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Phosphorus Application Decreased Copper Concentration but Not Iron in Maize Grain

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Abstract: Copper (Cu) and iron (Fe) are essential micronutrients for plants and animals. How phosphorus (P) application affects Cu and Fe concentrations in maize grain still remains unclear. Two-year field studies were conducted in a long-term experiment with six P levels (0, 12.5, 25, 50, 100, and 200 kg·ha^{-1} P) on calcareous soil. Phosphorus application significantly decreased the average grain Cu concentration by 12.6% compared to no P treatment, but had no effect on grain Fe concentration. The copper content increased as the P application rate increased from 0 to 25 or 50 kg·ha^{-1}, but then decreased, while Fe content kept increasing. As the P application rate increased, the specific Cu uptake by the roots decreased, but not for Fe. The root length density in response to P application had a positive relationship with shoot Cu and Fe content. The shoot Cu content and grain Cu concentration decreased with the reduction in the arbuscular mycorrhizal fungi (AMF) colonization of roots due to increasing P application. The reduction in grain Cu concentration with increasing P rates could be partly explained by the decreasing uptake efficiency.

Keywords: grain; Cu; Fe; phosphorus; AMF colonization

1. Introduction

Maize is an important ingredient in animal feed and industrial products [1], providing not only substantial levels of calories and protein [2], but also micronutrients such as copper (Cu), iron (Fe), and zinc (Zn) [3,4]. The improper management of maize crops, however, results in low contents of micronutrients in grain, such that the grain nutrients in the feed do not meet animal requirements [5]. As a result, exogenous micronutrients must be added to the feed to increase animal body weight [6]. The addition of large amounts of micronutrients, and especially Cu, to the feed can result in substantial soil and water pollution [7,8]. It is therefore important to increase the micronutrients supplied to animals by increasing the micronutrient contents in the grain rather, than using chemical additives directly in the feed.

The application of phosphorus (P) fertilizer in intensive agriculture production is a crucial measure for increasing grain yield [9,10], but it may affect the micronutrient contents in grain. The antagonistic effects between P-Zn and P-Cu have long been observed before now [11,12]. Whereas these studies mostly reported on how P application affected the Cu and Zn nutrients in the seeding stage through pot experiments, less attention was focused on grain. With regard to grain micronutrients, the mobilization from root to grain, including the uptake by roots and translocation within plants, are of great importance. Recently, long-term field experiments indicated that the application of P fertilizer significantly decreased grain Zn concentration in maize [13] and wheat [14], and the inhibition mainly occurred in the uptake by root, but not in the translocation process. It is still not clear, however, to what extent P application affects grain Cu nutrition, and the influence of P application on Cu transportation from root to grain should be receiving much more attention. Quantitative study is required in the field to analyze whether P fertilizer application influences Cu uptake efficiency by roots, or the translocation to grain.

Efficient uptake by the roots is the key to acquiring soil nutrients, and root growth (especially root morphology) is thought to significantly affect the root uptake of mineral elements [15,16]. Although the uptake of mineral elements can also increase as root quantity increases [17,18], a high concentration of P in the shoots may reduce the total and specific root length (the ratio of root length to root dry mass) of maize [19]. Teng et al. [20] showed that, under field conditions, the root dry weight and root length density (length of roots/unit volume of soil) increased as the P application rate increased up to a critical level, but did not increase further once the rate exceeded that critical level. However, whether the changes in maize root morphology caused by P application influence Cu uptake has not been reported. Maize is easily colonized by arbuscular mycorrhizal fungi (AMF) [21], and under P-deficient conditions, the AMF colonization of roots enhances P uptake [22,23]. AMF colonization can also increase the uptake of some micronutrients, especially Cu [24]. Lambert et al. [25] found that the Cu concentration in maize shoots was significantly greater in mycorrhizal than in non-mycorrhizal plants. Subsequent research confirmed that the total Cu content in shoots was higher in mycorrhizal than in non-mycorrhizal plants [26,27]. Deng et al. [28] found that the AMF colonization of field maize plants was severely inhibited when the soil P level was above the agronomic critical level. Under field conditions, however, it remains unclear whether excessive P application reduces the AMF colonization of maize roots, and thereby decreases Cu uptake in roots and shoots.

In addition, the effect of P application on the concentration of Fe in maize grain is less understood. It is clear that under Fe-deficient conditions, maize secretes mugineic acid family phytosiderophores through the phytosiderophore efflux transporter, and takes up Fe in the form of Fe(III)-phytosiderophore complexes [29–31]. The secreted amount of phytosiderophore is not only related to nutrient status, such as deficiencies of Fe, Mn, Cu and Zn in soil and hydroponic growth conditions [32], but also to root traits. A study from Ptashnyk et al. [33] indicated that root density has a large effect on Zn uptake per unit of phytosiderophore secreted. It is unclear if the changes in maize root morphology caused by P application influence Fe uptake, and if P fertilizer application affects Fe translocation to grain.

