



Long-term changes in organic and inorganic phosphorus compounds as affected by long-term synthetic fertilisers and pig manure in arable soils

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Received: 5 January 2021 / Accepted: 10 November 2021 / Published online: 16 January 2022
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Abstract

Background Manures and synthetic fertilisers can affect soil pH and plant-microbe processes and thus influence P forms and composition.

Aims The objective of this study was to examine the effects of the 40-year application of synthetic fertilisers or pig manure combined with synthetic fertilisers on the P forms and composition and the response of corn growth and yield to changes in P availability.

Methods A long-term field experiment with unfertilised, urea, urea+superphosphate, pig manure+urea and pig manure+urea+superphosphate fertilised soils was studied. ³¹P nuclear magnetic resonance spectroscopy was used to quantify P compounds.

Results The maximum corn yield was found when adding pig manure+urea, despite Olsen-P

was lower than on adding manure+superphosphate. Other monoester P compounds, typically related to microbial biomass and processes were highest on adding pig manure+urea. When adding superphosphate+urea+pig manure a reduction of these P compounds was observed as well as the highest accumulation of inositol hexakisphosphate (IHP). Synthetic fertilisers increased Olsen-P, orthophosphate and other monoester and total IHP and largely decreased soil pH. The addition of pig manure greatly increased the stereoisomers of IHP (myo-IHP, scyllo-IHP, neo-IHP, and D-chiro-IHP) and the concentration of orthophosphate diesters. D-chiro-IHP was not found in unfertilised soils while neo-IHP was not detected in synthetically fertilised soils.

Conclusions P compounds related to microbial processes, such as other monoester, increased when adding pig manure. Additions of superphosphate on top of manures reduced other monoester, while they increased on adding synthetic fertilisers alone.

Responsible Editor: N. Jim Barrow.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-021-05225-z>.

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Unfertilised soils were virtually free of P compounds related to microbial processes.

Keywords Nitrogen · Manure · Corn growth · Phosphorus form · ^{31}P -NMR · Organic P

Introduction

The use of phosphorus (P) in agriculture has increased over the last decades (Lun et al. 2018), mineable P is a non-renewable resource confined to a handful of countries including China (Obersteiner et al. 2013). However, P inputs in China are among the highest in the world and the use of organic fertilisers such as pig manures makes up more than half of the P inputs in Chinese fertilisation plans. Pig manure in China amounts 1.15 billion t y^{-1} (Liu et al. 2019) it comes from intensive pig farms feed by a protein rich and P rich diet including rice bran, wheat bran, rapeseed cake, peanut cake, distiller's grins, brain grains, fish meal and bone-meal, pig manure is thus P rich and is composed by faces and urine. It is interesting to note that most P in manures is generally held in inorganic form (Sharpley and Moyer 2000; Koopmans et al. 2007) so it may be readily available to plants and microbes. On the other hand, organic matter richness of manures can increase inorganic P solubility (Haynes and Mokolobate 2001).

In many agricultural soils most P is held in soils unavailable precipitated in primary minerals or adsorbed in organically complexed forms (Pierzynski et al. 2005; Stutter et al. 2012). Most P in agricultural soils is in inorganic forms although orthophosphate monoester makes a relevant pool of about one third of the total (Menezes-Blackburn et al. 2018) although it can range from 5 to 95% of soil P (Sharpley 1985). Organic P is made of orthophosphate monoester, orthophosphate diester, organic polyphosphate and Phosphonates (Condon et al. 2005). In natural soils a large proportion of organic P may be found in microbial biomass (Turner et al. 2013), so in organic matter rich soils organic P may be a very dynamic pool. Organic P can be transformed to inorganic via mineralization by root or microbial-released phosphatases (Bünemann 2015) while inorganic P can be derived from microbial processes or by addition of plant or manure residues (Romanyà et al. 2017).

Organic matter additions can affect soil microbial biomass and activity in soils (Ye et al. 2019) and likely have effects on P transformations. In low P soils adding manures has been found to decrease organic P pools while in high P have increased organic P (Romanyà and Rovira 2009a). Such changes have been attributed to soil microbial processes associated to soil organic matter and to root exudates to mobilize nutrients held in soil organic matter (Keiluweit et al. 2015). N additions can also have effects on soil microbiota and on P mineralization processes by altering extracellular enzyme activities and by promoting soil C mobilization (Xiao et al. 2021). Short term quantification of P changes in organic P generally are difficult to measure in the laboratory studies due to the rapid sorption of released P (Bünemann 2015). In field studies it has been shown that the size of microbial population is the main driver of P changes rather than the P forms (Liebisch et al. 2014). Long-term field studies can be a way to gain information on transformation of inorganic and organic P forms and how the changes in organic P composition can be related to management and or to soil properties.

Analysing ^{31}P in soil extracts by nuclear magnetic resonance (^{31}P -NMR) is a very effective way of obtaining detailed information on soil P forms to understand the distribution of P species in different types of soils (Ward et al. 2017). The most extensively-used method for extracting soils and fertilisers is the NaOH ethylenediamine tetra acetic acid (NaOH-EDTA) method (Cade-Menun and Liu 2014). Additionally, ^{31}P -NMR is the most effective method to obtain information of organic P compounds as it provides the most accurate and detailed information of almost all the known forms of organic P in soil (Doolette and Smernik 2011). In the present study, we used ^{31}P -NMR to study long-term soil P changes over a 40-year field experiment testing the effects of adding N and pig manure. Our hypothesis was that increased soil organic matter may increase soil microbial biomass and activity and thus promote changes in composition and distribution of organic P forms while in soils depleted with organic matter will have less microbial biomass and less capacity to change composition and distribution of soil P. Adding N may influence microbial populations and activity and thus it may have affected changes in soil P forms and compounds. The aim of this study was to investigate

the effects of long-term application of N or manure fertiliser, on soil P composition by analysing P availability and by identifying P compounds by ^{31}P -NMR spectroscopy.

Materials and methods

Study sites and field investigation

The study was carried out in a long-term field experiment in arable soils. According to the United Nations FAO classification, the soil was haplic luvisol (FAO 1998). The study area was located in the centre of the southern Song Liao Plain (40° 48' N, 123° 33' E, altitude 83.6 m), Shenyang County, Liaoning Province, China. Details of the site are available in Li et al. (2020). This experiment was established in 1979 to test the effects of synthetic and manure-based fertilisers in a corn-corn-corn-soybean crop rotation. The experiment was divided into three blocks: unfertilised, synthetic-fertilised, and manure-fertilised. Each block was divided into nine plots, 160 m² in area (10×16 m). The treatments selected for the current experiment were unfertilised soils, unfertilised, synthetic-fertilised soils (N and N+P), and soils fertilised with pig manure in combined with N or with N+P. On average, the pig manure (dry) used as manure fertiliser for the past 40 years has contained 83.5 g kg⁻¹ (average from 1979 to 2018) organic carbon (C), 7.2 g kg⁻¹ N, 3.8 g kg⁻¹ P, and 8.3 g kg⁻¹ K. All fertilisers were applied once as a base fertiliser. N

was applied as urea and P as calcium superphosphate. Before planting, the soil was fertilised and ploughed to a depth of 20 cm. The yearly applications of N and P and P balances are shown in Table 1.

Soil and plant sampling and analyses

For this experiment, we analysed soil samples collected after the autumn harvests of 2006 and 2018 (corn planting years) from 0–20 cm, 20–40 cm and 40–60 cm depth. Initial soil collected before the experiment started in 1979 was also obtained from 0 to 20 cm and 20–40. In 2018, in each 10–16 m plot each whole corn cob as well as the corn stem and leaf were weighed, and the total corn yield and weight of the stem and leaf per corn were calculated. Corn height and stem diameter were also measured. Soil organic C and total N were determined by an elemental analyser (Elemental III, Germany). The soil was extracted with the ammonia nitrogen in the extract was immediately analysed by a flow analyser (Kachurina et al. 2000; Auto Analyser 3-AA3, Seal Analytical). Soil pH was determined with a glass electrode using a soil-to-water ratio of 1:2.5.

