EFFECTS OF PHOSPHORUS APPLICATION METHODS ON NUTRITION UPTAKE AND SOIL PROPERTIES OVER 12-YEAR FIELD MICRO-PLOT TRIALS: II. SOIL-AVAILABLE MICRONUTRIENTS AND THEIR RELATIONSHIP WITH SOIL PROPERTIES

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ABSTRACT

In a long-term P micro-plot experiment with meadow brown soil conducted since 1997 in Shenyang, China, changes in soil-available micronutrients (Zn, Fe, Cu and Mn) were investigated and statistically related to soil properties of Olsen-P, total P, SOC, total N and pH. Four fertilization methods were included in the micro-plot experiment: unfertilized control, N fertilization annually, N and P fertilization annually, N fertilization annually and P fertilization once every 6 years. Compared to the unfertilized treatment, each of the fertilization methods increased the concentrations of available Mn, Fe and Cu but not Zn, throughout the trial period. Both P application rates and methods influenced soil-available Zn and Fe but not Cu and Mn. It was concluded that P application once every year and once every six years contributed to the availability of soil Zn and Fe, respectively. When taken as a canonical variable, the soil properties could well forecast the available Mn but poorly predict the other micronutrients. Path analysis showed that soil pH, total P and SOC were the three main factors directly influencing the soil-available micronutrients, while Olsen-P played an indirect role via them.

KEYWORDS:
Soil properties; P application; micronutrients; availability

1. INTRODUCTION

Soil-available micronutrients, such as Zn, Fe, Cu and Mn, play important roles in crop growth [1, 2]. But agricultural activities, such as P fertilizer application or fungicide use, can cause soil contamination by changing concentrations of soil-available micronutrients [3]. It was reported that the availability of soil micronutrients is influenced by soil properties, cropping and fertilization practices [4, 5], but the conclusions are not consistent. Some long-term fertilization experiments have shown that application of inorganic fertilizer, manure alone, or inorganic fertilizer with manure, increased the availability of soil micronutrients Zn, Fe, Mn and Cu [6-8]. However, an increase in the availability of P was expected to reduce zinc availability through precipitation of Zn₃(PO₄)₂ [9, 10], and this was verified in soil with P applied above 75 mg·kg⁻¹ [11]. P application also reduces the availability of Mn in acidic soil [12].

Fertilization practices in long-term field experiments can cause changes in both soil properties and nutrient availability. It was also reported that fertilization alters soil certain properties, such as soil pH, organic matter content, available P, and CaCO₃ which, in turn, affect soil micronutrient levels [5, 13, 14]. Similarly, Shuman [12] reported related changes in soil-available micronutrients to soil pH and surface charge, due to P fertilization.

Various hypotheses have been suggested to explain changes of soil-available micronutrients caused by fertilization. According to Wei et al. [5, 15], soil organic matter exerts a significant and direct impact on the availability of soil micronutrients; other factors like pH, available P and CaCO₃ have an indirect effect. However, other studies have shown that soil pH as the most important factor [16] affects the availability of micronutrients primarily through affecting soil charge characteristics and absorption of hydrolysable metals [1]. Thus, for a better understanding of soil-available micronutrients regarding fertilization treatments, a long-term P micro-plot trial was made to investigate the changes in soil-available micronutrients Zn, Fe, Cu and Mn, and to explore the relationship of soil-available micronutrients and soil certain properties.
2. MATERIALS AND METHODS

2.1 Soil sampling and analysis

The soil used in the experiment was collected from the surface layer (0-20 cm) at the 1st, the 4th and the 6th year of each 6-year period. Also the soil from the lower layer (20-40 cm) was collected at the last year of each 6-year period to compare the impact of P application in both soil layers.

Soil-available Zn, Fe, Mn, and Cu were extracted by the DTPA procedure developed for near-neutral and calcareous soil [18]. DTPA-extractable soil Zn, Fe, Mn, and Cu was obtained by shaking 10 g of air-dried soil with 20 ml of extractant for 2 h at 25 °C. After that, the leachate was centrifuged, and filtered for analysis of Zn, Fe, Mn and Cu by atomic absorption spectrometry (AAS). The background values of soil-available micronutrients are shown in Table 1.

