



## Research article

# Long-term effects of crop diversification on soil health, crop yield and resilience of tropical agroecosystems

Maurício Roberto Cherubin<sup>a,b,\*</sup>, Lucas Pecci Canisares<sup>a,b</sup>, Lucas Nogueira Souza<sup>a,b</sup>, Carlos Roberto Pinheiro Junior<sup>a,b</sup>, Moacir Tuzzin de Moraes<sup>a,b</sup>, Felipe Bertol<sup>c</sup>, Larissa Bortolo<sup>c</sup>, Rafael Braghieri Menillo<sup>a</sup>, Carlos Eduardo Pellegrino Cerri<sup>a,b</sup>

<sup>a</sup> Luiz de Queiroz College of Agriculture – University of São Paulo, Piracicaba, SP, Brazil

<sup>b</sup> Center for Carbon Research in Tropical Agriculture, University of São Paulo, Piracicaba, SP, Brazil

<sup>c</sup> Fundação Mato Grosso, Rondonópolis, Mato Grosso, Brazil

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## ABSTRACT

Crop diversification strategies can improve soil health while maintaining high yields. Healthy soils perform multiple functions that can also increase resilience to climate change. However, these benefits have yet to be demonstrated in the Brazilian savannah (Cerrado biome), one of the world's largest agricultural production regions and also one of the most vulnerable to climate change. Thus, this study evaluates the long-term (16 years) effects of crop diversification systems, including crop successions and rotations, and soybean monocropping (control), on soil health, grain yield, and yield stability in the Brazilian savannah. The experiment involved seven cropping systems with soybean as the main cash crop, integrating pearl millet, ruzigrass, sunn hemp, and/or maize into the different successions and rotations. Soil health data includes 23 indicators (8 chemicals, 10 physicals and 5 biologicals). Crop yield and weather data from the last 6 years (two rotation cycles) were analyzed. Soybean yields were higher under crop diversification compared to monocropping (soybean-fallow) (3948 - 4321 kg ha<sup>-1</sup> vs. 2282 kg ha<sup>-1</sup>), with reduced yield variability (5–9 % vs 19 %). Results showed that crop successions and rotations enhanced soil health indicators (mainly biological and physical) compared to soybean-fallow monocropping, resulting in higher soil health index and soil's multifunctionality. We observed a higher amplitude of grain yield in drier and warmer years and observed a positive linear relationship between soil health index and water use efficiency. Treatments with higher soil health index result in higher water use efficiency (3.1–3.5 vs. 1.8 kg ha<sup>-1</sup> mm<sup>-1</sup> of soybean grain) than monocropping, with successions and rotations enhancing systems' resilience compared to monocropping. In conclusion, long-term crop diversification, particularly including cover crops with high biomass accumulation, was an effective strategy to enhance soil health, productivity, and resilience in tropical agroecosystems in the Brazilian savannah. In addition, we suggest that the soil health index be used as a comprehensive indicator for assessing the effectiveness of climate change adaptation strategies in agroecosystems.

## 1. Introduction

Climate change increasingly affects the stability and productivity of agricultural systems, posing significant risks to global food security. This is particularly evident in tropical regions, which, despite their high agricultural potential, are highly vulnerable to climate extremes such as droughts, hail, floods, heat waves, and fires (Su et al., 2021; Ren et al., 2022; Feron et al., 2024). As global demand for food continues to grow, the challenge is to maintain and increase agricultural productivity in a

changing climate while reducing agriculture's impact on greenhouse gas emissions.

Healthy soils maintain chemical, physical, and biological functions that support crop growth and other vital ecosystem services (Adhikari and Hartemink, 2016; Kraamwinkel et al., 2021; Lehmann et al., 2020). Soil multifunctionality, which is the soil capacity to develop its multiple functions such as carbon sequestration, nutrient cycling, water regulation, crop productivity and climate regulation. In this context, healthy soils are the basis for designing more productive and resilient

\* Corresponding author. Department of Soil Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil.

E-mail address: [cherubin@usp.br](mailto:cherubin@usp.br) (M.R. Cherubin).

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agroecosystems that reduce crop vulnerability to adverse weather events (Qiao et al., 2022; Mahmood et al., 2023; Teng et al., 2024; Yang et al., 2024). Tropical countries, such as Brazil, have the potential to not only offset the adverse impacts of climate changes on crop yields through the adoption of sustainable agricultural practices (Jat et al., 2016; Souza et al., 2025) but also to mitigate climate change by enhancing soil health, and carbon sequestration (Lal, 2004, 2021; Bonetti et al., 2023; Yang et al., 2024; Souza et al., 2025).

Conservation management practices have been tested worldwide to enhance soil health (e.g., Cherubin et al., 2016; Wood and Bowman, 2021; Liptzin et al., 2022; Nwaogu and Cherubin, 2024; Silva et al., 2024; Yang et al., 2024) and crop yield and stability (Gaudin et al., 2015; Anghinoni et al., 2021; Nouri et al., 2021; Qiao et al., 2022; Balbinot Junior et al., 2024; Mattiello et al., 2024; Teng et al., 2024; Souza et al., 2025). For example, Wood and Bowman (2021) showed a positive effect of cover cropping across four of six soil health indicators in dozens of on-farm assessments in North America. Most of the strategies adopted to increase soil health are focused on increasing the number of plant residues left in the field or the reduced mineralization rate of the organic compounds, thus increasing soil C is typically limited by the photosynthesis of croplands (Janzen et al., 2022). Therefore, shifting from monocropping (with a winter fallow) to different successions and rotations (with cash crops or cover crops) is critical to increase plant biomass inputs (Mattiello et al., 2024) and promote soil multifunctionality (Schipanski et al., 2014). Further adding complexity, Yang et al. (2024) showed that increasing the diversity of crop rotation system improved the soil health index and grain yield while reducing the greenhouse gas emissions. Thus, introducing cover crops during the off-season and increasing the complexity of crop rotations may be a potential alternative to enhance soil health while sustaining food production.

In the Brazilian savannah, tropical agroecosystems benefit from weather conditions that allow soybean to be grown as the main crop, followed by a second cash crop (usually maize or cotton) or different types of cover crops (single, mixed, or intercropped with cash crop). Although the soybean–maize rotation is the most common double cropping system in tropical Brazil, fallow after soybean is still a common practice. According to CONAB - Companhia Nacional de Abastecimento (2025), of the approximately 30 million hectares of soybean cultivated in the tropical region, only about 14 million hectares are followed by maize in a second season. Increasing the net primary productivity of an agroecosystem by introducing more biodiversity into the system is crucial to increase soil C cycling (Cates and Jackson, 2019; Amelung et al., 2020) while enhancing soil health and cash crop yields (Anghinoni et al., 2021; Balbinot Junior et al., 2024; Mattiello et al., 2024; Souza et al., 2024). In this way, management strategies can be adopted to prolong the presence of plant species with complementary functional traits throughout the year, thereby sustaining complementary functions to agroecosystems. It can be achieved by crop succession (reduced diversity and complexity) or crop rotation (increased diversity and complexity). While long-term experiments support that complex rotations are crucial to enhance resilience and yield stability (Gaudin et al., 2015; Bowles et al., 2020; Li et al., 2019; Peng et al., 2024), evidence that the proportion of time with crop cover (including cash crops, cover crops or forage leys) had stronger impact than crop diversity (Garland et al., 2021). In tropical systems, keeping the soil covered for as long as possible (regardless of species) is even more critical to reduce the effects of climate stressors.

We hypothesized that crop diversification (both successions or rotations) would enhance soil health and thereby increase crop yield and resilience of tropical agroecosystems to weather variation across the years compared to soybean-fallow monocropping. To test this hypothesis, we evaluated a long-term (16-year) experiment containing seven cropping systems with gradients of intensification and diversification (quantity and quality) of plant residues within each crop system. By examining soil health, crop yield and stability, our research aims to test if crop successions and rotations can be implemented to bolster long-

term crop production through enhancing soil health in a changing climate.

## 2. Material and methods

### 2.1. Site characterization and experimental design

A long-term no-tillage experiment was established to evaluate different cropping systems (successions and rotations) at the *Fundação de Apoio à Pesquisa Agropecuária de Mato Grosso* (Fundação MT) research farm in Itiquira, MT, Brazil (17° 09' 27" S, 54° 45' 12" W), at an altitude of 490 m above sea level. The location and experimental conditions are typical of the Brazilian savannah (Cerrado biome). The soil is classified as "Latossolo Vermelho distrófico" according to the Brazilian Soil Classification System (Santos et al., 2018), corresponding to a Rhodic Ferralsol (IUSS Working Group WRB, 2022). This soil contains approximately 664 g kg<sup>-1</sup> clay, 167 g kg<sup>-1</sup> silt, and 169 g kg<sup>-1</sup> sand. The field experiment started in February 2008, when a composite soil sample from the 0–20 cm depth was collected and analyzed for soil characterization (Table 1).

All crops used in treatments were established using a no-tillage system. The study included soybean monocropping as a control treatment, where no crop was grown after soybean (Fallow; one species) three crop successions treatments with soybean followed by pearl millet (*Pennisetum glaucum*, CS1; two species), ruzigrass (*Urochloa ruziziensis*, CS2; two species), and maize (*Zea mays*, CS3; two species); and three treatments with rotations involving pearl millet, sunn hemp (*Crotalaria ochroleuca*), ruzigrass, and maize-ruzigrass intercropping (CR1; five species, CR2; four species, and CR3; four species) following soybean. The crop sequence of each treatment is shown in Fig. 1. The experiment followed a randomized complete block design with four replicates over 16 years, with each plot measuring 560 m<sup>2</sup>. Detailed descriptions of the treatments and experimental design are provided in Tables S1–S6.

In the first year of the experiment, the area was prepared using a subsoiler, plow harrow, and leveling harrow. Basal fertilization for soybean included 45 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>, 38 kg ha<sup>-1</sup> of Ca<sup>2+</sup>, and 25 kg ha<sup>-1</sup> of S, with additional rate of K<sub>2</sub>O applied after planting. Similar fertilization protocols were followed for maize, with variations in N and Zn application rates. Lime and gypsum applications were made periodically to correct soil acidity and increase calcium content. Pest, weed, and disease control measures were implemented as needed, with specific treatments applied to control ruzigrass using glyphosate and mechanical methods.

