	58 a	19 bc	14.2 b	7.9 a	72 a	16.7 ab	2.9 a	3.9 b
	50	3.7 bc	13.8 cd	2.4 c	2.3 b	14.8 bc	4.5 c	11.6 b	3.6 b	1.0 ab	1.5 b	25.2 a	187 ab	62 a	21 ab	15.5 a	6.0 b	75 a	16.5 ab	3.1 a	4.0 b
	100	3.6 bc	12.5 d	1.8 c	2.0 b	13.5 c	4.5 c	11.4 b	3.5 b	0.8 bc	1.2 c	25.2 a	186 ab	60 a	20 ab	14.3 ab	6.3 b	76 a	17.5 a	2.9 a	4.1 ab
	200	2.3 c	13.6 cd	1.8 c	2.0 b	13.7 c	3.5 d	11.1 b	3.4 b	0.5 c	1.2 c	24.8 a	202 a	63 a	23 a	14.5 ab	6.0 b	76 a	15.3 bc	3.0 a	4.4 a
2013	0	11.7 a	29.9 a	10.2 a	7.7 a	22.2 a	6.6 a	14.3 a	5.0 a	2.4 a	1.9 a	33.3 c	207 b	76 a	18 b	18.5 ab	9.5 a	105 ab	23.5 bc	3.6 a	5.8 b
	12.5	7.2 b	21.7 b	7.8 b	7.8 a	21.0 ab	5.9 a	12.4 b	4.5 abc	2.2 a	1.5 b	35.6 c	226 a	76 a	25 a	19.4 a	8.0 a	97 b	22.5 c	3.9 a	6.1 ab
	25	6.4 bc	19.1 c	6.4 bc	5.2 b	20.3 ab	5.2 b	12.6 b	4.6 ab	2.1 ab	1.4 bc	40.8 b	236 a	66 a	26 a	17.0 bc	9.0 a	103 ab	24.7 abc	3.9 a	6.5 a
	50	6.0 bcd	18.3 cd	5.9 bc	3.9 b	18.9 bc	4.8 b	12.6 b	4.2 bcd	1.9 b	1.2 cd	42.7 ab	232 a	74 a	25 a	15.8 c	8.5 a	107 ab	26.4 a	4.0 a	6.2 ab
	100	5.0 cd	16.7 d	5.2 c	3.7 b	17.7 c	4.0 c	11.9 b	3.9 cd	1.6 c	1.1 d	44.5 a	238 a	78 a	26 a	18.5 ab	9.5 a	117 a	27.4 a	3.9 a	6.2 ab
	200	4.3 d	14.5 e	4.4 c	3.1 b	17.4 c	3.7 c	10.4 c	3.6 d	1.2 d	1.0 d	41.7 ab	240 a	74 a	25 a	17.3 ab	8.4 a	108 ab	25.7ab	4.1 a	6.2 ab
2014	0	7.9 a	14.6 a	7.9 a	8.0 a	18.3 a	5.5 ab	12.2 a	4.6 a	2.5 a	2.0 a	32.6 d	323 b	61 a	25 b	20.2 a	6.5 a	88 ab	18.8 d	3.3 b	4.1 c
	12.5	5.2 b	9.8 b	5.7 b	5.1 b	15.7 b	5.8 a	9.4 b	4.0 b	2.5 a	1.9 ab	32.6 d	337 a	64 a	28 a	20.5 a	5.2 ab	72 c	19.8 cd	3.9 a	4.6 b
	25	4.7 bc	9.7 b	5.4 bc	4.0 bc	15.3 b	5.4 ab	9.6 b	4.1 b	2.3 a	1.9 ab	34.6bcd	346 a	60 a	28 a	20.5 a	6.3 ab	81 bc	20.7 bc	3.6 ab	4.6 b
	50	4.4 bc	8.8 b	4.2 cd	3.1 cd	13.0 c	4.5 bc	8.8 bc	3.9 b	2.4 a	1.8 bc	35.5 ab	343 a	61 a	29 a	19.5 a	6.2 ab	93 a	21.4 ab	3.5 b	4.4 bc
	100	4.3 bc	8.1 b	3.5 d	2.0 d	13.1 c	4.1 c	7.6 cd	3.8 bc	1.8 b	1.7 cd	37.7 a	340 a	63 a	29 a	18.8 a	5.1 b	75 c	22.5 a	3.5 b	4.6 b
	200	3.6 c	7.9 b	3.1 d	1.9 d	12.8 c	3.8 c	6.5 d	3.5 c	1.5 b	1.5 d	34.9 bc	339 a	61 a	27 a	19.4 a	5.4 ab	79 bc	20.5 bc	3.5 b	5.2 a
Source of variation																					
P application (P)		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.136	0.000	0.133	0.056	0.054	0.000	0.545	0.001
Year (Y)		0.001	0.000	0.000	0.000	0.000	0.718	0.000	0.01	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P*Y		0.071	0.103	0.783	0.178	0.280	0.286	0.053	0.352	0.101	0.061	0.053	0.941	0.062	0.000	0.075	0.366	0.102	0.143	0.186	0.140

