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Phosphorus forms and their distribution under long-term no tillage systems

Kai Wei[1], *Zhenhua Chen*[1], *Xiaoping Zhang*[2], *Lijun Chen*[1]*

[1]*Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, P.R. China*
[2]*Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, P.R. China*

**Corresponding author: ljchenchina@hotmail.com; ljchen@iae.ac.cn*

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Abstract: Phosphorus (P) stratification in no-tillage (NT) systems has important implications for crop growth and potential P loss, but little is known about P forms and their distribution when mineral P fertilizers are placed to the depth of 5 cm in NT soil. A 10-year field experiment was used to study the effect of NT and mouldboard plough (MP) on soil P forms at three depths (0–5, 5–10 and 10–20 cm) and their relationship with Fe and Al oxides. The results indicated that stratification of organic P forms occurred under NT treatment, and Fe oxides may have a stronger capacity for adsorbing the P forms. When mineral P fertilizers were placed to the depth of 5 cm under NT treatment, there was no significant difference in P forms or crop yield between NT and MP treatment, and orthophosphate did not show any significant difference under NT treatment between 0–5 cm and 5–10 cm depth. Overall, the agricultural management practice that mineral P fertilizers are placed to the depth of 5 cm under NT treatment could result in stratification of P forms, while the changes in the distribution of P forms in soil profiles might help reduce potential P loss in surface runoff and do not make any difference to crop growth.

Keywords: nutrient; cMonoester; cDiesters; ^{31}P nuclear magnetic resonance; agriculture

Soil phosphorus (P) is an essential nutrient for crops in agricultural ecosystems. Tillage can affect soil P stratification, which has important implications for crop growth (Vu et al. 2009). However, although P stratification in agricultural soils has been widely studied, it is still necessary to study P forms and their distribution at different soil depths as well as their response to tillage practices, since it has been reported that different P forms in soils play different roles in providing P nutrient for crops (Murphy et al. 2009), and the response of P forms to tillage is more sensitive than soil total P (Wei et al. 2014).

Soil P forms can be characterized by extracting samples with NaOH-EDTA and analysing the solution using ^{31}P nuclear magnetic resonance (NMR) spectroscopy (Turner et al. 2003a,b). To date, only a few studies have investigated the effect of tillage practices on the distribution of P forms as determined by ^{31}P NMR at different soil depths (Cade-Menun et al. 2010, Abdi et al. 2014, Cade-Menun et al. 2015). The results obtained by Abdi et al. (2014) and Cade-Menun et al. (2015) indicate that the distribution of P forms in the soil profile can be affected by tillage, and the response of different P forms at soil depths to tillage may vary with different research areas. Moreover, the fertilization pattern adopted by the above studies is that mineral fertilizers or organic manure were placed on the surface soil (0–5 cm) of no-tillage (NT) treatment. The results showed NT stratified fertilizer P and increased potential P loss in surface runoff (Abdi et al. 2014, Cade-Menun et al. 2015). Therefore, it is necessary to place P fertilizers below the soil surface under NT treatment (Cade-Menun et al. 2015). However, little is known regarding the changes in the distribution of P forms in the soil profile when fertilizers are placed to the depth of

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5 cm under NT treatment, and whether the changes affect crop yield or potential P loss in surface runoff. Moreover, previous studies also found that P forms can be strongly adsorbed by Fe and Al oxides in acidic soil (Celi et al. 2001, Ohno et al. 2011), but the effect of Fe and Al oxides on P forms may be different in different research areas (Turner et al. 2003b, Murphy et al. 2009). Therefore, the objectives of this study were (1) to investigate the distribution of soil P forms determined by ^{31}P NMR at three depths (0–5, 5–10 and 10–20 cm) when mineral P fertilizers are placed to the depth of 5 cm under NT treatment; (2) to compare the relative importance of oxalate-extractable Fe and Al in maintaining P forms in the rainfed agroecosystem of the Northeast China.

MATERIAL AND METHODS

Study site. This study was conducted at the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences experimental station (44°12'N, 125°33'E) in the Dehui County, Jilin province, China. The station has a continental monsoon climate with a mean annual temperature of 4.4°C and mean annual precipitation ranging from 500 mm to 600 mm. The soil used at this study site was a clay loam soil, which was classified as a Phaeozem (FAO 1998). Before this tillage experiment, the region experienced more than ten years of conventional tillage management for continuous maize cultivation (Liang et al. 2007).