### 2.2. Crop management and fertilization

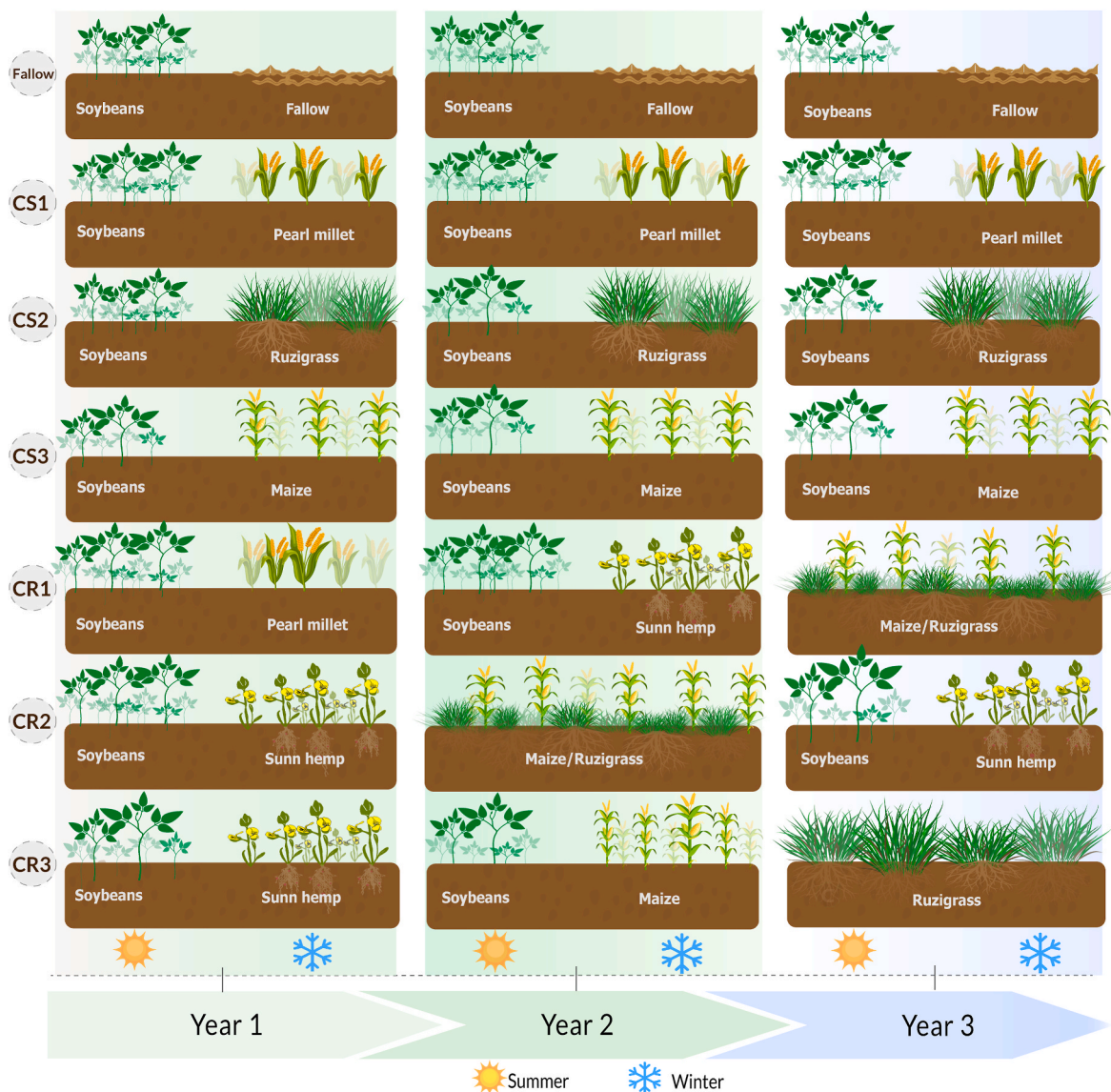
Over the years, the crops utilized in the main season included different cultivars of soybean and hybrid of first season maize across the growing season according to the local recommendation (Table S1). Additionally, second season maize, pearl millet, sunn hemp, and ruzigrass (Table S2). Sowing dates ranged from October 4–28 (soybean, Table S1), November 21–December 22 (first season maize, Table S1), February 5–26 (second season maize, Table S2), February 27–March 28 (pearl millet, Table S2), February 27–March 26 (sunn hemp, Table S3), and February 27–March 23 (ruzigrass, Table S3).

Lime was applied at 2 Mg ha<sup>-1</sup> on the soil surface in treatments during 2012/2013, 2014/2015, 2016/2017, and 2018/2019. In 2017/2018, all treatments received 3.2 Mg ha<sup>-1</sup> gypsum (Table S3). Soybean received a basal application of 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 38 kg Ca<sup>2+</sup> ha<sup>-1</sup>, and 25 kg S ha<sup>-1</sup> as simple superphosphate, followed by 90 kg K<sub>2</sub>O ha<sup>-1</sup> as KCl at planting (Table S5). Maize was fertilized with 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 40 kg N ha<sup>-1</sup>, and 2.5 kg Zn ha<sup>-1</sup> at planting, plus 50 kg K<sub>2</sub>O ha<sup>-1</sup> immediately after planting and 120 kg N ha<sup>-1</sup> at the V4 stage (Table S5). Off-season maize received 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 40 kg N ha<sup>-1</sup>, and 2.5 kg Zn ha<sup>-1</sup> at planting, followed by 30 kg K<sub>2</sub>O ha<sup>-1</sup> and 60 kg N ha<sup>-1</sup> at the V4 stage (Table S6). Fertilizer sources for maize included monoammonium

**Table 1**  
Soil chemical characterization in the topsoil layer in 2008, before the experiment installation.

Depth	pH (CaCl <sub>2</sub> )	P	K <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H + Al	CEC <sub>pH7</sub>	BS	SOC
cm		mg dm <sup>-3</sup>		cmol <sub>c</sub> dm <sup>-3</sup>					%	g kg <sup>-1</sup>
0–20	5.1	20	86	2	0.9	0	6.1	7.6	46	15.7

H + Al: the potential acidity; CEC<sub>pH7</sub>: cation exchange capacity at pH = 7.0; SOC: soil organic carbon; BS: Ca-Mg-K saturation.



**Fig. 1.** Crop sequence of the seven different cropping systems, including a soybean-fallow, three crop successions (CS1, CS2, and CS3), and three crop rotations (CR1, CR2, and CR3) within a three-year rotation in the Brazilian savannah region. Detailed management practices can be found in the supplemental material.

phosphate (MAP; 11 % N, 52 % P<sub>2</sub>O<sub>5</sub>), urea (45 % N), potassium chloride (KCl; 60 % K<sub>2</sub>O), and zinc sulfate (6 % S, 25 % Zn). Cover crops were not fertilized. Pest, weed, and disease control was performed to avoid interfering factors (Tables S12 and 13). All cover crop residues were left on the soil surface (Table S14), with no removal or incorporation, detailed information about the residue biomass production are described in Mattiello et al. (2024). The cover crops were managed through a combination of mechanical (knife roller) and chemical (Glyphosate at 3.5 L ha<sup>-1</sup>) methods, and terminated three months before planting the cash crops.

### 2.3. Grain yield and yield stability

During the whole experiment, the stand of soybean and maize was determined considering 4.5 m<sup>2</sup> of the two central rows of each plot, where the average values were extrapolated in plants ha<sup>-1</sup>. The grain yield of soybean and maize (season and off-season) was measured using 16 m long and 5 m wide areas within each plot using a harvester where the grain weight and moisture were determined. The grain yield was then converted to 13 % moisture and expressed in Mg ha<sup>-1</sup>. For this study, we used the grain yield of the last six years, corresponding to the last two complete crop rotation cycles (growing seasons from 2018/2019 to 2023/2024) to calculate the average grain yield production of

each treatment and also the yield resilience, represented by the coefficient of variation, as demonstrated in the following equation:

$$\text{Yield resilience (C.V.)} = \frac{\sqrt{\sum \frac{(GY_i - GY_{\text{mean}})^2}{n}}}{GY_{\text{mean}}} * 100 \quad (1)$$

The yield resilience is represented by the C.V. and presented in percentage, where the  $GY_i$  is the grain yield for individual plots,  $GY_{\text{mean}}$  is the average of the grain yield for the treatment corresponding to the individual plot and  $n$  is the number of observations for each treatment within each growing season.

Crop yield sensibility to the variation of precipitation and temperature was explored using the amplitude of grain yield among all the plots within each of the last 6 years as affected by the cumulative precipitation and mean air temperature measured within each growing season. The daily precipitation and air temperature were obtained from a weather station nearby the field experiment. We performed linear regressions using the amplitude ( $\Delta$ ) of annual grain yield (i.e., highest yield - lowest yield) the  $\Delta$  precipitation and  $\Delta$  mean air temperature, which were calculated according to the following equations:

$$\text{Amplitude of annual grain yield} = P_{\text{max,year}} - P_{\text{min,year}} \quad (2)$$

$$\Delta \text{ Precipitation (\%)} = \frac{\text{Precipitation}_{\text{GS}}}{\text{Precipitation}_{\text{GS-avg}}} * 100 \quad (3)$$

$$\Delta \text{ Mean air temperature (\%)} = \frac{\text{Mean air temperature}_{\text{GS}}}{\text{Mean air temperature}_{\text{GS-avg}}} * 100 \quad (4)$$

To calculate the amplitude of annual grain yield,  $P_{\text{max, year}}$  is the highest observed productivity within each year,  $P_{\text{min, year}}$  is minimum observed productivity within each year across all treatments. The  $\Delta$  precipitation (%) is the ratio of precipitation in each growing season ( $\text{Precipitation}_{\text{GS}}$ ) and the average precipitation of all growing seasons for this experiment ( $\text{Precipitation}_{\text{GS-avg}}$ ). The  $\Delta$  mean air temperature (%) is the ratio of the mean air temperature in each growing season ( $\text{Mean air temperature}_{\text{GS}}$ ) and the average air temperature of all growing seasons for this experiment ( $\text{Mean air temperature}_{\text{GS-avg}}$ ). These ratios were multiplied by 100 to be expressed in percentages. The closer  $\Delta$  precipitation and  $\Delta$  mean air temperature are to 100 %, the more similar the year's precipitation and temperature were to the averages of the previous years of the experiment.

