





## Article

# Organic Phosphorus Fractions in Relation to Soil Aggregate Fractions of Black Soil

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**Abstract:** Knowledge of long-term phosphorus behavior is essential to improve soil structure, nutrient supply potential, and the sustainability of cropping systems. A 45-year long-term experimental trial was used to observe organic phosphorus fractionation and its effects on soil aggregation and nutrient distribution at three depths (0–20, 20–40, and 40–60 cm) in Vojvodina Province, Serbia, under maize monoculture and maize/barley rotation. Five fertilizing systems were studied, including Control, NPK, NPK + maize remains, NPK + manure, and NPK + manure in rotation. Soil aggregates were fractionated into four size categories (>2000, 2000–250, 250–53, and <53 μm) using a wet sieving method. The samples were analyzed for main indicators, including different forms of phosphorus, total and available (PT and PA), as well as its organic forms (Labile Po, Biomass Po, Mod. Labile Po, Fulvic acid Po, Humic acid Po, and Resistant Po), and other fertility parameters. Significant differences in total and available phosphorus as well as all observed organic phosphorus fractions were evident between treatments with and without organic amendments, particularly in the 0–20 and 20–40 cm soil layers. Moderately labile P forms were dominant across all treatments, while labile forms constituted a smaller proportion. The most notable differences between treatments were observed in the labile and moderately labile forms, as well as in the resistant form of organic phosphorus. Manure application led to increased nutrient content in macroaggregates (>250 μm) compared to microaggregates. Microaggregates (<250 μm) were predominant across all depths, while stable structural aggregates did not show a significant increase after manure application. PCA highlighted significant correlations between soil characteristics, including total and available P, total organic carbon, clay content, and enzyme activity, across different aggregate sizes and organic P fractions. Overall, long-term mineral fertilization combined with organic amendment application induced variations in phosphorus fractions and the content of carbon, nitrogen, and phosphorus associated with aggregates in the first two soil layers, except for aggregate size classes.

**Keywords:** long-term experiment; fertilizing systems; P organic fractionation; soil aggregates; black soil



**Citation:** Milić, S.; Ninkov, J.; Vasin, J.; Zeremski, T.; Jakšić, S.; Živanov, M.; Šeremešić, S.; Milić, D. Organic Phosphorus Fractions in Relation to Soil Aggregate Fractions of Black Soil. *Agronomy* **2024**, *14*, 1022. <https://doi.org/10.3390/agronomy14051022>

Academic Editor: Jiafa Luo

Received: 3 April 2024

Revised: 30 April 2024

Accepted: 1 May 2024

Published: 11 May 2024



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## 1. Introduction

In the 20th century, agricultural production became heavily reliant on phosphate ore as a primary source of phosphorus, shifting away from the utilization of phosphorus derived from organic sources. Approximately 40 million tons of P is being used from the deposition of 7000 million tons of rock P every year [1], with almost 90% reserved for use in the agriculture industry [2]. According to [3], there is an assumption that the current reserves of phosphate ore will be depleted within the next 50 to 100 years. In order to sustain crop production and animal feed and meet the nutritional requirements of the human population, it is imperative to prioritize phosphorus recycling, as suggested

by [4]. Therefore, it is crucial to understand the challenges posed by phosphorus in soil, the different sources of P, and the impacts of its application and use. This knowledge is essential for conserving phosphorus and ensuring the future of agricultural production.

According to availability, phosphorus in soil can be categorized into four forms: (1) easily accessible P via plant roots for uptake; (2) labile soil organic P; (3) P in inorganic solid phases; and (4) stable organic P, which is less readily available for plant uptake [5].

Organic phosphorus ( $P_O$ ) has the potential to constitute a significant proportion of the total phosphorus content in the soil, ranging from 4% to 90% [6]. Organic matter present in the soil interacts with phosphorus and influences the availability of phosphorus in the soil solution, either directly or indirectly. The direct effects of dissolved organic matter on phosphorus availability can be attributed to three fundamental processes: (1) competition between organic compounds and phosphorus for adsorption sites, (2) the enhancement of phosphorus dissolution and the control of phosphorus adsorbed in the mineral phase, and (3) the release of phosphorus into the solution from organic fractions [7]. Indirect impacts include improvements in soil structure, moisture regulation [8], and partial influence on soil pH through the reduction in the sorption capacity of iron and aluminum oxides.

Quantification of the various known  $P_O$  compounds in soil has been described in numerous studies in the past and is advocated by [9] as a means of fractionating soil organic phosphorus ( $P_O$ ). The fractionation scheme involves a sequence of extractions that separates soil ( $P_O$ ) into labile, moderately labile, and nonlabile fractions. In recent years, this scheme has been widely used to evaluate P cycling in different ecosystems and also in diverse soils under varying management [10–14].

Soil agricultural practices, specifically management practices, can influence the enhancement of soil aggregation and organic matter content, leading to increased retention and stability of phosphorus in the soil. The impact of soil aggregation on the capacity to retain labile forms of phosphorus chemical fractions can demonstrate how land use practices affect the distribution and stability of phosphorus [15]. Differentiating soils into aggregates of varying sizes can offer valuable insights into the impacts of different land uses, as well as changes in phosphorus and its availability. Aggregates play a crucial role in preserving organic matter, as they can impede its decomposition [16,17]. Conventional tillage practices typically diminish soil aggregation and enhance the turnover (formation and degradation rate) of macroaggregates when compared to reduced tillage methods. Consequently, this can lead to the depletion of organic carbon and nutrients from the soil [18–20].

The investigation of soil structure and aggregate stability is at the forefront of current research, considering international initiatives for soil protection, as they are crucial for soil fertility and several key aspects of soil functioning. They affect the soil's water, air, and heat dynamics, which play a crucial role in regulating plant growth and nutrient availability. Additionally, soil structure and aggregate stability impact soil erodibility, as well as its ability to sequester carbon, which provides insights into soil fertility but also serves as an indicator of its overall health and resilience [21–28]. Many studies have shown that the addition of organic amendments (e.g., manure and crop residues) to soil over time could maintain or increase soil fertility, nutrient contents (N, P, and K), and soil organic carbon accumulation and improve aggregation [29–32]. Contrary, several studies have reported that aggregate stability did not increase following organic amendments (manure, vermicompost, and lantana compost), [33]. Alterations in aggregate stability and nutrient content within agricultural soil occur gradually, often reflecting the impact of soil management practices over time [34–36]. In the context of rising production costs, finite resources, and the need for environmental conservation in fertilizer usage, effectively managing nutrient cycling while maximizing benefits has become a crucial aspect of agroecosystems [37].

This study is based on a 45-year-long consistent experimental field trial and aims to investigate the influence of diverse fertilization and agricultural practices on the distribution of organic phosphorus fractions in the soil over a long-term period. Furthermore, it aims to

assess the resulting effects on soil aggregate distribution, as well as the concentrations of carbon, nitrogen, and phosphorus within soil aggregates.

## 2. Materials and Methods

### 2.1. Site Description

The experimental trial was located in the northern part of Serbia—Vojvodina Province—in the southeastern part of the Pannonian (Carpathian) Basin. Vojvodina is a typical agricultural region in which arable land covers 73% of the utilized area, roughly 1,470,000 ha. Regarding the distribution of soil types in Vojvodina, as much as 60% of the area is black soil (chernozems) which is considered ideal for agricultural production according to its physical and chemical properties [38]. The field experiment was conducted in the experimental field of the Institute of Field and Vegetable Crops in Novi Sad, in a long-term trial established in 1965 (45°32'51" N; 19°84'77" W; A84 m). The soils are classified as a calcareous chernozem, CH-cc-ai.lo.ph (IUSS Working Group WRB, 2022). The experimental setup included 5 fertilizing systems in maize monoculture: Control (I), NPK (II), NPK + maize remains (III), NPK + manure (IV), and a 2-year rotation of maize/barley NPK + manure (V). Treatments utilizing mineral fertilizers were applied; NPK mineral fertilizers at a ratio of 15:15:15 were applied in autumn at a rate of 400 kg ha<sup>-1</sup>, followed by a spring application of 130 kg ha<sup>-1</sup> of urea (46% N). In two-crop and single-crop trial variants, 25 t ha<sup>-1</sup> of manure was applied in autumn every second year. In the treatment incorporating maize remains, the applied quantity by year varied from 8 to 10 t ha<sup>-1</sup>, depending on the average yield. The trial followed a three-factorial design with four replicates based on the plan of divided plots with a randomized block design, each covering 300 m<sup>2</sup> area. A detailed view of the experimental site and setup, location, experimental design, fertilizer application, partial nutrient balance, and soil chemical properties in the period of trial establishment are given in [39].

### 2.2. Soil Sampling and Analysis

The analyzed dataset consisted of 120 samples and was statistically processed with respect to three soil depths (0–20, 20–40, and 40–60 cm). The samples were collected during the fall of 2009 and 2010. Laboratory studies performed in this investigation represent the extension of previous research provided by [39]. The collected soil samples were air-dried and milled to <2 mm particle size, according to [40]. Soil pH was determined in soil suspension with water (active acidity) and water suspension in 1M KCl (substitution acidity), according to [41]. Total phosphorus (P<sub>T</sub>) in the soil was analyzed by ICP-OES, VistaPro Varian apparatus, in accordance with the US EPA method [42]. Readily available phosphorus (P<sub>A</sub>) was determined by extracting ammonium lactate [43], whereby detection was performed spectrophotometrically at a wavelength of 830 nm in a UV/VIS spectrophotometer, Cary 3E Varian. Fractionation of organic phosphorus followed the procedures developed by [10] and modified by [12,13]. The analysis procedure used in this investigation was described in detail by [14]. Organic phosphorus (PO) fractionation becomes evident by distinguishing three primary forms related to changes in solubility. The first is the labile fraction (Labile Po), defined by organic phosphorus adsorbed on soil particle surfaces, extractable by using 0.5M NaHCO<sub>3</sub>, and phosphorus bound to microbiological biomass (Biomass Po). The second is the moderately labile fraction, which includes organic phosphorus extracted using 1.0 M HCl (Mod. Labile Po), and phosphorus bound to fulvic acid-resistant components (Fulvic acid Po). Lastly, the third fraction involves highly resistant, non-labile forms, exemplified by phosphorus bound to humic acids (Humic acid Po) and residual organic phosphorus (Resistant Po).

Fractionation of soil aggregates was performed using a wet sieving procedure [44,45]. Approximately 100 g of air-dried soil was capillary-wetted to field capacity to prevent slaking following immersion. The wetted soil was immersed in water on a nest of sieves (2000, 250, and 53 μm) and shaken vertically over 3 cm 50 times during a 2 min period. Soil aggregates retained on sieves were collected, oven-dried at 50 °C, and weighed to a

constant mass. Aggregate size fractions included large macroaggregates (>2000  $\mu\text{m}$ ), small macroaggregates (250–2000  $\mu\text{m}$ ), microaggregates (53–250  $\mu\text{m}$ ), and silt- and clay-associated particles (53  $\mu\text{m}$ ). Whole-soil samples and subsamples of aggregate size fractions were subjected to chemical analyses of  $P_T$ , TOC, and  $N_T$ . The total nitrogen and total organic carbon (TOC) content in soil were determined through elemental analysis using a CHNS VarioEL III analyzer, according to the AOAC Official Method 972.43:2000 and [46], respectively. The methods used for determining the activity of acid and alkaline phosphatase (phosphomonoesterases) in the soil was described by [47].