Experimental design. The field experiment consisted of no-tillage and mouldboard plough (MP) treatments that were initiated in the fall of 2001. The experiment was arranged in a randomized complete block design with four replicates. However, due to an operation error that occurred in one of the MP replications, only three replications under MP treatment were used in this study. Each plot was 5.2 m × 20 m for maize-soybean rotation. The MP treatment involved one fall mouldboard ploughing (20 cm deep) after the crop harvest, spring disking (7.5 cm to 10 cm deep) before planting and inter tillage after planting. For the NT treatment, the soil was not disturbed except for planting. A KINZE-3000 NT planter (Williamsburg, USA) was used for the spring seeding for all treatments. All of the crop residues were returned to the soil surface. Each year, 100 kg N/ha, 45.5 kg P/ha, and 78 kg K/ha of urea, ammonium dibasic phosphate, and potassium sulfate fertilizers, respectively, were applied to the maize as basal fertilizers. An additional 50 kg N/ha was applied as a top dressing during the V-6 stage (40 days after planting). For soybeans, all of the fertilizers, including 40 kg N/ha, 60 kg P/ha, and 80 kg K/ha, were applied as basal fertilizer. Under the NT treatment, the basal fertilizers were applied concurrently with seeding at the 5–10 cm depth using the banding attachment on the NT planter, and additional urea as the top dressing was applied to the soil surface without incorporation into the soil. MP treatment had the same basal fertilizers application method as NT treatment. However, the top dressing under MP treatment was applied concurrently with inter tillage when the maize was at the V-6 stage.

Soil sampling and analyses. Soil sampling was carried out before the maize harvest in September 2011. In each tillage plot, ten soil cores (2.6 cm in diameter) were sampled randomly at 0–5, 5–10 and 10–20 cm depths using an auger. After visible root and plant residues were removed, the soil cores from the same depth were combined to make composite samples from the same plot. Then, the composited samples were placed in plastic bags in the field and transported to the laboratory for chemical analysis.

The soil samples were air-dried, sieved (< 2 mm), and stored at ambient laboratory temperature before chemical analysis. The soil pH was measured using a 1:2.5 (w:v) soil: water suspension ratio. Oxalate-extractable Fe and Al were extracted with ammonium oxalate/oxalic acid (pH 3.0) and determined using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Schoumans 2000). Soil P forms were characterized by extracting samples with NaOH-EDTA and analysing the solution using ^{31}P nuclear magnetic resonance spectroscopy as described by Wei et al. (2018). The solution ^{31}P NMR spectra was acquired using a JEOL ECA 600 spectrometer (Tokyo, Japan) operating at 243 MHz with a 30° pulse, 0.675-s acquisition time; 2.50-s delay time; 20 000 scans and no proton decoupling. Although T1s were not measured for these samples, this delay time was estimated to be sufficient based on the ratio of P/(Fe + Mn) in the extracts (McDowell et al. 2006). All of the results were expressed by the oven-dry (105°C) weight of soil.

Statistical analysis. The normality of the data was tested by fitting it to a normal distribution and assessing the goodness of fit with the Shapiro-Wilks *w* test. A log (*n*) transformation was used to normalize the data before statistical analysis, but means and standard deviations in the tables were calculated from the untransformed data. The Duncan's test at the $P = 0.05$

Table 1. Distribution of soil pH, oxalate-extractable Fe, and Al at the 0–5, 5–10 and 10–20 cm depths and crop yield under mouldboard plough (MP) and no-tillage (NT) treatments

Tillage system	Depth (cm)	Soil pH	Oxalate-extractable Fe (g/kg)	Oxalate-extractable Al (g/kg)	Crop yield (kg/ha)
	0–5	5.54 ± 0.16^{c}	2.47 ± 0.05^{Ba}	2.02 ± 0.01^{a}	
MP	5–10	5.89 ± 0.10^{Ab}	2.21 ± 0.04^{Bb}	1.84 ± 0.03^{Bb}	11 437 ± 325
	10–20	6.32 ± 0.12^{a}	2.08 ± 0.09^{Bc}	1.87 ± 0.09^{Bb}	
	0–5	5.32 ± 0.16^{c}	2.62 ± 0.02^{Aa}	2.06 ± 0.07^{ns}	
NT	5–10	5.65 ± 0.01^{Bb}	2.51 ± 0.07^{Ab}	2.06 ± 0.06^{A}	12 446 ± 605
	10–20	6.28 ± 0.05^{a}	2.29 ± 0.07^{Ac}	1.98 ± 0.04^{A}	

