





No-tillage system combined with cover crops to improve phosphorus availability in the short term

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ABSTRACT: Phosphorus (P) is an essential macronutrient for plant growth, and its availability is often influenced by management systems adopted over time. Adopting management systems, such as no-tillage, combined with cover crops, can influence soil P availability through factors including soil organic matter accumulation. This study aimed to evaluate how management systems and cover crops influence different P fractions and organic matter. Furthermore, the effect of the time since the implementation of management systems on phosphorus (P) availability and soil organic matter accumulation was assessed. The study was conducted at an organic farm in Seropédica, Rio de Janeiro, Brazil. The soil was classified as Argissolo Amarelo with a sandy texture in the surface layer, corresponding to Ultisols. The experimental design was a randomized complete block design with three replications in a 2 × 6 factorial scheme, with two main plots representing the management systems (no-tillage and conventional tillage) and six subplots within each main plot: monocultures of pearl millet *Pennisetum glaucum*, sunn hemp (*Crotalaria juncea*), and jack bean (*Canavalia ensiformis*), two cover crop mixtures (100 and 50 % seed ratios of the aforementioned species), and a control subplot with spontaneous vegetation. Soil organic matter (SOM) particle size fractionation and P fractionation were analyzed at two-time points: the beginning of the experiment, in 2019, and four years later, in 2023. Four years later, the management systems increased the soluble phosphorus fraction (114-161 % across the evaluated layers) and decreased the occluded fraction (-23 to -43 %). However, within the management systems, higher values were observed for the occluded fraction and organic phosphorus, which may be correlated with the increase in soil organic matter fractions over time. Cover crops did not affect SOM accumulation and P availability. Soil management systems and time since adoption influenced phosphorus availability and soil organic matter accumulation. However, the timeframe was too short to observe the effects of cover crops.

Keywords: conservation system, cover crops, land use planning, organic carbon, phosphorus management.

INTRODUCTION

Phosphorus (P) is an essential element for biochemical processes in plants, such as photosynthesis, through the generation of chemical energy in the form of ATP and NADPH, which are indispensable for photosynthesis (Lovio-Fragoso et al., 2021). Soils in tropical regions, such as Brazil, often have low reserves of this nutrient. Furthermore, their mineral composition, predominantly comprised of iron and aluminum oxides, leads to the fixation of this nutrient within the soil, reducing its availability to crops and requiring phosphate fertilizer applications to meet crop demands. However, soil-applied P can be readily adsorbed or lost through erosion of P-containing sediments, leading to its deposition in riverbeds and subsequent water pollution (eutrophication) (George et al., 2016; Zhu et al., 2018; Santos and Medeiros, 2023).

Phosphorus adsorption can be influenced by factors such as soil texture, clay content and type, pH, and organic matter content. Soil organic matter (SOM) influences P availability through two mechanisms: by maintaining P in solution and reducing P adsorption through the occupation of adsorption sites (Pereira et al., 2021). Soil organic matter can complex iron and aluminum ions, which typically form insoluble phosphorus compounds, thus preventing precipitation and enhancing P availability to plants (Hinsinger, 2001). Furthermore, organic matter enhances soil microbial activity, promoting processes such as inorganic phosphorus solubilization, which further increases its availability (Oberson and Joner, 2005).

Adoption of conservation agriculture systems, such as no-tillage, is essential for increasing SOM levels, which can enhance P availability. Studies show that the *status* of soil P availability may be correlated with soil SOM and its different fractions (Pereira et al., 2010; Fink et al., 2016; Kawkins et al., 2022). These fractions include both labile forms, such as particulate organic carbon (POC), and more recalcitrant forms, such as mineral-associated organic carbon (MAOC). This correlation allows for the identification of how P is linked across different organic matter fractions, highlighting the importance of management systems that maintain SOM levels to ensure P availability in the soil (Spohn, 2024). Santos et al. (2011) observed an increase in SOM in the surface layers of areas after four years of no-tillage (NT) adoption. They noted that in the early years of NT management, SOM levels can increase, reaching intermediate values between those found under natural vegetation and conventional tillage.

No-tillage systems are based on three fundamental principles: maintaining soil cover, crop rotation, and minimal soil disturbance. This management system is one of the primary factors responsible for SOM accumulation (Bertol, 2016; Tiecher et al., 2020). No-tillage systems combined with the use of cover crops are an important strategy for maintaining and/or increasing SOM content, especially in soils in tropical climates, since these conditions accelerate decomposition rates and hinder SOM accumulation. Additionally, this system optimizes phosphate fertilizer applications, enhancing the longevity of P sources (Torres et al., 2014; Navarro-Pedreño et al., 2021; Pereira et al., 2021; Pavinato et al., 2022).

In Brazil, the use of cover crops to enhance P availability can reduce phosphate fertilizer imports, as the country relies heavily on international markets to meet its phosphate fertilizer demands. The Russia-Ukraine war has impacted Brazilian agriculture through rising fertilizer costs, as Russia is a major fertilizer supplier to Brazil (Sipert and Cohim, 2020; Russo et al., 2023). The demand for phosphate fertilizer imports may decline as the use of cover crops for P supply increases. Based on the above, cover crops combined with NT systems can aid P management. Furthermore, assessing soil P fractions based on their lability and recalcitrance can inform P fertilization management, thus reducing phosphate fertilizer use (Mogollón et al., 2018; Gatiboni and Condron, 2021).

The effects of NT systems on P availability can be observed over time, starting from the transition phase (5-10 years), when organic matter accumulation and soil property changes begin to occur (Alvarenga et al., 2001; Freixial et al., 2013). Rotta et al. (2015) studied an area under NT systems for 7, 11, and 16 years and observed increased P concentrations and stocks over time.

Therefore, developing soil management research that enhances P availability across different management systems is crucial. We hypothesized that cover crop diversity combined with soil management practices enhance SOM content and P availability in sandy soils. This study aimed to evaluate the influence of management systems and cover crops on different P fractions and organic matter. Additionally, we assessed the influence of time since the adoption of management systems and cover crops on P availability and SOM accumulation.

MATERIALS AND METHODS

Experimental area and design

The study was conducted at an organic farm (Sítio do Sol) in Seropédica, Rio de Janeiro, Brazil (22° 49' 20.3" S, 43° 44' 19.4" W, 26 m altitude). The organic farm is certified by ABIO (Rio de Janeiro State Association of Organic Farmers) and registered with CNPO (National Registry of Organic Producers), bearing organic certification through SisOrg (Brazilian Organic Conformity Assessment System) and undergoing regular inspections by OPAC (Participatory Organic Conformity Assessment Bodies) to maintain its certification status (Figure 1).

