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## Long-term rotation fertilisation has differential effects on soil phosphorus

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**Abstract:** The application of organic fertiliser is a commonly used method to improve the utilisation efficiency of phosphorus (P) fertiliser. However, long-term over-fertilisation will cause environmental pollution. This study analysed a field test was conducted to study the impacts of P forms in soil under long-term (40 years) rotation fertilisation, to evaluate the contribution of different fertilisation to the available P form, to clarify how to apply pig manure to improve the concentration of available P forms. The results show that the main P compounds were orthophosphate and phosphate monoester. The combination of a high amount of manure and NPK fertilisers can increase the content of orthophosphate that can be absorbed and utilised by crops, the orthophosphate was 477.2 mg/kg, and phosphate monoester was 190.4 mg/kg. The long-term addition of nitrogen fertiliser significantly reduced the pH value of soil, and the addition of manure slowed down the trend of pH reduction. Furthermore, manure in combination with NPK fertiliser, can extremely increase the total P extracted by NaOH-EDTA by changing soil pH. The NaHCO<sub>3</sub>-soluble P (Ca<sub>2</sub>-P), NH<sub>4</sub>Ac-soluble P (Ca<sub>8</sub>-P), NH<sub>4</sub>F-soluble P (Al-P), and occluded-P (O-P) were positively correlated with orthophosphate, while middle stable and high stable organic P was positively correlated with monophosphate.

**Keywords:** organic manure; soil P availability; P form change; plants available P; <sup>31</sup>P nuclear magnetic resonance

The increase in soil phosphorus (P) content was observed in recent years as a result of increased P fertiliser application owing to its positive effect on crop yields and low P fertiliser prices (Xu et al. 2008). However, excessive fertilisation has become a serious issue that causes environmental pollution (Gao et al. 2006). Additionally, animal manure has begun to accumulate in large quantities owing to high animal populations. In an attempt to improve P efficiency, some places use crop straw and animal manure in large quantities, leading to soil pollution and other problems (Li et al. 2015). Therefore, reducing the use of mineral P fertiliser and partially supplementing

it with an organic fertiliser such as manure could be beneficial to the environment if done correctly. To achieve this goal, it is necessary to understand the forms of P in soil and how they are affected by long-term fertilisation strategies.

The distribution of different forms of P affects the transformation of soil P and absorption by crops (Condon et al. 2005, Cade-Menun 2017). <sup>31</sup>P nuclear magnetic resonance (NMR) is an effective method for widely characterising the forms of extractable soil P, and it could provide detailed information on different forms of soil P (Cade-Menun 2017). There are many forms of organic P and inorganic P in the

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soil; however, only the soluble orthophosphate anion can be directly absorbed and utilised by plant roots (Condrón et al. 2005, Richardson et al. 2011). Organic P can be divided into several phosphate-binding compounds, such as the common phosphate monoester and phosphate diester (Annaheim et al. 2015). Many studies have shown that different long-term fertilisation practices can change the composition of soil P (Stutter et al. 2015, Deiss et al. 2016); among them, the proportion of soil orthophosphate after long-term application of mineral P fertiliser is large (Ahlgrén et al. 2013). Some studies also showed that the concentration of orthophosphate increases with an increase in mineral P fertiliser or organic P fertiliser; however, the concentration of phosphate monoester remains unchanged (Ahlgrén et al. 2013). In this study, using a 40-year field experiment, we studied the effect of pig manure as an organic fertiliser on different forms of P in the soil to understand the correlation between fertilisation and specific forms of P and provide effective measures for environmental protection.

## MATERIAL AND METHODS

**Experimental site and design.** In this study, a long-term site-based experiment was conducted which was established in 1979 in Shenyang Agricultural University (41°48'N, 123°33'E), China, and could be confirmed that the change in nutrients has a major role in the potency of soil P. The climate is mid-temperate. The annual average temperature over 7.0–8.1 °C, and annual precipitation is 574–684 mm. According to the FAO classification, soils were Haplic Luvisol (FAO/UNESCO 1996). The mode of crop rotation is corn-corn-soybean. A total of 32 plots