Determination of P (Olsen-P, solution ^{31}P nuclear magnetic resonance)

Soil Olsen-P (Olsen-P as labile P or available P; Olsen and Sommers 1982) was determined by the

Table 1 Annual inputs of N, P, inorganic P, organic P from synthetic fertilisers and pig manure under all treatments, and abbreviation

Treatment	P amount kg ha ⁻¹	Nutrients from mineral fertilisers		Nutrients from pig manure		Organic P amount kg ha ⁻¹	Inorganic P amount kg ha ⁻¹	P balance kg ha ⁻¹ yr ⁻¹	Abbreviation
		kg ha ⁻¹		kg ha ⁻¹					
		N	P	N	P				
initial soil	0	0	0	0	0	0	0		
unfertilised	0	0	0	0	0	0	0	-3.6	P _o ⁰ P _i ⁰
N fertiliser	0	120	0	0	0	0	0	-5.3	P _o ⁰ P _i ⁰ -N
N+P fertilisers	26	120	26	0	0	0	26	8.9	P _o ⁰ P _i ²⁶ -N
pig manure+N fertiliser	102.6	120	0	194.4	102.6	4.2	98.4	23.9	P _o ⁴ P _i ⁹⁸ -N
pig manure+N+P fertilisers	128.6	120	26	194.4	102.6	4.2	124.4	29.9	P _o ⁴ P _i ¹²⁴ -N

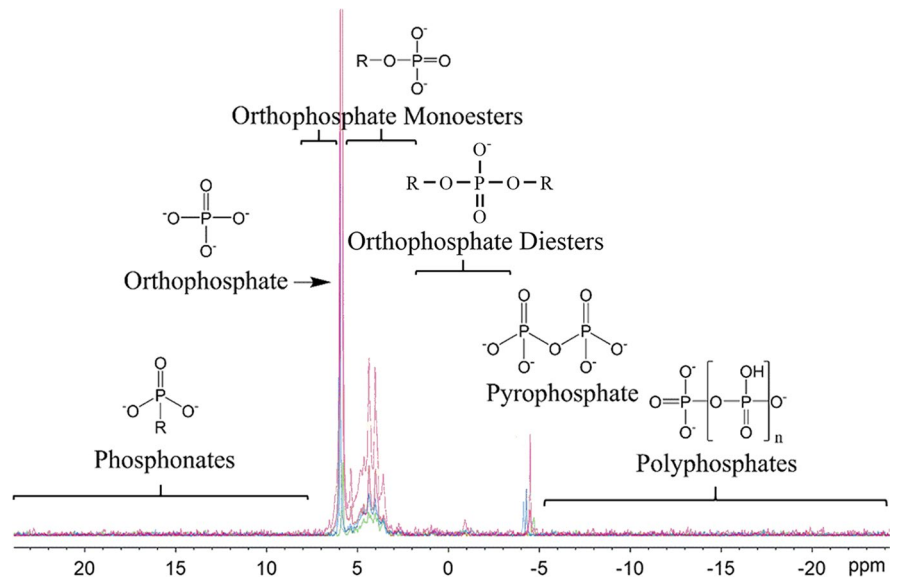
initial soil obtained before planting in 1979. P_o^xP_i^y: the application amount of organic P (Po) is x and the application amount of inorganic P (Pi) is y. From year 1992 on pig manure was not applied in soybean years (once every three years)

Olsen method. We based the extraction on previous methods (Murphy and Riley 1962; Cade-Menun and Preston 1996; Turner et al. 2003a; Jiang and Arai 2018). Extraction by NaOH-EDTA and determination of P compounds (NaOH-EDTA retained P) by ^{31}P -NMR were conducted as follows: 3 g air-dried soil samples (0.15 mm) were mixed with 60 mL 0.05 M EDTA +0.25 M NaOH, then oscillated at 22 °C for 16 h. No acid was used because with NaOH-EDTA extraction, the ^{31}P -NMR method can provide the most accurate estimate of soil organic P (Turner et al. 2003a). The extracts were centrifuged (10,000×g, 30 min), diluted at 1:100, and submitted to 880-nm molybdenum-antimony colorimetric assay (Murphy and Riley 1962). After rapid freezing at −80 °C and freeze-drying, 300 mg samples of NaOH-EDTA extracts were dissolved in 0.1 ml of 10 M NaOH and 0.6 ml D_2O , reacted for 30 min, oscillated occasionally, then centrifuged (1500 mg, 20 min) and transferred to NMR tubes of 10-mm diameter. They were stored at 4 °C and, within 24 h, a 10-mm wide-frequency probe was installed with a spectrometer (GE Omega 500 MHz), and the ^{31}P -NMR spectra were determined (Turner et al. 2003b). A pulse of 90°, detection time of 0.68 s, pulse lag of 4.32 s, and temperature of 20 °C were used; 85% H_3PO_4 was used as the standard sample, and each sample passed through 8000 scans. The P concentrations were determined by means of reduced phosphomolybdenum blue and inductively coupled plasma-mass spectrometry (ICP-MS) (these were used only to determine the content of total P of the soil extracted by the EDTA+ NaOH extract). In this study, organic P (Po) was the total organic P in the soil determined by ^{31}P -NMR spectroscopy, and included orthophosphate monoester (which is an ester derivative of phosphoric acid, also known as primary phosphoric acid ester or hydrocarbyl phosphoric acid), orthophosphate diester (which is an ester derivative of phosphoric acid, also known as secondary phosphoric acid ester), and Phosphonates. In this study, inorganic P was the total inorganic P in the soil determined by ^{31}P -NMR spectroscopy, including orthophosphate (which has a high affinity for the edges of metal oxides and clay minerals, and often forms relatively insoluble metal-P complexes in the soil), pyrophosphate (a type of P compound produced by the reaction between pyrophosphoric acid and anhydrous ammonia or potassium hydroxide.), and polyphosphate. Turner et al. (2003a)

argued that NaOH-EDTA extraction and ^{31}P -NMR provide the most accurate estimation of soil organic P. The relative proportions of all compositions in a spectrum were determined by MestRec and Chem-Draw software.

In the spectra of all samples, orthophosphate peaks were identified at 6 ppm. Additionally, there were peaks of orthophosphate monoester, neo-inositol hexakisphosphate (neo-IHP), D-chiro-inositol hexakisphosphate (D-chiro-IHP; an optically active isomer of inositol which may be derived from microorganisms and plants), myo-inositol hexakisphosphate (myo-IHP; cyclohexanol hexahol-dihydrogen phosphate is mainly found in the seeds, roots, and stems of plants, among which legumes seeds, grains of bran, and germ have the highest contents), scyllo-inositol hexakisphosphate (scyllo-IHP; derived from microbial synthesis), choline phosphate, α -glycerophosphate, β -glycerophosphate, and glucose 6-phosphate. Specifically, α -glycerophosphate and β -glycerophosphate are products of orthophosphate diester degradation, so the orthophosphate monoesters were corrected with consideration for these (Schneider et al. 2016). ^{31}P -NMR data correction was performed using NMR Utility T conversion software (NUTS, Acorn NMR, Livermore CA) to calculate the peak area of the integrated spectrum; the area of the known orthophosphate diester degradation compound (α -glycerophosphate and β -glycerophosphate) was subtracted from the total area of orthophosphate monoesters and add then added to the total area of orthophosphate diesters (Schneider et al. 2016). The ^{31}P -NMR spectra of the total NaOH-EDTA soil extract solutions collected from the six samples at a depth of 0–20 cm is shown in Fig. 1. The ^{31}P -NMR peaks detected in this study fell between 20 and −20 ppm. Furthermore, inorganic P forms were detected: orthophosphate (the main peak in all spectra), pyrophosphate, and polyphosphate; as were organic P forms: orthophosphate monoester, orthophosphate diester, and Phosphates. Orthophosphate monoester was detected with a 3.5 to 6.7 ppm chemical shift; the ^{31}P -NMR spectra indicate that the stereoisomers of inositol hexakisphosphate (IHP) accounted for the majority of the orthophosphate monoesters (Fig. 2). Four peaks of stereoisomers were identified as neo-IHP, D-chiro-IHP, myo-IHP, and scyllo-IHP (Fig. 2). There was no peak of D-chiro-IHP in the initial soil and unfertilised treatments, and

Fig. 1 Solution ^{31}P nuclear magnetic resonance (^{31}P -NMR) spectroscopy spectrum showing the range of P compounds detected in the topsoil (0–20 cm) of the six treatments



no peak of neo-IHP in the NF and NP treatments (Figs. 2 and 3). Four peaks of IHP (myo-IHP, scylo-IHP, neo-IHP, and D-chiro-IHP) were found in the pig manure treatments, and highest in pig manure

+N+P fertiliser treatment. In addition, the myo-IHP (phytate inositol) peak was the most frequent IHP peak observed in the six treatments (Figs. 2 and 3).