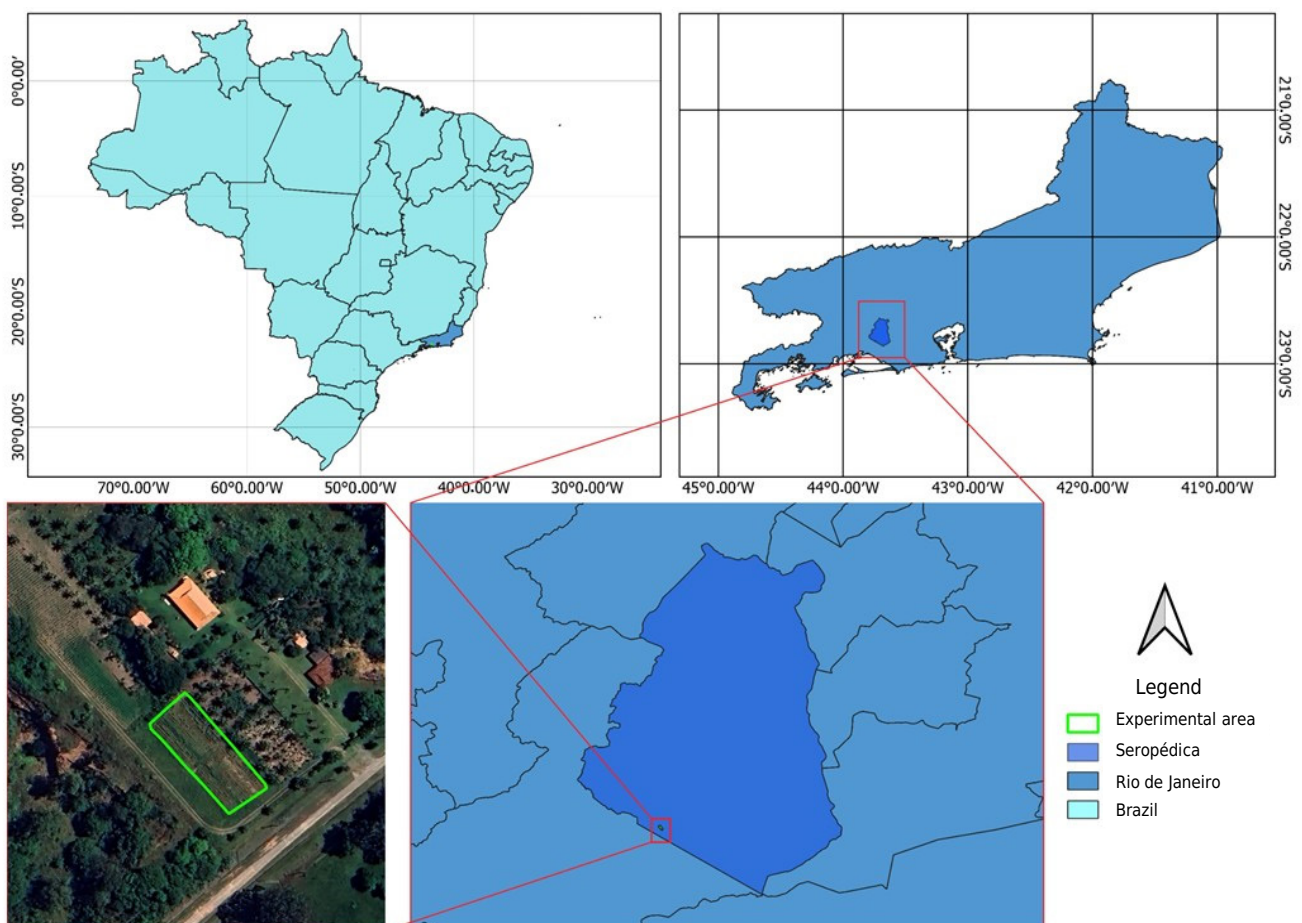


Figure 1. Location map of the area in the municipality of Seropédica - Rio de Janeiro State.

Annual accumulated precipitation is approximately 1,245 mm (Inmet, 2024), with a tropical climate classification (Aw) characterized by rainy summers and dry winters, and average temperatures around 24 °C, according to Köppen classification system (Köppen and Geiger, 1928). The soil was classified as an Argissolo Amarelo with sandy texture in the surface layer (0.00-0.20 m) (Santos et al., 2018), corresponding to Ultisols (Soil Survey Staff, 2022). Preparation of the experimental area began in 2018, with the removal of existing pasture and sowing of black oat (*Avena strigosa*) to achieve uniform field conditions. In 2019, the black oat cover crop was terminated, and the experiment with different management systems was established. The cover crop was terminated and left on the soil surface in the NT system. In the conventional tillage (CT) system, the soil was tilled, and the cover crop was incorporated. Following the implementation of these management systems, sweet corn (*Zea mays*) was sown, maintaining an annual cycle of cover crops followed by cash crops, prioritizing crop rotation.

The experiment was arranged in a randomized complete block design with three replications, using a 2 × 6 split-plot scheme. The main plots consisted of two soil management systems (no-tillage – NT, and conventional tillage – CT), and the subplots comprised six cover crop treatments: pearl millet (*Pennisetum glaucum*) (PM); sunn hemp (*Crotalaria juncea*) (SH); jack bean (*Canavalia ensiformis*) (JB); a mixture of 100 % of the recommended seeding rates of the cover crops (C1); a mixture of 50 % of the recommended seeding rates of the cover crops (C2); and spontaneous vegetation maintained in a fallow area (SV) (Figure 2).

Soil management system plots were each 144 m² (24 × 6 m). In the CT system plots, soil preparation was performed using a Brazilian-made Yanmar TC14 Super rotary tiller, equipped with rotating blades to simulate soil disturbance. In the NT system plots, the cover crops were simply mowed, and the residue was left on the surface as mulch. Within both the CT and NT treatments, subplots designated for cover crop treatments were 24 m² (6 × 4 m). Total area of all plots combined was 864 m². Phosphorus fertilization during the crop cycle consisted of thermophosphate application at a rate of 100 kg ha⁻¹, following the recommendations of the Rio de Janeiro State Fertilization and Liming Manual (Freire et al., 2013). This fertilization practice complies with Normative Instruction No. 61 of July 8, 2020, for organic production.