TABLE 1 - Background values of soil-available micronutrients Zn, Fe Cu and Mn.

<table>
<thead>
<tr>
<th>Variables</th>
<th>0-20 cm layer</th>
<th>20-40 cm layer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available Zn (mg·kg⁻¹)</td>
<td>1.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Available Fe (mg·kg⁻¹)</td>
<td>35.4</td>
<td>21.6</td>
</tr>
<tr>
<td>Available Cu (mg·kg⁻¹)</td>
<td>2.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Available Mn (mg·kg⁻¹)</td>
<td>72.5</td>
<td>37.8</td>
</tr>
</tbody>
</table>

Other soil properties that may be linked to the availability of soil micronutrients were analyzed. Soil-available P was determined by Olsen method, and the extract P was analyzed colorimetrically, using the method of Murphy and Riley [19]. Total P was analyzed by molybdenum-ascorbic acid method after digestion with H₂SO₄ and H₂O₂. Soil total N was analyzed by the Kjeldahl method. Soil pH was measured with a glass electrode using a 1:2.5 soil/water ratio [20]. Soil organic carbon was analyzed using a multi N/C-3100 TOC analyzer (Analytik Jena, Germany).

2.2 Statistical analysis

The data were analyzed using SAS statistical package (SAS 9.13). Analyses of variance and least square differences (LSD at P ≤ 0.05) were used to determine the statistical significance among the treatment means. Moreover, in order to explore the relationship between soil properties and soil-available micronutrients, correlation analysis, canonical correlation analysis, and path analysis were used.

3. RESULTS AND DISCUSSION

3.1 Changes of soil-available micronutrients under long-term fertilization experiment

3.1.1 Soil-available zinc

Among various fertilization treatments, P application caused different changes of available Zn in the 0-20 cm soil layer, while had little effect in the 20-40 cm layer. Compared with the background values, the long-term P application did not cause a decrease but an increase of available Zn in the topsoil at the end of the second 6-year period, except T6. This is consistent with previous studies [21, 22]. The formation of phosphate-Zn complexes at the high P application [6] might cause the decreased availability of Zn in T6 treatment, in the year 2008. The contents of available Zn, ranging from 0.72 to 2.08 mg·kg⁻¹ in the 0-20 cm soil layer, were sufficient to meet the crop need but had not yet reached the toxic level [23]. In contrast, the contents of DTPA-zinc in the 20-40 cm soil layer were near the critical value 0.5 mg·kg⁻¹ implying possible Zn deficiency in this soil layer.

In the surface layer, compared with P application once per 6 years (T4 and T6), available Zn was more likely to increase with the P application once every year (T3 and T5), at the end of the second 6-year period (Fig. 1). In addition, its level was significantly affected by the rate of P (p<0.05) when applied once every year. This demonstrated that, compared to P application once every 6 years, that once every year is a better way to improve the availability of soil Zn. Available Zn decreased at first, and then increased in the treatment without P application (T1 and T2) during each of the two 6-year periods. However, in the P application treatments (T3, T4, T5 and T6), its level was increased at the beginning, and then decreased during the first 6-year period, and vice versa, in the second period. The general trend of fluctuation of available Zn might mainly result from inter-annual variations of crop uptake, suggesting that P practice did influence the availability of soil Zn.

A number of reasons are responsible for the influence of P application on the availability of soil Zn in the top-soil. One possible reason is that suitable level of fertilization application significantly decreased Zn contents (bound by carbonate, organic and Fe oxide), while increased the exchangeable and amorphous Fe oxide-bound soil Zn. The reason may be further verified with fertilization of N, K and organic manure at appropriate levels which also increased the availability of soil Zn in other studies [24-27]. Apart from fertilization, available Zn may also be influenced by the contents of various particle-size fractions [28], and fluctuations of soil water contents under field conditions [29]. Therefore, to sum up, we tend to draw a preliminary conclusion: fertilization does affect the availability of soil Zn, but other factors, like water content or particle composition, may also contribute to available Zn.

