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Effects of application of biochar and straw on sustainable phosphorus management

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Abstract

Excessive use of phosphorus (P) in farmland soil and improper disposal of crop residues such as straws accelerate the consumption of P resources and cause a high level of air and water pollution, which are the main limiting factors for sustainable agricultural development. The most important alternative is the introduction of organic fertilizers to replace mineral P fertilizer. However, the type of organic fertilizers and management methods differ significantly. In this study, we used solution 31-P nuclear magnetic resonance spectra and Hedley fractionation method to characterize the P compounds in the initial soil (in 2013; CK0), long-term unfertilized (CK) soil, and the soils treated with N+P+K mineral fertilizer (NPK), biochar in combination with NPK fertilizer (CNPK), and corn straw in combination with NPK fertilizer (SNPK). The results showed that adding biochar significantly increased the concentration of P. However, Olsen-P was found to be the highest (21.88 mg kg^{-1}) in SNPK. The concentration of Hedley-P was the highest (574.76 mg kg^{-1}) in CNPK. The inorganic P forms were significantly increased by adding biochar (up to 183.9%). The concentration of orthophosphate is positively relative to Resin-P, $NaHCO_3$-inorganic P (Pi; organic P (Po)), NaOH-Po, and Residual-P, which are absorbed and utilized to plants and microorganisms. Adenosine monophosphate (AMP) and inositol hexakisphosphate (IHP) are potential sources of P. Compared to the direct application of straw, adding biochar increases the available P in the different soil and reduces environmental pollution.

Keywords

Biochar, Straw, Phosphorus resources, Field, Nuclear magnetic resonance, Phosphorus forms and Hedley-P

1. Introduction

Phosphorus (P) is a main limiting factor of plant productivity and plays an important role in maintaining the ecological balance of the system. The abundance and supply of P in the soil directly affect the productivity of plants. P has complex conversion relationships due to human activities and geochemical processes (Okin, et al., 2004). Among them, farmland P is mainly affected by different fertilization methods and environmental conditions. However, continuous large-scale P fertilizer application leads to the enrichment of P in farmland soil and decreases its effectiveness. Thus, even though the P content in farmland soil is high, a large amount of P fertilizer is still applied every year in order to ensure a good crop yield. Therefore, it is crucial to study the different forms of P in the soil for the sustainable management of P.

The content of the forms of P in the soil is affected by many factors such as soil pH, soil properties, organic matter, and microbial activity (Brucker, et al., 2020). Various P forms in the soil affect the P uptake by microorganisms and crops (Slazak, et al., 2010). P is generally divided into inorganic P and organic P forms in the soil (Haygarth, et al., 2018), but only part of the P forms are absorbed by the crops (Smolders et al., 2020). Some studies have shown that the types of fertilizers and fertilization practices alter the soil P forms (Deiss et al., 2016). Straw is the most abundant organic resource (Karami, et al., 2012), which enhances the soil quality, microbial activity, soil pH, and activates fixed P (Niu, et al., 2011) on its application to the fields. It plays an important role in the efficient utilization of P. Earlier studies have indicated that the application of residual straw on the field can increase the available P and total P contents in the soil. However, the P activation coefficient of the applied straw is lower than that of the unutilized straw (Zheng et al., 2019), and thus increases the conversion of organic P to inorganic P in the soil (Li, et al., 2019). In addition, the straw is converted to biochar and applied to the field.

On the one hand, it solves the problem of air pollution caused by burning straw caused by a large number of straws that are nowhere to be placed. On the other hand, it has shown great application potential in improving agricultural soil and other aspects. Ever since the introduction of biochar in the 1960s, its significance on the environment and agriculture has been widely recognized. Studies have shown that adding biochar reduces the fixation of P in different soil, promotes the activation of soil that cannot directly accommodate P, and affects the distribution of P in the soil (Lehmann et al., 2003). The biochar carries P by itself and thus results in a simple mechanism of P release. The carbonization process of biochar promotes the release of phosphate from the woody tissues of plant residues, thereby acting as an available source of soil-soluble and exchangeable P (Gundale and DeLuca, 2006). The addition of crop straw and its conversion to biochar for application on the field can reduce environmental pollution caused by the burning of straw, improve the efficient use of resources (Wang et al., 2017), and increase the soil enzyme activity, microbial biomass carbon (C), nitrogen (N) and P contents, and microorganisms. Diversity suddenly improves the environment of the microorganisms, which influences the soil ecological environment (Wang et al., 2020). Therefore, to better understand how biochar addition adjusts the P forms in the field soil is very important for sustainable P resource management (McBeath, et al., 2012).

NMR spectroscopy is widely used to obtain detailed information about soil P (Cade-Menun and Liu, 2014). In addition, Hedley et al. (1982) proposed a new P grading technique. Compared to the traditional grading methods, this technique can better reflect the dynamic changes in the soil P. It resolves the limitations of the traditional grading methods that cannot consider both inorganic and organic P forms and is thus adopted by many scholars (Wang et al., 2020). Obtaining the information of the grading of soil P and forms of P can more clearly exemplify the biological cycle of P, change in the P forms due to the process of soil formation, and effects of tillage, fertilization, and environmental factors on the soil P forms (Zamuner, et al., 2008). However, it is a lack of information on the effect of adding biochar to the field on the grading of P and forms of P.

In China, a large volume of straws is burned that causes environmental pollution. In recent years, to prevent air pollution, straws have been directly returned to the field or made into biochar and added to the field. It has been found that biochar can improve the soil environment. The resource utilization efficiency can repair or avoid certain environmental pollution, and reduce greenhouse gas emissions. So far, the soil environmental effects of biochar have attracted much attention. The effects of biochar in carbon sequestration, emission reduction, soil improvement and fertilization have been well studies. However, the effects of direct adding straw and biochar on the soil P compounds and the relationship between Hedley-P and P forms are still unclear. In addition, to the different properties of biochar used in different studies, the complex interaction between biochar and soil with different physical and chemical characterizations makes the comprehension of the results difficult. In this study, the application of the Hedley fractionation method and NMR spectroscopy were used to determine the P compounds in different types of soils, namely unfertilized soil and soils treated with mineral fertilizers, straw, and biochar. The purposes of present study were to 1) the changes of P component and P form in the soil under the direct return of straw to the field and the production of biochar; and 2) the relationship between Hedley-P and distribution of different P forms in the soil. We studied the reduction of environmental pollution caused by stubble burning, rational use of resources, sustainable and comprehensive P management.

2. Materials and Methods

2.1. Test Site

This study was performed in the field location set for the biochar consumption test (40°48′N, 123°33′E) in Shenyang Agricultural University, China, which was established in 2013. The test area is located in the southern center of the Songliao Plain, which has a temperate humid-semi-humid monsoon climate with an average annual rainfall of 736 mm, an average annual temperature of 7.5 °C. According to the FAO classification, the soil in the region belongs to the Haplic Luvisol profile (FAO, 1996). The continuous cropping of spring corn, namely "Dongdan 6531", was taken as the experimental planting mode. This crop variety was cultivated in one season per year.

Different fertilizations were used as CK0, CK, NPK, CNPK, and SNPK. Here, CK0 represents the initial soil in 2013; and CK is the unfertilized soil in 2018; NPK (mineral N, P, and K fertilizer); CNPK (biochar + N, P, and K fertilizer); SNPK (corn straw + N, P, and K fertilizer). The biochar was applied at a rate of 3000 kg ha^{-1}. The straw was applied at a rate of 9000 kg ha^{-1}. The N, P, and K fertilizers were applied at a rate of 195 kg ha^{-1}, 39.3 kg ha^{-1}, and 62.2 kg ha^{-1}, respectively. Each treatment was repeated thrice. The fertilizers to be tested were urea (N 46%), superphosphate (P 5%), and potassium chloride (K 42%). Biochar and straw were obtained using corn straw as the raw material. The basic physical and chemical characteristics of biochar (the temperature required for the formation of carbon (C) is about 450 - 600 °C) were as follows: pH of 8.8, total C concentration of 49.08%, total N concentration of 1.44%, total P_2O_5 concentration of 0.85%, total K_2O concentration of 3.20%, the specific surface area of 26.92 m^2 g^{-1}, pore volume of 0.0425 cm^3 g^{-1}, pore size of 7.12 nm, and ash content of 33.5%. The basic physical properties of straw were as follows: total C concentration of 42.08%, total N concentration of 0.96%, total P_2O_5 concentration of 0.72%, and total K_2O concentration of 0.87%.