Using the agronomic concept of partial factor productivity for water (Cassman et al., 2003), as described in the following equation:

$$\text{Water use efficiency (WUE)} = \frac{\text{Grain Yield}}{P_{\text{cum}}} \quad (5)$$

where we considered the ratio of the annual average grain yield of each treatment and the cumulative precipitation ( $P_{\text{cum}}$ ) of each growing season as the water use efficiency (WUE) for precipitation in each treatment because it relates the output (grain yield) with a single input (water from precipitation) expressed in  $\text{kg ha}^{-1} \text{mm}^{-1}$ .

#### 2.4. Soil sampling and analysis

Soil sampling was carried out in March 2024, after 16 years of experiment establishment. Mini-trenches (30 x 30 x 30 cm) were open within each plot. Disturbed samples were taken for 0–10, 10–20 and 20–30 cm layers, while undisturbed samples were taken for the same layers (another trench wall) using a volumetric ring (5 x 5 cm, ~100  $\text{cm}^3$ ). The soil samples taken at this moment were then subject to further analysis. Soil field-saturated hydraulic conductivity (Kfs) was measured at three locations across the plot recording the time to infiltrate 330 mL of water in a 14.5 diameter PVC ring according to the 'simplified falling-head' methodology proposed by Bagarello et al. (2004). Available phosphorus (P), potassium ( $\text{K}^+$ ), calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ), pH ( $\text{pH CaCl}_2$  0.01  $\text{mol L}^{-1}$ ), potential acidity (H + Al), and potential cation

exchange capacity at pH 7 ( $\text{CEC}_{\text{pH7}}$ ) were determined using analytical methods described by Raji et al. (2001). Bulk density (BD) was determined using the core method with 100  $\text{cm}^3$  cylinders, as the ratio between dry soil mass and ring's volume. Wet macroaggregate stability was determined using a vertical oscillator (Yoder, model MA-148) with three sieves (2000, 250, and 53  $\mu\text{m}$ ) moving at a speed of 30 oscillations per min for 10 min. The percentage of macroaggregates (MA) was calculated by summing aggregate mass for >2000 and 250  $\mu\text{m}$  classes, dividing by the total mass, and multiplying by 100. Mean weight diameter (MWD) was calculated as the sum of the proportion of aggregates in each size fraction, with each proportion weighted by the mean diameter of aggregates in that size fraction. Total porosity (TP) was calculated as the mass difference of the saturated and dry soil in the volumetric ring. Soil water contents at matric potential of -6 kPa and -10 kPa were determined using tension tables as described by Ball and Hunter (1988). Soil macroporosity (MaP) was computed as the difference between soil water content at saturation and at -6 kPa. Water-filled pore space (WFPS) was calculated by dividing volumetric moisture at -6 kPa by total porosity as indicated in Wienhold et al. (2009). We also calculated two indexes suggested by Reynolds et al. (2002): i) soil water storage capacity (SWSC) defined as the ratio between water content at field capacity (FC, -10 kPa soil water potential) and TP ( $\text{SWSC} = \text{FC}/\text{TP}$ ); and ii) soil aeration capacity (SAC) calculated as the ratio between drained pores at soil water potential of -10 kPa (ACt) and TP ( $\text{SAC} = \text{ACt}/\text{TP}$ ). Soil penetration resistance (SPR) was obtained with the moisture of the sample at matric potential of -10 kPa. The equipment used was an electronic bench penetrometer with a 4-mm diameter cone and 30° of angle tip and a constant penetration rate of 10  $\text{mm s}^{-1}$ . Measures were obtained in the center of undisturbed soil samples. Measures from the upper (1 cm) and lower portions (1 cm) were excluded from averaging. Part of the soil subsamples were kept at 4 °C for a week and used to measure potential enzymatic activities of  $\beta$ -glucosidase (BG) and arylsulfatase (ARYL) were measured as described by Tabatabai (1994), the autoclave citrate extracted protein (ACE-protein) content was determined based on the method described by Wright and Upadhyaya (1996), and used as a biological indicator of soil health (Huriisso and Culman, 2021; Naasko et al., 2024). Soil organic carbon (C) and total nitrogen (N) were determined by dry combustion on a LECO® CN-2000 elemental analyzer (furnace at 1350 °C in pure oxygen).

##### 2.4.1. Soil health assessment

Soil health was assessed using the multifunctionality approach (Garland et al., 2021) to develop a soil health index (SHI) composed of 23 indicators distributed into three main components (i.e., chemical, physical, and biological). The minimum dataset for the chemical component was composed by the eight measured indicators (soil  $\text{pH}_{\text{CaCl}_2}$ , available  $P_{\text{resin}}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Al}^{3+}$ , potential acidity, and  $\text{CEC}_{\text{pH7}}$ ). The minimum dataset for the soil physical component was composed by 10 measured indicators (field-saturated hydraulic conductivity, weighted mean diameter of aggregates, percentage of macroaggregates, macroporosity, total porosity, soil water storage capacity, water-filled pore space, soil aeration capacity, soil penetration resistance, bulk density). Finally, the minimum dataset of soil biological components was composed of five measured indicators (arylsulfatase and  $\beta$ -glucosidase activity, soil C content, soil N content, and ACE-protein content).

Soil indexing processes were composed of three sequential steps. First, soil indicators of each component (chemical, physical, and biological) were separately subjected to a principal components analysis (PCA) using *prcomp* function and visualized with the *fviz.pca.biplot* function of the factoextra package (Kassambara and Mundt, 2017). In this method, each indicator was centered and scaled (mean = 0; s.d. = 1). For each component, the PCA generates coordinates for each axis in each soil layer and each soil sample. The scores of each axis were weighted by multiplying the proportion of variation explained by each

axis. The score of each sample and each component was calculated by averaging the weighted score for all axes, as represented by equation (4):

$$\text{subSHI}_{\text{score}} = \frac{1}{n} \sum_{i=1}^n (p_i * PC_i) \quad (6)$$

where  $n$  is the number of principal components (PCs),  $p_i$  is the proportion of the eigenvector for the  $i$ -th PC axis,  $PC_i$  is the score of the  $i$ -th principal component axis. The sum represents the product of the eigenvector proportions and their corresponding PC axis scores, summed over all PCs. The entire expression is averaged by dividing by  $n$  to represent the mean.

Sequentially, we performed a Spearman correlation analysis to verify the direction (positive or negative) of the relationship between each indicator with the final SHI score for chemical, physical, and biological components. The values of the eigenvector vector were considered that larger positive values reflects higher SHI for the variables known as overall positive ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{CEC}_{\text{pH}7}$ , P,  $\text{K}^+$ ,  $\text{Al}^{3+}$ , C, N,  $\beta$ -glucosidase, arylsulfatase, ACE-protein, MaP, WFPS, SAC, SWSC, Total porosity, macroaggregation, MWD and Kfs) and larger negative values reflects higher SHI for variables known as overall negative ( $\text{Al}^{3+}$ ,  $\text{H}^+ + \text{Al}^{3+}$ , SPR and BD) as observed by the correlation of the measured indicators with the final score of chemical (Table S7), biological (Table S8) and physical (Table S9) components. To facilitate the interpretation, the *sub*-SHI for each component was obtained by rescaling the weighted score within each component to vary between 0 and 1, where 1 represents the highest value obtained for each category and 0 is the minimum score.

Finally, the three *sub*-SHIs calculated by component (chemical, physical and biological) were integrated into an overall soil health index (SHI) using a weighted additive approach (Equation (5)).

$$\text{Soil health index (SHI)} = \sum_{i=1}^n (\text{subSHI}_i * W_i) \quad (7)$$

where, *sub*-SHI<sub>*i*</sub> is the final score for each component (chemical, physical, and biological) and  $W_i$  the weighted value of each component. Regardless of the number of indicators of each component, each *sub*-SHI had an equal weight (33.33 %) in the final index (Cherubin et al., 2016).

This approach using 23 indicators aligns with the conceptual foundation of composite indices, where the main goal is to capture the emergent properties of the system rather than maximize the separation for each individual variable for agronomic recommendation. Therefore, the inclusion of such indicators support the robustness of the SHI by integrating both dominant and subtle contributors to soil function, reflecting in an ecologically meaningful assessment of soil health.

## 2.5. Data analysis

We used the R software (R Core Team, 2024) to perform our data processing and analysis. A linear mixed-effects model was fitted to the data using the *lmer* function from the *lme4* package in R (Bates et al., 2015), in which average values (for each soil indicator, *sub*-SHI, SHI, and soybean yield and the C.V. soybean yield) for each plot as dependent variables while the treatments were considering the fixed effect and the replicates the random effect of the model. To assess the significance of the treatments, an analysis of variance (ANOVA) was performed on the fitted model using the *anova* function, model diagnostics were conducted by plotting the residuals of the fitted model to assess the assumptions of the linear mixed-effects model, such as normality and homogeneity of variance. To further investigate the differences between treatments, pairwise comparisons were performed using the *emmeans* package (Lenth, 2020), the treatments were considered different according to Tukey's test ( $p < 0.05$ ). To investigate the resilience of the system through the impact of weather variation across the year on crop performance, we fitted a linear regression where the amplitude of grain yield as explained by the  $\Delta$  precipitation and  $\Delta$  mean air temperature. In

addition, a linear regression was performed to understand how the average grain yield observed for each treatment across all years of the experiment is explained by the annual precipitation. Finally, the WUE for precipitation of each treatment was used to perform another linear regression to explore the relationship between soil health index and yield response to variation in precipitation over time.

## 3. Results

### 3.1. Soil health indicators

Overall, the results indicate that crop diversification strategies, through both crop succession and rotation after soybean, enhanced multiple soil health indicators compared to the soybean monocropping (Fallow) (Table 2). Most of the treatment effects were concentrated in the topsoil layer (0–10 cm), showing treatment effects in more than half of the measured indicators. On the other hand, only two indicators showed treatment effects on the 10–20 and 20–30 cm layers (Figs. S1–S8). Therefore, the results of topsoil (0–10 cm) were the central focus of discussion in this study.

