

## Article

# Phosphorus Distribution within Aggregates in Long-Term Fertilized Black Soil: Regulatory Mechanisms of Soil Organic Matter and pH as Key Impact Factors

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**Abstract:** Understanding soil phosphorus (P) distribution and its key drivers is fundamental for sustainable P management. In this study, a 21-year fertilization experiment on black soil was carried out, setting up five fertilization treatments: unfertilized control (CK), nitrogen and potassium (NK), nitrogen, P and potassium (NPK), NPK plus straw (NPKS), and NPK plus manure (NPKM). The distribution and effecting factors of P pools within soil aggregates were investigated. Compared to CK, the NK and NPK treatments decreased calcium-associated P concentration in all aggregate fractions. Meanwhile, the NPK treatment significantly increased the organic P extracted from NaOH in unaggregated particles (<0.053 mm). This was mainly due to the reduction in soil pH. The NPKS and NPKM treatments increased almost all P forms in aggregates, especially Ca-P. For the NPKM treatment, inorganic P extracted from resin, NaHCO<sub>3</sub>, and NaOH increased as aggregate size increased. This was mainly because straw or manure addition promoted soil organic carbon (SOC) storage in aggregates, creating more sorption sites via association with amorphous metallic minerals, and, thus, facilitating P accumulation. In conclusion, decreasing soil pH by chemical fertilizers is an effective strategy for mobilizing soil P, whereas increasing SOC by straw or manure facilitates P accumulation.

**Keywords:** phosphorus forms; soil aggregates; long-term fertilization; soil organic carbon; soil pH



**Citation:** Zhang, N.; Wang, Q.; Chen, Y.; Zhang, S.; Zhang, X.; Feng, G.; Gao, H.; Peng, C.; Zhu, P. Phosphorus Distribution within Aggregates in Long-Term Fertilized Black Soil: Regulatory Mechanisms of Soil Organic Matter and pH as Key Impact Factors. *Agronomy* **2024**, *14*, 936. <https://doi.org/10.3390/agronomy14050936>

Academic Editor: Domenico Ronga

Received: 1 April 2024

Revised: 25 April 2024

Accepted: 26 April 2024

Published: 30 April 2024



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## 1. Introduction

Phosphorus (P) is an essential element for growing crops and plays a critical role in ensuring food production to meet the demands of the growing global population [1]. Over the past few decades, high P fertilizer input and low P-use efficiency have caused P surpluses in many croplands [2]. Excess P in soil layers increases the risk of P leaching, contributing to the eutrophication of water bodies [3]. Meanwhile, P fertilizer is mainly from nonrenewable fossil P resources, which will likely be depleted within a century due to continued mining [4]. When facing the global challenges related to P, a full comprehension of the characteristics of the soil P pool and its influential factors is important for sustainable P management in agriculture.

Almost all soil P forms will be depleted when no P fertilizer is input [5], while soil P forms will be selectively accumulated when P fertilizer is input with a surplus [6]. The changes in P forms depend on the abundance of P-fixing chemicals and the impact of soil properties, especially soil organic matter (SOM) and pH [7,8]. Oliveira et al. [9] found

that SOM and total P had similar spatial distributions at the soil surface when fertilizer was broadcast. Takamoto et al. [10] found that lower-density soil particles obtained by soil density fractionation were rich in SOM and accompanied by high P accumulation. Zhang et al. [11] further found that SOM concentration was positively correlated with almost all P fractions in three non-acidic soils. The main mechanism behind these phenomena may be the binding of SOM to metal ions such as  $\text{Fe}^{3+}$ ,  $\text{Al}^{3+}$ , and  $\text{Ca}^{2+}$ , which promotes the formation of SOM–metal–P ternary complexes and contributes to the accumulation of soil P [10,12,13]. Additionally, the association of SOM with soil minerals promotes the aggregation of soil particles, and the formation of soil aggregates facilitates P accumulation [14–16].

Although SOM is an important factor affecting P accumulation, the form of accumulated P is usually pH-dependent, i.e., it is predominantly bound to Fe in acidic soils with low pH, to Al in weakly acidic soils, and to Ca in alkaline soils with high pH [7,17]. Therefore, the change in soil pH controls the transformation of P forms, and a typical example is that the decrease in soil pH promotes the solubilization of calcium phosphate minerals and increases the Fe/Al-associated P [18,19]. Generally, both chemical fertilizer and chemical fertilizer combined with organic fertilizer increase SOM levels [20], which could be due to fertilization practices increasing production and promoting the input of more biomass, as well as the direct input of organic materials contributing to increasing SOM, while changes in soil pH vary by fertilization practices [21,22]. At present, there is a lack of assessment and mechanistic studies focusing on the impact of SOM and pH on soil P form transformation and distribution under different fertilization practices.

As the basic functional units, soil aggregates are primarily the product of SOM and soil mineral interactions [13,23,24]. Generally, the concentration of SOM and associated soil minerals varies among aggregate size fractions [25,26]. The size-grouped soil aggregates create favorable conditions for understanding P forms, distribution, and their influential factors [17,27,28]. Long-term fertilization is a crucial external driver of the changes in soil aggregate structure and properties [25,26,29]. Therefore, soil aggregates under long-term different fertilization practices contribute to elucidating the effects of SOM and pH on P forms.

Based on a 21-year fertilization experiment on black soil, we assessed the effect of fertilization practices on soil P pools and explored the causes of differences in P pools among fertilization practices and aggregate size fractions. We focused on the effects of changes in SOM and soil pH caused by fertilization on the forms and distribution of P pools. We hypothesized the following: (1) the discrepancies in P pools among various fertilization practices and aggregate size fractions would mostly be explained by SOM and soil pH and (2) SOM would mainly govern the accumulation of P forms, while soil pH would mainly control the mobilization of P forms. The results of the study could enhance the understanding of the distribution characteristics and contributing factors of soil P pools and provide theoretical support for rational fertilization of black soil.

## 2. Materials and Methods

### 2.1. Study Site and Soil Sampling

The study site is located in Gongzhuling City, China (124.809° E, 43.506° N). The fertilization experiment began in 1989 and continues to this day. The soil type is black soil (Mollisol in USDA Soil Taxonomy and Phaeozem in World Reference Base for Soil Resources) with loamy clay texture, and the parent material is quaternary loess-like sediments. The phrase “black soil” is a term used in the Genetic Soil Classification of China [30]. The average annual temperature ranges from 4 °C to 5 °C. The annual precipitation ranges from 450 mm to 650 mm. The characteristics of the surface soil (0–20 cm) before the experiment were as follows: pH was 7.6, bulk density was  $1.2 \text{ g cm}^{-3}$ , soil organic matter (SOM) was  $20 \text{ g kg}^{-1}$ , total nitrogen (N) was  $1.34 \text{ g kg}^{-1}$ , total P was  $0.61 \text{ g kg}^{-1}$ , and total potassium (K) was  $16.36 \text{ g kg}^{-1}$ . The available N, P, and K were 114, 11.8, and  $158.3 \text{ mg kg}^{-1}$ , respectively.