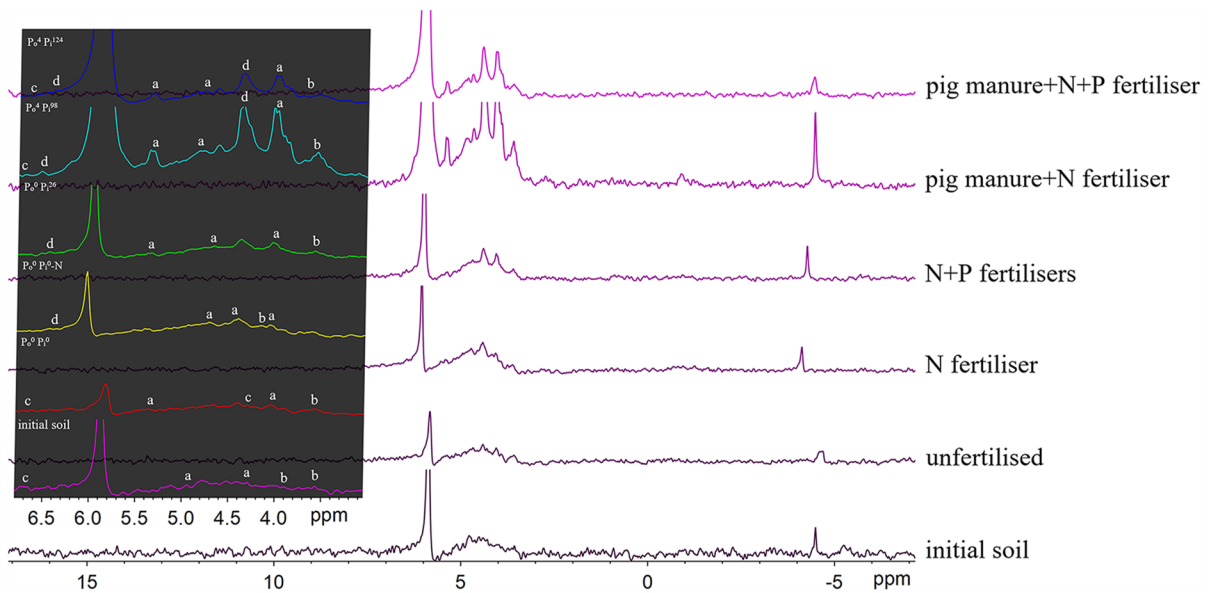


Fig. 2 Solution ^{31}P nuclear magnetic resonance (^{31}P -NMR) spectra of NaOH-EDTA soil extracts collected from the six treatments showing the inositol hexakisphosphate (IHP) in the orthophosphate monoester region in detail (6.7–3.5 ppm). **a**

myo-inositol hexakisphosphate, **b**) scylo-inositol hexakisphosphate, **c**) neo-inositol hexakisphosphate, and **d**) D-chiro-inositol hexakisphosphate

Statistical analyses

Statistical analyses involved two-way ANOVAs performed using SPSS 21.0 (SPSS Inc., Chicago, Illinois, USA). ^{31}P -NMR spectral analysis was conducted using MestReNova software version 11.0. The data were presented as in Figure (Barrow 2021). The ^{31}P -NMR data were centred-log-ratio-transformed prior to statistical analysis (Abdi et al. 2014).

Results

Soil chemical properties, corn growth, and yield

Soil pH decreased with time in most treatments; the exception was the treatment with added pig manure (Fig. 4). The largest decrease was in the N treatment and the next largest the N+P treatment. There were large increases in soil total organic C in the pig manure+N treatment. This effect was marked

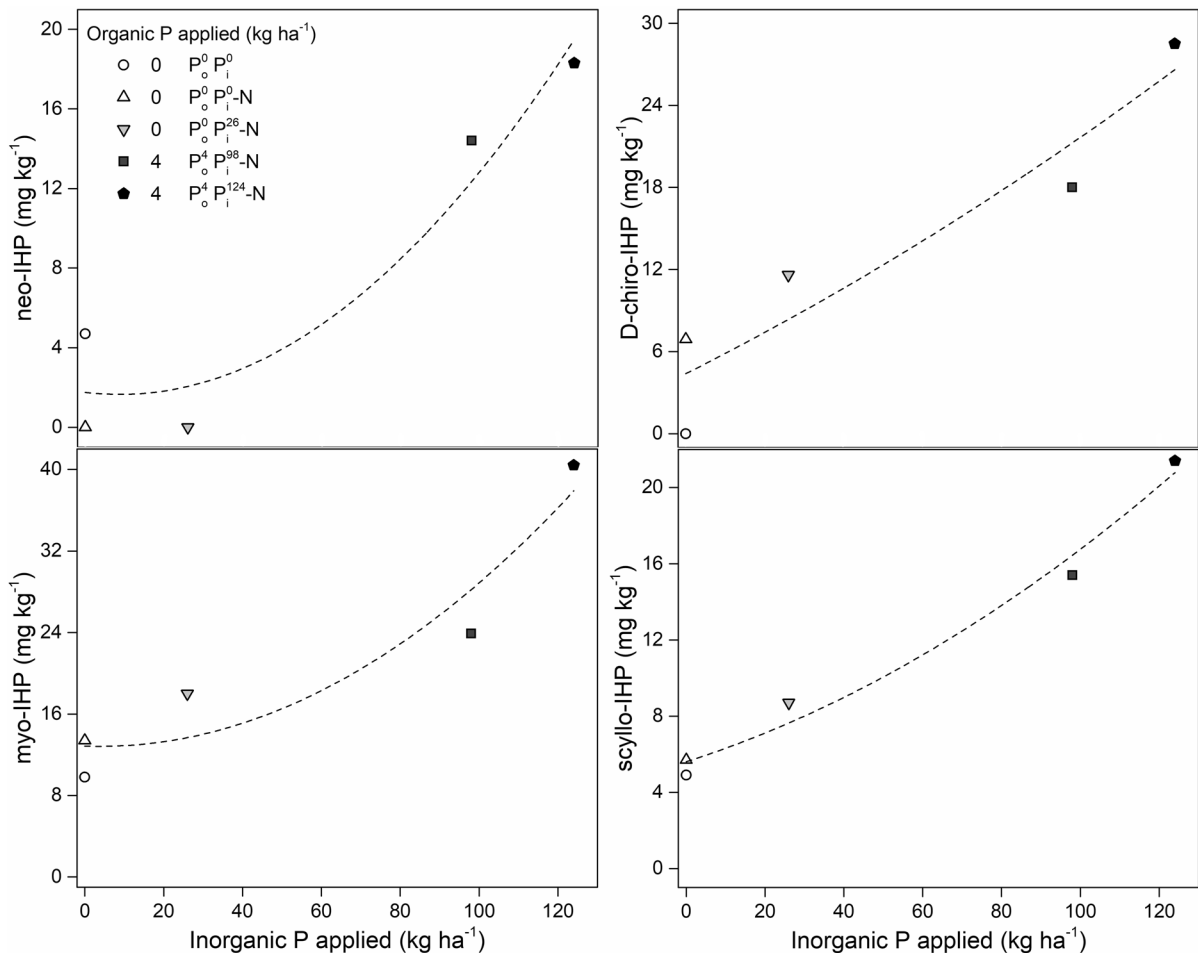


Fig. 3 The concentrations of IHP (neo-IHP, D-chiro-IHP, myo-IHP, and scyllo-IHP) in different treatments in 2018. neo-IHP: neo-inositol hexakisphosphate; D-chiro-IHP: D-chiro-inositol hexakisphosphate; myo-IHP: myo-inositol hexakispho-

sphate; scyllo-IHP: scyllo-inositol hexakisphosphate. (Values for R^2 are: neo-IHP 0.924, D-chiro-IHP 0.900, myo-IHP 0.908, scyllo-IHP 0.984). Po^xPi^y : the application amount of organic P (Po) is x and the application amount of inorganic P (Pi) is y

in 2018. There were smaller increases in the pig manure+N+P treatment; little change in the N and N+P treatment; and a decrease in the unfertilised treatment (Fig. 4). There was little change in total N through time for the unfertilised treatment but large increases with time for the other treatments. Largest increases on total N occurred following application of pig manure and especially pig manure+N+P treatment (Fig. 4). Total P, inorganic P and Olsen-P were greatly increased by treatments that supplied P especially, with the addition of pig manure either with N or N+P fertilisers. The largest increases were in pig manure+N+P treatment. Organic P increased in treatments with P additions but in this case the largest increase occurred in pig manure+N treatment with lowest P additions than pig manure+N+P treatment. Adding only N increased both organic and inorganic P and slightly increased Olsen-P in year 2018 as compared to unfertilised treatment. Total, Olsen and organic P decreased in unfertilised treatment.

The stem diameter, corn height, stem and leaf weight, and yield values were influenced by the fertiliser treatments (Fig. 5). All measured corn growth parameters (stem diameter, corn height, stem and leaf weight, and yield) were the highest in the treatment of pig manure+N+P fertilisers, but the stem and leaf weight and corn height were also the highest in the treatment of pig manure+N fertiliser. Furthermore, the corn height was lowest in N fertiliser, N+P fertiliser and unfertilised treatments. N+P fertiliser treatment increased stem and leaf weight and yield.