### 2.3. Statistical Analysis

This study utilized factorial ANOVA, a method based on linear modeling, to examine variations among variables such as the fertilization system, sampling date, and sampling depth. Fisher's test for the least significant difference was employed to determine significant differences between means. To ensure normality, all variables underwent a log transformation with the formula  $\log(x + 1)$ , guided by the outcomes of Shapiro-Wilk normality tests ( $p < 0.05$ ). For statistical analyses, Microsoft Excel [48] and Statistica 14 [49] commercial software packages were used, along with the correlation package R version 4.2.2 (R Core Team). Unless otherwise stated, the level of significance referred to in the results is  $p < 0.05$ .

In this article, we employed principal component analysis (PCA) as a statistical procedure to enhance interpretability while minimizing information loss. PCA is a well-established method commonly utilized to reduce the dimensionality of complex datasets that may be challenging to interpret. Our study utilized PCA to obtain valuable insights from the soil quality dataset and determine the most influential factors among the analyzed parameters within the investigated treatments.

## 3. Results and Discussion

### 3.1. Soil Characteristic

Basic statistical parameters of selected physical and chemical properties of the soil samples are given in Table 1. A detailed analysis of soil fertility and its interaction are given in [39]. The soils belonged to the neutral to slightly alkaline and low organic matter class. Calcium carbonate varied from 0 to 23.2%. Clay content in treatments ranged between 21.2 and 30.6%. Available P varied wildly from 2 to 690  $\text{mg kg}^{-1}$ , whereas total phosphorous was in the range from 521 to 1359  $\text{mg kg}^{-1}$  with an average of 807  $\text{mg kg}^{-1}$ . According to the data obtained from the long term investigation in a biological trial conducted by [50], optimal levels of available phosphorous in soil for field crop production are in the range between 150 and 250  $\text{mg kg}^{-1}$ . When the available phosphorus levels fall below the recommended 150  $\text{mg kg}^{-1}$ , it is suggested to use ameliorative doses of P fertilizers.

**Table 1.** Basic statistical parameters of selected physical and chemical properties of soil samples.

Parameters	Clay	Silt %	Sand	pH (KCl)	CaCO <sub>3</sub> %	OM %	Avail. P $\text{mg kg}^{-1}$	Total P $\text{mg kg}^{-1}$
Mean	27.03	30.42	42.55	7.05	4.71	2.29	129.0	807.96
Min.	21.20	22.56	37.12	5.72	0.00	1.47	2.0	521.0
Max.	30.56	37.16	48.72	7.76	23.22	4.77	690.0	1359.0
Mediana	27.02	27.36	42.44	7.15	8.11	2.26	48.0	757.0
Std. Dev.	16.11	1.84	3.15	0.47	6.36	0.47	16.1	211.1
Coef. Var.	124.8	6.8	7.4	6.6	135.0	20.7	124.8	26.1

### 3.2. Forms of Phosphorus and Its Organic Fractions in Soils

#### 3.2.1. Total Content of Phosphorous

Following 45 years of the application of different fertilizers pattern, the total phosphorus content ( $P_T$ ) exhibited significant variations among the investigated treatments.

The lowest  $P_T$  content was observed in the control variant, measuring  $585 \text{ mg kg}^{-1}$ , while the highest content was recorded in the treatment involving manure application and crop rotation, amounting to  $1211 \text{ mg kg}^{-1}$  in the surface soil layer (Table 2). Notably, a statistically significant difference was observed between the first two soil depths and the 40–60 cm layer in all treatments, except for the control variant, where no depth-based differences were evident. Moreover, the treatments involving manure application exhibited statistically significant distinctions from all other treatments. Particularly noteworthy were the significantly elevated values found in variants with applied manure IV and V in the top two soil layers (0–20 and 20–40 cm) compared to deepest layer (40–60 cm) (Table 2). Conversely, no statistical differences were established between treatments II and III. The increase in  $P_T$ , when compared to the control, ranged from 30% to 97%, depending on the specific treatment and soil depth under investigation. These findings align with previous research by many authors such as [51–55], who also documented a substantial rise in total phosphorus content in the arable soil layer due to the excess fertilization practices.

### 3.2.2. Content of Available Phosphorus

The variations in available phosphorus content, as influenced by the tested treatments, mirror those observed in the total phosphorus concentrations. The highest statistically significant content was detected in treatments where manure was applied (treatments IV and V), particularly in the surface layer of the soil ( $327.0$  and  $478.8 \text{ mg kg}^{-1}$ ). Conversely, the lowest values were identified in the control variant ( $13.7 \text{ mg kg}^{-1}$ ). In relation to the soil's classification according to the national categorization of nutrient supply [50] for readily available phosphorus, the arable soil layer in treatments I, II, and III is characterized as having a very low (I) and low (II and III) levels, while treatments IV and V are classified as high. Our findings are in line with those reported by [56], who observed higher available P contents in an arable soil in Germany fertilized with organic manure compared with a soil fertilized with mineral NPK.

Long-term application of P fertilizers, exceeding the plant's annual requirements, can significantly increase both organic and inorganic forms of phosphorus content [29]. Furthermore, if we examine the proportion of available phosphorus within the total phosphorus content in the arable soil layer (0–20 and 20–40 cm), we can observe a wide range, varying from 2.2% to 39.5%. The lowest values were observed in the control treatment and treatments with solely mineral fertilizers (II and III), while the highest proportion was recorded in the treatment involving manure application and crop rotation (V). Long-term application of animal manures and other organic amendments has been shown to increase soil total, available, and soluble P concentrations extending to various/applied soil depths [57,58].

### 3.2.3. Distribution of P Organic Fraction and Soil Phosphatase Activity

Fractionation of organic phosphorus ( $P_O$ ) can be shown through three main forms in relation to solubility (transformation): labile, moderately labile, and resistant (Table 2). Each of these fractions represents different levels of availability of specific organic phosphorus components to plants, signifying the strength of their binding to the soil matrix [59].

In our analysis, we observed statistically significant main effects ( $p < 0.05$ ) for both factors, treatment and depth, indicating that these factors individually have a significant impact on the outcome of all investigated variables. Furthermore, we found a significant interaction between those factors for the variables  $P_T$ ,  $P_A$ , labile forms (Labile Po and Biomass Po), moderately labile form (Mod. Labile Po), and alkaline phosphatase. The results of the analyses suggest that the combined effect of these factors is greater than would be expected from their individual effects alone (Table 2).

**Table 2.** Distribution of total ( $P_T$ ), available ( $P_A$ ), and organic phosphorus fractions ( $\text{mg kg}^{-1}$ ) and content of alkaline and acid phosphatase ( $\mu\text{g p-np g}^{-1} \text{ s}^{-1}$ ) depending on applied fertilization system and investigation depth.

Treatment (A)	Depth (B) (cm)	$P_T$	$P_A$	Labile		Moderately Labile		Nonlabile			Alkaline Phosphatases	Acid Phosphatases
				Labile Po	Biomass Po	Mod. Lab. Po (HCl)	Fulvic Acid Po	Humic Acid Po	Resistant Po			
I Control (single-crop system)	0–20	612.83 *fg	13.7 g	9.89 c	9.07 ef	143.92 bcd	160.19 cde	117.73 cd	62.50 ef	255.33 cde	135.33 b	
	20–40	620.50 fg	13.5 g	9.48 c	6.33 ef	142.58 bcd	155.76 de	116.33 cd	55.00 ef	254.67 cde	127.75 bc	
	40–60	585.67 g	13.8 g	5.22 c	3.73 f	128.05 de	160.95 cde	92.80 ef	50.00 f	217.50 ef	84.50 de	
II NPK (single-crop system)	0–20	802.67 c	82.5 ef	14.54 c	9.09 ef	170.15 bc	175.00 abc	125.83 bc	98.33 bcde	218.67 def	71.67 de	
	20–40	792.00 c	59.3ef	11.67 c	6.04 ef	138.52 cd	185.08 a	114.09 cd	88.33 cdef	235.50 cde	66.83 e	
	40–60	665.83 def	10.0 g	11.76 c	2.66 f	108.52 e	144.55 ef	75.87 g	85.00 def	156.17 f	33.08 ef	
III NPK + maize remains (single-crop system)	0–20	796.00 c	95.2 e	10.93 c	13.76 de	181.40 bc	162.92 cde	126.67 bc	65.00 ef	283.50 bc	133.33 b	
	20–40	765.83 cd	72.0 ef	10.88 c	8.66 ef	175.82 bc	165.27 bcd	135.15 ab	65.00 ef	258.00 cde	112.92 bc	
	40–60	646.00 efg	10.2 g	11.39 c	3.09 f	141.67 cd	135.95 f	90.72 fg	69.18 def	165.17 g	51.00 ef	
IV NPK + manure (single-crop system)	0–20	1046.30 b	327.0 c	32.78 ab	35.66 b	291.35 a	172.92 abc	123.75 bc	129.17 abc	274.00 c	175.25 a	
	20–40	983.83 b	270.3 d	26.20 b	23.28 c	287.35 a	185.42 a	107.08 de	137.50 ab	266.83 cd	173.92 a	
	40–60	699.17 de	39.5 fg	11.67 c	4.58 f	173.03 bc	164.17 bcd	83.75 fg	112.50 bcd	170.17 fg	72.58 e	
V NPK + manure (two-crop rotation— maize/barley)	0–20	1211.80 a	478.8 a	31.67 b	46.96 a	301.22 a	182.39 ab	149.28 a	170.83 a	369.50 a	114.67 bc	
	20–40	1160.80 a	408.7 b	36.48 a	38.68 b	306.27 a	184.20 a	143.30 a	163.33 a	326.83 ab	101.67 cd	
	40–60	729.83 cd	40.5 fg	12.22 c	5.11 f	155.15 bcd	150.76 def	97.30 ef	136.67 ab	151.83 g	43.67 f	
A ** <i>p</i> value B AxB		$<2 \times 10^{-16}$	$<2 \times 10^{-16}$	$5.47 \times 10^{-7}$	$3.05 \times 10^{-13}$	$6.36 \times 10^{-16}$	0.0489	0.0199	$8.06 \times 10^{-13}$	$1.02 \times 10^{-5}$	$6.54 \times 10^{-12}$	
		$1.44 \times 10^{-14}$	$2.87 \times 10^{-11}$	0.000874	$1.65 \times 10^{-11}$	$4.90 \times 10^{-10}$	$6.64 \times 10^{-5}$	$3.11 \times 10^{-9}$	0.289	$2.90 \times 10^{-10}$	$1.24 \times 10^{-15}$	
		$3.62 \times 10^{-6}$	$2.39 \times 10^{-14}$	0.020531	$3.39 \times 10^{-9}$	0.000111	0.2549	0.8175	0.965	0.0256	0.0922	

\* LSD Fisher test: significance at the 0.05 F level of probability. Factor levels marked with the same letter do not differ; \*\* The effect was significant for *p* values less than 0.05.

