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Phosphorus affects enzymatic activity and chemical properties of cotton soil

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Abstract: Pot experiments were conducted in 2017 with two cotton cultivars (CCRI 79 and LMY 28) and three phosphorus (P) levels: 3, 8 and 12 mg P_2O_5/kg as P_0, P_1 and P_2, respectively. In this study, the soil water-soluble organic carbon content increased as the soil available P (AP) increased, while there were no significant variations for soil total organic matter content among the three AP levels. The activities of invertase, cellulase and urease in cotton soil decreased significantly in the P_0. There were positive correlations between invertase and cellulose activities with soil organic carbon and inorganic-nitrogen (N); these correlated negatively with soil C/N ratio and AP level. In addition, high soil AP can raise soil AP and enhance alkaline phosphatase activity, which had a significant negative relationship with the soil C/P ratio. Urease activity had a significant positive relationship with soil NH_4^+-N, C/P and N/P, as well as a negative correlation with soil C/N. Moreover, soil NH_4^+-N and NO_3^--N in the P_1 and P_2 were lower than in the P_0, which might be an effect of high AP on soil N availability.

Keywords: soil available phosphorus; soil enzymes; C, N and P availability; plant nutrition; fertilization; soil microorganisms

Phosphorus (P) is an important nutrient in the soil; it limits plant growth and metabolism due to its low mobility and bioavailability. Low P availability is frequently a primary limiting factor for crop production in many soils (Shen et al. 2011, Mühlbachová et al. 2018). Indeed, it has been estimated that 5.7 billion hectares worldwide contain insufficient available P (AP) for optimal crop production (Cordell and White 2013, Niu et al. 2013), and about 30% of cultivated land in China has only 3–5 mg AP/kg soil (Wu 2013).

The management of soil quality is crucial to improve agricultural productivity and environmental quality. In the absence of fertilizer inputs, soil organic carbon (OC) is a primary source of plant nutrients. P application can increase soil C, N and P (Griffiths et al. 2012), especially soil AP for plants (Djodjic and Mattsson 2013), and subsequently reduce the C/P ratio in the rhizosphere. In addition, increasing soil AP can affect the C/P ratio by decreasing the carbohydrate contents released per unit of plant root (Schilling et al. 1998, DeForest et al. 2012). However, little is known about soil C, N and P availability in low AP soil for cotton. Soil total organic carbon (TOC) can promote the accumulation of soil nutrients such as alkali-hydrolyzed nitrogen, AP and available potassium by soil enzymes (Bowles et al. 2014). Thus, the soil enzymes are generally used to evaluate the factors affecting fertilizer application, soil quality and plant litter decomposition (Bowles et al. 2014, Mi et al. 2018). These soil enzymes associated with

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the C, N, and P cycles are invertase, β-glucosidase, and cellulase, urease, and phosphatase (Adetunji et al. 2017).

Invertase acts by the organic compounds degrading to cut down their molecular size and make smaller organic structures, thus promoting microbial enzymatic activities (Li et al. 2014). β-glucosidase can hydrolyze the cellobiose residue during the final stage of the cellulose degradation process (Adetunji et al. 2017). Cellulase catalyzes the hydrolysis of cellulose to glucose. Urease is a crucial enzyme regulating nitrogen (N) conversion in soil and is mainly derived from microorganisms and plants (Li et al. 2014). Alkaline phosphatases (ALP) act by hydrolyzing simple phosphomonoesters and acquiring orthophosphate (Fraser et al. 2017), and are only produced by soil microorganisms (Spohn and Kuzyakov 2013). It is of great significance to study the response characteristics of these soil enzymatic activities to different soil AP levels and their relationships with soil chemical properties in order to reveal the rules for transformation and utilization of soil C, N and P, and in addition to improve the utilization efficiency of P fertilizer in cotton soil.