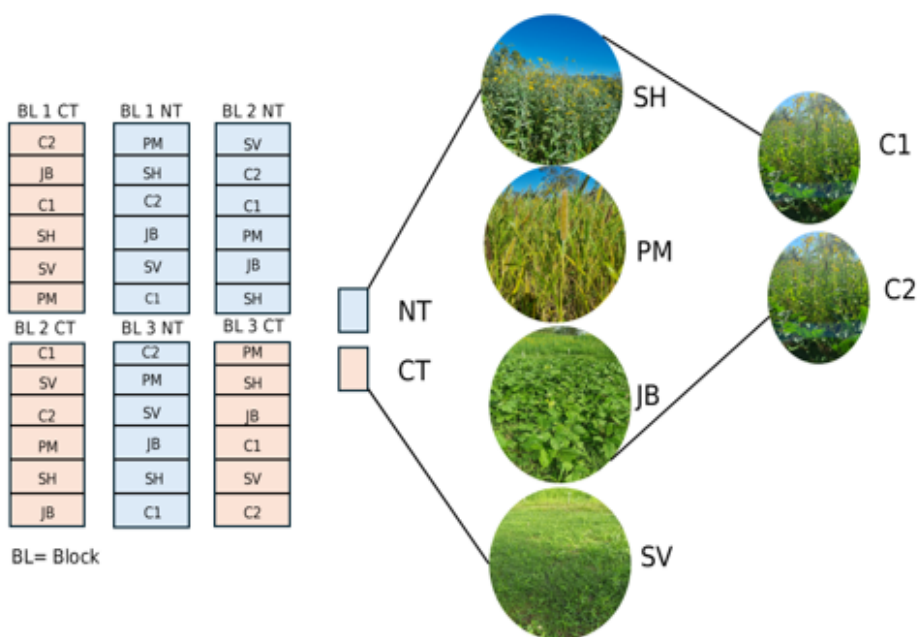


Figure 2. Layout of the treatments in the plot and sub-plot and the cover crops grown alone and in a consortium.

Laboratory analysis

Phosphorus fractionation and remaining phosphorus (P-rem) analyses were performed using samples collected at the beginning of the experiment in 2019 and compared with samples from 2023. Five individual soil samples were collected at each of the layers of 0.00-0.05 and 0.05-0.10 m, and then combined to form a composite sample, which was then air-dried, broken up, and passed through a 2 mm mesh sieve to obtain air-dried fine earth (ADFE).

Phosphorus (P) fractions were extracted following the method proposed by Gatiboni and Condron (2021). Five P fractions with varying degrees of lability and availability potential were sequentially extracted from 0.50 g of ADFE: labile P extracted with 0.01 mol L⁻¹ CaCl₂ solution (P_{SOL}); labile P extracted with Mehlich-3 solution (P_{M3}) (Mehlich, 1984); moderately labile inorganic P and autoclaved P extracted with 0.5 mol L⁻¹ NaOH solution (P_{OH} and P_{autoclave}, respectively); and moderately labile P extracted with 1 mol L⁻¹ HCl (P_{HCl}). Phosphorus concentrations in each fraction were determined colorimetrically (Murphy and Riley, 1962). Organic P extracted with NaOH (P_{OH}) was determined to be the difference between P_{autoclave} and P_{OH}. Occluded P (P_{OCL}) (non-labile), comprising highly recalcitrant Pi and Po forms not extracted by any previous extractants (CaCl₂ 0.01 mol L⁻¹, Mehlich-3, 0.5 mol L⁻¹ NaOH, and 1 mol L⁻¹ HCl), was also quantified. The P_{OCL} was determined by subtracting the T_P of the P_{SOL}, P_{M3}, P_{OH}, P_{OH}, and P_{HCl}, which was obtained through sulfuric acid digestion following the method of Tedesco et al. (1995).

P-rem levels were determined according to Alvarez and Fonseca (1990), with the modifications made by Wadt and Silva (2011). The analysis used 5 cm³ of TFSA in contact for 16 h with a solution of 0.01 mol L⁻¹ CaCl₂ containing 60 mg L⁻¹ of P. After homogenization, solid and liquid phases were separated, and P-rem concentration in the equilibrium solution was determined by the ascorbic acid method using spectrophotometry (Braga and Defelipo, 1974).

Carbon (C) content was determined by wet oxidation of organic matter with potassium dichromate (K₂Cr₂O₇) 0.167 mol L⁻¹ in sulfuric medium, followed by titration with 0.2 mol L⁻¹ aqueous ferrous ammonium sulfate (NH₄)₂Fe(SO₄)₂(6H₂O) using ferroin as an indicator, according to the method proposed by Yeomans and Bremner (1988).

The physical fractionation of organic matter, as proposed by Cambardella and Elliot (1993), separates soil carbon fractions based on particle size, distinguishing between particulate organic carbon (POC; >53 µm) and mineral-associated organic carbon (AMOC; bound to silt [2-53 µm] and clay [0-2 µm] particles). After separating the POC fraction, organic carbon was analyzed according to Yeomans and Bremner (1988), and MAOC was calculated as the difference between TOC and POC.

Statistical analysis

Sampling design comprises spatially replicated and autocorrelated plots with low within-plot variability. Therefore, a linear mixed-effects model (R packages lme4 v. 1.1.23 and lmerTest v. 3.1-2) was used to assess significant differences in P variables and SOM fractions between management systems (NT and CT) and among cover crops over time. For model construction, cropping systems, cover crops, and time since adoption were considered fixed effects, while sampling blocks were treated as random effects. Type II Wald chi-square tests and least squares mean with false discovery rate correction for multiple comparisons (R package v. 3.0-10) were used to test the significance of the estimated parameters in the models.

Principal component analysis (PCA) was performed on the evaluated properties for management systems and time using the Pearson correlation matrix, given that most cover crops showed no statistical differences in mixed models. All statistical analyses were performed using R software version 4.2.2 (R Core Team, 2022) with the Openxlsx, ExpDes.pt, and ggplot2 packages.

RESULTS

Differences were observed between the time since the adoption of management systems and the interaction between system type and year for P fractions in both evaluated soil layers. Cover crops had no significant effect (Tables 1 and 2). For the 0.00-0.05 m layer, significant differences in P_{sol} , P_{iOH} , and P_{HCl} fractions were only observed as a function of time since adoption of management systems, with increases in fraction contents (1.19 mg kg^{-1} , 0.24 mg kg^{-1} , 0.46 mg kg^{-1} , respectively) after four years (Table 1).