Our previous studies have focused on P reducing grain Zn and the possible mechanisms in maize [13]. As such, the aims of the current study are continually (1) to quantitatively determine how the P application rates affect grain Cu and Fe concentrations, (2) to ascertain the Cu and Fe uptake and translocation processes from root to grain as correlated to varied P rates, and (3) to elucidate the roles of root morphology and AMF colonization in Cu and Fe uptake under varying P rates.

2. Materials and Methods

2.1. Field Experiment

A field experiment with summer maize (*Zea mays* L.) was conducted from June to October in 2014 and 2015 at the Quzhou experiment station, Hebei Province, on the North China Plain. The soil of the experiment site is a typical calcareous alluvial soil and the initial pH was 8.0 (1:2.5 *w*/*v* in water). Before maize was sown and fertilizer was applied in 2014, the soil bicarbonate-extractable (Olsen method) P concentration [34] was 6.0 mg·kg^{-1}, and the average soil diethylene triamine pentacetic acid (DTPA)-extractable Cu and Fe concentrations [35] were 0.90 and 7.78 mg·kg^{-1}, respectively.

The field experiment included six P application levels: 0, 12.5, 25, 50, 100, and 200 kg of P ha^{-1}. Each treatment had four replicate plots, and the area of each plot was 75 m^2 (10 m × 7.5 m). The cultivar of summer maize was "Zhengdan 958", and the planting density was 67,000 plants ha^{-1} in 2014 and 75,000 plants ha^{-1} in 2015. In each year, 75 kg of nitrogen (N) ha^{-1} as urea (46% N), 60 kg of K_2O ha^{-1} as potassium (K) sulfate, and P fertilizer at the indicated rates as calcium superphosphate were broadcast before sowing. At the 6-leaf stage (V6), another 150 kg of N ha^{-1} was topdressed. The plots were irrigated only one time, i.e., at the V6 stage. A pre-emergence herbicide was applied to control weeds, and Omethoate (Planck Biochemical Industry, Zhengzhou, China) was applied when the plants were at the 12-leaf (V12) stage to control aphids.

2.2. Plant and Soil Sampling

At the physiological maturity stage (R6), to evaluate the maize grain yield, the ears from a 7.2 m^2 (3 m × 2.4 m) area in the center of each plot were harvested (the grain yield was expressed on a 15.5% moisture basis). Meanwhile, shoot samples for nutrient analysis (4 plants per plot) were collected and separated into grain and straw. At the tasseling (VT) stage in 2014, the root dry weight, root length and AMF colonization were determined for roots collected from one 0.18 m^3 block of soil from each plot. Each soil block was 120 cm long, 25 cm wide, and 60 cm deep. The block was located between two plants in adjacent rows (the row spacing was 60 cm). All roots in each block were collected by washing the soil over sieves. The roots from the 0–20 cm section of topsoil were used to measure AMF colonization, and all roots were assessed by root dry weight and root length. The root dry weight (g m^{-2}) used in this text meant total weight from the 60 cm soil layer. The root length density meant the root density per unit volume (cm^3). At the maturity stage of maize, six subsamples of soil in each plot were taken from a 0–20 cm soil depth with a stainless steel auger then composited into a single sample.

2.3. Plant and Soil Analyses

All plant samples included roots and shoots that were sampled from the same plant at tasseling, and the grains and straws that were taken from the same plant at the maturity stage were firstly washed with tap water and then with deionized water before they were dried at 60–65 °C to a constant weight. A microwave-accelerated reaction system (CEM, Matthews, NC, USA) was used to digest the plant samples with HNO_3-H_2O_2. The copper and Fe concentrations in the digested solutions were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, OPTIMA 3300 DV, PerkinElmer Inc, Boston, MA, USA). To verify the digestion procedures and to calibrate the ICP-OES, the IPE684 grain and IPE126 straw samples (Wageningen University, Wageningen, The Netherlands) were used as the reference materials. The Olsen-P concentrations of soil from 0–20 cm were measured by the molybdovanadate phosphate method [34] with a colorimeter (Macy UV-1700, Macy Instrument Industry, Shanghai, China) after being extracted with 0.5 M $NaHCO_3$ at pH 8.5 (180 rpm, 25 °C). The DTPA-Fe and Cu concentrations of soil were measured by ICP–OES (Agilent 5110, Agilent Technologies Co. Ltd., Palo Alto, CA, USA) after being extracted with 0.005 mol L^{-1} DTPA.