Five fertilization practices were selected for the investigation: (i) unfertilized control (CK); (ii) imbalanced application of chemical N and K fertilizers (NK); (iii) balanced application of chemical N, P, and K fertilizers (NPK); (iv) chemical N, P, and K fertilizers plus maize straw return (NPKS); and (v) chemical N, P, and K fertilizers in combination with manure (NPKM). N, P, and K indicate chemical fertilizers, i.e., nitrogen (urea), phosphorus (triple superphosphate), and potassium (muriate of potash), respectively. The manure applied under the NPKM treatment was pig manure in 1990–2004 and cattle manure in 2005–2010. The planting system was a continuous maize monoculture, and the fertilization treatments were distributed in completely randomized block form with three replicates. The area of each plot was 130 m<sup>2</sup>. For the CK, NK, NPK, and NPKM treatments, maize straw on the ground was removed after harvest each year. For the NPKS treatment, maize straw was used to cover over the topsoil layer by crushing after harvest. The details of nutrient inputs are summarized in Table 1. The recent literature provides more details of the plot design and soil characteristics [11,31]. The experiment was set up with inconsistent amounts and types of P inputs due to historical reasons, but some pairwise comparisons between fertilization treatments were valuable, e.g., between the NK and NPK treatments, highlighting the difference between with and without chemical P, and between the NPK and NPKS treatments, mainly reflecting the role of straw addition. Furthermore, we also focused on comparing the differences in the distribution of P forms among aggregate size fractions. Thus, this historical experimental design created different scenarios for exploring the role of SOM and pH in the distribution of P forms. After harvest in October 2010, five 0–20 cm topsoil cores in each plot were randomly drilled and mixed to form one sample. The soil samples were transported indoors for natural air drying and stored in plastic bottles, avoiding crushing and vibration to maintain the natural structure.

**Table 1.** Details of fertilizer application rates (kg ha<sup>−1</sup>) associated with different fertilization treatments.

| Treatments | Nutrient Inputs of Chemical Fertilizer |    |    | Nutrient Inputs of Straw or Manure |    |    |
|------------|--|----|----|------------------------------------|----|----|
|            | N                                      | P  | K  | N                                  | P  | K  |
| CK         | 0                                      | 0  | 0  | 0                                  | 0  | 0  |
| NK         | 165                                    | 0  | 68 | 0                                  | 0  | 0  |
| NPK        | 165                                    | 36 | 68 | 0                                  | 0  | 0  |
| NPKS       | 112                                    | 36 | 68 | 53                                 | 6  | 58 |
| NPKM       | 50                                     | 36 | 68 | 115                                | 39 | 77 |

CK: unfertilized control; NK: chemical N and K fertilizers; NPK: chemical N, P, and K fertilizers; NPKS: chemical N, P, and K fertilizers plus maize straw; and NPKM: chemical N, P, and K fertilizers plus manure. The application rate of maize straw under the NPKS treatment was approximately  $7.5 \times 10^3$  kg ha<sup>−1</sup>. The application rate of pig manure (1990–2004) and cattle manure (2005–2010) under the NPKM treatment was  $2.3 \times 10^4$  kg ha<sup>−1</sup>.

## 2.2. Soil Aggregate Sieving

Classification of soil aggregates using the wet-sieving procedure [23]. Briefly, the bulk soils were slightly broken apart, filtered using an 8 mm sieve, and then weighed and 100 g placed on the top of a group of five sieves with different pore sizes (2, 1, 0.5, 0.25, and 0.053 mm). After being soaked in deionized water (10 min), the soil was shaken vertically for 30 min at 30 shakes per minute, moving up and down over a distance of 4 cm. At the end of the shaking, the soil particles on each sieve were transferred into a beaker to collect the aggregate fractions (>2, 2–1, 1–0.5, 0.5–0.25, and 0.25–0.053 mm). The silt and clay in the barrel (<0.053 mm) were allowed to settle for 48 h and then transferred to a beaker. The aggregates were dried (60 °C) and weighed to calculate their proportions. Soil samples were sieved (0.15 mm) and used for subsequent analysis. R<sub>0.25</sub> represents the percentage of >0.25 mm aggregates; a higher value indicates better soil aggregate stability [32]. According to the analysis, a small amount of water-soluble P was lost during the aggregate sieving, and we ignored the effects it caused in subsequent analyses.

### 2.3. P fractions Obtained by Chemical Sequential Fractionation

Phosphorus fractions were extracted by a modified Hedley sequential fractionation procedure that is described in detail by [33]. Briefly, 0.5 g of soil samples were weighed in 50 mL centrifuge tubes and then extracted sequentially with anion exchange resin in deionized water, 0.5 M NaHCO<sub>3</sub> (pH 8.5), 0.1 M NaOH, 1 M HCl, and 11.3 M HCl. Finally, soil residues were digested with 30% H<sub>2</sub>O<sub>2</sub> and 18 M H<sub>2</sub>SO<sub>4</sub> in an electric furnace at 360 °C. After extraction, the extracts and soil solids were separated by centrifugation, and the supernatants were filtered through a 0.45 µm membrane for direct determination of inorganic P (P<sub>i</sub>). To determine total P (TP) in extracts, the supernatants from the NaHCO<sub>3</sub>, NaOH, and 11.3 M HCl extractions were digested with H<sub>2</sub>SO<sub>4</sub>-(NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub> in an autoclave (121 °C and 103 kPa for 1 h). The P concentration in the solution was determined according to the molybdenum blue method [34]. The extraction procedure is summarized in Table 2. Organic P (P<sub>o</sub>) was calculated as the difference between TP and P<sub>i</sub>. The sum of all P fractions was the total P concentration in aggregates.

**Table 2.** The extraction procedure scheme of P fractions.

| Steps | Extraction Agent  | Volume (mL) | Condition   | P Fractions  |
|-------|---|-------------|---|--|
| 1     | Resin   | -           | Shake 16 h (25 °C)  | Resin-P <sub>i</sub>   |
| 2     | 0.5 M NaHCO <sub>3</sub> (pH 8.5)                                       | 30          | Shake 16 h (25 °C)  | NaHCO <sub>3</sub> -P <sub>i</sub><br>NaHCO <sub>3</sub> -P <sub>o</sub> = TP-P <sub>i</sub> |
| 3     | 0.1 M NaOH  | 30          | Shake 16 h (25 °C)  | NaOH-P <sub>i</sub><br>NaOH-P <sub>o</sub> = TP-P <sub>i</sub>                               |
| 4     | 1 M HCl   | 30          | Shake 16 h (25 °C)  | Dil. HCl-P <sub>i</sub>  |
| 5     | 11.3 M HCl  | 15          | Water bath (80 °C for 10 min), then standing 1 h<br>(vortex every 15 min) | Con. HCl-P <sub>i</sub><br>Con. HCl-P <sub>o</sub> = TP-P <sub>i</sub>                       |
| 6     | 30% H <sub>2</sub> O <sub>2</sub> + 18 M H <sub>2</sub> SO <sub>4</sub> | -           | Digestion at high temperature   | Residual-P   |

### 2.4. P Species Identified by XANES Spectroscopy

The possible P species within the soil aggregates were identified using P K-edge XANES spectroscopy at beamline 4B7A equipped with a 13-element Si(Li) detector in the Beijing Synchrotron Radiation Facility. All references and soil aggregate samples were thinly placed across P-free and double-sided carbon tape for spectral collection [35]. The spectra of P references and soil aggregate samples were acquired in total electron yield mode and partial fluorescence yield mode, respectively. The P references included compounds of FePO<sub>4</sub>, AlPO<sub>4</sub>, CaHPO<sub>4</sub>·(DCPD), Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> (MCP), Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>·(TCP), Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>OH (HAP), and inositol hexakisphosphate (IHP). The spectra were processed and analyzed by Athena software (Version 0.9.26, Inc., Boston, MA, USA). The energy range of the linear combination fitting (LCF) was 2140–2180 eV. The combination of the highest number of P reference standards was selected as the possible P species that existed in soil aggregates.

