Soil Advances 4 (2025) 100084

Contents lists available at ScienceDirect

Soil Advances

journal homepage: www.sciencedirect.com/journal/soil-advances

Can no-tillage and crop diversification sustain nutrient stocks in acidic and poorly-fertilized soils? Evidence from 32 years of real-world agricultural management in Paraguay

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ARTICLE INFO

Keywords:
Agricultural systems
Crop rotation
Soil fertility
Soil health
Macronutrient
Micronutrient
Sustainable agriculture

ABSTRACT

Intensification under technical and economic constraints has driven widespread soil fertility decline in Paraguay. In this context, whether no till with crop diversification alone can maintain fertility under low-input conditions remains uncertain. Our objective was to evaluate how contrasting agricultural systems defined by types of soil tillage and crop sequence affect soil nutrient stocks under long-term low-input management. We measured stocks of macro and micronutrients to 30 cm and assessed associations between pH and nutrients. Diversified no till helped maintain higher stocks of total N (+15 %) and of available P (+40 %), exchangeable K (+27 %), Ca (+72 %) and Mg (+43 %) relative to conventional tillage. In contrast, extractable concentrations of redox sensitive micronutrients (Cu, Fe, Mn) were higher under conventional tillage, consistent with disturbance induced transient mobilization. Depth resolved analyses (0–10, 10–20, 20–30 cm) indicated that most between system differences in total stocks were concentrated in the surface layer. Although this study focused on nutrient stocks, consistent relationships between pH and nutrients were observed while pH remained below the minimum threshold recommended for the regional commercial crops (pH 5.5). Thus, diversified no till farming conserves fertility in low input contexts, but the benefits are limited due to soil acidity. Finally, integrating periodic soil diagnosis with amendments, particularly liming, offers a practical pathway to sustaining production in Paraguay.

1. Introduction

Global agriculture is at a decisive crossroads, increasingly pressured by climate change and the unsustainable exploitation of soil resources (Feng et al., 2025; Poppiel et al., 2025). Each year, around 12 million hectares of arable land lose agricultural suitability due to soil degradation, eroding the productive foundation of global food systems (Rickson et al., 2015). Instead of reversing this trajectory by rehabilitating lands depleted by intensive cultivation and poor management, agricultural expansion continues to advance into natural. This pattern is clearly reflected in Paraguay, where nearly 39 % of the Atlantic Forest has been lost in recent decades (Huang et al., 2007). Consequently, these processes destabilize the climate (You et al., 2025), weaken essential soil functions such as nutrient cycling, impose severe constraints on the prospects for sustainable agricultural development, and threaten food security (Gao et al., 2025; Poppiel et al., 2025; Van Dijk et al., 2021; Xing et al., 2025a).

Against this backdrop, preserving soil fertility has become a central

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https://doi.org/10.1016/j.soilad.2025.100084
Received 31 July 2025; Received in revised form 6 October 2025; Accepted 11 October 2025
Available online 15 October 2025

concern, with growing emphasis on the strategic role of nutrient reserves. Maintaining a stable supply of essential elements can reduce reliance on synthetic fertilizers and minimize environmental externalities (Nthebere et al., 2025; Tang et al., 2023; Xing et al., 2025b). However, this issue remains only partially understood, as nutrient dynamics are simultaneously regulated by a complex interplay of soil properties such as pH, texture, mineralogy, organic matter content, and biological activity (Devi et al., 2024; Dhaliwal et al., 2019; Hartemink and Barrow, 2023), while each nutrient exhibits distinct chemical behaviors. Soil nitrogen (N), for instance, is highly dynamic and prone to volatilization, denitrification and leaching (Islam et al., 2024), whereas phosphorus (P), iron (Fe), zinc (Zn), copper (Cu), boron (B), and manganese (Mn) tend to be immobilized through adsorption or precipitation, limiting their plant availability (Friedel and Ardakani, 2021; Gavelaki et al., 2025). Potassium (K), calcium (Ca), and magnesium (Mg), largely present in exchangeable forms, are susceptible to leaching and strongly influenced by the ionic balance of the soil exchange complex (Hartikainen, 1978; Meetei et al., 2020). In this context, promoting conservation agriculture practices that enhance both above- and below-ground biomass inputs and consequently accumulate soil organic matter is one of the most effective approaches to maintaining the stocks of most nutrients in the agricultural system (Lal, 2020). Stabilizing carbon in the soil is critical to the cation exchange capacity of tropical soils, and therefore to nutrient storage and availability (Soares and Alleoni, 2008). However, agricultural management practices may assume a dual role, either promoting nutrient retention and internal cycling within the soil-plant system or accelerating nutrient depletion (Abed et al., 2024).

Mechanical soil disturbance in agricultural systems, particularly through conventional and reduced tillage practices, significantly accelerates nutrient losses due to increased erosion, leaching, and rapid organic matter mineralization, processes exacerbated by structural degradation and reduced soil aggregate stability (Chen et al., 2024; Rogovska et al., 2025; Zhang et al., 2023). In contrast, no-tillage systems, defined by minimal soil disturbance, continuous soil cover, and crop rotations, are increasingly recognized as ecologically sustainable strategies to conserve soil functionality and curb nutrient depletion across diverse agroecosystems, chiefly by maintaining surface cover and the physical integrity of the soil matrix, thereby supporting nutrient retention and longer-term soil function (Jiang et al., 2025; Sankar et al., 2025; Souza et al., 2025).

Paraguay is one of the world's leaders in no-tillage adoption (Blanco-Canqui and Ruis, 2018; Kassam, 2021), a practice that has positioned the country among the main suppliers of food in international markets (CAPECO, 2025). Nevertheless, more than 52 % of its agricultural land, approximately 21 million hectares, is degraded (Antúnez, 2018). This contrast highlights persistent bottlenecks that hinder the uptake of complementary best practices: Paraguayan producers face financial constraints and limited access to technical assistance, a common challenge in developing countries (Derpsch et al., 2016; Gurmessa, 2021; Michelson et al., 2023), and these limitations constrain the efficient use of external inputs (Abay et al., 2022). In prevailing farm practices, fertilization is often standardized by crop and applied without prior soil analysis, correction of soil acidity is virtually absent, and cash crop successions biased toward monocultures persist (Derpsch et al., 2016). Under these conditions, no tillage alone cannot guarantee long term soil fertility (Alves et al., 2023; Andrade et al., 2025; Bellinaso et al., 2021; Du et al., 2024a; Mulualem et al., 2024; Pandian et al., 2024), with adverse effects on national development, given the economy's reliance on agriculture (González et al., 2019), and it jeopardizes progress toward the United Nations Sustainable Development Goals.

In the face of these constraints, crop diversification emerges as an economically and agronomically viable pathway to strengthen soil fertility (Awiti et al., 2022; Bonini Pires et al., 2020; Rigon et al., 2022; Zabala et al., 2023). More diverse sequences adjust the quantity and stoichiometry of residues, promote soil organic matter formation, and increase cation exchange capacity while moderating acidity and improving base cation retention (Jiang et al., 2018; Locatelli et al., 2025). The inclusion of legumes supplies biologically fixed N (Mondal and Chakraborty, 2022), whereas deep rooted grasses mobilize nutrients from subsoil layers and redistribute them to the surface, strengthening internal recycling (Han et al., 2021). The diversity of root traits intensifies rhizosphere processes, such as organic acid exudation and mycorrhizal associations, which solubilize and mobilize previously fixed nutrients, particularly P and micronutrients (Hallama et al., 2022). These mechanisms collectively could help stabilize soil nutrient stocks and decrease the rate of agricultural soil depletion under conditions of limited external inputs (Hoffmann et al., 2020; Isbell et al., 2017).