2.2. Test Soil

The soil samples were taken from a soil layer of 0-20 cm after being harvested in 2018. The "S" five-point sampling was about 500 g, and the soil was evenly mixed to remove the plant roots and senescent plant tissue residues. The soil was naturally air-dried and sieved through 20 and 100 mesh sieves.

2.3. Laboratory analysis

The available N in the soil was analyzed using 1.0 mol L^{-1} of NaOH by the alkali diffusion method (Xiong, et al., 2008). Olsen-P was analyzed using 0.5 mol L^{-1} of $NaHCO_3$ by the molybdenum-antimony anti-colorimetric method (Olsen, et al., 1954). The available K was evaluated using 1.0 mol L^{-1} of NH_4OAc via the flame photometric method (Carson, 1980). The pH of water: soil was 2.5:1, which was measured using the Lei Magnetic PHS-3C type pH meter, China. Total P content was determined by sodium hydroxide melting-molybdenum-barium colorimetric method (Lu et al., 1996). The soil C and N contents were determined by the elemental analyzer (Elemental III, Germany).

2.4. Determination of phosphorus fraction

In prtesent study, the Hedley-P fractions were grouped into seven soil P fractions (Table 2) on the basis of the previous studies (Tiessen and Moir, 1993). 1) About 0.5 g (100 mesh) of the soil samples were taken in 50 ml screw-cap centrifuge tubes. To these samples, 30 mL deionized water and two saturated HCO_3^- anion resin membranes were added and shaken for 16 h. The resin bag was removed and shaken in 0.5 mol L^{-1} HCl solution for 16 h. The inorganic phosphorus was determined by the molybdenum blue method and labeled as Resin-P. 2). The soil residue was centrifuged in the centrifuge tube at 0 °C for 10 min at 25000 ×g, which was filtered and rinsed with 30 ml $NaHCO_3$. The solution was shaken for 16 h and then centrifuged for 10 min. The supernatant was divided into two parts, among which 0.9 mol L^{-1} H_2SO_4 was added to one portion, labeled as $NaHCO_3$-Pi ($NaHCO_3$ extracts inorganic P, mainly adsorbed on the surface of the soil, and this part of the P is effective.), which was frozen and centrifuged for further analysis. To another portion, ammonium sulfate was added and heated to 121 °C in an autoclave for 1 h, which was labeled as $NaHCO_3$-Po ($NaHCO_3$ extracts organic P, which is easy to mineralize and can be used by plants in a short period of time). 3) The soil was washed with 30 mL NaOH on the filter membrane into the centrifuge tube. The sample was shaken for 16 h, centrifuged for 10 min, and filtered. The supernatant was divided into two parts, among which 0.9 mol L^{-1} of H_2SO_4 was added to one portion, which was further frozen and centrifuged for analysis and labeled as NaOH-Pi (Inorganic P extracted with NaOH, chemically adsorbed to the soil Fe, Al compounds and on the surface of clay particles). To another portion, ammonium sulfate was added and heated to 121 °C in an autoclave for 1.5 h and labeled as NaOH-Pt (NaOH extracted total P). NaOH-Po (organic P) = NaOH-Pt–NaOH-Pi. 4) The soil

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was washed with 30 mL of 1 mol L^{-1} HCl using a filter membrane into the centrifuge tube. The sample was shaken for 16 h, centrifuged for 10 min, and filtered. The supernatant was labeled as HCl-Pi and taken for analysis. 5) About 10 ml of concentrated HCl was added to the soil residual sample. This was kept in a water bath heated up to 80 °C for 10 min. About 5 ml of concentrated HCl was added to the sample and centrifuged for 10 min. The supernatant was taken for analysis and labeled as HCl-Pt (HCl extracted total P). HCl-P = HCl-Pt–HCl-Pi. 6) To the soil residue, 5 mL of concentrated H_2SO_4 was added. This was treated with H_2O_2 and digested repeatedly at 360 °C. The sample was shaken, filtered, and left undisturbed for some time. The supernatant was labeled as Residual-P.

2.5. Spectral processing

The liquid-state ^{31}P NMR spectroscopy of the soil samples (extracts) was analyzed using the AVANCE III Bruker-500MHz nuclear magnetic resonance instrument manufactured by Swiss Bruker installed in Jilin University and Shenyang Agriculture University (Abdi, et al., 2014; Li et al., 2020). The pretreatment of the soil sample was performed as follows: About 3.00 g of the sample that was passed through a 2 mm sieve into a 100 ml centrifuge tube was taken, which was treated with a 60 mL mixture of 0.25 mol L^{-1} NaOH and 0.05 mol L^{-1} Na_2EDTA extractant at a water-soil ratio of 20:1. After mixing, the sample was shaken at 20 °C for 16 h, centrifuged (20 °C, 10000 g, 20 min), and filtered the supernatant using a 0.45-micron filter membrane. About 15 mL of the extract was frozen. The lyophilized sample was redissolved with 1 mL of 0.25 mol L^{-1} NaOH and centrifuged (4 °C, 10000 g, 5 min). The supernatant was separated out. About 0.6 mL of the supernatant was treated with 0.05 mL of D_2O and transferred to a 5 mm NMR tube to perform the analysis. At the same time, 5 ml of the filtered extract was digested using the H_2SO_4-$HClO_4$ mixture, and the total phosphorus content of the soil was determined by ICP-OES. The 85% orthophosphoric acid was used as the standard substance (Cade-Menun et al., 2015), and its chemical shift was set to 6 ppm. Although the ^{31}P-NMR spin-lattice relaxation times were not measured for these samples, the delay time was estimated to be sufficient on the basis of the ratio of P/ (Fe + Mn) in these extracts (Cade-Menun and Liu, 2014). Also, the MestReC software was used to plot the nuclear magnetic resonance spectra and calculate the integrals and peak areas.

2.6. Statistical analysis

In this study, the statistical analyses were performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). In addition, the data was presented as the arithmetic mean with standard deviations.

The one-way ANOVA was performed to compare the effects of the five treatments on the soil properties and P extraction efficiencies of NaOH and EDTA. The least significant difference (LSD) test was performed to determine the significance ($P < 0.05$) of the differences in the P compounds during different treatments. The correlations between the P fractions and P forms were examined by calculating Pearson's correlation coefficients.

3. Results

3.1. Soil characterizes in all the treatments

The concentration of organic C and the total amount of N, P, and Olsen-P in the soil were extremely increased by fertilization (Table 1). Compared to the initial soil (CK0), the content of available N in the soil was significantly decreased by long-term cropping or fertilization. However, compared to CK, the concentration of the available N was extremely increased by adding biochar. The application of NPK fertilizer or biochar and straw on the field had significant effects on the efficiency of extraction of P by NaOH and EDTA. Besides, the application of biochar gave the most significant results as the total P content was the highest in CNPK (Table 1). The soil pH was decreased by long-term planting or the addition of NPK fertilizers, but the application of straw or biochar (NPK+ Straw, NPK+ Biochar) effectively reduce the decline in pH (Table 1).