In our analysis, we observed statistically significant main effects (P) for both treatment and depth factors, indicating that these factors individually have a significant impact on all investigated variables. Additionally, we found a significant interaction between these factors for the variables PT, PA, labile forms (Labile Po and Biomass Po), moderately labile form (Mod. Labile Po), and alkaline phosphatase. The results of the analyses suggest that the combined effect of these factors is greater than would be expected from their individual effects alone (Table 2).

In our study, significant differences in all observed  $P_O$  fractions and depths can be separated into two fundamental groups: one with the utilization of organic amendments and another without. This demarcation is evident as the highest values were precisely recorded in the treatment with organic amendment application. The studies of [60–62], also report an increase in organic phosphorus fractions in soils with continuous application of organic fertilizers. In our investigation, the differences are most pronounced in the first two test depths (0–20 and 20–40 cm), while the 40–60 cm depth values are the lowest in most observed treatments and fractions and statistically differ, especially in the treatments with manure application (IV and V). Increased  $P_O$  values in the surface layer were also reported by [63], which can be attributed to the deposition and accumulation of organic components in this layer.

Significant increases in the labile phosphorous (Labile Po) and phosphorus bound to the microbiological mass (Biomass Po) are evident in treatments with continuous application of both organic and mineral fertilizers (IV and V). These values surpass two to four times the typical values observed in I control, II NPK, and III NPK + maize remains treatments (Table 2). Studying various fertilization systems [64], also observes an elevation in the labile fraction in treatments utilizing manure. This increase is attributed to the transformation of a portion of the moderately labile fraction toward the formation of labile organophosphorus compounds.

The moderately labile fraction content (Mod. Labile Po) exhibits a significant increase in treatments incorporating organic matter in the first two depths of the test (from 287.35 to 306.27 mg kg<sup>-1</sup>), in line with the findings of [65,66]. Likewise, [67] propose that a major portion of the (Mod. Labile Po) fraction originates from microorganisms, with phospholipids dominating the extraction step in isolating this phase. This suggests that the accumulation of this fraction occurs in soil layers where the plant root system is most prominent, as microbiological activity is most intense in the root zone [68]. The content of the Fulvic acid Po demonstrates statistically minimal deviations among all observed treatments at the same depth. The study by [13] also highlight the stability of this fraction. Conversely, [48], in a 30-year examination of mineral and organic fertilizer application, report no distinct changes in the moderately labile fraction of phosphorus across the observed treatments.

Considering the distribution of the accessibility of individual organic phosphorus fractions across treatments collectively, it is evident that the moderately labile forms (Mod. Labile Po and Fulvic acid Po) constitute the most prominent form in all tested variants (Table 2). In contrast, the labile fraction (Labile Po and Biomass Po) represents the smallest share of total organic phosphorus. Similar observations are made by [64,65,69]. According to [70] the content of the labile and moderately labile fraction of organic phosphorus determined by a similar method can serve as a useful index for assessing soil phosphorus and organic matter availability.

The concentration of fractions, particularly labile and moderately labile fractions (Labile Po, Biomass Po, and Mod. Labile Po), in treatments involving organic fertilizers (IV and V) corroborates the obtained values of microbiologically bound Po and alkaline phosphatase content. This supports the notion that phosphorus transformation occurs between these two phases through intermediaries. The treatments with manure exhibit the statistically highest values of Biomass Po, particularly in soil depths of 0–20 and 20–40 cm (from 23.28 to 46.96 mg kg<sup>-1</sup>) (Table 2). The study by [71] has demonstrated that the introduction of organic materials activates the inorganically fixed phosphorus,

with this form largely transforming into an organic reservoir through the phosphorus of microbial biomass.

The activity of alkaline phosphatase is slightly more pronounced than that of acid phosphatase, which is expected considering the prevailing alkaline to neutral soil reaction. The highest values for both phosphatases are associated with manure application (treatments IV and V). Furthermore, the increase in the alkaline phosphatase value is most notable in treatment V (Table 2), indicating that crop changes and the introduction of manure significantly affect the activity of microorganisms involved in phosphorus cycling. Research of [72] assert that the phosphatase enzyme activity is notably influenced by crop rotation, with the highest value obtained in the rotation of maize and rye. Moreover, long-term studies on manure application suggest that its continuous use enhances the activity of both alkaline and acid phosphatase in the soil [73,74].

Based on the presented findings, the nonlabile fraction related to humic acids in organic phosphorus (Humic acid Po) exhibits a relatively stable characteristic among treatments. The limited impact of long-term fertilization, whether with mineral or organic fertilizers, on this fraction aligns with the results of [64,65,69]. Notably, the treatment V stands out as significant, being statistically separated from all other treatments in the first two observation layers (Table 2). This differentiation is likely a consequence of the increased phosphorus content in this treatment attributed to the specific cultivation system and the quantity of mineral and organic fertilizers applied. The dynamics of the Humic acid Po across soil depths indicate no substantial differences between the observation depths in the 0–20 and 20–40 cm layers. However, in the 40–60 cm layer, differences are evident across all treatments compared to the first two observation depths, suggesting the low mobility of the organic phosphorus fraction in this soil depth, indicative of its retention in the application zone.

Analyzing the highly resistant form of nonlabile organic phosphorus (Resistant Po) reveals two distinct groups: the first comprising monoculture without manure application (I, II, and III), and the second involving treatments with continuous manure application (IV and V). Treatment V, with manure and the application of mineral fertilizer in a two-crop rotation, exhibits the statistically highest values (Table 2). Research of the [75] also note that the highest content of the residual fraction of organic phosphorus (Resistant Po) occurs in soils exclusively treated with manure (organic production). Similarly, [76] have found in their long-term study on phosphorus fertilizers that the introduction of organic matter significantly increases the residual fraction, along with other organic fractions (labile and partially labile), except for the resistant form, where no differences were observed. The application of mineral fertilizers, consistent with our study, does not show any noticeable impact on any group of organic fractions.

### 3.3. Aggregate Stability and C, P, and N Concentration under Long-Term Fertilization

#### 3.3.1. Soil Aggregate Distribution following Wet Sieving

Investigating the influence of the fertilization system and the investigation depth on soil aggregate distribution, we observed statistically significant main effects for both factors. Furthermore, we identified a significant interaction between these factors for all variables except microaggregates <53  $\mu\text{m}$ . These findings underscore the importance of considering both the fertilization system and the investigation depth in understanding aggregate size formation (Table 3).

The analysis of soil aggregate fractions (Table 3) revealed the prevalence of microaggregates (<250  $\mu\text{m}$ ) across all three investigated depths. Depending on the treatment variant, the sum of <53 and 53–250  $\mu\text{m}$  ranged from 58.85% to 84.49%. Statistically, the highest occurrence of small microaggregates (<53  $\mu\text{m}$ ) was observed in treatments II (42.46%) and V (45.16%), and they were particularly apparent at depths of 0–20 and 20–40 cm, indicating soil dispersion in the arable layer (Table 3). Across all three soil depths (0–60 cm), the percentage share of large macroaggregates (>2000  $\mu\text{m}$ ) was the lowest. This aligns with the findings by [77], who determined that the share of macroaggregates constitutes



approximately 1% of the soil's dry mass. Control treatments (I) exhibited the highest content of macroaggregates (>2000  $\mu\text{m}$ ) at 5.30%, followed closely by monoculture with the application of manure and the NPK fertilizer (IV) at 5.04%. Despite organic matter being considered an important soil stabilizer, alterations in the representation of water-resistant aggregates in various tillage systems are not solely determined by its content [78].

**Table 3.** Soil aggregate distribution (%) depending on the applied fertilization system and the investigation depth.

Aggregate Size ( $\mu\text{m}$ )/Treatment (A)	I Control	II NPK	III NPK + Maize Remains	IV NPK + Manure	V NPK + Manure	
<b>** <i>p</i> value A</b>						
<b>0–20 cm (B)</b>						
$3.18 \times 10^{-16}$	>2000	5.30 *a	1.96 bc	1.83 bcd	5.04 a	1.35 cd
$1.75 \times 10^{-9}$	250–2000	35.17 ab	16.74 ef	21.60 de	18.67 def	13.66 f
$9.34 \times 10^{-6}$	53–250	34.92 ef	39.16 cdef	44.586 a	37.40 cdef	39.83 abcde
$2.49 \times 10^{-16}$	<53	23.93 ef	42.46 ab	31.99 c	39.86 b	45.16 abc
<b><i>p</i> value B</b>						
<b>20–40 cm</b>						
$1.04 \times 10^{-5}$	>2000	4.64 a	1.55 cd	2.77 b	4.80 a	1.17 cd
$7.74 \times 10^{-8}$	250–2000	31.91 abc	27.98 c	31.92 abc	22.33 d	18.20 def
$7.63 \times 10^{-16}$	53–250	38.40 bcdf	37.53 cdef	39.52 bcde	40.66 abcde	40.37 abcd
$4.04 \times 10^{-9}$	<53	23.64 f	32.94 c	25.79 def	32.43 c	40.26 ab
<b><i>p</i> value AxB</b>						
<b>40–60 cm</b>						
0.000158	>2000	4.54 a	0.81 d	0.91 cd	1.09 cd	0.81 d
0.00159	250–2000	35.89 a	30.15 bc	30.66 abc	28.60 c	34.07 abc
0.000694	53–250	34.30 f	37.75 cdef	42.46 ab	41.75 abc	35.84 def
0.126	<53	21.92 f	31.28 c	25.96 def	28.90 cde	29.29 cd

\* LSD Fisher test: significance at the 0.05 F level of probability. Factor levels marked with the same letter do not differ \*\* The effect was significant for *p* values less than 0.05.

The analysis of structural properties indicated that in the crop rotation where manure was applied, this did not lead to a significant increase in the proportion of water-stable aggregates (macroaggregates). This finding contradicts our expectations and runs counter to numerous research reports highlighting the positive influence of manure and cropping systems on macroaggregate improvement and stability [79–82]. One interpretation for the decrease in macroaggregate participation and the rise in microaggregates in treatments with manure is provided by [83]. They suggest that manure contains substantial amounts of monovalent cations (primarily Na and K),  $\text{NH}_4$ , and positively charged organic anions, which are known to disperse soil colloids and may contribute to the breakdown of larger soil aggregates [84,85]. Moreover, [86] found that both organic and mineral fertilization treatments increased the proportion of the silt and clay fraction (<53  $\mu\text{m}$ ) and decreased the proportion of macroaggregates (250–2000  $\mu\text{m}$ ), which supports our findings. Additionally, the elevated value of macroaggregates in the control treatment could result from the stabilization of organic matter, reflecting the establishment of balanced organic matter conditions over an extended period [39,87,88]. It is important to consider that the impact of manure is subject to modification, with the primary effects on the soil structure being influenced by the physical characteristics of soil, the intensity and frequency of soil processing, stronger microbial activity, crop rotation, and root secretions [36,44,89]. Study by [90] found that aggregate stability and moisture content are highly correlated with organic matter (OM) content, and the decay rate of both macro- and microaggregates is highly influenced by the intensity of tillage.