At present, few studies have been published on the effects of soil AP levels on soil enzymatic activities and active organic matter in cotton soil (Chen et al. 2014). The present study used soil with three different soil AP levels to evaluate the effect of soil AP levels on soil enzymatic activities and chemical properties, to provide data support and a scientific basis for evaluation of cotton soil quality, establishment of a reasonable fertilization system and improvement of land productivity.

MATERIAL AND METHODS

Experimental design. A pot experiment was conducted in 2017 at the Baibi station, Anyang, Henan, China (36°06'N, 114°21'E). In a depth of 0–20 cm before sowing cotton, the TOC and total N, available N and available K of the soil were 16.2 g/kg, 1.0 g/kg, 71.2 mg/kg and 106.7 mg/kg, respectively. Cotton seeds of cvs. CCRI 79 and LMY 28 were planted into plastic pots on April 19th, 2017. The quantitative irrigation was applied as needed to minimize the moisture stress during each season.

The treatments for the pot experiments consisted of three AP (P) levels: (i) < 3 ± 0.5 mg/kg (P_0, P deficiency), (ii) 8 ± 0.5 mg/kg (P_1), and (iii) 12 ± 0.5 mg/kg using triple superphosphate (P_2, moderate P under the AP in this experiment) (Zheng et al. 2015), as a control. In each plot at the flower and boll stage and the boll-opening stage, three soil samples from 0–20 cm depth were chosen from three replicate plots randomly. The samples were then used for the measurement of soil enzymes and soil chemical properties.

Determination of soil chemical properties and soil enzymes. Soil TOC was measured using the dichromate oxidation method (Hai et al. 2010). Soil water-soluble organic carbon (WSOC) was measured using the dichromate oxidation method. Soil water-soluble total nitrogen (WSTN) was measured using the Kjeldahl acid-digestion method after extraction with sodium hydroxide-potassium persulfate. Soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) were analysed with the automated discrete analyser (SmartChem 200, AMS Alliance, Rome, Italy) after soil samples were extracted with potassium chloride, and then shaken at 200 rpm for 1 h. Soil AP was analysed using the Olsen-P method (Yang and Jacobsen 1990). Kits for invertase, β-glucosidase, cellulose, urease and ALP were acquired from the Suzhou Coming Biotechnology, Suzhou, China.

Statistical analysis. Data were handled by SPSS V24.0 (IBM corp., New York, USA), and the differences between mean values with $P < 0.05$ were determined as significant. The ratios of WSOC to WSTN (C/N), SOC to AP (C/P), WSTN to AP (N/P) were also calculated.

RESULTS

Effect of phosphorus levels on soil enzymatic activity

Soil enzymes related to C cycle. During cotton growth, the activity of invertase in the soil increased (Figure 1a,d). With increasing soil AP level, soil invertase activity decreased significantly ($P < 0.05$). Compared to the P_0, the soil invertase activity in the P_1 and P_2 for cv. CCRI 79 decreased by (–1.8)–15.0% and 25.3–37.6%, respectively, and by 20.5–25.4% and 35.1–69.0% for cv. LMY 28, respectively. The changing trend of soil cellulase activity was similar to that of invertase (Figure 1c,f).

The activity of β-glucosidase exhibited an increasing trend with cotton growth (Figure 1b,e). There were no significant differences in soil β-glucosidase activity by soil AP level. However, the soil β-glucosidase activity initially increased and then decreased as the soil AP level increased. Compared to the P_0, soil β-glucosidase activity for cv. LMY 28 in the P_1 and P_2 increased by 8.1–27.1% and 1.0–13.6%, respectively.

