INDICATIVE SOIL BIOLOGICAL PARAMETERS IN LONG-TERM CONVENTIONAL AND CONSERVATION TILLAGE EXPERIMENT

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DOCTORAL (Ph.D.) DISSERTATION

PRIYO ADI NUGROHO

Supervisor: Dr. Zsolt Kotroczó
Associate Professor

Co-Supervisor: Dr. Katalin Juhos
Senior Lecturer

Magyar Agrár- és Élettudományi Egyetem
Hungarian University of Agriculture and Lifesciences
Agrárkörnyezettani Tanszék
Agro-Environmental Studies Department

Budapest, 2024

Hungarian University of Agriculture and Life Sciences

The doctoral school: Doctoral School of Horticulture Sciences
Institute: Environmental Science
Department: Agro-Environmental Studies

Head of doctoral school:	**Zámboriné Dr. Németh Éva, Professor, DSc** Hungarian University of Agriculture and Life Sciences, Institute of Horticulture, Department of Medicinal and Aromatic Plants
Supervisor:	**Zsolt Kotroczó, Associate professor, PhD** Hungarian University of Agriculture and Life Sciences, Institute of Environmental Science, Department Agro-Environmental Studies
Co-Supervisor:	**Katalin Juhos, Senior lecturer, PhD** Hungarian University of Agriculture and Life Sciences, Institute of Environmental Science, Department Agro-Environmental Studies

Approval signature of the head of the doctoral school and supervisors:
The candidate has fulfilled all the conditions prescribed by the doctoral school of Hungarian University of Agriculture and Life Sciences. The comments and suggestions at the thesis workshop were taken into consideration when revising the thesis so the dissertation could be submitted to a public debate.

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Supervisor Co-Supervisor

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Head of Doctoral School

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I. INTRODUCTION AND OBJECTIVES

1.1 Background

The growth of the world population has been predicted to reach 9.2 billion people in 2050 and positively correlated with the increase in food demand (Lal, 2009). To anticipate this growth, the high-quality agricultural land that can support plant growth and production must be well prepared. According to the Food and Agriculture Organization (FAO), 25 percent of the total land area has been degraded globally. Mismanagement is a predominant issue that has led to land degradation in general.

Intensive agriculture, which has been carried out for 100 years, has reduced the capacity of agricultural land significantly (Kopittke et al., 2019). The use of agricultural machinery and high input of chemicals are the characteristics of intensive agriculture. Mechanization in agriculture effectively reduces labor costs and is more efficient in time. However, it potentially decreases plant production in the long-term due to soil physical disruption. In intensive agriculture, soil tillage is a phase that most frequently uses machinery. Research indicated that intensive tillage leads to soil aggregate/structure deterioration, stimulating organic matter (OM) decomposition and accelerating CO_2 emission to the atmosphere (Buragienė et al., 2019). OM is important in soil biological activity, particularly in providing soil substrate for microorganisms. Hence, the decrease in OM caused by soil aggregate damage affects soil health.

Soil health has become a concern in the last two decades because it is closely related to the processes in the soil, such as nutrient cycling, water relations (drainage, flow, and storage of water and solutes), habitat for biodiversity (variety of plants, animals, and soil microorganisms), filtering (protect the quality of water, water, and other resources), and physical stability and support (plant root medium and anchoring support for human structures) (Lehmann et al., 2020). The high frequency of drought in several parts of the world due to climate change is also a challenge to the world's food supply. Water is essential for life on our earth. Land degradation decreases the capacity of soil to retain rainwater, so most of the water will losses either through runoff or percolation.

Conservation agriculture (CA) is an alternative agricultural system that is expected to maintain the sustainability of plant production and be environmentally friendly. CA covers three aspects, i.e., minimum mechanical disturbance (CT), species diversification (crop rotation), and permanent soil organic cover (crop residue and/or cover crops) (FAO, 2022). This experiment is more focused on the conservation tillage (CT). CT has been established in Hungary since the 1970s and has expanded continuously until recently (Birkás et al., 2017). Besides the farmers' awareness of implementing sustainable agriculture practices, the EU's incentive approach to land with reduced tillage is increasingly attracting the interest of farmers and companies to apply CT.

The ability of CT to improve soil physical properties, reduce soil erosion, and promote earthworm activities has received considerable attention from Hungarian scientists (Jakab et al., 2017; Dekemati et al., 2019). The effect of CT on yield has been partially documented (Madarasz et al., 2016; Bramdeo and Rátonyi, 2020). However, limited attention has been given to the effect of CT practice on soil microbiological activity and consequent plant nutrition potential. According to our hypothesis, CT with lower water, carbon, and nutrient loss results in a higher and temporally more balanced microbiological activity in the soil compared to plowing. This positive change can improve the plants' water and nutrient absorption capabilities and partially or fully compensate for the agrotechnical disadvantages of CT (e.g., greater weed pressure and soil-dwelling pests).

1.2 Objectives

Our study aimed to investigate the dynamic of soil microbiological activities and plant nutrition potential after the long-term practice of conservation tillage compared to intensive tillage.

In connection with these, we conducted our investigations between 2021 and 2023 in a research area continuously undergoing conservation and conventional soil management for 20 years.
Our specific questions were:

1. How does the available and reserved nutrient content of the soil change as a result of long-term conservation tillage? Does this show up in crop yields?
2. As a result of long-term conservation tillage, what dynamics does the microbial activity of the soil show during the growing season compared to conventional tillage? What differences emerged in the vertical distribution of microbial activity between the two types of tillage?
3. Can the ability of plants to absorb nutrients be increased by additionally increasing the microbiological activity of the soil on the given soil type (Luvisols)?

II. MATERIALS AND METHODS

2.1 Experimental background

2.1.1 Field experiment

The research area was located near Szentgyörgyvár, Zala county, Southwest Hungary (N 46°44'53.32" E 17° 8'48.54"E). A small farm operated by the Geographical Institute, Research Centre for Astronomy and Earth Sciences (CSFK) Hungary was selected (Fig. 6).

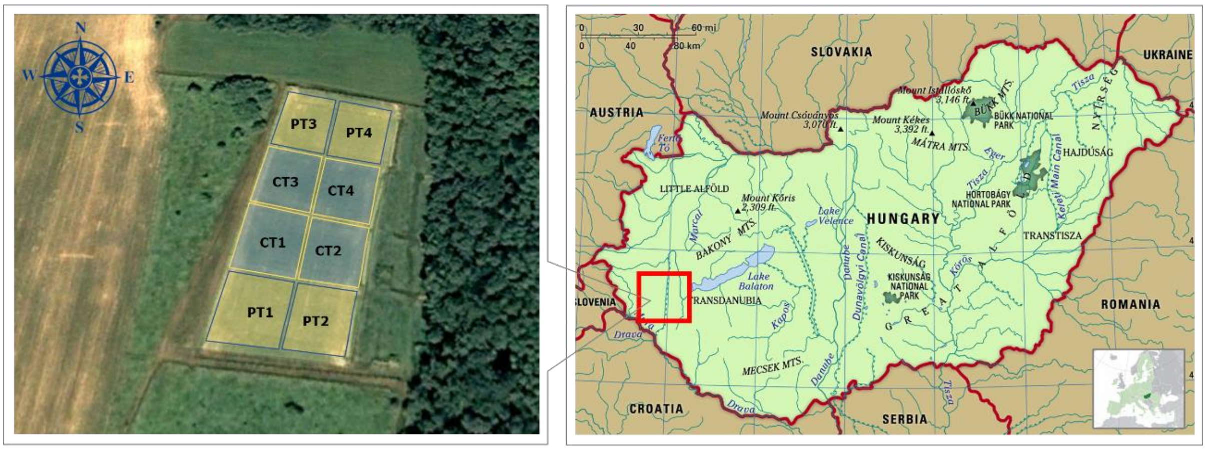


Figure 6. Research location and the layout of the field experiment. CT: conservation tillage, PT: ploughing tillage

The site elevation is 150 m above sea level at a 10% incline. The climate is classified as warm-summer humid continental (Köppen, 1936). The mean annual precipitation and air temperature during the study periods (2021-2023) were 633 mm and 11.79 °C, respectively. The monthly distribution of precipitation and air temperature is shown in Fig. 7.

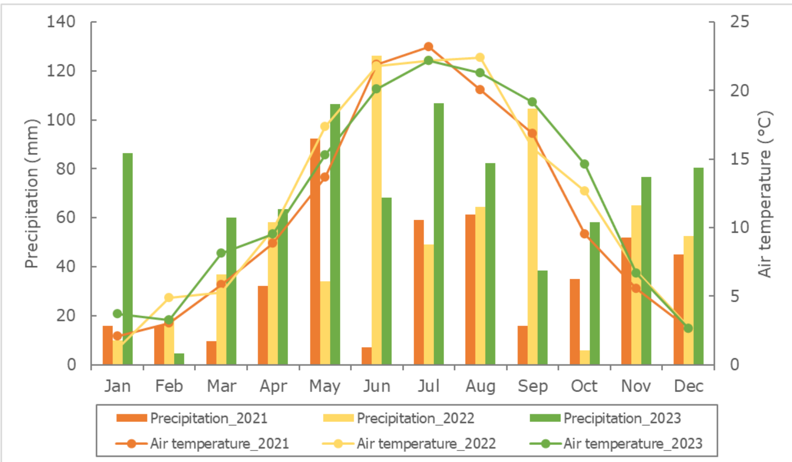


Figure 7. Monthly precipitation and air temperature (2021-2023) at the experimental site (Hungarian Meteorological Service, Sármellék station).