### 2.5. Determination of Chemical Properties

The concentration of soil organic carbon (SOC) in aggregates was analyzed by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> oxidation method [36]. The soil pH was obtained using a pH meter [soil:water; 1:2.5 (m:v)]. Poorly crystalline Fe (Fe<sub>o</sub>) and Al (Al<sub>o</sub>) were extracted using 0.2 M ammonium oxalate at a pH of 3 [37]. Organically complexed Fe (Fe<sub>p</sub>) and Al (Al<sub>p</sub>) were extracted using 0.1 M sodium pyrophosphate [38]. Exchangeable Ca<sup>2+</sup> (Ca) and Mg<sup>2+</sup> (Mg) were extracted with 1 M NH<sub>4</sub>OAc [39]. Metal ion concentrations in the extracts were analyzed by ICP-OES (Varian 715-ES).

### 2.6. Statistical Analysis

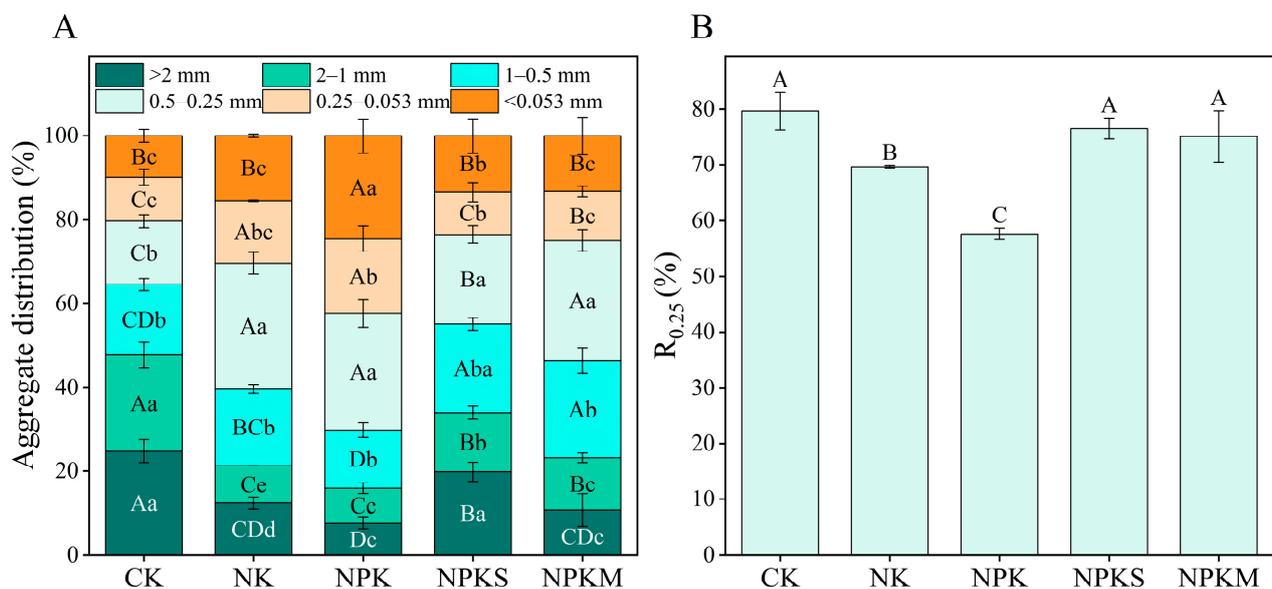
The differences in the distribution of aggregates, R<sub>0.25</sub>, soil properties, and P fractions among fertilization treatments and aggregates were tested using one-way ANOVA in R software v4.1.3 [40]. Post hoc tests were carried out with Fisher's least significant difference (LSD) with a significance level of 0.05. Principal component analysis (PCA),

Pearson's correlation heatmap, and linear fitting were performed in OriginPro 2023 software (OriginLab Corporation, Northampton, MA, USA).

### 3. Results

#### 3.1. Soil Aggregate Distribution

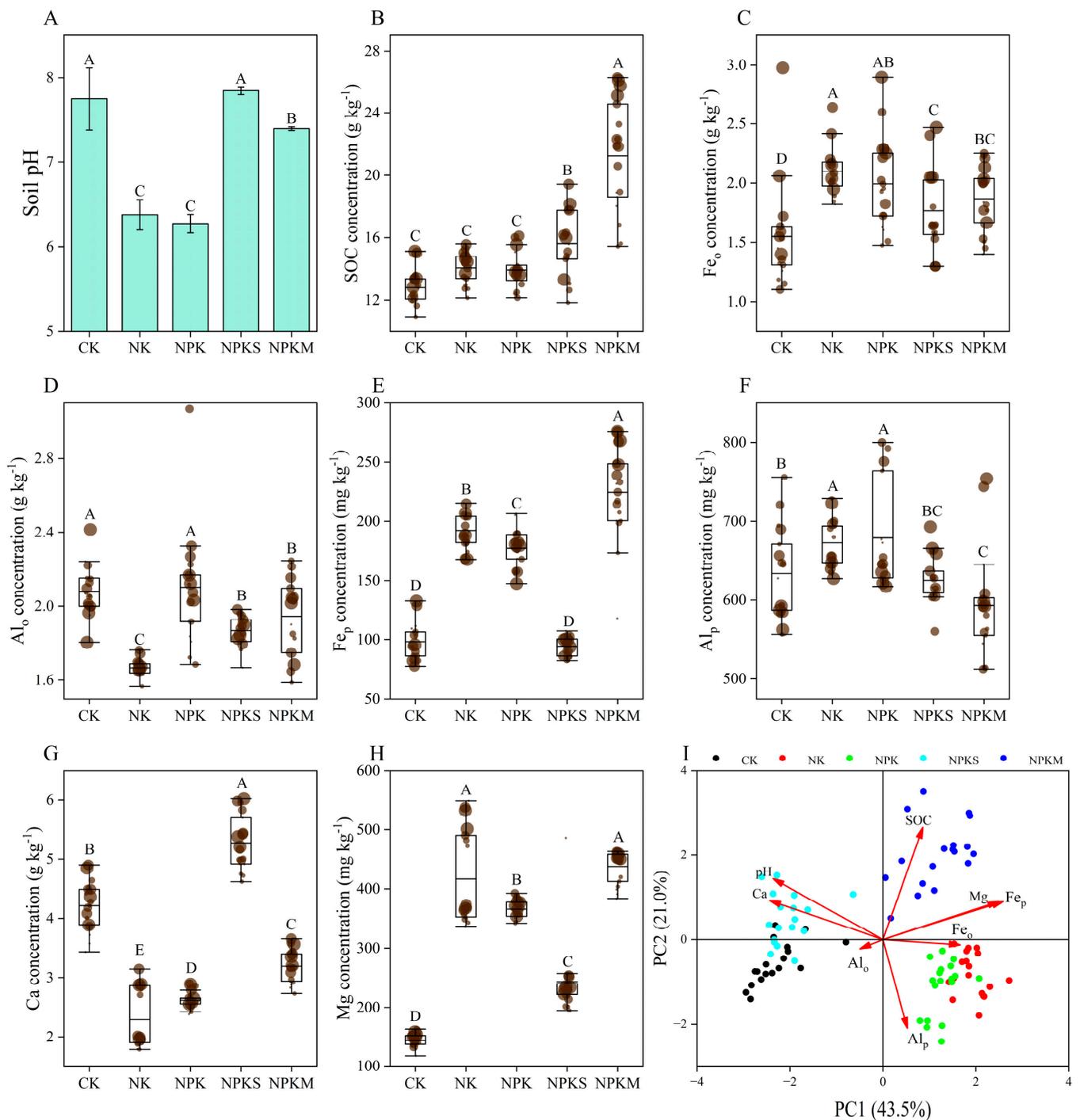
The distribution of soil aggregates was impacted by long-term fertilization (Figure 1). Compared to the unfertilized control (CK), all fertilization treatments (NK, NPK, NPKS, and NPKM) significantly decreased the proportion of >2 and 2–1 mm aggregate fractions by 19.98–69.11%, while the proportion of 0.5–0.25 mm aggregate fractions was significantly increased by 41.59–98.03% (Figure 1A). Overall, the NK and NPK treatments significantly decreased the percentage of  $R_{0.25}$  (Figure 1B), indicating that long-term chemical fertilizer inputs reduced soil aggregate stability.