In terms of chemical indicators, the response pattern is unclear among the treatments. For example, crop rotation (CR3) with sunn hemp or maize following soybean and another year with ruzigrass instead of soybean exhibited lower concentration of phosphorus (P) ( $21.1 \text{ mg dm}^{-3}$ ) when compared to fallow, and to other two rotations, CR1 and CR2 ( $45.1$ ,  $33.9$  and  $35.9 \text{ mg dm}^{-3}$ , respectively). Soybean followed by ruzigrass (CS2), and all three crop rotations had higher  $\text{Mg}^{2+}$  content, resulting in an average increase of 1.69-fold compared to fallow. For  $\text{CEC}_{\text{pH}7}$ , the three treatments that involve ruzigrass (single or in intercropping) CS2, CR2, and CR3) increased the  $\text{CEC}_{\text{pH}7}$  of the soil by 1.27-fold compared to fallow (Table 2).

Regarding physical indicators, the mean weight diameter (MWD) of aggregates and percentage of macroaggregates (MA) were higher in all successions and rotations compared to the fallow, increasing MWD by 1.90-fold and the proportion of macroaggregates by 1.10-fold (Table 2). The total porosity (TP) increased by 1.03-fold when comparing CR3 relative to CS3, while CS3 had higher BD than CR3. The soil penetration resistance (SPR) was higher in CS3 than CR2. The field-saturated hydraulic conductivity was 14.7-fold higher in the CS2 than in the fallow treatment. The CS2, CR1, CR3 had on average 1.20-fold more water-filled pores space (WFPS) than the fallow (Table 2).

As for the indicators of biological activity, the treatment with soybean followed by ruzigrass (CS2) and CR1 resulted in the highest potential activity of arylsulfatase and  $\beta$ -glucosidase as well as C and N content of the soil compared to the fallow treatment and the CS3. The CS2 treatment showed higher values of potential arylsulfatase activity (11.6-fold),  $\beta$ -glucosidase (5.6-fold), C (1.6-fold), and N (1.8-fold) compared to the fallow treatment, and 3.2-, 2.4-, 1.5, and 1.6-fold higher than CS3, respectively. Similarly, CR1 resulted in increases of 9.0-, 5.8-, 1.3-, and 1.5-fold compared to fallow and 2.5-, 2.3-, 1.2-, and 1.4- compared to CS3.

Overall, the soybean-ruzigrass crop succession (CS2) also resulted in the highest soil C and N contents, being 1.62 and 1.77-fold higher than the fallow treatment, respectively, or 1.48 and 1.64-fold higher than CS3 (Table 2). Crop rotation increased on average 1.32 and 1.38-fold soil C and N content compared to the fallow. Fallow had also the lowest value of ACE-protein content, but a significant difference was only detected with CR2, which includes sunn hemp twice in the three-year rotation (Table 2).

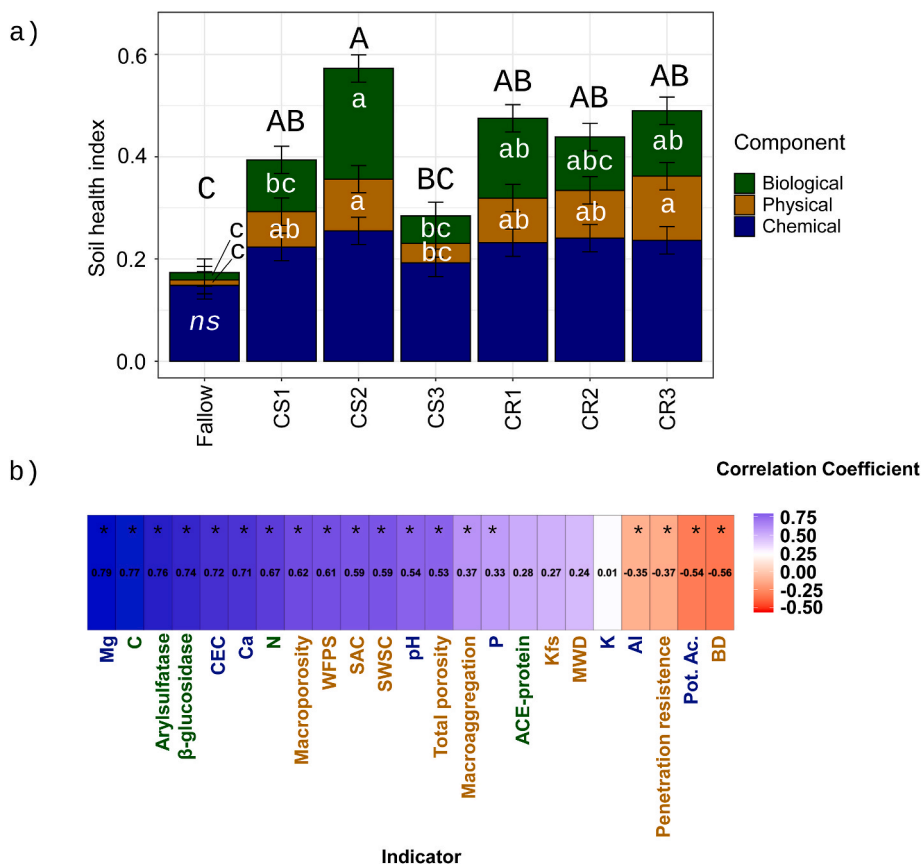
### 3.2. Soil health index

The soil health index (SHI), which combines biological, physical, and chemical components, revealed notable differences among treatments for the topsoil (0–10 cm) (Fig. 2a). Changes detected in SHI were driven by the biological and physical components, while the integrated

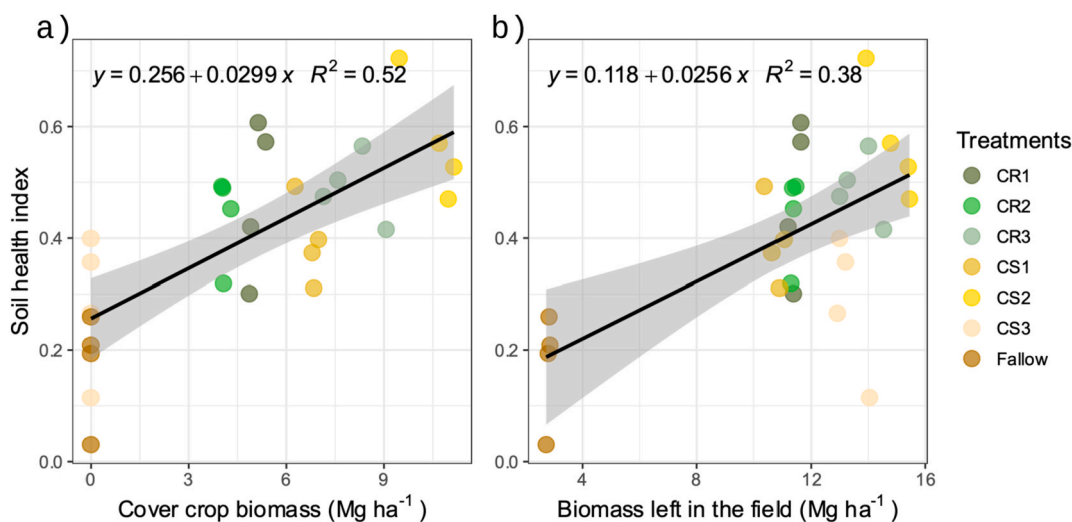
**Table 2**  
Mean values of soil chemical, physical, and biological indicators at 0–10 cm depth for each cropping system.

Cropping systems	Chemical indicators								CEC <sub>pH7</sub>		
	pH	P	Ca <sup>2+</sup>	Mg <sup>2+</sup>	K <sup>+</sup>	Al <sup>3+</sup>	H + Al				
		mg dm <sup>-3</sup>	mmol <sub>c</sub> dm <sup>-3</sup>								
Fallow	5.75 <sup>ns</sup>	45.1a	45.5 <sup>ns</sup>	18.7b	2.40 <sup>ns</sup>	0.30 <sup>ns</sup>	31.3 <sup>ns</sup>		98.0b		
CS1	6.04	27.9 ab	61.6	28.3 ab	3.27	0.00	20.6		115 ab		
CS2	5.98	29.4 ab	67.5	34.1a	3.09	0.00	23.4		129a		
CS3	5.75	29.5 ab	54.9	25.5 ab	1.83	0.10	30.3		113 ab		
CR1	5.93	33.9a	61.0	30.4a	2.49	0.00	23.6		117 ab		
CR2	6.00	35.9a	64.2	30.4a	2.92	0.00	22.2		120a		
CR3	5.88	21.1b	63.7	31.8a	1.69	0.00	25.7		123a		
Physical indicators											
	MWD	MA	MaP	TP	WFPS	SWSC	SAC		SPR	BD	Kfs
	mm	%	m <sup>-3</sup>	m <sup>3</sup>	%	m <sup>-3</sup>	m <sup>3</sup>		MPa	Mg m <sup>-3</sup>	mm h <sup>-1</sup>
Fallow	1.48b	81.6b	0.09 <sup>ns</sup>	0.53 ab	0.13d	0.75 <sup>ns</sup>	0.18 <sup>ns</sup>		3.10 ab	1.31 ab	4.0b
CS1	2.53a	90.7a	0.11	0.55 ab	0.17bcd	0.78	0.21		2.89 ab	1.28 ab	33.7 ab
CS2	2.77a	91.5a	0.12	0.56 ab	0.23a	0.82	0.24		2.79 ab	1.23 ab	58.9a
CS3	2.46a	89.3a	0.09	0.53b	0.14cd	0.76	0.12		3.66a	1.33a	15.9 ab
CR1	3.06a	91.9a	0.11	0.55 ab	0.19 ab	0.81	0.21		2.71 ab	1.27 ab	41.2 ab
CR2	2.98a	90.6a	0.11	0.56 ab	0.17bcd	0.78	0.21		2.49b	1.23 ab	31.8 ab
CR3	3.06a	92.8a	0.14	0.58a	0.17bc	0.79	0.25		2.97 ab	1.19b	33.3 ab
Biological indicators											
	ARYL	BG	ACE-Protein		C	N					
	μgPNF g <sup>-1</sup> h <sup>-1</sup>	μgPNF g <sup>-1</sup> h <sup>-1</sup>	mg g <sup>-1</sup>		g kg <sup>-1</sup>	g kg <sup>-1</sup>					
Fallow	15.3d	17.7c	1.69b		22.6d	1.3d					
CS1	88.1bcd	66.3abc	1.95ab		27.9bcd	1.7bcd					
CS2	177.0a	105.0a	2.41ab		36.5a	2.3 a					
CS3	55.6cd	44.6bc	2.09ab		24.7cd	1.4cd					
CR1	138.0 ab	103.0a	2.33ab		30.3b	1.9 ab					
CR2	86.6bcd	69.3abc	2.74a		29.0bc	1.7bcd					
CR3	104.0bc	81.2ab	2.40ab		30.8b	1.7bc					

H + Al = Potential acidity (H<sup>+</sup> + Al<sup>3+</sup>), CEC<sub>pH7</sub> = Cation exchange capacity, MWD = Mean weight diameter, MA = Macroaggregation, MaP = Macroporosity (>50 μm), TP = Total porosity, WFPS = Water-filled pore space, SWSC = Soil water storage capacity, SAC = Soil air capacity, SPR = Soil penetration resistance at water matric potential of -10 kPa, BD = Bulk density, Kfs = field-saturated hydraulic conductivity, ARYL = Arylsulfatase activity, BG = β-glucosidase activity, ACE-protein = autoclave citrate-extracted protein. Fallow represents the treatment soybean-fallow. CS1, CS2 and CS3 represent the crop succession soybean-pearl millet, soybean-ruzigrass and soybean-maize, respectively. CR1 represent the crop rotations with soybean-pearl millet, soybean-sunn hemp and maize-ruzigrass (intercropping); CR2 the rotation with soybean-sunn hemp, maize-ruzigrass (intercropping) and soybean-sunn hemp; CR3 the rotation soybean-sunn hemp, soybean-maize and ruzigrass. \*Means followed by different letters indicate statistical difference among treatment according to Tukey's test ( $p < 0.05$ ).