Values represent the mean of four replicates. Within a column, different letters denote significant difference at $P < 0.05$ by Duncan's multiple comparison test.

Table 2

Distribution of Zn, Cu, Fe, and Mn in tissues of summer maize as affected by P application rate on calcareous soil.

Cropping years	P rates (kg P/ha)	Distribution of micronutrient (%)																			
		Zn					Cu					Fe					Mn				
		Stem	Leaf	Bract	Rachis	Grain	Stem	Leaf	Bract	Rachis	Grain	Stem	Leaf	Bract	Rachis	Grain	Stem	Leaf	Bract	Rachis	Grain
2012	0	7.3 a	29.6 a	2.1 a	2.2 ab	58.9 b	20.6 a	54.3 ab	5.4 ab	1.8 ab	17.9 bc	8.3 ab	66 ab	5.4 b	2.9 ab	17.0 a	6.1 ab	75.6 a	4.9 b	1.5 a	12.0 a
	12.5	6.5 a	26.9 ab	1.7 ab	2.8 a	62.2 b	19.9 a	56.7 a	4.7 b	1.7 ab	16.9 c	7.3 b	70 a	5.0 b	2.7 b	15.2abc	5.8 ab	74.7 a	6.1 a	1.5 a	11.9 a
	25	6.9 a	25.5 abc	2.2 a	2.5 ab	62.9 b	20.5 a	53.8 ab	5.7 a	1.5 ab	18.5abc	7.4 b	68 ab	6.9 a	3.0 ab	14.7 bc	6.7 a	75.3 a	5.5 ab	1.2 a	11.2 a
	50	5.5 ab	21.6 c	1.3 bc	1.7 bc	69.9 a	19.6 a	51.6 b	5.7 a	2.2 a	20.8 a	8.5 ab	65 b	6.8 a	3.5 a	16.6 ab	5.8 ab	74.7 a	5.8 a	1.5 a	12.2 a
	100	6.4 a	22.6 bc	1.0 c	1.5 c	68.6 a	20.6 a	53.0 ab	6.0 a	1.6 ab	18.9abc	8.9 a	67 ab	6.9 a	2.9 ab	14.6 bc	6.2 ab	75.4 a	5.6 ab	1.2 a	11.7 a
	200	3.7 b	24.8 abc	1.0 c	1.5 c	69.0 a	15.9 a	57.1 a	5.6 a	1.2 b	20.3 ab	7.5 b	69 ab	6.8 a	3.5 a	13.6 c	5.1 b	76.3 a	4.9 b	1.3 a	12.3 a
2013	0	9.2 a	27.7 a	4.0 a	3.9 ab	55.2 d	19.7 a	50.2 a	7.4 a	4.5 a	18.2 a	8.7 bc	63 ab	10.0 a	3.0 b	15.4 ab	5.8 a	74.7 a	7.1 a	1.4 a	11.1 b
	12.5	6.0 b	22.2 b	3.4 ab	4.4 a	64.0 c	19.3 a	49.8 a	7.9 a	4.7 a	18.4 a	8.1 c	63 ab	9.4 a	3.6 ab	16.1 a	4.8 a	72.6 ab	7.4 a	1.5 a	13.6 a
	25	6.2 b	21.0 bc	2.9 b	3.2 ab	66.7 bc	18.8 ab	52.0 a	8.0 a	4.7 a	16.6 a	9.9 ab	65 a	7.7 a	3.8 a	14.0 b	5.6 a	72.1 ab	7.3 a	1.5 a	13.6 a