**Figure 1.** Soil aggregate distribution (A) and  $R_{0.25}$  (B). Different lowercase letters indicate significant differences between aggregate size fractions in the same fertilization treatment ( $p < 0.05$ ), while different uppercase letters indicate significant differences between different fertilization treatments ( $p < 0.05$ ). Values showed mean  $\pm$  sd ( $n = 3$ ). CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure.

#### 3.2. Chemical Properties in Soil Aggregates

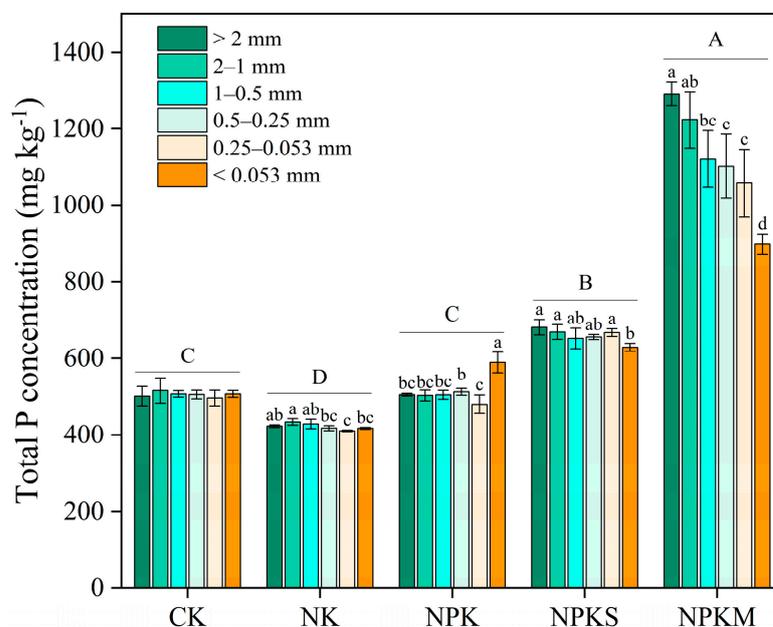
Compared to CK, the NK, NPK, and NPKM treatments significantly decreased soil pH by 17.68, 19.06, and 4.56%, respectively (Figure 2A). The mean concentrations of SOC in aggregates under the NPKS and NPKM treatments were higher than those under other fertilization treatments (Figure 2B). All fertilization practices significantly increased the mean concentrations of  $Fe_o$  and Mg (Figure 2C,H). The NK, NPK, and NPKM treatments significantly increased the mean concentrations of  $Fe_p$  (Figure 2E), and the NPKS treatment significantly increased the mean concentrations of Ca (Figure 2G). The NK, NPKS, and NPKM treatments significantly decreased the mean concentrations of  $Al_o$  (Figure 2D), meanwhile, the NPKM treatment significantly decreased the mean concentrations of  $Al_p$  (Figure 2F). Principal component analysis showed that soil pH and Ca in all aggregates were dominant under the CK and NPKS treatments,  $Al_p$  and  $Fe_o$  in aggregates were dominant under the NK and NPK treatments, and SOC in aggregates was dominant under the NPKM treatment (Figure 2I). Moreover, it was observed that the concentrations of SOC,  $Fe_p$ , Ca, and Mg exhibited uniform and strong differences under the NPKM treatment, i.e., higher concentrations occurred in aggregates with larger sizes (Figure 2B,E,G,H).



**Figure 2.** Effects of long-term fertilization practices on soil properties. (A) pH in bulk soil, values showed mean  $\pm$  sd ( $n = 3$ ). (B–H) Chemical properties of soil aggregates (data points of different aggregate sizes were presented with circles varying in size); the middle lines, lower and upper of the boxes, and bars represent mean values, 25th and 75th, 5th and 95th percentiles of all data ( $n = 18$ ), respectively. (B) SOC, soil organic carbon; (C)  $\text{Fe}_o$ , poorly crystalline Fe; (D)  $\text{Al}_o$ , poorly crystalline Al; (E)  $\text{Fe}_p$ , organically complexed Fe; (F)  $\text{Al}_p$ , organically complexed Al; (G) Ca, exchangeable  $\text{Ca}^{2+}$ ; and (H) Mg, exchangeable  $\text{Mg}^{2+}$ . (I) Principal component analysis (PCA) based on the soil aggregate properties. Different uppercase letters indicate significant differences between different fertilizer treatments ( $p < 0.05$ ). CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure.

### 3.3. P Fractions

The long-term fertilization treatments affected the total P distribution in aggregates (Figure 3). Compared to CK, the mean concentration of total P in aggregates under the NK treatment significantly decreased by 16.62%, whereas it significantly increased by 30.1% and 118.44% under the NPKS and NPKM treatments, respectively. In particular, the total P concentration increased as aggregate size increased under the NPKM treatment.