in the experimental area were divided into 8 treatment groups (4 replicates for each treatment), and the random block design was adopted, with a total of 160 m<sup>2</sup>. The original soil in 1979 and the arable soil collected after the autumn harvest in 2018 (corn planting year) were selected as soil samples. The treatment selected for this test: (1) CK0 (the soil before planting in 1979); (2) CK (an unfertilised control in 2018); (3) NPK (mineral fertiliser NPK); (4) M1NPK (low amount of manure fertiliser plus NPK); (5) M2NPK (high amount of manure fertiliser plus NPK). Table 1 shows the basic properties of all treatments. The rate of low and high manure fertiliser was 13.5 t/ha/year and 27 t/ha/year for maize. Because the continuous application of manure tended to reduce the yields of soybean, soybean planting year had no manure fertiliser since 1992. The rate of N-P-K was 120-26-50 kg/ha/year for maize and 30-39-75 kg/ha/year for soybean. Mineral fertiliser: N – urea (N 46%); P – calcium superphosphate (P 5%); K – potassium sulfate (K 42%). Pig manure (dry) as manure fertiliser contains 83.5 g organic C/kg; 7.2 g N/kg; 3.8 g P/kg; 8.3 g K/kg on a 40-year average. All the fertilisers were applied basally at one time before sowing.

**Soil sampling and analysis.** Randomly selected five points each plot with an "S" shape and collected 20 cm depth of soil mixed the five soil samples as a composite soil sample. Total P was determined by NaOH fusion (Sommers and Nelson 1972). Olsen-P was extracted with 0.5 mol/L NaHCO<sub>3</sub>, then determined using the molybdenum blue method (Olsen et al. 1954). The inorganic P of the soil sample was determined by a sequential fractionation method developed (Jiang and Gu 1989, Samadi and Gilkes

Table 1. Topsoil properties (0–20 cm) of the long-term fertilisation experiment in all treatment

Treatment	pH	Organic C	Total N	Total K
		(g/kg)		
CK0	6.50 ± 0.02	9.22 ± 0.10	0.80 ± 0.01	20.10 ± 1.54
CK	5.63 ± 0.05	9.39 ± 0.22	0.78 ± 0.02	20.06 ± 2.12
NPK	5.36 ± 0.02	10.20 ± 0.37	0.84 ± 0.02	22.29 ± 1.30
M1NPK	5.63 ± 0.03	12.98 ± 1.01	1.03 ± 0.02	23.38 ± 1.37
M2NPK	6.01 ± 0.02	14.28 ± 0.48	1.16 ± 0.01	23.58 ± 0.47

Means (± standard error) followed among different soil treatments under each column. 10 g of the composite soil sample for measuring pH in the soil-to-water ration of 1:2.5. The soil organic C and total N were determined using an elemental analyser (Elementar III, Bremen, Germany). CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

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1999); the soil inorganic P fractions determined were 0.1 mol/L  $\text{NH}_4\text{OH}$ -soluble P: Ca bound P ( $\text{Ca}_2\text{-P}$ ), 0.5 mol/L  $\text{NH}_4\text{Ac}$ -soluble P: Ca bound P ( $\text{Ca}_8\text{-P}$ ), 0.5 mol/L  $\text{NH}_4\text{F}$ -soluble P: Al bound P (Al-P), 0.1 mol/L  $\text{NaOH-Na}_2\text{CO}_3$ -soluble P: Fe bound P (Fe-P), 0.3 mol/L sodium citrate + 1 g  $\text{Na}_2\text{S}_2\text{O}_4$  + 0.5 mol/L  $\text{NaOH}$ : occluded-P (O-P), 0.25 mol/L  $\text{H}_2\text{SO}_4$ -soluble P: Ca bound P ( $\text{Ca}_{10}\text{-P}$ ), respectively. Bowman and Cole (1986) classification method for soil organic P, they were divided into four groups: labile organic P (LOP): 0.5 mol/L  $\text{NaHCO}_3$ , moderately labile organic P (MLOP): 1 mol/L  $\text{H}_2\text{SO}_4$  + 0.5 mol/L  $\text{NaOH}$ , moderately resistant organic P (MROP): 0.5 mol/L  $\text{NaOH}$  no precipitation, highly resistant organic P (HROP): 0.5 mol/L  $\text{NaOH}$  precipitation. The P activation coefficient (PAC) is used to characterise soil P availability. The higher PAC, the higher the soil P availability,  $\text{PAC} = \text{Olsen-P (mg/kg)}/\text{total P (mg/kg)} \times 100\%$ .