To measure root length, all root samples from the 0–60 cm soil layer were washed with tap water and kept at −20 °C before images were captured with an optical scanner (Epson V850Pro, Seiko Epson Corporation, Nagano-ken, Japan); then the images were analyzed with WinRHIZO software (Quebec, QC, Canada) to calculate the total root length. For the determination of AMF colonization, the root samples (0.5 g per plot) were cut into 1 cm segments, and the segments were placed in 10% (*w*/*v*) KOH at 90 °C in a water bath for 1 h. The root samples were then placed in 2% HCl for 5 min and stained with trypan blue. For each sample, 15 root segments were evaluated for AMF colonization on a scale from 0% (no sign of infection) to 100% (heavily infected) [23].

2.4. Statistical Analysis

For each year, one-way analyses of variance (ANOVAs) were used to test the effects of the P application rate on the dependent variables. When the ANOVAs were significant, the means were compared with an LSD test at $p < 0.05$. SAS software (SAS 8.0, SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. Relationships between shoot Cu content, shoot Fe content and root length density, and relationships between shoot Cu content, grain Cu concentration and AMF colonization of root were quantified by regression models.

In this report, nutrient 'content' is equal to nutrient concentration multiplied by biomass. 'Shoot' indicated the above-ground part of the maize plant. Specific Cu uptake was calculated from shoot Cu content divided by root length, and specific Fe uptake was calculated in the same way.

3. Results

3.1. Cu and Fe Concentrations in Grain and Straw as Affected by P Fertilization

In both years, the grain Cu concentration was reduced significantly with the increase in P applications (Figure 1a). In 2014, compared to P0, the grain Cu concentration decreased by 3.1%, 7.4%, 12.0%, 16.4% and 25.6% in P12.5, P25, P50, P100 and P200, respectively. In 2015, the grain Cu concentration continually reduced from 1.78 mg·kg^{-1} with P0 to 1.40 mg·kg^{-1} with P200. Phosphorus application also significantly reduced the straw Cu concentration (Figure 1c). Varying the P treatment had no effect on the Fe concentration in grain and straw in both years, and the grain Fe concentration almost stayed within a certain range from 18.8 to 21.2 mg·kg^{-1} (Figure 1b,d).

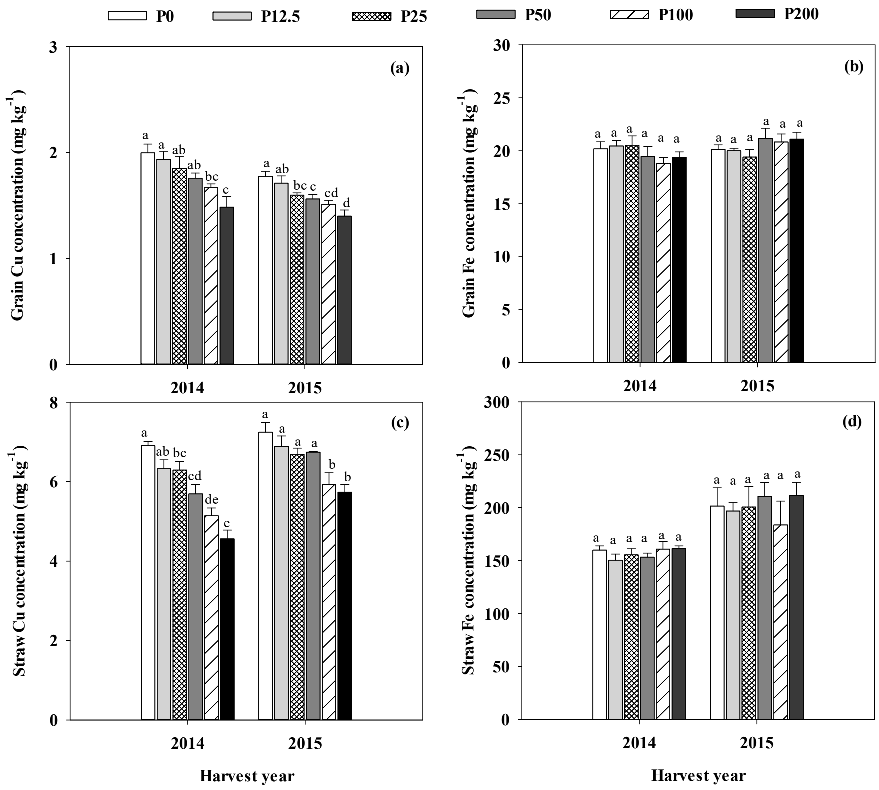

Figure 1. Cu concentration in grain (**a**) and straw (**c**), Fe concentration in grain (**b**) and straw (**d**), in 2014 and 2015 of maize as affected by P application rate. P0, P12.5, P25, P50, P100, and P200 equal 0, 12.5, 25, 50, 100, and 200 kg·ha^{-1} P applied, respectively. Values are means + SE of four replications. Within each year, means with the same letters indicate no significant difference according to an LSD test ($p < 0.05$).