	50	5.8 b	20.6 bc	2.9 b	2.4 b	68.4 ab	17.4 ab	54.2 a	7.9 a	4.3 ab	16.2 a	10.1 ab	64 ab	8.9 a	3.6 ab	13.9 b	5.0 a	72.3 ab	7.8 a	1.5 a	13.6 a
	100	5.3 b	19.3 bc	3.0 b	2.7 ab	69.7 ab	16.3 b	53.6 a	8.5 a	4.6 a	17.0 a	10.4 a	60 b	9.7 a	4.1 a	15.8 ab	5.4 a	71.9 ab	8.3 a	1.5 a	13.0 a
	200	5.1 b	18.1 c	2.7 b	2.3 b	71.9 a	17.6 ab	52.3 a	8.8 a	3.7 b	17.7 a	10.1 ab	62 ab	9.3 a	3.8 a	14.9 ab	5.2 a	71.3 b	8.3 a	1.6 a	13.8 a
2014	0	7.5 b	21.8 a	5.1 a	4.5 a	61.1 b	19.6 a	46.9 a	9.0 a	4.1 ab	20.4 a	8.5 a	66.7 c	5.2 a	3.1 a	16.5 ab	5.2 a	71.4 c	9.6 a	1.5 a	12.3 b
	12.5	10.1 a	19.8 b	3.8 b	2.9 b	63.4 b	20.1 a	45.1 a	8.1 b	4.3 a	22.4 a	8.7 a	66.2 b	5.4 a	2.4 b	17.3 a	4.2 ab	72.1 c	9.2 a	1.6 a	12.9ab
	25	5.9 b	18.9 b	3.6 bc	2.4 b	69.2 a	19.8 a	47.7 a	7.9 b	4.0 ab	20.6 a	8.1 a	67.7 ab	5.5 a	2.3 bc	16.4 ab	4.7 ab	72.9 bc	8.1 ab	1.3 b	13.0 ab
	50	7.2 b	18.4 b	3.3 bcd	2.2 b	68.9 a	19.3 a	47.7 a	8.2 b	4.5 a	20.3 a	8.7 a	68.1 ab	5.5 a	2.5 b	15.2 b	4.5 ab	74.4 a	7.4 b	1.1 b	12.6 ab
	100	6.4 b	16.6 c	2.6 cd	1.4 c	73.0 a	17.8 a	47.6 a	8.3 b	3.7 b	22.6 a	8.3 a	69.1 a	5.1 a	2.3 bc	15.2 b	3.8 b	73.2 b	8.4 ab	1.3 b	13.3 ab
	200	5.7 b	17.9 bc	2.4 d	1.4 c	72.6 a	19.9 a	47.5 a	8.0 b	4.3 a	20.3 a	8.2 a	68.2 ab	5.5 a	2.0 c	16.1 ab	4.1 ab	73.2 b	7.7 b	1.4 b	13.6 a
Source of variation																					
P application (P)		0.000	0.004	0.000	0.000	0.000	0.074	0.642	0.357	0.054	0.886	0.051	0.055	0.637	0.263	0.072	0.090	0.431	0.096	0.156	0.075
Year (Y)		0.580	0.000	0.000	0.001	0.000	0.245	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.000	0.005	0.031
P*Y		0.055	0.061	0.054	0.111	0.065	0.057	0.260	0.058	0.266	0.110	0.000	0.007	0.000	0.302	0.054	0.827	0.079	0.098	0.128	0.056

Values represent the mean of four replicates. Within a column, different letters denote significant difference at $P < 0.05$ by Duncan's multiple comparison test.