Table 1. Phosphorus fractions in the soil in different management systems and covers as a function of time in the 0.00-0.05 m layer, Seropédica - Rio de Janeiro State

Fractions	Sys.	Year	Layer 0.00-0.05 m							F-val.
			Cover crops							
			SV	C2	C1	PM	JB	SH	p-val.	
P _{sol} (mg kg ⁻¹)	NT	2019	3.07 B	2.34 B	3.61 B	3.94 B	2.94 B	3.61 B	ns	0.20
	CT		4.88 B	0.88 B	3.48 B	3.08 B	8.95 B	4.55 B		
	NT	2023	6.79 A	5.71 A	12.52 A	7.30 A	6.51 A	5.20 A	ns	
	CT		9.26 A	9.84 A	8.25 A	9.70 A	7.67 A	10.42 A		
P _{M3} (mg kg ⁻¹)	NT	2019	54.73 A	73.02 A	45.69 A	34.46 A	70.65 A	40.99 A	*	2.1
	CT		74.86 A	64.84 A	55.44 A	77.94 A	69.76 A	76.78 A		
	NT	2023	24.08 B	21.36 B	29.86 B	36.34 B	33.17 B	20.51 B	ns	
	CT		46.54 B	22.12 B	26.01 B	50.21 B	31.02 B	36.92 B		
Pi _{OH} (mg kg ⁻¹)	NT	2019	70.71 A	78.71 A	60.79 A	85.73 A	75.02 A	59.74 A	ns	0.17
	CT		83.28 A	76.60 A	63.69 A	89.42 A	73.97 A	67.12 A		
	NT	2023	54.20 B	49.64 B	51.13 B	56.75 B	60.36 B	43.85 B	ns	
	CT		59.92 B	52.10 B	61.94 B	61.32 B	62.02 B	59.92 B		
Po _{OH} (mg kg ⁻¹)	NT	2019	130.4Bb	135.9Bb	139.9Ba	148.9Bb	138.0Bb	140.3Bb	ns	5.10
	CT		155.3Ba	163.1Aa	137.3Bb	164.0Ba	150.0Ba	157.6Aa		
	NT	2023	151.0Ab	156.0Aa	152.2Ab	155.7Ab	151.7Ab	157.7Ab	.	
	CT		202.9Aa	147.6Bb	162.0Aa	195.1Aa	172.4Aa	150.7Ba		
P _{HCl} (mg kg ⁻¹)	NT	2019	6.98 B	5.94 B	7.32 A	12.90 A	8.56 B	4.69 B	ns	0.005
	CT		6.07 B	5.08 B	5.81 B	5.61 B	9.55 A	13.88 A		
	NT	2023	12.76 A	13.10 A	5.35 B	4.82 B	16.77 A	14.28 A	ns	
	CT		10.21 A	6.46 A	18.61 A	20.06 A	6.33 B	6.59 B		
P _{OCL} (mg kg ⁻¹)	NT	2019	410 Ab	550 Ab	540 Ab	390 Ab	490 Ab	530 Ab	**	7.70
	CT		660 Aa	650 Aa	800 Aa	560 Aa	590 Aa	640 Aa		
	NT	2023	360 B	370 B	400 B	390 B	330 B	380 B	ns	
	CT		340 B	400 B	390 B	370 B	380 B	350 B		
Prem(mg L ⁻¹)	NT	2019	36.57	34.25	33.89	33.90	31.22	36.71	ns	0.70
	CT		31.01	35.30	35.86	35.37	35.02	38.05		
	NT	2023	31.36	39.87	37.27	34.67	37.63	35.51	ns	
	CT		32.14	36.64	36.43	34.60	35.37	36.78		

Averages of different capital letters were significant between years and different lowercase letters were significant between systems. Sys: systems; NT: no-till system; CT: conventional tillage system; P_{sol} : soluble phosphorus; P_{M3} : phosphorus extracted with Mehlich-3; P_{iOH} : inorganic phosphorus; P_{oOH} : organic phosphorus; P_{HCl} : phosphorus extracted with HCl; P_{OCL} : occluded phosphorus; Prem: remaining phosphorus; val.: value; $p < 0.10$. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; $p < 0.10$.

Table 2. Phosphorus fractions in the soil in different management systems and covers as a function of time in the 0.05-0.10 m layer, Seropédica – Rio de Janeiro State

Fractions	Sys.	Year	Layer 0.05-0.10 m							F-val.
			Cover crops							
			SV	C2	C1	PM	JB	SH	p-val.	
P _{sol} (mg kg ⁻¹)	NT	2019	2.34B	2.48 B	1.88 B	2.28 B	2.48 B	2.14 B	ns	2.42
	CT		2.94 B	0.81 B	2.74 B	2.54 B	3.21 B	0.94 B		
	NT	2023	5.71 Ab	3.32 Ab	4.84 Aa	4.04 Ab	3.32 Ab	2.74 Ab	*	
	CT		6.51 Aa	4.91 Aa	4.26 Ab	5.06 Aa	6.65 Aa	7.16 Aa		
P _{M3} (mg kg ⁻¹)	NT	2019	43.01 A	37.91 A	22.08 A	30.53 A	34.37 A	37.19 A	ns	1.05
	CT		38.98 A	38.01 A	23.91 A	39.38 A	51.83 A	53.75 A		
	NT	2023	7.72 B	13.49 B	14.25 B	6.11 B	21.45 B	15.77 B	ns	
	CT		12.91 B	8.75 B	10.85 B	18.41 B	14.97 B	13.45 B		
Pi _{OH} (mg kg ⁻¹)	NT	2019	63.69 A	50.87 A	47.80 A	45.02 A	60.62 A	56.23 A	ns	1.39
	CT		63.08 A	49.11 A	42.44 A	63.96 A	63.52 A	66.59 A		
	NT	2023	38.13 B	35.77 B	35.15 B	44.69 B	41.65 B	40.95 B	ns	
	CT		34.98 B	31.46 B	39.72 B	39.54 B	37.61 B	37.08 B		
Po _{OH} (mg kg ⁻¹)	NT	2019	135.0Bb	115.5Bb	136.6Bb	112.9Bb	103.6Bb	144.1Bb	***	4.85
	CT		159.7Aa	142.2Ba	142.1Ba	152.4Ba	163.9Ba	174.5Aa		
	NT	2023	153.3 A	167.6 A	149.9 A	148.1 A	156.2 A	144.6 A	ns	
	CT		142.3 B	155.9 A	153.1 A	183.0 A	163.5 A	148.9 B		
P _{HCl} (mg kg ⁻¹)	NT	2019	5.28	15.79	4.95	4.56	5.02	5.41	ns	1.09
	CT		6.13	4.62	4.82	4.82	9.02	12.37		
	NT	2023	23.53	7.71	4.95	5.74	10.73	5.08	ns	
	CT		3.25	8.30	3.84	10.67	7.51	5.28		
P _{oCL} (mg kg ⁻¹)	NT	2019	450 Ab	650 Ab	600 Ab	400 Ab	570 Ab	420 Ab	*	6.52
	CT		610 Aa	660 Aa	740 Aa	710 Aa	710 Aa	620 Aa		
	NT	2023	440 B	320 B	370 B	360 B	450 B	260 B	ns	
	CT		370 B	360 B	380 B	390 B	400 B	400 B		
Prem(mg L ⁻¹)	NT	2019	36.57	34.25	33.90	33.90	31.22	36.71	ns	0.38
	CT		31.01	35.30	35.86	35.37	35.02	38.05		
	NT	2023	31.36	39.87	37.27	34.67	37.62	35.51	ns	
	CT		32.14	36.64	36.43	34.60	35.37	36.78		

Averages of different capital letters were significant between years and different lowercase letters were significant between systems. Sys: systems; NT: no-till system; CT: conventional tillage system; P_{sol}: soluble phosphorus; P_{M3}: phosphorus extracted with Mehlich 3; P_{iOH}: inorganic phosphorus; P_{oOH}: organic phosphorus; P_{HCl}: phosphorus extracted with HCl; P_{oCL}: occluded phosphorus; Prem: remaining phosphorus. *** p<0.001; ** p<0.01; * p<0.05; · p<0.10.