The soil is classified as Luvisols with low soil organic matter content (SOM) that was developed from sandy Loess, the parent material (IUSS Working Group-WRB, 2015). Soil texture is dominated by silt, followed by sand and clay. The soil acidity is categorized as neutral. The soil properties of the experimental plots are shown in Table 3.

Table 3. Physical properties in 2003 and chemical properties in 2019 of the 0–45 cm layers of a soil profile representative of the experimental field (Madarász et al., 2021)

Depth (cm)	pH (H_2O) -	pH $_{(KCl)}$ -	BD (g cm^{-3})	Clay	Silt	Sand	SOC-PT	SOC-CT
				------------%------------			-----------%-----------	
0-15	6.25	4.80	1.37	3.94	59.63	36.43	1.32	1.90
15-30	6.28	4.57	1.57	3.68	57.20	39.12	1.19	1.19
30-45	6.36	4.72	1.59	4.80	58.46	36.74	0.26	0.26

SOC= Soil organic carbon, BD= Bulk density

Two types of tillage systems, CT and PT, have been established at the study site since 2003 as a part of the SOWAP (Soil and surface water protection using CT in Northern and Central Europe) project. The PT cultivation comprised a moldboard ploughing (to a depth of 25–30 cm), harrowing, and seed-bed preparation every year. On the other hand, a plough-reduction, non-inversion tillage practice, and leaving ~30 % of crop residues covering the soil surface were implemented in the CT. A cultivator machine (8-10 cm depth) was operated for weed control. The cultivation of both plots, PT and CT, was across the land slope. After harvesting, the plant residues were left in both CT and PT. This study compared two soil tillage practices, CT and PT. Each tillage practice had four replication plots (25 m long × 24 m wide) (Fig. 6).

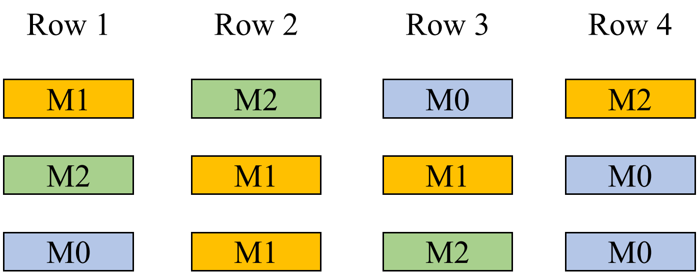
Crop rotation has been implemented since 2003, characterizing the usual grain-oriented Central European intensive agriculture systems. The crops that were planted were: maize (10 times), winter wheat (4 times), sunflower (3 times), oilseed rape (2 times), and spring barley (1 time). In addition, cover crops were planted during the five growing seasons in the CT plot from 2015 to 2018, 2020, and 2022. The present investigation was conducted in three years of growing seasons; the crops were maize in 2021 (maize I), sunflower in 2022, and maize in 2023 (maize II).

2.1.2 Pot experiment

A pot experiment study was conducted at Hungarian University of Agricultural and Life Sciences, Budai Campus. In the spring season, soil material from Szentgyörgyvár Luvisols was taken at 0-20 cm depth in the CT and PT plots. A randomized design was employed with two factors (tillage system and molasses concentration). Molasses is a type of simple sugar rapidly available for microorganism activity. Molasses were preferred for use by the soil microbes as an available substrate. There were six treatment combinations with four replications (24 experimental units/pot total). 1 kg soil was packed in the plastic pot, and three maize seeds were sown and watered regularly and cared for up to 8 weeks (Fig. 8).

Figure 8. Soil material in the pot experiment.

A week after sowing and the young plant had emerged, culling was done by selected one plant with superior growth. Pots were then put on the building terrace to open space. Three levels of molasses concentration, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2), were applied every 7-8 days. The combination of treatment and the layout of the experiment is shown in Fig. 9.



M= Molasses concentrations, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2).

Figure 9. The layout of the pot experiment

Dehydrogenase and β-glucosidase activity, permanganate oxidizable carbon concentration, plant height, and dry weight biomass were measured at the end of the experiment (after eight weeks).

2.2 Soil sampling and analysis

In the field experiment, soil samples were collected three times during the growing season and represented the growth stage of the crops. For the maize of 2021 and 2023 growing season, soil samples were collected in the initial vegetative stage (V3) on May 27, 2021, and May 20, 2023; middle vegetative stage (V7) on June 22, 2021, and July 05, 2023; and the end of a vegetative stage (VT) August 13, 2021, and August 23, 2023. The growth stages are determined following the most common way method, the "collar" method. The collar is defined as the condition where the leaf sheath and leaf blade join, as described by Reed (2017) in table 5.

Table 5. The growth stage of corn when the soil sampling

Stage	Description
V3	Third leaf collar is visible, plant begins to photosynthesize and rely on nodal root system.
V7-V(n)	Seventh to ninth leaf collars are visible, period of very rapid growth.
VT	Tasseling, tassel is emerged, transitioning to reproductive phase.

When the crop was sunflowers, the soil samples were taken on the initial vegetative stage (V4) on May 07, 2022, the generative stage (R5) on July 21, 2022, and after harvesting (H) on October 28, 2022. The growth stage of sunflowers (Table 6) refers to Schneiter & Miller (1981).

Table 6. The growth stage of sunflowers when the soil sampling

Stage	Description
V4	The number of true leaves (at least 4 cm in length) is four.
R5	This stage is the beginning of anthesis. The mature ray flowers are fully extended, and all disk flowers are visible.

Soil was sampled by soil auger at 0-5, 10-15, and 20-25 cm of depth in the CT and PT plots. The 0-5 cm samples represent the rapidly drying surface soil layer most exposed to the environment in the case of both treatments. This illustrates the potential differences between the two treatments, as the CT treatment left 30% plant residue on the soil surface, which protects the surface and reduces exposure. In the case of CT, the 10-15 cm samples characterize the layer directly under shallow cultivation, while the 20-25 cm samples characterize the undisturbed layer. In the case of PT, the 10-15 cm samples represent the middle of the plowed layer, while the 20-25 cm samples represent the denser layer directly under cultivation. Thus, for both treatments, these levels are located in the middle of the typical depth that best represents the depth interval. The advantage of this sampling is that the layers characterized by different environmental conditions are clearly separated during the tests. However, the disadvantage of this sampling is that it does not continuously represent the entire depth. The soil sample was a composite of four random sampling points. A composite sample weighing about 100 g was then put in a sealed plastic bag and refrigerated at 4 °C to keep it fresh until the analysis of soil biological properties; the maximum preservation is four weeks (Lee et al., 2007). Before preserving, soil water content (SWC) (w/w %) was determined using the gravimetric method. The soil sample was dried in an oven at 105 °C for 24 hours. For soil chemical analysis, another 100 g of composite soil sample was aired in a room with the temperature ±20 °C until the sample reached the air-dried condition.

For the pot experiment, soil samples were taken after harvesting and two days before the watering was stopped to avoid waterlogging. A 100 g soil was sampled from each pot and represented the whole part of the pot. Soil samples were then prepared for analysis as in the field experiment. Soil biological parameters, including soil enzyme activity and soil physicochemical properties, i.e., bulk density and soil nutrient concentration, were measured in the soil laboratory of Department Agro-environmental studies, Hungarian University of Agriculture and Life Sciences, Budai Campus.

Another research related to soil and water conservation, including erosion, was also conducted and published in the same plot. We used the information by Madarász et al. (2016; 2021) to enhance the discussion section of this study.

2.2.1 Physicochemical parameters

Parameters	Description
Bulk density (BD)	Ring/cylinder method (Blake and Hartge 1986).
Total organic carbon (TOC) Permanganate oxidizable carbon (POXC)	Combustion method at 900 °C (Jakab et al., 2016) $KMnO_4$ oxidation method (Weil et al., 2003)
Ammonium (NH_4-N) and nitrate (NO_3-N) concentrations	Salicylate extraction method (Kempers & Zweers, 1986). NH_4-N and NO_3-N are quantified by a spectrophotometer at 655 nm and 410 nm wavelength (absorbance mode) respectively.
Easily available P, potential available P, and total P concentrations	Easily available P by $CaCl_2$ extraction (Houba et al., 2000), Potential available P by P-Bray extraction (Bray & Kurtz, 1945) P concentration then is quantified by a spectrophotometer at 438 nm wavelength. Total P by $CaCl_2$ extraction vanadate-molybdenum reagent (Pardo et al., 2003) P concentration then is quantified by a spectrophotometer at 400 nm wavelength.
K_2O and CaO concentrations	Ammonium acetate solution method, K_2O and CaO concentrations were measured by a flame photometer