Determination of P compounds by  $^{31}\text{P}$  NMR using the improved Cade-Menun and Preston (1996) program (McDowell et al. 2006), to obtain the detailed information of P composition, 5 g of air-dried composite soil samples (< 2 mm) were shaken for 16 h at 20 °C with a 1:20 solution of 100 mL 0.25 mol/L  $\text{NaOH}$  + 0.05 mol/L EDTA, followed by centrifugation for 30 min at approximately  $10\,000 \times g$ ; the supernatant was freeze-dried at  $-80$  °C. A 1 g sample of the freeze-dried material was dissolved in 0.4 mL 10 mol/L  $\text{NaOH}$  and 2.6 mL  $\text{D}_2\text{O}$  (deuterium oxide) solution, the reaction lasted for 30 min, with occasional oscillation, and then centrifuged at  $1\,500 \times g$  for 20 min, the supernatant after centrifuging was analysed by NMR. All experiments were carried out in a dual resonance probe using a 10 mm NMR tube. Spectra were acquired on a 500 MHz spectrometer (General Electric, Fremont, USA) equipped with a 10 mm broadband probe. The experimental parameters for NMR were as follows: pulse with  $90^\circ$  flip angle used, detection time of 0.68 s, pulse lag of 4.32 s, the temperature of 20 °C, each sample passed more than 8 000 scans, chemical shifts were determined relative to an external 85% orthophosphoric acid standard. The Peak areas were corrected for diester degradation by subtracting the areas of  $\alpha$ - and  $\beta$ -glycerophosphate and mononucleotides from the phosphate monoester area and adding them to the phosphate diester area (Schneider et al. 2016). The relative proportion of all compounds in a spectrum was determined by ChemDraw and MestRec software. Determination of total P in the extract by ICP-OES. In the spec-

tra of all the treatments, peaks were identified as orthophosphate (6 ppm); phosphonates (7.8, 8.67, 18.95); phosphate monoester (3–6 and 6–7.5 ppm); phosphate diester (–0.95, –0.73, 1.95, 3.58); pyrophosphate (–4.49) and polyphosphate (–4.23). Additionally, the peaks of phosphate monoester were identified as myo-inositol hexakisphosphate (myo-IHP) (4.01, 4.39, 4.55, 5.38), scyllo-inositol hexakisphosphate (scyllo-IHP) (3.56), neo-inositol hexakisphosphate (neo-IHP) (4.28, 6.72), and D-chiro-inositol hexakisphosphate (D-chiro-IHP) (4.36, 6.45),  $\alpha$ -glycerophosphate (5.11),  $\beta$ -glycerophosphate (4.8), glucose 6-phosphate (5.13), and choline phosphate (4.04). The content of P compounds in the pig manure (2018 year): 872.0 mg/kg orthophosphate; 5.5 g/kg pyrophosphate; 34.8 g/kg phosphate monoester; 2.7 g/kg phosphate diester.

**Statistical analysis.** The data was presented as the arithmetic mean values with standard errors. One-way analysis of variance procedure was used to test the effects of the different treatments on TP (total P), Olsen-P, inorganic P, organic P, PAC (P activation coefficient) and  $\text{P}_{\text{rec}}$  (P recovered by  $\text{NaOH-EDTA}$  extraction), and the least significant difference (*LSD*) test was performed to establish whether the differences between the treatments were significant ( $P \leq 0.05$ ). The correlation between inorganic P and organic P with TP, Olsen-P, and PAC was examined by calculating the Pearson correlation coefficients. The statistical analyses were performed with SPSS software, version 21.0 (IBM Corp., Armonk, USA). There was using MestRec software (Galicia, Spain) to draw NMR spectra.

## RESULTS AND DISCUSSION

**Correlation between soil P and P components in different fertility soils.** Forty years of N application decreased soil pH, but the addition of manure can slow down this phenomenon (Table 1). Long-term application of manure can significantly increase the contents of total P, Olsen-P, inorganic P, and organic P; besides, it was extremely increased the rates of P activation coefficient and P recovered by  $\text{NaOH-EDTA}$  extraction (Table 2). There was a significant positive correlation between total P and available P, inorganic P, and organic P ( $P < 0.01$  or  $P < 0.05$ ) (Table 3). In contrast to soil pH, an increase in the contents of organic P was followed by M2NPK, M1NPK, NPK, CK, and CK0 in turn. Presumably, because there were significant negative correlations

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Table 2. Phosphorus (P) content, P activation coefficient, and P recovered by NaOH-EDTA extraction on different fertiliser treatments