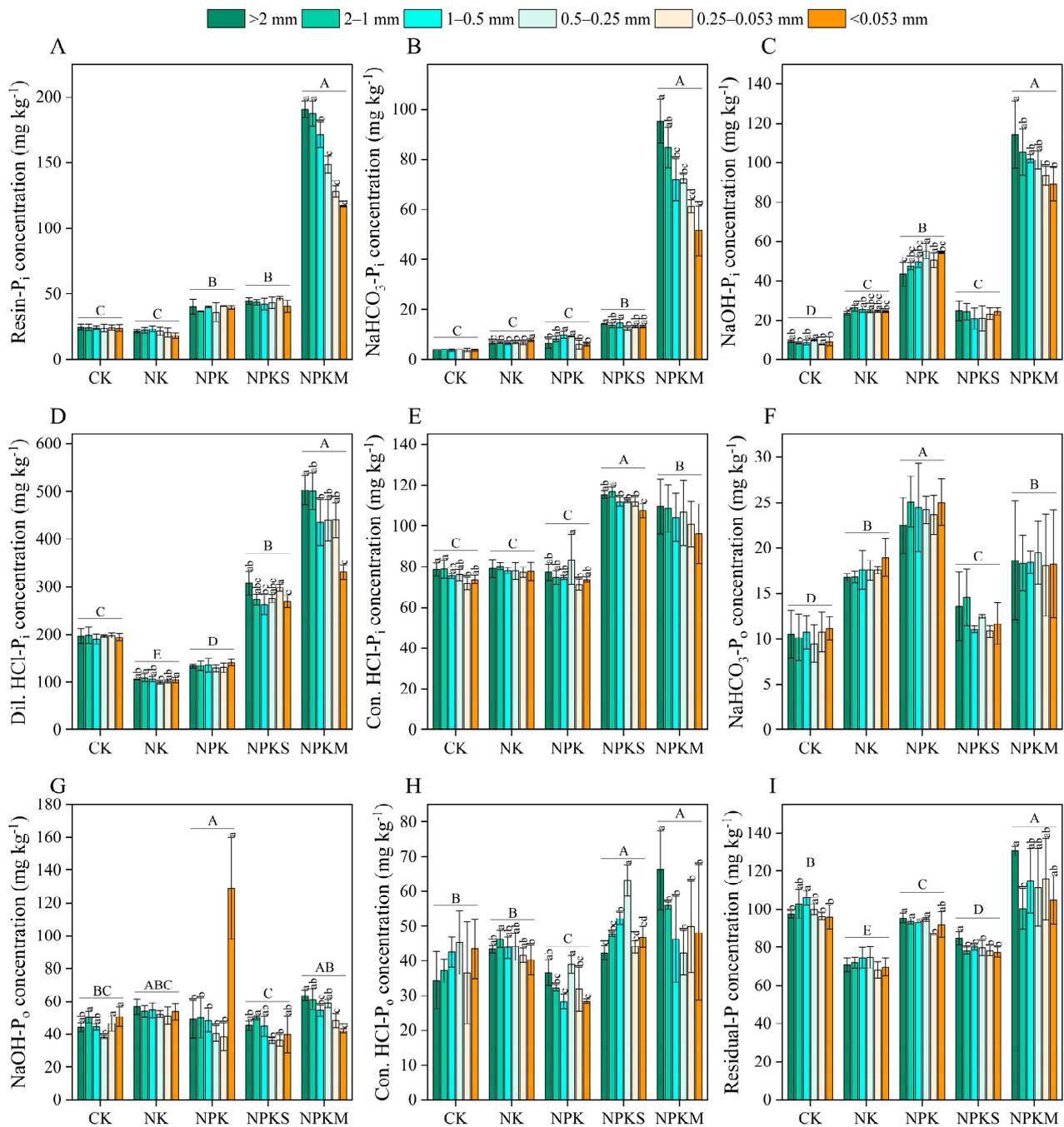
**Figure 3.** Total P in soil aggregates. Values showed mean  $\pm$  sd ( $n = 3$ ). Different lowercase letters indicate significant differences between aggregate size fractions in the same fertilizer treatment ( $p < 0.05$ ), while different uppercase letters indicate significant differences between fertilization treatments ( $p < 0.05$ ). CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure.

Compared to CK, the NK and NPK treatments significantly increased  $\text{NaOH-P}_i$  and  $\text{NaHCO}_3\text{-P}_o$  concentrations in aggregates (Figure 4C,F), while Dil.  $\text{HCl-P}_i$  and Residual-P concentrations significantly decreased (Figure 4D,I). Moreover, the NPK treatment also significantly increased Resin- $\text{P}_i$  and  $\text{NaOH-P}_o$  concentrations and decreased Con.  $\text{HCl-P}_o$  concentration in aggregates (Figure 4A,G,H). Except for  $\text{NaOH-P}_o$  and Residual-P under the NPKS treatment and  $\text{NaOH-P}_o$  under the NPKM treatment, the NPKS and NPKM treatments significantly increase other P fraction concentrations in aggregates (Figure 4A–I). For the NPKM treatment, Resin- $\text{P}_i$ ,  $\text{NaHCO}_3\text{-P}_i$ , and  $\text{NaOH-P}_i$  concentrations increased as aggregate size increased (Figure 4A–C). Notably, the  $\text{NaOH-P}_o$  concentration in  $< 0.053$  mm particles under the NPK treatment was highest among all aggregate size fractions, which was 162.34–234.07% higher than other aggregates (Figure 4G).

### 3.4. P Species Identified by XANES

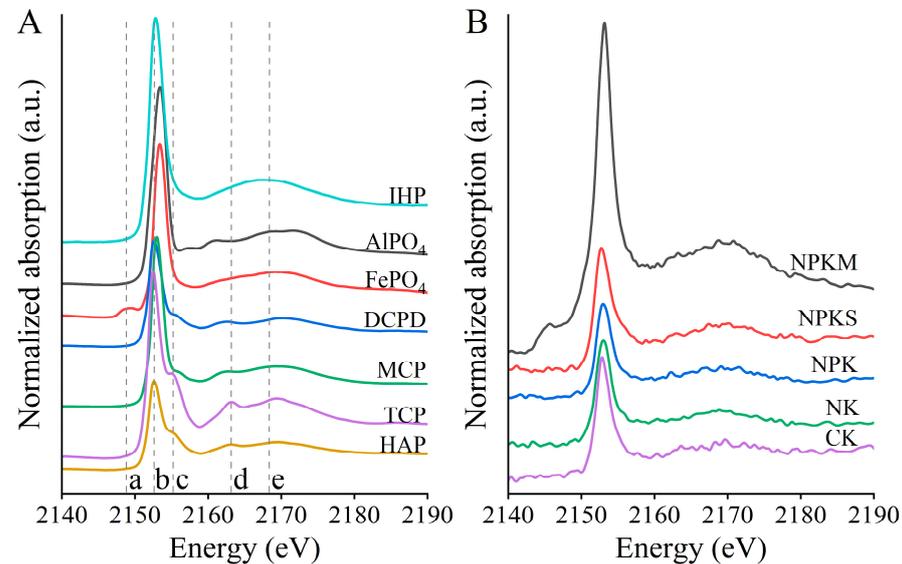
The XANES spectra of the P standards exhibited unique characteristic peaks, such as the peaks at 2148, 2155, 2165, and 2169 eV, which represent P associated with Fe (hydro)oxides, Ca-P species, P associated with Al (hydro)oxides, and oxygen oscillation, respectively (Figure 5A). Based on this, the P species of the soil aggregates were identified (Figure 5B), and their abundance was estimated (Table 3). Except for 0.5–0.25 mm aggregates under the NK treatment, Ca-P mainly contained  $\text{CaHPO}_4$ ,  $\text{Ca}_3(\text{PO}_4)_2$ , and  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$  and was predominant in soil aggregates under the NK, NPKS, and NPKM treatments, and its proportion was 43.92–84.77%. Except for  $< 0.053$  mm soil particles

under the NK treatment,  $\text{AlPO}_4$  and IHP existed in other aggregates under all fertilization treatments, ranging from 5.45% to 19.82% and from 4.58% to 50.62%, respectively. The proportions of  $\text{CaHPO}_4$ ,  $\text{Ca}_3(\text{PO}_4)_2$ , and IHP in <0.053 mm particles were the lowest among all aggregate size fractions under the NPKM treatment, indicating that more of those P species occurred in aggregates under chemical fertilizer in combination with manure.