Recent evidence indicates that the effects of diversification on soil fertility are heterogeneous and strongly context dependent across soils, climates, and rotation design (Beillouin et al., 2021; Bowles et al., 2020; Tamburini et al., 2020). Consequently, its efficacy varies among regions and is not transferable without local adjustment. In Paraguay, evidence from long term experiments that consider national agroclimatic conditions remains limited (Villalba Algarin et al., 2024). To our knowledge, no study has evaluated or synthesized whether no tillage and crop diversification, in highly weathered soils managed with low input, are sufficient to sustain soil nutrient stocks and maintain soil chemical quality. This gap limits understanding of the long-term performance of agricultural practices and reduces the country's capacity to anticipate fertility decline and design evidence based management strategies for sustainable and resilient production.

To address this critical knowledge gap, we evaluated a 32-year field experiment conducted under conditions representative of prevailing farmers' practices in the Eastern Region of Paraguay. Moving beyond conventional assumptions about the benefits of no-tillage, we hypothesized that its effects on the long-term maintenance of soil nutrients and the mitigation of constraints in low-input systems are amplified by more diverse crop rotations and accumulate over time. Accordingly, we investigated how contrasting agricultural systems, defined by types of soil tillage and crop sequence, influence soil nutrient stocks under long-term low-input management. We also aimed to discuss how no-tillage and a more biodiverse biomass cover might help stabilize soil organic carbon, thereby improving nutrient retention, availability, and internal cycling. These are key goals of the 4 per 1000 initiative (https://4p1000.org), which aims to sustain soil fertility for regenerative, productive, and climate-resilient agricultural systems worldwide.

2. Material and methods

2.1. Experimental site description

The long-term field experiment was conducted at the Capitán Miranda Research Center of the Paraguayan Institute of Agricultural Technology (IPTA), located in the district of Capitán Miranda, Itapúa Department, Eastern Paraguay (27°17′S, 55°29′W; 196 m above sea level), which represents the country's main agricultural production region (Fig. 1). The region has a humid subtropical climate (Cfa), as classified by the Köppen–Geiger system (Peel et al., 2007), with a mean annual temperature of 21.6 °C and an average annual precipitation of 1950 mm, based on two decades of records from the IPTA Agrometeorology Department (Fig. S1). The topography is flat to gently undulating, with slopes of less than 4 %. The soil is classified as an Oxisol (Soil Survey Staff., 2022), with a clayey texture consisting of 683 g kg^{-1} clay, 100 g kg^{-1} silt and 217 g kg^{-1} sand. The experiment was established in 1991, when a composite soil sample from the 0–20 cm depth was collected for the chemical characterization of the soil (Table 1).

2.2. Experimental design and treatments

In this study, we considered five treatments representing agricultural systems relevant to Paraguay. The first three corresponded to the most

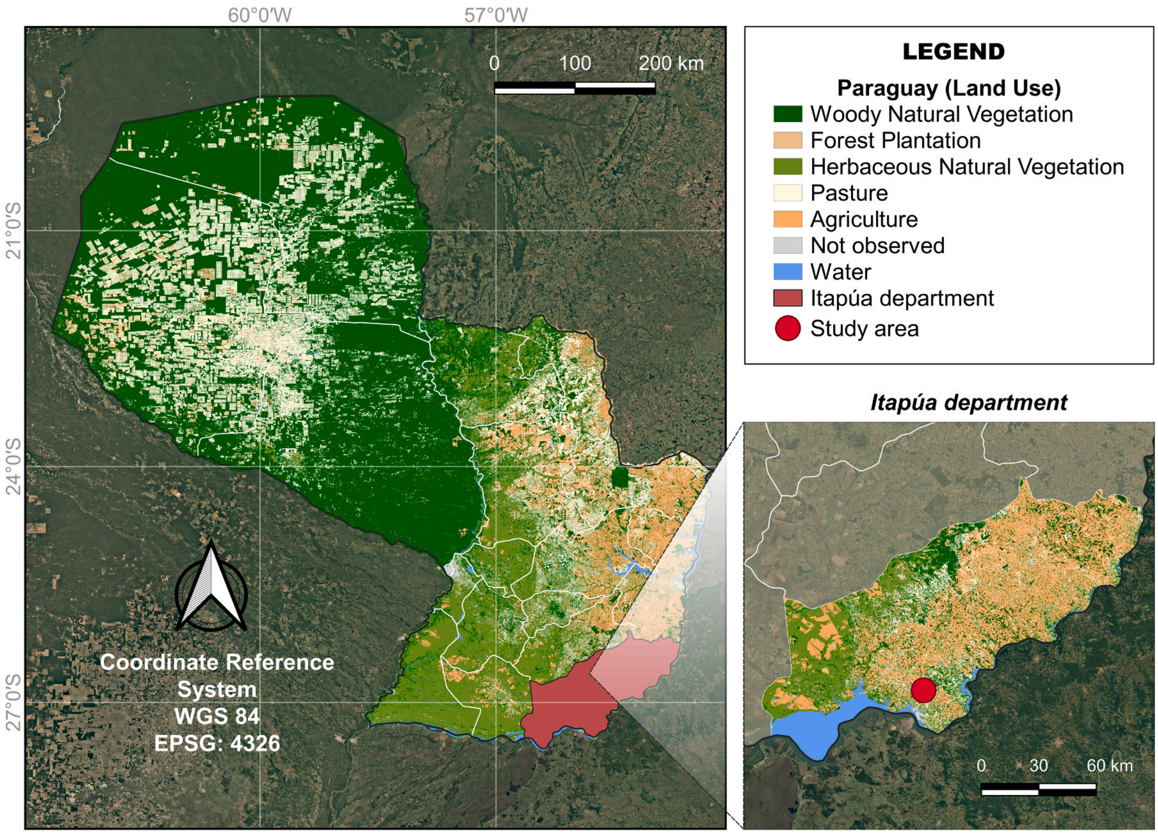

Fig. 1. Location of the long-term field experiment in Capitán Miranda, Itapúa Department, Paraguay.

Table 1
Initial soil chemical characterization (0–20 cm) at the beginning of the experiment in 1991.

Depth	MO	pH	P	Ca2 +	Mg2 +	K+	Al+ 3
cm	%		mg dm-3	cmolc dm-3			
0–20	1.74	6.03	9.00	5.41	0.67	0.54	0.00

*OM: soil organic matter (Walkley–Black); pH (H_2O, 1:2.5); P: available phosphorus (Bray II); Ca^{2+}, Mg^{2+}, K^{+}: exchangeable cations (Schollenberger); Al^{3+}: exchangeable aluminum (KCl extraction).

Table 2
Main management practices in the different crops of the experiment.

Crop	Season	Seeding rate kg ha^{-1}	Fertilization kg ha^{-1}	Harvest method
Soybean	October-March	60	Planting: N (6), P (45), K (15)	Experimental harvester
Maize	October-March	25	Planting: N (6), P (45), K (15) Cover: N (28)	Manual harvest
Wheat	May-September	120	Planting: N (27), P (69), K (0) Cover: N (28)	Experimental harvester
Black oats	May-August	80	Without fertilization	knife roller
Vetch	May-August	60	Without fertilization	knife roller

predominant succession system, which includes wheat (*Triticum aestivum* L.)–soybean (*Glycine max* L.), under different levels of soil preparation: conventional tillage (CT), reduced tillage (RT), and no-tillage (NT1). In addition, two diversified no-tillage rotations with increasing adoption in the region were included: NT2, composed of black oat (*Avena strigosa* Schreb.)–soybean, wheat–soybean, and black oat–soybean; and NT3, consisting of wheat–soybean, vetch (*Vicia villosa* Roth)–maize (*Zea mays* L.), and black oat–soybean. These crop sequences were implemented continuously in three-year cycles over the 32-year study period (Fig. 2).

The adopted experimental design was a randomized complete block design, with three replicates per treatment, totaling 15 experimental plots. Each plot measured 20 × 8 m (160 m^2), separated laterally by 3 m-wide alleys and by 12 m alleys between blocks, to minimize edge effects and facilitate operations.

2.3. Management practices of the agricultural systems

Management practices remained unchanged since 1991 until the present study. In CT treatment, soil preparation consisted of one pass with a moldboard plow followed by a disk harrow. In RT, only a shallow tillage operation was performed using a single pass with a disk harrow. In NT systems, crops were established directly on the residues of the previous crop, with no soil disturbance. The cropping calendar included two growing seasons per year: winter (May to September) and summer (October to March).