Fig. 2. Grain yield (A) and harvest index (B) of summer maize as affected by P application rate in the three year. Values are means $\pm$ standard error ($n=4$).

Fig. 3. Shoot biomass of summer maize as affected by P application rate on a calcareous soil in (A) 2012, (B) 2013, and (C) 2014. Values are means $\pm$ standard error ($n=4$). Within each growth stage means followed by different lowercase letters are significantly different by Duncan's multiple comparison test ($P < 0.05$). The different capital letters indicate a significant difference between growth stage ($P < 0.05$).

increased Zn distribution in grain and decreased the distribution in other shoot tissues and the year also had a significant effect on Zn distribution. Phosphorus application had no effect on Cu distribution while the year had effect on Cu concentration in leaf, bract, rachis, and grain. The response of Fe concentration in stem, leaf, and bract to increasing P rates varied with year (P value of interaction < 0.05). Phosphorus application had no effect on Mn distribution while the year significantly affected Mn distribution.

3.4. Molar ratios of P to micronutrients in grain and stover

The molar ratios of P/Zn, P/Cu, P/Fe, and P/Mn in grain and stover of mature maize were significantly affected by P application rate and cropping year. Overall, grain and stover molar ratios of P/Zn, P/Cu, P/Fe, and P/Mn increased as the P application rate increased from 0 to 200 kg ha⁻¹ (Table 3).

3.5. Accumulation of P, Zn, Cu, Fe, and Mn pre- and post-anthesis

The percentages of the total quantity of minerals accumulated in maize differed pre- vs. post-anthesis (Table 4). Pre-anthesis accumulation accounted for $> 80\%$ of the total Cu and Mn, 50–70% of the total P, Zn, and Fe. Phosphorus application increased the ratio of

Zn accumulation but slightly decreased Fe accumulation ratio pre-anthesis. The ratio of Mn and Cu accumulations of pre-anthesis vs. post-anthesis was not affected by P application rates (Table 4).

3.6. Effect of P application on soil Olsen-P concentration and DTPA-Fe, -Mn, -Cu, and -Zn concentration

Phosphorus application significantly increased soil Olsen-P concentration ($P = 0.0012$). The soil DTPA-Fe, -Mn, and -Cu concentrations was not affected by P application rates while soil DTPA-Zn concentration was increased with P application from 0 to 200 kg ha⁻¹ (Fig. 6).

4. Discussion

4.1. Shoot biomass and yield

In agreement with previous report (Banaj et al., 2006; Kovačević et al., 2008), maize grain yield and shoot biomass were significantly increased with P application. It was reported that the critical soil available P concentration to reach the yield plateau was varied in different areas due to the maize cultivars, soil type, pH and soil organic matter content (Bai et al., 2013). In view of absolute value

Fig. 4. Mineral concentrations in the shoots of summer maize as affected by P application rate on a calcareous soil. P concentration in (A) 2012, (B) 2013 and (C) 2014; Zn concentration in (D) 2012, (E) 2013 and (F) 2014; Cu concentration in (G) 2012, (H) 2013 and (I) 2014; Fe concentration in (J) 2012, (K) 2013 and (L) 2014; and Mn concentration in (M) 2012, (N) 2013 and (O) 2014. Values are means + standard error ($n=4$). Within each growth stage means followed by different lowercase letters are significantly different by Duncan's multiple comparison test ($P<0.05$). The different capital letters indicate a significant difference between growth stage ($P<0.05$).

of shoot biomass accumulation, P application also had significant effect on biomass accumulation at early growth stage, indicating that a sufficient P supply at the early growth stage was equally important for maize growth. The grain yield and shoot biomass in 2013 was lower than that in 2012 and 2014, which could be

partly explained by differences in climate (precipitation and mean temperature) among the three years. In July of 2013, the higher precipitation accompanied with lower temperature may partly attributed to the decrease in shoot biomass among the stage from V12 to VT stage.

