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Comparison of soil phosphorus and phosphatase activity under long-term no-tillage and maize residue management

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Abstract: The migration and transformation of soil phosphorus (P) are essential for agricultural productivity and environmental security but have not been thoroughly elucidated to date. A 10-year field study was conducted to explore the effects of conventional tillage (CT) and no-tillage with maize residue management (NT-0, NT-33%, NT-67% and NT-100%) on P contents and phosphatase activities in soil layers (0–5, 5–10, 10–20 and 20–40 cm). The results showed that soil available P content and phosphatase activities were higher in no-tillage with maize residue than CT. Soil moisture and pH were significantly positively correlated with soil available P. Higher organic P contents and lower inorganic P contents in the 0–5 cm soil layer were found in the treatment NT-67% compared with other treatments. According to the structure equation model, the source of available P was inorganic P in NT-33%, while organic P in NT-67%. This study demonstrated that the variation of dominant mechanisms involved in soil P migration and transformation were dependent on residue input amounts, and NT-67% might play an important role in the maintenance and transformation of soil organic P.

Keywords: P cycle; nutrient; soil fertility; sustainable development; agriculture

In Northeast China, one of the major mollisol areas in the world was over cultivated, and its fertility has been reduced seriously. Conventional tillage has been used for cereal production in this region for a long time. In this process, postharvest residues of maize were removed or burnt. Then, the soil was plowed prior to sowing the new season's maize, and the process has led to serious soil degradation (Jiang et al. 2018). In recent years, conservation tillage has gradually replaced conventional tillage to protect and recover soil quality. Conservation tillage involves the two key components of minimal soil disturbance (e.g., reduced tillage or no-tillage) and soil cover (e.g., residue retention), which has potential applications in minimizing soil erosion and degradation (Powlson et al. 2016). Postharvest residues are sources of nutrients and primary organic matter (Torma et al. 2018).

Phosphorus (P) represents one of the major soil nutrients limiting agricultural production (Mühlbachová et al. 2018). Organic P is the predominant form of total P in most soils, accounting for 50% or more of total P content, but most organic P cannot be utilized directly by crops (Garg and Bahl 2008). Organic P acquired by plants and microorganisms must primarily be mineralized by specific phosphatases (Nannipieri et al. 2011), such as phosphomonoesterase and phosphodiesterase. At present,

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low utilization rate of soil P and excessive use of P fertilizer cause the loss of P, which results in the eutrophication of surface water (Leinweber et al. 1999). Therefore, it is necessary to study how to increase soil P storage and the utilization efficiency of P by adopting optimized soil agricultural management. Straw coverage is an effective residue management that regulates the availability of P in soil by affecting the microbial community and P level with increasing carbon source (Margenot et al. 2017).

No-tillage and maize straw coverage were both expected to improve P availability by reducing soil P fixation and increasing available P in plants and supporting organic P storage and its mineralization via greater phosphatase activities due to microorganism release. Therefore, the objectives of this study were (1) to evaluate the impact of no-tillage and maize residue management after 10 years on soil P migration and transformation; (2) to provide a suitable amount of maize straw coverage to increase our understanding of the variation mechanism of soil P transformation in the mollisol area of northeastern China.

MATERIAL AND METHODS

Study site and experimental design. The study site was located at the Lishu Conservation Tillage Research and Development Station of the Chinese Academy of Sciences (43°19'N, 124°14'E), Jilin province, which is located in northeastern China. The site has a temperate subhumid continental monsoon climate with a mean annual precipitation of 614 mm and mean annual temperature of 6.9°C. The soil is classified as mollisol (IUSS working group WRB, 2007). The initial properties of the 0–20 cm soil layer were 6.55 g/kg soil organic carbon, 1.2 g/kg total N, 0.38 g/kg total P and pH_{H_2O} 7.1 in 2007.

In May 2007, the experiment employed a randomized complete block design with four replicates. The size of each plot in each replication was 261 m^2 (8.7 m × 30 m). The treatments were no-tillage without maize straw coverage (NT-0), no-tillage with 2.5 t/ha maize straw coverage (NT-33%), no-tillage with 5 t/ha maize straw coverage (NT-67%), and no-tillage with 7.5 t/ha maize straw coverage (NT-100%). Background fertilizer inputs were 100 kg N/ha, 45.5 kg P/ha and 78 kg K/ha of urea, diammonium hydrogen phosphate and potassium chloride, respectively. Conventional tillage (CT) served as a control. The amount of total P added was 1.8, 3.6 and 5.4 g/kg in NT-33%, NT-67% and NT-100%, respectively. The no-tillage plots were left undisturbed, except for the surface drilling of the spring maize, and maize straw was evenly distributed over the field surface after each year's harvest. The CT plots were rotary tilled to a depth of 25 cm to 30 cm before the crops were planted, and maize straw was removed from the field after harvest (Jiang et al. 2018).