The level of soil-available Zn in the 20-40 cm soil layer underwent a dramatic decrease compared with the background value, and was reduced by about 80%, 70% at the ends of the first and second 6-year periods, respectively. The reduction might be a result from uptake by crop roots. The level of available Zn was obviously lower (p<0.05) than that of the 0-20 cm soil layer, but not significantly changed by fertilization treatments. Our finding suggested that the long-term experiment had little impact on soil-available Zn in the 20-40 cm soil layer.
3.1.2 Soil-available iron

The level of available Fe ranged from 27.2 to 59.6 mg·kg\(^{-1}\) at the 0-20 cm soil layer, and from 26.1 to 41.8 mg·kg\(^{-1}\) at the 20-40 cm soil layer. According to the classification criteria for Fe content in soil [36], the level of soil-available Fe in this soil was relatively high, and available Fe deficiency is less likely to happen to affect plant growth. Soil-available Fe content in the 0-20 cm layer was increased in each treatment during each 6-year period. Soil-available Fe content in the 0-20 cm layer was increased in each treatment during each 6-year period. Compared with the unfertilized treatment, fertilization increased the level of soil-available Fe. When P was applied at low levels (T3 and T4), it did not cause significant differences in soil-available Fe. However, compared with P application at 75 mg·kg\(^{-1}\) annually, P application at 450 mg·kg\(^{-1}\) once every 6-years period greatly increased the level of soil available Fe (p<0.01) in the first 4 years during each 6-year period (Fig. 1). This suggested that P application once a 6-year period at high rates contributes to improve the availability of soil Fe.

The greater influence on the availability of Fe by the larger dose of P might be indirectly exerted through changing soil pH, and reacting with soil solution components by P application [12]. A larger dose of applied P was expected to cause greater changes in certain soil properties, such as soil pH, cation exchange capacity (CEC), and SOC to increase the level of available Fe [30, 31]. However, an increase of available Fe content, found at the end of each 6-year period in the N treatment T2 of our study, was different from that reported by other researchers [26], possibly due to variations in N application rates, experiment time and soil types.

In the 20-40 cm soil layer, compared to the background values, soil-available Fe content of each treatment increased during the trial periods. Moreover, the increments of treatment T2, T3, T4, T5 and T6 were significantly higher than in treatment T1 (p<0.01). This demonstrated that long-term fertilization practices can increase the availability of soil Fe in the subsoil layer.

3.1.3 Soil-available copper

Available Cu, ranging from 2.6 to 3.7 mg·kg\(^{-1}\) at the 0-20 cm soil layer and from 2.7 to 3.1 mg·kg\(^{-1}\) at the 20-40 cm soil layer, is more than adequate to meet plant need, according to the critical value of 0.2 mg·kg\(^{-1}\) for available Cu under which to affect crop growth [18]. Copper can be used as fungicide to cause soil contamination [3]. For our experiments, Cu was never used for this purpose. In the 0-20 cm soil layer, the fertilization treatments (T2, T3, T4, T5 and T6) slightly increased the level of available Cu compared to the unfertilized treatment T1. However, it did not reach a significant level (p<0.05) until the second 6-year period. It varied similarly among the fertilization treatments, in a pattern of continuously increasing in the first 6-year period, but decreasing first and then increasing in the second 6-year period. The fluctuation of available Cu during the two 6-year periods might mainly originate from the impact from climatic fluctuation and inter-annual variations of crop uptake.

The insignificant impact of methods of P application on soil-available Cu in the topsoil layer were also observed in others’ studies [5, 32]. One possible reason might be that Cu was primarily bound by soil organic matter, possibly through formation of complexes between Cu and humus substances [33]. Other studies also demonstrate that soil-available Cu is significantly increased with organic matter-containing materials [26, 34, 35]. Compared to the unfertilized control, the availability of Cu was not significantly increased with the application of N alone, or N and P simultaneously, until the second 6-year period. Hence, the inconspicuous effect of fertilization on available Cu may become more obvious as time goes by.

Soil-available Cu content in the 20-40 cm layer was significantly lower than in the 0-20 cm layer (p<0.01) of each treatment. However, soil-available Cu content in the 20-40 cm layer was increased compared with the background value (Fig. 2). This demonstrated that long-term fertilization practices can increase the availability of soil Cu in the subsoil layer.