Values followed by different uppercase letters (between tillage systems) and lowercase letters (at different soil depths in each tillage systems) within a column indicate the difference ($P < 0.05$). ns – not significant ($P > 0.05$)

level and one-way ANOVA were used to analyse the effect of soil depth on soil parameters and crop yield. The independent-samples *T* test was applied to analyse whether tillage had significant effects on the soil parameters and crop yield. The correlation between oxalate-extractable Fe, Al and P forms was based on the Pearson's correlation coefficients. Differences at the $P < 0.05$ level were considered significant. All of the statistical analyses were conducted with the SPSS 16.0 software (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Soil properties and crop yield. In this study, soil pH increased with increasing soil depth in both NT and MP soils (Table 1). Compared with MP treatment, NT significantly decreased soil pH at the 5–10 cm depth, which could be attributed to the placement of fertilizers in this study, since the basal fertilizers under NT treatment were placed below 5 cm in soil without mechanical mixing of soil, and a previous study found that fertilizer application in agricultural systems could result in soil acidification (Jacobsen and Westerman 1991). However, although basal fertilizers under NT treatment were placed to the depth of 5 cm in soil, the soil pH in NT soil was higher at the 5–10 cm depth than that at the 0–5 cm depth, which could be related to the decomposition of applied crop residues at the surface soil, since crop residues decomposition could generate some acidic compounds (Sun et al. 2015).

Additionally, the application of additional urea as the top dressing at the soil surface without incorporation might also partly contribute to soil acidification at the 0–5 cm depth.

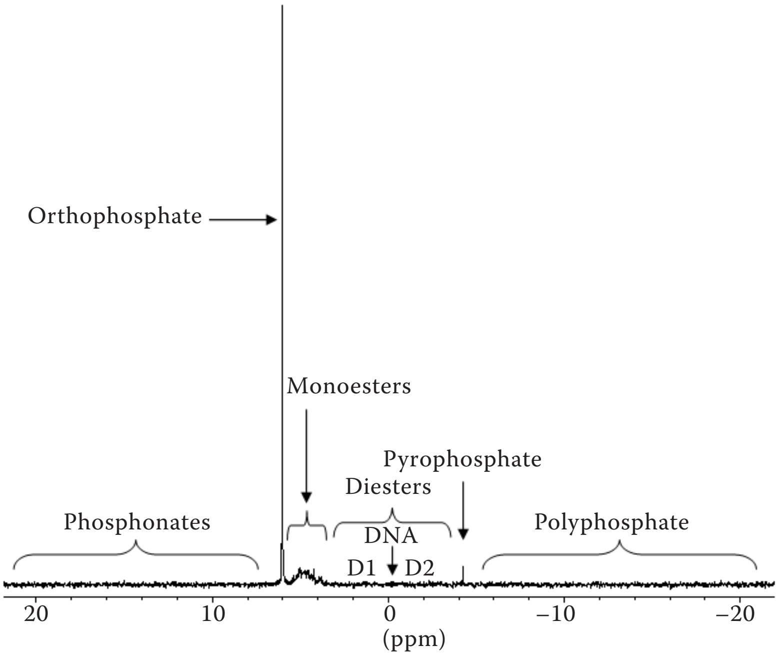

Figure 1. Solution ^{31}P nuclear magnetic resonance spectra of one sample at the 5–10 cm depth under mouldboard plough treatment

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In contrast to soil pH, oxalate-extractable Fe and Al showed higher concentrations at the soil surface than at depths under both NT and MP treatments (Table 1). The differences in oxalate-extractable Fe and Al between NT and MP treatments and their distribution trends with depth were related to soil pH, since the solubility of iron and aluminum increased with low soil pH (Finzi et al. 1998). Moreover, our study observed no significant difference in crop yield between NT and MP treatments (Table 1).