Soil sampling. Soil samples were collected in late April 2018. Five randomly placed soil cores (0–5, 5–10, 10–20 and 20–40 cm depth) were taken from each subplot using a soil auger (3 cm in diameter) to form one composite sample. After removing visible plant residues and stones, each sample was homogenized and passed through a 2-mm sieve. A subsample was stored in a plastic bag at 4°C in the field and immediately frozen at –20°C after it was returned to the laboratory for biological analysis. The remainder of the soil samples was air-dried for the measurement of abiotic properties.

Soil property analysis. Soil pH was determined at a 1:5 soil/deionized water ratio using a glass electrode. Soil moisture was measured using the oven-dried method (105°C for constant weight). Soil total organic acid was measured by colorimetric determination (Montgomery et al. 1962). The measurement for cation exchange capacity (CEC) followed the methods described in Page et al. (1982). Total carbon (TC) and total nitrogen (TN) levels in soils were determined via the dry combustion method using an automatic element analyzer (Analyzer Vario Micro cube, Elementar, Hanau, Germany) (Zhang et al. 2012).

Soil P and phosphatase activity assays. Total P (TP) was first combusted by a muffle furnace at 550°C for 1 h. Then, P was extracted with 1 mol/L H_2SO_4. Inorganic P (IP) was extracted with 0.5 mol/L H_2SO_4 (1:25 soil-to-solution ratio) (Kuo 1996). Available P (AP) was extracted with 0.5 mol/L $NaHCO_3$ (Olsen et al. 1954). All of the produced orthophosphate was detected by molybdate colorimetry at 880 nm. Organic P (OP) was calculated as the difference between TP and IP. Phosphatase activities including acid phosphomonoesterase (AcP), alkaline phosphomonoesterase (AlP) and phosphodiesterase (PD) were investigated with a modified version of the fluorimetric microplate assay described in Sinsabaugh et al. (2000).

Statistical analysis. All values were expressed based on the oven-dried soil (105°C) weight. The differences among means for soil P and phosphatase activity in no-tillage with straw coverage croplands were evaluated separately by a one-way general analysis of variance (ANOVA) with a Duncan's test at the

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P = 0.05 level. The correlations among soil P, phosphatase activities and soil property variables were based on Pearson's correlation coefficients. All statistical analyses were conducted using SPSS 22.0 (SPSS, Chicago, USA). The graphs were generated using Origin Pro 8.5 (OriginLab Corp., Northampton, USA).

Structural equation modeling (SEM) was established by AMOS (version 17.0, IBM, SPSS, New York, USA) to better understand the relationships between available P and organic P or inorganic P. The model fit was assessed using the maximum likelihood chi-squared test (χ^2; the model has a good fit when χ^2 was low and the P value ≤ 0.05), comparative fit index (CFI; the model has a good fit when 0.9 ≤ CFI ≤ 1), goodness of fit (GFI; the model has a good fit when 0.9 ≤ GFI ≤ 1), and root square mean error of approximation (RMSEA; the model has a good fit when 0 ≤ RMSEA ≤ 0.05). To obtain the most parsimonious models, uninformative weak pathways were removed stepwise in the final models.

RESULT AND DISCUSSION

Soil P contents. In 0–5 cm, NT-33% showed a higher IP, while NT-67% exhibited a lower value of IP and exerted the greatest effect on OP (Figure 1). Compared with CT, no-tillage with maize straw coverage significantly increased AP levels in four soil layers, and NT-67% had a higher soil AP at 0–5 cm. This model explained 79% of the variation in AP in NT-33%, 94% in NT-67% and 84% in NT-100% (Figure 2). This model revealed that IP had the strongest direct effect on AP (path coefficient = 0.89**) in NT-33% and a stronger direct effect on AP (path coefficient = 0.50*) in NT-100%. However, the effect of OP on AP was significant (path coefficient = 0.64**) in NT-67%.