A significant interaction between the management system and time since adoption was observed only for P_{M3}, P_{oOH} and P_{oCL} fractions (Table 1). When analyzing the breakdown of the P_{M3} fraction under different management systems, statistical differences were observed only in 2019, with CT systems showing higher mean values. In 2023, no differences were observed between the management systems. The P_{M3} fraction decreased over time, with lower mean values quantified in 2023. For the P_{oOH} fraction, an interaction between the management system and time since the adoption of the management system was observed. In 2023, differences were observed between systems, with CT system generally showing higher mean values. A significant increase in this fraction was observed from the experiment installation year (2019) to the final evaluation year (2023).

Through analysis of the P_{oCL} fraction interaction breakdown, significant differences were observed in the management system in 2019, with the highest mean values recorded for the CT system (Table 1). No significant differences were observed among management systems in 2023. Regarding the effect of cover crops, the mean values of the P_{oCL} fraction were higher in 2019 compared to 2023.

Regarding remaining P (P-rem), no significant differences were observed in the interaction between management system and year, or between cover crops, in either soil layer (Tables 1 and 2). In the 0.05-0.10 m layer, the P_{M3} and P_{iOH} fractions showed significant differences only across years, with higher mean values observed in 2019, followed by a reduction after four years (Table 2). The interaction between management systems and the year was only observed for P_{sol} , P_{oOH} , and P_{oCL} fractions (Table 2). For the P_{sol} fraction, management system effect was only observed in 2023, with the highest mean values under CT system. Regarding time since adoption, P_{sol} fraction increased from 2019 to 2023 (Table 2).

For the P_{oOH} fraction, the breakdown by management system was only significant in 2019, with higher mean values under NT system, while in 2023, no differences were observed between management systems (Table 2). Analyzing the system-year interaction, a reduction in soil P_{oCL} fraction was observed, with the highest mean values quantified in the CT system in 2019. No statistical differences were observed among management systems in 2023. A general upward trend in this fraction was observed over the years. No statistical differences were observed in the P_{HCl} fraction across management systems, cover crops, or years (Table 2). The fractions decreased in the following order: $P_{oCL} > P_{oOH} > P_{iOH} > P_{M3} > P\text{-rem} > P_{HCl} > P_{sol}$. The highest values were observed for the occluded fraction and the lowest for the soluble fraction in both evaluated layers (Tables 1 and 2).

Soil organic matter fractionation revealed increased levels across different fractions in both evaluated soil layers. In the surface layer, TOC content analysis at the system level revealed higher mean values under NT compared to CT in both 2019 and 2023. The TOC content increased under the management systems over time, with peak values recorded in 2023. The distribution of the POC fraction at the system level was significant in the two years evaluated, with the highest mean values quantified in the NT system in 2019 and in the CT system in 2023. Higher POC contents were observed, with the highest mean values quantified in 2023, and a reduction of this fraction under NT.

At the 0.05-0.10 m soil layer, the TOC breakdown at the system level revealed significant differences among systems only in 2019. Overall, higher mean values were observed for the NT system; however, in 2023, no significant differences were detected between the systems. The TOC content increased from 2019 to 2023. The MAOC fraction also showed an increasing pattern in both evaluated layers (Table 3).

Regarding the percentage increase in P and SOM fractions in each management system, increases were observed across all layers for P_{sol} , P_{oOH} , TOC, POC, and MAOC fractions, while decreases were noted in P_{M3} , P_{iOH} , and P_{oCL} fractions. In the 0.0-0.05 m layer, the P_{HCl} fraction increased in both systems; however, in the 0.05-0.10 m layer, this fraction decreased in the CT system. No changes were observed in the P-rem fraction in the CT system in both evaluated layers. Conversely, in the 0.05-0.10 m layer, a reduction in P-rem was observed in the NT system. The dynamics of the different P fractions and SOM during the sampling periods are shown in table 4. The percentage variation revealed an increase in all SOM fractions and in most P fractions (Table 4).

The PCA was used to evaluate the contribution of each attribute to the management systems across years, selecting those with correlation values between $-0.50 \leq r \leq 0.50$ (moderate correlation) to form the PCAs of the management systems (Table 5). The PCAs were performed for the management systems across years and layers (Figure 3). In the 0.00-0.05 m layer during 2019, the cumulative variance contribution reached 63.3 %, with the principal axis accounting for 36.6 %. This revealed a clear distinction between management systems, where P_{sol} , P_{oCL} , P_{M3} , P_{iOH} and P_{oOH} were associated with CT, while organic matter fractions were associated with NT (Figure 3a). In 2023, the axes explained 62 % of the total variance, with the primary axis accounting for the highest contribution (41.9 %). This demonstrates system differentiation based on attribute associations, where P_{M3} , P_{iOH} , POC, and P_{oCL} were associated with CT, while P-rem, TOC, and MAOC were linked to NT (Figure 3b).

Table 3. Soil organic matter fractions in different management systems and covers over two years, Seropédica - Rio de Janeiro State