Fig. 5. Mineral contents in shoots of summer maize as affected by P application rate on a calcareous soil. P content in (A) 2012, (B) 2013 and (C) 2014; Zn content in (D) 2012, (E) 2013 and (F) 2014; Cu content in (G) 2012, (H) 2013 and (I) 2014; Fe content in (J) 2012, (K) 2013 and (L) 2014; and Mn content in (M) 2012, (N) 2013 and (O) 2014. Values are means + standard error ($n=4$). Within each growth stage means followed by different lowercase letters are significantly different by Duncan's multiple comparison test ($P<0.05$). The different capital letters indicate a significant difference between growth stage ($P<0.05$).

4.2. Shoot micronutrient concentration

As the maize plants grew, the concentrations of micronutrients in the shoots generally decreased but their contents increased. At each growth stage, however, the effects of P application rate on the

concentration and content of each mineral differed. As P application rate increased, the shoot P concentration substantially increased while the shoot Zn concentration significantly decreased. In previous studies of wheat, maize, soybean, and cotton, P application increased the concentration of P (Colomb et al., 2007) but decreased

Table 3

The effect of P application rate on the molar ratios of P/Zn, P/Cu, P/Fe, and P/Mn in grain and stover of field maize at maturity.

Cropping years	P rates (kg P/ha)	Molar ratio of phosphorus to micronutrient							
		Grain				Straw			
		P/Zn	P/Cu	P/Fe	P/Mn	P/Zn	P/Cu	P/Fe	P/Mn
2012	0	273 e	2990 d	298 bc	1050 b	92 f	153 d	14 cd	34 c
	12.5	303 de	3240 cd	287 c	1058 b	132 e	173 d	13 d	37 bc
	25	357 cd	3498 cd	308 bc	1109 ab	169 d	220 c	15 bcd	39 bc
	50	397 bc	3792 bc	322 b	1222 a	233 c	253 bc	16 abc	43 b
	100	442 ab	4128 ab	358 a	1221 a	265 b	262 b	17 ab	44 b
	200	456 a	4362 a	372 a	1199 a	308 a	335 a	18 a	51 a
2013	0	222 d	2559 e	230 d	719 c	75 e	153 e	11 d	25 d
	12.5	307 c	4105 d	283 c	888 ab	129 d	216 d	13 cd	33 c
	25	319 bc	4732 c	328 abc	851 b	174 c	256 c	14 c	36 bc
	50	360 ab	5695 b	373 a	923 ab	186 c	262 c	14 c	35 bc
	100	391 a	6156 ab	325 bc	945 a	251 b	348 b	17 b	39 b
	200	399 a	6479 a	343 ab	939 a	349 a	474 a	20 a	51 a
2014	0	185 c	1657 d	144 d	908 c	106 c	206 d	6 c	39 b
	12.5	286 b	2276 c	192 c	1071 b	217 b	267 c	7 c	47 b
	25	335 b	2740 bc	207 c	1221 a	232 b	247 cd	9 abc	46 b
	50	409 a	2953 b	264 a	1322 a	259 b	247 cd	8 bc	40 b
	100	402 a	3110 ab	274 a	1264 a	361 a	359 b	10 ab	58 a
	200	456 a	3702 a	242 ab	1245 a	413 a	422 a	11 a	65 a
Source of variation									
P application (P)		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Year (Y)		0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000
P*Y		0.162	0.090	0.145	0.052	0.061	0.102	0.178	0.128

Values represent the mean of four replicates. Within a column, different letters denote significant difference at $P < 0.05$ by Duncan's multiple comparison test.**Table 4**

Ratio of content at pre-anthesis vs. post-anthesis to total shoot content of P, Zn, Cu, Fe, and Mn of summer maize on a calcareous soil as affected by P application rate.