Treatment	Total P (g/kg)	Olsen-P	Inorganic P	Organic P	PAC	P <sub>rec</sub>
		(mg/kg)			(%)	
CK0	0.42 ± 0.13 <sup>d</sup>	7.9 ± 2.59 <sup>e</sup>	231.4 ± 23.7 <sup>c</sup>	164.18 ± 24.94 <sup>d</sup>	1.91 ± 0.21 <sup>e</sup>	39.6 ± 0.9 <sup>d</sup>
CK	0.25 ± 0.05 <sup>e</sup>	19.96 ± 1.92 <sup>d</sup>	178.37 ± 13.26 <sup>d</sup>	191.56 ± 17.95 <sup>c</sup>	7.65 ± 1.35 <sup>c</sup>	20.6 ± 0.7 <sup>e</sup>
NPK	0.72 ± 0.27 <sup>c</sup>	28.2 ± 3.07 <sup>c</sup>	227.83 ± 13.77 <sup>c</sup>	233.55 ± 22.23 <sup>b</sup>	4.25 ± 1.22 <sup>d</sup>	48.7 ± 1.2 <sup>c</sup>
M1NPK	1.08 ± 0.31 <sup>b</sup>	107.59 ± 12.74 <sup>a</sup>	576.48 ± 29.98 <sup>b</sup>	342.45 ± 22.25 <sup>a</sup>	8.32 ± 11.46 <sup>a</sup>	60.3 ± 2.0 <sup>b</sup>
M2NPK	1.34 ± 0.38 <sup>a</sup>	125.47 ± 11.84 <sup>b</sup>	655.19 ± 40.22 <sup>a</sup>	346.88 ± 32.06 <sup>a</sup>	12.14 ± 2.26 <sup>b</sup>	87.5 ± 1.8 <sup>a</sup>

Means (± standard error) followed by different letters denote significant differences according to the Tukey test ( $P < 0.05$ ; Duncan's test) among different soil treatments under each column. PAC – P activation coefficient; P<sub>rec</sub> – P recovered by NaOH-EDTA extraction; CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

between pH and organic P extracted by a sequential fractionation scheme (Tiessen et al. 1984). There was a negative correlation between the PAC of CK0 and the P components (Table 3). The application of mineral

fertiliser or manure fertiliser combined can significantly increase the soil P content, but a large amount of inorganic P and organic P have more ineffective P, so it can not effectively activate the total P (Table 2).

Table 3. Results of analysis of Pearson's correlation ( $P$ -values) for inorganic phosphorus (P) and organic P with total P, available P, and P activation coefficient on different fertilisers

Treatment	Item	Inorganic P						Organic P			
		Ca <sub>2</sub> -P	Ca <sub>8</sub> -P	Al-P	Fe-P	O-P	Ca <sub>10</sub> -P	LOP	MLOP	MROP	HROP
CK0	TP	0.90**	0.96**	0.76*	0.73*	0.69*	0.69*	0.85**	0.97**	0.68*	0.61*
	AP	0.70*	0.82**	0.85**	0.84**	0.59*	0.62*	0.97**	0.88**	0.79*	0.64*
	PAC	-0.64*	-0.84**	-0.71*	-0.58*	-0.33	-0.30	-0.94**	-0.73*	-0.59*	-0.51*
CK	TP	0.97**	0.97**	0.96**	0.95**	0.86**	0.75*	0.91**	0.75*	0.87**	0.95**
	AP	0.95**	0.95**	0.90**	0.95**	0.87**	0.83**	0.96**	0.80**	0.90**	0.97**
	PAC	0.92**	0.92**	0.88**	0.94**	0.90**	0.88**	0.94**	0.82**	0.93**	0.97**
NPK	TP	0.97**	0.94**	0.82**	0.95**	0.94**	0.90**	0.97**	0.97**	0.96**	0.97**
	AP	0.91**	0.86**	0.80**	0.88**	0.87**	0.87**	0.95**	0.98**	0.84**	0.86**
	PAC	-0.77*	-0.90**	-0.70*	-0.89**	-0.82**	-0.70*	-0.80**	-0.76*	-0.94**	-0.92**
M1NPK	TP	0.85**	0.97**	0.89**	0.79*	0.80**	0.57*	0.86**	0.89**	0.76*	0.87**
	AP	0.83**	0.71*	0.89**	0.92**	0.94**	0.98**	0.85**	0.90**	0.97**	0.76*
	PAC	-0.71*	-0.89**	-0.69*	-0.63*	-0.56*	-0.29	-0.75*	-0.73*	-0.52*	-0.82**
M2NPK	TP	0.87**	0.89**	0.85**	0.85**	0.87**	0.92**	0.95**	0.93**	0.85**	0.95**
	AP	0.82**	0.97**	0.97**	0.98**	0.61*	0.89**	0.91**	0.70*	0.64*	0.86**
	PAC	-0.77**	-0.71*	-0.59*	-0.60*	-0.96**	-0.73*	-0.82**	-0.90**	-0.92**	-0.90**