Fractions	Systems	Year	Cover crops							F-value
			SV	C2	C1	PM	JB	SH	p-value	
Layer 0.00-0.05 m										
TOC (g kg ⁻¹)	NT	2019	11.83Ba	7.25Bb	13.09Ba	10.73Ba	14.66Ba	11.81Ba	**	4.90
	CT		7.25 Bb	9.17Ba	7.50 Bb	3.85 Bb	8.29 Bb	5.95 Bb		
	NT	2023	27.10Aa	30.60Aa	30.12Aa	28.91Aa	23.73Ab	27.47Aa	***	
	CT		24.34Ab	28.07Ab	19.88Ab	28.07Ab	24.34Aa	22.4 Ab		
POC (g kg ⁻¹)	NT	2019	2.56 Ba	4.21 Ba	4.50 Ba	3.87 Ba	3.27 Bb	4.24 Ba	.	7.06
	CT		0.66 Bb	3.11 Bb	1.16 Bb	1.68 Bb	3.81 Ba	0.39 Bb		
	NT	2023	11.19Ab	10.34Aa	6.68Ab	8.30 Ab	9.92 Aa	8.68 Ab	.	
	CT		12.52Aa	6.13Ab	9.41Aa	15.12Aa	9.67Ab	14.7 Aa		
MAOC (g kg ⁻¹)	NT	2019	6.24 B	5.38 B	9.06 B	6.32 B	8.00 B	5.77 B	ns	0.75
	CT		4.16 B	6.90 B	4.83 B	5.33 B	4.81 B	4.86 B		
	NT	2023	14.74 A	18.47 A	14.96 A	18.03 A	18.20 A	13.60 A	ns	
	CT		18.65 A	15.67 A	13.44 A	16.27 A	19.88 A	19.87 A		
Layer 0.05-0.10 m										
COT (g kg ⁻¹)	NT	2019	9.13Ba	7.87Bb	11.49Ba	8.53Ba	11.31Ba	7.85 Ba	**	4.36
	CT		5.16Bb	8.20Ba	6.63Bb	6.33Bb	6.41Bb	6.53 Bb		
	NT	2023	21.76A	24.81A	20.07A	25.05A	22.16 A	18.71 A	ns	
	CT		23.37A	21.92A	19.35A	24.65A	24.73 A	24.25 A		
COp (g kg ⁻¹)	NT	2019	2.89 B	2.49 B	2.43 B	2.20 B	3.32 B	2.09 B	ns	1.79
	CT		1.00 B	1.30 B	1.80 B	1.00 B	1.60 B	1.67 B		
	NT	2023	7.03 A	6.34 A	5.11 A	7.03 A	3.96 A	5.11 A	ns	
	CT		4.73 A	6.26 A	5.92 A	8.39 A	4.85 A	4.39 A		
COam (g kg ⁻¹)	NT	2019	6.25 B	5.39 B	9.06 B	6.33 B	8.00 B	5.77 B	ns	2.08
	CT		4.17 B	6.90 B	4.83 B	5.33 B	4.81 B	4.87 B		
	NT	2023	14.74A	18.47A	14.97A	18.03A	18.21A	13.60A	ns	
	CT		18.65A	15.67A	13.44A	16.27A	19.88A	19.87A		

Averages of different capital letters were significant between years and different lower case letters were significant between systems. NT: no-till system; CT: conventional tillage system; TOC: total organic carbon; POC: particulate organic carbon; MAOC: organic carbon associated with minerals. *** p<0.001; ** p<0.01; * p<0.05; . p<0.10.

Table 4. Percentage variation of the fractions in each management system in the two layers evaluated, in the different sampling periods, Seropédica - Rio de Janeiro State

Fractions	Variation 2019-2023			
	Layer 0.00-0.05 m		Layer 0.05-0.10 m	
	NT	CT	NT	CT
	%			
P _{sol}	125	114	76	161
P _{M3}	-48	-49	-61	-67
P _{iOH}	-27	-21	-26	-37
P _{oOH}	11	11	23	1
P _{HCl}	45	48	41	-7
P _{oclu}	-23	-42	-40	-43
Prem	3	0	-2	0
TOC	138	263	136	252
POC	143	522	124	314
MAOC	120	164	140	235

NT: no-till system; CT: conventional tillage system; P_{sol}: soluble phosphorus; P_{M3}: phosphorus extracted with Mehlich-3; P_{iOH}: inorganic phosphorus; P_{oOH}: organic phosphorus; P_{HCl}: phosphorus extracted with HCl; P_{oclu}: occluded phosphorus; Prem: remaining phosphorus; TOC: total organic carbon; POC: particulate organic carbon; MAOC: organic carbon associated with minerals.

Table 5. Matrix of the principal component analysis (PCA). The relative contribution corresponds to the Pearson correlation (r) between each principal component (PC, axis) and the variables

Fractions	0.00-0.05 m		0.05-0.10 m	
	PC1	PC2	PC1	PC2
Year 2019				
P _{sol}	0.06	0.62	0.37	-0.04
P _{M3}	0.69	-0.15	0.61	0.62
Pi _{OH}	0.52	-0.63	0.47	0.65
Po _{OH}	0.51	-0.51	0.62	0.45
P _{HCl}	0.22	0.09	0.51	-0.04
P _{OCL}	0.25	0.77	0.27	0.31
Prem	0.13	0.47	-0.20	0.27
TOC	-0.86	-0.16	-0.78	0.54
POC	-0.34	0.07	-0.56	0.28
MAOC	-0.81	-0.20	-0.71	0.53
Year 2023				
P _{sol}	0.14	0.32	0.34	0.53
P _{M3}	0.54	0.52	-0.12	0.68
Pi _{OH}	0.72	0.32	-0.04	0.62
Po _{OH}	0.14	0.12	-0.03	0.70
P _{HCl}	0.47	-0.00	0.68	0.06
P _{OCL}	0.03	-0.54	0.48	0.02
Prem	-0.54	0.05	0.71	0.14
TOC	-0.55	0.70	0.84	-0.14
POC	0.79	0.22	0.35	0.45
MAOC	-0.89	0.36	0.62	-0.39

P_{sol}: soluble phosphorus; P_{M3}: phosphorus extracted with Mehlich-3; Pi_{OH}: inorganic phosphorus; Po_{OH}: organic phosphorus; P_{HCl}: phosphorus extracted with HCl; P_{OCL}: occluded phosphorus; Prem: remaining phosphorus; TOC: total organic carbon; POC: particulate organic carbon; MAOC: organic carbon associated with minerals.

In the subsurface layer (0.05-0.10 m) of the management systems in 2019, the axes explained 65.5 % of the total variance, with the principal axis showing the highest contribution at 39.6 %. The pattern observed in this layer was similar to the surface layer, with NT associated with SOM fractions and CT associated with Po_{OH}, P_{M3}, Pi_{OH}, and P_{HCl} fractions (Figure 3c). In 2023, PCA analysis did not reveal a clear separation between the systems based on the evaluated attributes (Figure 3d).

Regarding the year, PCAs revealed a clear separation of attributes along the most relevant principal axis, accounting for 36.2 and 40.2 % of the total variance in the surface and subsurface layers, respectively. In both layers, the P_{OCL}, P_{M3}, and Pi_{OH} fractions were associated with 2019, while Po_{OH}, P_{HCl}, POC, AMOC, TOC, P_{sol}, and P-rem were associated with 2023 (Figure 4a and 4b).

DISCUSSION

Management systems and time since adoption influenced the availability of P and MOS fractions. After four years of experiment implementation, the NT system, with minimal soil disturbance, cover crops, and crop rotation, increased labile phosphorus fractions (P_{sol} and Po_{OH}), which may correlate with higher TOC content and organic matter fractions (POC and MAOC), while moderately labile (Pi_{OH}, P_{M3}) and non-labile P_{OCL} fractions decreased (Dortzbach et al., 2020).