3.2. Determination of phosphorus using the Hedley fractionation method in soil with mineral fertilizer, biochar, and straw

Due to long-term fertilization or cropping, the P accumulated in the soil occurred in different forms. Each form was influenced and restricted by the other to a certain extent and always acquired a dynamic balance. The content and distribution of various P forms in different treatments were determined (Table 2 and Figure 1). Among them, the concentration of Resin-P was increased up to 2.2 times that of CK treatment by adding biochar. In addition, the proportions of stable P components (HCl-P and Residual-P) were lowest in CNPK-treated soil (Figure 1). Moreover, the proportions of labile P components (Resin-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po) were highest in CNPK-treated soil, which were increased by 121.8%, 61.7%, and 28.0%, respectively, compared to the CK treatment. Specifically, the content of Resin-P was highest in CNPK-treated soil that was easily absorbed by the plant. Also, the content of $NaHCO_3$-P (Pi and Po) was increased by the application of biochar, which was twice the value of CK-treated soil. Accordingly, the concentration of labile and moderately labile P components

(NaOH-Pi and NaOH-Po) differed among the treatments. On fertilization, the effect was found to be in the following order: CNPK > SNPK > NPK > CK0 > CK (Table 2).

3.3. Identification of phosphorus compounds by 31-phosphorus NMR

In this study, five P compounds (orthophosphate, pyrophosphate, polyphosphate, monoester, diester, and phosphonate) were found in all the soil samples (Figure 2). Among these compounds, the concentrations of phosphonates, pyrophosphates, diesters, and monoesters were higher in different treatments. In addition, the concentrations of the monoesters were different in all the P compounds during the five treatments (Figure 3). The AMP peaks appeared in the samples undergoing all the treatments except for those labeled as CK0. Furthermore, no DNA peak was detected in the CK-treated soil. Inositol hexakisphosphate (IHP) accounted for a vast majority (5.6–3.3 ppm) of the processed monoesters. Four types of IHPs were identified, namely, myo-inositol hexakisphosphate (myo-IP_6), scyllo-inositol hexakisphosphate (scyllo-IP_6), neo-inositol hexakisphosphate (neo-IP_6), and D-chiro-inositol hexakisphosphate (D-IP_6), among which myo-IP_6 constituted the highest proportion. Neo-IP_6 (4.28 ±0.01 ppm) was detected in the soils undergoing CK0 and SNPK treatments. D-IP_6 (4.35 ±0.01 ppm) was detected in the CK0, and CNPK treated soils.

3.4. The distribution of phosphorus forms in different treatments

In most of the treatments, most of the inorganic P in the soil is used up by plants and microorganisms. The organic P in the soil included inositol phosphate, phospholipids, nucleic acids, a small amount of phosphoprotein, phosphate sugar, and microbial P. The organic P compounds were easily decomposable and acted as the source of available P in the soil. The components and their distribution and increase in the content of inorganic P were highly significant for understanding the supply status of soil P. The LSD results of the concentration of P compounds in the treated soils are shown in Table 3. The results showed that the highest concentration of orthophosphate was observed in CNPK-treated soil, followed by the NPK-treated type. IHP was found to be the major component among the monoesters. The total concentration of inorganic P showed the maximum value in CNPK-treated soil (79.8%, 173.2 mg kg^{-1}). In addition, the total concentration of organic P in the SNPK-treated soil was 2.3 times that undergoing CK treatment. The concentration of orthophosphate varied up to 166.2 mg kg^{-1} and 91.6 mg kg^{-1} in CNPK- and CK0-treated soils, respectively. The concentration of pyrophosphate were the lowest (0.8% and 1.7 mg kg^{-1}) in CNPK-treated soil and highest in the soil treated with straw, which were 5.4 times and 4.9 times that of the NPK-treated soil, respectively. After correction, there was no significant difference between the soil samples undergoing CK0 and SNPK treatments. However, the concentration values of monoester before and after correction were obtained in SNPK-treated soil. The lowest values for the concentration before the diester correction were observed in CK-treated soil. After correction, the lowest value was still observed in CK-treated soil, while the highest value was observed in the CK0-treated type. The Mo/Di ratio was decreased after the correction. The maximum concentration in the SNPK-treated soil was 41.2 mg kg^{-1}. Long-term non-fertilization leads to the disappearance of DNA. The concentration of DNA were extremely decreased by long-term fertilization. However, the application of biochar remarkably increased these values compared to other fertilization treatments (Table 3).

3.5. Correlations between the phosphorus forms and their fractions

The correlation analysis of the concentration of different P forms with their fractions indicated (Table 4) that the orthophosphate showed a significant positive correlation with all the soil P fractions (P<0.01), except for NaOH-Pi and HCl-P. Pyrophosphate exhibited a negative correlation with all the P fractions and a significant correlation with Resin-P, $NaHCO_3$-Po, and Residual-P (P<0.05 or P<0.01). The monoester after correction showed a significant negative correlation with NaOH-P and Residual-P (P<0.05 or P< 0.01). Furthermore, the diester after correction had an insignificant correlation with the six P fractions. Moreover, IHP was positively correlated to NaOH-Pi and Residual-P (P<0.05). As a result, AMP showed a significant positive correlation with $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pi, and NaOH-Po (P<0.01 or P<0.05). Among all the P fractions, the content of DNA was significantly positively relative to labile P ($NaHCO_3$-Po; P<0.05).

4. Discussion

4.1. Effects of mineral fertilizers, biochar, and straw on soil properties

The results of present study resemble the findings of Schjønning et al. (1994), who reported that the soil treated with mineral fertilizers had the lowest pH, while the addition of straw or biochar slowed down the decrease in the pH value. Due to a large number of mineral carbonates and a surface rich in acidic functional groups, biochar is generally alkaline. Therefore, the continuous large-scale adding biochar significantly slowed down the acidification of the soil. The concentration of soil P and its availability increased by the application of biochar or straw (Steiner et al., 2007). There was a long-term supply of P to the soil attributing to the high proportion of soluble phosphate residues in the biochar prepared by low-temperature pyrolysis, its stable physical and chemical properties, and strong anti-decomposition and anti-oxidation capabilities. Therefore, the continuous application of biochar inevitably leads to the accumulation of available P in the soil. In addition, biochar has high anion and cation exchange capacities. An increase in the anion exchange capacity can affect the interaction between the soil and external P, resulting in an increase in the availability of P (Uzoma et al., 2011). Also, the concentration of Olsen-P was the highest in the soil treated with straw, indicating the potential of straw in the activation of P in the soil compared to NPK. Due to the abundance of C in biochar and straw, their addition increased the organic C content in the soil. In addition to the large concentration of organic matter, biochar showed unique physical properties. The results of this study revealed that the concentration of N pool in the soil was significantly increased by the application of mineral fertilizer compared to the treatment with biochar or straw. This may be attributed to the increase in the uptake of N by the high yields of the crop obtained on the addition of biochar or straw to the soil. In addition, the activity of N was increased by the addition of biochar, attributing to its strong retention effect on N (Dandamudi et al., 2021). On the one hand, the porous characteristics and huge specific surface area of biochar affected the concentration of N in the soil, resulting in its adsorption. On the other hand, the addition of biochar directly or indirectly affected the microbial diversity, abundance, and activity during the soil turnover process and further affected the N cycle of the soil (Spokas and Reicosky, 2009). Doydora et al. (2011) showed that the combination of biochar with organic fertilizers significantly reduced the volatilization of ammonia in the soil. Taghimadeh-Toosi et al. (2012) used the isotope tracing method to conclude that the biochar showed not only obvious adsorption capacity for NH_3 but also improved the utilization of N by the plant.