Throughout the 32 years of experimentation, no liming or application of soil acidity amendments was carried out, regardless of the management system. Moreover, mineral fertilization was not applied in the systems that incorporated cover crops. These choices reflect common practices adopted by farmers in the region. For commercial crops, fertilizer application rates remained unchanged over time: soybean and maize received 150 kg ha^{-1} of NPK 4–30–10 at sowing, while wheat was fertilized with 150 kg ha^{-1} of NPK 18–46–0 at sowing, followed by a topdressing of 28 kg ha^{-1} of N in the form of urea. These fertilizer rates typically met less than 60 % of the nutrient requirements of the commercial crops in this experiment, according to soil fertility levels and the

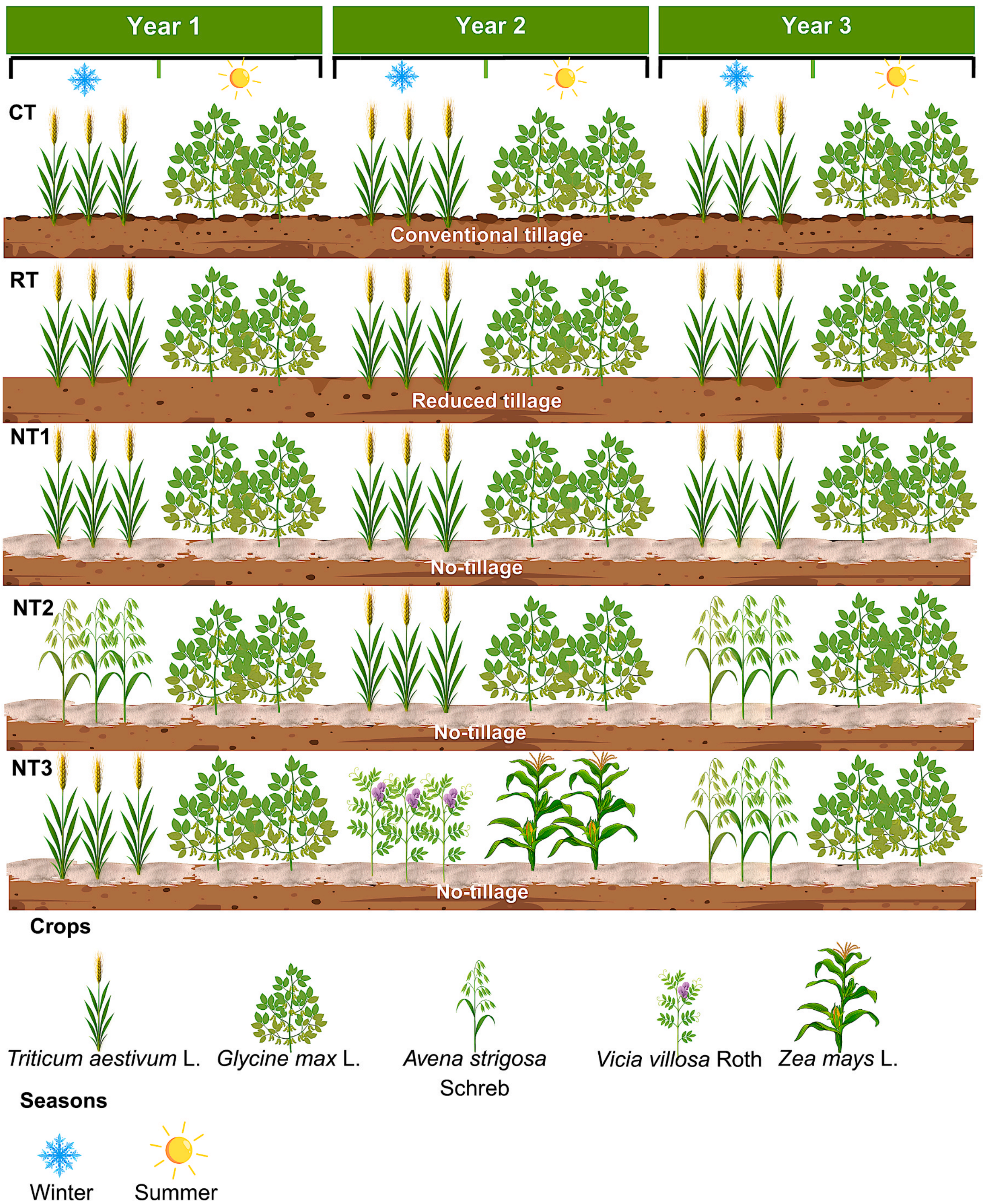

Fig. 2. Schematic representation of the treatments, showing soil disturbance level and crop sequence in each agricultural system. The sequences consisted of three-year cycles repeated continuously over 32 years. The treatments represented are: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean). Symbols indicate the crops grown in each season (winter and summer) and the tillage level used in each agricultural system.

regional recommendation bulletin (Cubilla et al., 2012). Given the absence of additional macronutrient and micronutrient inputs, this configuration was classified and referred to throughout the study as a low-input system.

Weed control in wheat, soybean, and maize was carried out using two herbicide applications per season, supplemented by manual weeding. For cover crops, a single manual weeding operation was performed per cycle. Phytosanitary treatments were applied only when required, guided by phenological crop stages and monitored thresholds of pest populations or disease incidence, in accordance with integrated pest management protocols. Wheat and soybean were machine-harvested, while maize was manually harvested. Cover crops were mechanically rolled at the milky grain stage, leaving their residues on the soil surface. A summary of the main agronomic practices is provided in Table 1.

2.4. Soil sampling

Soil sampling was conducted in October 2023, following the winter harvest and prior to summer crop sowing. Both disturbed and undisturbed samples were collected at three depths: 0–10, 10–20, and 20–30 cm. Disturbed samples were taken using a tubular auger, extracting eight subsamples per plot (four within the previous crop rows and four between rows), which were homogenized by depth to form one composite sample per treatment, intended for nutrient concentration analysis. Undisturbed samples were collected by opening two trenches per plot (30 × 30 × 30 cm), from which soil was extracted using 100 cm^3 metal cylinders (Kopeck rings) at each depth (*i.e.,* 0–10, 10–20, and 20–30 cm), in order to determine soil bulk density (BD).

2.5. Soil analysis and stock calculations

The soil used for chemical analyses was air-dried, ground, and sieved through a 2 mm mesh. Available P and exchangeable Ca, Mg, and K were extracted using ion-exchange resin; P was determined with a UV-Vis spectrophotometer (BEL Photonics UV-M51) and cations with an atomic absorption spectrophotometer (PerkinElmer AAnalyst 400); B was determined by hot-water extraction with microwave heating; and micronutrients (Cu, Fe, Mn, and Zn) were analyzed by the DTPA method, also measured by atomic absorption (PerkinElmer AAnalyst 400). All these analyses followed the procedures described by Raij et al. (2001). Total N was determined by dry combustion in an elemental analyzer (Carlo Erba EA 1110 CHNS, CE Instruments), after soil subsamples of approximately 5 g were air-dried and finely ground (< 149 μm).

BD was determined by dividing the oven-dry mass of the soil (dried at 105 °C for 24 h) by the volume of the sampling cylinder (100 cm^3). Soil pH was measured in a 0.01 mol L^{-1} $CaCl_2$ solution, using a soil-to-solution ratio of 1:2.5 (w/v), using a digital pH meter (Digimed DM-22). Both analyses were performed according to the protocol of Teixeira et al. (2017), with BD being used for the calculation of nutrient stocks and pH included to examine its influence on soil nutrient dynamics.