2.2.2 Monitoring of the biological properties

Parameters	Description
Dehydrogenase activity (DHA)	A 2,3,5 triphenyl tetrazolium chloride solution (TTC) method (Veres et al., 2013). DHA is measured by spectrophotometer at a wavelength of 546 nm.
β-glucosidase activity (GLU)	P-nitrophenol glucosidase (PNP-G) method. GLU is measured by spectrophotometer at a wavelength of 410 nm (Sinsabaugh et al., 1999)
Phosphatase activity (PHOS)	P-nitrophenyl phosphate (PNP-PO_4) method. PHOS is measured by spectrophotometer at a wavelength of 410 nm.
Glomalin concentration	Easily extracted glomalin related soil proteins (EE-GRSP) were measured by the Bicinchoninic acid method (BCA) proposed by Stoscheck (1990). Glomalin concentration was assessed spectrophotometrically at 562 nm wavelength.
Mycorrhiza colonization assay	Microscopic staining procedure (Phillips and Hayman, 1970)
Total fungi	Most probable number (MPN) method (Libisch et al., 2010)

2.3 Data analysis

We employed two statistical software for the data analysis. The analysis of variance (ANOVA), repeated measures multivariate analysis of variance (R-MANOVA), principal component analysis (PCA), Boxplot, and Pearson correlations were performed by IBM SPSS version 29.0 (IBM Corp., 2019). The p-value of the analysis was <0.05. The assumption test was checked for the whole data set. For the R-MANOVA analysis, three assumption checks were normality, homogeneity of variance, and sphericity. In case, the value of Greenhouse Geisser (ε) in the sphericity test > 0.6, the MANOVA should be used instead of R-MANOVA. Bonferroni's test was applied to compare the subject effects pairwise. For other analyses, only the normality and homogeneity of variance were checked. The normality of data can be proved by Kolmogorov-Smirnov test, the Shapiro-Wilk test, skewness and kurtosis, and the d'Agostino test. Data transformation can help overcome abnormal data issues in a particular situation. Furthermore, the homogeneity of variance test will determine the post-hoc test method, a Tukey's test or Games howell's test. Additionally, for the PCA, the Kaiser-Meyer-Olkin (KMO) check was performed to assess the appropriateness of the model.

A Random Forest-based (RF) approach by R software (Core Team, 2018), was employed to our study. First, the dataset was reviewed, and the Box-Cox methods treated and transformed the outliers. RF algorithm method was used for the data analysis because of the best accuracy of RF (Accuracy>0.70; Kappa>0.70) among four other algorithm methods (i.e., linear discriminant analysis, classification and regression trees, k-nearest neighbors, and support vector machines). This comparison is important to generate predictive models through the 10-fold cross-validation training in three iterations. RF indicated the best accuracy for all three factors of the variables tested. The three environmental factor RF model was performed with the package: 'randomForest' "randomForest', 'caret', "caret', and 'rfPermutate' "rfPermutate'). Moreover, the plots were generated using the 'ggplot2''‘ggplot2' package. The accuracy of the RF model was evaluated using OOB (Out-Of-Bag). The mtry and ntree parameters are optimized according to OOB. For mtry optimization, we used the 'tuneRF''‘tuneRF' package. The optimization of the two parameters was considered good when the OOB was the smallest. However, when estimating the importance of the variables, more reliable results are obtained with a larger number of ntrees. For our data, the final settings were ntree=500 or 550 and mtry=2 or 5. The importance of variables was calculated based on the overall mean deviation of accuracy (MDA) and categories. The significance of the important metrics was assessed by the 'rfPermute''‘rfPermute' package, estimating the null distribution of important metrics for each predictor variable and the observed p-value.

III. RESULTS AND DISCUSSION

3.1 The effect of conservation tillage on plant development

The plant development and yield overview during the three years of study indicated inconsistent results (Table 7). The application of CT slightly affected plant height parameters in maize I and II. However, it considerably influences stem and flower diameters in sunflowers.

Table 7. Plant development and production under different tillage systems in each crop for three years of growing season.

Crops	Plant parameters	CT	PT
Maize I	Plant height_V7 stage (cm)	117.49±4.47 ^{A}	109.75±10.90 ^{A}
	Yield (tonnes ha^{-1})	8.00±0.51 ^{A}	8.10±0.23 ^{A}
Sunflowers	Plant diameter_R5 stage (cm)	35.06±1.00 ^{B}	28.00±1.94 ^{A}
	Flower diameter_R5 stage (cm)	22.67±0.46 ^{B}	17.60±1.46 ^{A}
	Yield (tonnes ha^{-1})	3.38±0.35 ^{A}	3.42±0.74 ^{A}
Maize II	Plant height_VT stage (cm)	284.73±15.75 ^{A}	280.67±21.91 ^{A}
	Yield (tonnes ha^{-1})	9.78±0.19 ^{A}	12.36±0.38 ^{A}

Different capital letters (A and B) indicate significant differences between CT and PT ($p<0.05$)

The minimum soil disturbance in CT undoubtedly improved the physical and soil biological activity, which will be discussed in detail in the next sub-chapter. However, this situation did not contribute much to the growth and production, especially in maize. The establishment of cover crop plants (CC) in the spring of 2020 and 2022 probably resulted in a more conducive environment for maize growth, which in turn somehow affected the plant height of maize in the CT (Table 7). In addition, by covering the ground, the CC breaks the precipitation energy and assists the water seeping into the soil, which in turn hinders soil and nutrient loss. The canopy of CC shades the soil, reducing evaporation and inhibiting weed germination. The root of CC weaves through and loosens the soil, taking the microflora to a deeper layer. The CC root attracts symbiont bacteria (N-fixation) and mycorrhizal fungi (produce glomalin). When the CC dies, the CC roots will become habitats and food for the decomposing organisms (Koudahe et al., 2022).

We also measured the root capacity to assess how the CT application affects the maize growth. The root capacity was assessed with a Volt craft LCR-300 instrument, which provided a representation of the root's current functional condition and showed a significant association with root biomass. The root capacity of CT was significantly higher (10.63±2.79 nanoFarad) than PT (7.43±1.36 nanoFarad), indicating the improved soil physical properties and higher amount of organic carbon stimulated the root development in the CT.

Even though there was a tendency for better growth, CT application does not have a significant effect on crop yields. There are many variables that influence crop production, apart from soil fertility factors. Plant management is also quite important in determining the success of plant production. In the case of our investigations, the higher proportion of perennial weeds and the larger weed population may be the reason that there was not a significantly higher crop yield on the CT plots. This situation was apparent during the soil sampling time. Winkler et al. (2023) reported that weed is a common issue in CT practice; therefore, it has a high dependency on the use of herbicides in weed control. The pest attack, especially snails and mice in this experiment, also contributed to the gap in crop yield in the CT and PT. This result was not in line with our previous 10-year investigation in the same soil type. The crop yield in CT increased by 12.7% compared to in PT (Madarász et al. 2016), suggesting that the response to CT implementation varies, determined by environmental and field management factors.

3.2 Vertical and temporal changes affect the soil biological parameters

Soil biological indicators also responded differently to temporal variability. As a kind of temporal variation, the growth stage is fascinating to discuss, even though it is difficult to obtain the single effect of root activity from the effect of the plant growth stages. A previous study by Deng et al. (2019) and Zhang et al. (2005) revealed the variation of soil biological parameters in different vegetative growth periods. Soil biological parameters may be affected differently by the interaction of tillage and the crop growth stage, and it all depends on the depth at which the soil is examined. For this reason, the investigation involved two years (2021 and 2022) of data on soil biological activity to reveal the effect of vertical (soil depth) and temporal (growth stages) variability on soil biological indicators in a long-term CT experiment has been conducted; the results are shown in Table 8.

Effect of the interaction of tillage × growth stage on soil biological parameters

We assessed the effect of the interaction of the tillage system (CT and PT) × growth stages (V3, V7, VT, V4, R5, H) on the soil enzyme activities and POXC. The effect of tillage and growth stage interaction was significant only in the case of SWC ($p<0.05$). However, our results indicated that the activities of DHA, GLU, and PHOS in the PT soil were more responsive to the temporal variability, suggested by the higher coefficient of variation (CV). Conversely, the temporal variation did not change POXC concentration and SWC, in which the CV value was less than 18% (Table 8).

A higher POXC concentration occurred in the CT than in PT in the whole growth stages, excluding the V3 stage of maize and the V4 stage of sunflower (Table 8). CT stimulated a higher DHA (Table 8), although it was insignificant in all growth stages. In the CT and PT plots, the R5 growth stage of sunflower had the lowest DHA and SWC among all crop growth stages measured, but at the maize growth stage, V7 had the highest DHA. The largest GLU was recorded in the V4 stage, and the lowest activity was indicated in the V7 stage in CT and PT (Table 8). SWC was changed over time. We did not find a significant difference in SWC between the CT and PT treatments in the examined years. PHOS may trend slightly higher in CT than in PT (Table 8). The most increased PHOS of the maize and sunflower growth stages emerged in the V7 and V4 stages, respectively, in both tillage systems, CT and PT.

Effect of the interaction of tillage practice × soil depth on soil biological parameters

We also examined the effect of the interaction of two factors, tillage system (CT and PT) × soil depth (0-5; 10-15, and 20-25 cm), on the soil enzyme activities and POXC. The interaction was not significant in the case of SWC (Fig. 10e). The effect of tillage system × soil depth interaction was significant on the DHA ($p<0.001$) (Fig. 10a). In the case of PT practice, DHA did not change significantly with soil depth. On the other hand, in the CT treatment, DHA showed a tendency to decrease with soil depth. DHA differed significantly only in the 0-5 cm layer between CT and PT ($p<0.0001$). A significant effect of tillage on the DHA only in 0-5 cm depth ($p <0.0001$) in this study confirms the previous study by Álvaro-Fuentes et al. (2013).