4.2. Effects of the addition of chemical fertilizers, biochar, or straw on the phosphorus content of the soil

The results of this study showed that the proportion of Residual-P (stable P, which is extremely difficult to be used by plants under normal conditions) in the soil was decreased by fertilization. However, the concentrations of $NaHCO_3$-Pi (the effective P mainly adsorbed on the soil surface and similar in ratio to that of Olsen-P) and NaOH-Pi (the amount of P on the surface of Fe, Al compounds and clay particles due to its chemical adsorption to the soil) were increased (Figure 1). Fertilization increased the activity of P in the soil, which was consistent with the results obtained by Lee et al. (Lee, et al., 2004). Also, the concentration of Residual-P in the soil was not reduced by long-term planting or fertilization. This is attributed to the stable state of Residual-P that is not easily absorbed by the plants. The application of NPK highly increased the content of HCl -P and promoted the accumulation of available P in the soil. The effect of the addition of biochar notably increased the organic P fractions ($NaHCO_3$-Po and NaOH-Po) in the soil. This may be attributed to the high proportion of soluble organic P in the soil, which retains the concentration of P during the pyrolysis process and mostly exists in the soluble form. The carbonization process of biochar promotes the release of P from the woody tissues of the plant residues, thereby acting as a direct source of soluble P in the soil (Gundale and DeLuca, 2006). The influence of biochar on the effectiveness of P can be achieved by changing the adsorption of P by the soil, which cannot be obtained from the results of the current study. Mukherjee (2011) speculated that the bridging effect of cations on the surface of biochar may also affect the effectiveness of P in the soil. The effect of biochar on the adsorption capacity of soil P was significantly correlated to many factors such as soil pH, background value of P, cation concentration, and microbial activity. $NaHCO_3$-P is an effective source of P that can be easily absorbed by the crops. The study showed no significant difference in the fraction of $NaHCO_3$-P by the addition of NPK fertilizer, biochar, or straw to the soil. This is attributed to the high crop yield and increased absorption of $NaHCO_3$-P by the plants. Long-term planting without fertilization does not affect the change in the content of $NaHCO_3$-P in the soil. In present study, long-term planting without fertilization of the soil was found to decrease the concentration of NaOH-Pi, which was highly increased by the application of mineral fertilizer. There was no significant difference in its concentration by the application of biochar and straw, as the concentration of inorganic P was mainly increased by the addition of mineral fertilizer to the soil (Jing, et al., 2019). Besides, the addition of biochar and straw can effectively activate the P in the soil (Huang et al., 2019) and increase crop absorption. The concentration of Resin-P was significantly increased by the addition of biochar compared to that of straw. This may be attributed to the special structure of biochar, which can stimulate stable P conversion with the increase in concentration, by adding biochar to increase the concentration of that can be absorbed by plants, or to stimulate the transformation of P form, it can effectively reduce the application of chemical fertilizers to the soil, reduce the consumption of resources, and reduce the water pollution caused by excessive application of P fertilizers.

4.3. Effects of the addition of NPK fertilizers, biochar, and straw on the soil P forms and buildup

Total P content of the soil by the addition of straw was significantly lower than those obtained by the other treatments. This may be attributed to the increase in the absorption of P by the high yield of crops obtained by the addition of straw. The concentration of inorganic P was significantly increased by the addition of biochar. The biochar is rich in organic matter and long-chain molecular structures that possess good constraining ability toward the mineral particles of the soil. The adsorption capacity of the soil varies with pH. In acidic soil, the adsorption capacity of soil P increases (Hale et al., 2013). The influence of biochar on the adsorption capacity of soil P is significantly correlated with various factors such as the soil pH, P pool, cation concentration, and microbial activity. The total concentration of organic P was the highest in SNPK-treated soil. The application of mineral fertilizers converts the organic P in the soil to inorganic P forms (Nobile, et al., 2020). AMP appeared in the soil after long-term planting (Figure 2B). AMP is mainly related to microorganisms and is generally obtained by the partial degradation of diesters (He, et al., 2011). In this study, crops were planted for a long duration, and the corn stubbles were plowed into the soil to provide nutrients for microbial activities before the planting process (Koller, et al., 2013). The highest proportion of IHP in the soil was attributed to myo-IP_6, as it was mainly derived from crop residues. In this experiment, long-term planting and application of straw resulted in plant residues. This was inconsistent with the results reported by Noack (2014) and Annaheim (2015). Neo-IP_6 and D-IP_6 are considered to be the products of microbial action (Turner, 2007), and their abundance reflect the resistance of these isomers to enzymatic hydrolysis (Cosgrove, 1970). These compounds were detected in CK0-treated soil. However, Neo-IP_6 was only detected in SNPK-treated soil, and D-IP_6 was only detected in the CNPK-treated sample. This may be attributed to the consumption of Neo-IP_6 and D-IP_6 present in the soil on long-term planting. The addition of NPK fertilizer does not affect their concentration. However, the addition of straw resulted in the detection of Neo-IP_6, and the application of biochar resulted in the detection of D-IP_6, the P nutrient (Neo-IP_6, D-IP_6), which may be taken up by the plants and microorganisms (Giles, et al., 2011). The porous structure of biochar provided a living environment for the microorganisms in the soil. The biochar soils that are rich in organic matter tend to be better than mineral soils. The organic matter in the soil increases its recovering ability under the compressed state and thus exhibits elasticity. At the same time, organic matter promotes the growth of microorganisms and crop roots, and their life activities further promote the formation of micro-aggregates in the soil (Frey, 2019). The concentration of the monoesters exhibited the same trend, which was similar to those shown by the proportion and concentration of diesters before and after calibration. The concentration of α- and β-forms showed the least values upon the addition of straw. This is attributed to α- and β-forms being classified as phosphate diesters. The stable diester is destabilized by the addition of straw. Besides, they are easily converted to monoesters or exist in low concentrations.

4.4. Phosphorus form and composition

This study indicated that Resin-P, $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Po, and Residual-P had a good correlation with orthophosphate. This may be attributed to the availability of these P fractions and their incompatibility with plants and microorganisms. The available states in plants and microorganisms were the effective components of soil P that were directly absorbed by the crops or used by the microorganisms in a short duration (Zheng, et al., 2003). The incompatible states included NaOH-Po and Residual-P. These forms were not directly used by plants within a short duration. However, the change in the external environment may transform them into a form that can be used by the plants and microorganisms (Wang, et al., 2019), such as orthophosphates (Schneider et al., 2016). Interestingly, pyrophosphate was negatively correlated to all the P fractions and showed a significant relationship with Residual-P, Resin-P, and $NaHCO_3$-Po. The same correlation was observed in the case of orthophosphate. When the content of orthophosphate was high, the content of pyrophosphate was lower. All the fractions except HCl-P were positively correlated to orthophosphate, indicating a balance in the proportion of pyrophosphate and orthophosphate in the soil. The content of pyrophosphate in the fertilized soil was low, which was consistent with the previous studies (Hu, et al., 2015). The correlation of the P fraction with IHP and was the same as that with the corrected monoester, as it is the major monoester in the soil (Noack et al., 2014). AMP is also a monoester, but its correlation with the P fraction is different from that of IHP. This may be attributed to the fact that AMP is formed by the partial degradation of diester (Doolette et al., 2009). Therefore, the existence of AMP must be considered comprehensively. Due to its significant positive correlation with $NaHCO_3$-Pi (Po) and NaOH-Pi (Po), the AMP can be indirectly or directly absorbed and utilized by the plants. DNA was significantly relative to $NaHCO_3$-Po due to their similar contribution toward utilization by the plants. The organic P compounds are not easily absorbed by plants (Cade-Menun, 2017). In addition to Residual-P, the corrected monoester showed a significant correlation with NaOH-Pi, while the corrected diester indicated a remarkable correlation with $NaHCO_3$-Po and HCl-P. This may be attributed to the changes caused by the α- and β-forms before and after correction. Thus, it is indirectly inferred that the α- and β-forms may be related to NaOH-Pi and $NaHCO_3$-Po and act as effective P forms.