$r$ -value was shown. \* $P < 0.05$ ; \*\* $P < 0.01$ . Indicating significance ( $P < 0.05$ ; Person coefficient). The inorganic P of the soil sample was determined by a sequential fractionation method developed (Jiang and Gu 1989, Samadi and Gilkes 1999). Ca<sub>2</sub>-P – NaHCO<sub>3</sub>-soluble P; Ca<sub>8</sub>-P – NH<sub>4</sub>Ac-soluble P; Al-P – NH<sub>4</sub>F-soluble P; Fe-P – NaOH-Na<sub>2</sub>CO<sub>3</sub>-soluble P; O-P – occluded-P; Ca<sub>10</sub>-P – H<sub>2</sub>SO<sub>4</sub>-soluble P. The organic P of soil sample was determined by Bowman Cole (1986); LOP – labile organic P; MLOP – moderately labile organic P; MROP – moderately resistant organic P; HROP – highly resistant organic P; TP – total P; AP – Olsen-P; PAC – P activation coefficient; CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

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Table 4. Concentrations of phosphorus (P) compound classes (mg/kg) by solution  $^{31}\text{P}$  NMR (nuclear magnetic resonance) spectroscopy

Treatment	Orthophosphate	Pyrophosphate	Monoester	Diester	Phosphonates	M:D	c Monoester	c Diester	c M/D
CK0	77.0	5.0	65.9	2.6	0.0	25.8	48.5	20.0	2.4
CK	15.4	7.0	49.5	0.6	0.0	86.1	35.2	14.9	2.4
NPK	73.4	2.3	79.6	3.1	0.2	25.7	57.7	25.0	2.3
M1NPK	346.2	7.7	138.5	7.0	3.5	19.8	98.9	46.6	2.1
M2NPK	477.2	15.6	190.4	6.7	6.7	28.4	136.9	60.2	2.3

c Monoester, c Diester – phosphate monoester and diester, respectively, denote the correction for degradation products; M:D – monoester:diester ratio; c M:D – monoester:diester ratio, corrected for degradation products. CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

**Distribution of mineral forms of P under different treatments.** Table 4 shows the spectrum of all treatments had the highest content is orthophosphate. According to the  $^{31}\text{P}$  NMR peak and its integral strength, the mineral forms of P at the chemical shift are orthophosphate, with an abundance of 15.4–477.2 mg/kg. The overall performance is that the concentration of phosphate diester is low in abundance, CK is the lowest, because 40 years no fertilisation, the original phosphate diester content is very low and easy to decompose into phosphate monoester, resulting in the inability to detect phosphate diesters clearly in CK. The monoester:diester (M:D) ratio is thought to be a measurement of P lability (Schneider et al. 2016), with no correction for diester degradation, the content of diester was low in all the treatments, but the ratios of M:D varied largely (Table 2). After the correction for diester degradation, there was no significant difference in corrected ratios of the M:D in all the treatments (Table 2), but the content of diester was increased, which was highest in M2NPK. Compared with CK0, fertilisation alone does not increase the concentration of orthophosphate, but the application of manure fertiliser increases the concentration. Interestingly, we found that the content of the orthophosphate of CK0 was still lower, but the content of phosphate diester was increased by applying mineral fertiliser. A strong relationship between pH and the composition of P forms (Chen et al. 2019), in this study, the application of N fertiliser reduced the soil pH; furthermore, some compounds, including DNA and phosphonates, accumulate preferentially in the soil with low pH (Condon et al. 2005). And the concentration of pyrophosphate could be increased by applying mineral fertiliser compared with other treatments except for CK. The maximum strength

content of pyrophosphate in M2NPK was 15.6 mg/kg, which was lowest in NPK (2.3 mg/kg). Moreover, the long-term application of manure fertiliser will increase the number of microorganisms and enzyme activity in the soil (Popelářová et al. 2008, Wang et al. 2013). The addition of manure increased the contents of orthophosphate and phosphate monoester, while the addition of mineral fertiliser and manure changed the soil pH; besides, the inositol phosphate in soil showed a pH-dependent variation in stabilisation in soil (Celi and Barberis 2007). This is important because phosphate monoesters are mainly composed of inositol compounds (Xin et al. 2019), and they may be available to plants in a long-time range, while orthophosphate is easily and immediately available (Yu et al. 2019); further tests are needed for confirmation. It is suggested that the application of manure fertiliser can improve the available P form of crops and increase the yield of crops.