3.2. Cu and Fe Content, Ratio of Grain to Shoot Cu and Fe Content as Affected by P Fertilization

The copper content (g·ha^{-1}) in the grain and straw increased as the P application rate increased from 0 to 25 kg·ha^{-1}, but then decreased at higher rates in 2014 (Figure 2a). In 2015, a similar trend was evident, in which the Cu content in grain and straw also increased first and then decreased with increasing P application, and it was highest at the 50 kg·ha^{-1} P level (Figure 2a). Phosphorus application increased the contents of Fe in grain and straw in both cropping seasons (Figure 2b). The ratio of grain to shoot Cu content was less affected by P application in both cropping seasons, and averaged 26% and 25% in the first and second cropping season, respectively (Figure 2c). Phosphorus application also had little effect on the ratio of grain to shoot Fe content in the two cropping seasons, while a significant difference was only found between the P12.5, P50 and P100 treatments in 2014 (Figure 2d).

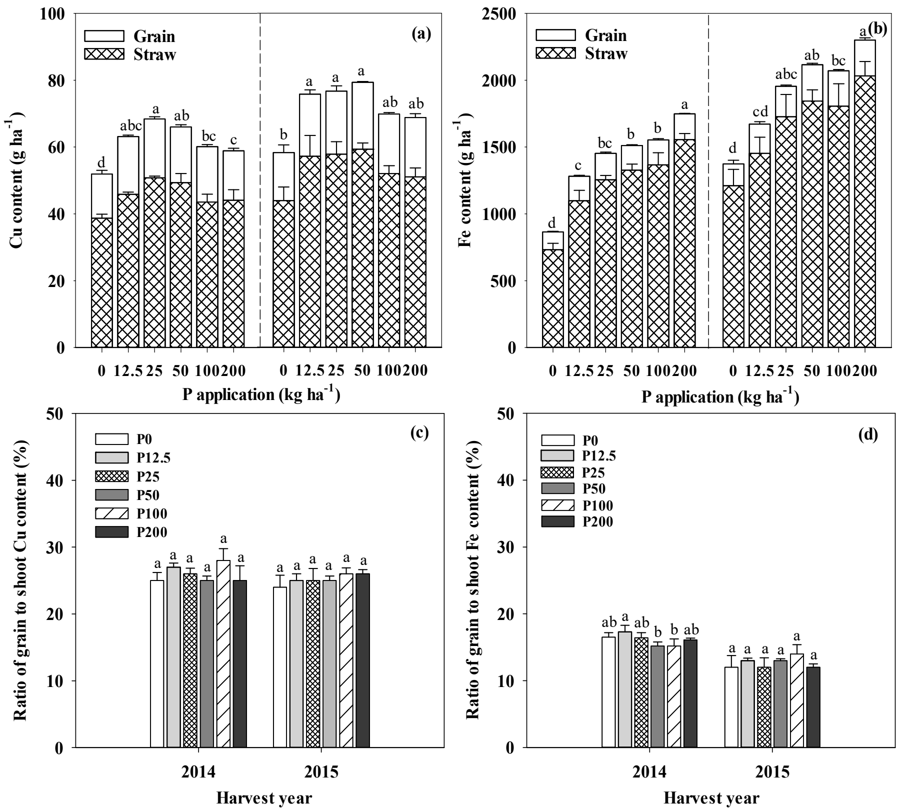

Figure 2. Content of Cu (**a**) and Fe (**b**) in grain and straw at maturity in 2014 and 2015, and ratio of grain to shoot Cu content (**c**) and Fe content (**d**) at maturity as affected by P application rate. P0, P12.5, P25, P50, P100, and P200 equal 0, 12.5, 25, 50, 100, and 200 kg·ha^{-1} P applied, respectively. Values are means + SE of four replications. Within each year, means with the same letters indicate no significant difference according to an LSD test ($p < 0.05$).

3.3. Root Morphology, Specific Cu and Fe Uptake as Affected by P Fertilization

A significant increase in root dry weight was recorded between P0 and P25. (Figure 3a). Root length density (cm of root per cm^3 of soil) had an increasing tendency as the P application rate increased from 0 to 25 kg·ha^{-1}, while no difference was found between P25, P50, P100 and P200 (Figure 3b). Phosphorus application reduced the specific Cu uptake (Figure 3c), but did not affect the specific Fe uptake (Figure 3d).