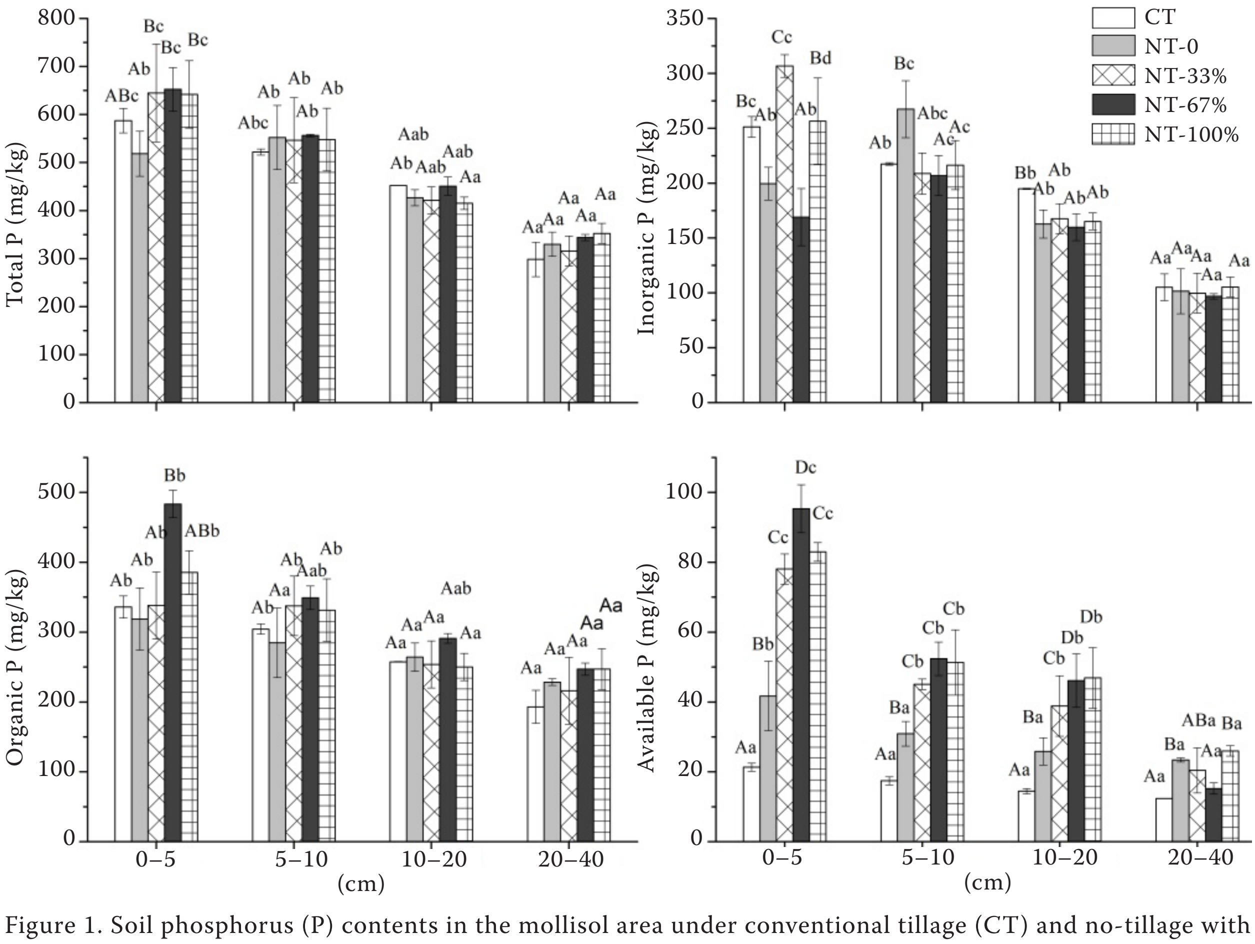

Figure 1. Soil phosphorus (P) contents in the mollisol area under conventional tillage (CT) and no-tillage with maize residue management. Error bars represent standard deviation. Uppercase letters indicate differences among different treatments in the same soil layers. Lowercase letters indicate differences among different soil layers in the same treatment. NT-0 – no-tillage without maize straw coverage; NT-33% – no-tillage with 2.5 t/ha maize straw coverage; NT-67% – no-tillage with 5 t/ha maize straw coverage; NT-100% – no-tillage with 7.5 t/ha maize straw coverage