**Identification of forms of P in soil by  $^{31}\text{P}$  NMR.**  $^{31}\text{P}$  NMR spectra showed that orthophosphate and monoesters (mineral forms of P) were the main samples (Figure 2). Phytic acid is the most easily detected component of all processed phosphate monoesters. It may be that phytic acid originated from plants, especially seeds (Noack et al. 2014). The phytic acid in CK0, CK, and NPK mainly comes from the input of plants (seeds, weeds, roots, and residues). In addition to M1NPK and M2NPK, some of them come from manure fertiliser (Xin et al. 2019). However, Anaheim et al. (2015) showed that there was no significant difference in the concentration of phytic acid in the soil with long-term application of fertiliser. Different results may be due to different sources of manure fertiliser. It may also be an effective nutrient for plants in the long run (Richardson et al. 2007).

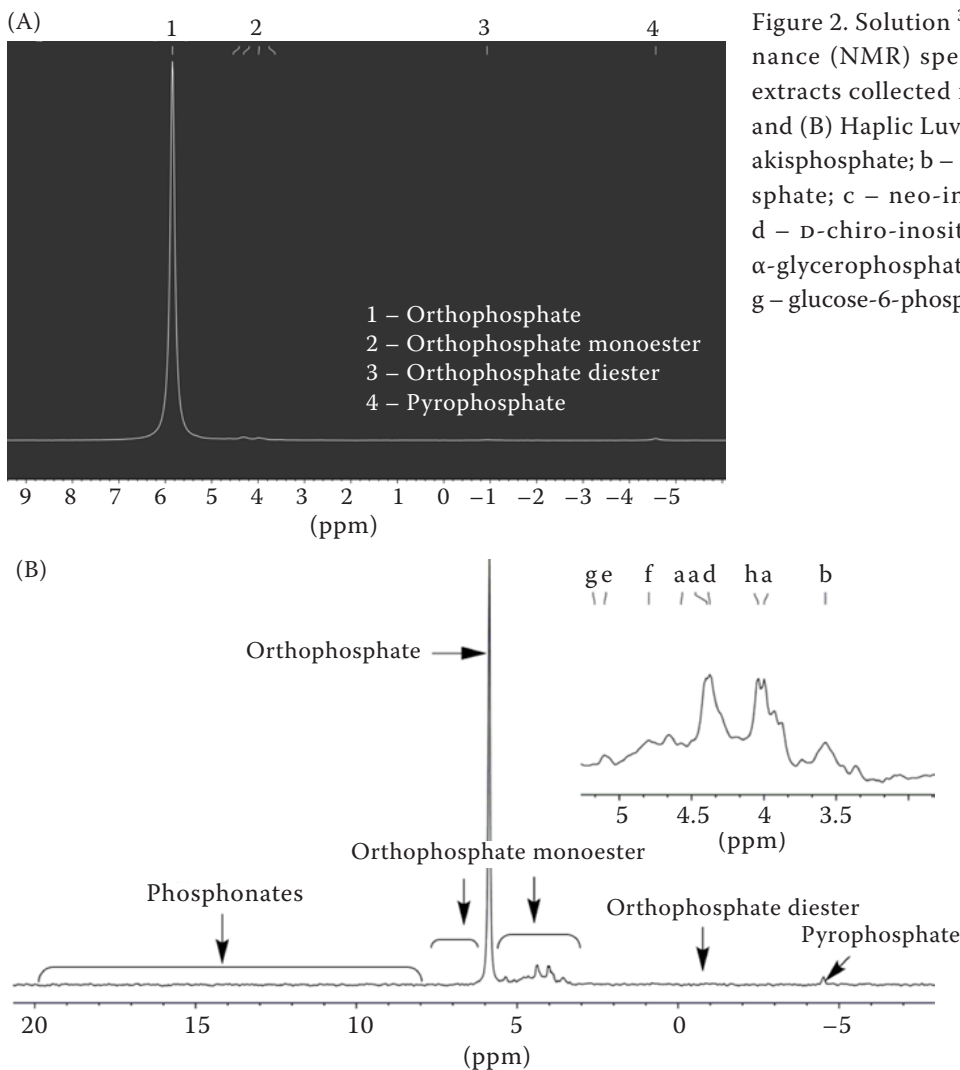
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Figure 2. Solution  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectra of NaOH-EDTA soil extracts collected from (A) manure fertiliser and (B) Haplic Luvisol. a – myo-inositol hexakisphosphate; b – scyllo-inositol hexakisphosphate; c – neo-inositol hexakisphosphate; d – D-chiro-inositol hexakisphosphate; e –  $\alpha$ -glycerophosphate; f –  $\beta$ -glycerophosphate; g – glucose-6-phosphate; h – choline phosphate

A small amount of pyrophosphate was also found, is also an active and plant available compound. While phosphate diester, phosphonate, and polyphosphate (mineral forms of P) were rarely found in the soil.