5. Conclusions

In this study, the soil P pool was found to increase by the addition of biochar obtained by the conversion of straw rather than the direct application of straw to the soil. The concentrations of labile P (Resin-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po) and moderate labile P (NaOH-Pi and NaOH-Po) were notably increased by the application of biochar. In addition, the concentration of inorganic P forms and AMP were significantly increased. The concentration of IHP were found to increase remarkably on the treatment of the soil with straw. The P fractions that mainly contributed to orthophosphate in the soil were Resin-P, $NaHCO_3$-Pi (Po), NaOH-Po, and Residual-P. AMP showed a significant correlation with labile P ($NaHCO_3$-Pi and $NaHCO_3$-Po) and moderate labile P (NaOH-Pi and NaOH-Po). Thus, AMP was indirectly inferred to be a potential source of P. IHP had a remarkable correlative with NaOH-Pi and Residual-P. This indicated that the addition of biochar efficiently increased the content of plant-available P and potential P sources in the soil. The direct addition of straw can increase the potential source of P in the soil. The study showed that converting straw into biochar as a partial replacement fertilizer can solve the environmental pollution caused by a large amount of straw accumulation and straw burning. Further, it can improve resource utilization, slow down the consumption of effective P resources, and provide nutrients for crop growth. This is turn will improve the efficiency of fertilizer utilization and reduce the risk of water pollution caused by excessive application of chemical P.

Conflict of interest

The authors declare no competing financial interest.

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References

Abdi, D., Cade-Menun, B.J., Ziadi, N., Parent, L.É., 2014. Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by 31P nuclear magnetic resonance spectroscopy. *Journal of Environmental Quality,* 43, 1431-1441. https://doi.org/10.2134/jeq2013.10.0424.

Annaheim, K.E., Doolette, A.L., Smernik, R.J., Mayer, J., Oberson, A., Frossard, E., 2015. Long-term addition of organic fertilizers has little effect on soil organic phosphorus as characterized by 31P NMR spectroscopy and enzyme additions. *Geoderma,* 257-258, 67-77. https://doi.org/10.1016/j.geoderma.2015.01.014.

Brucker, E., Kernchen, S., Spohn, M., 2020. Release of phosphorus and silicon from minerals by soil microorganisms depends on the availability of organic carbon. *Soil Biology and Biochemistry,* 143, 107737. https://doi.org/10.1016/j.soilbio.2020.107737.

Cade-Menun, B., Liu, C.W., 2014. Solution phosphorus-31 nuclear magnetic resonance spectroscopy of soils from 2005 to 2013: A review of sample preparation and experimental parameters. *Soil Science Society of America Journal,* 78, 19-37. https://doi.org/10.2136/sssaj2013.05.0187dgs.

Cade-Menun, B.J., 2017. Characterizing phosphorus forms in cropland soils with solution 31 P-NMR: past studies and future research needs. *Chemical and Biological Technologies in Agriculture,* 4, 19. https://doi.org/10.1186/s40538-017-0098-4.

Cade-Menun, B.J., He, Z., Zhang, H., Endale, D.M., Schomberg, H.H., Liu, C.W., 2015. Stratification of phosphorus forms from long-term conservation tillage and poultry litter application. *Soil Science Society of America Journal,* 79, 504-516. https://doi.org/10.2136/sssaj2014.08.0310.

Carson, P., 1980. Recommended potassium test. In W. Dahnke (Ed.), *Recommended chemical soil test procedures for the north central region, Bulletin 499*. Fargo: North Dakota Agricultural Experiment Station.

Cosgrove, D., 1970. Inositol phosphate phosphatases of microbiological origin. Inositol phosphate intermediates in the dephosphorylation of the hexaphosphates of myo-inositol, scyllo-inositol, and D-chiro-inositol by a bacterial (Pseudomonas sp.) phytase. *Australian Journal of Biological Sciences,* 23, 1207-1220. https://doi.org/10.1071/bi9701207.

Dandamudi, K.P.R., Mathew, M., Selvaratnam, T., Muppaneni, T., Seger, M., Lammers, P., Deng, S.G., 2021. Recycle of nitrogen andphosphorus in hydrothermal liquefaction biochar from Galdieria sulphuraria to cultivate microalgae. Resources, Conservation and Recycling, 171, 105644. https://doi.org/10.1016/j.resconrec.2021.105644.

Deiss, L., de Moraes, A., Dieckow, J., Franzluebbers, A.J., Gatiboni, L.C., lanzi Sassaki, G., 2016. Soil phosphorus compounds in integrated crop-livestock systems of subtropical Brazil. *Geoderma,* 274, 88-96. https://doi.org/10.1016/j.geoderma.2016.03.028.

Doolette, A., Smernik, R., Dougherty, W., 2009. Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. *Soil Science Society of America Journal,* 73, 919-927. https://doi.org/10.2136/sssaj2008.0192.

Doydora, S.A., Cabrera, M.L., Das, K.C., Gaskin, J.W., Sonon, L.S., Miller, W.P., 2011. Release of nitrogen and phosphorus from poultry litter amended with acidified biochar. *International Journal of Environmental Research and Public Health,* 8, 1491-1502. https://doi.org/10.3390/ijerph8051491.

FAO., 1996. World reference base for soil resources. *Rome, Italy.*

Frey, S.D., 2019. Mycorrhizal fungi as mediators of soil organic matter dynamics. *Annual Review of Ecology, Evolution, and Systematics,* 50, 237-259. https://doi.org/10.1146/annurev-ecolsys-110617-062331.

Giles, C., Cade-Menun, B., Hill, J., 2011. The inositol phosphates in soils and manures: abundance, cycling, and measurement. *Canadian Journal of Soil Science,* 91, 397-416. https://doi.org/10.4141/CJSS09090.

Gundale, M.J., DeLuca, T.H., 2006. Temperature and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal. *Forest Ecology and Management,* 231, 86-93. https://doi.org/10.1016/j.foreco.2006.05.004.

Hale, S.E., Alling, V., Martinsen, V., Mulder, J., Breedveld, G., Cornelissen, G., 2013. The sorption and desorption of phosphate-P, ammonium-N and nitrate-N in cacao shell and corn cob biochars. *Chemosphere,* 91, 1612-1619. https://doi.org/10.1016/j.chemosphere.2012.12.057.

Haygarth, P.M., Harrison, A., Turner, B., 2018. On the history and future of soil organic phosphorus research: a critique across three generations. *European Journal of Soil Science,* 69, 86-94. https://doi.org/10.1111/ejss.12517.

He, Z., Olk, D.C., & Cade-Menun, B.J., 2011. Forms and lability of phosphorus in humic acid fractions of Hord silt loam soil. *Soil Science Society of America Journal,* 75, 1712-1722. https://doi.org/10.2136/sssaj2010.0355.

Hedley, M., & Stewart, J., 1982. Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry,* 14, 377-385. https://doi.org/10.1016/0038-0717(82)90009-8.

Huang, X.-f., Li, S.-q., Li, S.-y., Ye, G.-y., Lu, L.-j., Zhang, L., 2019. The effects of biochar and dredged sediments on soil structure and fertility promote the growth, photosynthetic and rhizosphere microbial diversity of Phragmites communis (Cav.) Trin. ex Steud. *Science of the Total Environment,* 697, 134073. https://doi.org/10.1016/j.scitotenv.2019.134073.

Jing, J., Christensen, J.T., Sørensen, P., Christensen, B.T., Rubæk, G.H., 2019. Long-term effects of animal manure and mineral fertilizers on phosphorus availability and silage maize growth. *Soil Use and Management,* 35, 323-333. https://doi.org/10.1111/sum.12477.

Karami, A., Homaee, M., Afzalinia, S., Ruhipour, H., Basirat, S., 2012. Organic resource management: impacts on soil aggregate stability and other soil physico-chemical properties. *Agriculture, Ecosystems & Environment,* 148, 22-28. https://doi.org/10.1016/j.agee.2011.10.021.

Koller, R., Robin, C., Bonkowski, M., Ruess, L., Scheu, S., 2013. Litter quality as driving factor for plant nutrition via grazing of protozoa on soil microorganisms. *FEMS Microbiology Ecology,* 85, 241-250. https://doi.org/10.1111/1574-6941.12113.