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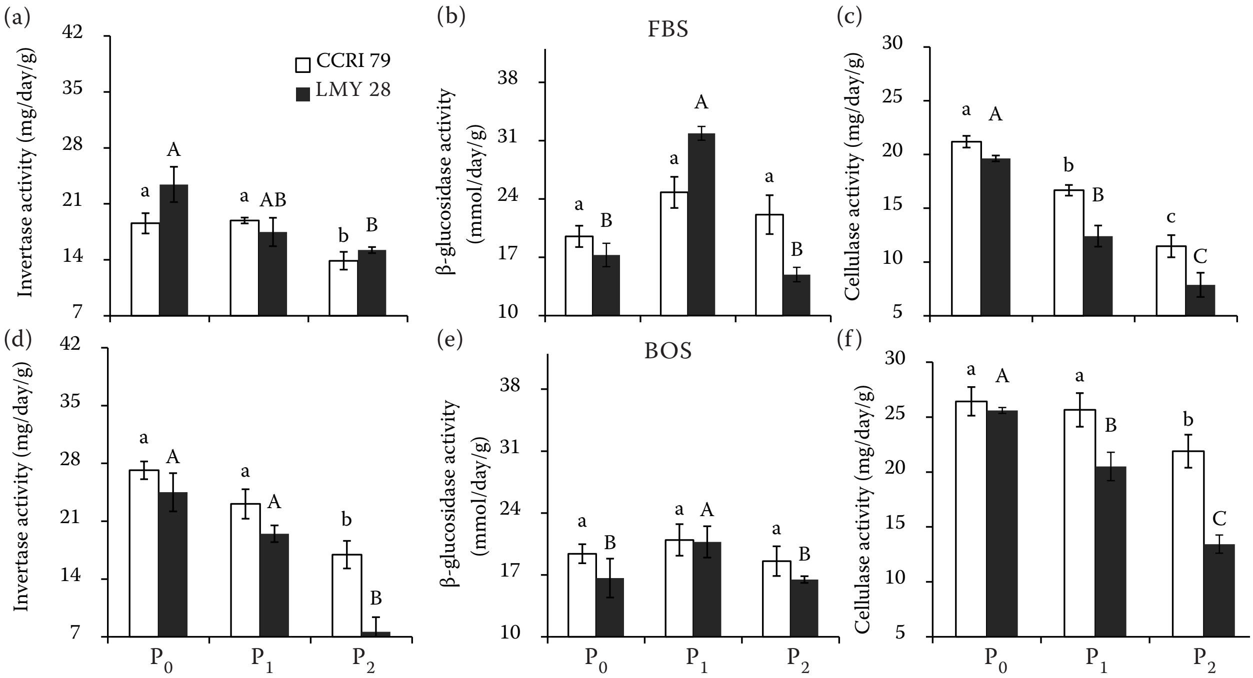

Figure 1. Effect of phosphorus levels on soil invertase activity, β-glucosidase activity and cellulase activity. Values followed by a different letter within the same figure mean significant difference at 0.05 probability level. Lowercase letters and uppercase letters indicate cvs. CCRI 79 and LMY 28, respectively. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg; FBS – the flower and boll stage; BOS – the boll-opening stage

Soil enzyme related to N cycle-urease. Soil urease activity increased with cotton growth and decreased significantly as soil AP increased ($P < 0.05$, Figure 2). Analysis of variance showed that there was no significant difference in soil urease activity between the P_0 and P_1. During the flowering and boll stage, soil urease activity in the P_2 treatment decreased by 14.2% for cv. CCRI 79 and by 21.7% for cv. LMY 28, compared with the P_0. However, the soil urease activity of cv. CCRI 79 was not significantly affected by soil AP level during the boll-opening stage, and only the soil urease activity of cv. LMY 28 decreased by 27%.