Depth distribution of NaOH-EDTA P forms and ^{31}P -NMR compounds

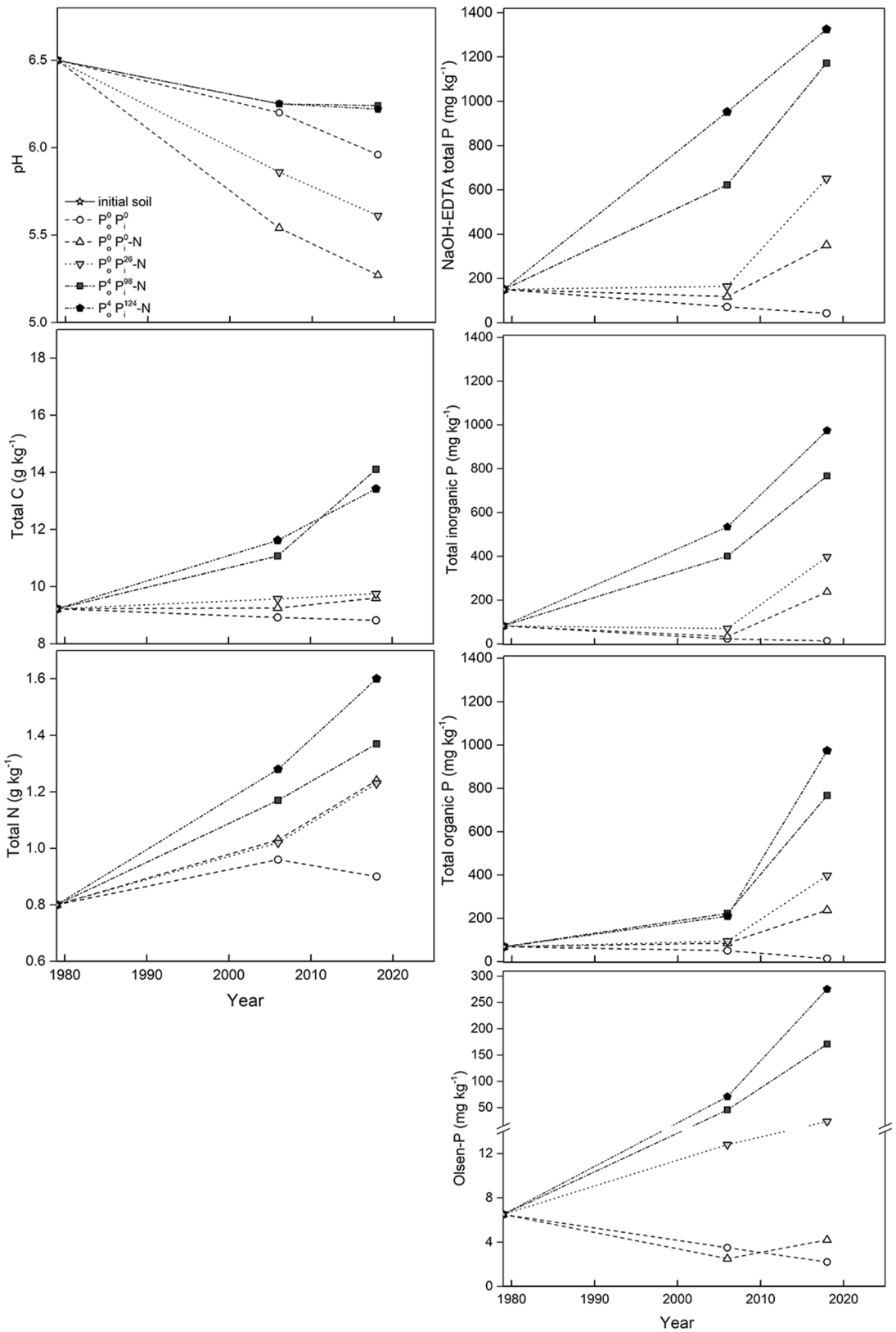
According to the ^{31}P -NMR peaks we selected five P compounds based on the overtime range of variation in the concentration of each soil P compound from 20 to -20 ppm (Fig. 1). In Figs. 7 and 8 we present inorganic and organic P forms from NaOH-EDTA extracts and five ^{31}P -NMR P compounds 27 years after the onset of the fertilisation regimes. The addition of fertilisers markedly changed the concentrations of P forms and compounds in different soil layers (Fig. 6). The concentrations of total organic P, orthophosphate monoester, and diesters largely increased when adding pig manure. The largest increases occurred in pig manure+N fertiliser treatment. However, the largest IHP increases were observed in pig manure+N+P treatment, similar

to inorganic P, orthophosphate and pyrophosphate. Increases of total organic P in N+P fertiliser treatments were observed also in 20-40 cm layer and coincided with increases of orthophosphate monoester and total IHP. In this treatment diester were only increased in the second layer. Total organic P increases in N fertiliser treatment only occurred in 0-20 cm layer coinciding with an increase of orthophosphate monoester and total IHP. In this treatment, both orthophosphate monoester and total IHP also increased in 20-40 cm layer. The reduction of organic P observed in unfertilised soils was only observed in 0-20 cm and affected orthophosphate monoester and diester compounds while IHP was not reduced. In unfertilised soils 40-60 cm layer orthophosphate monoester, diester and total IHP were not detected.

The largest increases of total inorganic P, orthophosphate and pyrophosphate were observed in pig manure+N+P treatment in 0-20 cm layer (Fig. 6). In contrast, in the 20-40 cm layer the largest increases of total inorganic P and pyrophosphate were observed in manure+N treatment. The lowest values of pyrophosphate of this later treatment were observed in 40-60 cm layer. In 0-20 cm layer, total inorganic P decreased in treatments with no or low P additions (unfertilised, N fertiliser and N+P fertiliser). No effects on inorganic P and orthophosphate in layers in 20-40 cm layer were found in these treatments. Orthophosphate behaved similarly than total inorganic P. In contrast, pyrophosphate did not change in 0-20 cm layer. In treatments with no or low P additions, this compound increased in 20-40 cm layer.

Over time variation of ^{31}P -NMR P compounds under different fertilisation regimes

The most abundant P compounds, orthophosphate and orthophosphate monoester, increased over time (Figs. 7 and 8). In treatments with low P addition (N fertilised and N+P fertilised) such increase mainly occurred from 2006 onwards. Unfertilised treatment did not increase. Somewhat surprisingly, in pig manure fertilised treatments total IHP decreased after the year 2006 (Fig. 7). This contrast with a large increase in low P addition treatments. This increase in total IHP coincides with an increase in Pi/Po ratio in these treatments, while in pig manure treatments this ratio did not change much after the year 2006.



◀**Fig. 4** Soil parameters and NaOH-EDTA total P in top-soil (0–20 cm) as affected by different treatments. Initial soil obtained before planting in 1979. $P_o^xP_i^y$: the application amount of organic P (Po) is x and the application amount of inorganic P (Pi) is y

The less abundant P compounds, pyrophosphate and diester, in pig manure treatments, decreased after the year 2006. The largest decreases of these compounds at the later stages of the experiment occurred in the treatment in the highest P addition treatment (pig manure+N+P), in pig manureN treatment pyrophosphate in 2018 was lower the initial value. After 2006 pyrophosphate increased only in N fertiliser treatment. In contrast, diesters increased only in N+P fertilised treatment. In 2018, the concentrations of orthophosphate, orthophosphate monoester, and pyrophosphate in the unfertilised treatment were much lower than those of the initial soil (Figs. 7 and 8).

Discussion

Changes in Olsen-P and plant response

Long-term changes in Olsen-P, were only observed in treatments in which P was added at rates higher than 26 kg ha^{-1} year. Treatments with highest P additions coincided with high additions of organic matter and showed levels of Olsen-P extremely high and the highest levels of total C. Organic matter additions have been often reported as a way to increase P mobilization (Haynes and Naidu 1998) and to increase pH (Haynes and Mokolobate 2001). Adding only N, in urea form, did increase in Olsen-P, although it produced the largest decrease in soil pH. According to Barrow et al. 2020, lowering pH to 5 may have promoted plant P uptake. However, this has not been seen in our experiment, probably due to strong depletion of P caused by 40 years of negative balances ($-5.3 \text{ kg ha}^{-1} \text{ yr}^{-1}$).