Soil organic P forms determined by ^{31}P NMR. The organic P forms determined by ^{31}P NMR consisted primarily of monoesters, diesters, and phosphonates (Figure 1). In the monoesters region, *myo*-inositol hexakisphosphate (*myo*-IHP), α-glycerophosphate (α-gly), β-glycerophosphate (β-glyc), mononucleotides (Nucl) and *scyllo*-inositol hexakisphosphate (*scyllo*-IHP) were identified (Table 2). By spiking experiments, the peaks for *myo*-IHP were identified at 5.57, 4.68, 4.21 and 4.14 ppm. The peaks for α-gly, β-glyc and Pchol were identified at 4.90, 4.67 and 3.98 ppm, respectively. Based on the chemical shift of Pchol, the peak at 3.85 ppm was assigned to *scyllo*-IHP. An example spectrum of the monoester region from the MP treatment at the 5–10 cm depth is shown in Figure 2. Since previous studies have found that diesters, such as phospholipids and ribonucleic acid (RNA), could be degraded to produce monoesters during NMR extraction analysis (Doolette et al. 2009, Cade-Menun 2015), the monoesters and diesters determined by ^{31}P NMR in this study were corrected to cMonoesters and cDiesters, respectively (Table 3). After correction for degradation, both cMonoesters and cDiesters showed higher concentrations in the surface soil (0–5 cm) than in deeper layers (10–20 cm) under NT treatment (Table 3). This finding could be attributed to (1) the accumulation of crop residues in the surface of NT soil; (2) the adsorption of higher metal oxides in the surface soil (0–5 cm) of NT treatment on monoesters and diesters. Previous studies found that the effect of Fe and Al oxides on P forms was different in different research areas (Turner et al. 2003b, Murphy et al. 2009). In this study, Fe oxides might play a more important role in maintaining monoesters and diesters than Al oxides in NT soil, since cMonoesters and cDiesters were only significantly positively correlated with oxalate-extractable Fe under NT treatment, while there was no significant correlation with oxalate-extractable Al (Table 4). Unlike the distribution of organic forms along the NT soil profile, organic P forms were uniformly distributed in soil layers under the MP treatment (Table 3), which was attributed to the mechanical mixing of soil during tillage.

Soil inorganic P forms determined by ^{31}P NMR. The inorganic P forms determined by ^{31}P NMR consisted of orthophosphate, pyrophosphate, and polyphosphate (Figure 1). In the three inorganic P forms, stratification

Table 2. Mean concentration (mg/kg) and the standard deviation of soil P forms determined by ^{31}P NMR spectroscopy

Tillage system	Depth (cm)	Inorganic P forms			Organic P forms												
		Ortho	Pyro	Poly	myo-IHP	scyllo-IHP	Pchol	α-Glyc	β-Glyc	Nucl	Phon	M1	M2	M3	D1	D2	DNA
MP	0–5	98.1 ± 25.7	2.9 ± 0.8	1.0 ± 0.3^{B}	5.9 ± 1.5^{B}	1.0 ± 0.3^{B}	1.0 ± 0.3	1.0 ± 0.3	2.0 ± 0.5	4.9 ± 1.3	2.7 ± 1.1	4.4 ± 1.6	53.8 ± 7.4	6.0 ± 3.1	3.1 ± 0.5	2.0 ± 1.0	1.0 ± 0.3^{B}
	5–10	77.3 ± 17.3	2.7 ± 0.1	1.3 ± 0.4	5.5 ± 0.5	1.3 ± 0.5	0.8 ± 0.2^{B}	1.0 ± 0.2	1.7 ± 0.1	5.2 ± 0.1^{B}	2.0 ± 0.5	3.2 ± 1.8	49.8 ± 1.9	6.2 ± 0.8	2.3 ± 1.0	1.6 ± 0.7	0.8 ± 0.2
	10–20	61.5 ± 11.8	2.1 ± 0.7	1.6 ± 0.3	4.8 ± 1.3	1.2 ± 0.2	0.6 ± 0.1	1.0 ± 0.3	1.4 ± 0.1	4.1 ± 1.1^{B}	1.3 ± 0.8	4.2 ± 0.6	55.2 ± 4.4	3.8 ± 2.2	2.5 ± 0.5	1.5 ± 0.9	0.6 ± 0.1
NT	0–5	134.2 ± 42.2^{a}	2.6 ± 1.0	2.1 ± 0.6^{A}	9.1 ± 1.5^{Aa}	1.9 ± 0.6^{Aa}	1.3 ± 0.4	1.3 ± 0.4	2.3 ± 1.1	7.7 ± 1.6^{a}	4.4 ± 0.8^{a}	6.2 ± 2.5	52.9 ± 4.8	8.0 ± 2.1	4.0 ± 1.5^{a}	2.6 ± 1.0^{a}	1.5 ± 0.3^{Aa}
	5–10	114.3 ± 33.8^{ab}	2.7 ± 0.4	1.3 ± 0.3	6.9 ± 2.0^{a}	1.3 ± 0.3^{ab}	1.3 ± 0.3^{A}	1.4 ± 0.5	2.5 ± 0.7	7.3 ± 1.1^{Aab}	3.1 ± 1.6^{ab}	4.7 ± 3.9	50.7 ± 3.9	5.4 ± 3.0	1.7 ± 1.0^{ab}	1.3 ± 0.3^{b}	1.1 ± 0.3^{ab}
	10–20	67.1 ± 4.1^{b}	2.2 ± 0.4	1.3 ± 0.6	4.0 ± 0.2^{b}	0.8 ± 0.3^{b}	0.8 ± 0.4	1.2 ± 0.3	2.0 ± 0.6	5.7 ± 0.6^{Ab}	2.2 ± 1.0^{b}	3.1 ± 1.5	46.5 ± 4.2	6.3 ± 1.8	2.7 ± 1.1^{b}	1.5 ± 0.4^{b}	0.8 ± 0.4^{b}