Table 8. Descriptive statistics of the measured soil parameters in the average of 0-25 cm depth on the CT and PT plots (mean ± standard deviation, n= 216).

Crop stages	DHA (TPF μg g^{-1} dry soil)		GLU (μg mol^{-1} $hour^{-1}$)		PHOS (μg mol^{-1} $hour^{-1}$)		POXC (mg kg^{-1})		SWC (w/w %)	
	CT	PT	CT	PT	CT	PT	CT	PT	CT	PT
V3	0.57±0.35[b]	0.32±0.19[a]	1.92±1.47[a]	1.46±0.89[a]	0.95±2.40[a]	0.46±1.08[a]	505.47±155.93[a]	406.22±76.12[a]	30.44±3.80[a]	29.81±3.13[a]
V7	1.31±1.00[a]	0.70±0.52[a]	1.06±1.41[a]	0.73±0.77[a]	27.06±10.78[a]	28.23±20.66[a]	562.64±102.18[b]	448.40±120.70[a]	15.95±7.58[a]	15.11±7.77[a]
VT	0.57±0.35 [b]	0.32±0.19[a]	1.86±0.96[b]	1.11±0.73[a]	4.28±1.57[a]	3.55±2.62[a]	381.34±53.33[b]	310.53±47.79[a]	13.96±2.36[a]	11.86±2.79[a]
V4	0.29±0.171[a]	0.28±0.13[a]	2.18±0.41[a]	1.82±1.08[a]	14.32±2.67[a]	13.15±5.60[a]	415.41±89.85[a]	365.34±36.80[a]	11.78±4.51[a]	13.87±5.14[a]
R5	0.10±0.09[b]	0.04±0.05[a]	1.21±0.37[a]	1.02±0.40[a]	3.71±1.20[a]	3.41±2.41[a]	434.66±84.98[b]	333.35±85.51[a]	8.10±2.12[a]	6.69±1.92[a]
H	0.76±0.32[b]	0.48±0.21[a]	1.82±0.81[b]	1.05±0.38[a]	3.65±0.64[a]	3.26±0.70[a]	568.86±59.55[b]	392.00±42.54[a]	13.99±0.69[a]	12.96±0.89[a]
AV	0.57	0.33	1.67	1.20	8.99	8.68	478.06	375.97	19.77	18.98
SD	0.18	0.17	0.72	0.62	3.13	5.63	69.24	65.42	2.30	2.74
CV (%)	31.79	52.73	42.77	51.63	34.75	64.92	14.48	17.40	11.64	14.44

DHA= Dehydrogenase activity, GLU= β-glucosidase activity, PHOS= Phosphatase activity, POXC= Permanganate oxidizable carbon, SWC= Soil water content
Different lowercase letters (a and b) indicate significant differences between CT and PT (p<0.05)

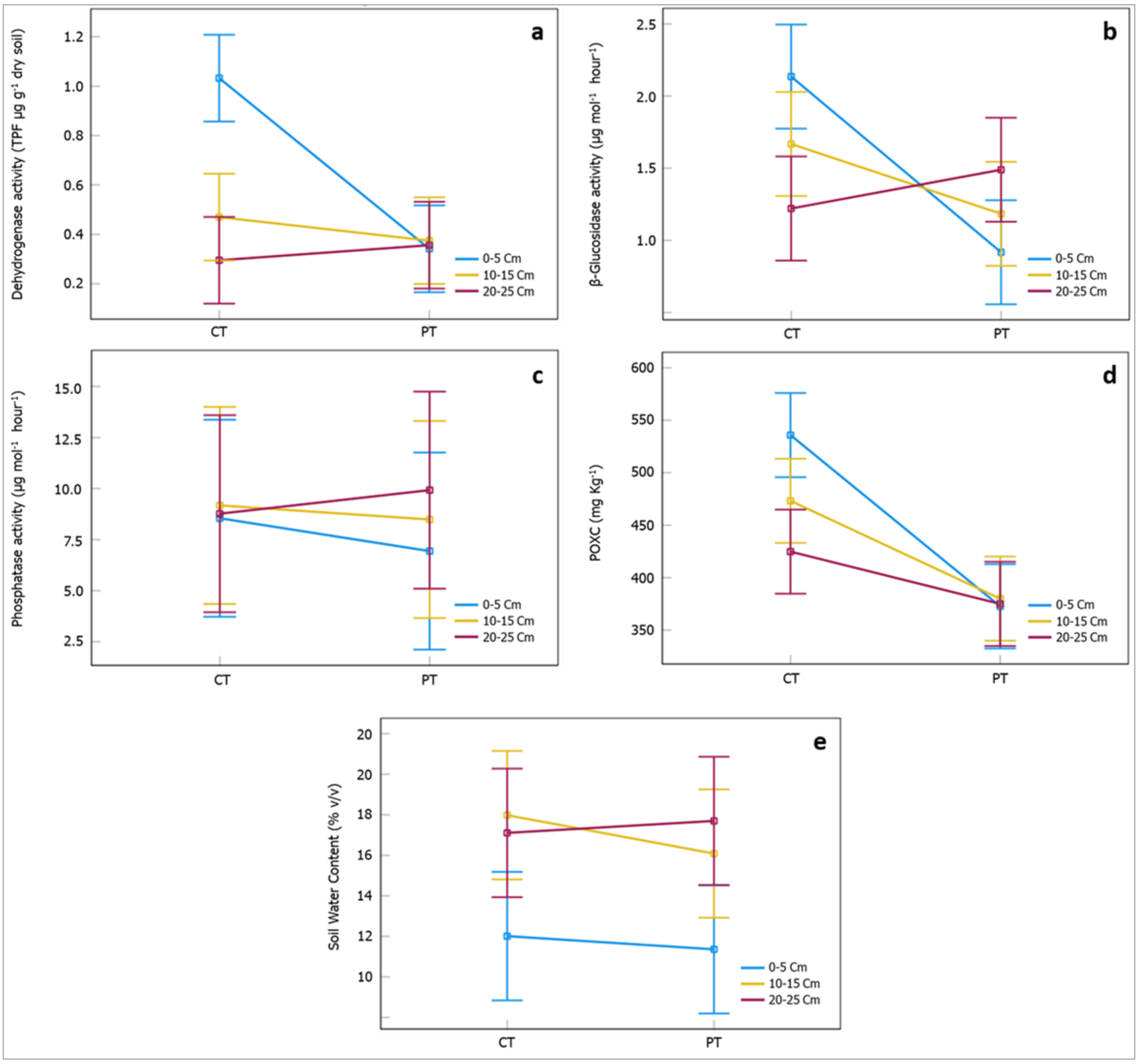


Figure 10. Interaction diagram showing the combined effect of soil depth (0-5; 10-15, and 20-25 cm) and tillage system (CT and PT) on the soil enzymes, POXC, and SWC.

A significant interaction ($p<0.0001$) of GLU was demonstrated in the CT in 0-5 and 20-25 cm depths (Fig. 10b). This means that a reverse vertical trend can be observed between the two tillage treatments. That is, in the case of CT, GLU decreasing from the upper layer to the lower layers is characteristic, while in the case of PT, increasing values can be observed from top to bottom. In the GLU, a decrease was shown over the soil depth (up to 10-15 cm) from CT to PT practice, and conversely, the GLU from CT to PT increased in the deeper soil depth (20-25 cm).

No significant interaction of tillage system × soil depth in the PHOS was revealed (Fig. 10c). The effect of tillage system × soil depth interaction was significant on the POXC ($p<0.01$) (Fig. 10d). A significant interaction was observed in the 0-5 and 10-15 cm depth of CT. The concentration of POXC increased in the whole soil depth when the tillage system shifted from PT to CT. While there was an increasing trend of POXC from the lower to the upper layers in the CT soil, in the case of PT, there was no significant difference between the soil layers, in which the POXC was the same in all investigated layers.

3.3 Sensitivity of soil biological parameters under different tillage practice

Three data of soil biological parameters from 2021 to 2023, i.e., soil enzyme activity, POXC, glomalin, and SWC, were employed to assess the sensitivity of the soil biological parameters under different tillage practices (Table 9). DHA of the three-year experiment ranged from 0.21 to 1.49 TPF µg g^{-1} dry soil. DHA was more active under CT treatment than PT, especially in 0-5 and 10-15 cm depths of all crops. The average of DHA in the sunflower season was 0.32 TPF µg g^{-1} dry soil, significantly lower than maize I and II (0.68 and 0.63 respectively TPF µg g^{-1} dry soil), suggesting crop rotation affected the DHA. Generally, GLU was highest in the surface layer and tended to decrease with the soil depth in tillage systems. The CT resulted in higher GLU than PT; however, it was significant only in the 0-5 cm layer of all crops and the 10-15 cm layer of maize II and sunflower. The significant effect of CT treatment was not found in the deepest layer of all crops. The GLU value ranged from 0.35 to 1.99 µg mol^{-1} $hour^{-1}$. Tillage treatments had no significant effect on the PHOS of all crops. We recorded the range of PHOS from 2.54 to 12.57 µg mol^{-1} $hour^{-1}$. The trend of PHOS diversified against the depth, sometimes with no increase, like in maize I and sunflower, and the reduction coincides with the depth as indicated in maize II.