Soil enzyme related to P cycle-ALP. The soil ALP activity increased significantly as the soil

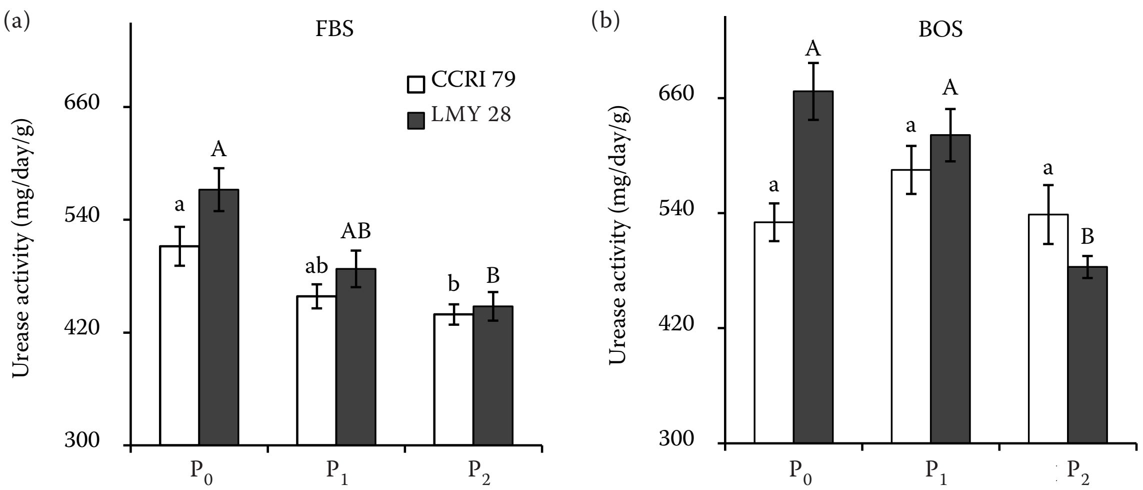

Figure 2. Effect of phosphorus levels on soil urease activity. Values followed by a different letter within the same figure mean significant differences at 0.05 probability level. Lowercase letters and uppercase letters indicate cvs. CCRI 79 and LMY 28, respectively. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg; FBS – the flower and boll stage; BOS – the boll-opening stage

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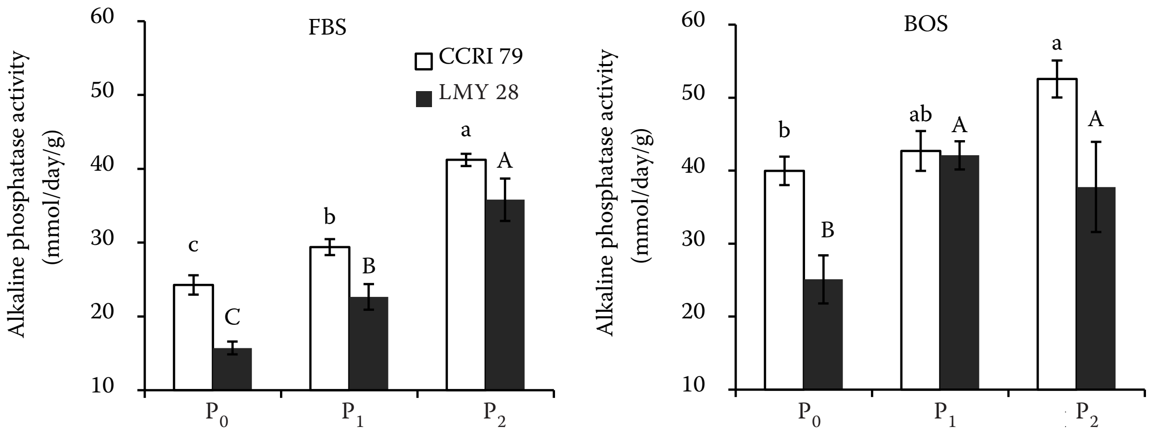

Figure 3. Effect of phosphorus levels on soil alkaline phosphatase activity. Values followed by a different letter within the same figure mean significant differences at 0.05 probability level. Lowercase letters and uppercase letters indicate cvs. CCRI 79 and LMY 28, respectively. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg; FBS – the flower and boll stage; BOS – the boll-opening stage

AP increased, and the range by which it changed during flowering and boll period was higher than that during boll-opening period ($P < 0.05$, Figure 3). With the increase of soil AP, the soil ALP activity for cv. CCRI 79 and cv. LMY 28 increased by 21.2–69.9% and 44.0–127.7%, respectively. In addition, the soil ALP activity for cv. CCRI 79 was significantly higher than that for cv. LMY 28.