3.1.4 Soil-available manganese

The content of soil-available Mn varied from 34.5 to 85.5 mg·kg\(^{-1}\) in the 0-20 cm soil layer, and from 19.8 to 41.9 mg·kg\(^{-1}\) in the 20-40 cm soil layer. The high values of available Mn in the soil indicated that Mn was extremely abundant in this district. In such circumstance, further investigation on the status of Mn in crops is needed. Similar to other micronutrients, the level of available Mn fluctuated during the trial period. In the 0-20 cm soil layer, soil-available Mn from the control treatment T1 decreased at the beginning, and then rose in the first 6-year period and continuously decreased in the second 6-year period. In the fertilization treatments T2, T3, T4, T5 and T6, it increased firstly, and then declined in the first 6-year period and, vice versa, in the second 6-year period.

The fluctuation of soil-available Mn in the 0-20 cm soil layer was related to climatic fluctuation, plant uptake, and possibly fertilization [32]. Nevertheless, different P fertilization did not cause significant variations (p>0.05) of soil-available Mn in the treatments T3, T4, T5 and T6 during the trial period. Wei et al. [5] and Richards et al. [35] also found that long-term application of inorganic P had little effect on availability of soil Mn, despite differences among soil type, management, microenvironment and climate. In contrast, application of organic fertilizers could increase availability of soil Mn [26, 32, 35]. Moreover, in the second 6-year period, available Mn concentration in the treatments T3, T4, T5 and T6 was significantly lower than in the treatment T2, suggesting that simultaneous application of N and P compared to N application only somewhat decreased the availability of soil Mn.

Values of available Mn in the 20-40 cm soil layer were significantly lower than in the 0-20 cm soil layer.
Available Mn contents in the 20-40 cm soil layer were higher in the treatments T5 and T6 than in the treatments T1, T2, T3 and T4, and became significantly different at the end of the second 6-year period while available Mn levels were not significantly different among the treatments T1, T2, T3 and T4, indicating that only a relatively high level of P application can influence soil-available Mn in the 20-40 cm soil layer.

### 3.2 Relationship between soil prosperities and available micronutrients

The components of a soil interact with each other to affect its physical, chemical and biological properties. Therefore, certain soil properties, including Olsen-P, total P, SOC, total N and pH, were measured to explore their possible impacts on soil micronutrients during the long-term experiment. Correlation analysis showed that soil-available Zn, Fe, Mn and Cu were negatively correlated with soil pH while positively correlated with other soil properties, except the poor correlation of Zn with SOC and Olsen-P (Table 2). The coefficients were all significant at p<0.05. This indicates that changes in soil-available micronutrients were not only dependent on fertilization, but also dependent on the variation of soil properties. Similarly, Gupta and Aten [36] found that soil CEC and organic matter had positive effects on the availability of micronutrients.

![Figure 1 - Soil-available Zn, Fe, Cu and Mn in the 0-20 cm layer during the long-term experiment.](image)

The capital letter is used to indicate the difference of soil-available micronutrient under different treatments. Values with the same letter are not significantly different at p < 0.05).
Canonical correlation analysis reflected the overall correlation among pairs of canonical variables. The canonical variables, in this paper, were soil properties (SP), including Olsen-P, total P, SOC, total N and pH, and soil-available micronutrients (SAM), including soil-available zinc, iron, copper and manganese. The canonical coefficients of the first pair of canonical variables (SP1, SAM1) and the second (SP2, SAM2) were 0.799, 0.472 separately, and the canonical correlations were both significant at p<0.01. The relationship between canonical variables and the corresponding concrete variables was represented by the following 4 equations:
According to the squared multiple correlations of canonical redundancy analysis, the prediction effect of canonical variables SP1, SP2 on SAM1, SAM2 declined in the order of Mn (0.5794) > Cu (0.2359) > Fe (0.0686) > Zn (0.0034), and Mn (0.5861) > Cu (0.2408) > Fe (0.2124) > Zn (0.1385), respectively. The soil properties, taken as a canonical variable, can well predict the availability of soil Mn but not Fe, Cu, and especially Zn.