In the research [79] argues that establishing a direct correlation between water-resistant aggregates and OM is not always straightforward. He suggests that OM may not be the sole cementing material in the soil and emphasizes that the quality, rather than the quantity, of introduced OM plays a crucial role. Besides these factors, mineral fertilizers can also significantly influence aggregate stability. In a study on the impact of fertilizers and plant

residues on organic matter stabilization in soil aggregates [91] notes that the effect of mineral fertilizer tends to increase the presence microaggregates, aligning with the results obtained in our experiment. Changes in the representation of individual aggregates may be attributed more to the accelerated microbial decomposition of organic binding agents within the aggregates than to the influence of mechanical processing forces, indicating that synthetic fertilizers play a somewhat less pronounced yet similar role in aggregation compared to tillage effects [19]. The observed stability of aggregates in our research lends support to these changes. Specifically, in treatments involving mineral fertilizer and mineral fertilizer with manure (crop rotation), a more pronounced formation of microaggregates in relation to macroaggregates is evident compared to the control variant and treatments with mineral fertilizer. Considering the significant presence of enzymatic and total microbiological activity, coupled with a high concentration of nutrients observed in treatments involving fertilizers, the minimal alteration in the share of macroaggregates and the changes in aggregate stability become more pronounced. From this observation, we can reasonably deduce that the hypothesis regarding the breakdown of binding agents is likely accurate.

### 3.3.2. Concentration of C, P, and N in Soil Aggregates

In our study on the impact of fertilizer systems (A) and soil depth (B) on the concentration of C, P, and N in soil aggregates, we observed significant main effects ( $p < 0.05$ ) for both factors, with the exception of the variable total organic carbon (TOC) content in microaggregates ( $<53 \mu\text{m}$ ). Additionally, we identified a significant interaction between fertilizer systems and soil depth for the majority of variables, except for the content of  $P_T$ , TOC, and  $N_T$  in microaggregates  $<53 \mu\text{m}$ . These findings underscore the importance of considering both fertilizer systems and soil depth in relation to soil aggregate stability.

By examining the levels of organic carbon, total phosphorus, and nitrogen across various treatments and aggregate sizes, the highest values were identified in the initial two depths of the study (0–20 and 20–40 cm). Comparing the treatments within those two soil layers, statistically significant higher values were recorded for particle sizes  $>2000 \mu\text{m}$ , 250–2000  $\mu\text{m}$ , and 53–250  $\mu\text{m}$  in treatments IV and V. Conversely, the control and treatments with mineral fertilizer (II and III) registered the lowest values for all parameters (Table 4).

The findings of numerous studies on manure application and nutrient content in aggregate fractions support an increased concentration of total C, N, and P in macroaggregates ( $>250 \mu\text{m}$ ) compared to microaggregates [92–96]. Our results align with these findings. Conversely, some research reports have different outcomes, indicating a higher concentration of organic C and total P in microaggregates ( $<0.1 \text{ mm}$ ) in relation to macroaggregates [97–99]. This is partially consistent with our results, as the values in microaggregates in most treatments without manure application (I, II, and III), for all analyzed elements, were either equal or slightly higher ( $<53 \mu\text{m}$ ), although without statistical significance between aggregate sizes and observed depths. However, since macroaggregates are typically less abundant in the soil, their content does not necessarily correspond to the total level of organic matter in the soil [88]. Macroaggregate fractions ( $>2000$  and 250–2000  $\mu\text{m}$ ), depending on the treatment, constitute 1.17–5.30% and 13.7–36.8% of the arable soil layer in an individual sample (Table 4).

Examinations of total organic carbon (TOC) and total nitrogen ( $N_T$ ) content in aggregates across all treatments reveal a high dependence between these two parameters as well as similar trends in both the tested treatments and the various depths and sizes of aggregates (Table 4). In the 0–20 cm layer, the analysis indicates that the application of manure leads to an increased content of TOC and  $N_T$  in macroaggregates, corroborating the findings of [30,81]. Our study identifies the positive impact of manure on the accumulation of TOC and N in macroaggregates up to depths of 0–40 cm, whereas this effect diminishes in the deeper layer since manure is applied at the ploughing depth.

**Table 4.** The distribution of total phosphorous (P<sub>T</sub>), total organic carbon (TOC), and total nitrogen (N<sub>T</sub>) depending on the size of the aggregates and the fertilization system.

Treatment (A)	Depth (B) (cm)	P <sub>T</sub> Content mg kg <sup>-1</sup>				TOC Content %				N <sub>T</sub> Content %			
		>2000	250–2000	53–250	<53	>2000	250–2000	53–250	<53	>2000	250–2000	53–250	<53
<b>I Control</b> (single-crop system)	0–20	742.8 *d	760.0 d	689.8 d	822.0 bc	1.487 cd	1.74 defg	1.37 c	1.60 ab	0.162 de	0.186 cde	0.147 bc	0.160 b
	20–40	751.5 cd	707.5 d	660.2 de	782.3 bc	1.566 bcd	1.71 defg	1.35 c	1.42 bc	0.167 de	0.175 cde	0.146 c	0.161 b
	40–60	689.8 d	674.3 d	596.9 ef	728.9 cd	1.384 cd	1.37 efg	1.14 d	1.14 d	0.157 de	0.149 de	0.124 d	0.118 cd
<b>II NPK</b> (single-crop system)	0–20	730.3 d	873.1 d	671.6 de	734.7 cd	1.859 bcd	1.91 def	1.39 c	1.40 bc	0.185 de	0.192 cde	0.152 bc	0.159 b
	20–40	680.9 d	709.4 d	640.0 de	731.0 cd	1.618 bcd	1.74 defg	1.38 c	1.37 c	0.177 de	0.190 cde	0.148 bc	0.158 b
	40–60	598.3 d	599.3 d	521.3 f	596.5 e	1.291 d	1.25 fg	1.16 d	1.01 de	0.136 e	0.127 e	0.112 d	0.098 d
<b>III NPK + maize</b> <b>remains</b> (single-crop system)	0–20	794.2cd	848.0 d	731.9 cd	809.7 bc	1.860 bcd	2.23 bcd	1.48 bc	1.53 abc	0.208 cde	0.218 bc	0.165 b	0.175 ab
	20–40	814.8 cd	772.8 d	706.7 cd	788.5 bc	1.914 bcd	2.06 cde	1.43 c	1.45 abc	0.218 cde	0.212 cd	0.163 bc	0.168 ab
	40–60	640.8 d	617.1 d	552.4 ef	653.6 de	1.284 d	1.18 g	1.04 d	1.10 de	0.152 e	0.144 e	0.125 d	0.117 cd
<b>IV NPK + manure</b> (single-crop system)	0–20	1264.7 bc	1763.3 b	1147.1 a	1169.1 a	2.134 bc	2.82 b	1.74 a	1.53 abc	0.248 cd	0.366 a	0.200 a	0.184 a
	20–40	1423.0 b	1459.5 c	1066.5 ab	1135.7 a	2.261 b	2.65 bc	1.57 b	1.49 abc	0.284 bc	0.283 b	0.190 a	0.184 a
	40–60	912.3 bcd	875.7d	782.6 c	871.1 b	1.389 cd	1.51 efg	1.06 d	1.02 de	0.164 de	0.157 cde	0.119 d	0.122 c
<b>V NPK + manure</b> (two-crop rotation— maize/barley)	0–20	3612.7 a	2380.1 a	1070.1 ab	1136.5 a	3.307 a	3.72 a	1.75 a	1.63 a	0.375 ab	0.384 a	0.196 a	0.175 ab
	20–40	3176.0 a	2048.8 a	1062.5 b	1115.5 a	4.019 a	4.02 a	1.76 a	1.57 ab	0.442 a	0.402 a	0.198 a	0.182 a
	40–60	861.22 cd	873.8 d	661.6 de	786.0 bc	1.344 d	1.30 fg	1.05 d	0.94 e	0.151 e	0.142 e	0.115 d	0.104 cd
<b>A</b>		$7.24 \times 10^{-11}$	$6.95 \times 10^{-14}$	$<2 \times 10^{-16}$	$2.49 \times 10^{-16}$	$6.09 \times 10^{-5}$	$2.21 \times 10^{-7}$	$7.73 \times 10^{-5}$	0.514	$3.17 \times 10^{-5}$	$5.66 \times 10^{-8}$	$9.34 \times 10^{-6}$	0.00718
<b>** p value B</b>		0.00131	$4.21 \times 10^{-7}$	$1.02 \times 10^{-11}$	$4.04 \times 10^{-9}$	0.000114	$1.99 \times 10^{-9}$	$<2 \times 10^{-16}$	$9.99 \times 10^{-15}$	0.000192	$3.92 \times 10^{-9}$	$7.63 \times 10^{-16}$	$<2 \times 10^{-16}$
<b>AxB</b>		$2.94 \times 10^{-5}$	$4.53 \times 10^{-5}$	0.00411	0.126	0.0273	0.00117	$2.32 \times 10^{-5}$	0.625	0.0288	0.000392	0.000694	0.83721

\* LSD Fisher test: significance at the 0.05 F level of probability. Factor levels marked with the same letter do not differ; \*\* The effect was significant for p values less than 0.05.

Statistically, the highest values of TOC and N content are observed in fractions  $>2000$  and  $250\text{--}2000$   $\mu\text{m}$  compared to other fractions. These values are most pronounced in the treatments V NPK + manure and IV NPK + manure, as well as in the treatment III NPK + manure maize remains, particularly for the fraction  $250\text{--}2000$   $\mu\text{m}$  in relation to all other tested varieties. These differences are not observed in other treatments and are consistent across all investigated treatments for the depth of  $40\text{--}60$  cm. In the research [100], using soil physical fractionation and  $\delta^{13}\text{C}$ , suggest that the TOC content in soil aggregates, where organic fertilizer was applied, decreases proportionally with particle size. Work of [95], based on a multi-year experiment with NPK fertilizer and manure ( $20\text{ t ha}^{-1}\text{ year}^{-1}$ ), argued that mineral fertilizers, unlike manure, do not impact the increase in C content in microaggregates, aligning with our results obtained from treatments without the use of manure (Table 4). In addition to its pronounced impact on carbon content, the formation of soil aggregates combined with other soil properties significantly influences the sorption and bioavailability of phosphorus in the soil [101–103]. Our research reveals that the phosphorus content in the treatments and specific fractions of the aggregate closely mirrors the organic carbon content (Table 3). The distribution of phosphorus and its forms concerning aggregate fractions may vary based on the type and application method of fertilizer, as well as agricultural management in land cultivation [83,92,104]. Regarding phosphorus supply, treatments utilizing manure stand out in nearly all tested structural aggregates, with the V treatment characterized by the statistically highest values across different aggregate sizes and soil depths. Similar findings were reported by [83], who noted that the content of total phosphorus and carbon increases with the application of manure.

It is crucial to note that, similar to organic carbon and nitrogen, phosphorus content values differ primarily in the first two test depths ( $0\text{--}20$  and  $20\text{--}40$  cm), corresponding to the zone of soil cultivation and nutrient deposition. At a soil depth of  $40\text{--}60$  cm, the absence of statistical significance in structural aggregates is characteristic, as observed with most examined parameters. The observed distribution pattern with depth aligns with expectations, considering that the increase in the content of TOC, N, and P due to manure application is significant in the arable soil layer [83,105–107]. The lack of statistically significant differences in the control treatment, both in terms of different fractions and soil depth, can be attributed to the stabilization or state of equilibrium of conditions for aggregate formation as mentioned above.