3.4. Shoot Cu and Fe Content, Grain Cu and Fe Concentration Related to Root Length Density and AMF Colonization

Figure 4 shows that a positive relationship was found between root length density and shoot Cu and Fe content. Although a regression relationship could be found between grain Cu concentration and root length density (Figure 4c), it was not appropriate to present the relationship, because it was illogical that the root length density had a negative effect on grain Cu concentration. As a result, the linear relationship was not shown. The arbuscular mycorrhizal fungi colonization of roots was positively related to the shoot Cu content and grain Cu concentration (Figure 5a,c), but was independent of shoot Fe content and grain Fe concentration (Figure 5b,d). It is also illogical to show the relationship between the shoot Fe content and AM colonization within the regression model (Figure 5b).

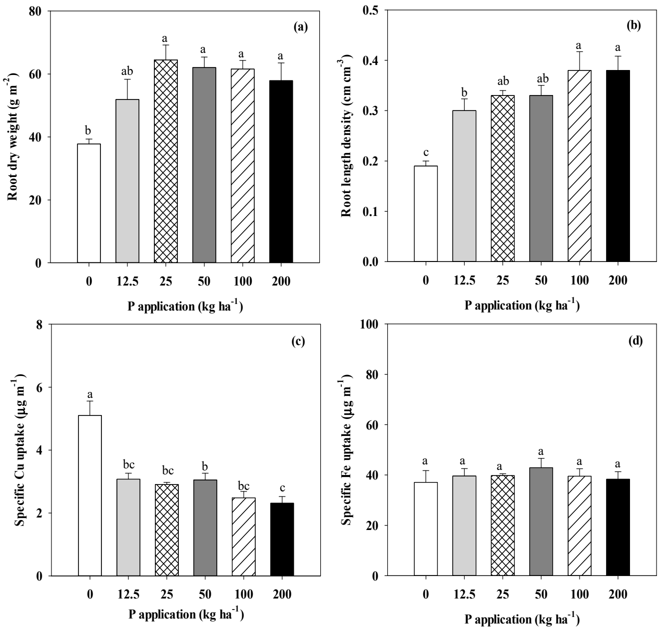

Figure 3. Root dry weight (**a**), root length density (**b**), specific Cu uptake (**c**), and specific Fe uptake (**d**) at tasseling as affected by P application rate in 2014 (these data were not collected in 2015). Values are means + SE of four replications. Within each panel, means followed by the same letters indicate no significant difference according to an LSD test ($p < 0.05$).

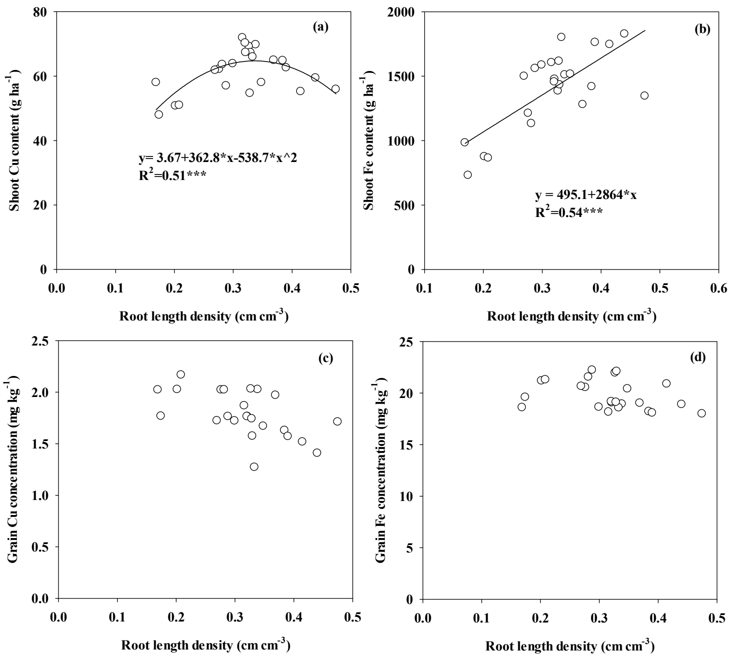

Figure 4. Relationship between root length density and shoot Cu content (**a**), shoot Fe content (**b**), grain Cu concentration (**c**) and grain Fe concentration (**d**) at tasseling in 2014 (these data were not collected in 2015). *** indicates that the regression is significant at $p < 0.001$.