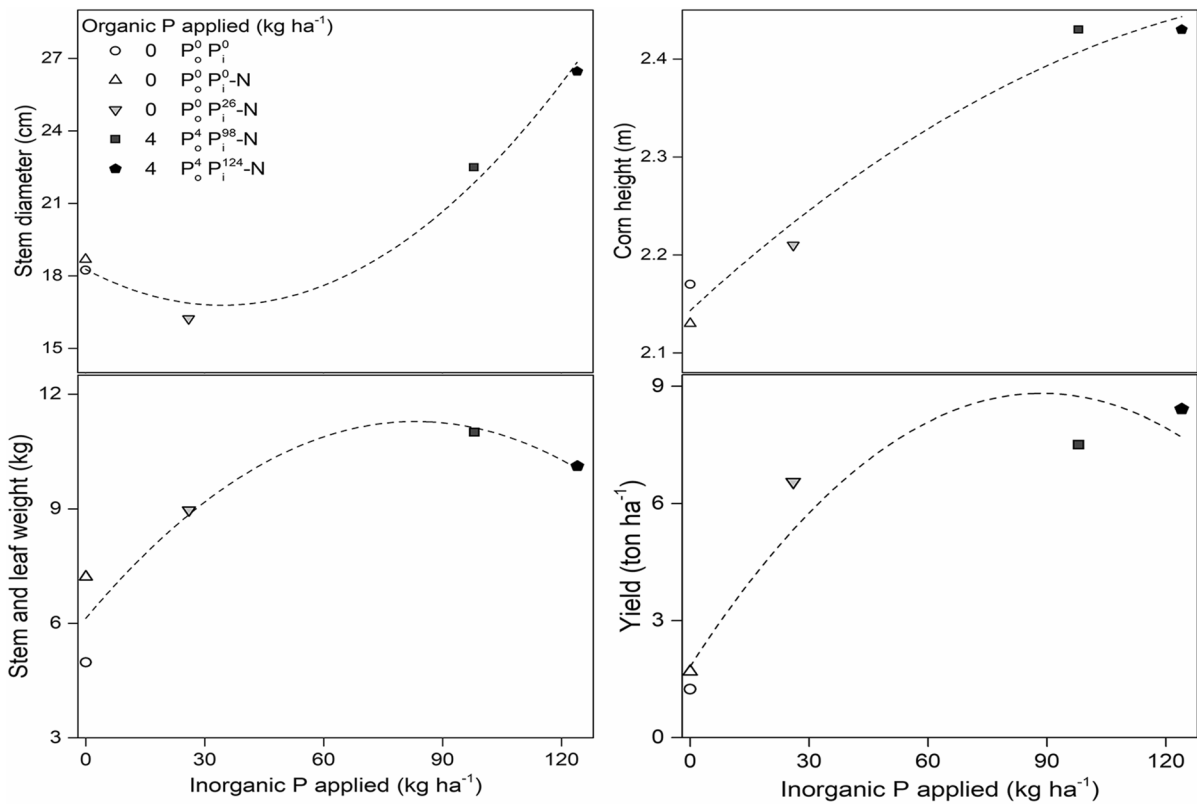
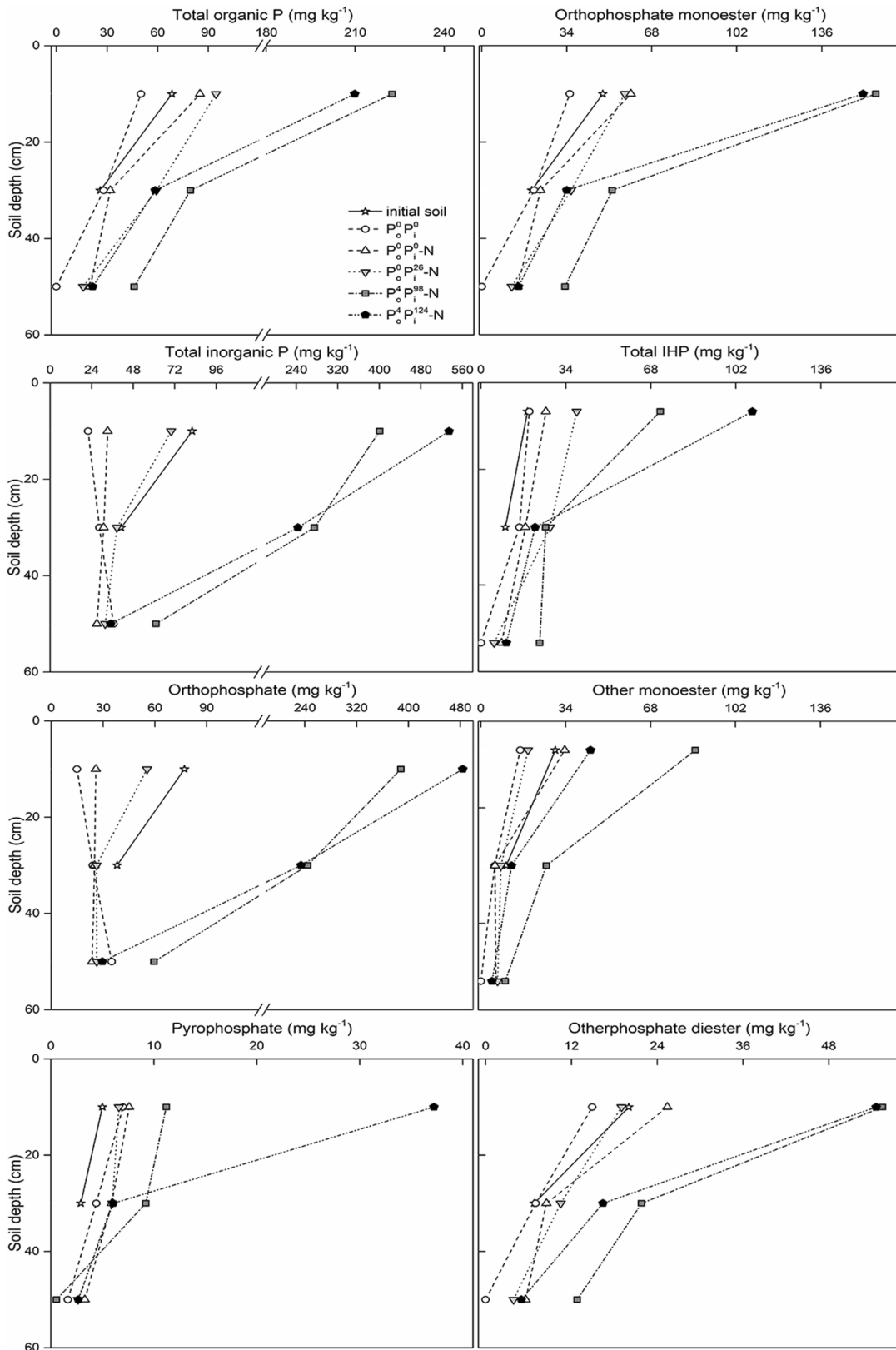


Fig. 5 Stem diameter, corn height, stem and leaf weight, and yield as affected by different treatments in 2018. (Values for R^2 are: stem diameter 0.983, corn height 0.975, stem and leaf

weight 0.900 and yield 0.919). $P_o^xP_i^y$: the application amount of organic P (Po) is x and the application amount of inorganic P (Pi) is y



◀**Fig. 6** Distributions of total inorganic P, total organic P, orthophosphate, pyrophosphate, orthophosphate monoesters (corrected for monoester degradation), orthophosphate diesters (corrected for diester degradation), other monoester, and total IHP (inositol hexakisphosphate) at various soil depths under the six treatments in 2006. initial soil obtained before planting in 1979. $P_o \times P_i$: the application amount of organic P (Po) is x and the application amount of inorganic P (Pi) is y

Changes in soil reserves of C, N and P

After 40 years, total P decreased in unfertilised treatments. In contrast total N stayed unchanged. The increases of N in N fertiliser and N + P fertiliser treatments were exactly the same, while as expected N + P fertiliser treatment relatively increases in total P. Total C slightly increased in N + P treatment as compared to N fertiliser treatment. This may be related to the increased plant production observed in N + P treatment. Adding manures largely increased the reserve of all nutrients, C, N, and P.

Organically fertilised plots largely increased of both inorganic and organic P reserves. During the first 27 years the increase of inorganic P was much higher than that of organic P. During this period of time treatments with no P additions and with low P additions showed the opposite trend as in these treatments the share of organic P increased. However, for the last 13 years these treatments relatively reduced organic P, while in high input treatments organic P share stayed virtually constant (pig manure+N+P fertiliser) or slightly increased (manure+N fertiliser). This may indicate that organic P reserves may have been mobilized by plants and microbiota in low P input treatments only when N and or P were added. Organic P share in the unfertilised treatment stayed very high until the end of the experiment. In high P input treatments, it appears that organic P share can be stabilized or slightly increased after a few decades of increasing the share of inorganic P. Organic P reserves can be mobilized in soils with low P input (Romanyà and Rovira 2009b; Romanyà et al. 2017) as is our case occurred at the last decade of the experiment. In P depleted soils, such as the case of our unfertilised soils, the mobilization of organic P by plant and soil microbiota seems to be reduced because in this case P may be retained in the physically protected fractions of soil organic matter (Stahr et al. 2018). It is

interesting to note that adding only N (N fertiliser treatment) increased total inorganic and organic P forms and also Olsen-P only at the last decade of the experiment.