Cropping years	P rates (kg P/ha)	Ratio of minerals content at pre-anthesis and post-anthesis (%)									
		P		Zn		Cu		Fe		Mn	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
2012	0	56 b	44 a	49 b	51 a	95 a	5 a	71 a	29 a	88 a	12 a
	12.5	67 a	33 b	62 a	38 b	97 a	3 a	70 a	30 a	83 a	17 a
	25	65 a	35 b	62 a	38 b	99 a	1 a	68 a	32 a	83 a	17 a
	50	64 a	36 b	63 a	37 b	96 a	4 a	64 a	36 a	82 a	18 a
	100	66 a	34 b	65 a	35 b	96 a	4 a	66 a	34 a	84 a	16 a
	200	63 a	37 b	69 a	31 b	97 a	3 a	65 a	35 a	83 a	17 a
2013	0	66 a	34 a	35 b	65 a	92 a	8 a	51 a	49 b	77 a	23 a
	12.5	58 a	42 a	44 ab	56 ab	93 a	7 a	48 ab	52 ab	77 a	23 a
	25	60 a	40 a	44 ab	56 ab	88 a	12 a	46 ab	54 ab	72 a	28 a
	50	67 a	33 a	45 ab	55 b	99 a	1 a	46 ab	54 ab	72 a	28 a
	100	58 a	42 a	46 a	54 b	93 a	7 a	45 ab	55 ab	63 a	37 a
	200	58 a	42 a	47 a	53 b	96 a	4 a	41 a	59 a	68 a	32 a
2014	0	67 a	33 ab	52 b	48 a	90 a	10 a	74 a	26 b	88 a	12 a
	12.5	63 a	37 ab	65 a	35 b	86 a	14 a	55 b	45 a	89 a	11 a
	25	62 a	38 a	67 a	33 b	84 a	16 a	54 b	46 a	87 a	13 a
	50	67 a	33 ab	64 a	36 b	65 a	35 a	53 b	47 a	79 a	21 a
	100	71 a	29 b	68 a	32 b	92 a	8 a	52 b	48 a	82 a	18 a
	200	62 a	38 a	73 a	27 b	88 a	12 a	49 b	51 a	77 a	23 a
Source of variation											
P application (P)		0.275	0.275	0.000	0.000	0.641	0.641	0.000	0.000	0.174	0.174
Year (Y)		0.066	0.066	0.000	0.000	0.007	0.007	0.000	0.000	0.006	0.006
P*Y		0.240	0.240	0.962	0.962	0.210	0.210	0.250	0.250	0.919	0.919

Values represent the mean of four replicates. Within a column, different letters denote significant difference at $P < 0.05$ by Duncan's multiple comparison test.

the concentration of Zn in stover and grain (Izsáki, 2014; Zhang et al., 2012). At all growth stages in the current study, the Cu concentration in shoots had a similar response as Zn to P application rate. Similar results were reported by Zhang et al. (2012) in winter wheat and by Haldar and Mandal (1981) in rice for both shoots and roots. Researchers have often discussed possible explanations for decreased Zn and Cu concentrations with increased P rates. Shukla and Singh (1979) suggested that P application reduced Cu availability in soil because of the formation of $\text{Cu}_3(\text{PO}_4)_2$, which is insoluble.

In the current study, soil DTPA-Zn and DTPA-Cu were not decreased by P application (Fig. 6B), indicating that the current P supply did not affect Zn and Cu solubility in soil but may have affected Zn and Cu uptake at the root surface or movement through the plant after uptake (Goh et al., 1997). Another possible explanation is that P application often reduces the colonization of roots by arbuscular mycorrhizal fungi (AMF) (Ova et al., 2015). In previous studies, Zn and Cu concentrations in shoots were reduced when AMF were absent (Kucey and Janzen, 1987). The current study also indicated

Fig. 6. The effect of P application on mean soil Olsen-P concentration (A, $\pm$ standard error) and soil mean DTPA-Fe, -Mn, -Cu and -Zn concentration (B, $\pm$ standard error) at maturity. Values are the mean of three years. NS means not significant at $P > 0.05$. *** indicates a significant difference among treatments at $P < 0.0001$.

that the decrease in shoot Zn and Cu concentrations with increases in P application rate could not be explained by a “dilution effect” (in which concentrations decline as biomass increases), as suggested in a previous study (Zhu et al., 2002). This explanation was not supported because shoot biomass did not further increase but shoot Zn and Cu concentrations continued to decrease as the P application rate exceeded 12.5 kg P ha⁻¹. As noted earlier, P application may have decreased Zn uptake by roots or Zn translocation from roots to shoots.