Nutrient stocks were calculated for each of the three sampled layers (0–10, 10–20, and 20–30 cm) following Eq. 1. Given that these layers were defined without prior knowledge of BD, and considering that cropping systems can modify this property by altering soil structure, a correction by equivalent soil mass was applied according to the methodology proposed by Ellert and Bettany (1995). This correction, based on the BD obtained from a native forest adjacent to the experimental site, prevented overestimation of the stocks and standardized the calculation basis, allowing for more accurate comparisons among treatments.

$$SXS = (TX \times BD \times h) \quad (1)$$

where *SXS* represents the stock of each evaluated nutrient: N, Ca, Mg, and K (Mg ha^{-1}); and P, B, Cu, Fe, Mn, and Zn (kg ha^{-1}); *TX* corresponds to the nutrient concentration in the soil: N, Ca, Mg, and K in (g kg^{-1}), and P, B, Cu, Fe, Mn, and Zn in (mg kg^{-1}), following the same procedures as described by Canisares et al. (2025); *BD* is the soil bulk density (g cm^{-1}); and *h* is the thickness of the soil layer adjusted by equivalent mass (cm).

2.6. Data analysis

All statistical analyses and figure preparation were performed using R software v. 4.4.1 (R Studio Team, 2024). Initially, the data were subjected to the Shapiro-Wilk test to assess normality. Subsequently, an analysis of variance (ANOVA) was applied to evaluate the effect of the cropping systems on nutrient stocks. When significant differences were detected ($p < 0.05$), means were compared using Duncan's test at a 5 % significance level. To identify multivariate patterns and explore the relationship between cropping systems and soil nutrient stocks, a principal component analysis (PCA) was conducted. All variables were standardized (z-score) and the PCA was performed on the correlation matrix "FactoMineR" package (Lê et al., 2008). The number of components to retain was determined using parallel analysis using the "psych" package (Revelle, 2017), ensuring that only components exceeding random or expected eigenvalues were interpreted. To evaluate the stability of loadings, a bootstrap procedure (B = 10000 resamples) was applied, providing 95 % confidence intervals. In this study, PCA was employed as an exploratory tool to synthesize multivariate nutrient patterns across treatments. Additionally, Pearson correlation analysis was employed to assess how soil pH affects nutrient availability within these systems.

3. Results

3.1. Effect of agricultural systems on soil macronutrient stocks

Cropping systems significantly influenced the stocks of primary macronutrients (N, P, and K), with contrasting patterns across the soil profile (Fig. 3a-c). At the profile scale (0–30 cm), NT2 and NT3 sustained the greatest totals, contrasting with the markedly lower stocks under CT and RT. NT3 accumulated 4.9 Mg ha^{-1} of N and 360 kg ha^{-1} of P, representing increases of 15 % and 40 %, respectively, relative to CT. Potassium followed a similar trend, with NT2 and NT3 reaching up to 3.2 Mg ha^{-1} , at least 23 % higher than CT and RT.

By depth, patterns differed among nutrients. For N, NT3 showed higher stocks at 0–10 cm, while below this depth stocks declined across all treatments, with no clear system effects. For P, the greatest accumulation occurred in the 0–10 cm layer, and the effect persisted up to 10–20 cm, where NT2 and NT3 maintained stocks about 25–30 % greater than CT. For K, the stratified analysis did not reveal significant differences among systems, although positive trends were observed under no-tillage, mainly up to 20 cm, which explains the greater stocks achieved in the total profile.

The agricultural systems also influenced secondary macronutrient stocks (Fig. 4a-b). NT2 exhibited the highest values in the 0–30 cm profile, with 72 % more Ca (3.38–5.80 Mg ha^{-1}) and 43 % more Mg (1.08–1.54 Mg ha^{-1}) compared to CT, while NT3 also sustained elevated values, comparable to NT2. Stratification showed that NT2 accumulated more Ca in the 0–10 and 10–20 cm layers, whereas NT3 was superior in the 20–30 cm layer. For Mg, higher stocks were observed in the 0–10 cm layer under NT2 and NT3, while at 10–20 cm the highest values were recorded under NT2, with no system effects in the deepest layer (20–30 cm).

In general, NT1 showed a positive trend compared to CT and RT systems, but at 0–30 cm it presented lower values than the diversified no-tillage systems (NT2 and NT3) (N: 4.65 Mg ha^{-1}; P: 314 kg ha^{-1}; K: 2.24 Mg ha^{-1}; Ca: 4.87 Mg ha^{-1}; Mg: 1.39 Mg ha^{-1}).

3.2. Influence of agricultural systems on soil micronutrient stocks

Micronutrient responses to cropping systems were more variable (Figs. 5 and 6). At the profile scale (0–30 cm), CT sustained the highest Cu stocks, reaching 450 kg ha^{-1} , nearly double the values observed under no-tillage systems (e.g., 228 kg ha^{-1} in NT1). Cu was also the micronutrient showing the greatest variation across soil layers. For Fe, CT presented the highest values in the full profile (708 kg ha^{-1}), with NT2 recording the lowest (518 kg ha^{-1}); the same pattern was observed in the 0–10 cm layer, where CT was the highest and NT2 the lowest, whereas no effects were detected in deeper layers. For Mn, contrasts appeared mainly at the profile scale, with CT accumulating the greatest values (5130 kg ha^{-1}) and no-tillage systems presenting the lowest (≈4461 kg ha^{-1}). For Zn and B (Fig. 6a-b), no consistent effects were observed: Zn ranged from 415 to 600 kg ha^{-1} , with slightly higher values in CT and RT, while B was modestly greater in NT2 (17.6 vs. 14.8 kg ha^{-1} in CT).

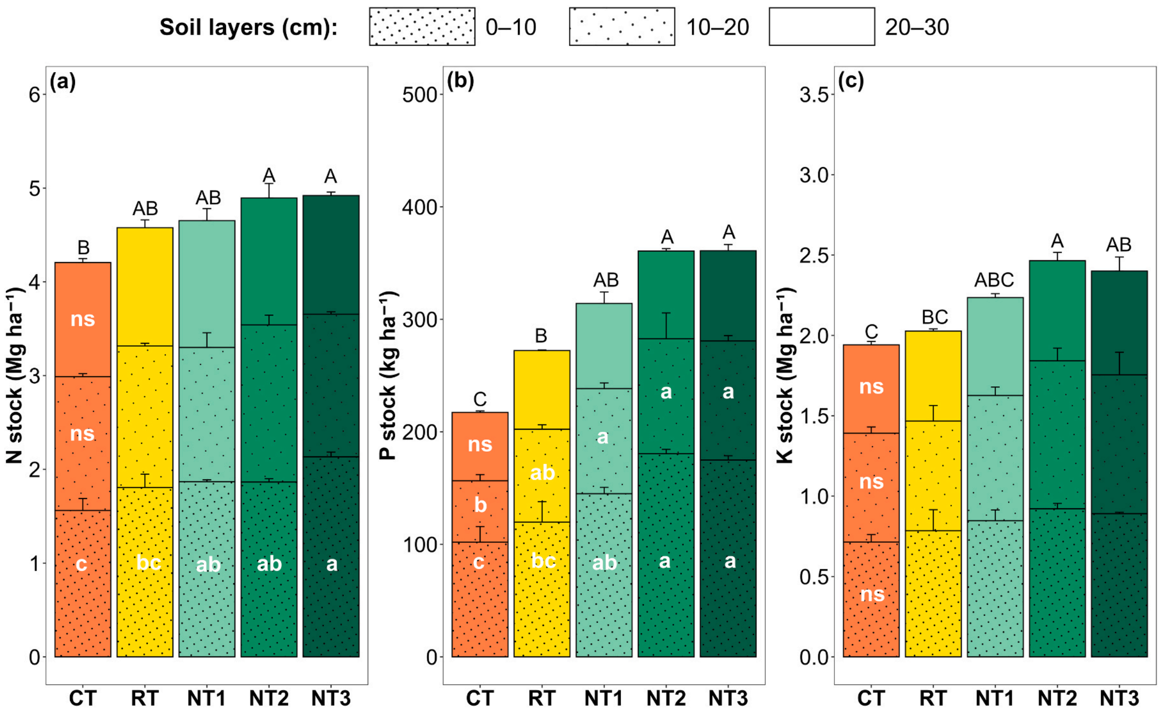

Fig. 3. Stocks of total nitrogen (a), phosphorus (b), and potassium (c) in different soil layers (0–10, 10–20, and 20–30 cm) under agricultural systems: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean). Bars represent the mean ± standard error ($n = 3$). Different lowercase letters indicate significant differences among treatments within each soil depth, whereas different uppercase letters indicate significant differences among treatments considering the total 0–30 cm layer; ns: not significant, according to Duncan's test ($p < 0.05$).