**Fig. 2.** Soil health index at the topsoil (0–10 cm) composed of biological, physical, and chemical components (a) and the heatmap of the correlation between measured indicators and the final soil health index (b). CS1, CS2 and CS3 represent the crop succession soybean-pearl millet, soybean-ruzigrass and soybean-maize, respectively. CR1 represents the crop rotations with soybean-pearl millet, soybean-sunn hemp and maize-ruzigrass (intercropping); CR2 the rotation with soybean-sunn hemp, maize-ruzigrass (intercropping) and soybean-sunn hemp; CR3 the rotation soybean-sunn hemp, soybean-maize and ruzigrass. The capital letter denotes statistical significance of soil health index among treatments according to Tukey’s test ( $p < 0.05$ ). The lower-case letter denotes statistical significance among treatments within soil health components according to Tukey’s test ( $p < 0.05$ ). The spearman coefficients are represented in the heatmap (bluish colors represent positive coefficient and the negative for reddish colors) with the indicators color coded for each component.

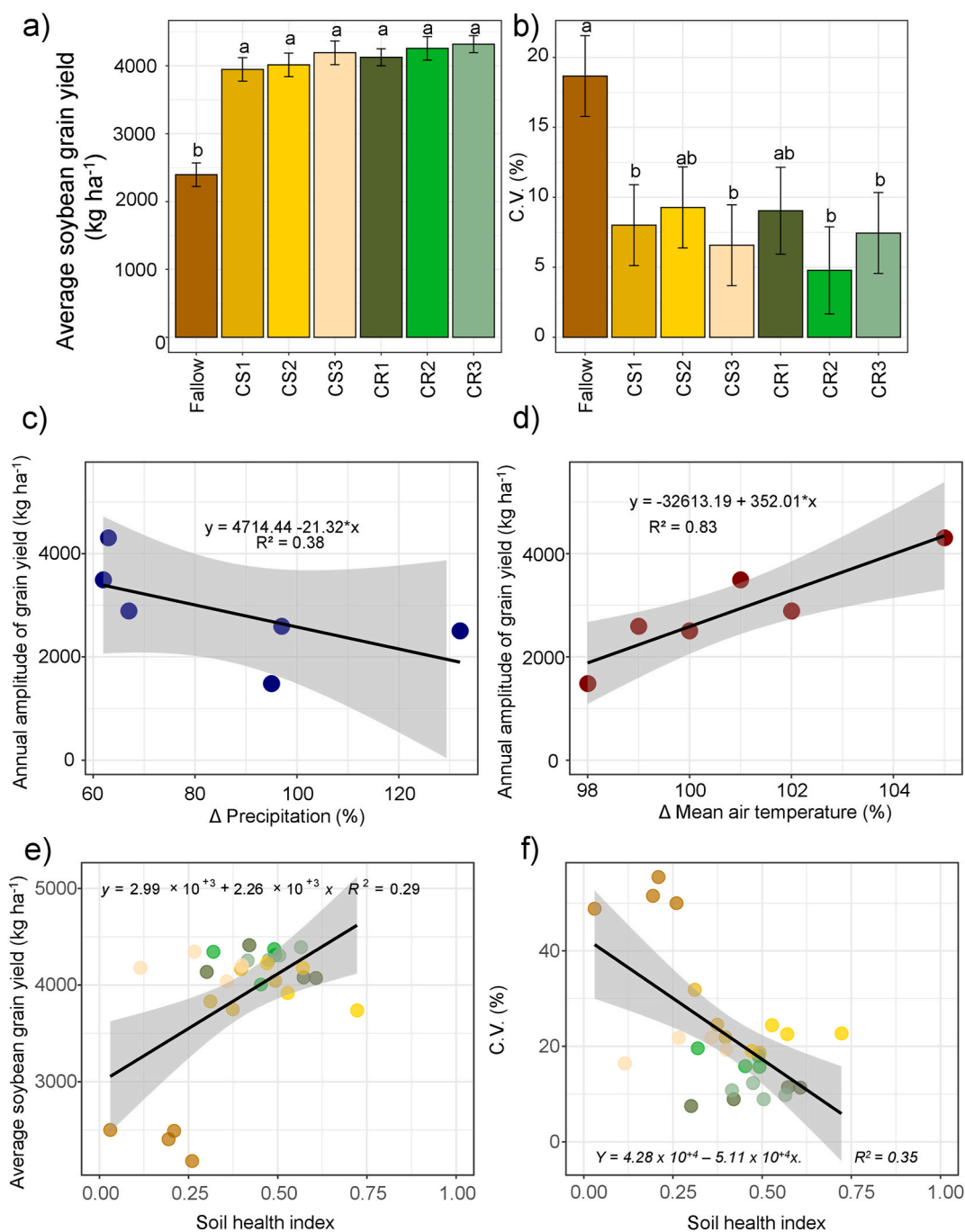


**Fig. 3.** Linear regression between the soil health index (SHI) and average cover crop biomass produced across the different treatments (a). Linear regression between SHI and average cover dry mass left in the field across the different treatments (b). CS1, CS2 and CS3 represent the crop succession soybean-pearl millet, soybean-ruzigrass and soybean-maize, respectively. CR1 represent the crop rotations with soybean-pearl millet, soybean-sunn hemp, and maize-ruzigrass (intercropping); CR2 the rotation with soybean-sunn hemp, maize-ruzigrass (intercropping) and soybean-sunn hemp; CR3 the rotation soybean-sunn hemp, soybean-maize, and ruzigrass.

chemical component was not sensitive to changes. The soybean-fallow monoculture consistently exhibited the lowest SHI score ( $p < 0.05$ ) compared to the crop succession (CS) and crop rotation (CR) treatments, considering both species diversity and sequencing. The succession of soybean-ruzigrass (CS2) achieved the highest SHI value of 0.56, differing from soybean-fallow and soybean-maize (CS3). The other crop

succession and all rotations (CS1, CR1, CR2, and CR3) resulted in SHI score higher than the fallow.

Interestingly, among all measured indicators, the magnesium ( $Mg^{2+}$ ) showed the highest positive correlation coefficient (0.79) with the SHI, followed by organic C (0.77), arylsulfatase activity (0.76),  $\beta$ -glucosidase activity (0.74),  $CEC_{pH7}$  (0.72) and  $Ca^{2+}$  (0.71) (Fig. 2b). These