CT application significantly increased the POXC in the 0-5 cm layer of maize I and maize II; meanwhile, in sunflowers, POXC was remarkably higher in the whole soil layer of CT. The POXC was also affected by crops, in which POXC in the whole depth of maize II was relatively greater than maize I and sunflowers. Although the average SWC of CT was not significantly bigger than PT, the significant tillage effect only occurred in the surface layer of maize I and maize II. Crops significantly impacted the SWC, where maize I and maize II had SWC that were bigger than sunflowers. The glomalin concentration was significantly affected by the tillage intensity in 0-5 cm depth, whereas in the deeper layer, the glomalin concentration in CT treatment was somewhat larger than in PT. Crops considerably impacted the glomalin concentration in the whole layer, in which the concentration in maize II was higher than in maize I and sunflowers.

Principal component analysis (PCA) was performed based on the correlation coefficients of all measured indicators to determine the sensitivity of soil biological indicators under different tillage practices. PCA resulted in two principal components (PCs) with eigen values larger than 1. According to PCA analysis, the value of the models was 0.60-0.82, indicating the model is adequate (Beavers et al., 2013) with a significant level <0.001. The PCs manage a maximum of 75.03% of the total variability (Table 10). The PCA test of six soil parameters suggested a discrepancy in each crop and three-year studies, shown in Fig. 11.

Table 9. Descriptive statistics of the measured soil parameters in 0-25 cm depth under different soil tillage and crop (mean ± standard deviation).

Crops	Tillage	Depth (cm)	DHA TPF µg g^{-1} dry soil	GLU µg mol^{-1} $hour^{-1}$	PHOS µg mol^{-1} $hour^{-1}$	POXC (mg kg^{-1})	SWC (% w/w)	GLOM (mg kg^{-1})
Maize I	CT	0-5	1.49±0.84 ^{BX}	2.28±1.62 ^{BX}	10.57±13.34 ^{AX}	577.16±144.83 ^{BZ}	14.24±8.98 ^{BY}	0.22±0.05 ^{AZ}
	PT		0.37±0.25 ^{AX}	0.85±0.86 ^{AX}	9.17±14.75 ^{AX}	363.01±104.90 ^{AY}	13.60±10.25 ^{AY}	0.18±0.02 ^{AZ}
	CT	10-15	0.63±0.36 ^{BX}	1.46±1.18 ^{BX}	11.10±14.08 ^{AX}	485.03±95.79 ^{AY}	23.30±8.26 ^{BX}	0.21±0.01 ^{AZ}
	PT		0.54±0.54 ^{AX}	1.19±0.78 ^{AX}	10.50±17.16 ^{AX}	397.78±98.61 ^{AY}	20.67±7.61 ^{AX}	0.19±0.02 ^{AZ}
	CT	20-25	0.34±0.20 ^{AX}	1.10±0.87 ^{BX}	10.62±13.73 ^{AX}	387.26±80.72 ^{AY}	22.81±6.99 ^{AY}	0.20±0.01 ^{AZ}
	PT		0.45±0.27 ^{AX}	1.26±0.86 ^{AY}	12.57±20.53 ^{AX}	404.37±108.30 ^{AY}	22.51±8.14 ^{AX}	0.20±0.02 ^{AZ}
Sunflower	CT	0-5	0.58±0.42 ^{BY}	1.99±0.57 ^{BY}	6.53±4.80 ^{AX}	494.80±114.30 ^{BY}	9.79±4.67 ^{AY}	0.52±0.06 ^{BY}
	PT		0.32±0.31 ^{AY}	0.99±0.48 ^{AY}	5.41±4.81 ^{AX}	382.52±47.22 ^{AXY}	9.13±4.12 ^{AX}	0.40±0.03 ^{AY}
	CT	10-15	0.31±0.25 ^{BY}	1.88±0.74 ^{BY}	7.78±5.83 ^{AX}	461.62±87.55 ^{BY}	12.67±2.70 ^{BX}	0.46±0.07 ^{AY}
	PT		0.21±0.17 ^{AY}	1.18±0.67 ^{AX}	6.94±6.70 ^{AX}	362.38±76.48 ^{AXY}	11.51±2.75 ^{AX}	0.41±0.07 ^{AY}
	CT	20-25	0.25±0.29 ^{AXY}	1.35±0.61 ^{AX}	7.37±5.79 ^{AX}	462.51±112.15 ^{BY}	11.41±3.31 ^{AY}	0.45±0.05 ^{AY}
	PT		0.26±0.21 ^{AY}	1.72±0.96 ^{AX}	7.47±6.11 ^{AX}	345.79±59.48 ^{AX}	12.89±5.64 ^{AX}	0.41±0.06 ^{AY}
Maize II	CT	0-5	1.51±1.14 ^{BY}	0.90±0.90 ^{AXY}	2.54±2.90 ^{AX}	832.53±66.46 ^{BX}	15.80±1.76 ^{BX}	1.57±0.11 ^{BX}
	PT		0.57±0.74 ^{AY}	0.35±0.34 ^{AXY}	2.73±1.77 ^{AX}	541.02±53.58 ^{AX}	14.78±4.26 ^{AX}	1.30±0.23 ^{Ax}
	CT	10-15	0.74±0.75 ^{AY}	0.85±0.45 ^{AY}	3.88±3.17 ^{AX}	709.58±92.05 ^{AX}	15.61±1.65 ^{AX}	1.38±0.19 ^{AX}
	PT		0.40±0.50 ^{AY}	0.46±0.66 ^{AX}	3.55±2.16 ^{AX}	562.94±71.78 ^{AX}	16.01±4.48 ^{AX}	1.29±0.15 ^{AX}
	CT	20-25	0.44±0.50 ^{AY}	0.77±0.76 ^{AX}	4.42±3.43 ^{AX}	620.12±113.79 ^{AX}	15.88±4.68 ^{AX}	1.27±0.19 ^{AX}
	PT		0.45±0.57 ^{AY}	0.58±0.64 ^{AXY}	4.55±3.23 ^{AX}	536.57±73.82 ^{AX}	14.52±4.48 ^{AX}	1.38±0.13 ^{AX}

Different capital letters (A and B) indicate significant differences between CT and PT (p-value < 0.05).
Different capital letters (X and Y) indicate significant differences between the crops in the same depth (p-value < 0.05).

Table 10. The results of the principal component analysis of each crop and three years study

Soil biological parameters	Maize I			Sunflower			Maize II		
	PC 1	PC 2	Communality	PC 1	PC 2	Communality	PC 1	PC 2	Communality
DHA	**0.878**	-0.164	0.797	**0.766**	0.024	0.587	**0.832**	0.248	0.753
GLU	-0.433	**0.753**	0.755	**0.710**	0.372	0.643	**0.766**	-0.459	0.797
PHOS	0.084	**0.770**	0.600	0.054	**0.934**	0.876	-0.430	**0.626**	0.576
POXC	**0.914**	-0.029	0.836	**0.656**	0.383	0.577	**0.944**	0.074	0.897
SWC	-0.134	**0.625**	0.409	**0.833**	-0.128	0.710	**0.761**	-0.124	0.595
GLOM	**0.925**	-0.178	0.887	**0.712**	0.362	0.637	0.343	**0.875**	0.882
Eigen values	3.01	1.28		3.04	1.01		3.06	1.44	
Cummulative explained variance (%)	50.11	71.42		50.61	67.17		51.08	75.03	

DHA= Dehydrogenase activity, GLU= β-glucosidase activity, PHOS= Phosphatase activity, POXC= Permanganate oxidizable carbon, SWC= Soil water content, GLOM= Glomalin concentration

PC= Principal component, Rotation method: Varimax with Kaiser normalization. Rotation converged in six literations.