Changes in P compounds in high P treatments

In high P treatments P has been mostly added by pig manure, mostly inorganic P, and in pig manure+N+P treatment also as synthetic fertiliser. In both cases large amounts of C and N organic have been also added on top of the synthetic N and/or P. As expected, high P additions have increased largely the amounts of P in soils with effects down to 40 cm depth. During the first 27 year of the experiment treatment with the highest P inputs (pig manure+N+P fertiliser) showed the highest increases of orthophosphate, and total IHP while increases of orthophosphate monoester was the same as in pig manure N fertiliser treatment. Pyrophosphate concentration was much lower than that orthophosphate monoester and decreased with depth. Similar results have been presented by other authors (Abdi et al. 2014; Deiss et al. 2016; Xin et al. 2019). Pig manure+N+P fertiliser treatment also showed high pyrophosphate and orthophosphate diester indicating high microbial biomass (Turner et al. 2003a) while pig manure+N fertiliser treatment only showed high orthophosphate diester and a small increase in pyrophosphate. This may indicate that microbial processes may be different in this later case. In the pig manure+N+P treatment, at the end of the experiment orthophosphate increased at a faster rate than during the first 27 years while pig manure+N treatment showed the same rate. Orthophosphate monoester showed an increased rate for both treatments indicating that inorganic P reserve was relatively more important in the treatment with the highest P balance. This coincides with lower other monoester for the both 0–20 and 20–40 cm layer. The degradation products of microbial biomass are important sources of orthophosphate monoester in soils (Bünemann et al. 2004). Indeed, other orthophosphate monoester are made up of microbial products readily mineralizable compounds such as α -Glycerophosphate, β -Glycerophosphate, Choline phosphate, Glucose-6-phosphate among other (Xin et al. 2019). These compounds may be related to presence of microbial cells (Bünemann et al. 2008) and thus may be an

Fig. 7 Orthophosphate monoester (corrected for monoester degradation), orthophosphate diesters (corrected for diester degradation), and total IHP (inositol hexakisphosphate), other monoester (orthophosphate monoesters other than total IHP) in the different treatments' conditions in the topsoil (0–20 cm) obtained from 1979 to 2018. initial soil obtained before planting in 1979. $P_o^xP_i^y$: the application amount of organic P (P_o) is x and the application amount of inorganic P (P_i) is y

indication of microbial biomass. Other indicators of microbial biomass such as pyrophosphate and orthophosphate diester decreased in both cases at the end of the experiment. Such decreases were larger in pig manure+N+P fertiliser, that at the end of the experiment sowed lowest orthophosphate diester values than pig manure+N treatment. It appears therefore that this treatment had reduced microbial P transformations than the pig manure+N treatment. Indeed, this later treatment showed the higher share of organic P and higher organic C and lower total IHP than pig manure +N+P treatment. This may suggest that adding high amounts of P ($29.9 \text{ kg ha}^{-1} \text{ yr}^{-1}$) may reduce P compounds associated to microbial processes and increase total IHP.

Changes in P compounds in low P treatments

In 0–20 cm layer low P treatments after 27 years of experiment showed a reduction of orthophosphate. This was so even in N+P fertiliser treatment showing a positive P balance ($8.9 \text{ kg ha}^{-1} \text{ yr}^{-1}$). These effects were not observed in the 20–40 cm layer. Adding only N showed lower decrease in orthophosphate when compared to unfertilised treatment. In 0–20 cm layer total IHP and other monoester also increased on adding only N, but no increases on orthophosphate diester and pyrophosphate were found at this time. No monoester increase was observed at the second layer except for the total IHP. 20–40 m. At the end of the experiment, somewhat surprisingly, large increases of orthophosphate were observed in N fertiliser treatment. The increases in inorganic P were much larger than increases in organic P compounds (total IHP, and other monoester), and coincided with increases in pyrophosphate. Increases of pyrophosphate and other monoester may indicate increased microbial activity at the later stages of the experiment. I

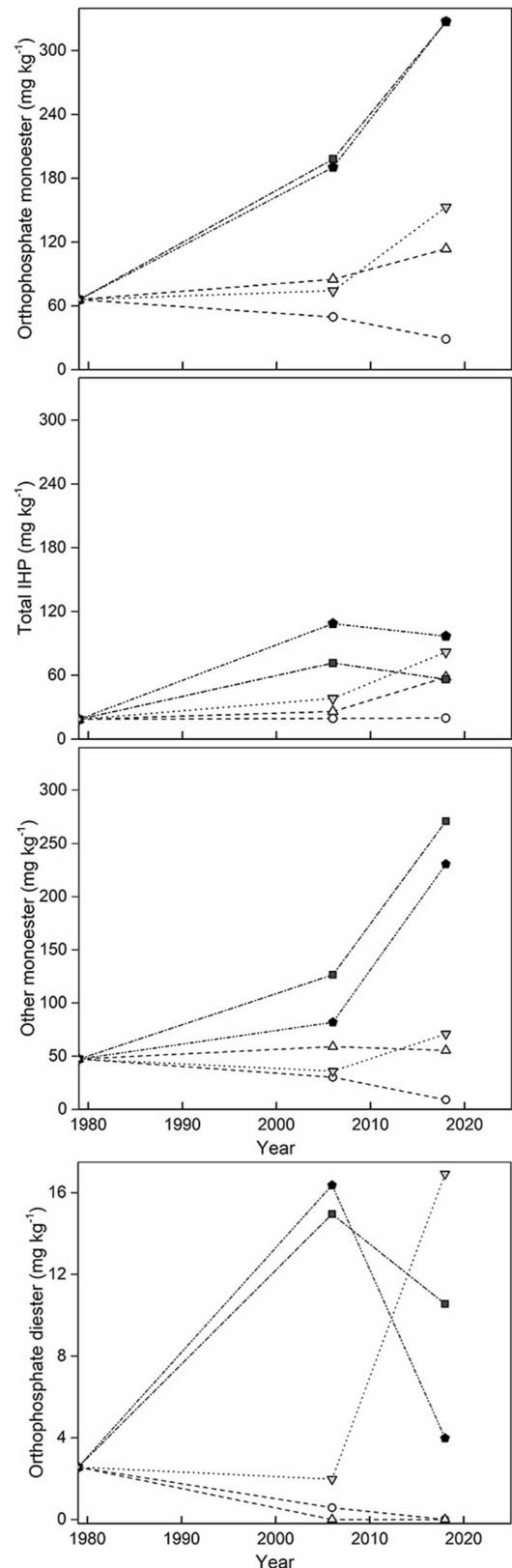
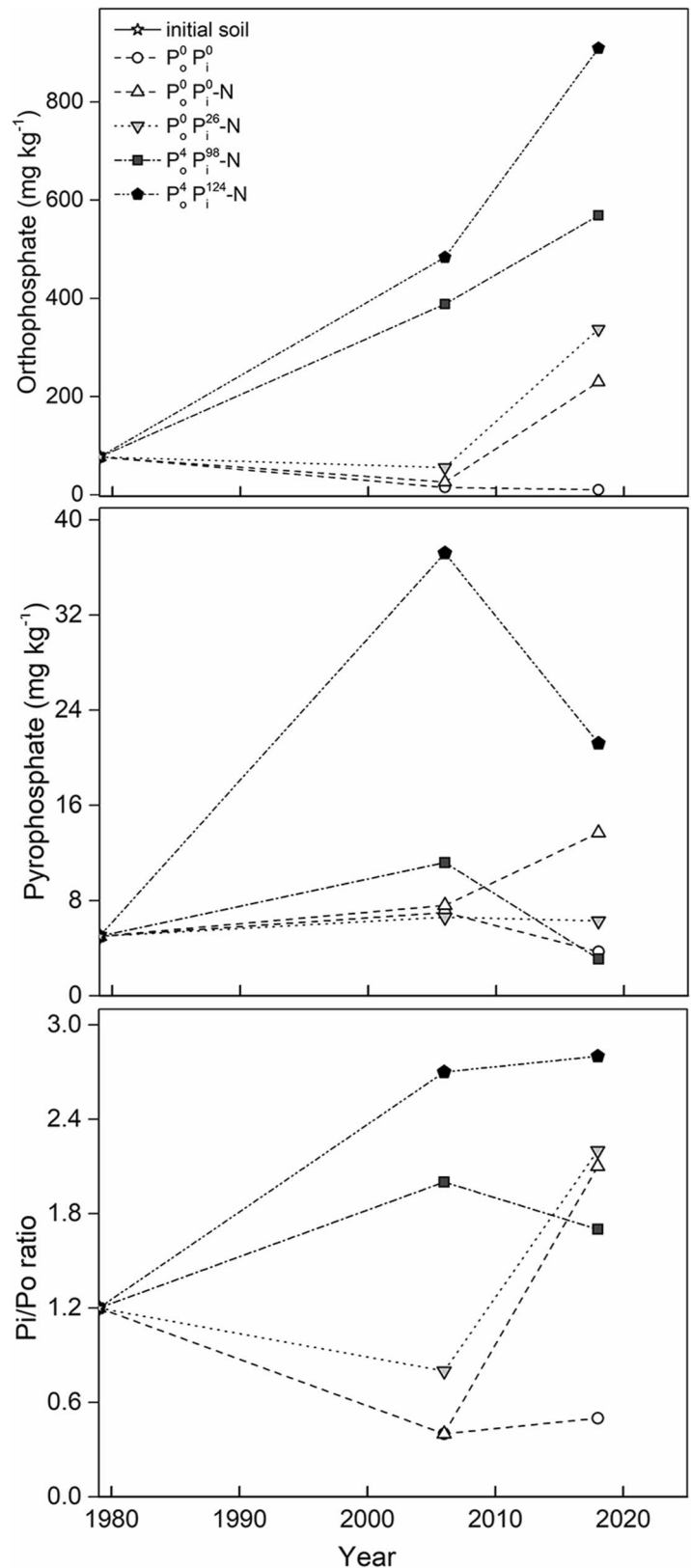