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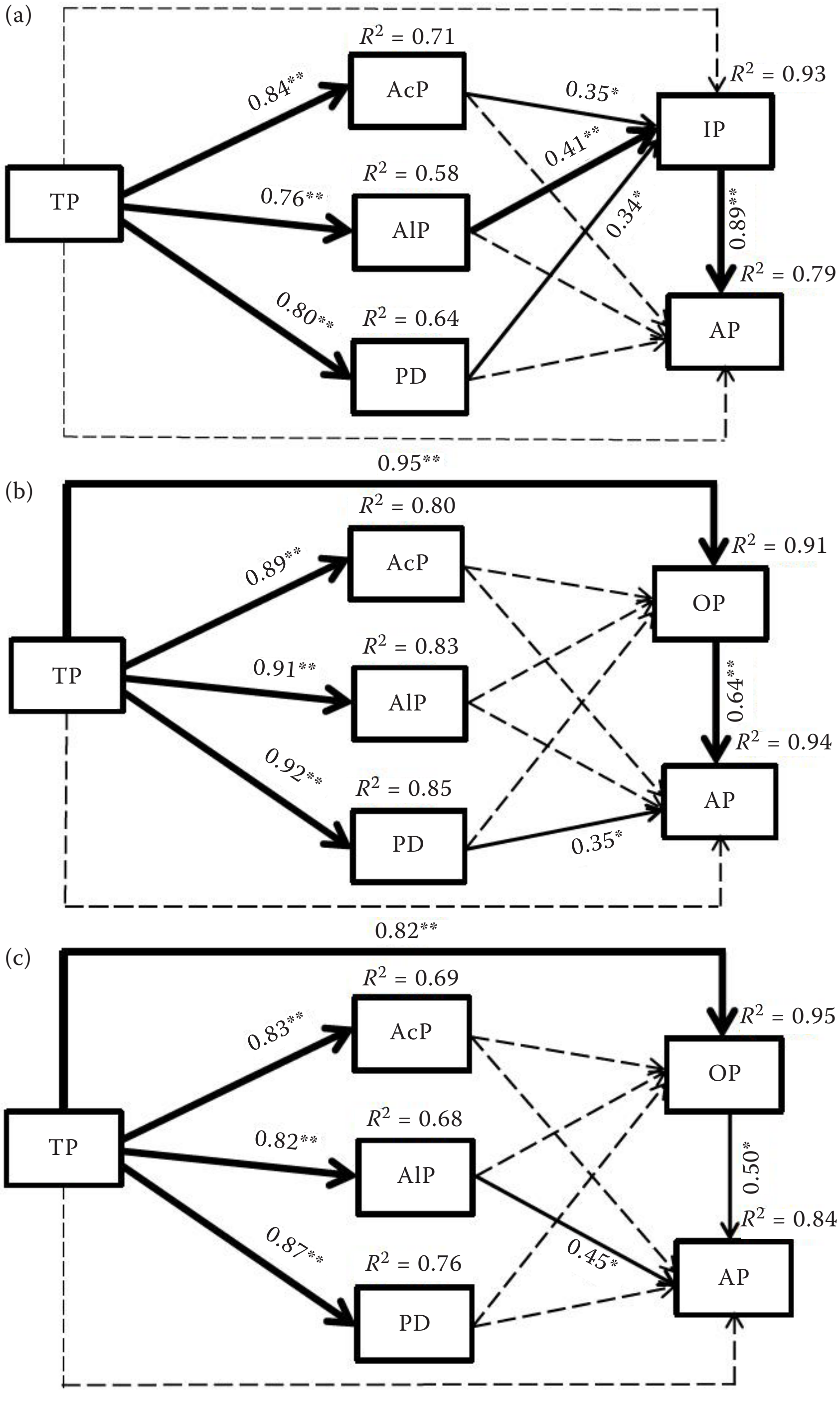