### 3.4. Principal Component Analysis and Correlation

To enhance our comprehension of soil characteristics, particularly focusing on different forms of phosphorus, across the examined treatments, investigated depths, and varying aggregate sizes with respect to their content of  $P_T$ , TOC, and  $N_T$ , we conducted an evaluation of these variables using principal component analysis (PCA) and factor analysis. One potential use of PCA is the examination of alternative soil amendment effects on soil's chemical and biological properties [108].

The first two principal components (PCs), each with an eigenvalue greater than 1, had the most significant influence on the variation in investigated soil properties and were consequently utilized for further interpretation of the results. PC1 accounted for 51.07%, 51.25%, and 26.35% of the soil variation in the three analyzed soil layers (Table 5, Figure 1). The results exhibited significant correlations within PC1 for depths of  $0\text{--}20$  and  $20\text{--}40$  cm, notably between soil factors such as  $P_T$ ,  $P_A$ , TOC,  $N_T$ , clay content, alkaline phosphatase, and the content of  $P_T$ , carbon, and nitrogen in the majority of aggregates, except for the content of TOC and  $N_T$  in aggregate sizes  $<53$   $\mu\text{m}$ . Additionally, pronounced correlations within PC1 at the same depths were observed for labile P (labile P and biomass P), Fulvic acid  $P_o$ , and resistant  $P_o$  (Table 5). PC1, with the highest total variance, emphasized the importance of its parameters in assessing variations in different treatments, phosphorus organic forms, and nutrient content in different aggregate sizes. However, PC2 also provided valuable insights, describing additional gradients in the dataset, with total variance ranging from 11.29% to 16.21% across depths. PC2 was mainly correlated with variables

related to aggregates size >2000  $\mu\text{m}$ , acid phosphatase, and soil pH. Furthermore, ref. [31] confirm that principal component analysis is an effective method for describing, illustrating, and evaluating soil chemical parameters in various fertilization systems, noting that the application of manure and its combinations with chemical fertilizers results in higher soil electrical conductivity (EC), cation exchange capacity (CEC), TOC, total nitrogen ( $\text{T}_\text{N}$ ), and soil enzyme activity. Similarly, [109] specify spatial separation through PCA of manured treatments in all studied soils in relation to all forms of phosphorus and soil organic carbon (SOC) content, which aligns with our findings.

The scatter plots of the first two PCs illustrate the positions of the analyzed treatments and variables in the majority of soil properties, indicating spatial separation between treatments. The scatter plots for the 0–20 and 20–40 cm soil layers exhibit a similar pattern. In contrast, at the 40–60 cm soil depth, clear differentiation between the analyzed treatments is not evident (Figure 1). PC1 facilitated the distinction between the treatments IV and V, with other treatments primarily based on different forms of phosphorus, enzymatic activities, and the distribution of carbon, nitrogen, and phosphorus in soil aggregates. PCA indicated that manured soils were distinct from soils without manure application, underscoring the importance of manure application in enhancing soil nutrients and enzyme activities, which is also confirmed by the research findings of [31,110].

The abundances of total and available P, alkaline phosphatase activity, TOC,  $\text{N}_\text{T}$ , and available K were strongly positively correlated with almost all forms of mineral phosphorous and the majority of organic forms (labile P, biomass P, Fulvic acid Po, and resistant Po), whereas no obvious correlation was found with forms Fulvic and Humic Po (Figure 2). The similarity in the relationship between total P and the fraction of organic P was also observed by [59]. A negative correlation was observed for the variables clay, pH, and aggregate size >2000  $\mu\text{m}$  and 250–2000  $\mu\text{m}$  with both mineral and organic forms of P (Figure 2). The correlation patterns observed in the 0–20 and 20–40 cm soil layers were consistent, whereas the depth of 40–60 cm did not exhibit similarly pronounced correlations.

**Table 5.** Principal component loadings for soil properties and P fractionation in soil samples.

Depth (cm)	PC/Factor 1			PC/Factor 2		
	0–20	20–40	40–60	0–20	20–40	40–60
$\text{P}_\text{T}$	* <b>−0.95</b>	<b>−0.91</b>	−0.36	−0.12	−0.14	−0.40
$\text{P}_\text{A}$	<b>−0.95</b>	<b>−0.91</b>	−0.22	0.03	0.08	−0.68
>2000 $\mu\text{m}$	0.23	0.20	−0.53	<b>0.85</b>	<b>0.78</b>	0.48
250–2000 $\mu\text{m}$	0.55	0.65	−0.55	0.56	−0.24	0.34
53–250 $\mu\text{m}$	−0.17	−0.08	0.31	−0.48	−0.07	−0.20
<53 $\mu\text{m}$	−0.48	−0.62	0.53	−0.44	0.07	−0.43
Pt in >2000 $\mu\text{m}$	<b>−0.84</b>	<b>−0.88</b>	−0.48	0.04	−0.11	−0.67
Pt in 250–2000 $\mu\text{m}$	<b>−0.88</b>	<b>−0.95</b>	−0.21	0.16	0.05	<b>−0.79</b>
Pt in 53–250 $\mu\text{m}$	<b>0.83</b>	<b>−0.77</b>	0.61	0.42	0.22	−0.40
Pt in % <53 $\mu\text{m}$	<b>−0.81</b>	<b>−0.80</b>	−0.49	0.40	0.40	<b>−0.71</b>
TOC in >2000 $\mu\text{m}$	<b>−0.85</b>	<b>−0.79</b>	−0.63	−0.09	−0.24	−0.17
TOC in 250–2000 $\mu\text{m}$	<b>−0.90</b>	<b>−0.88</b>	−0.62	−0.07	−0.14	−0.20
TOC in 53–250 $\mu\text{m}$	<b>−0.85</b>	<b>−0.90</b>	−0.40	0.24	0.15	0.33
TOC in <53 $\mu\text{m}$	−0.36	−0.58	−0.21	0.32	0.03	0.09
$\text{N}_\text{T}$ in >2000 $\mu\text{m}$	<b>−0.90</b>	<b>−0.79</b>	−0.33	−0.07	−0.21	−0.33
$\text{N}_\text{T}$ in 250–2000 $\mu\text{m}$	<b>−0.87</b>	<b>−0.91</b>	−0.59	0.16	−0.13	−0.24
$\text{N}_\text{T}$ in 53–250 $\mu\text{m}$	<b>−0.81</b>	<b>−0.87</b>	−0.52	0.22	0.14	0.09
$\text{N}_\text{T}$ in <53 $\mu\text{m}$	−0.46	−0.61	−0.70	0.28	0.07	−0.09
Clay	<b>0.73</b>	<b>0.73</b>	−0.50	0.49	0.60	0.04
Alk. Phos.	<b>−0.75</b>	<b>−0.71</b>	−0.54	−0.09	−0.22	0.42
Acid Phos.	−0.27	−0.22	<b>−0.70</b>	<b>0.82</b>	<b>0.74</b>	0.02
TOC %	<b>−0.82</b>	<b>−0.91</b>	<b>−0.83</b>	−0.02	−0.05	0.31
$\text{N}_\text{T}$	<b>−0.82</b>	−0.38	<b>−0.82</b>	−0.02	0.03	0.21

Table 5. Cont.

Depth (cm)	PC/Factor 1			PC/Factor 2		
	0–20	20–40	40–60	0–20	20–40	40–60
pH in KCl	−0.28	−0.20	0.36	<b>−0.83</b>	<b>−0.82</b>	−0.19
OM	−0.50	<b>−0.71</b>	−0.56	−0.01	0.05	0.39
Labil Po	<b>−0.74</b>	<b>−0.83</b>	0.40	0.37	0.08	−0.29
Biomass Po	<b>−0.85</b>	<b>−0.87</b>	0.18	0.03	0.05	−0.25
Mod. Lab. Po	<b>−0.90</b>	<b>−0.87</b>	−0.43	0.16	0.18	−0.64
Fulv. Po	−0.37	−0.41	−0.58	0.05	0.29	0.11
Humic Po	−0.51	−0.25	−0.62	−0.39	<b>−0.71</b>	0.14
Rezist. Po	<b>−0.77</b>	−0.72	0.13	0.02	0.16	<b>−0.78</b>
<b>Total Variability (%)</b>	<b>51.07</b>	<b>51.25</b>	<b>26.35</b>	<b>13.03</b>	<b>11.29</b>	<b>16.76</b>

\* Values in bold indicate significance and provide information about the contribution of variables to the components.