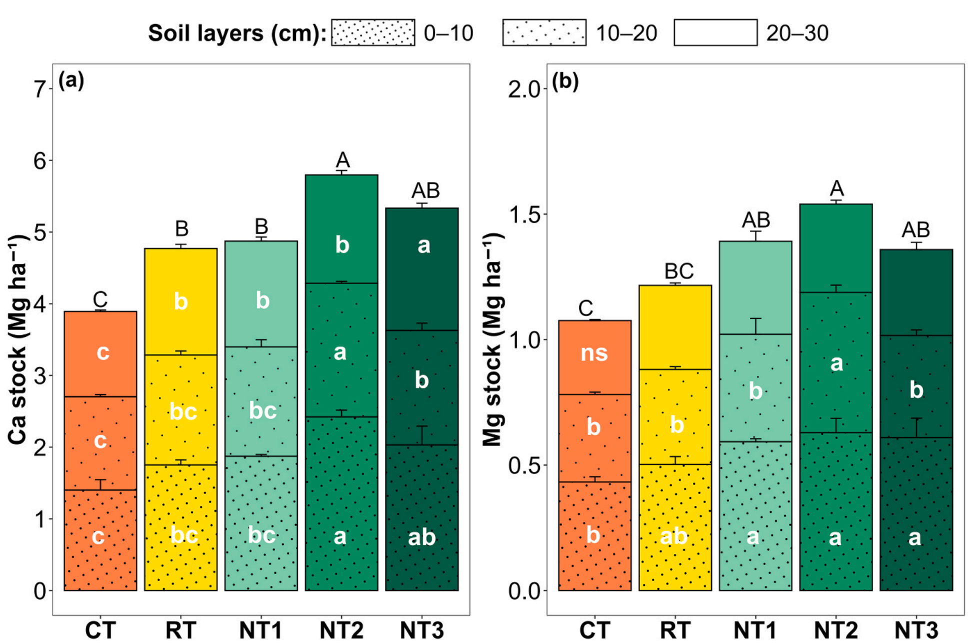

Fig. 4. Stocks of calcium (a) and magnesium (b) in different soil layers (0–10, 10–20, and 20–30 cm) under agricultural systems: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean). Bars represent the mean ± standard error ($n = 3$). Different lowercase letters indicate significant differences among treatments within each soil depth, whereas different uppercase letters indicate significant differences among treatments considering the total 0–30 cm layer; ns: not significant, according to Duncan's test ($p < 0.05$).

3.3. Exploring the relationship between nutrient stocks and soil acidity in agricultural systems

We performed a principal component analysis (PCA) to discriminate between agricultural systems based on soil nutrient stocks (0–30 cm). Parallel analysis (Fig. S2) indicated retaining two components; together they explained 81.4 % of the variation (Dim1 = 69.8 %; Dim2 = 11.6 %) (Fig. 7), and components beyond Dim2 fell below the threshold

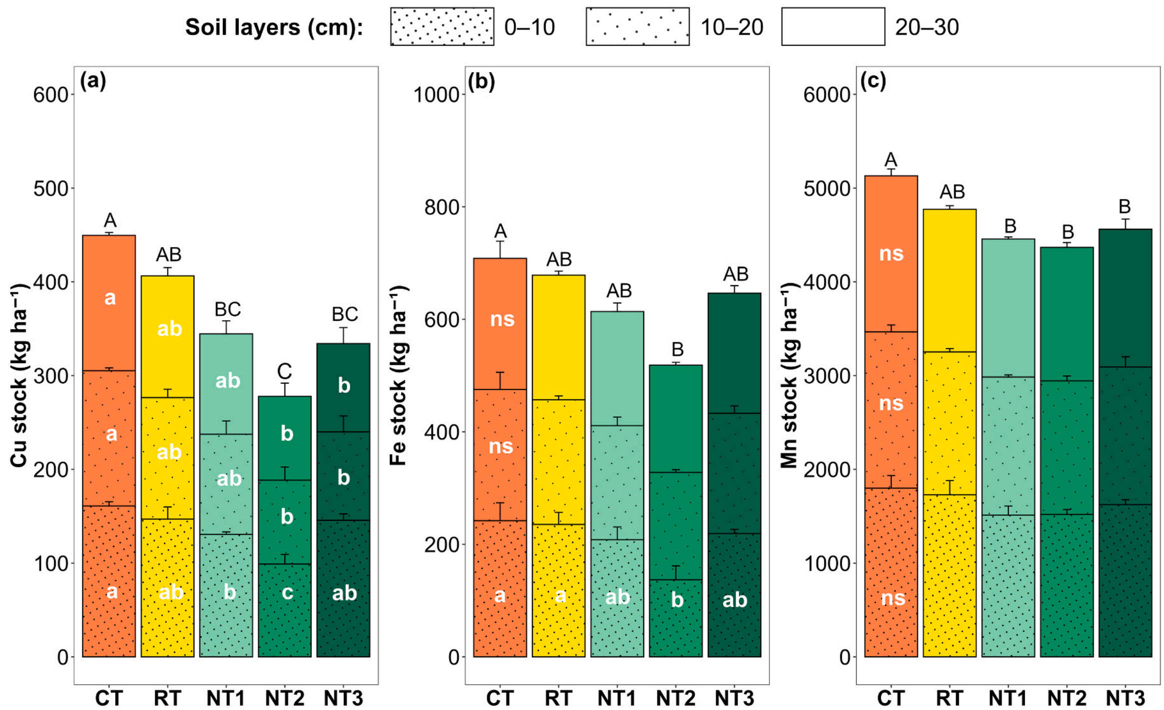

Fig. 5. Stocks of copper (a), iron (b), and manganese (c) in different soil layers (0–10, 10–20, and 20–30 cm) under agricultural systems: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean). Bars represent the mean ± standard error ($n = 3$). Different lowercase letters indicate significant differences among treatments within each soil depth, whereas different uppercase letters indicate significant differences among treatments considering the total 0–30 cm layer; ns: not significant, according to Duncan's test ($p < 0.05$).

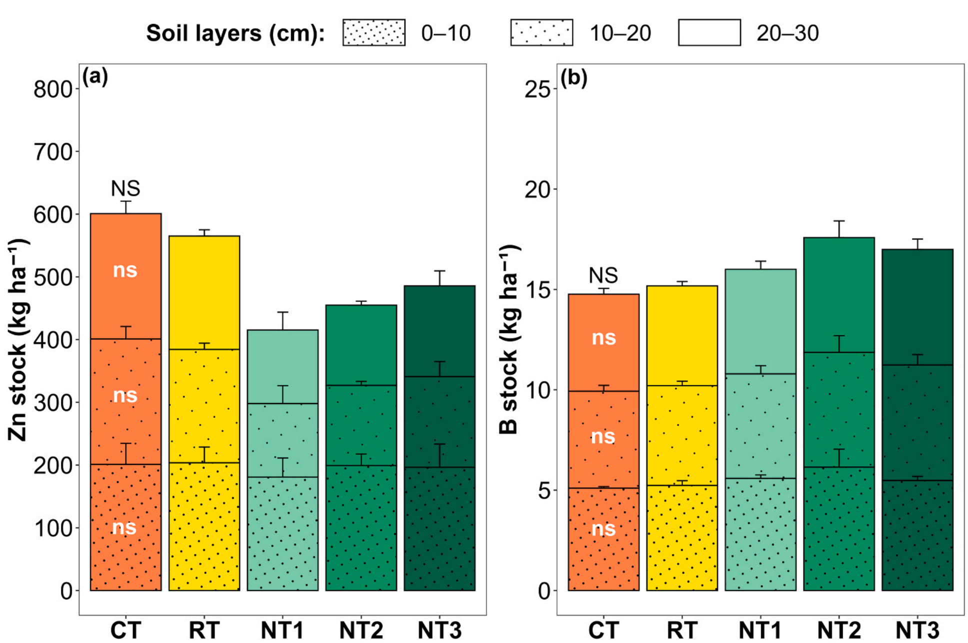

Fig. 6. Stocks of zinc (a) and boron (b) in different soil layers (0–10, 10–20, and 20–30 cm) under agricultural systems: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean). Bars represent the mean ± standard error ($n = 3$). ns indicates no significant differences among treatments within each soil layer, whereas NS indicates no significant differences among treatments considering the total 0–30 cm layer, according to Duncan's test ($p < 0.05$).