Path analysis described the effects of soil properties on available micronutrients, and differentiated them into direct and indirect effects. A direct effect means an immediate impact of a soil property on available micronutrients via nothing, while an indirect effect specifies that through other properties. Effects on each micronutrient from soil properties by path analysis are shown in Table 2. For Zn, the direct impact mainly came from SOC, followed by soil total P and pH. Soil total P and pH directly influenced Fe most, and soil pH and SOC directly affected Cu most. Available Mn was influenced in the order of SOC > total N > total P > pH. Olsen-P had little direct effects on the micronutrients but exerted its influence mainly via SOC, total P and pH (data had not shown). We speculated that it was not soil Olsen-P but other forms of soil P (likely organic, Fe-bound P) that directly influence the availability of micronutrients. The negative direct path coefficients from soil pH on all micronutrients implied that the decrease of pH would directly increase their availability. The other properties played a positive role in the availability of soil micronutrients, except SOC for available Zn and total N for available Fe.

Total effects of soil properties on available micronutrients were listed in orders of total P > total N > pH > SOC > Olsen P for Zn, pH > total P > Olsen P > SOC > total N for Fe, SOC > pH > total P > Olsen P > total N for Cu, and SOC > total N > pH > total P > Olsen P for Mn, respectively. In general, soil pH, SOC and total P were the three important factors to directly influence the available micronutrients, and Olsen P played an indirect role via them, while soil total N had direct and indirect effects on available Zn and Mn, respectively, and no effect on available Fe and Cu. Many previous studies also demonstrated that long-term P practice caused changes in soil pH, soil organic carbon (SOC) and cation exchange capacity (CEC) [37-39], which, in turn, affect soil micronutrient contents [5, 32, 35].

An indirect effect means the path coefficient of soil properties to soil micronutrient via certain other soil properties, while a direct effect means the path coefficients of soil properties to soil micronutrient via nothing. Total effect is the sum of indirect and direct effects. TP = soil total phosphorus; Olsen P = soil-available phosphorus; TN = soil total nitrogen; SOC = soil organic carbon. *, ** = Values significant at p <0.05 and 0.01, respectively.

\[
\begin{align*}
SP1 & = 0.1105 \text{ Olsen P} - 0.102 \text{ pH} + 0.1581 \text{ total P} + 0.209 \text{ total N} + 0.7497 \text{ SOC} \\
SAM1 & = -0.2601 \text{ Zn} + 0.0131 \text{ Fe} + 0.2016 \text{ Cu} + 0.0936 \text{ Mn} \\
SP2 & = 0.0837 \text{ Olsen P} - 0.8525 \text{ pH} + 0.5493 \text{ total P} + 0.1607 \text{ total N} - 0.8026 \text{ SOC} \\
SAM2 & = 0.3636 \text{ Zn} + 0.9986 \text{ Fe} - 0.5648 \text{ Cu} - 0.0112 \text{ Mn}
\end{align*}
\]
4. CONCLUSION

Long-term P practice did not cause a decrease or insufficiency of available micronutrients in the topsoil. In contrast, their contents were still at high levels, especially for Mn and Cu. The contents of soil-available micronutrients in the 0-20 cm soil layer were higher than that in the 20-40 cm soil layer. Compared to the unfertilized control, long-term fertilization increased the availability of all available micronutrients, except Zn. Rates and methods of P application had great influence on micronutrients Zn and Fe, but not on soil Cu and Mn. It was concluded that P application once every year and once every 6 years contributed to the availability of soil Zn, Fe, respectively. Besides fertilization, soil properties also had significant influences on micronutrients. The soil micronutrients were obviously correlated with the soil properties, when taken as a canonical variable. And the soil properties can well predict the availability of soil Mn. Further path analysis showed that soil total P, pH and SOC were the three main factors which directly influence soil micronutrients, while Olsen-P exerts an indirect impact through them.

ACKNOWLEDGEMENTS

This work was financially supported by National Natural Science Foundation of China (Grant No. 41271317) and Science and Technology Infrastructure Program of the Ministry of Science and Technology of P. R. China (2012BAD14B02).

The authors have declared no conflict of interest.

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