**The effects of different fertilisation soils on the components changes of inorganic P and organic P.** Orthophosphate content increased when the inorganic P components were increased (Figure 1A). On the whole, the orthophosphate content followed the following order: CK < CK0 < NPK < M1NPK < M2NPK. The orthophosphate content in CK was the lowest, which was 15.4 mg/kg. M2NPK had the most significant effect and the highest content, and the orthophosphate content was 477.2 mg/kg. Orthophosphate content in CK0 (77.0 mg/kg) was similar to that in NPK (73.4 mg/kg). Orthophosphate has a high correlation with  $\text{Ca}_2\text{-P}$ , Al-P, and O-P (Table 5).

Fertilisation can extremely increase organic P components and phosphate monoester contents (Figure 1B).

Furthermore, except for CK0, the phosphate monoester content of other treatments increased significantly with the increase of organic P components, the phosphate monoester content followed the following order: CK < CK0 < NPK < M1NPK, M2NPK. No significant change in LOP content. The MLOP content of CK0 was lower than that of CK, but the phosphate monoester content is the opposite. It may be related to their pH because the contents of organic P increased through an accumulation of some P forms such as phosphate monoester (inositol hexakisphosphate) (Turner et al. 2013), and indicating that most of the organic P components in CK have a small contribution to the phosphoric acid monoester, although it can be expected that the long-term addition of organic fertiliser will increase the soil organic P pool (Song et al. 2011, Gardini et al. 2012), only MLOP and HROP have a high contribution (Figure 1B, Table 6). Phosphate monoester content in

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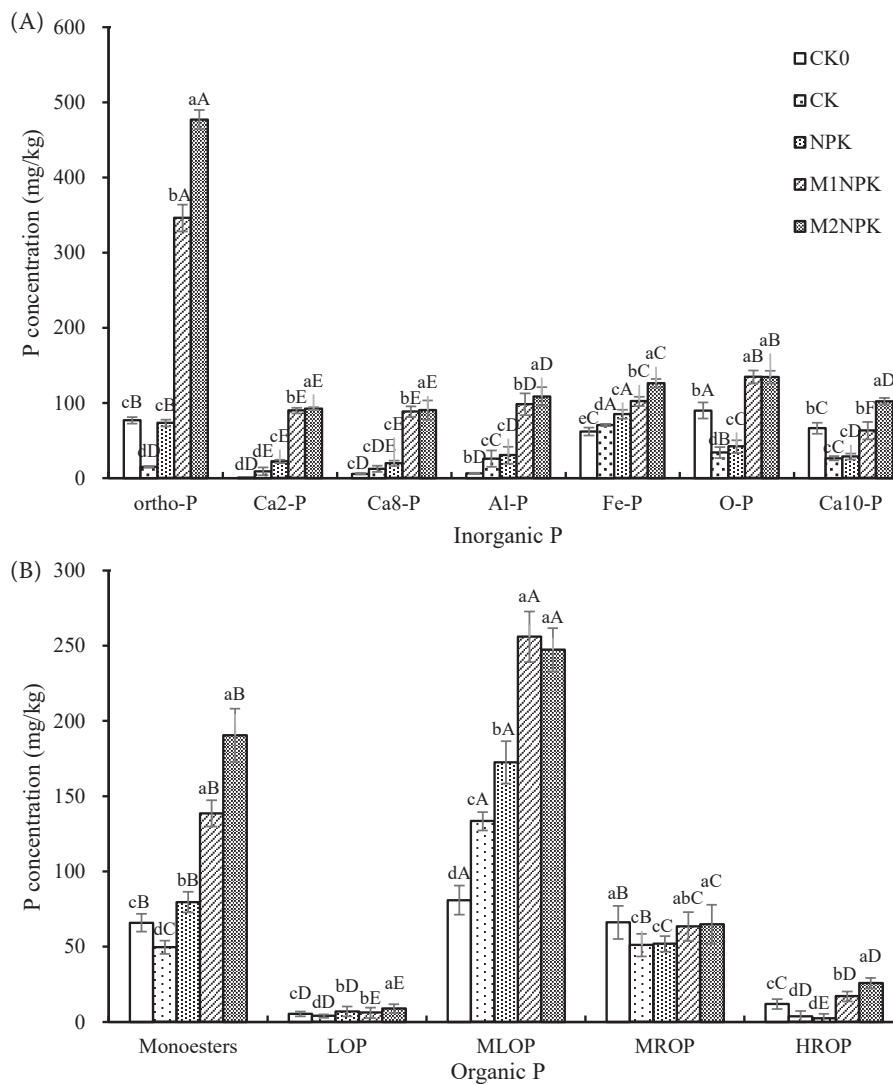