and were not retained (Table S2). The ordination primarily separated systems along Dim1: CT and RT plotted on the negative side, aligned with higher stocks of Cu, Mn, Zn, and Fe; bootstrap 95 % confidence intervals for the corresponding loadings are provided in Fig. S2. Conversely, NT2 and NT3 were located on the positive side of Dim1, closely aligned with greater stocks of macronutrients (N, P, K, Ca, Mg) and B, with confidence intervals not crossing zero (Fig. S3). NT1 remained near the origin, in an intermediate position between CT/RT and NT2/NT3.

Soil pH was also higher under no-tillage systems with crop

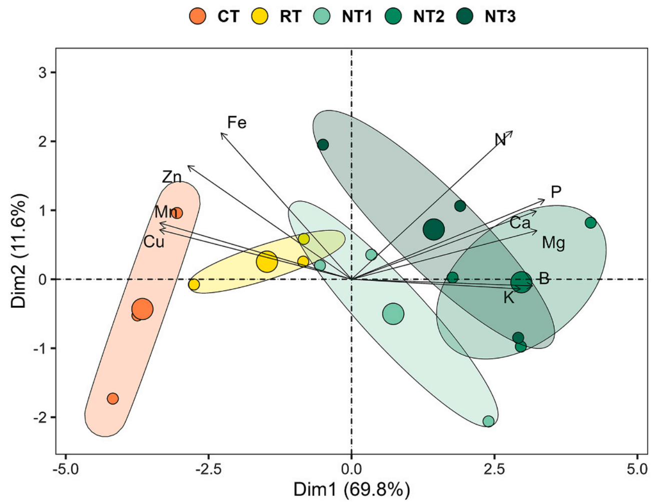

Fig. 7. Principal component analysis (PCA) of soil nutrient stocks (0–30 cm) under different agricultural systems. The evaluated treatments were: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean).

diversification than in systems with soil disturbance and crop succession (mean pH 5.29 vs. 4.95, respectively) (Fig. 8a). When assessing relationships between pH and nutrient stocks (Fig. 8b), we found strong positive correlations with P ($r = 0.95$, $p < 0.001$), Mg ($r = 0.90$, $p < 0.001$), Ca ($r = 0.85$, $p < 0.001$), and N ($r = 0.85$, $p < 0.001$). Moderate positive correlations were also observed for K ($r = 0.72$, $p < 0.001$) and B ($r = 0.65$, $p < 0.01$). In contrast, negative correlations were found for Cu ($r = -0.72$, $p < 0.001$), Mn ($r = -0.69$, $p < 0.001$), Zn ($r = -0.49$, $p < 0.05$), and Fe ($r = -0.45$, $p < 0.05$), respectively.

4. Discussion

4.1. Overall effect of no-tillage and crop diversification on macro- and micronutrient stocks

Our findings show that no-tillage (NT) and crop diversification are key drivers of long-term soil nutrient conservation in Paraguayan agriculture, supporting soil health and food security through sustainable land management. After three decades of implementation, diversified NT systems consistently maintained higher macronutrient stocks in the 0–30 cm soil layer than conventional tillage (CT). In this context, previous studies have demonstrated that NT systems with organic matter inputs from multiple plant species support distinct biological niches in the soil, effectively enhancing SOM in its various forms (Qiu et al., 2024). These conservation practices promote the accumulation of both labile SOM, which improves nutrient cycling by supplying resources to plants and functional microbes (Cotrufo et al., 2013; Tiemann et al., 2015), and stabilized SOM, which associates with soil minerals, increasing cation exchange capacity and nutrient retention, especially in tropical soils (Ramos et al., 2018). Although SOM was not directly assessed in this study, we believe its improved cycling plays a central role in explaining the higher nutrient stocks (N, P, Ca, Mg) observed in diversified NT systems. This interpretation is supported by studies demonstrating that SOM acts as a nutrient reservoir, gradually releasing nutrients through mineralization to support plant productivity and the sustainability of agroecosystems (Cherubin et al., 2021; Gerke, 2022; Villalba Algarin, 2025).

Even with a lower diversification level (*i.e.,* wheat–soybean succession), our results show higher macronutrient stocks under NT1 than under CT, particularly in the upper two soil layers (0–10 cm and 10–20 cm). This pattern is primarily attributed to the absence of soil disturbance and preservation of soil structure under NT. Earlier studies have shown that adopting NT after CT enhances the formation of stable aggregates and increases porosity, thereby improving soil structure (Dai et al., 2025; Zhang et al., 2021). Well-structured soils are less prone to nutrient losses via erosion, denitrification, and leaching (Komissarov and Klik, 2020). They support microenvironments that are favorable for functional and healthy microbial activity (Wen et al., 2025), which is essential for efficient nutrient recycling, with minimized losses (Souza et al., 2025).

More diversified no-tillage systems (NT2 and NT3) maintained higher soil pH compared to conventional and reduced tillage systems (CT and RT), where soils were significantly more acidified. Although the absolute difference was modest (approximately 0.4 units; 5.3 vs. 4.9), pH emerged as a key correlated soil property, exhibiting a synergistic relationship with macronutrient stocks and, like them, responding positively to NT and crop diversification. In contrast, pH correlated

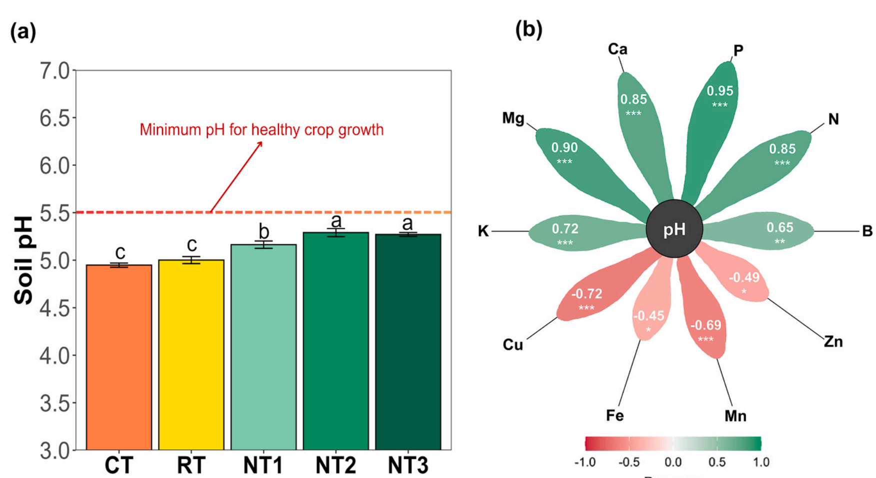

Fig. 8. Soil pH (a) and Pearson correlation with nutrient stocks (b) in the 0–30 cm soil layer under agricultural systems: CT (conventional tillage: wheat–soybean), RT (reduced tillage: wheat–soybean), NT1 (no-tillage: wheat–soybean), NT2 (no-tillage: black oat–soybean, wheat–soybean, black oat–soybean), and NT3 (no-tillage: wheat–soybean, vetch–maize, black oat–soybean). In (a), bars represent the mean ± standard error (n = 3), and the red dashed line marks the minimum pH threshold (5.5) recommended for regional commercial crops (Cubilla et al., 2012). Different letters denote significant differences among treatments according to Duncan's test ($p < 0.05$). In (b), petal length represents correlation magnitude and petal color indicates its direction (green = positive, red = negative). Asterisks indicate significance levels (*p < 0.05; **p < 0.01; ***p < 0.001).

negatively with micronutrient stocks. This pattern aligns with classical nutrient availability curves, where increasing pH toward neutrality (6.0–7.0) enhances the availability of macronutrients but reduces the solubility of several micronutrients. Still, raising the soil pH toward neutrality represents improving overall nutrient availability for plant uptake and development (Hartemink and Barrow, 2023). Therefore, our findings demonstrate that conservation soil management and crop diversification are effective strategies for enhancing three key soil chemical functions in Paraguayan agricultural systems: regulating acidity, improving nutrient availability, and promoting long-term nutrient retention within the agroecosystem. By fostering SOM accumulation, maintaining soil structure, and buffering pH within a more favorable range, these practices improve soil health and the sustainability of nutrient cycling in low-input tropical systems.