Shoot Fe and Mn concentrations were not significantly affected by P application rates at all growth stages. This agrees with previous reports concerning Fe in wheat (Zhang et al., 2012) and Mn in barley (Zhu et al., 2002). Although an increase in soil P availability slightly increased the Mn concentration in barley roots, Zhu et al. (2002) suggested that an increase in P supply may reduce Mn transportation from roots to shoots.

4.3. Shoot micronutrient accumulation

As the maize plants grew in the current study, the shoots accumulated increasing quantities of P, Fe, Zn, Cu, and Mn irrespective of P application rate, but the amount accumulated (i.e., the content) at each growth stage differed depending on P application rate. Within each growth stage, P accumulation in shoots increased as the P application rate increased, but Cu and especially Zn accumulation in shoots decreased as the P application rate increased. The reduction in Zn accumulation in response to increases in P application differed from previous reports with wheat (Zhang et al., 2015). Zhang et al. (2015) found that Zn accumulation in wheat at the flowering and maturity stage increased as the P application rate increased from 0 to 50 kg P ha⁻¹ and then decreased as the application rate increased further. In the current study, in contrast, Zn accumulation in maize shoots decreased as the P application rate increased from 0 to 200 kg ha⁻¹ during all growth stages. Cu accumulation in maize shoots was only slightly affected by P application rates increased from 0 to 25 kg ha⁻¹ and then decreased at higher application rates. Differences between wheat and maize growing under similar environmental conditions may be ascribed to different nutrient requirements and different nutrient uptake efficiency. In this regard, maize is more tolerant to low P availability in soil than wheat (Postma and Lynch, 2011). This is ascribed at least in part to the stimulated root hair production (Ma et al., 2001) and

mycorrhizal colonization of roots (Hagh et al., 2016) under P deficient conditions, which also enhance Zn and Cu uptake. Decreased mycorrhizal colonization with increased P rates may result in an impaired Zn uptake by maize plants (Kothari et al., 1991). Our results indicate that the decline in Zn and Cu concentration is greater than the increase in shoot biomass with P application; thus it seems that there is an effect of P fertilization on the concentration of these nutrients which is not ascribed to a dilution effect.

Shoot Fe and Mn accumulation steadily increased with P application rate from 0 to 200 kg P ha⁻¹, which is consistent with a previous study of cowpea crop (Nyoki and Ndadidem, 2014). The increases of Fe and Mn accumulation in shoot were greatly ascribed to the increase in shoot biomass rather than nutrient concentration. The ratios of pre-anthesis to post-anthesis mineral content were not affected by P application rate, which agrees with previous results in wheat (Zhang et al., 2015). In addition the competition between ions may also affect the uptake and accumulation of micronutrients. It was reported that ZRT-, IRT-like protein (ZIPs) and yellow stripe-like proteins (YSLs) are responsible for root Zn uptake and that these transport proteins can simultaneously transport Fe, Cu, Mn (Curie et al., 2009). The decrease of Zn accumulation may be caused by the increase of Fe and Mn accumulation which competed the same absorption site (Zhang et al., 2012).