Figure 1. The response of (A) inorganic phosphorus (P) and (B) organic P. Values were means  $\pm$  standard deviation ( $n = 3$ ). Values with different uppercase letters within different treatments and different lowercase letters within different P forms indicated statistically significantly different at  $P < 0.05$  level. Ca<sub>2</sub>-P – NaHCO<sub>3</sub>-soluble P; Ca<sub>8</sub>-P – NH<sub>4</sub>Ac-soluble P; Al-P – NH<sub>4</sub>F-soluble P; Fe-P – NaOH-Na<sub>2</sub>CO<sub>3</sub>-soluble P; O-P – occluded-P; Ca<sub>10</sub>-P – H<sub>2</sub>SO<sub>4</sub>-soluble P. LOP – labile organic P; MLOP – moderately labile organic P; MROP – moderately resistant organic P; HROP – highly resistant organic P. CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

M2NPK (190.39 mg/kg) increased extremely and was the highest. As a whole, the phosphate monoester in the soil is high correlated to the MLOP and HROP (Table 6).

Table 5. Correlations orthophosphate (ortho-P) with inorganic phosphorus (P) of different fertiliser treatments

Inorganic P	Significance Ortho-P	Marginal means for treatments (mg/kg)				
		CK0	CK	NPK	M1NPK	M2NPK
Ca <sub>2</sub> -P	*	0.90	9.06	22.17	89.89	92.62
Ca <sub>8</sub> -P	**	5.81	12.31	19.57	88.28	90.56
Al-P	*	6.43	25.93	30.45	98.31	108.82
Fe-P	ns	62.03	70.59	84.98	102.09	126.28
O-P	*	90.01	34.08	41.99	134.76	134.47
Ca <sub>10</sub> -P	ns	66.40	26.40	28.67	63.16	102.44

\* $P < 0.05$ ; \*\* $P < 0.01$ ; ns – not significant. Indicating significance ( $P < 0.05$ ; Person coefficient). The inorganic P of the soil sample was determined by a sequential fractionation method developed (Jiang and Gu 1989, Samadi and Gilkes 1999). Ca<sub>2</sub>-P – NaHCO<sub>3</sub>-soluble P; Ca<sub>8</sub>-P – NH<sub>4</sub>Ac-soluble P; Al-P – NH<sub>4</sub>F-soluble P; Fe-P – NaOH-Na<sub>2</sub>CO<sub>3</sub>-soluble P; O-P – occluded-P; Ca<sub>10</sub>-P – H<sub>2</sub>SO<sub>4</sub>-soluble P; CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

Table 6. Correlations phosphate monoester (monoesters) with organic phosphorus (P) of different fertiliser treatments

Organic P	Significance Monoesters	Marginal means for treatments (mg/kg)				
		CK0	CK	NPK	M1NPK	M2NPK
LOP	ns	5.32	3.84	6.95	6.07	8.86
MLOP	**	80.88	133.35	172.48	255.95	247.32
MROP	ns	66.13	50.94	51.85	63.45	64.92
HROP	*	11.85	3.42	2.27	16.98	25.78

ns – not significant; \* $P < 0.05$ ; \*\* $P < 0.01$  indicating significance ( $P < 0.05$ ; Person coefficient). The organic P of the soil sample was determined by Bowman Cole (1986). LOP – labile organic P; MLOP – moderately labile organic P; MROP – moderately resistant organic P; HROP – highly resistant organic P; CK0 – soil before planting in 1979; CK – unfertilised control in 2018; NPK – NPK mineral fertiliser; M1NPK – NPK mineral fertiliser plus low pig manure; M2NPK – NPK mineral fertiliser plus high pig manure

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