4.2. Nutrient-specific responses under different soil management and crop diversification levels

4.2.1. Macronutrients

The most diversified NT system (NT3), which included wheat, soybean, vetch, maize, and black oat, contained 640 kg ha^{-1} more N in the 0–30 cm soil layer compared with CT under the simple wheat–soybean rotation. Because soil N is closely linked to SOM and strongly correlated with soil C (Li et al., 2012), it can serve as a reliable proxy for soil C accumulation. Amorim et al. (2022) reported a C:N ratio of 12 ± 0.4 for well-drained tropical soils, indicating low variability. Based on this ratio, transitioning from low-diversity CT to a highly diversified NT system would increase soil C stocks by approximately 7.68 Mg C ha^{-1} over 32 years, corresponding to an average accumulation rate of 0.24 Mg C ha^{-1} yr^{-1}. These findings suggest that diversified NT systems contribute to greenhouse gas mitigation by sequestering both C and N in the soil. Supporting this, a literature review on smart-climate agri-systems in Brazil by Bieluczyk et al. (2024) reported that biodiverse intensification under NT enhances microbial communities that retain N in the soil while suppressing those responsible for N_2O emissions, consistent with our observation of higher soil N storage in diversified systems.

Furthermore, meta-analytical evidence, conducted by Mondal and Chakraborty (2022), indicates that N accumulation is 16–21 % greater under NT than under CT, particularly in cereal-legume rotation systems. Nonetheless, persistent acidity may still constrain N stabilization at depth by limiting root growth and aggregate formation (Dai et al., 2025; Liu et al., 2024), while lower pH, likely under our CT system, may suppress diazotrophs and disrupt N-cycling microbial groups (Xu et al., 2025).

Available phosphorus (P) stocks were significantly greater under NT2 and NT3 in the 0–10 and 10–20 cm soil depths. This improvement in P availability through conservation management is particularly relevant in highly weathered tropical soils, such as the clayey Oxisol studied here, where soluble P is rapidly adsorbed onto Fe and Al oxides, constraining its accessibility to plants and soil biota (Pavinato et al., 2020). Although tillage may temporarily increase P availability through the accelerated mineralization of organic matter (Karlen et al., 2013), it disrupts gradual P cycling in organic and labile forms (Cherubin et al., 2021; Gu et al., 2024; Suñer et al., 2018) and exacerbates P losses through soil erosion (Alewell et al., 2020). In contrast, no-tillage minimizes soil detachment and enhances physical protection of labile organic P (Uusitalo et al., 2018). The slightly higher pH we found under NT may suppress Fe and Al activity, thus reducing P fixation and favoring accumulation in more plant-available forms (Hartemink and Barrow, 2023; Yang et al., 2024). Enhanced rhizodeposition and organic P formation in subsurface layers, as reported with cover crop rotations (Bowman and Halvorson, 1997; Hallama et al., 2022), may also have contributed to our findings. Lastly, each tillage event under CT likely destroyed most mycorrhizal networks, impairing their contribution to P uptake (Angon et al., 2023), whereas under NT, their preservation supports more efficient biological acquisition (Peng et al., 2024). Given the high P fixation capacity of tropical soils and the global decline in phosphate reserves, these findings underscore the role of conservation systems in enhancing P-use efficiency under low-input tropical agriculture.

Potassium (K) dynamics also improved under NT and crop diversification, with the highest stocks observed in the intermediate level of diversification (NT2). The greater K stocks under NT2 likely result from continuous inputs of high-quality residues, especially from Poaceae cover crops such as black oat, which is known for its efficient K cycling under NT (Echer et al., 2023; Sharma et al., 2018). As a nonstructural element, K exists predominantly in ionic form within plant tissues and is readily released from senescent biomass (Sun et al., 2021), a process well synchronized with plant demand under NT. In contrast, CT accelerates OM decomposition and favors nutrient leaching, processes to which K is highly susceptible due to its rapid release from plant material. In our CT system, this certainly led to K losses and disrupted the synchronization between nutrient release and uptake by the subsequent soybean crop. Moreover, previous studies have also shown that root diversification in NT rotations supports K mobilization through rhizosphere exudation and microbial interactions (Hinsinger et al., 2005; Zhao et al., 2021). Although K is highly leachable, NT may reduce losses by increasing cation retention at higher pH levels and improved aggregate stability (Karasawa, 2024; Pesini et al., 2025). Collectively, these conditions could have promoted a better K use efficiency in the two more diversified NT systems of our experiment.

We found that exchangeable calcium (Ca) and magnesium (Mg) stocks in the 0–30 cm layer were over 30 % higher in NT2 and NT3 compared to CT, while reduced tillage and the less diversified no-tillage system (NT1) showed intermediate values. In agricultural systems, Ca and Mg are almost entirely supplied through liming, which, however, was never applied in this long-term experiment. Our results suggest that conservation agriculture practices, particularly those involving crop diversification, help sustain soil Ca and Mg stocks over time. Both nutrients were highly positively correlated with soil pH ($r > 0.85$; $p = 0.001$), and thus with lower negative impacts of acidity on crops. Reduced acidity promotes the retention of basic cations and decreases Al toxicity, a key advantage in highly weathered soils (Hartemink and Barrow, 2023). Previous studies support our findings, showing that rotations involving both grasses and legumes increase soil Ca and Mg retention compared to monocultures (Li et al., 2025; Tahir et al., 2022). Improvements in rhizosphere chemistry under undisturbed conditions, such as increased dissolved organic carbon and reduced acidity, may have further sustained Ca and Mg in an active and healthy NT soil environment (De Conti et al., 2018). Given that Ca and Mg have essential structural and physiological roles in plant metabolism (Maathuis, 2009), conserving these elements through diversified no-tillage systems is a promising strategy for a more sustainable crop production.

4.2.2. Micronutrients

Despite greater macronutrient conservation and reduced acidity achieved under diversified no-tillage systems, extractable Cu, Fe, and Mn stocks were consistently higher under CT, whereas Zn and B remained unchanged. We interpret these patterns as tillage-induced mobilization and transient pH shifts, rather than improved micronutrient fertility. Tillage disrupts soil aggregates, increases O_2 diffusion and CO_2 efflux, and can locally acidify the soil, potentially releasing micronutrients previously protected within organo-mineral complexes or occluded in microaggregates (Dhaliwal et al., 2019; Palmer et al., 2023). It also accelerates SOM oxidation and raises redox potential in aerated zones, increasing the exposure and reactivity of Fe and Mn (oxyhydr)oxides (Li et al., 2021). Furthermore, repeated tillage often promotes the formation of a compacted subsurface layer (plough pan), typically at depths of approximately 15–25 cm, which restricts water infiltration and creates zones of alternating oxic and anoxic conditions in the upper profile (Batey, 2009). During saturation (anoxic conditions), ferric [Fe^{3+}] and manganic [Mn^{4+}] oxides are reduced to the more soluble ferrous [Fe^{2+}] and manganous [Mn^{2+}] forms (Liu et al., 2022), directly increasing their DTPA extractability. Simultaneously, this environment favors greater dissolution of organic C, which may facilitate the desorption of Cu from organo-mineral complexes (Violante et al., 2010), thereby expanding its extractable pool. Upon re-aeration, Fe^{2+} and Mn^{2+} reoxidize to less soluble forms, while Cu tends to re-adsorb onto SOM (Liu et al., 2022), reducing solubility and extractability.