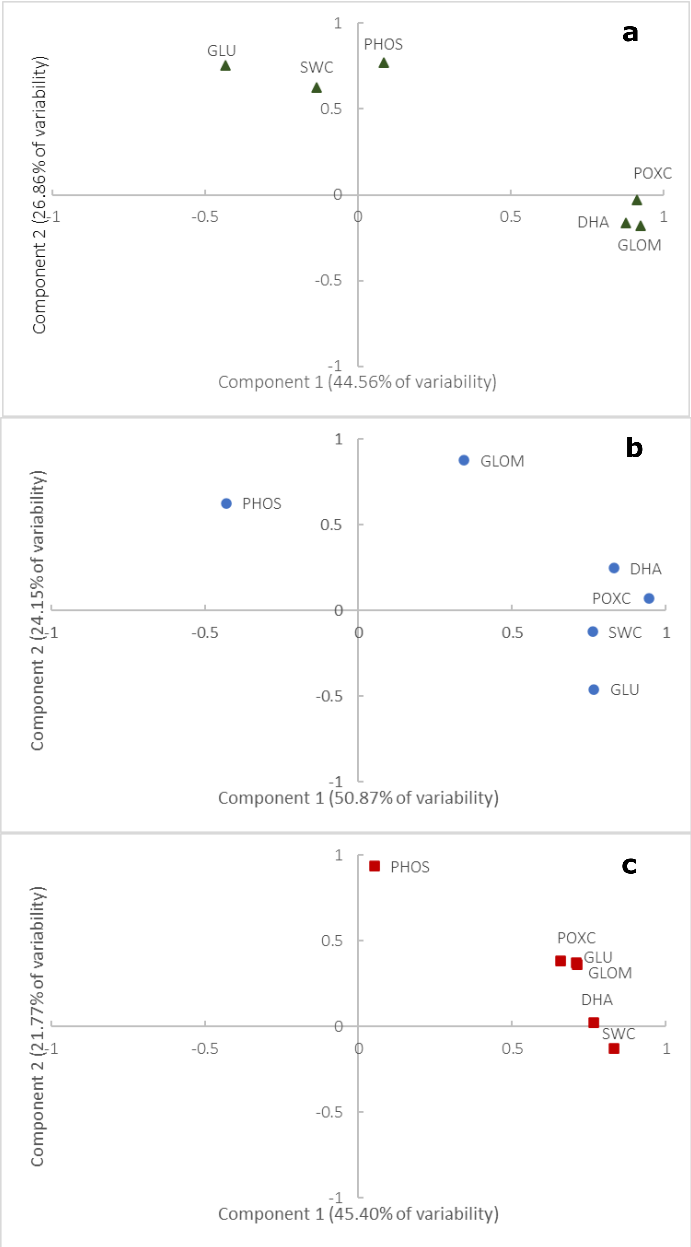


Figure 11. Principal Components Analysis in 0-25 cm depth of (a) maize I, (b) sunflower, and (c) maize II. *DHA= Dehydrogenase activity, GLU= β-glucosidase activity, PHOS= Phosphatase activity, POXC= permanganate oxidizable carbon, SWC= Soil water content, GLOM= Glomalin concentration.*

In maize I, the glomalin, POXC, and DHA described the first component; meanwhile, the other three soil parameters, PHOS, GLU, and SWC, defined the second component of PCA. In maize II, glomalin and PHOS explained the second component, and the other four, POXC, DHA, GLU, and SWC, were in the first component. The result of PCA in sunflowers also showed a different pattern. Only PHOS was the second component; otherwise, the DHA, GLU, POXC, SWC, and glomalin expressed the first component of PCA.

The PCA components reflected the sensitivity of the six soil parameters under long-term soil tillage experiments. DHA and POXC were two parameters consistent in the first component of each crop (Fig. 11); therefore, these parameters were more sensitive to soil disturbance than the other four parameters. Our findings also suggested that DHA was significantly correlated with POXC in each crop. The smallest correlation coefficient (r) occurred in sunflowers with a value of r=0.36, while the largest correlation coefficient is found in maize, r=0.78. This correlation was associated with the POXC concentration, which was relatively higher in maize than in sunflower (Table 9). Chen et al. (2016) also reported the type of crop residue influencing the soil organic fraction. The different types of residues will differentiate the quality of crop residue (Poeplau & Don, 2015), the rate of decomposition (Schmatz et al., 2017), and the biochemical composition (Almagro et al., 2021) impacted the soil carbon mineralization. Maize contains more easily decomposable chemicals such as protein, monosaccharide, and starch. Otherwise, sunflowers comprise more heavily decomposable compounds (lignin) (Dębska et al., 2012). The higher content of lignin resulted in slower nutrient mineralization (Vahdat et al., 2011).

DHA reflects soil biological activity (Kucharski et al., 2009). Generally, DHA was directly proportional to GLU. These two enzymes were related to carbon mineralization in soil. In CT and PT, tillage activity has caused a disturbance in soil properties, leading to the alteration of DHA, GLU, and POXC concentrations. Organic material under PT will be easily exposed and accelerate decomposition, which in turn results in carbon losses to the atmosphere as CO_2. Meanwhile, the other fraction of unstable organic material will also easily be lost through runoff and transported to the deeper layer by water infiltration. The depletion of organic material induced the amount of available substrate, diminishing soil biological activity.

The soil tillage system was sensitive to glomalin, but the level of sensitivity was lower than DHA and POXC. Glomalin is remarkably correlated with POXC concentration in maize and sunflower. The Pearson correlation coefficient (r) was 0.755, 0.52, and 0.34 for maize I, sunflowers, and maize II. These results verified Šarapatka et al. (2019) and Staunton et al. (2020) investigations. Glomalin can protect soil organic carbon from degradation due to its large amount of insoluble hydrophobic glycoproteins—a glycoprotein acts as a glue to bind the soil particle, resulting in a more stable aggerate. Soil aggregation physically protects SOC within aggregates to inhibit microbial activity (Goebel et al., 2009; Liu et al., 2020). The previous investigation on our study site suggested the value of WSA was remarkably higher in CT than in PT. This also confirmed the study of Wilkes et al. (2021) that revealed a stronger correlation between WSA and glomalin zero tillage.

Like glomalin, GLU and SWC were not always consistent in the first component of PCA, so the sensitivity was lower than DHA and POXC. SWC can change due to tillage operation. The porosity of soil will increase and accelerate soil moisture losses by evaporation (Dalmago & Bergamaschi, 2017). The evaporation caused the soil to be drier and increased the soil temperature in the surface layer, inducing the biological activity of the soil (Borowik & Wyszkowska, 2016). DHA, GLU, and glomalin are significantly affected by the POXC concentration, and in general, SWC considerably impacted the GLU (r= 0.4-0.67).

Unlike the other five parameters, PHOS was consistent in component II. It was found in each crop, so it can be assumed that PHOS has a lower sensitivity than component I. Some previous studies indicated that soil tillage does not directly and significantly impact PHOS (Erdel & Şimşek, 2023). PHOS is closely related to the stoichiometry of the amount of available P in the soil. PHOS becomes more active when the P content in the soil decreases. Other previous experiments also suggested that the soil organic matter's decline significantly affectsecreasing PHOS (Lemanowicz & Krzyżaniak, 2015; Azene et al., 2023). Conversely, our study indicated that soil carbon concentration (POXC) and SWC did not affect PHOS in all crops.

Evaluation of the importance of soil biological parameters using Random Forest modeling

The overall importance of the soil parameters in differentiating the growth stage, soil depth, and tillage system was analyzed using the RF classification model (Fig. 12). PHOS denoted the highest mean predictor importance (MDA) by 55% in explaining the growth stage, followed by SWC, DHA, POXC, and GLU (Fig. 12a). POXC and GLU explain the growth stage to a much lesser extent. However, their importance in the classification was still significant. SWC indicated the highest MDA (35%) in illustrating soil depth to soil parameters, followed by DHA. At the same time, the importance of PHOS, POXC, and GLU in the classification was not significant (Fig. 12b). POXC showed the highest MDA (30%) in confirming soil tillage to soil parameters, then GLU and SWC (Fig. 12c). The explanatory power of DHA and PHOS were not significant in the RF classification model.

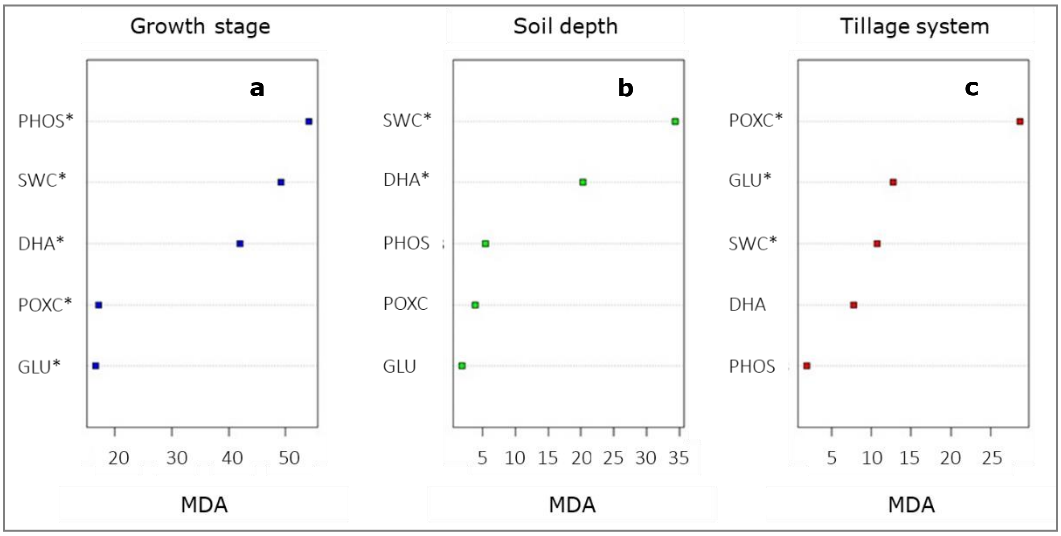


Figure 12. The overall importance of the soil parameters in differentiating the growth stage, soil depth, and tillage system was calculated as the mean decrease in accuracy (MDA) using the Random Forest classification model. '*' denotes a significantly different ($p<0.05$) of the soil parameters according to the growth stage, soil depth, and tillage system. *DHA= Dehydrogenase activity, GLU= β-glucosidase activity, PHOS= Phosphatase activity, POXC= permanganate oxidizable carbon, SWC= Soil water content*