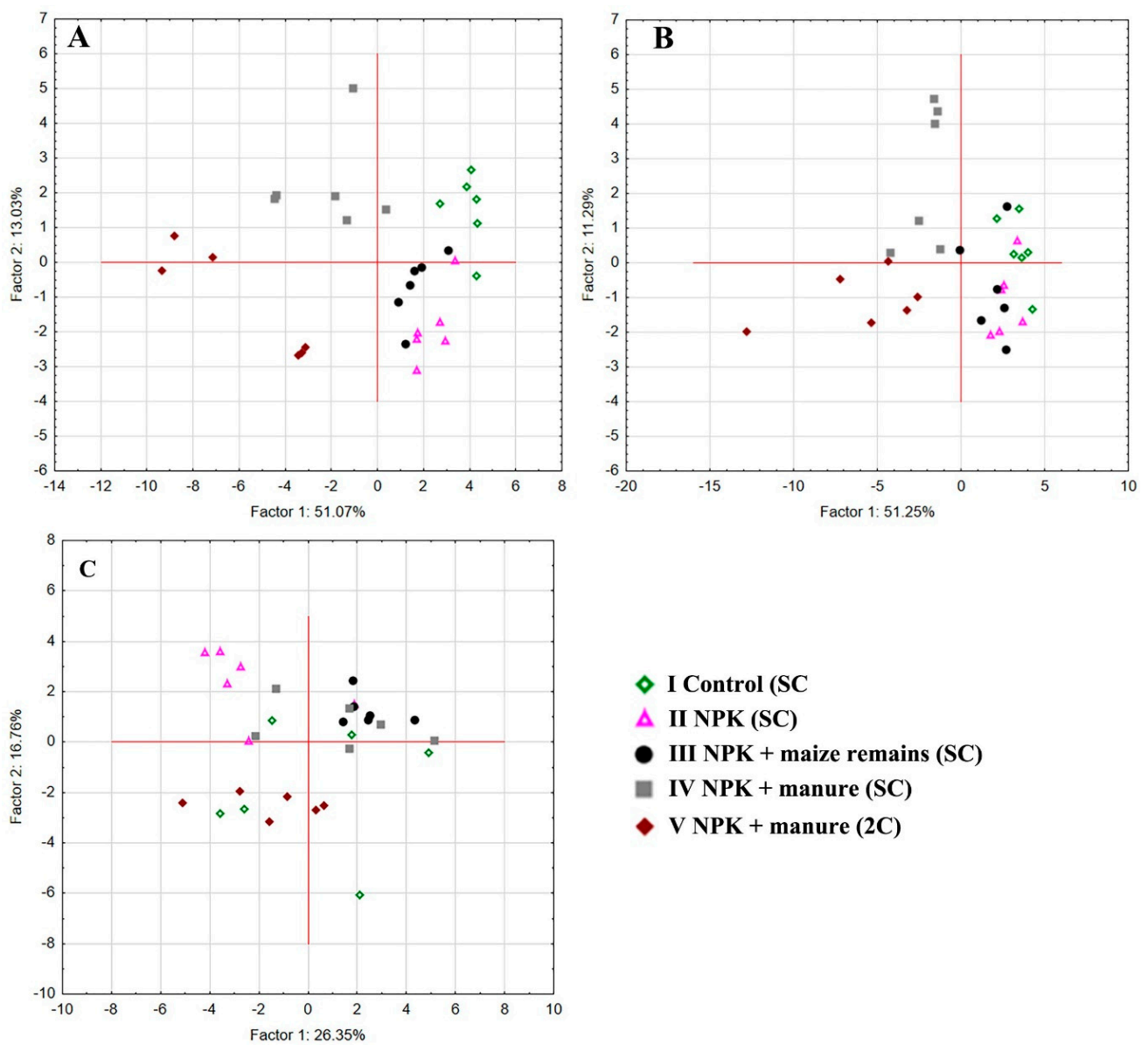
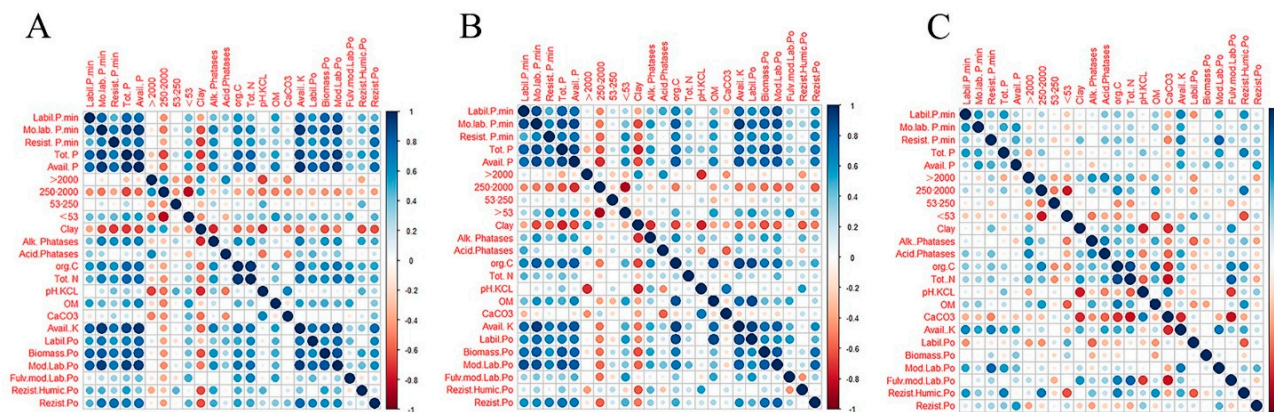


Figure 1. Separation of treatments by principal component analysis for three soil depths (0–20 cm (A); 20–40 cm (B); 40–60 cm (C)).



**Figure 2.** Relationship between PT, PA, NT, TOC, clay content, pH, alkaline and acid phosphatase, CaCO<sub>3</sub>, organic and mineral phosphorus forms, size of soil aggregates, and concentration of TOC, N, and P in soil aggregates for depths of 0–20 cm (A), 20–40 cm (B), and 40–60 cm (C).

#### 4. Conclusions

After 45 years of continuous application of both mineral fertilizers and organic amendments, our findings lead us to the following conclusions:

- The variations in fertilization regimes and soil depths and their interactions significantly influenced phosphorus organic fractions and the content of carbon, nitrogen, and phosphorus within aggregates. Contrary to expectations, the distribution of aggregate size classes remained mainly unaffected by these regimes, with the domination of small aggregates observed.
- The treatments with added manure notably increased all forms of phosphorus, particularly labile and moderately labile organic forms, enriching the nutrient deposition zone. Statistical analysis identified phosphorus accumulation and distribution of its organic forms as the primary distinctions among treatments.
- Other soil properties, such as total organic carbon, total nitrogen, clay content, and phosphatase activity, also significantly influenced differences among treatments across various aggregate sizes.
- Understanding the dynamics of organic phosphorus in different cropping systems requires analyzing sensitive parameters like labile and moderately labile organic P forms, microbial biomass, and phosphatase activity.
- Combined application of organic amendments and mineral fertilizers appears to be an effective agronomic practice for enhancing soil fertility and phosphorus status in black soils.
- Future research should address the effects of more different fertilization treatments and sources of organic amendments on phosphorus forms and cycling.

**Author Contributions:** Conceptualization, S.M., J.N. and S.Š.; methodology, S.M. and T.Z.; validation, J.N. and J.V.; formal analysis, S.M. and D.M.; investigation, S.M., J.V. and M.Ž.; resources, S.J.; writing—original draft preparation, S.M.; writing—review and editing, J.N., J.V., T.Z., S.J., M.Ž., S.Š. and D.M.; visualization, J.N. and D.M.; supervision, S.Š.; project administration, S.M.; funding acquisition, S.J. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was financially supported by the Provincial Secretariat for Higher Education and Scientific Research of Autonomous Province of Vojvodina (project title: Environmental DNA—biomarker of soil quality in Vojvodina; grant no. 142-451-3478/2023-01/2). This study was also supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant number: 451-03-66/2024-03/200032.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author due to the legal reasons.

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

## References

1. Azam, H.M.; Alam, S.T.; Hasan, M.; Yameogo, D.D.S.; Kannan, A.D.; Rahman, A.; Kwon, M.J. Phosphorous in the environment: Characteristics with distribution and effects, removal mechanisms, treatment technologies, and factors affecting recovery as minerals in natural and engineered systems. *Environ. Sci. Pollut. Res.* **2019**, *26*, 20183–20207. [CrossRef] [PubMed]
2. Smil, V. Phosphorus in the environment: Natural flows and human interferences. *Annu. Rev. Energy Environ.* **2000**, *25*, 53–88. [CrossRef]
3. Steen, I. Phosphorus availability in the 21st Century: Management of a nonrenewable resource. *Phosphorus Potassium* **1998**, *217*, 25–31.
4. Shu, L.; Schneider, P.; Jegatheesan, V.; Johnson, J. An economic evaluation of phosphorus recovery as struvite from digester supernatant. *Bioresour. Technol.* **2006**, *97*, 2211–2216. [CrossRef] [PubMed]
5. Johnston, A.; Steen, I. *Understanding Phosphorus and Its Use in Agriculture*; European Fertilizer Manufacturers Association: Brussels, Belgium, 2002.
6. Khan, M.S.; Zaidi, A.; Wani, P.A. Role of phosphate-solubilizing microorganisms in sustainable agriculture—A review. *Agron. Sustain. Dev.* **2007**, *27*, 29–43. [CrossRef]
7. James, H. Chemical Characterization of Dissolved Organic Matter: Competitive Effects on Phosphorus Sorption to Minerals. Mater's Thesis, 2006; p. 1135. Available online: <https://digitalcommons.library.umaine.edu/etd/1135> (accessed on 30 April 2024).
8. Wang, X.; Yost, R.; Linquist, B. Soil Aggregate Size Affects Phosphorus Desorption from Highly Weathered Soils and Plant Growth. *Soil Sci. Soc. Am. J.* **2001**, *65*, 139–146. [CrossRef]
9. Kuo, S. Phosphorus. In *Methods of soil Analysis Part 3*; Chemical Method No.5. 869–919; Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., Eds.; Soil Science Society of America, American Society of Agronomy: Madison, WI, USA, 1996.
10. Bowman, R.A.; Cole, C.V. An exploratory method for fractionation of organic phosphorus from grassland soils. *Soil Sci.* **1978**, *125*, 95–101. [CrossRef]
11. Hedley, M.J.; Stewart, J.W.B.; Chauhan, B.S. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Sci. Soc. Am. J.* **1982**, *46*, 970–976. [CrossRef]
12. Sharpley, A.N.; Smith, S.J. Fractionation of Inorganic and Organic Phosphorus in Virgin and Cultivated Soils. *Soil Sci. Soc. Am. J.* **1985**, *49*, 127–130. [CrossRef]
13. Ivanoff, D.B.; Reddy, K.R.; Robinson, S. Chemical fractionation of organic phosphorus in selected histosols1. *Soil Sci.* **1998**, *163*, 36–45. [CrossRef]
14. Kovar, J.L.; Pierzynski, G.M. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*, 2nd ed.; Southern Coop Series Bull No. 408; Virginia Tech University: Blacksburg, VA, USA, 2009; pp. 19–25.
15. Wright, A.L. Phosphorus sequestration in soil aggregates after long-term tillage and cropping. *Soil Tillage Res.* **2009**, *103*, 406–411. [CrossRef]
16. Jastrow, J.D. Soil aggregate formation and the accrual of particulate and mineral associated organic matter. *Soil Biol. Biochem.* **1995**, *28*, 665–676. [CrossRef]
17. Six, J.; Elliott, E.; Paustian, K.; Doran, J.W. Aggregation and Soil Organic Matter Accumulation in Cultivated and Native Grassland Soils. *Soil Sci. Soc. Am. J.* **1998**, *62*, 1367–1377. [CrossRef]
18. Six, J.; Elliott, E.T.; Paustian, K. Aggregate and Soil Organic Matter Dynamics under Conventional and No-Tillage Systems. *Soil Sci. Soc. Am. J.* **1999**, *63*, 1350–1358. [CrossRef]
19. Six, J.; Paustian, K.; Elliott, T.E.; Combrink, C. Soil structure and Organic matter: I. Distribution of aggregate size classes and aggregate-associated carbon. *Soil Sci. Soc. Am. J.* **2000**, *64*, 681–689. [CrossRef]
20. Wright, A.L.; Hons, F.M. Tillage impacts on soil aggregation and carbon and nitrogen sequestration under wheat cropping sequences. *Soil Tillage Res.* **2005**, *84*, 67–75. [CrossRef]
21. Dexter, A.R. Physical properties of tilled soil. *Soil Tillage Res.* **1997**, *43*, 41–63. [CrossRef]
22. Hillel, D. *Environmental Soil Physics*; Academic Press: Cambridge, MA, USA; Elsevier: London, UK, 1998; pp. 173–198.
23. Six, J.; Elliott, E.T.; Paustian, K. Macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biol. Biochem.* **2000**, *32*, 2099–2103. [CrossRef]
24. Shi, P.; Arter, C.; Liu, X.; Keller, M.; Schulin, R. Soil aggregate stability and size-selective sediment transport with surface runoff as affected by organic residue amendment. *Sci. Total. Environ.* **2017**, *607–608*, 95–102. [CrossRef]
25. Lal, R. Soil degradation by erosion. *Land Degrad. Dev.* **2001**, *12*, 519–539. [CrossRef]
26. Pagliali, M.; Vignozzi, N.; Pellegrini, S. Soil structure and effect of management practices. *Soil Tillage Res.* **2004**, *79*, 131–143. [CrossRef]
27. Zhang, P.; Wei, T.; Jia, Z.K.; Han, Q.F.; Ren, X.L. Soil aggregate and crop yield changes with different rates of straw incorporation in semiarid areas of northwest China. *Geoderma* **2014**, *230*, 41–49. [CrossRef]
28. Toosi, E.R.; Kravchenko, A.N.; Mao, J.; Quigley, M.Y.; Rivers, M.L. Effects of management and pore characteristics on organic matter composition of macroaggregates: Evidence from characterization of organic matter and imaging. *Eur. J. Soil Sci.* **2017**, *68*, 200–211. [CrossRef]