**Figure 4.** The distribution of P fractions in soil aggregates. (A) Resin-P<sub>i</sub>, inorganic P extracted from resin; (B) NaHCO<sub>3</sub>-P<sub>i</sub>, inorganic P extracted from 0.5 M NaHCO<sub>3</sub>; (C) NaOH-P<sub>i</sub>, inorganic P extracted from 0.1 M NaOH; (D) Dil. HCl-P<sub>i</sub>, inorganic P extracted from 1 M HCl; (E) Con. HCl-P<sub>i</sub>, inorganic P extracted from 11.3 M HCl; (F) NaHCO<sub>3</sub>-P<sub>o</sub>, organic P extracted from 0.5 M NaHCO<sub>3</sub>; (G) NaOH-P<sub>o</sub>, organic P extracted from 0.1 M NaOH; (H) Con. HCl-P<sub>o</sub>, organic P extracted from 11.3 M HCl; (I) Residual-P, residual P extracted from 30% H<sub>2</sub>O<sub>2</sub> + 18 M H<sub>2</sub>SO<sub>4</sub>. Values showed mean ± se (n = 3). Different lowercase letters indicate significant differences between aggregate size fractions in the same

fertilization treatment ( $p < 0.05$ ), while different uppercase letters indicate significant differences between different fertilization treatments ( $p < 0.05$ ). CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure.



**Figure 5.** P K-edge XANES spectra of standards (A) and 2-1 mm soil aggregate samples (B). IHP, inositol hexakisphosphate;  $\text{AlPO}_4$ , berlinite;  $\text{FePO}_4$ , iron phosphate; DCPD, dicalcium phosphate dihydrate ( $\text{CaHPO}_4$ ); MCP, monocalcium phosphate [ $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ]; TCP, tricalcium phosphate [ $\text{Ca}_3(\text{PO}_4)_2$ ]; and HAP, hydroxyapatite [ $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ ]. Peak a, b, c, d and e represent P associated with Fe (hydr)oxides (2148 eV), the absorption edge (2153 eV), Ca-P species (2155 eV), P associated with Al (hydr)oxides (2165 eV), and oxygen oscillation (2169 eV), respectively. CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure.

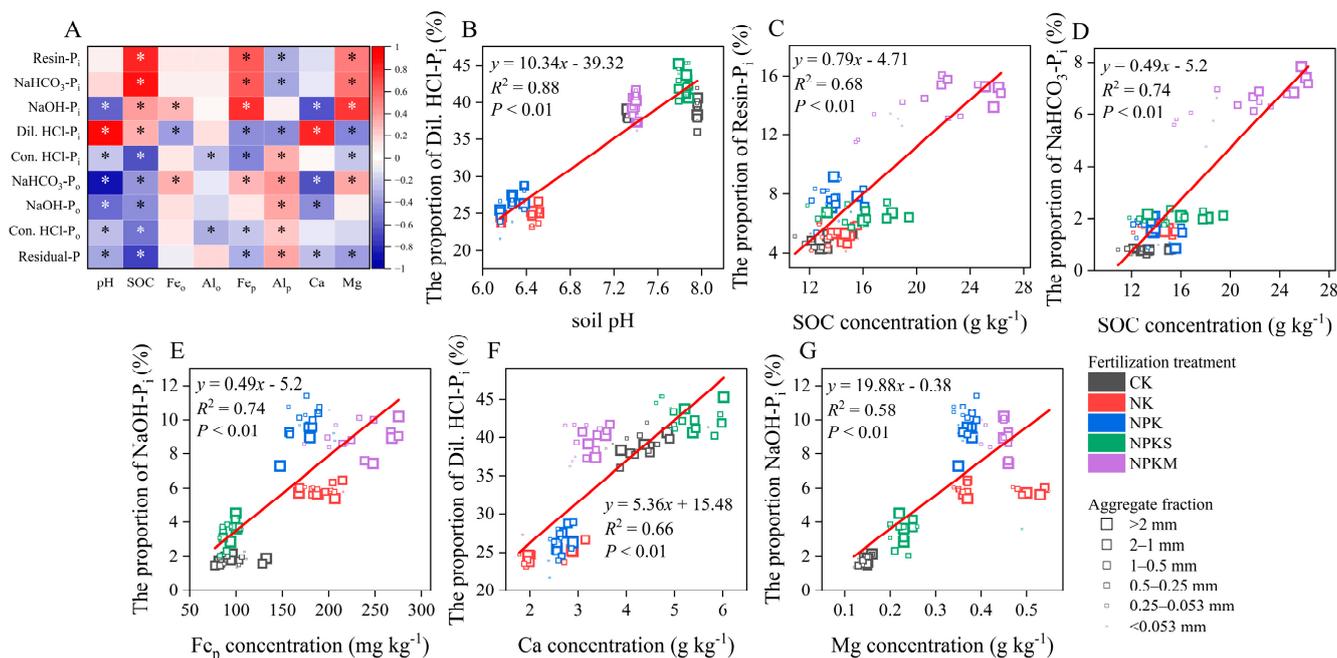
**Table 3.** Phosphorus K-edge XANES fitting results show the relative percentage (%) of each P species in the studied soil aggregate samples.

| Treatments | Aggregate Size (mm) | $\text{CaHPO}_4$ | $\text{Ca}(\text{H}_2\text{PO}_4)_2$ | $\text{Ca}_3(\text{PO}_4)_2$ | $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ | Ca-P  | $\text{AlPO}_4$ | $\text{FePO}_4$ | IHP   | R-Factor |
|------------|---------------------|------------------|--------------------------------------|------------------------------|---------------------------------------|-------|-----------------|-----------------|-------|----------|
| CK         | 2-1                 | 9.99             | 26.19                                |                              |                                       | 36.18 | 16.76           | 10.77           | 36.29 | 0.058    |
|            | 0.5-0.25            |                  |                                      | 21.35                        | 16.28                                 | 37.63 | 29.82           | 15.23           | 17.32 | 0.027    |
|            | <0.053              | 5.83             |                                      | 21.94                        | 3.03                                  | 30.8  | 21.00           |                 | 48.19 | 0.027    |
| NK         | 2-1                 | 15.04            |                                      | 22.13                        | 28.03                                 | 65.2  | 15.25           | 10.20           | 9.34  | 0.042    |
|            | 0.5-0.25            |                  | 6.61                                 | 19.14                        |                                       | 25.75 | 28.86           | 5.73            | 39.65 | 0.015    |
|            | <0.053              | 5.11             |                                      | 20.66                        | 40.64                                 | 66.41 | 25.20           | 8.40            |       | 0.038    |
| NPK        | 2-1                 | 13.02            |                                      | 19.24                        | 20.12                                 | 37.31 | 23.9            | 3.79            | 19.92 | 0.034    |
|            | 0.5-0.25            | 9.48             |                                      | 18.52                        | 9.48                                  | 37.48 | 23.37           | 3.31            | 23.70 | 0.015    |
|            | <0.053              | 13.58            |                                      | 20.69                        | 13.58                                 | 47.85 | 18.97           | 6.14            | 18.00 | 0.012    |
| NPKS       | 2-1                 | 14.70            |                                      | 32.48                        | 37.59                                 | 84.77 | 8.63            | 2.02            | 4.58  | 0.019    |
|            | 0.5-0.25            | 16.20            |                                      | 28.98                        | 30.73                                 | 75.91 | 10.63           | 3.51            | 9.95  | 0.019    |
|            | <0.053              | 14.99            |                                      | 23.48                        | 8.99                                  | 47.46 | 15.68           |                 | 36.86 | 0.016    |
| NPKM       | 2-1                 | 14.18            |                                      | 28.18                        | 21.56                                 | 63.92 | 9.51            |                 | 26.57 | 0.0075   |
|            | 0.5-0.25            | 16.65            |                                      | 24.69                        | 2.58                                  | 43.92 | 5.45            |                 | 50.62 | 0.0059   |
|            | <0.053              | 13.27            | 7.39                                 | 22.95                        | 21.96                                 | 65.57 | 15.57           |                 | 18.86 | 0.0072   |

CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure. Ca-P, the sum of  $\text{CaHPO}_4$ ,  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ,  $\text{Ca}_3(\text{PO}_4)_2$  and  $\text{Ca}_5(\text{PO}_4)_3\text{OH}$ . IHP, inositol hexakisphosphate.