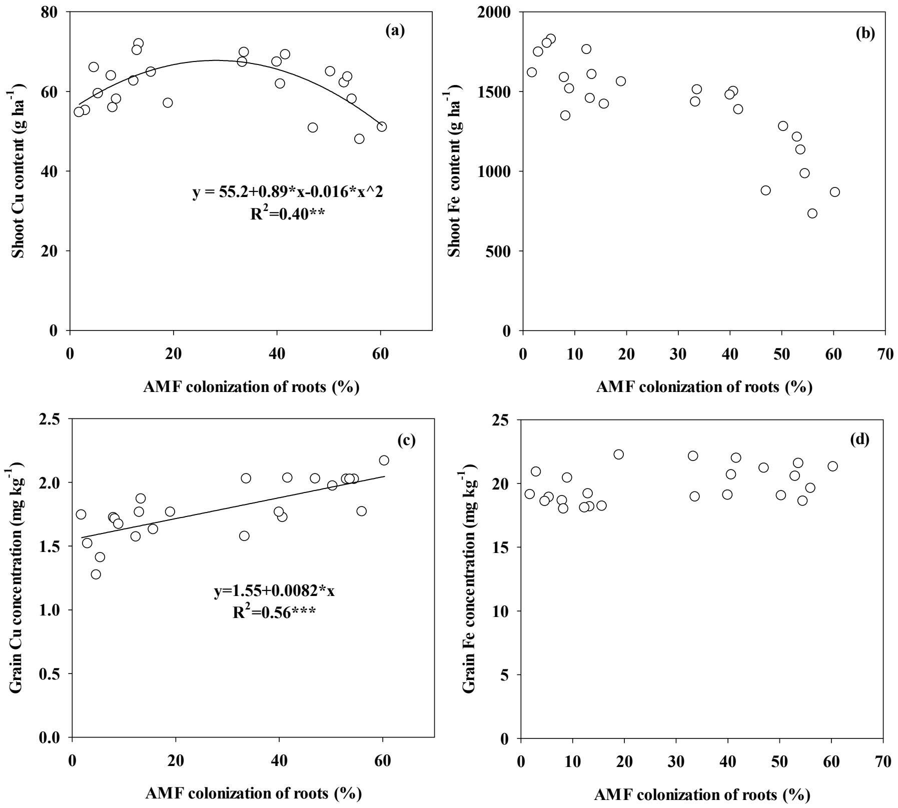

Figure 5. Relationship between AMF colonization of roots and shoot Cu content (**a**), shoot Fe content (**b**), grain Cu concentration (**c**) and grain Fe concentration (**d**) at tasseling in 2014 (these data were not collected in 2015). ** and *** indicates that the regression is significant at $p < 0.01$ and $p < 0.001$, respectively.

3.5. Soil Availability of P, Cu and Fe Concentrations as Affected by P Fertilization

Compared to P0, P fertilizer application significantly increased the soil Olsen-P concentration from an average of 9.6 mg·kg^{-1} under P12.5 to 63.8 mg·kg^{-1} under the P200 treatment in both years (Figure 6a). Phosphorus fertilizer application had no effect on soil DTPA-Cu (Figure 6b) or DTPA-Fe concentration (Figure 6c) in 2014 and 2015.

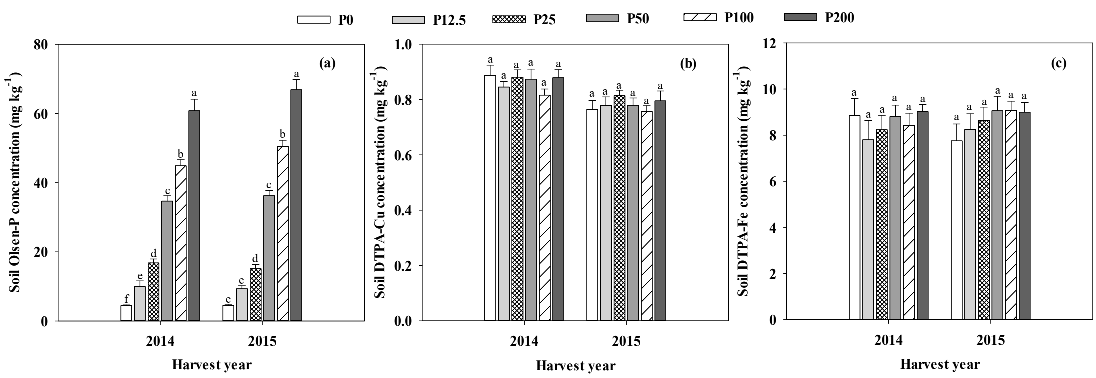

Figure 6. Concentrations of soil Olsen-P (**a**), DTPA-Cu (**b**) and DTPA-Fe (**c**) as affected by P application rate at maturity stage of summer maize. P0, P12.5, P25, P50, P100, and P200 equal 0, 12.5, 25, 50, 100, and 200 kg·ha^{-1} P applied. Values are means + SE of four replications. Within each year, means with the same letters indicate no significant difference according to an LSD test ($p < 0.05$).