29. Singh, G.; Jalota, S.; Singh, Y. Manuring and residue management effects on physical properties of a soil under the rice–wheat system in Punjab, India. *Soil Tillage Res.* **2007**, *94*, 229–238. [[CrossRef](#)]
30. Huang, S.; Peng, X.X.; Huang, Q.R.; Zhang, W.J. Soil aggregation and organic carbon fractions affected by long-term fertilization in a red soil of subtropical China. *Geoderma* **2010**, *154*, 364–369. [[CrossRef](#)]
31. Zhang, L.; Chen, W.; Burger, M.; Yang, L.; Gong, P.; Wu, Z. Changes in Soil Carbon and Enzyme Activity As a Result of Different Long-Term Fertilization Regimes in a Greenhouse Field. *PLoS ONE* **2015**, *10*, e0118371. [[CrossRef](#)] [[PubMed](#)]
32. Cai, A.; Xu, M.; Wang, B.; Zhang, W.; Liang, G.; Hou, E.; Luo, Y. Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil Tillage Res.* **2019**, *189*, 168–175. [[CrossRef](#)]
33. Abiven, S.; Menasseri, S.; Angers, D.A.; Leterme, P. Dynamics of aggregate stability and biological binding agents during decomposition of organic materials. *Eur. J. Soil Sci.* **2007**, *58*, 239–247. [[CrossRef](#)]
34. Xu, M.; Lou, Y.; Sun, X.; Wang, W.; Baniyamuddin, M.; Zhao, K. Soil organic carbon active fractions as early indicators for total carbon change under straw incorporation. *Biol. Fertil. Soils* **2011**, *47*, 745–752. [[CrossRef](#)]
35. Tirol-Padre, A.; Ladha, J.K. Assessing the reliability of permanganate-oxidizable carbon as an index of soil labile carbon. *Soil Sci. Soc. Am. J.* **2004**, *68*, 969–978.
36. Khan, A.; Guo, S.; Rui, W.; He, B.; Li, T.; Mahmood, U. The Impact of Long-term Phosphorus Fertilization on Soil Aggregation and Aggregate-Associated P Fractions in Wheat-Broomcorn Millet/Pea Cropping Systems. *J. Soil Sci. Plant Nutr.* **2023**, *23*, 2755–2769. [[CrossRef](#)]
37. Rengel, Z. *Nutrient Cycling in Terrestrial Ecosystems*; Springer: Berlin/Heidelberg, Germany, 2007; Volume 10, pp. 159–182.
38. Škorić, A.; Filipovski, G.; Ćirić, M. *Soil Classification of Yugoslavia*; Academy of Sciences and Arts of Bosnia and Herzegovina, Special Publications: Sarajevo, Bosnia and Herzegovina, 1985; Volume LXXXVIII, pp. 29–69.
39. Milić, S.; Ninkov, J.; Zeremski, T.; Latković, D.; Šeremešić, S.; Radovanović, V.; Žarković, B. Soil fertility and phosphorus fractions in a calcareous chernozem after a long-term field experiment. *Geoderma* **2018**, *339*, 9–19. [[CrossRef](#)]
40. *ISO 11464:2006*; Soil Quality—Pretreatment of Samples for Physico-Chemical Analysis. International Organization for Standardization: Geneva, Switzerland, 2006.
41. *ISO 10390:2021*; Soil Quality—Soil, Treated Biowaste and Sludge—Determination of pH. International Organization for Standardization: Geneva, Switzerland, 2021.
42. U.S. EPA. *Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry*; Revision 4.4; U.S. EPA: Cincinnati, OH, USA, 1994.
43. Egner, H.; Riehm, H.; Domingo, W.R. Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Boden, II: Chemische Extraktionsmethoden zu Phosphor und Kaliumbestimmung. *K. Landbrukshögskolans Ann.* **1960**, *26*, 199–215.
44. Elliott, E.; Cambardella, C. Physical separation of soil organic matter. *Agric. Ecosyst. Environ.* **1991**, *34*, 407–419. [[CrossRef](#)]
45. Cambardella, C.A.; Elliott, E.T. Carbon and Nitrogen Dynamics of Soil Organic Matter Fractions from Cultivated Grassland Soils. *Soil Sci. Soc. Am. J.* **1994**, *58*, 123–130. [[CrossRef](#)]
46. *SRPS ISO 10694:2005*; Soil Quality—Determination of Organic and Total Carbon after Dry Combustion (Elementary Analysis). Institute for standardization of Serbia: Belgrade, Serbia, 2005.
47. Tabatabai, M.A.; Bremner, J.M. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.* **1969**, *1*, 301–307. [[CrossRef](#)]
48. Microsoft Corporation. Microsoft Excel. 2013. Available online: <https://office.microsoft.com/excel> (accessed on 30 April 2024).
49. Statistica 14, TIBCO Software Inc. Data Science Workbench, Version 14. 2020. Available online: <http://tibco.com> (accessed on 30 April 2024).
50. Manojlović, S.; Ubavić, M.; Bogdanović, D. Current problems of the use of fertilizers with special reference to the possibility of soil pollution and proposals for solving them, through the introduction and functioning of the control system of soil fertility and the use of fertilizers. *Agrochemistry* **1988**, 393–442.
51. Blagojević, S.; Žarković, B. The solubility diagram as an important indicator of the phosphorus regime in a calcareous chernozem. *Zemljište I Biljka* **1997**, *46*, 25–32.
52. Lee, C.H.; Lee, I.B.; Kim, P.J. Effects of long-term fertilization on organic phosphorus fraction in paddy soil. *Soil Sci. Plant Nutr.* **2004**, *50*, 485–491. [[CrossRef](#)]
53. Liu, E.; Yan, C.; Mei, X.; He, W.; Bing, S.H.; Ding, L.; Liu, Q.; Liu, S.; Fan, T. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma* **2010**, *158*, 173–180. [[CrossRef](#)]
54. Horta, C.; Roboredo, M.; Carneiro, J.P.; Duarte, A.C.; Torrent, J.; Sharples, A. Organic amendments as a source of phosphorus: Agronomic and environmental impact of different animal manures applied to an acid soil. *Arch. Agron. Soil Sci.* **2017**, *64*, 257–271. [[CrossRef](#)]
55. Lu, X.; Mahdi, A.-K.; Han, X.-Z.; Chen, X.; Yan, J.; Biswas, A.; Zou, W.-X. Long-term application of fertilizer and manures affect P fractions in Mollisol. *Sci. Rep.* **2020**, *10*, 14793. [[CrossRef](#)] [[PubMed](#)]
56. Schliephake, W.; Garz, J.; Stumpe, H. Undispensability and limits of long-term fertilization experiments—A view of the experimental field in Halle. *Arch. Agron. Soil Sci.* **1997**, *42*, 319–334. (In German) [[CrossRef](#)]
57. Waldrip, H.M.; He, Z.; Griffin, T.S. Effects of Organic Dairy Manure on Soil Phosphatase Activity, Available Soil Phosphorus, and Growth of Sorghum-Sudangrass. *Soil Sci.* **2012**, *177*, 629–637. [[CrossRef](#)]