Ortho – orthophosphate; Pyro – pyrophosphate; Poly – polyphosphate; *myo*-IHP – *myo*-inositol hexakisphosphate; *scyllo*-IHP – *scyllo*-inositol hexakisphosphate; Phcol – choline phosphate; α-Glyc – α-glycerophosphate; β-Glyc – β-glycerophosphate; Nucl – various mononucleotides; Phon – phosphonate; Mono1, 2, 3 – various monoesters excluding the preceding identified peaks from 6.9 to 6.1 ppm, 5.9 to 4.0 ppm, 3.9 to 2.6 ppm, respectively; Di1 – unspecified diesters from 2.5 to –0.6 ppm; Di2 – unspecified diesters from –1.2 to –3.7 ppm; NMR – nuclear magnetic resonance

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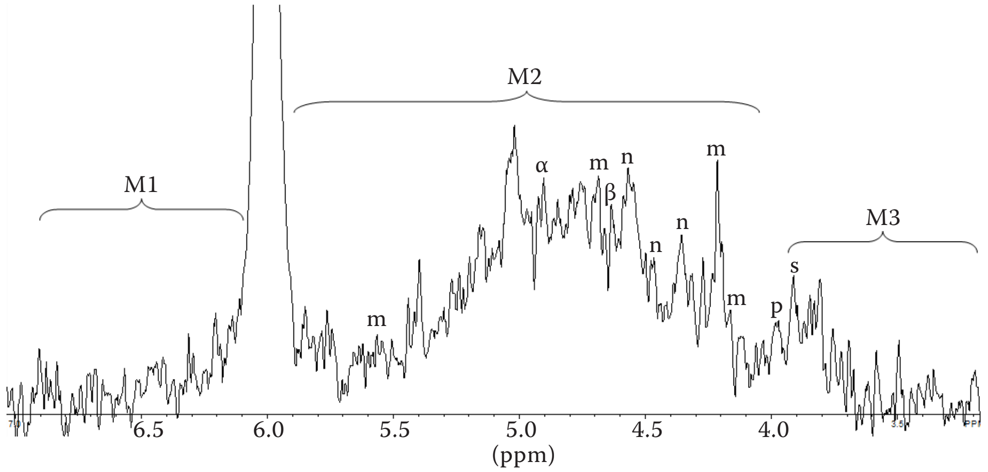

Figure 2. Solution ^{31}P nuclear magnetic resonance spectra of one sample at the 5–10 cm depth of mouldboard plough treatment showing the P forms detected in the monoester region. m – myo-inositol hexakisphosphate; α – α-glycerophosphate; β – β-glycerophosphate; n – mononucleotides; p – choline phosphate; s – scyllo-inositol hexakisphosphate; M1 – monoester 1; M2 – monoester 2; M3 – monoester 3