Figure 2. Structural equation modeling results for relationships between soil phosphorus (P) contents in soil and associated soil phosphatase activities under no-tillage with maize residue management in (a) NT-33% (χ^2 = 0.5044, *df* = 8, *P* = 0.753, CFI = 1.000, NFI = 0.944, and RMSEA = 0.000); (b) NT-67% (χ^2 = 4.870, *df* = 9, *P* = 0.845, CFI = 1.000, NFI = 0.961, and RMSEA = 0.000) and (c) NT-100% (χ^2 = 2.543, *df* = 8, *P* = 0.960, CFI = 1.000, NFI = 0.975, and RMSEA = 0.000). TP – total P; IP – inorganic P; OP – organic P; AP – available P; AcP – acid phosphomonoesterase; AlP – alkaline phosphomonoesterase; PD – phosphodiesterase; NT-0 – no-tillage without maize straw coverage; NT-33% – no-tillage with 2.5 t/ha maize straw coverage; NT-67% – no-tillage with 5 t/ha maize straw coverage; NT-100% – no-tillage with 7.5 t/ha maize straw coverage; CFI – comparative fit index; GFI – goodness of fit; RMSEA – root square mean error of approximation. Numbers in bold indicate the variance explained by the model (R^2). Numbers on arrows are standardized path coefficients. Bold arrows indicate statistically significant paths at $P < 0.05$, and thick bold arrows indicate statistically significant paths at $P < 0.01$. Dashed arrows indicate nonsignificant paths hat are removed in the final model

No-tillage increased available P contents (Sisti et al. 2004), which could be attributed to the notion that no-tillage treatment weakened P binding due to reduced contact between solution P and soil particles. The residue input amount increased available soil P contents; similar results were reported by previous studies (Alamgir et al. 2012). This finding may be attributed to the notion that residues containing high total P contents benefited P mineralization and thus increased available P (Nziguheba et al. 2000). Additionally, residue retention with organic matter addition could reduce P fixation due to increasing organic anion competition for P binding sites (Margenot et al. 2017). The no-tillage and residue input increased the soil total P, inorganic P and organic P in the soil surface layers (0–10 cm), which is consistent with findings from other researchers (Wei et al. 2014). However, the lack of a significant effect

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of the tillage and maize residue management on P contents in the deeper soil layers could be attributed to the dilution of deeper soil layers during sampling. Additionally, NT-33% exhibited the highest inorganic P concentration in the 0–5 cm layer, while NT-67% exhibited the lowest inorganic P concentration. In contrast, the organic P concentration exhibited different trends in response to NT-33% and NT-67% of the 0–5 cm layer. This result revealed that inorganic P (main leaching form) was reduced, and organic P was increased. These results indicated that the no-tillage and residue input system could reduce the risk of P leaching, and NT-67% was optimized for maize systems in this region. Moreover, organic P plays an important role in the soil P cycle after tillage and maize residue management, which was consistent with the findings of Margenot et al. (2017). Structural equation modeling demonstrated that the source of available P was inorganic P in NT-33%, while the source of available P was organic P in NT-67% (Figure 2). This finding also confirmed that the no-tillage and maize residue management system could change the soil P migration and transformation, in which residue inputs resulted in the different source of available P. Medium P residues (NT-67% with 3.6 g P/kg) increased the net mineralization of labile organic P (Alamgir et al. 2012). This finding indicates mineralization of organic P when the amount of P in the residues exhibited relative fitness, whereas the residues with lower and higher P resulted in the conversion of some mineralized P into stable P.

Soil properties and maize yield. Soil pH and moisutre in the treatment of NT-67% were significant higher than other that in CT (*P* < 0.05) (Table 1). TC

Table 1. Soil properties in the mollisol area under no-tillage with maize residue management