In contrast to CT, diversified NT systems typically maintain more stable chemical and physical conditions in the soil. These systems maintained a slightly higher soil pH compared to CT, which increases the adsorption of cationic micronutrients, such as Cu, Fe, and Mn (Peacock and Sherman, 2004; Moreira et al., 2017; Shiwakoti et al., 2019), thereby reducing their extractable forms in the soil. These systems also presented higher N stocks, indicating greater contents of SOM. Increased SOM inputs enhance soil microbial biomass and favor the formation of stable chelates and insoluble Cu complexes with humic substances (Sun et al., 2019; Carvalho et al., 2023; Klučáková and Enev, 2024), limiting Cu extractability. Indeed, extractable Cu was the micronutrient with the highest reduction in NT2 and NT3 systems compared to CT, by more than 43 % in the 0–30 cm soil layer. Together, improved soil structure (*e.g.,* stable macropores that facilitate water movement), along with higher SOM content and higher pH under NT systems, particularly the most diversified ones, favors the stabilization of cationic micronutrients into less labile pools, not extracted by DTPA. These findings suggest that conservation agriculture enhances long-term micronutrient cycling and retention in less labile forms by promoting chemical buffering, structural protection, and biological immobilization, thereby increasing the functional resilience of tropical agricultural soils.

4.2.3. General patterns of nutrients across the soil profile

Nutrient stock differences among agricultural systems were clearly stratified within the 0–30 cm soil profile, with the most pronounced contrasts in the upper 0–10 cm. Differences remained detectable at 10–20 cm for specific nutrients (*i.e.,* P, Ca, Mg, and Cu), but largely converged at deeper layers for most nutrients, except for Ca and Cu. These patterns suggest that the benefits of conservation agriculture are primarily concentrated near the surface, driven by residue inputs, reduced disturbance, and enhanced stabilization processes (Teng et al., 2024). However, they may not extend to deeper layers under low-input conditions (*i.e.,* no liming and suboptimal fertilization). Studies have demonstrated that NT systems can improve nutrient status compared to CT in the long term, even at depths below 20 cm, when soil lime is incorporated in the implementation and fertilizers are applied according to regional recommendations (Lv et al., 2023; Tiecher et al., 2023). Still, vertical attenuation is also commonly observed, as lime is frequently spread on the soil surface in NT, with improvements primarily restricted to the upper 20 cm, where biological activity is concentrated (*e.g.,* Antonangelo et al., 2022; Mühlbachová et al., 2024). Subsoil layers are typically more acidic and richer in mobile aluminum, which limits root growth and microbial activity (Caires et al., 2006), thereby hindering SOM accumulation (Wang et al., 2021), and thus its related benefits. The downward movement and accumulation of nutrients such as Ca, Mg, P, and N — often referred to as "soil profile buildup" by field practitioners — are also constrained under such conditions. This process has gained attention as a key strategy to enhance subsoil fertility and improve crop resilience under limiting conditions, such as prolonged droughts (Cherubin et al., 2025), which are becoming increasingly frequent due to climate change (IPCC, 2021).

4.3. Strengths, limitations, and future directions

This study reveals field-based, quantitative evidence under real farmer-managed, low-input conditions in Paraguay. Leveraging a 32-year experiment managed with fertilization and liming practices typical of regional producers, we combined depth-resolved measurements of key macro- and micronutrient stocks with contrasting tillage and rotation systems. We identified systems that alleviated low-input constraints and sustained soil nutrient stocks, also revealing conditions that might be limiting nutrient retention and plant availability in conservation-oriented systems. Our results provide a robust basis for targeted, practice-level adjustments to strengthen soil health, raise productivity on existing cropland, curb natural-resource degradation, and support national sustainability commitments.

We acknowledge several limitations of this study. The quantification of SOM and its granulometric fractions (*i.e.,* particulate and mineral-associated), as well as related microbes transforming SOM pools, would have provided deeper insights into nutrient cycling pathways by linking nutrient stocks to soil C stabilization. SOM plays a central role in nutrient retention and availability, particularly in tropical soils, where it is a key indicator of soil health (Lal, 2016). Liming and complete fertilization based on soil diagnosis should have been included to contrast the current low-input management with the potential of high-standard systems, aiming to maximize yields and long-term fertility. Moreover, the study was conducted at a single site and soil type, limiting the extrapolation of results to other soil and climate contexts in Paraguay. To address these gaps, future research should include comprehensive chemical and biological soil assessments, as well as evaluations of subsurface physical constraints such as compaction. We also recommend replicating the experiment across diverse soil types and regions of Paraguay, while incorporating site-specific liming, fully recommended fertilization, and mixed cover crops as a further step toward system diversification. These additions will help to fully uncover the benefits of conservation agriculture and support its scalability and long-term sustainability across varied socioeconomic farming contexts.

5. Conclusion and final remarks

Overall, our results partially support the hypothesis that no-till crop rotations help maintain macronutrient stocks relative to systems with soil disturbance and crop succession. In contrast, extractable concentrations of redox-sensitive micronutrients (Cu, Fe, Mn) were higher under conventional tillage, consistent with transient mobilization following disturbance. Depth-resolved analyses (0–10, 10–20, 20–30 cm) showed that most between-system differences in total stocks were concentrated in the two surface layers (0–10 and 10–20 cm). Although this study focused on nutrient stocks, we observed consistent relationships between pH and nutrients; future work should examine these linkages together with measurements of soil structure, microbial activity, and SOM fractions. Finally, in low-input contexts, no-till crop rotation promotes fertility conservation, but it does not ensure optimal pH by itself. Integrating periodic soil diagnostics with soil amendments, at least liming, into no-till rotations should be experimented with in the future, as it could offer a practical pathway to sustain higher standards of soil nutrient availability and agricultural production in Paraguay.

CRediT authorship contribution statement

Eduardo Mariano: Writing – review & editing, Methodology. **Wanderlei Bieluczyk:** Writing – review & editing, Conceptualization. **Maurício Roberto Cherubin:** Writing – review & editing, Conceptualization. **Cassio Carlette Thiengo:** Writing – review & editing, Writing – original draft, Data curation. **Carlos Alcides Villalba Algarin:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **José Lavres:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization. **Alodia Concepción González:** Writing – review & editing, Investigation. **Marcos Fabian Sanabria Franco:** Writing – review & editing. **Deoclecio Jardim Amorim:** Writing – review & editing, Formal

analysis, Data curation.

Funding

This research did not receive specific funding. However, the first author was supported by the National Scholarship Program Don Carlos Antonio López (BECAL), through a master's scholarship granted by the Government of Paraguay.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: CARLOS ALCIDES VILLALBA ALGARIN reports a relationship with IPTA that includes: non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was based on a long-term field trial maintained by the Instituto Paraguayo de Tecnología Agraria (IPTA), to which we express our gratitude for granting access and providing logistical support. We particularly thank the Soil Department of the Centro de Investigación Capitán Miranda for their institutional support during the experiment, especially Mr. Julio César Morel, for his valuable assistance in the field sampling campaigns and management of the experimental plots. We also acknowledge the Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (ESALQ/USP) and CCARBON (FAPESP process # 21/10573–4) for the scientific guidance, analytical infrastructure, and academic training. C.A. Villalba Algarin gratefully acknowledges the financial support provided by the National Scholarship Program Don Carlos Antonio López (BECAL – Paraguay) for his academic training in Brazil. J. Lavres and M. R. Cherubin are grateful to the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq) (Grant #303718/2020–0; 302249/2025–7).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.soilad.2025.100084.

Data availability

Data will be made available on request.

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