Effect of P levels on soil chemical properties

Soil AP content. Soil AP level increased significantly with increasing soil AP level (Figure 4), and its content was basically within the range of soil AP level set by the current research.

Soil TOC and WSOC content. There were no significant differences among the three soil AP levels for soil TOC content ($P > 0.05$, Figure 5a). Nevertheless, the soil WSOC content showed an increasing trend with increasing soil AP levels (Figure 5b). Compared to the P_0, the soil WSOC content in the P_1 and P_2 increased by 5.9–10.0% and 15.0–17.0%, respectively. The C/P ratio in soil was > 500 and increasing AP levels gradually decreased the soil C/P ratio.

Soil NH_4^+-N, NO_3^--N and WSTN content. The NH_4^+-N content decreased with the increase of soil AP (Figure 6a). Analysis of variance showed that the P_0 and P_1 had no significant difference for NH_4^+-N content in soil, and both reached significant levels compared with the P_2 ($P < 0.05$). Compared with the P_0, the NH_4^+-N content for cv. CCRI 79 and cv. LMY 28 in the P_2 reduced by 22.8% and 15.1%, respectively. The NO_3^--N content showed a significant downward trend with increasing soil AP (Figure 6b). Compared with the P_0, soil NO_3^--N content in the P_1 and P_2 decreased by 25.5–44.5% and 36.2–59.1%, respectively.

The WSTN content significantly decreased with increasing soil AP (Figure 6c). However, the soil WSTN for cv. CCRI 79 and cv. LMY 28 only showed a significant variation between the P_0 and P_2 ($P < 0.05$). Compared with the P_0, soil WSTN content in the P_2 was reduced by 21.2% for cv. CCRI 79, and by 13.7% for cv. LMY 28. However, soil C/N ratio showed the opposite trend as the soil AP increased (Table 1). In addition, the C/N ratio had a significant variation in

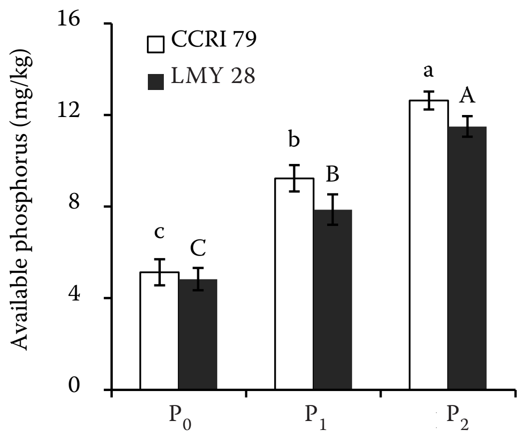

Figure 4. Effect of phosphorus levels on soil available phosphorus content. Values followed by a different letter within the same figure mean significant differences at 0.05 probability level. Lowercase letters and uppercase letters indicate cvs. CCRI 79 and LMY 28, respectively. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg

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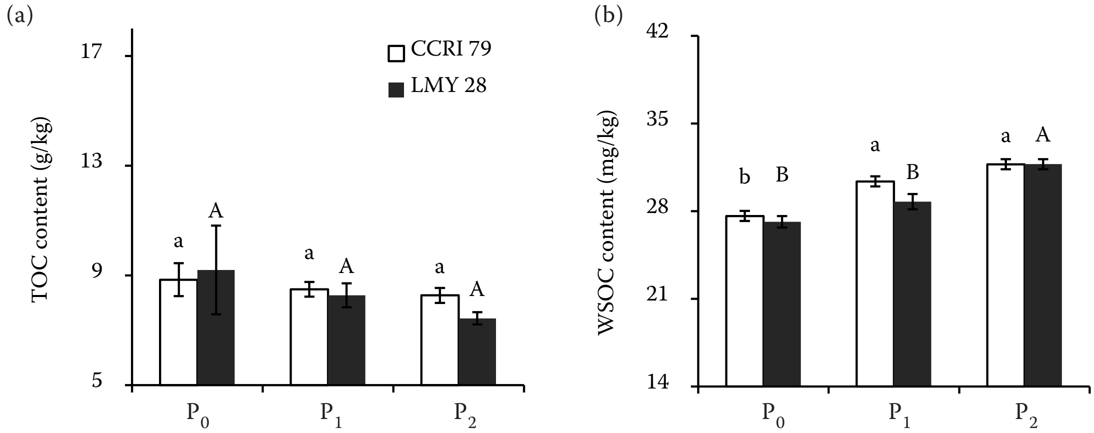
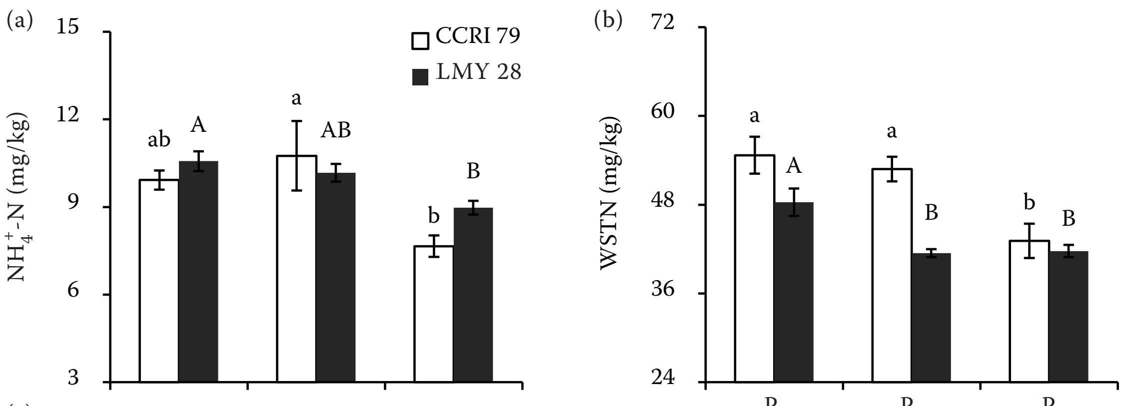


Figure 5. Effect of phosphorus levels on soil organic carbon (TOC) and water-soluble organic carbon (WSOC) content. Values followed by a different letter within the same figure mean significant differences at 0.05 probability level. Lowercase letters and uppercase letters indicate cvs. CCRI 79 and LMY 28, respectively. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg

cotton soil only between the P_0 and P_2. Moreover, the soil C/P ratio was gradually reduced with increasing AP levels.

Correlation analysis between soil enzymatic activities and soil chemical properties. Soil invertase, cellulose and urease had significantly positive correlations with soil TOC and NH_4^+-N, and negative correlations with soil WSOC and AP (Table 2). In addition, soil ALP had significantly positive correlations with soil WSOC and AP and negative correlations with NH_4^+-N. Soil cellulose was significantly correlated with NO_3^--N. Moreover, these four enzymes also significantly correlated with each other, because they belong to the same group of hydrolases. The



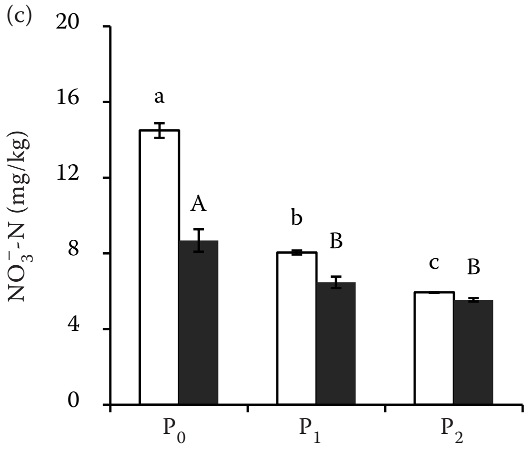


Figure 6. Effect of phosphorus levels on soil NH_4^+-N, NO_3^--N and water-soluble total nitrogen (WSTN) content. Values followed by a different letter within the same figure mean significant differences at 0.05 probability level. Lowercase letters and uppercase letters indicate cvs. CCRI 79 and LMY 28, respectively. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg

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Table 1. Effect of phosphorus levels on the ratio of C/P, C/N and N/P in cotton soil

Phosphorus level	C/P ratio		C/N ratio		N/P ratio	
	CCRI 79	LMY 28	CCRI 79	LMY 28	CCRI 79	LMY 28
P_0	1767[a]	1902[a]	0.51[b]	0.56[b]	10.89[a]	10.40[a]
P_1	926[bc]	1072[b]	0.58[b]	0.69[a]	5.79[b]	5.83[b]
P_2	656[c]	649[c]	0.74[a]	0.76[a]	3.41[b]	3.41[b]

Values followed by the different letters within the same index are significantly different at $P < 0.05$ probability level. P_0 – 3, P_1 – 8, P_3 – 12 mg P_2O_5/kg

correlation between β-glucosidase and soil chemical properties did not reach significant levels (data not shown).

DISCUSSION

Soil C, N and P availability. WSOC is the intermediate product of soil OC transformation and microbial metabolism. These two competing processes contribute to soil management and/or plant nutrient availability, to microbial activity and a series of enzymes that determine plant productivity and/or soil fertility (Powlson et al. 2011). In this study, soil WSOC increased with increasing soil AP (Figure 5b), while there were no significant differences for soil TOM among the three soil AP levels (Figure 5a). Generally, the application of fertilizers can directly increase the soil OC and release more WSOC when decomposition by microorganisms occurs (Pospíšilová et al. 2011, Burns et al. 2013). In addition, the C/N ratio in this study increased significantly with the increase of soil AP level (Figure 6c), indicating that the soil C/N ratio can respond sensitively to soil nutrient changes, and it also verified the importance of WSOC as an index for evaluating soil fertility. Moreover, because of higher soil C/N ratio and N fixation (Gu et al. 2018), soil N loss in the P_2 was less than that in the P_0 or P_1. For the above reasons, significantly higher soil N in the P_2 was observed than in the P_0 or P_1.

The mineralization of P by soil microorganisms is greater than the immobilization due to a low C/P ratio. However, more P is fixed than mineralized, leading to P competition between soil microorganisms and plants with higher C/P ratio (Zhang et al. 2014). High AP reduced the C/P ratio in the P_1 and P_2 soil with the direct P supply, indicating that high P may indirectly improve organic P mineralization mediated

Table 2. Correlation analysis between soil chemical properties and soil enzymatic activities

	OC	NH_4^+-N	NO_3^--N	AP	WSOC	WSTN	C/P	C/N	N/P	Invertase	Cellulase	Urease
NH_4^+-N	ns											
NO_3^--N	ns	ns										
AP	–0.507*	–0.636**	–0.704**									
WSOC	–0.556*	–0.517*	–0.633**	0.950**								
WSTN	0.476*	ns	0.748**	–0.512*	ns							
C/P	0.488*	0.471*	0.708**	–0.941**	–0.912**	ns						
C/N	–0.582*	–0.517*	–0.806**	0.782**	0.730**	–0.916**	–0.734**					
N/P	0.601**	0.483*	0.836**	–0.777**	–0.717**	0.927**	0.727**	–0.992**				
Invertase	0.693**	0.704**	ns	–0.754**	–0.739**	0.481*	0.653**	–0.680**	0.668**			
Cellulase	0.564*	0.511*	0.818**	–0.798**	–0.763**	0.747**	0.826**	–0.892**	0.880**	0.660**		
Urease	0.625**	0.505*	ns	–0.769**	–0.777**	ns	0.802**	–0.604**	0.590**	0.781**	0.666**	
ALP	ns	–0.623**	ns	0.893**	0.864**	ns	–0.817**	0.591**	–0.562*	–0.684**	–0.626**	–0.725**