Treatment	Soil layer (cm)	Soil pH	SM (%)	CEC ($mmol_+$/kg)	Organic acid (mg/g)	Total C (g/kg)	Total N (g/kg)	Maize yield (kg/ha)
CT	0–5	6.9 ± 0.06^{A}	15.9 ± 2.15^{Aa}	285 ± 0.23^{Aa}	1.3 ± 0.13^{Bb}	14.2 ± 0.70^{Ab}	1.4 ± 0.08^{Ab}	12 567 ± 608
	5–10	6.7 ± 0.02^{A}	17.9 ± 0.85^{Aa}	287 ± 0.44^{a}	0.6 ± 0.01^{a}	11.8 ± 0.47^{a}	1.2 ± 0.03^{a}	
	10–20	6.5 ± 0.06^{A}	20.1 ± 2.02^{Ab}	296 ± 0.07^{a}	0.9 ± 0.07^{Aa}	11.5 ± 0.19^{a}	1.2 ± 0.01^{a}	
	20–40	6.5 ± 0.05^{A}	20.0 ± 0.82^{Ab}	320 ± 1.27^{b}	1.0 ± 0.08^{Bab}	13.2 ± 0.53^{b}	1.1 ± 0.05^{a}	
NT-0	0–5	6.8 ± 0.02^{A}	18.7 ± 0.54^{AB}	265 ± 1.52^{Aa}	0.9 ± 0.20^{ABab}	15.8 ± 0.20^{Ab}	1.6 ± 0.03^{Ab}	13 428 ± 692
	5–10	7.1 ± 0.04^{AB}	18.9 ± 1.26^{A}	284 ± 2.71^{a}	0.7 ± 0.08^{a}	12.3 ± 0.06^{a}	1.3 ± 0.01^{a}	
	10–20	7.0 ± 0.07^{AB}	19.8 ± 1.36^{A}	283 ± 1.48^{a}	1.1 ± 0.16^{ABb}	11.5 ± 0.23^{a}	1.2 ± 0.03^{a}	
	20–40	6.7 ± 0.25^{A}	20.2 ± 0.45^{A}	305 ± 3.73^{b}	1.0 ± 0.11^{Bb}	12.3 ± 0.53^{a}	1.0 ± 0.03^{a}	
NT-33%	0–5	6.8 ± 0.07^{A}	21.3 ± 0.40^{B}	328 ± 6.97^{Bb}	0.7 ± 0.04^{Aa}	15.5 ± 0.75^{Ab}	1.6 ± 0.07^{Ab}	13 221 ± 1141
	5–10	7.2 ± 0.03^{AB}	21.2 ± 0.44^{AB}	286 ± 0.94^{a}	0.7 ± 0.07^{a}	11.7 ± 0.25^{a}	1.2 ± 0.04^{a}	
	10–20	7.2 ± 0.01^{AB}	21.6 ± 0.95^{A}	276 ± 1.71^{a}	1.5 ± 0.20^{Cb}	11.5 ± 0.46^{a}	1.1 ± 0.04^{a}	
	20–40	7.2 ± 0.03^{AB}	21.0 ± 0.45^{A}	305 ± 1.77^{a}	0.9 ± 0.08^{Aa}	12.2 ± 0.96^{a}	1.0 ± 0.01^{a}	
NT-67%	0–5	7.6 ± 0.01^{B}	24.1 ± 0.11^{B}	262 ± 2.47^{Aa}	0.7 ± 0.02^{Aa}	17.1 ± 1.27^{Bb}	1.7 ± 0.11^{Bb}	14 161 ± 593
	5–10	7.6 ± 0.04^{B}	23.9 ± 0.94^{B}	290 ± 0.46^{ab}	0.7 ± 0.04^{a}	12.3 ± 0.21^{a}	1.3 ± 0.02^{a}	
	10–20	7.7 ± 0.02^{B}	24.1 ± 1.34^{B}	298 ± 1.99^{ab}	1.3 ± 0.31^{Bb}	11.6 ± 0.20^{a}	1.2 ± 0.04^{a}	
	20–40	7.6 ± 0.11^{B}	23.7 ± 0.44^{B}	320 ± 1.89^{b}	0.8 ± 0.03^{Aa}	12.7 ± 0.30^{a}	1.1 ± 0.01^{a}	
NT-100%	0–5	7.9 ± 0.01^{B}	23.4 ± 0.07^{B}	277 ± 0.68^{Aa}	0.7 ± 0.04^{A}	14.7 ± 0.45^{Ab}	1.5 ± 0.04^{Ab}	12 962 ± 1060
	5–10	7.9 ± 0.01^{B}	23.7 ± 1.00^{B}	303 ± 0.08^{b}	0.7 ± 0.03^{a}	11.9 ± 0.65^{a}	1.2 ± 0.01^{a}	
	10–20	7.9 ± 0.01^{B}	24.2 ± 1.36^{B}	312 ± 0.33^{b}	0.9 ± 0.03^{A}	11.2 ± 0.25^{a}	1.1 ± 0.01^{a}	
	20–40	7.9 ± 0.05^{B}	23.1 ± 0.85^{B}	314 ± 0.35^{b}	0.8 ± 0.05^{A}	11.2 ± 0.78^{a}	1.0 ± 0.03^{a}	

CT – conventional tillage; NT-0 – no-tillage without maize straw coverage; NT-33% – no-tillage with 2.5 t/ha maize straw coverage; NT-67% – no-tillage with 5 t/ha maize straw coverage; NT-100% – no-tillage with 7.5 t/ha maize straw coverage; SM – soil moisture; CEC – cation exchange capacity. Data are presented as the mean ± standard deviation. Values followed by different uppercase letters within a column indicate differences (*P* < 0.05) among no-tillage with maize residue management in the same soil layers. Values followed by different lowercase letters within a column indicate differences (*P* < 0.05) among different soil layers in each treatment. Columns without letters indicate no significant difference (*P* > 0.05)