### 3.5. Correlations between P Fractions and Soil Properties

Pearson’s correlation heatmap revealed that most of the proportion of P fractions were correlated with aggregate properties (Figure 6A). In particular, some P fractions had the most positive correlation with soil pH and the concentrations of SOC, Fe<sub>p</sub>, Ca, and Mg (Figure 6A). Concretely, Dil. HCl-P<sub>i</sub> was positively correlated with pH and Ca concentrations and they were dominant under the CK and NPKS treatments (Figure 6B,F). Resin-P<sub>i</sub> and NaHCO<sub>3</sub>-P<sub>i</sub> were positively correlated with SOC and they were dominant under the NPKM treatment (Figure 6C,D). Importantly, for the NPKM treatment, it was also found that the proportion of Resin-P<sub>i</sub> and NaHCO<sub>3</sub>-P<sub>i</sub> and SOC concentration increased as aggregate size increased (Figure 6C,D). The proportion of NaOH-P<sub>i</sub> was positively correlated with Fe<sub>p</sub> and Mg and they were dominant under the NPK and NPKM treatments (Figure 6E,G).



**Figure 6.** Relationship between the relative proportion of P fractions to total P and soil aggregate properties. (A) Pearson’s correlation heatmap (n = 90). The intensity of color indicates the correlation coefficient (R) (blue and red indicate a negative and positive correlation, respectively). \* Indicates significant relationships at *p* < 0.05. (B–G) Linear correlations between the selected P fractions and soil aggregate properties according to the Pearson correlation heatmap (n = 90). SOC, soil organic carbon in aggregates; Fe<sub>o</sub>, poorly crystalline Fe; Al<sub>o</sub>, poorly crystalline Al; Fe<sub>p</sub>, organically complexed Fe; Al<sub>p</sub>, organically complexed Al; Ca, exchangeable Ca<sup>2+</sup>; and Mg, exchangeable Mg<sup>2+</sup>. CK, unfertilized control; NK, chemical N and K fertilizers; NPK, chemical N, K, and P fertilizers; NPKS, chemical N, K, and P fertilizers plus maize straw; and NPKM, chemical N, K, and P fertilizers plus manure.

## 4. Discussion

### 4.1. Effect of Fertilization Practices on Aggregate Distribution and Properties

The application of chemical fertilizers (NK and NPK) significantly decreased the soil pH (Figure 2A), which was mainly due to nitrate leaching caused by N fertilizer and the removal of base cations [41,42]. It provided evidence in this study that the NK and NPK treatments significantly reduced exchangeable Ca (Figure 2G). This may reduce the binding sites of organic matter to cations [43] and inhibit the formation of large-sized aggregates. The application of chemical fertilizers had little effect on total SOC (Figure 2B) but promoted the accumulation of mineral SOC rather than particulate SOC [44], which is the driver of large-sized aggregates [45]. These results could explain the decrease in the proportion of macroaggregates (>2 mm and 2–1 mm) under the NK and NPK treatments (Figure 1A). The

NK and NPK treatments had higher  $Fe_o$ ,  $Fe_p$ ,  $Al_p$ , and Mg concentrations in aggregates than CK (Figure 2C,E,F,H), which was related to the lower soil pH that promoted the production of amorphous metal minerals or oxides [19]. These amorphous metal minerals or oxides facilitated the formation of microaggregates via association with mineral SOC [46]; thus, there was an increase in the proportion of some smaller aggregates (0.5–0.25 mm and 0.25–0.053 mm) under the NK and NPK treatments (Figure 1A). Nonetheless, the greater reductions in large-sized aggregates (>2 mm and 2–1 mm) resulted in a reduction in water-stable aggregates (>0.25 mm) (Figure 1B). Therefore, chemical fertilizer application reduced soil aggregate stability in black soil.

The addition of straw (NPKS) also reduced the proportion of >2 mm and 2–1 mm aggregates (Figure 1A), which was similar to a previous report that straw retained on the soil surface reduced the proportion of >2 mm aggregates [47]. This was contrary to the results of most studies on straw return [48,49], which may depend on the method of straw return. The reduction in large-sized aggregates under manure application (NPKM) (Figure 1A), which was also reported in previous literature [50], might be due to the dispersion effect of  $Na^+$  derived from manure [51]. The manure applied in our experiments was pig manure in 1990–2004 and cattle manure in 2005–2010, which contained 0.25–0.31% and 0.13–0.34% Na, respectively [52]. Nonetheless, the addition of straw and manure maintained the proportion of water-stable aggregates (>0.25 mm) because of the greater increase in the proportion of some small macroaggregates (1–0.5 mm and 0.5–0.25 mm) (Figure 1A,B). The increased number of small macroaggregates originated from the disintegration of large-sized aggregates and were protected by increased SOC (Figure 2B) [50,53]. Meanwhile, the straw and manure substituted 32% and 70% of chemical N fertilizers (Table 1). Organic substitution could inhibit N leaching and regulate soil microorganisms, reducing soil acidification [54,55]. The increase in SOC might have also inhibited the decrease in soil pH because SOC is rich in functional groups and has a good pH-buffering capacity and cation retention capacity [15,56–59]. Correspondingly, the results showed that Ca was abundant under the NPKS treatment (Figure 2G), as well as that the concentration of SOC,  $Fe_p$ , Ca, and Mg increased as aggregate size increased under the NPKM treatment (Figure 2B,E,G,H).

#### 4.2. Effect of Chemical Fertilizer on Soil P Forms

Despite the absence of P inputs, this group has reported that maize grain yields under the NK treatment were notably higher than for CK [60], implying that the soil provided sufficient P for maize growth, which was found in this study to result mainly from the contribution of Dil.  $HCl-P_i$  (Figure 4D). Dil.  $HCl-P_i$  is clearly defined as Ca-associated P (Ca-P) in soil [33], and it can be solubilized with decreasing pH [18] for uptake by maize. More evidence showed that under the NPK treatment with P input, the Dil.  $HCl-P_i$  concentration was lower than that of CK due to the decrease in soil pH (Figures 2A and 4D). We observed that the mobilization of Ca-P by soil pH occurred in soils with relatively low SOC concentrations, which is similar to the research by Yang et al. [61], which showed that the effectiveness of some acidic materials (citric, oxalic, and malic acids) in enhancing soil P solubilization was most pronounced in soils with low SOC concentrations. This could be because SOC can prevent the diffusion of P into the soil micropores [62]; conversely, SOC also protects the P in the micropores from mobilization; thus, Ca-P is more prone to being solubilized in soils with low SOC.