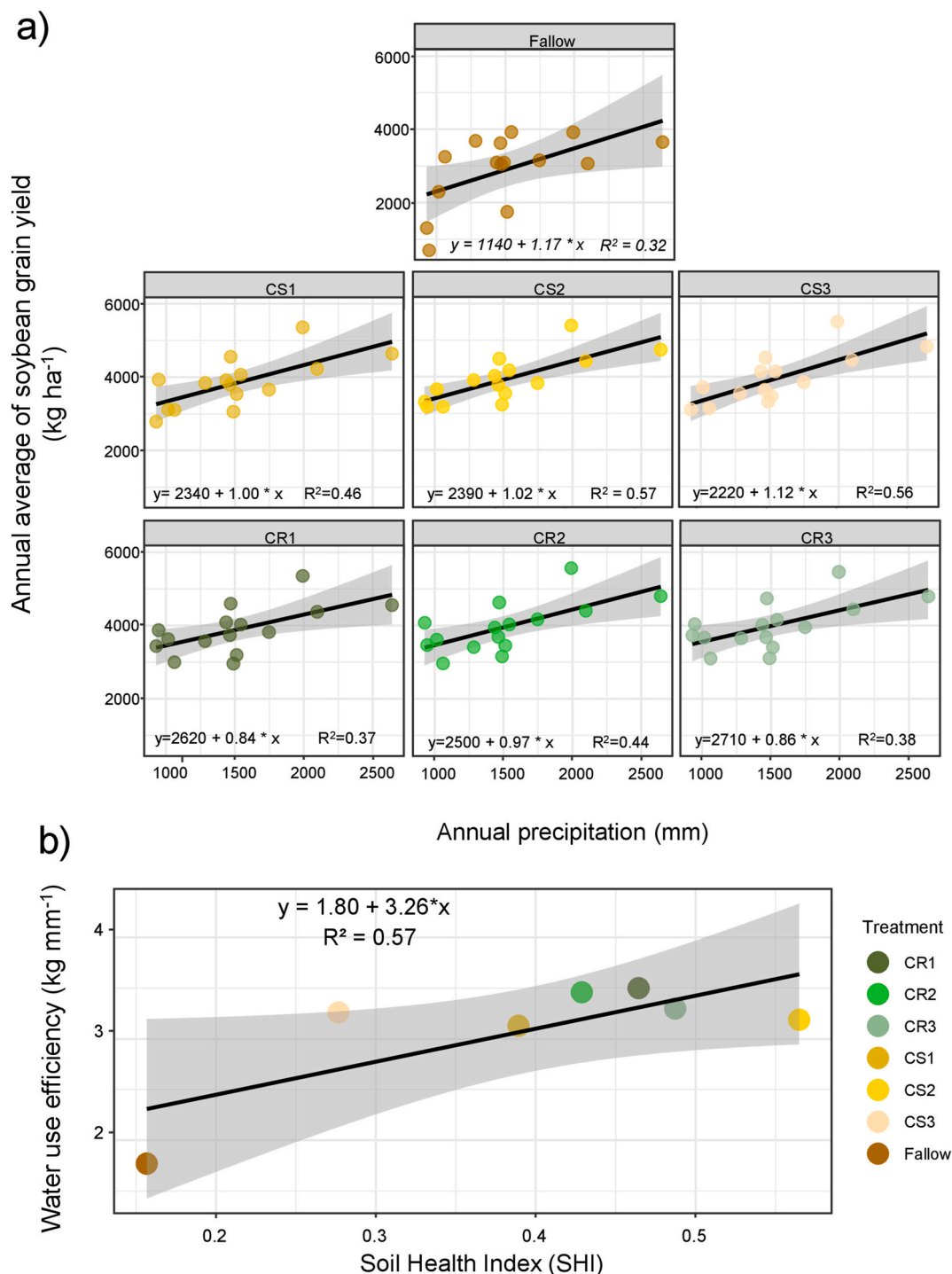


**Fig. 4.** Average soybean grain yield (kg ha<sup>-1</sup>) over the last six growing seasons (2018/2019 to 2023/2024) for each cropping system treatment (a). Yield resilience expressed as the mean annual coefficient of variation (C.V., %) of soybean yield within each year and averaged over the same period (b). Annual amplitude of soybean grain yield (difference between the highest and lowest yields across all plots within a growing season) as a function of  $\Delta$  precipitation (%), defined as the percentage deviation of precipitation in each growing season relative to the experimental average (c). Annual amplitude of soybean grain yield as a function of  $\Delta$  mean air temperature (%), defined as the percentage deviation of mean air temperature in each growing season relative to the experimental average (d). Linear regression between the soil health index (SHI) and average soybean yield across treatments (e). Linear regression between SHI and the average annual C.V. of soybean yield across treatments (f). CS1, CS2 and CS3 represent the crop succession soybean-pearl millet, soybean-ruzigrass and soybean-maize, respectively. CR1 represent the crop rotations with soybean-pearl millet, soybean-sunn hemp, and maize-ruzigrass (intercropping); CR2 the rotation with soybean-sunn hemp, maize-ruzigrass (intercropping) and soybean-sunn hemp; CR3 the rotation with soybean-sunn hemp, soybean-maize, and ruzigrass. Lower-case letters in panels (a) and (b) indicate statistically significant differences among treatments (Tukey's test,  $p < 0.05$ ).

indicators are closely linked to soil organic matter, microbial activity, and nutrient availability, storage, and cycling. Other indicators such as MP, WFPS, SAC, SWSC, pH, TP, MA, and P were positively correlated with the SHI, while ACE-protein, Kfs, MWD, and  $K^+$  were not correlated with the SHI. Conversely, some indicators were negatively correlated with the SHI, suggesting that lower values of these indicators are associated with better soil health. The strongest negative correlations were observed for SPR (-0.37),  $Al^{3+}$  (-0.35), H + Al (-0.54) and BD

(-0.56). These indicators are typically associated with soil compaction and acidity issues, which can adversely affect soil health.

When evaluating the influence of biomass inputs on soil health, the average cover crop biomass production of the previous 6 years emerged as a significant explanatory variable for SHI (Fig. 3), showing a positive relationship ( $R^2 = 0.52$ ). Similarly, the total biomass returned to the soil (comprising both cover crop and cash crop dry mass) also exhibited a positive relationship with SHI ( $R^2 = 0.38$ ).



**Fig. 5.** Annual average of the soybean grain yield as affected by the precipitation for each treatment (a) and the water use efficiency ( $kg\ mm^{-1}$ ), the slope of the linear regression for each treatment, as affected by the average soil health index of each treatment (b). CS1, CS2 and CS3 represent the crop succession soybean-pearl millet, soybean-ruzigrass and soybean-maize, respectively. CR1 represent the crop rotations with soybean-pearl millet, soybean-sunn hemp, and maize-ruzigrass (intercropping); CR2 the rotation with soybean-sunn hemp, maize-ruzigrass (intercropping) and soybean-sunn hemp; CR3 the rotation soybean-sunn hemp, soybean-maize, and ruzigrass.

### 3.3. Grain yield and yield stability

Crop succession and rotation strategies affected soybean grain yield (Fig. 4a). The crop rotation treatments average yields ranged from 4128 to 4321 kg ha<sup>-1</sup>, not differing from crop succession treatments that yielded between 3948 and 4192 kg ha<sup>-1</sup>. Soybean-fallow monocropping had the lowest yield (2282 kg ha<sup>-1</sup>). Fig. 4e shows a regression based on the average grain yield based on the average grain yield of each replicate (n = 28) across the six-year period. We observed a positive relationship between SHI and the average yields ( $R^2 = 0.29$ , Fig. 4e).

The annual amplitude of crop yield among treatments increased during growing seasons with lower  $\Delta$ precipitation and higher  $\Delta$ temperature (Fig. 4c and d). The average precipitation and mean air temperature during all growing seasons were 1507 mm and 26.3 °C. In two growing seasons where the precipitation was 60 % of the average precipitation observed during the soybean growing season, the amplitude of grain yield reached up to 4309 kg ha<sup>-1</sup>. In contrast, for the growing season with precipitation higher than the average (>100 %) the yield amplitude was reduced to 2507 and 2596 kg ha<sup>-1</sup>. Warmer growing seasons also increased the amplitude of grain yield, so the highest amplitude (4309 kg ha<sup>-1</sup>) was observed when the mean air temperature was 105 % of the historic average. The regression analyses in Fig. 4c and d were based on six observations (n = 6), each representing one growing season between 2018/2019 and 2023/2024.

The coefficient of variation (C.V.) of soybean grain yield over time varied significantly among treatments (Fig. 4b). The fallow treatment exhibited the highest C.V. at 19 %, indicating the greatest yield variability. In contrast, the C.V. values for the successions and rotations treatments ranged from 5 % to 9 %, suggesting more stable yields and resilient systems. However, the C.V. values for CS2 and CR1, both at 9 %, were not different from those of the fallow treatment or the other cropping system treatments. The regression shown in Fig. 4e was based on the average C.V. of each replicate of the studied treatments (n = 28) across the six-year period. We observed a negative relationship between SHI and the C.V. ( $R^2 = 0.35$ , Fig. 4f). A negative correlation ( $R^2 = 0.45$ ) was also observed between average yield and C.V. (Fig. S9). The regression analysis revealed that for every 1 % increase in C.V., the average yield decreased by 95 kg ha<sup>-1</sup>.

#### 3.3.1. The relationship between water use efficiency (WUE) for precipitation and soil health index

The impact of annual precipitation on soybean grain yield varies across different crop rotation systems and fallow conditions (Fig. 5a). Analysis of the regression slope coefficients highlighted these differences in yield responsiveness. Soybean-fallow monocropping exhibited the highest sensitivity to precipitation, with slope coefficients of 1.17 (Fallow). In comparison, crop succession treatments showed moderate responsiveness, with a slope coefficient ranging from 1.00 (CS1) to 1.12 (CS3). Crop rotation (CR) treatments showed the lowest sensitivity to precipitation changes, with slope coefficients of 0.84 (CR1), 0.86 (CR3), and 0.97 (CR2).

A significant positive linear relationship was observed between the SHI and WUE (Fig. 5b). This relationship suggests that as soil health index increases, soybean WUE also increases. The SHI values among the treatments ranged from 0.15 in the soybean-fallow to 0.56 in the soybean-ruzigrass (CS2) treatment (Fig. 2a). The WUE values were lower in soybean-fallow treatment (1.77 kg ha<sup>-1</sup> mm<sup>-1</sup> of soybean grain) than in the different crop succession and rotations that ranged from 3.13 (CS1) and 3.50 (CR1) kg ha<sup>-1</sup> mm<sup>-1</sup> of soybean grain (Table S11). This trend was especially notable considering that soybean-fallow treatment had the lowest WUE value (1.77 kg ha<sup>-1</sup> mm<sup>-1</sup> of soybean grain and the lowest SHI value (0.15) suggesting that crop diversification increases the use efficiency of precipitation for maintaining grain yield in healthier soil.

## 4. Discussion

### 4.1. Long-term effect of crop diversification on soil health indicators

Soil chemical indicators did not show a clear response pattern to crop diversification. The patterns of soil chemical change in croplands can be explained by several key factors, such as liming and fertilization management, nutrient exportation, the accumulation of organic matter, and the intensity of nutrient cycling driven by introduced species and the activity of soil organisms (Garland et al., 2021). For example, we observed that CEC<sub>pH7</sub> was lower in soybean-fallow monocropping (Table 2) than cropping systems with the introduction of ruzigrass (CS2, CR2 and CR3), a clear indication that lower C inputs led to reduced soil organic matter and consequently, reflects in a reduction of the negative charges responsible for the cations exchange capacity (CEC<sub>pH7</sub>, Soares and Alleoni, 2008). The soybean-maize succession did not differentiate from cropping system with the winter fallow or cropping systems with the introduction of ruzigrass (CS2, CR2 and CR3), which also sustained higher content of Mg<sup>2+</sup> than soybean-fallow, as previously observed by Crusciol et al. (2015), which may be related to the great capacity of this deep-rooted grass to extract and recycle this element from the deeper soil layers. However, this pattern was not exclusive because it was also observed in CS1 and CS3 without ruzigrass. Therefore, further research is needed to test not only this hypothesis, but other potential pathways, such as higher CEC due to soil organic matter, can contribute to increasing Mg<sup>2+</sup> content in the soil. Indeed, Mg<sup>2+</sup> showed positive correlation with SHI (r = 0.79, Fig. 3), not because it is the most sensitive nutrient management, but because it may be a proxy for improved nutrient availability and CEC in diversified systems. Soares and Alleoni (2008) showed that between 50 and 95 % of the CEC<sub>pH7</sub> in Oxisols is derived from soil organic matter sources. Thus, soil C can translate into higher retention of cation in the soil, including Mg<sup>2+</sup>. Finally, higher P content was observed in the soybean-fallow monocropping, which can be related to the reduced P exportation with grains (Bender et al., 2015), since this treatment presented the lowest grain yield across the years (Fig. 4). It is a trade-off of the system that can be carefully considered in an integrated assessment of soil health.