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and TN increased with the addition of maize straw coverage of 0 cm to 5 cm, and NT-67% exhibited a better effect. NT-33% showed a higher CEC in the 0–5 cm layer compared with other treatments. Soil organic acid decreased significantly in the 0–5 cm layer with maize straw coverage compared with CT ($P < 0.05$), whereas NT-33% had a higher soil organic acid in 10–20 cm. NT-67% showed higher maize yield compared to other treatments, but there was no significant difference in maize yield among different treatments. Soil TP, IP, OP and AP were significantly positively correlated with TC and TN, and P contents were significantly negatively correlated with soil organic acid and CEC (Table 2). Soil OP and AP both showed significant positive correlations with pH.

The increase in pH and soil moisture might result in shifts in composition and the size of the microbial community (Killam 1994, Aciego Pietri and Brookes 2008). It was widely believed that phosphatase is produced by microorganisms (fungus and bacterium) and plant roots (Zhang et al. 2012). Microorganisms enhanced soil P mineralization and immobilization and thus increased available P. The pH and soil moisture might be the indicators of P availability under conservation agriculture practices. Soil organic acid is negatively correlated with available P contents in this study. This finding was attributed to the fact that high P affected factors involved in internal plant and microorganism metabolism, such as ethanol dehydrogenase, and thus affected the synthesis of organic acid. Similar results were also reported by Neumann et al. (2000). This study also excluded the mineralization of P by organic acid in the migration and transformation of soil P under conservation agriculture practices.

Soil phosphatase activities. Soil phosphatase activities in the no-tillage with maize straw coverage were higher than that in CT (Figure 3). AlP and AcP both increased with the addition of maize straw coverage in each soil layer. The relationships between soil phosphatase activities and P contents (TP, OP, IP and AP) under no-tillage with maize straw coverage were analyzed separately by structural equation modeling (SEM) (Figure 2). The most parsimonious model revealed that TP had the strongest direct effect on phosphatase activities. The effects of phosphatase activities on AP contents were different in the three models. In NT-67%, PD showed a significant positive effect on AP, and AlP had a significant positive effect on AP in NT-100%. However, no significant effects of phosphatase activities on AP were noted in NT-33%. In addition, the models were rejected in the CT and NT-0 given that P-value < 0.05.

Phosphatase activities in the no-tillage systems were significant higher that other that in CT, which was attributed to no-tillage systems could protect soil porosity given reserved soil aggregates, thereby reducing decomposition of soil organic matter and increasing phosphatase activities (Wang et al. 2011). The activities studied here were also affected by maize residue management, and the activities increased with increasing residue input amounts. This was attributed to the increased substrates of soil phosphatases resulting from the maize residues application, and the increased microbial activities due to phosphatases in soils are derived primarily from

Table 2. Pearson's correlation coefficients (r) between soil properties and phosphorus (P) contents under different treatments

	TP	IP	OP	AP	OA	pH	TC	TN	SM	CEC
TP	1									
IP	0.836**	1								
OP	0.900**	0.513**	1							
AP	0.693**	0.470**	0.710**	1						
OA	–0.313*	–0.259*	–0.284*	–0.298*	1					
pH	ns	ns	0.256*	0.439**	ns	1				
TC	0.507**	0.322*	0.537**	0.558**	ns	ns	1			
TN	0.784**	0.654**	0.707**	0.653**	ns	ns	0.848**	1		
SM	ns	ns	ns	0.465**	ns	0.722**	ns	ns	1	
CEC	–0.335**	ns	–0.327*	ns	ns	ns	ns	–0.390**	ns	1

TP – total P; IP – inorganic P; OP – organic P; AP – available P; OA – organic acid; TC – total carbon; TN – total nitrogen; SM – soil moisture; CEC – cation exchange capacity. *$P < 0.05$; **$P < 0.01$; ns – not significant