4. Discussion

4.1. Grain Cu and Fe Nutrition as Affected by P Application and Its Effects for Feed

In these field experiments, P application significantly increased maize grain yield, and the 25 kg·ha^{-1} P input was optimal for ensuring grain yield, while excessive input had no benefit on maize yield [13]. Meanwhile, the stover yield of maize was also significantly enhanced with the increasing P fertilizer rate from 0 to 12.5 (or 25 kg·ha^{-1}), and excessive P fertilization had no benefit on stover yield [13]. Our findings showed that P application significantly decreased the grain Cu concentration by 3.4–23.4%, while an optimal P application (25 kg·ha^{-1}) decreased grain Cu concentration by 8.6%, and excess (200 kg·ha^{-1}) led to a grain Cu concentration reduction of 23.4%, whereas P application had no effect on grain Fe concentration. To our knowledge, this is the first time that a field experiment has reported that grain Fe nutrition is not influenced by P fertilizer application.

The decreasing Cu concentration in maize grain may affect the adjustment of feed copper nutrition—if the maize grains produced under higher P conditions are to be used as fodder and additional Cu needs to be supplemented. Based on the National Standard of China, the limited contents of Cu in feeds were 3–6 mg·kg^{-1} and 8 mg·kg^{-1} for swine and chicken, respectively [36,37]. However, the current grain Cu concentration under optimal (25 kg P ha^{-1}) and excessive (200 kg P ha^{-1}) P application was only 1.73 and 1.45 mg·kg^{-1}, respectively. The scenario analysis indicated that the current maize grain provided insufficient Cu for livestock and poultry. It still needs 1.7 to 4.6 times more chemical Cu addition under optimal P application to guarantee the feeding Cu requirement for swine and chicken, and a 2.1- to 5.5-fold increase in Cu is required under the overuse of P condition.

Although P fertilizer did not affect Fe concentrations in grain in the current study, the Fe bioavailability in grain, which means the Fe homeostasis in the animal intestine, may be largely reduced due to the high phytate concentration with increasing P rates [38,39]. In other words, the conventional manner of overuse of P fertilizer will severely reduce the Fe intake by the intestinal canal compared to under optimal P application.

In addition, P fertilization also affected the grain Zn concentration and content in this study. The grain Zn concentration significantly decreased with the increasing P fertilizer rate, while the grain Zn content increased first and then decreased [13]. The reasons for the decline in grain Zn concentration could be partly explained by the 'dilution effect' and the rhizosphere effect, such as root length density and AM fungi. P fertilization had no effect on grain manganese concentration, but increased manganese content due to the improvement of grain yield.

4.2. Does Grain Cu and Fe Nutrition Relate to Uptake or Translocation?

The copper content in grain and straw increased when low levels of P were applied to optimal P rates, but they then decreased with high levels of P in the current study. This result was inconsistent with a previous study, which indicated that the Cu content in rice decreased as the P application rate increased [40]. This discrepancy might be explained by the effects of P application on plant biomass and Cu concentration—from the low to the optimal P application rate, a combined effect with a fast increase in maize biomass and a slight reduction in Cu concentration resulted in the improvement of Cu accumulation, while, from optimal to excessive P application rates, a relatively unchanging shoot biomass and a gradual decline in Cu concentration led to a reduction in Cu content. It follows that a reasonable level of P application in intensive maize production could result in a high level of Cu content. Previous studies also reported that high levels of P in the soil had a tendency to reduce the uptake of Cu by maize roots [11,41,42], while the extent of decline in this study was substantial. Meanwhile, the results further showed that the ratio of grain to shoot Cu content was not affected by P application rates. These results therefore indicate that the grain Cu reduction may be not due to the inhibition of Cu re-translocation from straw to grain, but instead a synthesis effect of the reduction of root uptake and the potential dilution effect (high yield caused concentration decrease). With regard to the reasons for the 'P decrease of Cu', the 'dilution effect' and the Cu acquisition capability of the root together affect the Cu concentrations. With a low-available-P soil, increasing the P fertilizer rapidly enhances crop biomass or yield, and the 'dilution effect' may play an important role in the reduction of Cu concentration, especially when the amount of P fertilizer is less increased. On the other hand, with sufficient-available-P soil, increasing the P fertilizer has little or no effect on the further improvement of crop biomass or yield, but the Cu concentration is in continuous decline.

The iron content in the current study was increased with P application rates. The increase in Fe content mainly resulted from an increase in plant biomass rather than an increase in the Fe concentration in maize tissues. In other words, the increase did not result from a direct effect on Fe processing by the plant. The finding that the ratio of grain to shoot Fe content were not affected by P application indicated that the translocation of Fe from straw to grain was also not affected by P application, which was consistent with a previous result obtained with wheat [43]. These results indicate that P application might increase Fe uptake, and the re-translocation of Fe from straw to grain was not affected by P rates.