4.4. Micronutrient partitioning in maize tissues

At maturity, the concentrations of Cu, Fe, and Mn in leaves were higher than in other shoot tissues, while Zn concentrations in grain and leaves were higher than in other shoot tissues. These results agree with previous studies (Bender et al., 2013). Marschner (2012) indicated that Fe, Mn, Cu and Zn in plant were moderate phloem mobility. However, compared with Fe, Mn, and Cu, Zn remobilization from stem to grain is particularly critical for the accumulation of Zn in grain during reproductive growth in wheat (Kutman et al., 2012). For instance, it was reported that around 60–70% of the Zn content in grain was provided by Zn remobilization of pre-anthesis Zn uptake in wheat (Zhang et al., 2015). Zn and Cu concentrations in all tissues significantly decreased with increases in P application rate. P application increased the concentration of Fe in stem, leaf, and rachis and increased the concentration of Mn in bract but had no significant effects on Fe and Mn concentrations in other plant parts.

Irrespective of P rate, the average accumulation of Cu, Fe, and Mn in grain was 19, 16, and 13%, respectively, of that in shoots, and the accumulation of Zn was from 58 to 71% of that in shoots. This partitioning was similar to that previously reported (Bender et al., 2013). The results further showed that the partitioning of Zn was affected by P application rate. Zinc harvest index, i.e. the ratio of Zn content in grain to that in shoot, increased with increasing P rates. This was consistent with a previous finding that more Zn was translocated to the grain and less was retained in the stover when maize was treated with increasing quantities of P (Verma and Minhas, 1987). Nicotianamine (NA) has been reported to be involved in the phloem loading and translocation of Zn (Deng et al., 2013), and an increase in NA stimulated Zn translocation and seed-loading (Lee et al., 2011). However, identifying the pathway by which P application increases Zn translocation will require further research. The important fact is that P application promoted Zn translocation from stem, leaf, bract, and rachis to the grain of maize. Our results indicated that, even though P application decreased the Zn concentrations in grain and in all other shoot tissues of maize, it did not suppress Zn remobilization from vegetative tissues to grain, which agrees with results for wheat (Zhang et al., 2015).

4.5. Bioavailability of micronutrients in grain

The current study also showed that the average concentrations of Zn and Fe in grain were only 16.5 mg kg^{-1} and 17.4 mg kg^{-1} , respectively. These values are far below the target values for biofortification (38 mg kg^{-1} for Zn and 60 mg kg^{-1} for Fe) in maize (Bouis and Welch, 2010). The effects of P application on the concentrations, contents, and partitioning of P, Zn, Cu, Fe, and Mn in maize will affect the bioavailability of these minerals in grain. Phytic acid (PA) is the primary P storage compound in seeds and PA accounts for up to 80% of the total P content in seed, while it impaired biological utilization by binding nutritionally important mineral such as Zn (Febles et al., 2002). Previous report had showed that the grain PA concentration was highly correlated with total P concentration (Lolas et al., 1976). An increase in the P to Zn molar ratio indicated a decrease in the bioavailability of Zn in maize grain in response to P application, which is consistent with previous reports on wheat (Zhang et al., 2012). The bioavailability of Fe in maize grain was also reduced by a high P supply. Because a reduction in Zn and Fe bioavailability in maize grain will likely have negative effects on human nutrition, research is needed to determine how to manage P application so as to meet yield requirements while reducing the ratios of P/Zn and P/Fe in maize grain. To reduce the negative effects of P application rates on the Zn and Fe content in grain, growers should also consider applying Zn and Fe fertilizer in intensive maize production systems.

5. Conclusion

Phosphorus application significantly decreased shoot Zn and Cu concentrations but had no significant effects on shoot Fe and Mn concentrations at all growth stages. The ratios of pre-anthesis to post-anthesis nutrient accumulation were not affected by P application rates except Zn. P application had greater effects on the partitioning of Zn than on the partitioning of other nutrients and increased the partitioning of Zn to grain but decreased the partitioning of other nutrients to grain. This indicated that Zn remobilization from vegetative tissues (stem, leaf, rachis, and bract) to grain was not suppressed by higher rates of P application. The increase in the ratios of P/Zn and P/Fe in grain as the P application rate increased indicated that increases in P application will reduce Zn and Fe bioavailability. These results indicate that P and

micronutrients must be managed to meet both yield requirements and human nutritional requirements.

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