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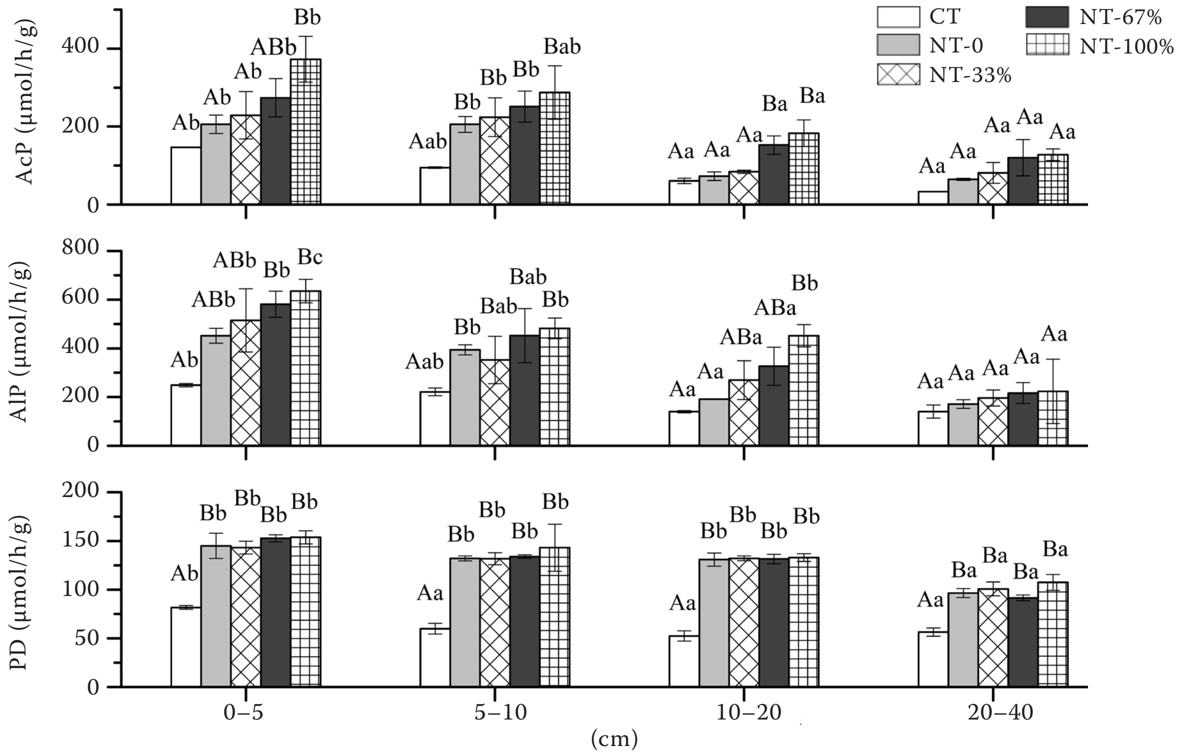

Figure 3. Soil phosphatase activities in the mollisol area under conventional tillage (CT) and no-tillage with maize residue management. AcP – acid phosphomonoesterase; AlP – alkaline phosphomonoesterase; PD – phosphodiesterase; NT-0 – no-tillage without maize straw coverage; NT-33% – no-tillage with 2.5 t/ha maize straw coverage; NT-67% – no-tillage with 5 t/ha maize straw coverage; NT-100% – no-tillage with 7.5 t/ha maize straw coverage. Error bars represent standard deviation. Uppercase letters indicate differences among different treatments in the same soil layers. Lowercase letters indicate differences in different soil layers in the same treatment

microorganisms (Turner and Haygarth 2005). The residue inputs with increasing C sources potentially enhance microbial activity, and phosphatase activities exhibit positive correlations with microbial biomass and activity in soil (Nannipieri et al. 2011). The effects of phosphatase activities on soil P contents (OP, IP and AP) were compared based on structural equation modeling (Figure 3), and the results showed that residue amounts affected phosphatase activity on soil P contents. In NT-67%, PD activity showed a significant correlation with AP ($P < 0.05$), while AlP had a significantly stronger effect on AP in NT-100% ($P < 0.01$). The results presented in this study could also be attributed to the differences in the origin, states, and/or persistence of different groups of enzymes affected by the different effects of tillage and maize residue management (Wei et al. 2014).

Overall, after a ten-year no-tillage and maize residue management experiment, soil P contents (OP and AP) and phosphatase activities were significantly increased that compared with tillage treatment. Under the no-tillage and residue inputs, pH and soil moisture were indicators of P availability. No-tillage and maize residue input played a greater role in these effects than tillage. In conclusion, this study suggested that no-tillage and residue input could reduce the risk of P leaching, and NT-67% might play an important role in the maintenance and transformation of soil organic P. The no-tillage and maize residue management plan could change the migration and transformation path of soil P, in which residue inputs resulted in different sources of available P.

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