The mean concentration of total P in aggregates was not significantly different under the NPK treatment compared to CK (Figure 3); however, Resin- $P_i$ , NaOH- $P_i$ , and  $NaHCO_3-P_o$  concentrations were significantly increased (Figure 4A,C,F), and Dil.  $HCl-P_i$ , Con.  $HCl-P_o$ , and Residual-P concentrations were significantly decreased (Figure 4D,H,I). The Resin- $P_i$ , NaOH- $P_i$ , and  $NaHCO_3-P_o$  belong to the labile and moderately labile P fractions, while the Con.  $HCl-P_o$  and Residual-P belong to the stable P fractions [63], indicating that the balanced application of chemical fertilizer improves soil P availability. This could be mainly explained by soil pH and SOC (Figure 6). The reduction in soil pH led to the solubilization

of Ca-P and, conversely, promoted the formation of Fe/Al-associated P [7,17], which was supported by our P fractions (NaOH-P<sub>i</sub>) and XANES results (Figure 4C and Table 3). The application of chemical fertilizer increased microbial carbon limitation; accordingly, microbial demand for carbon can drive stable P<sub>o</sub> mineralization with the condition of lack of carbon source [64]. Notably, the NPK treatment showed very high levels of P<sub>o</sub> extracted from NaOH in <0.053 mm soil particles (Figure 4G). Garland et al. [28] found that enhanced soil aggregation increased P<sub>o</sub> accumulation in microaggregates, thus, reducing organic P losses via sorption to the unaggregated soil particles (<0.053 mm). Conversely, the breakage of large macroaggregates under the NPK treatment led to the release of P<sub>o</sub> adsorbed on unaggregated particles, indicating that these P<sub>o</sub> are more susceptible to mineralization due to being unprotected by aggregates. In summary, these results demonstrate that chemical fertilizers mainly alter soil pH to mobilize and redistribute P in black soil.

#### 4.3. Effect of Chemical Fertilizer Combined with Straw or Manure on Soil P Forms

In this experiment, maize grain yields were also similar each year between the P application treatments (NPK, NPKS, and NPKM) [60]; therefore, P carried away by harvest had a small effect on the differences in soil P pools among those treatments. The annual input of P was only 6 kg P ha<sup>-1</sup> higher under the NPKS treatment than the NPK treatment, but after 21 years of fertilization, the NPKS treatment had higher concentrations of Dil. HCl-P<sub>i</sub>, Con. HCl-P<sub>i</sub>, and Con. HCl-P<sub>o</sub> in aggregates (Figure 4D,E,H). In contrast to the NK and NPK treatments, straw return maintained soil pH (Figure 2A) and facilitated the accumulation of Dil. HCl-P<sub>i</sub>. This was also confirmed by the highest percentages of Ca-P detected by XANES (Table 3). Furthermore, we found that Ca concentration was positively correlated with the proportion of Dil. HCl-P<sub>i</sub>, both of which were highest under the NPKS treatment (Figure 6F). The abundant SOC can bind to minerals (such as Fe<sup>3+</sup>, Al<sup>3+</sup>, and Ca<sup>2+</sup>) to form binary complexes, which promote the P adsorption capacity of soil and facilitate the accumulation of related P forms [8,65]. Straw return can also promote the biological immobilization of P, increasing P<sub>o</sub> levels [66], and the storage of SOC leads to the sequestration of high amounts of P<sub>o</sub> [67,68]. These results imply the importance of straw return in promoting soil P accumulation by increasing SOC.

Our results showed that almost all P fractions in aggregates had the highest concentrations under the NPKM treatment among all fertilization treatments (Figure 3). Similar to straw return, manure input increased the concentration of SOC and stabilized soil pH (Figure 2A,B), thus, favoring P accumulation in the case of high P input (75 kg ha<sup>-1</sup>). Our results further showed that SOC concentration was positively correlated with the proportions of Resin-P<sub>i</sub> and NaHCO<sub>3</sub>-P<sub>i</sub>, both of which were dominant under the NPKM treatment (Figure 6C,D). A previous study reported that the increase in SOC efficiently enhanced P availability by reducing the maximum P buffering and the strength of P adsorption in black soil [69]. Additionally, SOC promotes the formation of molecular organo-mineral bonds, stabilizing labile P<sub>i</sub> compounds and inhibiting the formation of stable P<sub>i</sub> compounds [70,71]. We found that the proportion of NaOH-P<sub>i</sub>, which was predominant under the NPKM treatments, was positively correlated with Fe<sub>p</sub> concentration (Figure 6E). The increase in SOC can also create additional P sorption sites by increasing amorphous Fe (hydr)oxides and reducing the aging of P by limiting its diffusion into micropores [62,72]. Interestingly, for the NPKM treatment, it was found that Resin-P<sub>i</sub>, NaHCO<sub>3</sub>-P<sub>i</sub>, and NaOH-P<sub>i</sub> concentrations increased with increasing aggregate size (Figure 4A–C), and SOC, Fe<sub>p</sub>, Ca, and Mg showed the same regularity (Figure 2B,C,G,H). This is further evidence that SOC association with amorphous metallic minerals created more sorption sites, promoting the storage of highly available P<sub>i</sub> fractions in larger-sized aggregates. Therefore, increasing SOC through straw or manure addition promoted P accumulation in soil aggregates.

## 5. Conclusions

Different long-term fertilization practices resulted in profound effects on the characteristics of the soil P pool, which could be explained by soil pH and the distribution and

properties of aggregates. Chemical fertilizers mainly reduced soil pH and the proportion of large-sized aggregates, promoting Ca-P dissolution and  $P_o$  mobilization. Chemical fertilizer combined with straw or manure application significantly increased SOC in aggregates, inhibited the reduction in soil pH, and maintained aggregate stability, promoting the accumulation of almost all P forms in aggregates. For manure addition treatment with high P input, SOC association with some amorphous metallic minerals promoted the storage of highly available  $P_i$  fractions in larger-sized aggregates. We suggest that an appropriate amount of chemical fertilizer application is an effective and maneuverable way to utilize soil surplus P, and the addition of straw or manure is suitable for increasing fertility in poor soil, especially for P.

**Author Contributions:** Conceptualization, N.Z., S.Z., G.F. and H.G.; methodology, N.Z., Q.W. and S.Z.; software, N.Z. and Q.W.; validation, Y.C. and X.Z.; formal analysis, N.Z.; investigation, N.Z. and Q.W.; resources, H.G., C.P. and P.Z.; data curation, N.Z.; writing—original draft, N.Z., Q.W. and S.Z.; writing—review and editing, N.Z., S.Z., Y.C. and H.G.; visualization, N.Z. and Q.W.; supervision, S.Z. and G.F.; project administration, S.Z. and X.Z.; funding acquisition, S.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the National Key Research and Development Program of China (2022YFD1500101, 2021YFD1500205) and the National Natural Science Foundation of China (41977103).

**Data Availability Statement:** The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Acknowledgments:** We gratefully acknowledge the help provided by the Jilin Academy of Agricultural Science and the assistance of the members in the laboratory.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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