Lee, C.H., Park, C.Y., Do Park, K., Jeon, W.T., Kim, P.J., 2004. Long-term effects of fertilization on the forms and availability of soil phosphorus in rice paddy. *Chemosphere,* 56, 299-304. https://doi.org/10.1016/j.agee.2011.10.021.

Lehmann, J., da Silva, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil,* 249, 343-357. https://doi.org/10.1023/A:1022833116184.

Li, F., Liang, X., Zhang, H., & Tian, G., 2019. The influence of no-till coupled with straw return on soil phosphorus speciation in a two-year rice-fallow practice. *Soil and Tillage Research,* 195, 104389. https://doi.org/10.1016/j.still.2019.104389.

Li, X., Wen, Q.-X., Zhang, S.-Y., Li, N., Yang, J.-F., Han, X., 2020. Long-term rotation fertilisation has differential effects on soil phosphorus. *Plant, Soil and Environment,* 66, 543-551. https://doi.org/10.17221/263/2020-PSE.

Liu, J., Hu, Y., Yang, J., Abdi, D., Cade-Menun, B.J., 2015. Investigation of soil legacy phosphorus transformation in long-term agricultural fields using sequential fractionation, P K-edge XANES and solution P NMR spectroscopy. *Environmental Science & Technology,* 49, 168-176. https://doi.org/10.1021/es504420n.

Lu, R., Liu, H., Wen, D., Qin, S., Zhen, J., Wang, Z., 1996. Nutrient cycling and balance of agricultural ecosystem in different typical regions of China. II Parameters of nutrient input to farm land. *Chin. J. Soil Sci,* 27, 151-154.

McDowell, R., Condron, L., Stewart, I., 2008. An examination of potential extraction methods to assess plant-available organic phosphorus in soil. *Biology and Fertility of Soils,* 44, 707-715. https://doi.org/10.1007/s00374-007-0253-3.

Mukherjee, A., 2011. *Physical and chemical properties of a range of laboratory-produced fresh and aged biochars*: University of Florida.

Niu, L.-A., Hao, J.-M., Zhang, B.-Z., Niu, X.-S., 2011. Influences of long-term fertilizer and tillage management on soil fertility of the North China Plain. *Pedosphere,* 21, 813-820. https://doi.org/10.1016/S1002-0160(11)60185-9.

Noack, S.R., McLaughlin, M.J., Smernik, R.J., McBeath, T.M., Armstrong, R.D., 2014. Phosphorus speciation in mature wheat and canola plants as affected by phosphorus supply. *Plant and Soil,* 378, 125-137. https://doi.org/10.1007/s11104-013-2015-3.

Nobile, C., Bravin, M., Becquer, T., Paillat, J.-M., 2020. Phosphorus sorption and availability in an andosol after a decade of organic or mineral fertilizer applications: Importance of pH and organic carbon modifications in soil as compared to phosphorus accumulation. *Chemosphere,* 239, 124709. https://doi.org/10.1016/j.chemosphere.2019.124709.

Okin, G.S., Mahowald, N., Chadwick, O.A., Artaxo, P., 2004. Impact of desert dust on the biogeochemistry of phosphorus in terrestrial ecosystems. *Global Biogeochemical Cycles,* 18. https://doi.org/10.1029/2003GB002145.

Olsen, S.R., Watanabe, F.S., Cosper, H.R., Larson, W., Nelson, L., 1954. Residual phosphorus availability in long-time rotations on calcareous soils. *Soil Science,* 78, 141-152. https://doi.org/10.1097/00010694-195408000-00008.

Schjønning, P., Christensen, B.T., Carstensen, B., 1994. Physical and chemical properties of a sandy loam receiving animal manure, mineral fertilizer or no fertilizer for 90 years. *European Journal of Soil Science,* 45, 257-268. https://doi.org/10.1111/j.1365-2389.1994.tb00508.x.

Schneider, K.D., Cade-Menun, B.J., Lynch, D.H., Voroney, R.P., 2016. Soil phosphorus formsfrom organic and conventional forage fields. *Soil Science Society of America Journal,* 80, 328-340. https://doi.org/10.2136/SSSAJ2015.09.0340.

Slazak, A., Freese, D., da Silva Matos, E., Hüttl, R.F., 2010. Soil organic phosphorus fraction in pine–oak forest stands in Northeastern Germany. *Geoderma,* 158, 156-162. https://doi.org/10.1016/j.geoderma.2010.04.023.

Smolders, E., Nawara, S., De Cooman, E., Merckx, R., Martens, S., Elsen, A., 2020. The phosphate desorption rate in soil limits phosphorus bioavailability to crops. *European Journal of Soil Science*. https://doi.org/10.1111/ejss.12978.

Spokas, K.A., Reicosky, D.C., 2009. Impacts of sixteen different biochars on soil greenhouse gas production. *Annals of Environmental Science,* 3, 179-193.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macêdo, J.L.V., Blum, W.E., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. *Plant and Soil,* 291, 275-

290. https://doi.org/10.1007/s11104-007-9193-9.

Taghizadeh-Toosi, A., Clough, T.J., Sherlock, R.R., Condron, L.M., 2012. Biochar adsorbed ammonia is bioavailable. *Plant and Soil,* 350, 57-69. https://doi.org/10.1007/s11104-011-0870-3.

Tiessen, H., Moir, J., 1993. Characterization of available P by sequential extraction. Soil Sampling and Methods of Analysis. *Ed. MR Carter. P*, 75-86.

Turner, B.L., 2007. Inositol phosphates in soil: amounts, forms and significance of the phosphorylated inositol stereoisomers. *Inositol phosphates: Linking agriculture and the environment*, 186-206.

Uzoma, K., Inoue, M., Andry, H., Fujimaki, H., Zahoor, A., Nishihara, E., 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. *Soil Use and Management,* 27, 205-212. https://doi.org/10.1111/j.1475-2743.2011.00340.x.

Wang, H., Yan, S., Ren, T., Yuan, Y., Kuang, G., Wang, B., 2020. Novel environmental factors affecting microbial responses and physicochemical properties by sequentially applied biochar in black soil. *Environmental Science and Pollution Research,* 27, 37432-37443. https://doi.org/10.1007/s11356-020-10081-y.

Wang, L.F., Chen, C., Zhu, X.Y., Chen, J.L., Fang, X., 2019. Characteristics of soil phosphorus pool at different vegetation restoration stages in the mid-subtropical region of china. *Journal of Soil and Water Conservation*.

Wang, X.L., Li, Z.J., Long, P., Yan, L.L., Gao, W.S., Chen, Y.Q., Sui, P., 2017. Sustainability evaluation of recycling in agricultural systems by emergy accounting. Resources, Conservation and Recycling, 117, 114-124. https://doi.org/10.1016/j.resconrec.2016.11.009.

Wei, K., Bao, H., Huang, S., Chen, L., 2017. Effects of long-term fertilization on available P, P composition and phosphatase activities in soil from the Huang-Huai-Hai Plain of China. *Agriculture, Ecosystems & Environment,* 237, 134-142. https://doi.org/10.1016/j.agee.2016.12.030.

Xiong, Y., Xia, H., Cai, X.a., Fu, S., 2008. Impacts of litter and understory removal on soil properties in a subtropical Acacia mangium plantation in China. *Plant and Soil,* 304, 179-188. https://doi.org/10.1007/s11104-007-9536-6.

Zamuner, E., Picone, L., Echeverria, H., 2008. Organic and inorganic phosphorus in Mollisol soil under different tillage practices. *Soil and Tillage Research,* 99, 131-138. https://doi.org/10.1016/j.still.2007.12.006.