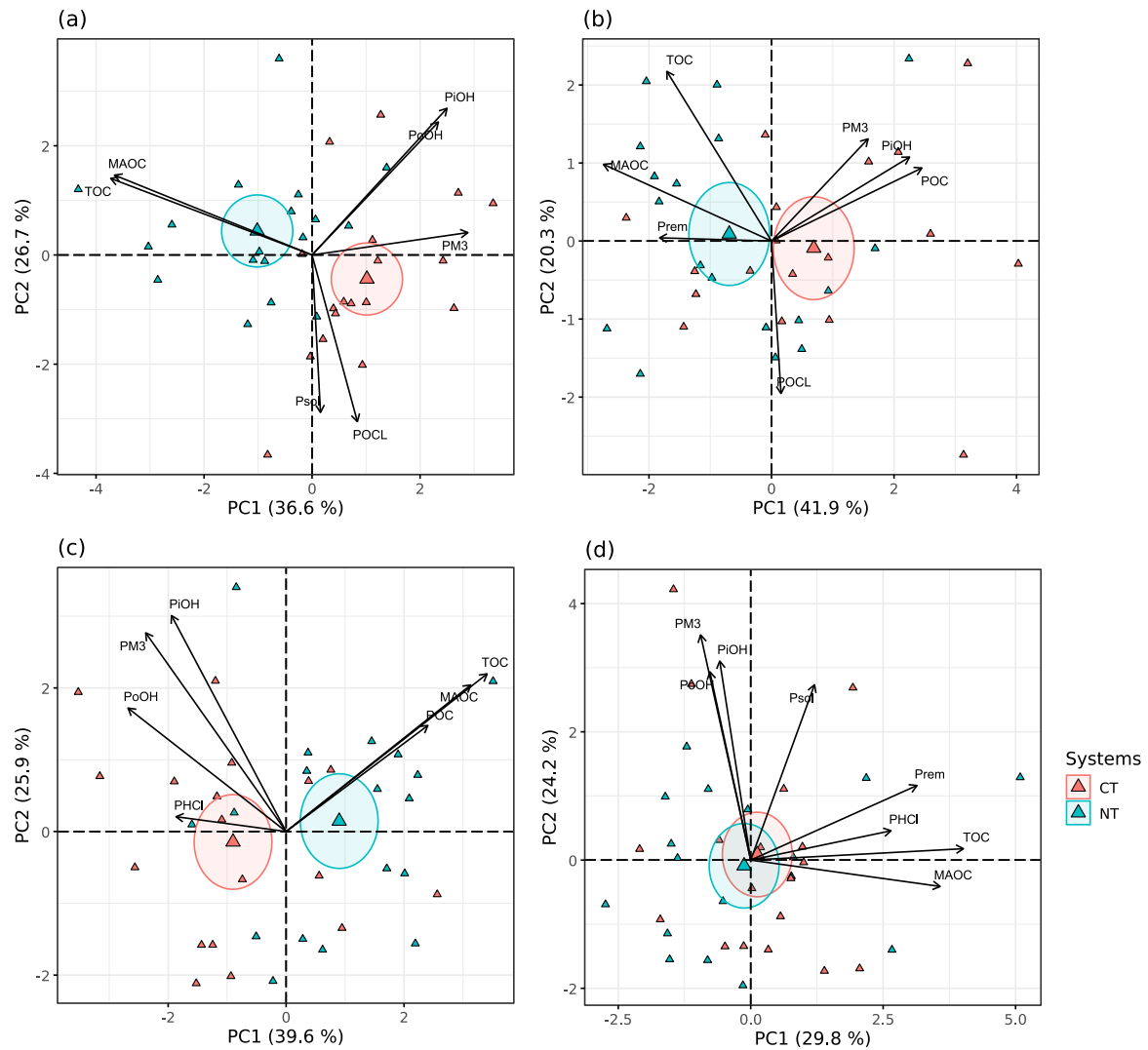


Figure 3. Principal Component Analysis in different management systems and year, Seropédica - Rio de Janeiro State. a: Systems in 2019 in the 0.00-0.05 m layer; b: Systems in 2023 in the 0.00-0.05 m layer; c: Systems in 2019 in the 0.05-0.10 m layer; d: Systems in 2023 in the 0.05-0.10 m layer.

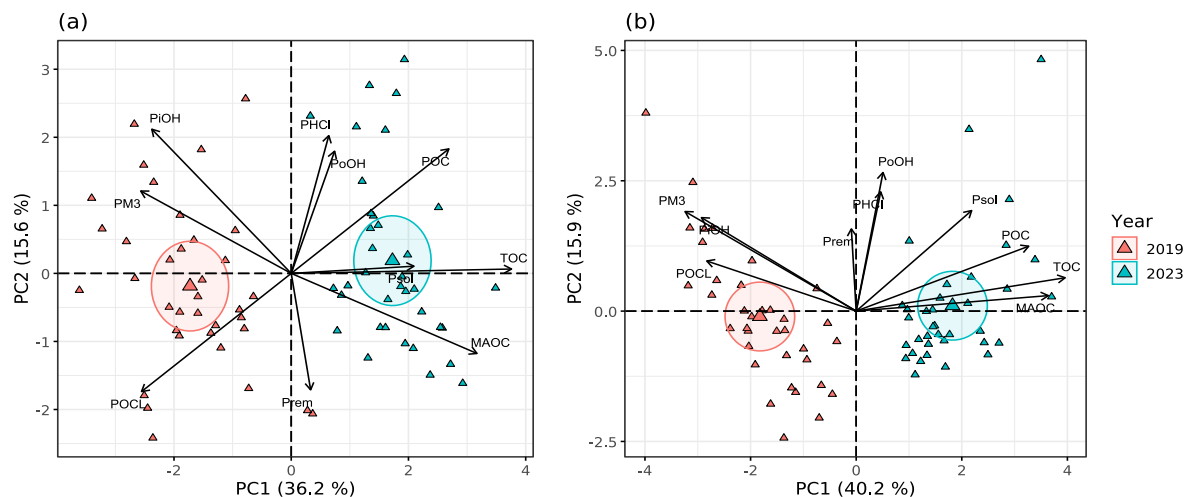


Figure 4. Principal Component Analysis in different years, Seropédica - Rio de Janeiro State. a: 2019 and 2023 in the 0.00-0.05 m layer; b: 2019 and 2023 in the 0.05-0.10 m layer.

The influence of SOM fractions on P availability may be linked to organic compounds occupying positive charges at adsorption sites. These sites would be responsible for P adsorption (Silva et al., 1997), and their occupation by organic compounds may thus contribute to increased P availability. However, the soluble fraction (P_{sol}) is characterized by its higher availability in the soil solution and weak association with soil particles, making it readily available for plant uptake, which likely explains its lower values compared to other fractions (Gatiboni and Condrón, 2021).