Fig. 8 Distributions of orthophosphate, pyrophosphate, and Pi/Po (inorganic P/ organic P) in the different treatments' conditions in the topsoil (0–20 cm) obtained from 1979 to 2018. initial soil obtained before planting in 1979. $P_o^x P_i^y$: the application amount of organic P (P_o) is x and the application amount of inorganic P (P_i) is y



appear therefore, that adding only N may promote plant and microbial mineralization and solubilization processes mobilizing occluded P. However, these effects did not increase crop production. Adding N can reduce microbial activity (Molina-Herrera and Romanyà 2015), however in a context of P starvation microbial activity can be increased to mobilize P reserves associated to soil organic matter and by increasing phosphatase activity (Daly et al. 2021). Some authors have found increases in phosphatase activity due to the increased N loadings (Chen et al. 2020).

It is interesting to note that in our experiment the N fertiliser treatment, with no P additions at all, behaved quite differently to unfertilised soils. This later treatment did not show any increase on P compounds during the 40 years period of study. This suggests that adding N may have induced plants and/or soil microbiota to mobilize and transform strongly retained soil P forms. Unfertilised treatment showed a strong decrease of orthophosphate and only a mild decrease of orthophosphate monoester that goes together with decreases of orthophosphate diesters, pyrophosphate and soil organic C. This suggests that in this treatment soil microbial activity may be strongly limited, with smaller capacity to mobilize organic P than inorganic P. Low organic matter levels in these soils may limit microbial processes and thus retain P and other nutrients mineral associated organic matter associated organic matter, as defined by Daly et al. (2021).

At year 27 adding low amounts of P (N+P fertiliser treatment) showed larger increases of total IHP and lower other monoester than N fertiliser alone. However, at the end of the experiment N+P fertiliser treatment increased other monoester and orthophosphate diester while N fertiliser treatment maintained the other monoester pool virtually unchanged and increased pyrophosphate. This suggests that P compounds associated to microbial biomass behave differently depending on whether N is applied alone or with P. Applying only N increases other monoester in the first measurement (27 years) while applying N and P shows a larger increase at the end of the experiment. However, both treatments largely increased the amount of orthophosphate in NaOH-EDTA extracts only at the end of the experimental period.

Changes in IHP stereoisomers

The results of this study indicated that the IHP stereoisomers resulting from different fertilisation treatments varied. Myo-IHP was the dominant form from all treatments (Liu et al. 2013). In many studies, it has been robustly confirmed that myo-IHP is mainly derived from seeds, is widespread in plants, and related to animal manure (Giaveno et al. 2010; Noack et al. 2014; Xin et al. 2019). In this study, due to long-term planting there was lot of plant tissue input mainly in treatments with high productivity. However, some studies have shown that addition of cow manure decreases myo-IHP content (Annaheim et al. 2015), in our study we have found myo-IHP increases on adding pig manure mainly in pig manure+N+P fertilised treatment while pig manure+N has shown a much lower increase. As both treatments have received the same amount of manure and held similar plant production, we suggest that plant-microbial processes may be responsible of this difference. The results of the present study also showed that the unfertilised treatment had no D-chiro-IHP. D-chiro-IHP typically comes from plants and microorganisms (Turner and Richardson 2004). As we discussed earlier, unfertilised treatment showed low microbial biomass indicators. This fact agrees with no presence of D-chiro-IP. It appears that the addition of synthetic fertiliser or manure to soil can promote the production of D-chiro-IHP. The content of neo-IHP in the soil treated with only synthetic fertiliser was low or constantly consumed, and was not detected. The addition of manure increased the content of myo-IHP, scyllo-IHP, neo-IHP, and D-chiro-IHP. This increase was even higher when adding mineral P on top of pig manure. This may be because manure can increase plant biomass and microbial biomass, and promote microbial activity (Wong and Selvam 2009). Adding pig manure largely increase the reserve of all IHP stereoisomers in the long-term. This increase is even larger when adding manure plus mineral P.

Conclusions

Increased organic matter in both high and low P additions soils coincided with increased other

monoester compounds. Some of these compounds can be directly related to microbial biomass or to microbial processes. In contrast, increases in IHP compounds were sustained until the end of the experiment only in low P additions soils fertilised with N and N + P. While in low P addition soils other monoester were of similar magnitude that IHP, in high P addition soils, other monoester were much higher than IHP. This may suggest greater share of P organic compounds in soil microbiota of high P addition soils. The reserve of organic P in unfertilised was mostly made up IHP, P compounds related to soil microbiota were extremely low in these soils. Unfertilised soils showed the lowest Pi/Po ratio that decreased overtime and organic P was mostly made up of IHP stereoisomers (neo-IHP, myo-IHP and scyllo-IHP). Olsen-P showed a similar pattern than orthophosphate and increased in all fertilised soils. Adding only N slightly increased Olsen-P at the end of the experiment coinciding with increases in orthophosphate, other monoester, total IHP and Pi/Po ratio. Such changes that mobilized large amounts of orthophosphate and increased pyrophosphate were likely related chemical changes related to decreases in soil pH and to soil microbial processes as indicated by increased levels of other monoester.

Acknowledgements We thank all of the individuals who helped collect and process the soil and plant samples in the Long-term Stationary Fertilisation Experimental Station of Brown Soil, Shenyang Agricultural University. We further thank the members of the Plant Nutrition and Fertilisation Technology Team, Shenyang Agricultural University. We wish to thank N. Jim Barrow for constructive comments on the manuscript. We thank Joan Romanyà for help in the revised version of the manuscript and for editing English. This work was supported by the National Key Research and Development Program of China (2018YFD0300308) and National Natural Science Foundation of China (30771291). We thank the editor and anonymous reviewers for their constructive comments which improved the manuscript.