only occurred under NT treatment for orthophosphate, and orthophosphate showed significantly higher concentration in the surface soil (0–5 cm) than in deeper layers (10–20 cm) (Table 2). Similar results were also reported by Abdi et al. (2014), who found that orthophosphate concentration was significantly higher at the 0–5 cm depth of NT-P_{35} treatment than that at the 5–10 cm and 10–20 cm depth, and which was partly attributed to the accumulation of mineral P fertilizer in the top 5 cm soil layer without incorporation. However, in this study, the mineral P fertilizers are placed below 5 cm under NT treatment. Since no exogenous mineral P fertilizer was added at a depth of 0–5 cm under NT treatment, the increased orthophosphate concentration in the surface soil might have resulted from the mineralization of organic P, which was supported by the significant positive correlation of orthophosphate with cMonoesters and cDiesters under NT treatment (Table 4). This also explained why the mineral P fertilizer was placed at the 5–10 cm depth in the NT soil studied here, while no significant difference was observed for orthophosphate concentration between 0–5 cm and 5–10 cm depth (Table 2). The above results also indicated the agricultural management practice that mineral P fertilizers placed to the depth of 5 cm under NT treatment could lower the P levels in the top 5 cm of the soil, which may help to reduce the potential P loss in surface runoff.

Overall, the agricultural management practice when mineral P fertilizers are placed to the depth of 5 cm under

Table 3. Mean concentration (mg/kg) and the standard deviation of compound classes of P forms determined by ^{31}P nuclear magnetic resonance spectroscopy

Tillage system	Depth (cm)	NaOH-EDTA extracted total P	Inorganic P	Organic P	Monoesters	Diesters	Deg	cMonoesters	cDiesters
MP	0–5	190.7 ± 42.0	102.0 ± 26.7	86.0 ± 14.2	79.9 ± 13.7	6.0 ± 1.2	34.8 ± 5.8	45.2 ± 8.0	40.8 ± 6.3
	5–10	162.6 ± 17.9	81.3 ± 17.2	79.3 ± 4.1	74.6 ± 2.9	4.7 ± 1.2	32.8 ± 1.0	41.8 ± 2.6	37.5 ± 1.8
	10–20	147.2 ± 16.4	65.2 ± 12.5	80.7 ± 4.2	76.2 ± 4.0	4.6 ± 0.5	34.0 ± 2.4	42.1 ± 3.0	38.6 ± 2.3
NT	0–5	242.4 ± 54.7^{a}	138.8 ± 42.4^{a}	99.2 ± 14.6^{a}	90.8 ± 12.6^{a}	8.4 ± 2.1^{a}	37.9 ± 4.7	52.9 ± 8.1^{a}	46.3 ± 6.7^{a}
	5–10	207.2 ± 48.9^{ab}	118.3 ± 34.2^{ab}	85.8 ± 15.1^{ab}	81.6 ± 14.0^{ab}	4.2 ± 1.5^{b}	36.5 ± 4.0	45.0 ± 10.5^{ab}	40.8 ± 4.8^{ab}
	10–20	148.5 ± 11.9^{b}	70.7 ± 4.6^{b}	75.6 ± 7.2^{b}	70.5 ± 6.2^{b}	5.1 ± 1.1^{b}	32.1 ± 3.0	38.3 ± 3.3^{b}	37.2 ± 3.8^{b}

Inorganic P – sum of inorganic P forms; organic P – sum of organic P forms; monoesters – sum of P forms in the monoester region; diesters – sum of P forms in the diester region; deg – sum of compounds in the monoester region that are believed to originate from diesters degraded during analysis (α and β-glycerophosphate, mononucleotides, half of Monoester 2 region); cMonoesters – monoesters minus Deg; cDiesters – diesters plus Deg

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Table 4. Correlations between oxalate-extractable Fe, Al and P forms under no-tillage treatments

	Oxalate-extractable Fe	Oxalate-extractable Al	cMonoesters	cDiesters	Orthophosphate
Oxalate-extractable Fe	1				
Oxalate-extractable Al	ns	1			
cMonoesters	0.655*	ns	1		
cDiesters	0.621*	ns	0.932**	1	
Orthophosphate	0.710**	ns	0.890**	0.746**	1

*$P < 0.05$; **$P < 0.01$; ns – not significant

NT treatment could result in stratification of P forms, while the changes in the distribution of P forms in soil profiles might help reduce potential P loss in surface runoff and do not make any difference to crop growth. Therefore, it is recommendable to place the mineral P fertilizers below the soil surface under NT treatment in the rainfed agroecosystem of Northeast China.

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