Zheng, B.-X., Ding, K., Yang, X.-R., Wadaan, M.A., Hozzein, W.N., Peñuelas, J., 2019. Straw biochar increases the abundance of inorganic phosphate solubilizing bacterial community for better rape (Brassica napus) growth and phosphate uptake. *Science of the Total Environment,* 647, 1113-1120. https://doi.org/10.1016/j.scitotenv.2018.07.454.

Zheng, Z., Parent, L., MacLeod, J., 2003. Influence of soil texture on fertilizer and soil phosphorus transformations in Gleysolic soils. *Canadian Journal of Soil Science,* 83, 395-403. https://doi.org/10.4141/S02-073.

Figure captions

Figure 1. The ratio of P compounds in the Hedley improvement method (1993) to the total phosphorus content in the soils undergoing different treatments.

Figure 2. Examples of solution 31-phosphorus nuclear magnetic resonance spectra for the five treatments.

Figure 3. The solution 31-phosphorus nuclear magnetic resonance spectra of the biochar-treated sample and the orthophosphate and monoester under different treatments in detail (5.4 to 3.0 ppm). a: adenosine monophosphate; b: myo-Inositol hexakisphosphate; c: scyllo-Inositol hexakisphosphate; d: neo-Inositol hexakisphosphate; and e: D-chiro-Inositol hexakisphosphate.

Table 1 Basic properties of soil used in experiment and efficiency of P in NaOH-EDTA extracts.

Item	Initial soil		No fertilizer		N+P+K fertilizer		NPK + Biochar		NPK + Straw	
pH	6.00 (0.01)	a	5.66 (0.01)	c	5.10 (0.01)	e	5.45 (0.01)	d	5.89 (0.04)	b
TOC (g kg^{-1})	9.87 (0.01)	c	9.27 (0.02)	e	9.59 (0.04)	d	10.85 (0.04)	a	10.03 (0.07)	b
TN (g kg-^{1})	0.90 (0.01)	b	0.77 (0.01)	d	0.98 (0.04)	a	0.87 (0.03)	b	0.87 (0.02)	c
TP (g kg^{-1})	0.51 (0.01)	c	0.50 (0.01)	c	0.56 (0.01)	b	0.58 (0.01)	a	0.54 (0.01)	b
Olsen-P (mg kg^{-1})	16.30 (0.33)	c	11.28 (0.55)	d	16.86 (0.58)	c	21.88 (0.35)	b	23.95 (0.84)	a
Available N (mg kg^{-1})	112.00 (0.85)	a	87.16 (0.99)	e	94.52 (1.21)	d	97.10 (0.99)	b	97.00 (1.22)	c
Available K (mg kg^{-1})	110.00 (0.89)	e	137.56 (1.47)	c	146.82 (1.34)	b	154.46 (1.06)	a	129.50 (0.90)	d
NaOH + EDTA-P$_{rec}$ (%)	31.00 (1.32)	bc	28.99 (1.33)	c	32.75 (2.57)	ab	37.42 (3.11)	a	33.02 (2.15)	ab

Values are means. Values in parentheses are standard deviations (n=3). Different letters in a column indicate significant differences at the 0.05 level. Same below.TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus; NaOH + EDTA-P$_{rec}$: the phosphorus extraction efficiency of NaOH + EDTA.

Table 2 Variation of soil P compounds contents in the Hedley improvement method (1993) to total phosphorus in five treatments.

Treatment	Concentration of soil P fraction (mg kg^{-1})						
	Resin-P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P	Residual-P
CK0	6.92 (1.02)	25.01 (0.77)	10.94 (2.16)	57.17 (5.42)	25.01 (1.67)	23.45 (2.18)	360.37 (8.10)
CK	6.25 (0.77)	23.89 (2.97)	9.16 (0.71)	43.99 (5.75)	31.04 (2.58)	19.21 (2.06)	364.88 (7.90)
NPK	9.60 (1.41)	42.65 (3.45)	11.17 (1.93)	69.67 (8.12)	36.18 (3.73)	23.67 (2.98)	362.58 (15.36)
CNPK	16.08 (2.74)	41.54 (2.88)	13.62 (2.22)	61.19 (3.72)	58.06 (5.84)	18.31 (3.01)	365.96 (17.75)
SNPK	8.61 (1.32)	40.72 (3.24)	10.53 (1.91)	65.79 (3.63)	37.36 (3.94)	20.63 (1.29)	355.23 (6.47)

CK0: the initial soil in 2013; CK: the unfertilized control; NPK: N+P+K mineral fertilizer; CNPK: biochar in combination with NPK mineral fertilizer; and SNPK: corn straw in combination with NPK mineral fertilizer. Resin-P: resin exchanged phosphorus; NaHCO3-P (Pi and Po): NaHCO3 extracted state inorganic phosphorus and organic phosphorus; NaOH-P (Pi and Po): NaOH extracted state inorganic phosphorus and organic phosphorus; HCl-P: 1 mol L dilute hydrochloric acid to extract phosphorus; and Residual-P: residual phosphorus. Same below.

Table 3 Concentrations and proportions of P compound classes by solution 31-phosphorus nuclear magnetic resonance spectra.

P composition	P content (mg kg^{-1})									
	CK0		CK		NPK		CNPK		SNPK	
Total Pi	94.2 (7.9)	d	114.3 (7.0)	c	143.8 (11.3)	b	173.2 (10.7)	a	107.4 (2.5)	cd
Total Po	63.6 (6.7)	a	30.6 (3.1)	c	39.6 (3.1)	b	43.8 (2.9)	b	71.1 (1.7)	a
Orthophosphate	91.6 (5.5)	c	106.2 (5.0)	c	136.8 (8.8)	b	166.2 (10.4)	a	98.1 (8.1)	c
Pyrophosphate	2.6 (0.3)	b	3.0 (0.3)	b	2.2 (0.1)	b	1.7 (0.1)	b	4.9 (1.4)	a
Mono	60.3 (6.4)	a	25.9 (3.0)	c	37.3 (2.9)	b	37.1 (2.3)	b	62.0 (7.7)	a
Diester	3.3 (0.4)	b	1.2 (0.1)	c	2.0 (0.2)	c	4.8 (0.3)	a	3.9 (1.1)	ab
Mo/Di	18.3 (1.5)		21.6 (1.8)		18.7 (2.2)		7.8 (0.8)		15.9 (0.6)	
Total IHP	37.6 (4.0)	a	21.2 (2.0)	c	28.2 (2.2)	b	31.0 (1.9)	b	41.2 (1.9)	a
AMP	0.0	c	2.8 (0.3)	b	7.5 (0.5)	a	7.0 (2.2)	a	6.3 (1.8)	a
DNA	3.1 (0.3)	a	0.0	d	0.9 (0.1)	c	2.4 (0.1)	b	2.1 (0.6)	b
c Mono	41.2 (4.4)	a	21.5 (2.5)	c	30.1 (2.4)	b	28.0 (1.7)	b	47.9 (3.7)	a
c Diester	22.4 (2.4)	a	5.7 (0.6)	d	9.4 (0.7)	cd	13.9 (0.9)	bc	18.0 (5.1)	ab
c Mo/Di	1.8 (0.1)		3.8 (0.3)		3.2 (0.3)		2.0 (0.2)		2.7 (0.2)	