58. Omara, P.; Macnack, N.; Aula, L.; Raun, B. Effect of long-term beef manure application on soil test phosphorus, organic carbon, and winter wheat yield. *J. Plant Nutr.* **2017**, *40*, 1143–1151. [[CrossRef](#)]
59. Braos, L.B.; da Cruz, M.C.P.; Ferreira, M.E.; Kuhnen, F. Organic phosphorus fractions in soil fertilized with cattle manure. *Revista Brasileira de Ciência do Solo* **2015**, *39*, 140–150. [[CrossRef](#)]
60. Sharpley, A.N.; McDowell, R.W.; Kleinman, P.J.A. Amounts, Forms, and Solubility of Phosphorus in Soils Receiving Manure. *Soil Sci. Soc. Am. J.* **2004**, *68*, 2048–2057. [[CrossRef](#)]
61. Reddy, D.D.; Rao, A.S.; Rupa, T. Effects of continuous use of cattle manure and fertilizer phosphorus on crop yields and soil organic phosphorus in a Vertisol. *Bioresour. Technol.* **2000**, *75*, 113–118. [[CrossRef](#)]
62. Zhang, Y.S.; Werner, W.; Scherer, H.W.; Sun, X. Effect of organic manure on organic phosphorus fractions in two paddy soils. *Biol. Fertil. Soils* **1994**, *17*, 64–68. [[CrossRef](#)]
63. Min, L.; Jing, Z.; Guangqian, W.; Haijun, Y.; Michael, J.W.; Sue, M.W. Organic phosphorus fractionation in wetland soil profiles by chemical extraction and phosphorus-31 nuclear magnetic resonance spectroscopy. *Appl. Geochem.* **2013**, *33*, 213–221.
64. Blagojević, S. The Effect of Multi-Year Application of Mineral and Organic Fertilizers on the Phosphorus Regime of Carbonate Chernozem of the Zemun field. Doctoral Dissertation, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia, 1987.
65. Sharpley, A.N.; Smith, S.J.; Stewart, B.A.; Mathers, A.C. Forms of Phosphorus in Soil Receiving Cattle Feedlot Waste. *J. Environ. Qual.* **1984**, *13*, 211–215. [[CrossRef](#)]
66. Pizzeghello, D.; Berti, A.; Nardi, S.; Morari, F. Phosphorus forms and P sorption in three alkaline soils after long-term mineral and manure applications. *Agric. Ecosyst. Environ.* **2011**, *141*, 58–66. [[CrossRef](#)]
67. Makarov, M.I.; Haumaier, L.; Zech, W. The nature and origins of diester phosphates in soils: A 31 P-NMR study. *Biol. Fertil. Soils* **2002**, *35*, 136–146. [[CrossRef](#)]
68. Perkins, R.; Underwood, G. The potential for phosphorus release across the sediment–water interface in an eutrophic reservoir dosed with ferric sulphate. *Water Res.* **2001**, *35*, 1399–1406. [[CrossRef](#)]
69. Blagojević, S.; Žarković, B. Effect of long-term fertilization on the organic phosphorus in a calcareous chernozem soil. *Zemljište I Biljka* **1998**, *47*, 1–8.
70. Zhang, J.H.; Tan, S.W.; Liu, X.Y.; Hong, F. A study on oxidation status and organic phosphorus components of organic substances in soils of different levels of fertility. *Chin. J. Soil Sci.* **1988**, *19*, 60–62.
71. Huang, M.; Xiao, H.A.; Huang, Q.Y.; Li, X.Y.; Wu, J.S. Effect of amendment of organic materials on transformation of P in red-earth soil under flood-drought cultivation. *Acta Pedol. Sin.* **2004**, *41*, 584–589.
72. Dick, W.A. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. *Soil Sci. Soc. Am. J.* **1984**, *48*, 569–574. [[CrossRef](#)]
73. Dick, R.P.; Rasmussen, P.E.; Kerle, E.A. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. *Biol. Fertil. Soils* **1988**, *6*, 159–164. [[CrossRef](#)]
74. Colvan, S.; Syers, J.; O'Donnell, A. Effect of long-term fertiliser use on acid and alkaline phosphomonoesterase and phosphodiesterase activities in managed grassland. *Biol. Fertil. Soils* **2001**, *34*, 258–263. [[CrossRef](#)]
75. Linqvist, B.A.; Ruark, M.D.; Hill, J.E. Soil order and management practices control soil phosphorus fractions in managed wetland ecosystems. *Nutr. Cycl. Agroecosyst.* **2010**, *90*, 51–62. [[CrossRef](#)]
76. Li, Y.-Y.; Yang, R.; Gao, R.; Wei, H.-A.; Chen, A.-L.; Li, Y. Effects of long-term phosphorus fertilization and straw incorporation on phosphorus fractions in subtropical paddy soil. *J. Integr. Agric.* **2015**, *14*, 365–373. [[CrossRef](#)]
77. Chen, Y.; Zhang, X.; He, H.; Xie, H.; Yan, Y.; Zhu, P.; Ren, J.; Wang, L. Carbon and nitrogen pools in different aggregates of a Chinese Mollisol as influenced by long-term fertilization. *J. Soils Sediments* **2009**, *10*, 1018–1026. [[CrossRef](#)]
78. Baldock, J.A.; Kay, B.D.; Schnitzer, M. Influence of cropping treatments on the monosaccharide content of the hydrolysates of a soil and its aggregate fractions. *Can. J. Soil Sci.* **1987**, *67*, 489–499. [[CrossRef](#)]
79. Skøien, S. Long-term effects of crop rotation, manure and straw on soil aggregation. *Nor. J. Agric. Res.* **1993**, *7*, 231–247.
80. Wortmann, C.S.; Shapiro, C.A. The effects of manure application on soil aggregation. *Nutr. Cycl. Agroecosyst.* **2008**, *80*, 173–180. [[CrossRef](#)]
81. Tripathi, R.; Nayak, A.K.; Bhattacharyya, P.; Shukla, A.K.; Shahid, M.; Raja, R.; Panda, B.B.; Mohanty, S.; Kumar, A.; Thilagam, V.K. Soil aggregation and distribution of carbon and nitrogen in different fractions after 41 years long-term fertilizer experiment in tropical rice–rice system. *Geoderma* **2014**, *213*, 280–286. [[CrossRef](#)]
82. Jiang, M.; Wang, X.; Liusui, Y.; Han, C.; Zhao, C.; Liu, H. Variation of soil aggregation and intra-aggregate carbon by long-term fertilization with aggregate formation in a grey desert soil. *CATENA* **2017**, *149*, 437–445. [[CrossRef](#)]
83. Whalen, J.K.; Chang, C. Macroaggregate Characteristics in Cultivated Soils after 25 Annual Manure Applications. *Soil Sci. Soc. Am. J.* **2002**, *66*, 1637–1647. [[CrossRef](#)]
84. Gillman, G. The influence of net charge on water dispersible clay and sorbed sulphate. *Soil Res.* **1974**, *12*, 173–176. [[CrossRef](#)]
85. Haynes, R.; Naidu, R. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: A review. *Nutr. Cycl. Agroecosyst.* **1998**, *51*, 123–137. [[CrossRef](#)]
86. Wan, W.; Li, X.; Han, S.; Wang, L.; Luo, X.; Chen, W.; Huang, Q. Soil aggregate fractionation and phosphorus fraction driven by long-term fertilization regimes affect the abundance and composition of P-cycling-related bacteria. *Soil Tillage Res.* **2020**, *196*, 104475. [[CrossRef](#)]

87. Wang, Y.; Hu, N.; Ge, T.; Kuzyakov, Y.; Wang, Z.-L.; Li, Z.; Tang, Z.; Chen, Y.; Wu, C.; Lou, Y. Soil aggregation regulates distributions of carbon, microbial community and enzyme activities after 23-year manure amendment. *Appl. Soil Ecol.* **2017**, *111*, 65–72. [[CrossRef](#)]
88. Šeremešić, S.; Ćirić, V.; Djalović, I.; Vasin, J.; Zeremski, T.; Siddique, K.H.; Farooq, M. Long-term winter wheat cropping influenced soil organic carbon pools in different aggregate fractions of Chernozem soil. *Arch. Agron. Soil Sci.* **2020**, *66*, 2055–2066. [[CrossRef](#)]
89. Guo, Z.; Zhang, L.; Yang, W.; Hua, L.; Cai, C. Aggregate Stability under Long-Term Fertilization Practices: The Case of Eroded Ultisols of South-Central China. *Sustainability* **2019**, *11*, 1169. [[CrossRef](#)]
90. Al-Kaisi, M.M.; Douelle, A.; Kwaw-Mensah, D. Soil microaggregate and macroaggregate decay over time and soil carbon change as influenced by different tillage systems. *J. Soil Water Conserv.* **2014**, *69*, 574–580. [[CrossRef](#)]
91. Fonte, S.J.; Yeboah, E.; Ofori, P.; Quansah, G.W.; Vanlauwe, B.; Six, J. Fertilizer and residue quality effects on organic matter stabilization in soil aggregates. *Soil Sci. Soc. Am. J.* **2009**, *73*, 961–966. [[CrossRef](#)]
92. Bhatnagar, V.K.; Miller, M.H. Sorption of carbon and phosphorus from liquid poultry manure by soil aggregates of differing size. *Can. J. Soil Sci.* **1985**, *65*, 467–473. [[CrossRef](#)]
93. Bhatnagar, V.K.; Miller, M.H.; Ketcheson, J.W. Reaction of fertilizer and liquid manure phosphorus with soil aggregates and sediment phosphorus enrichment. *J. Environ. Qual.* **1985**, *14*, 246–251. [[CrossRef](#)]
94. Mbagwu, J.; Piccolo, A. Carbon, nitrogen and phosphorus concentrations in aggregates of organic waste-amended soils. *Biol. Wastes* **1990**, *31*, 97–111. [[CrossRef](#)]
95. Aoyama, M.; Angers, D.A.; N'Dayegamiye, A.; Bissonnette, N. Protected organic matter in water-stable aggregates as affected by mineral fertilizer and manure applications. *Can. J. Soil Sci.* **1999**, *79*, 419–425. [[CrossRef](#)]
96. De Gryze, S.; Six, J.; Paustian, K.; Morris, S.J.; Paul, E.A.; Merckx, R. Aggregation and soil organic matter changes following possible land use changes in Michigan. *Glob. Change Biol.* **2004**, *10*, 1120–1132. [[CrossRef](#)]
97. He, Z.L.; Wilson, M.J.; Campbell, C.O.; Edwards, A.C.; Chapman, S.J. Distribution of phosphorus in soil aggregate fractions and its significance with regard to phosphorus transport in agricultural runoff. *Water Air Soil Pollut.* **1995**, *83*, 69–84. [[CrossRef](#)]
98. Wan, Y.; El-Swaify, S.A. Sediment Enrichment Mechanisms of Organic Carbon and Phosphorus in a Well-Aggregated Oxisol. *J. Environ. Qual.* **1998**, *27*, 132–138. [[CrossRef](#)]
99. Maguire, R.O.; Edward, A.C.; Wilson, M.J. Influence of cultivation on the distribution of phosphorus in three soils from NE Scotland and their aggregate size fractions. *Soil Use Manag.* **1998**, *14*, 147–153. [[CrossRef](#)]
100. Gerzabek, M.H.; Haberhauer, G.; Kirchmann, H. Soil organic matter pools and Carbon-13 Natural Abundances in Particle-Size Fractions of a Long-term Agricultural Field Experiment Receiving Organic Amendments. *Soil Sci. Soc. Am. J.* **2001**, *65*, 352–385. [[CrossRef](#)]
101. Nesper, M.; Bünemann, E.K.; Fonte, S.J.; Rao, I.M.; Velásquez, J.E.; Ramirez, B.; Hegglin, D.; Frossard, E.; Oberson, A. Pasture degradation decreases organic P content of tropical soils due to soil structural decline. *Geoderma* **2015**, *257–258*, 123–133. [[CrossRef](#)]
102. Wang, R.Z.; Creamer, C.A.; Wang, X.; He, P.; Xu, Z.W.; Jiang, Y. The effects of a 9-year nitrogen and water addition on soil aggregate phosphorus and sulfur availability in a semi-arid grassland. *Ecol. Indic.* **2016**, *61*, 806–814. [[CrossRef](#)]
103. Cui, H.; Ou, Y.; Wang, L.; Wu, H.; Yan, B.; Li, Y. Distribution and release of phosphorus fractions associated with soil aggregate structure in restored wetlands. *Chemosphere* **2019**, *223*, 319–329. [[CrossRef](#)]
104. Green, V.S.; Cavigelli, M.A.; Dao, T.H.; Flanagan, D.C. Soil physical properties and aggregate associate C, N, and P distributions in organic and conventional cropping systems. *Soil Sci.* **2005**, *170*, 822–831. [[CrossRef](#)]
105. Chang, C.; Sommerfeldt, T.G.; Entz, T. Soil chemistry after eleven annual applications of cattle feedlot manure. *J. Environ. Qual.* **1991**, *20*, 475–480. [[CrossRef](#)]
106. Chang, C.; Janzen, H.H. Long-term fate of nitrogen from annual feedlot manure applications. *J. Environ. Qual.* **1996**, *25*, 785–790. [[CrossRef](#)]
107. Miller, J.J.; Sweetland, N.J.; Chang, C. *Impact of Long-Term Manure Applications on Soil Physical Properties*; Final Tech. Report Project No. 970793; Farming for the Future Research Program: Lethbridge, AB, Canada, 1999.
108. Sena, M.M.; Frighetto, R.T.S.; Valarini, P.J.; Tokeshi, H.; Poppi, R.J. Discrimination of management effects on soil parameters by using principal component analysis: A multivariate analysis case study. *Soil Tillage Res.* **2002**, *67*, 171–181. [[CrossRef](#)]
109. Poblete-Grant, P.; Suazo-Hernández, J.; Condron, L.; Rumpel, C.; Demanet, R.; Malone, S.L.; Mora, M.d.L.L. Soil available P, soil organic carbon and aggregation as affected by long-term poultry manure application to Andisols under pastures in Southern Chile. *Geoderma Reg.* **2020**, *21*, e00271. [[CrossRef](#)]
110. Ekenler, M.; Tabatabai, M.A. Effects of liming and tillage systems on microbial biomass and glycosidases in soils. *Biol. Fertil. Soils* **2003**, *39*, 51–61. [[CrossRef](#)]

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