Long-term soybean-fallow monocropping but not soybean-maize succession (CS3) led to soil structure degradation over time (i.e., lower MWD and macroaggregation), which negatively affected the soil's capacity to conduct and storage water (i.e., lower Kfs and WFPS), compared to the more diversified cropping systems, regardless of species and sequence used (Table 2). The benefits of conservation agriculture practices on soil structure are well documented in the literature, primarily related to the key role of increased soil C content, higher abundance and diversity of roots and organisms in aggregate formation and stability (Tisdall and Oades, 1982; Six et al., 2004; Li et al., 2024). Locatelli et al. (2025) found that for the Brazilian savannah, complex cropping systems with cover crops can increase soil C and N stocks by approximately 20 % and 29 %, respectively, while doubling macroaggregation in the 5–10 cm and 10–20 cm depths compared to soybean-maize double cropping. Enhanced aggregation has been linked to increases in C content within macro- and micro-aggregates, contributing to improved soil structure and C stabilization. Soil structure of the diversified cropping system was also more functional, increasing soil's capacity to conduct and retain water, a critical function to increase the resilience to climate changes (Locatelli et al., 2025).

On the other hand, crop diversification did not result in positive effects on soil indicators related to soil compaction when compared to the soybean-fallow monocropping (i.e., bulk density, porosity, and soil penetration resistance). Therefore, root growth may be constrained by mechanical stress similarly in all systems, although soil aggregation is better in diversified systems. Despite the apparent contradiction, this result is strongly supported by previous studies under tropical and subtropical conditions (e.g., Moraes et al., 2016; Anghinoni et al., 2021; Sattolo et al., 2021). A higher frequency of machinery operations

required to manage a more diverse crop sequence may neutralize or even prevail over the benefits provided by crop diversification. Strategies to enhance the physical functionality of agroecosystem soils are critical in intensified agroecosystems (Anghinoni et al., 2021; Luz et al., 2023). Well-structured soil with lower compaction and improved water infiltration allows for deeper root growth, better access to nutrients and moisture, and greater resilience to drought and waterlogging.

Crop diversification improved soil biological indicators, particularly in the system with the introduction of ruzigrass (CS2 and CR1, CR2 and CR3). African grasses, such as ruzigrass, are well adapted to Brazilian savannah conditions, being widely cultivated as a single off-season crop, in cover crop mixes or intercropped with maize (Baptistella et al., 2020; Souza et al., 2024). Species of *Urochloa* genus are characterized by high aboveground biomass production and vigorous, abundant, and deep root system (Baptistella et al., 2020). Therefore, the introduction of ruzigrass increased C inputs in the systems (Mattiello et al., 2024), increasing soil C and N contents, and boosting microbial activity (higher activity of arylsulfatase and  $\beta$ -glucosidase and higher ACE-protein content) (Table 2). These benefits were also reported in other studies involving ruzigrass (Dieleman et al., 2012; Anghinoni et al., 2021; Damian et al., 2021; Barbosa et al., 2023).

It is particularly interesting that CR2 resulted in higher C and ACE-protein because this rotation was the only one where a legume cover crop was used twice within a 3-year rotation. ACE-protein, for example, has been reported as an indicator related to soil N cycling in the soil (Wade et al., 2020; Naasko et al., 2024) and is also part of the routine in some commercial soil health laboratories (Krishnan et al., 2020). This is likely because legume cover crops contribute to biologically  $N_2$  fixation and promote N cycling, thereby enhancing the pool of organically-bound nitrogen detected by the ACE protein test (Hurisso and Culman, 2021). Legume cover crops have also been associated with higher soil C stocks (Nicoloso and Rice, 2021) due to their potential to increase microbial activity (Hu et al., 2024). McDaniel et al. (2014) showed that adding one or more cover crops in rotation increased the total N by 13 % while only 8.5 % for total C, mainly as a result of the higher microbial biomass. These findings underscore the critical role of incorporating high-biomass grasses (ruzigrass) and legume cover crops (sunn hemp) in crop successions and rotations, particularly in systems like CS2 and CR1, which consistently improved biological indicators such as soil C and N, potential enzyme activities, contributing to enhanced nutrient cycling in agroecosystems.

As positive correlations were observed between biological indicators such as soil C and N, potential enzyme activities, with physical indicators such as Kfs (Fig. S11) highlights the intrinsic linkage between biological and physical functionality of the soil. Enzymes, such as arylsulfatase and  $\beta$ -glucosidase are sensitive indicators of microbial metabolic activity and organic matter turnover (Mendes et al., 2024), both of which are intimately connected to soil aggregation, porosity and hydraulic conductivity (Castioni et al., 2018). Higher microbial activity often reflects higher organic substrate availability (Soong et al., 2020), which is also related to soil aggregation (Six et al., 2004). Thus, these indicators that reflect biological substrate (organic C) can also improve pore continuity and connectivity, directly influencing water infiltration (Kfs). Enhanced biological functioning in diversified cropping systems is closely related to physical functioning.

Although the SHI was designed to capture soil multifunctionality by integrating 23 chemical, physical and biological indicators, it remains essential to understand how individual metrics correlates with this composite index. In this study, we observed key biological (arylsulfatase,  $\beta$ -glucosidase, soil C and N content), physical (Kfs and SAC) and chemical (CEC and  $Mg^{2+}$ ) indicators exhibited positive correlation with the overall SHI (Fig. S11), demonstrating their role as potential indicators of soil health. These findings validate the use of arylsulfatase,  $\beta$ -glucosidase, C, N, Kfs and CEC as key indicators in soil health assessment protocols involving a smaller minimum dataset in tropical agroecosystems (Cherubin et al., 2016; Mendes et al., 2024). Although

previous studies have consistently shown that simplified minimum datasets (including, for example, five indicators) can detect the effects of soil management on soil health (Cherubin et al., 2016, 2021; Mendes et al., 2024), a large number of indicators allows to better capture the interactions that define soil multifunctionality.

Indeed, despite of some indicators (e.g.  $Ca^{2+}$ , SAC and SWSC) did not differ across treatments, when analyzed univariately (Table 2), PCA clustered these indicators with other components that were well-correlated with the SHI. Thus, the inclusion of a wide range of indicators enhances the SHI ability to reflect emergent ecosystem properties, linking soil indicators to services such as crop yield and water-use efficiency (Figs. 2–4). In any case, integrating these indicators into a soil health index is a fundamental step in understanding the multifunctionality of soil, whether the number of indicators is large or small (Cherubin et al., 2025).

#### 4.2. Soil health index as a tool for understanding the changes in the soil multifunctionality of cropping systems

We sought to integrate all the measured indicators into one SHI to better understand how the different cropping successions and rotations affect the soil multifunctionality considering the complex interactions of biological, chemical, and physical indicators. Detecting soil changes in a variety of crop diversification experiments is not an easy task because the trade-offs and synergies associated with growing multiple crops occur simultaneously in complex ways and are also time-dependent, making it difficult to isolate factors and compare data (Hufnagel et al., 2020). Our results showed that introducing one or more crops, specially cover crops, into succession or rotation systems over the long term can increase multiple soil health indicators, such as soil C and N, and enzyme activities relative to monocropping. It corroborates that management strategies prolonging the presence of plant species with complementary functional traits throughout the year can be equal or even more important than plant diversity for maintaining soil multifunctionality (Garland et al., 2021). When increasing the diversity, all the crop rotations differed from the soybean-fallow treatment. However, soybean-ruzigrass cropping succession system sustained the greater soil multifunctionality score (56 %), with greater differences from the negative reference (soybean-fallow) and from soybean-maize, the most traditional crop succession in Brazilian Savannah. This highlights the crucial role of biodiversity and the continuous growth of plants, especially species such as ruzigrass year after year (Baptistella et al., 2020; Anghinoni et al., 2021; Silva et al., 2024), in enhancing soil health by increasing biological activity and improving soil structure, thereby promoting long-term soil health.

When considering increasing the plant biodiversity for enhancing soil health, it is important to consider the functional traits of plants introduced into agroecosystems. Our results showed that cropping succession with the same plant biodiversity but replacing maize by ruzigrass enhanced soil multifunctionality (higher SHI). Likely, these changes can be explained by two main mechanisms. First, it is important to consider not only the quantity of biomass the crop is returning to the soil but also the quality of the plant biomass (Barel et al., 2018). Second, the plant biomass allocation is different. While the breeding program focused on increasing the harvest index and reducing the C allocated in root systems for maize (Rinehart et al., 2024), tropical grasses produce a high amount of biomass and allocate a relatively higher proportion of the photosynthate into the root systems, likely an adaptation to be more resilient to water stresses. Rosolem et al. (2019) showed that the root biomass of the soybean-ruzigrass cropping system is majorly derived from ruzigrass, representing up to 80 % of the root biomass. Thus, quantity, quality, and the allocation of the plant residues left in different cropping systems with the same plant biodiversity lead to differences in soil health and functionality.

Among the measured soil indicators to compose the soil health index, biological indicators such as arylsulfatase,  $\beta$ -glucosidase and soil carbon

were the top contributors. Additionally, the physical indicators played an important role differentiating the soil health index of cropping systems (Fig. 2a). Biological indicators, such as arylsulfatase,  $\beta$ -glucosidase and SOC are sensitive indicators of soil health changes due to management practices, such as soil tillage, cover crops, organic amendment, and residue retention in an agroecosystem (Anghinoni et al., 2021; Liptzin et al., 2022; Barbosa et al., 2023; Brichi et al., 2023; Silva et al., 2024; Souza et al., 2025). Soil C is considered a key indicator of soil health and is the most frequently used indicator in soil health studies in Brazil (Simon et al., 2022; Cherubin et al., 2025) and globally (Bünemann et al., 2018). Soil enzymes are emerging indicators of soil health (Bünemann et al., 2018), and they are being incorporated into routine analysis in Brazilian soil laboratories (Mendes et al., 2019). Our results are consistent with recent advances in soil health assessment and interpretation approaches developed for Brazilian tropical soils. Mendes et al. (2024) proposed the framework for the Soil Bioanalysis (SoilBio) soil quality index, which includes the activity of  $\beta$ -glucosidase and arylsulfatase, in addition to other chemical indicators, to evaluate three soil functions (nutrient supply, nutrient storage, and nutrient cycling) in Brazilian savannah soils. Chaer et al. (2023) developed a four-quadrant model based on specific arylsulfatase and  $\beta$ -glucosidase activities, as well as soil C content, to interpret if the soils are in an equilibrium state (healthy or unhealthy soils) or in a transitional state (soils becoming healthier or unhealthy). Therefore, our results corroborate previous studies and underscore the importance of incorporating sensitive biological measures into soil health assessments to anticipate the detection of changes in soil multifunctionality (Canisares et al., 2024; Souza et al., 2025).