Phosphatase activity as an indicator of the root effect

The larger amount of TOC in CT did not induce PHOS in this study. Singh & Kumar (2021) also reported no significant difference in PHOS under long-term crop rotation and tillage. PHOS denoted the highest mean predictor importance in explaining the growth stage. However, the soil depth and the tillage factor did not significantly influence this enzyme. The highest PHOS emerged in the V7 and V4 stages, which are associated with the root density and distribution of fine-root and P-uptake as well, impacting PHOS (Mandal et al., 2007; Giles et al., 2018; Cabugao et al., 2021). These results indicated phosphorus uptake during the vegetative stage was high in maize and sunflower. The high uptake of P resulted in the P deficiency of the rhizosphere, activating the PHOS (Janes-Bassett et al., 2022). We found a weak-moderate negative linear correlation between soil moisture and PHOS ($r=-0.369$; $p<0.001$) (Table 11). In the growing season of 2021, the precipitation was less than 400 mm; this situation did not hinder the PHOS. Several studies indicated that the effect of SWC varies in PHOS in drought conditions (Brandt et al., 2011; Margalef et al., 2021). It is well understood that soil microorganisms and plant roots control PHOS. Root mucilage is the compound released by the root that significantly affects PHOS (Hu et al., 2019). Mucilage affects soil moisture by increasing water holding capacity and viscosity and decreasing the water tension (Carminati et al., 2017; Benard et al., 2018), which is very important to keep the PHOS (P-cycling) in the drought situation (Ahmed et al., 2018). It could be inferred that the root factor (likes, fine-root density, root length, P-uptake, etc.) was more determining the PHOS than tillage practice and soil depth (Cabugao et al., 2021).

Table 11. Correlation matrix (r) among soil enzyme activity, POXC, and SWC

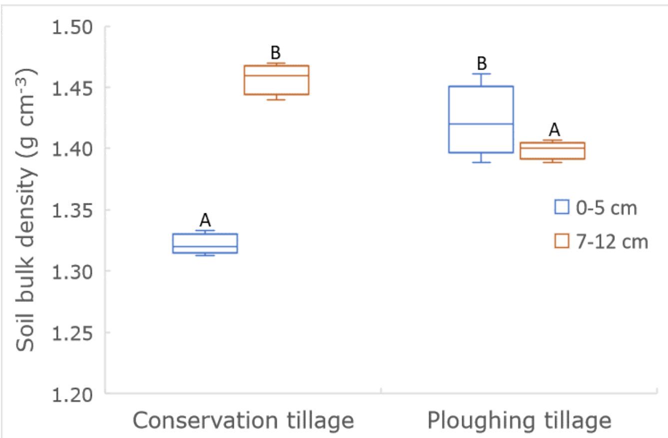
	DHA	PHOS	SWC	GLU
PHOS	0.191*			
SWC	0.216**	-0.369**		
GLU	0.186*	-0.085	0.090	
POXC	0.496**	0.018	0.099	0.188*

'*' and '**' are significantly indicated at 0.05 and 0.01 levels.
DHA= Dehydrogenase activity, GLU= β-glucosidase activity, PHOS= Phosphatase activity, POXC= permanganate oxidizable carbon, SWC= Soil water content

Dehydrogenase activity as an indicator of environmental effects

DHA was ranked as the third and second most important variable in our growth stage and the soil depth classification of the RF model, suggesting that DHA was more affected by environmental factors such as soil moisture, aeration, temperature, etc. (Bandyopadhyay & Maiti, 2021). DHA also varies significantly with soil depth. Since the oxygen near the surface depth is more available than in the deeper soil depth, it stimulates the DHA (Wolińska & Bennicelli, 2010; Weaver et al., 2012; Wolinska & Stepniewsk, 2012). According to BD measurement and the previous results in this experimental site, long-term CT operation resulted in a significantly lower BD in 0-5 cm depth (Fig. 13) and higher water water-stable aggregates (34% and 20%, respectively, for CT and PT) (Madarász et al., 2021). This situation promotes soil aeration and oxygen diffusion, resulting in the increase of the electron acceptor (O_2 concentration) for microbial respiration, which in turn stimulates the organic matter decomposition (Song et al., 2023). Besides that, the high substrate availability, POXC, positively impacts the DHA ($r=0.496$, $p<0.001$) (Table 11).

The high amount of substrate will contribute more protons and electrons transfer by a DHA to the ion acceptor (Brzeziñska et al., 2001), regulating organic matter decomposition and soil nutrient release (McLatchey & Reddy, 1998). However, DHA is more driven by environmental factors (water, air, and substrate), and changes in DHA over time are also influenced by plants (root system, root developments, and root density), as confirmed by (Kompała-Bąba et al., 2021; Jat et al., 2021). In our study, the different structures of maize and sunflower roots may impact soil enzyme activity. Sunflower is a dicot that essentially modified the diameter of its roots; in contrast, the monocot has a more significant increase in the number of existing roots (Goodman & Ennos, 1997). The difference in root morphology is also associated with the surface areas in which sunflower has a smaller root surface area than maize (Freundl et al., 1998), affecting the rhizosphere and microbial activity.



Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05)

Figure 13. Soil bulk density (g cm^{-3}) in the CT and PT at 0-12 cm depth for the growth stages V3 in the growing season of 2022, n=4.

β-glucosidase activity and POXC as indicators of tillage and management effects

The GLU and POXC were ranked as the most important factors in our soil tillage RF classification model. Both variables showed significant differences as a result of the interaction of soil depth × tillage. The significantly higher POXC in CT plots is strongly related to the increasing soil organic carbon content (Tobiašová et al., 2018; L. Zhang et al., 2020; Madarász et al., 2021). Our TOC analysis showed a significant increase in CT soils compared to PT by 1.1 mg g^{-1} and 0.8 mg g^{-1}, respectively, for CT and PT. This circumstance was related to the minimum soil disturbance and low erosion; therefore, CT can stabilize the form of carbon that remains in the soil for a longer period (Reicosky et al., 1997; Chowaniak et al., 2020). In the short termthe active labile carbon (POXC) content also increases faster under the influence of CT (Bongiorno et al., 2019).

The higher SOM, the better soil structure, and greater porosity, as a long-term effect of CT, provide more favorable soil conditions for microbial activity. High POXC concentration in the CT offers more available substrate for the mineralization of organic matter, designated by the increase of GLU ($r=0.188$, $p<0.05$) (Table 11). We found a reverse vertical trend for two tillage treatments in GLU, which is closely related to the decomposition of organic matter (García-Gil et al., 2000; Liu et al., 2022). The more available substrate accumulation and aerated condition in the surface layer by the tillage reduction have led to a higher GLU in the topsoil of CT. Conversely, in the PT, the inversion soil by moulboard ploughing and the incorporation of soil and crop residue resulted in the substrate being more uniformly distributed throughout the soil profile; therefore, the GLU in the deeper soil layer tended to increase (Fig. 14) (Hazarika et al., 2009).

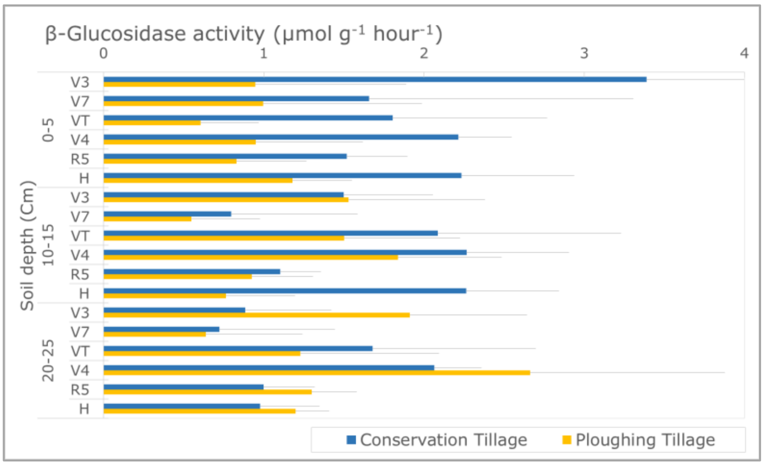
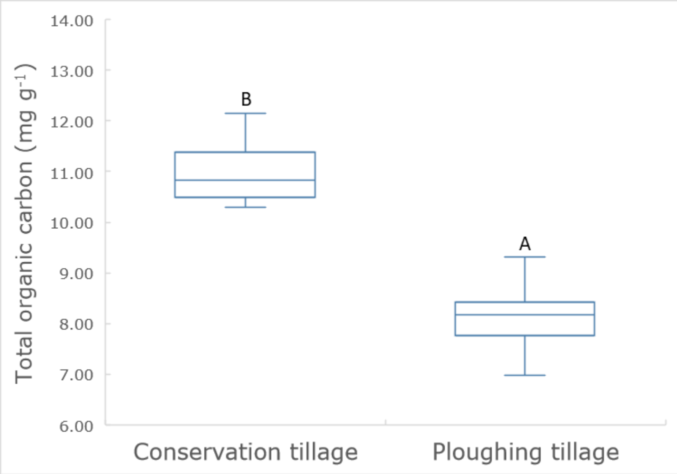


Figure 14. The dynamic of GLU is at 0-5, 10-15, and 0-25 Cm in CT and PT practice.