Phosphorus is not only found in inorganic form adsorbed to clay minerals and in organic form bound to organic compounds; in this study, the P_{oH} fraction increased over four years, which may be related to increased soil carbon content. The P_{oH} fraction is extracted from stable soil organic matter, and its increase can be considered an indicator of soil carbon accumulation, as observed in this study, where the highest TOC values were found in the MAOC fraction, considered the most stable fraction of organic matter (Pinto et al., 2020; Gatiboni and Condrón, 2021). Organic P compounds exhibit a strong affinity for mineral surfaces, leading to their preferential accumulation in the mineral-associated organic matter fraction, consequently reducing P_{iOH} , as observed in this study (Spohn, 2024).

Lower values of the P_{iOH} fraction indicate reduced P availability to plants, both due to the scarcity of inorganic orthophosphate for plant uptake and P occlusion in stable aggregates or in the more stable organic matter fraction, MAOC (Weihrauch and Opp, 2018). Silva et al. (2023) found that MAOC levels were higher than POC levels in soil aggregates at the study site. The increase in the recalcitrant fraction of organic matter within aggregates can be explained by its protection by clay and silt minerals inside the aggregates. This is considered a mechanism for stabilizing SOM that can occur through adsorption, complexation, or co-precipitation of SOM with the silt and clay fractions of the aggregates, resulting in slower decomposition (Li et al., 2023). Organic matter stabilization in stable aggregates, particularly in conservation systems such as no-tillage, enhances carbon storage, which can influence nutrient availability (Bayer et al., 2023).

The decrease in labile P_{M3} fraction after four years of experimental setup may be attributed to P uptake by plants and cover crops, which release P back into the soil through SOM decomposition. The P released from the decomposition of cover crops, characterized as organic phosphorus, is the fraction that plants do not directly absorb. For P to become available and absorbed by plants, hydrolysis and mineralization of P_{oH} must occur. This process is mediated by phosphatase enzymes, whose activity in the soil increases with the adoption of cover crops (Hallama et al., 2019). Although not significant, the benefits of cover crop adoption can be attributed to increased SOM and, consequently, cation exchange capacity, which influence nutrient availability and optimize fertilizer applications (Adetunji et al., 2020). The long-term use of cover crops influences P availability, particularly in P-deficient soils, due to increased exudation rates of organic acids, generally citrates, which are released by roots. This process maximizes P uptake and mobilizes soil P, thereby increasing its availability (Wang et al., 2021).

Based on these findings, cover crops play a vital role despite the absence of significant differences in phosphorus availability. This lack of differences may be attributed to the relatively short adoption duration (four years), as previous research indicates that benefits typically emerge during the consolidation phase of NT systems (10-20 years), when energy requirements stabilize, and soil improvements become more pronounced. During the adoption phase (0-5 years), higher energy expenditure is required for system equilibrium, reflecting low system organizational capacity (Addiscott, 1995, 2010; Pinto et al., 2023). Several studies (Alvarenga et al., 2001; Freixial et al., 2013; Inagaki et al., 2016; Fiorini et al., 2020; Wulanningtyas et al., 2021) have demonstrated that the benefits of conservation agriculture systems, such as no-tillage with cover crops, become evident during the transition phase (5-10 years), particularly regarding soil carbon accumulation. This may explain the lack of difference in P-rem values and cover crops, and the higher

phosphorus fraction values in CT systems compared to NT systems when differences between systems were observed in the fractions.

The higher values of P fractions in CT compared to NT can be attributed to the practice of soil tillage, which affects the structure and stability of macro- and microaggregates, accelerating SOM decomposition and consequently nutrient release (Topa et al., 2021; Wulanningtyas et al., 2021). This is true even for more occluded fractions, as observed with P_{OCL} , where the highest values were found under CT in both evaluated layers. In general, P and SOM fractions decreased with depth, which is attributed to the low mobility of P in soil (Bissani and Ernani, 2023). Another contributing factor to higher P levels may be the presence of crop residues on the soil surface, which was also reported by Teixeira et al. (2003). The authors found that soil organic matter accumulation in the topsoil (0.00-0.05 m) resulted in higher P concentrations in this layer.

Overall, most P is bound in the occluded, non-labile fraction; however, after four years, a gradual increase in P release was observed, with a reduction in this fraction when comparing 2019 and 2023. As the decomposition of the POC fraction progresses, evidenced by its decreasing values, more labile forms of P may be gradually released.

A slow P-release process for plant uptake was observed, attributed to lower values of labile/moderately labile fractions and higher values of P_{OCL} and P_{OH} fractions. The association of P_{OCL} and P_{OH} fractions with MAOC is crucial for P availability, as MAOC is characterized by slow decomposition and, consequently, gradual P release, which does not supply either the P_{iOH} fraction or the P_{sol} fraction (Villarino et al., 2023).

The slow P release in NT systems benefits agricultural production by ensuring long-term nutrient availability, since its low soil availability is characterized by reduced mobility and fixation (Meena et al., 2022). Management systems that ensure long-term P availability, such as NT systems, reduce the frequency of fertilizer applications and phosphate fertilizer acquisition costs.

Adoption of NT systems, with minimal soil disturbance and input from various cover crop biomass, promotes SOM accumulation, which influences soil microbial diversity responsible for P cycling and solubilization for plants (Oberson and Joner, 2005; Sekaran et al., 2020; Cappelli, 2022). Adopting conservation agriculture systems, such as no-tillage, is crucial for enhancing P availability, benefiting both agricultural productivity and the soil-plant-atmosphere continuum.

CONCLUSION

The adoption of the no-tillage and conventional systems over time, contributed to an increase in the soluble fraction and a decrease in the occluded fraction in the soil.

Higher levels of occluded P and organic P were observed in both evaluated layers under the soil management systems. These changes were associated with an increase in total organic carbon, particulate organic carbon, and mineral-associated organic carbon fractions.

Higher values of occluded and organic phosphorus fractions indicate lower phosphorus availability to plants. However, time since adoption played a crucial role in reducing occluding phosphorus and increasing soluble phosphorus in both evaluated layers, indicating gradual phosphorus release.

Cover crops pearl millet (*Pennisetum glaucum*), sunn hemp (*Crotalaria juncea*), and jack bean (*Canavalia ensiformis*), whether grown alone or in intercropping systems, did not affect phosphorus availability or organic matter accumulation over time. However, we recommend adopting a no-tillage system and using cover crops to supply soil organic matter and phosphorus. This is one more long-term agricultural strategy.







DATA AVAILABILITY






All data was generated or analyzed in this study.






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