Finally, we encourage further studies to test and/or develop more sensitive soil physical indicators. It is still unclear whether the lack of detectable changes due to crop diversification on most of the soil physical indicators is related to the limited effect of the treatments or the lack of sensitivity of the indicators currently used. For example, Favilla et al. (2020) concluded that physical indicators of intensity processes, such as  $K_{sat}$ , were  $\sim 10$  times more sensitive at detecting the effects of ruzigrass than the indicators of capacity processes, such as field capacity. It was observed in this study, where  $K_f$ s was more sensitive than MA or BD, for example. In addition, more refined techniques, such as X-ray (micro)tomography open new avenues to deeply explore changes in soil structure functions (Rabot et al., 2018), correlating changes in soil pore structure induced by crop diversification with soil C turnover and biochemical processes (e.g., Kravchenko et al., 2019; Lucas et al., 2022). Future research must bridge the microscale understanding of soil multifunctionality with field-scale agronomic practices to build climate-smart, resource-efficient, and stress-resilient agroecosystems (Hartmann and Six, 2023).

#### 4.3. Soil health effect on system resilience to weather stresses

Global environmental change is driven by multiple interacting factors that influence ecosystem functions, including soil health and agricultural productivity (Rillig et al., 2019). Enhancing soil health is key to maintaining soil multifunctionality in croplands in a changing climate (Qiao et al., 2022; Sünemann et al., 2023; Teng et al., 2024). Our findings highlighted a contrasting resilience of the cropping system between soybean-fallow monocropping and diversification strategies using crop successions and/or rotations. Long-term effects of soybean-fallow monocropping (Fallow) led to lower average soybean yields and exhibited the highest coefficient of variation (C.V.), indicating greater yield instability in conditions that were drier and warmer growing seasons. The contrasting resilience among cropping systems was also expressed by the increase in soybean yield amplitude among treatments as weather conditions became more unfavorable (drier and warmer seasons, Fig. 4d and e).

Our findings elucidating the benefits of crop diversification on soil health, crop yield and resilience in tropical agroecosystems are consistent

with recent discoveries reported for different cropping systems and environmental conditions around the world, such as soybeans, cotton and maize in the USA (Chalise et al., 2019; Nouri et al., 2019; Bowles et al., 2020; Kane et al., 2021), maize and soybean in Canada (Gaudin et al., 2015; Bowles et al., 2020; Renwick et al., 2021), soybean in Brazil (Balbinot Junior et al., 2024; Souza et al., 2025), maize in Malawi (Steward et al., 2019), maize, wheat and rice in China (Qiao et al., 2022; Teng et al., 2024) and southern Asia countries (Jat et al., 2020, 2023). For example, in a large study in China using a dataset of soil, climate, and associated yield (maize, wheat, and rice) observations for 12,115 site-years, Qiao et al. (2022) showed that healthier soils reduced the sensitivity of crop yield to climate variability leading to both higher mean crop yield ( $10.3 \pm 6.7\%$ ) and higher yield stability (decreasing variability by  $15.6 \pm 14.4\%$ ). Our results showed a positive relationship between SHI and average and variance of grain yield, explaining 29% and 35% of data variance, respectively. Thus, reinforcing the importance of soil health metrics to capture the emergent properties of the system rather than maximize the separation for each individual variable for agronomic recommendation.

Conservation agriculture practices that enhance soil health, such as cover cropping, reduced tillage, and diverse crop rotations, can help counteract the effects of precipitation variability on crop yields (Gaudin et al., 2015; Nunes et al., 2018; Yang et al., 2020). Chalise et al. (2019) concluded that cover crops were beneficial for improving soil properties, conserving soil moisture, and enhancing soybean yield by 14% compared with no cover crop (fallow) in the USA. This emphasizes that leaving soil exposed degrades soil health and increases the vulnerability to environmental stressors, particularly water shortages (Fang and Su, 2019; Maestrini and Basso, 2018; Su et al., 2021; Cárceles Rodríguez et al., 2022; Krupek et al., 2022). Renwick et al. (2021) highlighted that incorporating cover crops into soybean-maize systems can reduce drought-related yield losses by 17%, linking yield stability to increased soil organic matter levels. It was also reported in a recent global meta-analysis, in which Vendig et al. (2023) showed that incorporating cover crops compared to a soybean-fallow treatment can increase crop yield by 13%, which is also closely related to the increase in soil organic carbon. Additionally, these practices increase water-use efficiency and optimize nutrient cycling (Attia et al., 2024), increasing the resilience of the system.

We confirmed our hypothesis that increased crop diversification through both successions and rotations can enhance soil health, which in turn supports greater crop yield stability under variable precipitation. Specifically, we observed a clear gradient of SHI across the seven cropping systems, reflecting the differences in quantity and quality of plant residues. This gradient was associated with the capacity of the cropping systems to maintain yields during years with climatic stress, thereby demonstrating that diversified cropping systems are more resilient than soybean-fallow monocropping. Healthier soils with adequate cover not only reduce evaporation losses but also enhance water infiltration, optimizing water storage and availability for plants, and mitigating the stresses caused by droughts (Nouri et al., 2021) or warming (Teng et al., 2024). For instance, Magliano et al. (2017) demonstrated that plant residues can decrease soil water evaporation rates by up to  $5.6 \text{ mm day}^{-1}$ . Additionally, remaining residues and cover cropping systems have been associated with greater grain yield increases in dry climates compared to humid ones (Pittelkow et al., 2015). The higher water retention ensures a more consistent supply of water for plants, particularly during dry periods, optimizing photosynthesis and promoting plant growth. Moreover, higher water infiltration supports microbial activity, which is essential for the decomposition of organic matter and the release of key nutrients such as C, N, and P (Butcher et al., 2020; Ghezzehei et al., 2019; Kumar et al., 2017; Ye et al., 2022). On a larger scale, Fowler et al. (2024) showed not only the grain yield, but the yield stability zones are related to soil health metrics and soil C. These findings underscore the critical need for croplands to move beyond monocropping with fallow periods, especially in tropical environments

where soil degradation is accelerated. By improving soil structure, C sequestration and biological activity, and fostering resilience to environmental stresses, healthy soils are the foundation of higher and more stable crop yields in changing climate, promoting the sustainability of rainfed systems in the face of climatic vulnerability.

Overall, the results support that soil health is a key component of agricultural systems, as it directly influences crop productivity and ecosystem resilience (Qiao et al., 2022; Souza et al., 2025). The concept of soil multifunctionality is fundamental to combining the diverse ecosystem services that healthy soils provide, including food provision, carbon sequestration, nutrient cycling, and water regulation (Garland et al., 2021). However, a significant challenge in implementing management practices to promote soil health is the absence of standardized tools that account for regional variations in soil properties, climate, and land use. In the European Union, ongoing efforts aim to establish dynamic soil health benchmarks by comparing soils to high-performing reference systems (Schram et al., 2024). This benchmarking approach shifts the focus from a binary classification of soils as healthy or degraded toward a continuous improvement model that balances productivity and other ecosystem services. Such frameworks, as the SoilBio (Mendes et al., 2024), are crucial for guiding agricultural practices in tropical regions like Brazil, where soil health improvements through diversified cropping systems can enhance resilience to climate extremes while promoting other ecosystem services (e.g. carbon sequestration). By integrating robust soil indicators and standardized monitoring methods, policymakers and land managers can optimize soil conservation strategies, ultimately contributing to the broader goal of achieving resilient and climate-smart agroecosystems.

## 5. Conclusions

Our study highlights the significant impact of long-term crop diversification on soil health, yield, and resilience to reduce the vulnerability of tropical agroecosystems to climate adversity. This study measured 23 soil health indicators, and the results of the topsoil (0–10 cm) revealed that cropping systems with increasing diversification (e.g. successions and rotations), particularly those involving tropical grasses and legume cover crops, led to a notable increase in key soil health indicators such as arylsulfatase and  $\beta$ -glucosidase activity, soil organic C content, cation exchange capacity, magnesium, macroaggregation, WFPS and hydraulic conductivity, then, increasing the overall soil health index. These improvements were closely linked to critical functions such as soil C sequestration, nutrient supply and cycling, biological activity, soil structure stability, and water retention. These collectively enhance soil multifunctionality, soil security as well as crop performance and resilience.

This study emphasizes the critical role of enhancing soil health to improve the resilience and stability of crop yields, especially in a changing climate tropical agroecosystem. Long-term soybean-fallow monocropping results in lower and more unstable soybean yields compared to crop succession and rotation systems, particularly in drier and warmer seasons. Our results highlight the importance of crop diversification based on continuous soil cover with a greater diversity of species (preferentially including deep-rooted grasses) to enhance soil health and sustain high and stable yields in tropical rainfed agriculture of the Brazilian savannah. It offers a viable pathway for addressing the dual challenges of increasing food production, as well as mitigating and adapting to climate change. Finally, we suggest that soil health index, integrating biological, physical, and chemical indicators, could be used as a comprehensive metric for assessing the effectiveness of climate change adaptation strategies in agroecosystems.

## CRedit authorship contribution statement

**Maurício Roberto Cherubin:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation,

Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lucas Pecci Canisares:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation. **Lucas Nogueira Souza:** Writing – review & editing, Formal analysis, Conceptualization. **Carlos Roberto Pinheiro Junior:** Writing – review & editing, Formal analysis, Data curation. **Moacir Tuzzin de Moraes:** Writing – review & editing, Visualization. **Felipe Bertol:** Writing – review & editing, Validation, Funding acquisition, Data curation, Conceptualization. **Larissa Bortolo:** Writing – review & editing, Formal analysis, Data curation. **Rafael Braghieri Menillo:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Carlos Eduardo Pellegrino Cerri:** Writing – review & editing, Investigation, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2025.126845>.

## Data availability

Data will be made available on request.

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