ns – no significance at the $P < 0.05$; *$P < 0.05$; **$P < 0.01$; $n = 18$, $R_{0.05} = 0.4683$; $R_{0.01} = 0.5897$; OC – organic carbon; AP – available phosphorus; WSOC – water-soluble organic carbon; WSTN – water-soluble total nitrogen; ALP – alkaline phosphatases

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by soil microorganism. This resulted in a significant negative relationship between the soil AP and C/P ratio (Table 2). In addition, the osmotic metabolites, including sugars, amino acids and carboxylic acids, increase under AP deficient conditions in plants (Spohn and Kuzyakov 2013), possibly to significantly improve soil microbial-mediated mineralization of organic P to meet the demand of microorganism.

The soil N/P ratio can indicate the relative restriction of soil nutrients to plant growth. N is relatively sufficient for a higher N/P ratio and P is relatively sufficient for a lower N/P ratio (Liu et al. 2010). The P_2 had a high soil AP and low soil C/P or N/P ratios, suggesting that the supply of soil mineralized P was sufficient, and the N availability in cotton soil was low. Hence, WSOC had significant positive correlations with soil AP, and negative correlations with the C/P and N/P ratios (Table 2), suggesting that soil microorganisms were likely to be restricted by P.

Soil enzymes. Soil enzymes are often involved in nutrient cycling and availability, SOC decomposition and synthesis, and soil fertility and quality, as indicators for microbiological and biochemical processes (Burns et al. 2013, Moghimian et al. 2017). In this study, the activities of invertase, cellulase and urease in cotton soil decreased significantly in the P_0. It is possible that the P deficient in the P_0 would induce the growth of soil microorganisms, stimulate the activity of soil invertase, cellulose and urease, and increase the C and N utilization in soil, which is consistent with the previous studies (Moghimian et al. 2017). As positive relationships were observed between invertase and cellulose activities with OC in cotton soil, and inorganic-N (mainly NH_4^+-N); soil C/N ratio is negatively correlated with soil AP (Table 2). Although inorganic-P is given to have a feedback inhibition for ALP (Olander and Vitousek 2000), it was not observed to inhibit soil ALP activity with increasing soil AP (Figure 4). One possible reason is that most of the ALPs were not restrained by high P because they have stable interactions with colloids (Dilly and Nannipieri 2001).

ALPs can hydrolyse phosphate monoesters to obtain orthophosphate, and are only produced by soil microorganisms (Spohn and Kuzyakov 2013, Fraser et al. 2017). In addition, soil microorganisms, produced by ALPs, are more susceptible to P fertilization than other phosphatases, which is evident from the raised ALP activity due to P fertilization (Chen et al. 2014); this corresponds with the results of our study, which observed significant positive correlations between soil AP and ALP (Table 2). Hence, the soil C/P ratio had a significant negative correlation with the ALP activity.

Urease is mainly derived from plants and microorganisms; it has a crucial role in the N cycling process (Li et al. 2014). When NH_4^+ is the preferred N source, the synthesis of urease in cells is blocked (Geisseler et al. 2010). Nevertheless, an alternative N source or urea activates urease production (Mobley et al. 1995). In this study, the activity of urease had a significant positive correlation with soil NH_4^+-N, N/P and C/P, and a negative relationship with soil C/N, suggesting that the high soil urease activity for the P_0 indicates a lager N cycle potential due to a lower C/N ratio. In addition, soil NH_4^+-N and NO_3^--N for the P_1 and P_2 were lower than those for the P_0, possibly due to an influence of high AP on soil N availability.

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