The reduction in Cu and Fe concentration in grain and shoot of maize was also not due to the Cu and Fe availability in the soil. In fact, the increased P application did not change the available soil Cu and Fe in the current study (Figure 6b,c). In a study with wheat, Zhang et al. [43] also indicated that P application did not affect soil DTPA-Cu concentrations.

4.3. Cu and Fe Uptake Response to Root Length and AMF Colonization

Based on the above analysis, the Cu-uptake process by roots was limited under high levels of P. As the rate of P application increased, the root dry weight and root length density initially increased and then plateaued. The result was inconsistent with previous research, in which a high rate of P application reduced root length density [44]. Haynes and Ludecke [45] have explained the difference made by that large amount of shoot biomass as related to high root biomass to match the grain yield under field conditions. A study by Duncan et al. [46] also indicated that plants which received a co-application of N + P + K fertilizers had an increased overall root length, more root branches and greater root diameters. Another reason for the discrepancy may be that the level of available P in the soil was lower in the current study than in the study of Schroeder and Janos [44]. Regarding the latter possibility, Wen et al. [19] found that the total maize root length and specific root length decreased when the soil Olsen-P concentration ranged from 54 to 594 mg g^{-1} in a pot experiment. In the current field experiment, the soil Olsen-P concentration was <67 mg·kg^{-1}, which may have been too low to restrict root length [47]. Although P application did not reduce root length density, it did reduce specific Cu uptake. The results indicated that the ability of the roots to absorb Cu declined as the P application rate increased [42]. However, this reduction curve of root Cu uptake could not be explained simply by root length density, from the relationship between root length density and shoot Cu content (Figure 4a). Previous studies have shown that decreases in the AMF colonization of roots resulted in reduced Cu concentrations in plants. Li et al. [24] concluded that Cu uptake and transport by vesicular–arbuscular mycorrhizal hyphae may contribute to the Cu nutrition of host plants. Our previous results in this study also showed that the AMF colonization of maize roots significantly decreased as the P application rate increased [13], which was consistent with Liu et al. [48]. Furthermore, AMF colonization, shoot Cu content and grain Cu concentration were positively correlated in the current study, which indicated that a decrease in AMF colonization may be a main reason that explains why the Cu concentrations in grain and straw were reduced by P application.

The shoot Fe content in this study was improved by P application. Previous studies noted that an increase in root length would increase the plant's capability to obtain mineral nutrients, because it would increase the surface area available for acquiring minerals [49–51]. The current results support this view by indicating that root length density had a positive relationship with shoot Fe content. AMF colonization was unrelated to total Fe content and grain Fe concentration in the current study, which was consistent with Liu et al. [26], who reported that maize absorbed less Fe via the AMF pathway than via the non-mycorrhizal pathway. A meta-analysis also provided evidence that AMF had no effect on Fe nutrition in herb plants [52]. A report had indicated that the photosiderophores produced in maize partly sustained Fe acquisition [53]. This means that the photosiderophores production in maize roots was probably not affected by increasing P application, which needs further study.

4.4. Phosphorus Management Based on Yield, Grain Cu and Fe Nutrition

In an intensive maize production system, an appropriate P fertilizer application is beneficial to realizing higher maize grain yields compared to low P input [54,55], while excessive P fertilizer amounts, which exceed the growth requirement, are not advisable. The overuse of P application will not only lead to sharp declines in grain Cu concentration under the condition, while the yield will fail to increase compared to appropriate P application, but it will also cause a further reduced bioavailability of grain Cu, Fe, and Zn [56,57]. Meanwhile, excessive P application results in the reduction of AMF colonization [58], and then decreases in P efficiency [59]. From the mechanisms between P and Cu, Fe, biological traits and P management should be synchronously explored to achieve high grain yields and high grain Cu and Fe contents of maize, and then to reduce the extra Cu supplementation in industrialized feed production. Importantly, an optimal level of P fertilizer should be established based on soil P tests and crop requirements to explore the biological potential of crop roots and rhizosphere manipulation.

5. Conclusions

Phosphorus application significantly reduced Cu, but not Fe, concentration in grain. Phosphorus application had no effect on Cu or Fe distribution percentage in grain and straw. The reduction of Cu concentration could be partly explained by a decrease in the colonization of roots by AMF due to increasing P application. If the maize grains produced under the condition of the current P level (50–200 kg·ha^{-1}) are used as fodder, it will aggravate the Cu deficiency of livestock. Therefore, an appropriate P amount in the maize production system is necessary to maintain a higher yield and a suitably high concentration of Cu for feed.

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