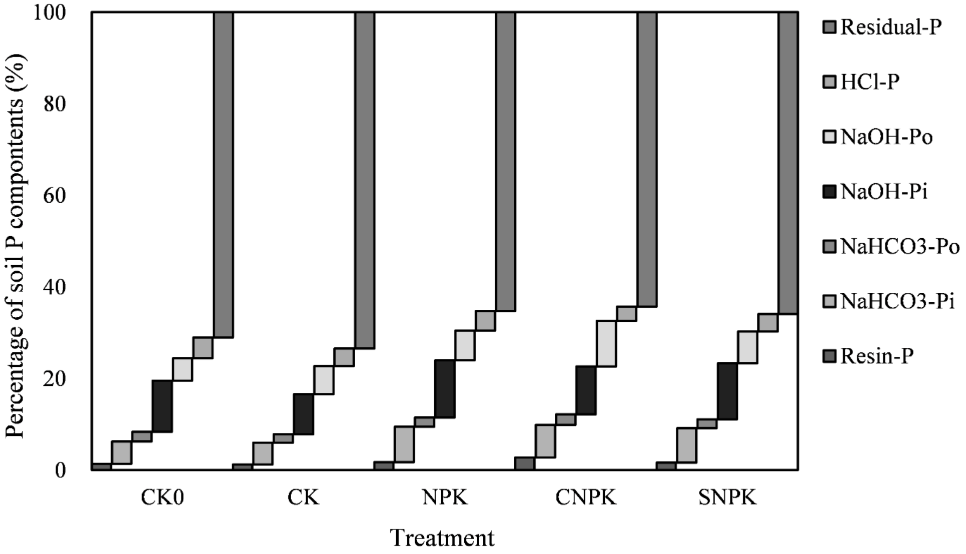
Total IHP: total inositol hexakisphosphate (sum: myo-Inositol hexakisphosphate; scyllo- Inositol hexakisphosphate; neo- Inositol hexakisphosphate; and D-chiro- Inositol hexakisphosphate.); Total Pi: total inorganic phosphorus; Total Po: total organic phosphorus; Mono: orthophosphate monoesters; Mo/Di: Monoester/Diester ratio; and c: denotes the correction for degradation products. Same below

Table 4 Correlation coefficients among P fractions in the Hedley improvement method (1993) and soil P compounds (n=15).

P fraction	Orthophosphate	Pyrophosphate	Total IHP	AMP	DNA	c Monoester	c Diester
Resin-P	0.907**	-0.484*	0.045	0.241	0.314	-0.181	0.006
$NaHCO_3$-Pi	0.630**	0.002	0.272	0.935**	0.121	0.192	-0.019
$NaHCO_3$-Po	0.799**	-0.549*	0.236	0.457*	0.568*	-0.003	0.277
NaOH-Pi	0.317	0.053	0.552*	0.651**	0.381	0.510*	0.332
NaOH-Po	0.883**	-0.342	-0.043	0.713**	0.144	-0.244	-0.144
HCl-P	-0.382	-0.024	0.290	-0.237	0.191	0.393	0.340
Residual-P	0.847**	-0.789**	-0.485*	0.252	-0.015	-0.691**	-0.332

c: denotes the correction for degradation products. r - value was shown. Indicating significance (*Person coefficient* are as follows: $^{*}P < 0.05$; $^{**}P < 0.01$).

665 **Figure 1**



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667 **Figure 2**

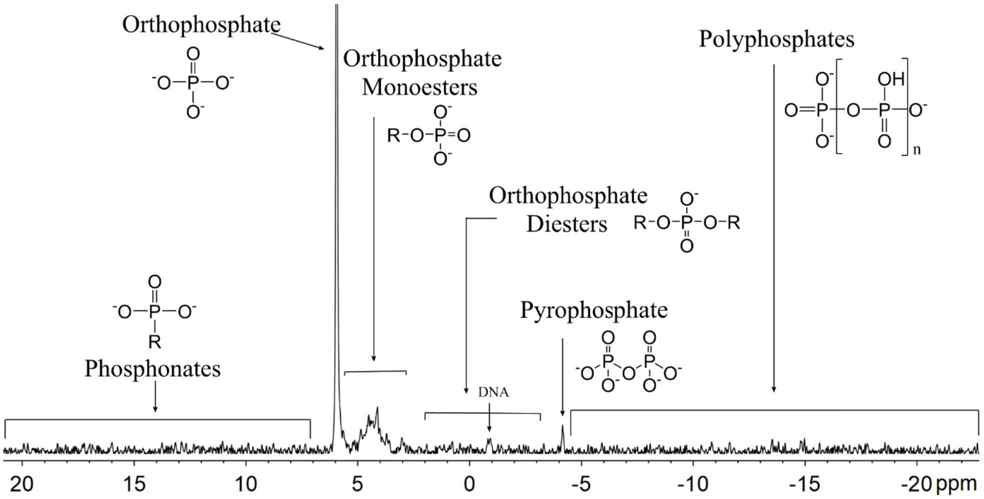

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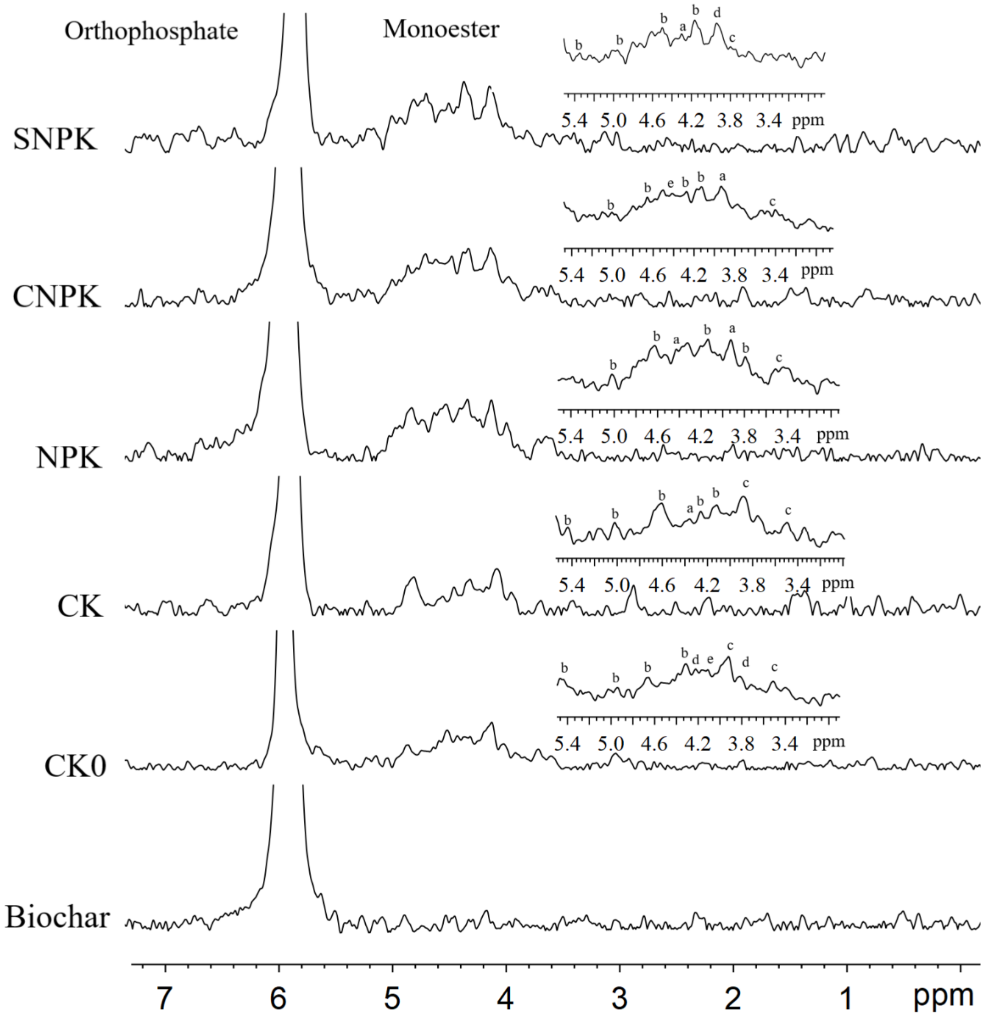
Figure 3


Orthophosphate
Monoester
SNPK
CNPK
NPK
CK
CK0
Biochar
7
6
5
4
3
2
1
ppm
5.4 5.0 4.6 4.2 3.8 3.4 ppm
a
b
c
d
e