References

- Abdi D, Cade-Menun BJ, Ziadi N, Parent L (2014) Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by P nuclear magnetic resonance spectroscopy. *J Environ Qual* 43:1431
- Annaheim KE, Doolette AL, Smernik RJ, Mayer J, Oberson A, Frossard E, Bünemann EK (2015) Long-term addition of organic fertilisers has little effect on soil organic phosphorus as characterized by P³¹ NMR spectroscopy and enzyme additions. *Geoderma* 257:67–77
- Barrow NJ (2021) Presenting data and distinguishing response curves. *Plant Soil* 462:1–5
- Barrow NJ, Debnath A, Sen A (2020) Measurement of the effects of pH on phosphate availability. *Plant Soil* 454:1–2
- Bünemann EK (2015) Assessment of gross and net mineralization rates of soil organic phosphorus - a review. *Soil Biol Biochem* 89:82–98
- Bünemann EK, Bossio DA, Smithson PC, Frossard E, Oberson A (2004) Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. *Soil Biol Biochem* 36:889–901
- Bünemann EK, Marschner P, Smernik RJ, Conyers M, McNeill AM (2008) Soil organic phosphorus and microbial community composition as affected by 26 years of different management strategies. *Biol Fertil Soils* 44:717–726
- Cade-Menun BJ, Liu CW (2014) Solution phosphorus-31 nuclear magnetic resonance spectroscopy of soils from 2005 to 2013: a review of sample preparation and experimental parameters. *Soil Sci Soc Am J* 78:19–37
- Cade-Menun BJ, Preston CM (1996) A comparison of soil extraction procedures for P-31 NMR spectroscopy. *Soil Sci* 161:770–785
- Chen J, Groenigen K, Hungate BA, Terrer C, Elsgaard L (2020) Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems. *Glob Chang Biol* 26:5077–5086
- Condon LM, Turner BL, Cade-Menun BJ (2005) Chemistry and dynamics of soil organic phosphorus. In: Sims JT, Sharpley AN (eds) *Phosphorus, agriculture and the environment*. Soil Science Society of America, Madison
- Daly AB, Jilling A, Bowles TM, Buchkowski RW, Frey SD, Kallenbach CM (2021) A holistic framework integrating plant-microbe-mineral regulation of soil bioavailable nitrogen. *Biogeochem* 154:211–229
- Deiss L, Moraes AD, Dieckow J, Franzluebbers AJ, Gatiboni LC, Sasaki GL, Carvalho PCF (2016) Soil phosphorus compounds in integrated crop-livestock systems of subtropical Brazil. *Geoderma* 274:88–96
- Doolette AL, Smernik RJ (2011) Soil organic phosphorus speciation using spectroscopic techniques. In: Bünemann EK, Oberson A, Frossard E (eds) *Phosphorus in action*. Springer, Heidelberg, pp 3–36
- FAO (1998) *World reference base for soil resources*. Italy, Rome
- Giaveno C, Celi L, Richardson AE, Simpson RJ, Barberis E (2010) Interaction of phytases with minerals and availability of substrate affect the hydrolysis of inositol phosphates. *Soil Biol Biochem* 42:491–498
- Haynes RJ, Mokolobate MS (2001) Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. *Nutr Cycl Agroecosyst* 59:47–63
- Haynes RJ, Naidu R (1998) Influence of lime, fertiliser and manure applications on soil organic matter content and soil physical conditions: a review. *Nutr Cycl Agroecosyst* 51:123–137

- Jiang X, Arai Y (2018) Effect of NaOH-EDTA extraction time on the degradation of phosphate compounds. *Geoderma* 324:77–79
- Kachurina OM, Zhang H, Raun WR, Krenzer EG (2000) Simultaneous determination of soil aluminum, ammonium and nitrate-nitrogen using 1M potassium chloride extraction. *Commun Soil Sci Plant Anal* 31:893–903
- Keiluweit M, Bougoure JJ, Nico PS, Pett-Ridge J, Weber PK, Kleber M (2015) Mineral protection of soil carbon counteracted by root exudates. *Nat Clim Chang* 5:588–595
- Koopmans GF, Chardon WJ, McDowell RW (2007) Phosphorus movement and speciation in a sandy soil profile after long-term animal manure applications. *J Environ Qual* 36:305–315
- Li X, Wen QX, Zhang SY, Li N, Yang JF, Han XR (2020) Long-term rotation fertilisation has differential effects on soil phosphorus. *Plant Soil Environ* 66:543–551
- Liebisch F, Keller F, Huguenin-Elie O, Oberson A, Frossard E, Bünemann EK (2014) Seasonal dynamics of microbial phosphorus in a permanent grassland. *Biol Fertil Soils* 50:465–475
- Liu J, Yang J, Cade-Menun BJ, Liang X, Hu Y, Liu CW, Zhao Y, Li L, Shi J (2013) Complementary phosphorus speciation in agricultural soils by sequential fractionation, solution ^{31}P nuclear magnetic resonance, and phosphorus K-edge X-ray absorption near-edge structure spectroscopy. *J Environ Qual* 42:1763–1770
- Liu XY, Wang XB, Li ST (2019) Phosphorus loading rates from livestock and faeces, and environmental evaluation in China. *J Agro-Environ Sci* 38:2594–2608
- Lun F, Liu J, Ciais P, Nesme T, Chang J, Rong W (2018) Global and regional phosphorus budgets in agricultural systems and their implications for phosphorus-use efficiency. *Earth Syst Sci Data* 10:1–18
- Menezes-Blackburn D, Giles C, Darch T, George TS, Blackwell M, Stutter M (2018) Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: a review. *Plant Soil* 427:5–16
- Molina-Herrera S, Romanyà J (2015) Synergistic and antagonistic interactions among organic amendments of contrasted stability, nutrient availability and soil organic matter in the regulation of C mineralisation. *Eur J Soil Biol* 70:118–125
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:678–681
- Noack SR, McLaughlin MJ, Smernik RJ, McBeath TM, Armstrong RD (2014) Phosphorus speciation in mature wheat and canola plants as affected by phosphorus supply. *Plant Soil* 378:125–137
- Obersteiner M, Peñuelas J, Ciais P, van der Velde M, Janssens IA (2013) The phosphorus trilemma. *Nat Geosci* 6:897–898
- Olsen SR, Sommers LE (1982) Phosphorus. In: A.L. Page (ed) *Methods of soil analysis. Part 2: chemical and microbiological properties*, 2nd edn. (Book Series No. 9) Soil Science Society of America, Madison, WI, pp. 403–429
- Pierzynski GM, McDowell RW, Sims JT (2005) Chemistry, cycling, and potential movement of inorganic phosphorus in soils. In: Sims JT, Sharpley AN (eds) *Phosphorus: agriculture and the environment. Vol phosphorus Agric. American Society of Agronomy*, pp 53–86
- Romanyà J, Rovira P (2009a) Organic and inorganic P reserves in rain-fed and irrigated calcareous soils under long-term organic and conventional agriculture. *Geoderma* 151:378–386
- Romanyà J, Rovira P (2009b) Organic and inorganic P reserves in rain-fed and irrigated calcareous soils under long-term organic and conventional agriculture. *Geoderma* 151:378–386
- Romanyà J, Blanco Moreno JM, Sans FX (2017) Phosphorus mobilization in low-P arable soils may involve soil organic C depletion. *Soil Biol Biochem* 113:250–259
- Schneider KD, Cade-Menun BJ, Lynch DH, Voroney RP (2016) Soil phosphorus forms from organic and conventional forage fields. *Soil Sci Soc Am J* 80:328–340
- Sharpley AN (1985) Phosphorus cycling in unfertilized and fertilized agricultural soils. *Soil Sci Soc Am J* 49:905–911
- Sharpley A, Moyer B (2000) Phosphorus forms in manure and compost and their release during simulated rainfall. *J Environ Qual* 29:146–1469
- Stahr S, Graf-Rosenfellner M, Klysubun W, Mikutta R, Prietzel J, Lang F (2018) Phosphorus speciation and C:N:P stoichiometry of functional organic matter fractions in temperate forest soils. *Plant Soil* 427:53–69
- Stutter MI, Shand CA, George TS, Blackwell MSA, Haygarth PM (2012) Recovering phosphorus from soil: a root solution? *Environ Sci Technol* 46:1977–1978
- Turner BL, Richardson AE (2004) Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Soc Am* 68:802–808
- Turner BL, Mahieu N, Condrón LM (2003a) The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy. *Org Geochem* 34:1199–1210
- Turner BL, Mahieu N, Condrón LM (2003b) Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Sci Soc Am J* 67:497–510
- Turner BL, Lambers H, Condrón LM, Cramer MD, Leake JR, Richardson AE (2013) Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant Soil* 367:225–234
- Ward D, Kirkman K, Hagenah N, Tsvuura Z (2017) Soil respiration declines with increasing nitrogen fertilization and is not related to productivity in long-term grassland experiments. *Soil Biol Biochem* 115:415–422
- Wong JWC, Selvam A (2009) Reduction of indicator and pathogenic microorganisms in pig manure through fly ash and lime addition during alkaline stabilization. *J Hazard Mater* 169:882–889
- Xiao H, Yang H, Zhao M, Monaco TA, Rong Y, Huang D (2021) Soil extracellular enzyme activities and the abundance of nitrogen-cycling functional genes responded

- more to N addition than P addition in an inner Mongolian meadow steppe. *Sci Total Environ* 759:143541
- Xin X, Zhang X, Chu W, Mao JD, Yang WL, Zhu AN, Zhang JB, Zhong XY (2019) Characterization of fluvo-aquic soil phosphorus affected by long-term fertilization using solution ^{31}P NMR spectroscopy. *Sci Total Environ* 692:89–97
- Ye G, Lin Y, Kuzyakov Y, Liu D, Luo J, Lindsey S (2019) Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: results of a 27-year field experiment. *Soil Biol Biochem* 134:15–24
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