Many investigations suggested that CT application promotes the availability of substrates for microbial activities by the slow C mineralization (Kan et al., 2020; Nugroho et al., 2023), corroborated by the fluctuation of soil enzyme activities by the growth stage and soil depth was relatively balanced in the CT soil, indicated by the lower CV values (Deng et al., 2019).

3.4 Plant-nutrition potential of CT and PT soils

Minimum soil disturbance and the abundance of crop residue impacted the TOC. The TOC concentration was found to be significantly higher for CT than in the PT plots (Fig. 15). However, the microbial biomass content (MBC) in our secondary data did not differ significantly, being 129.4 μg g^{-1} C and 166.3 μg g^{-1} C for CT and PT, respectively. TOC was strongly correlated with POXC and impacts the activity of soil enzymes (García et al., 1994).

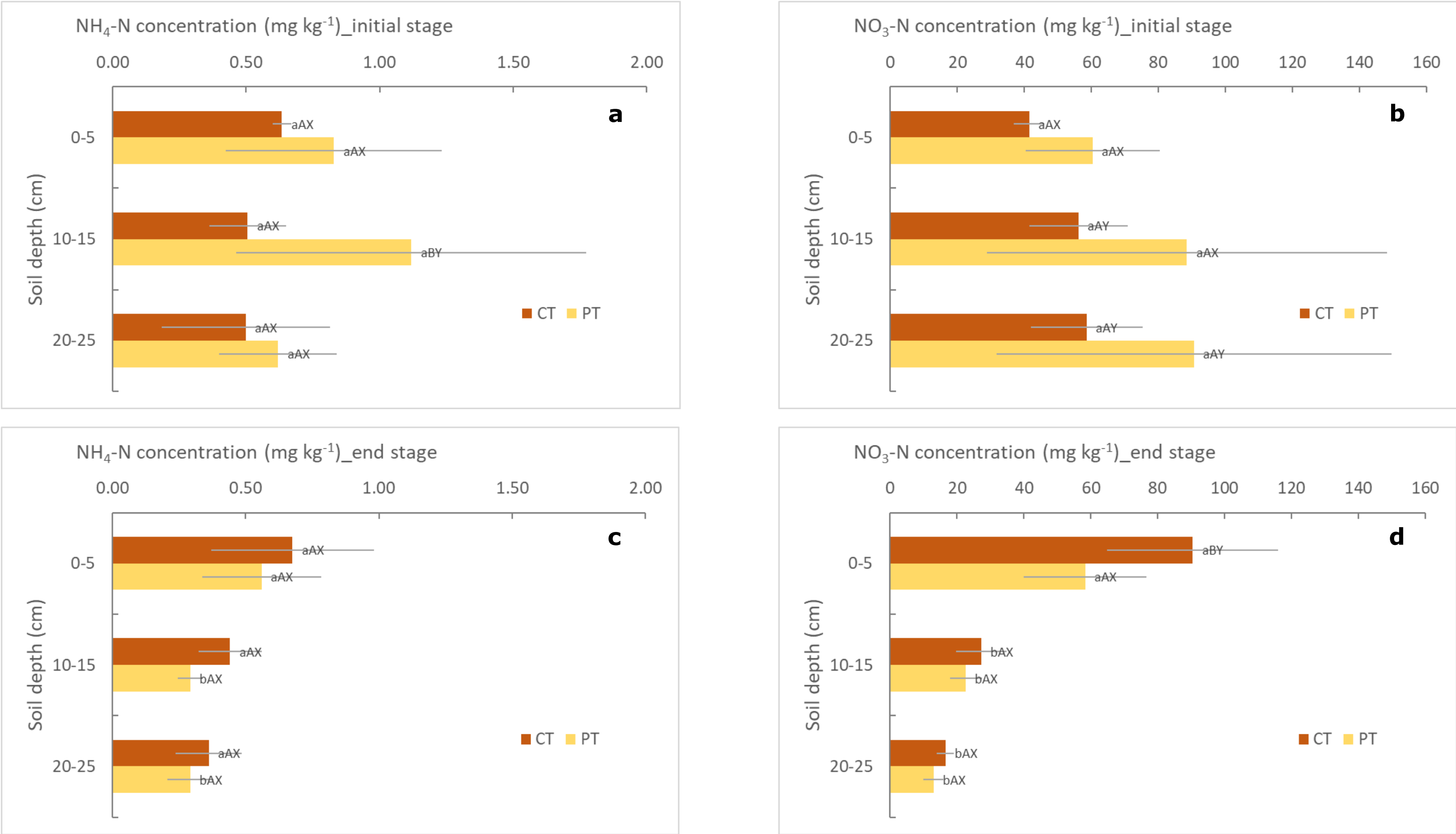


Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05)

Figure 15. Total organic carbon (mg g^{-1}) in the CT and PT at 0-20 cm depth, n=18.

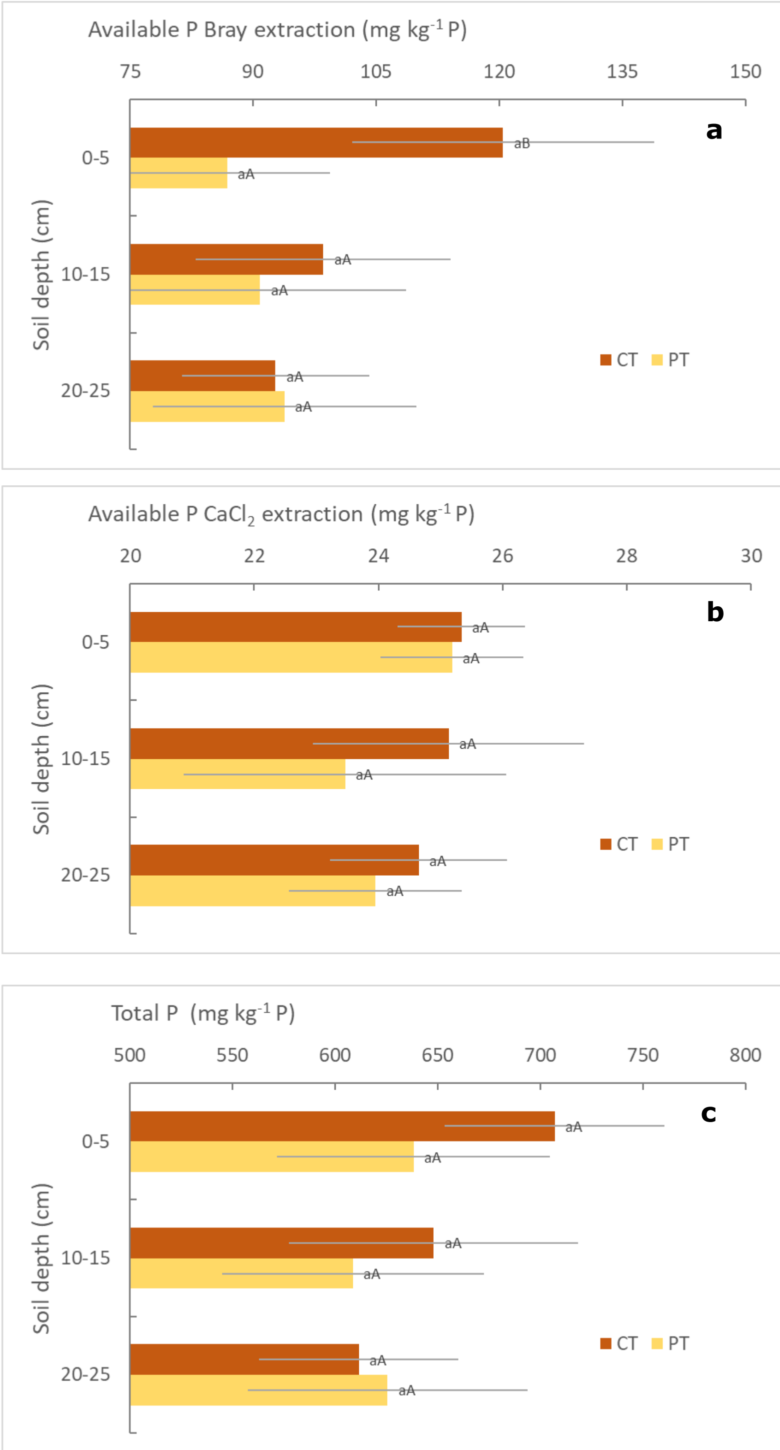
The improvement in physical soil properties in the CT plot resulted in less soil loss. This partly explained the higher TOC concentration in the CT plot than in the PT plot. On the other hand, in the case of CT, the mineralization of organic matter is probably slower, this stabilizing the form of carbon that remains in the soil for a longer period (Van Den Bossche et al., 2009; Cooper et al., 2021).

High TOC concentration induces microbial biomass activity and soil nutrient mineralization due to the sufficient energy source. The process of decomposition and mineralization responds to the presence of crop residues in the soil, and it is also linked to the soil enzyme activity associated with plant and soil microorganisms (Bandick & Dick, 1999; Gianfreda et al., 2002). To investigate the plant-nutrition potential of CT and PT soils, we used the growing season data of 2021. Below is the nutrient concentration of N, P, K, and Ca of CT and PT soils at the initial and the end of the growth stage.



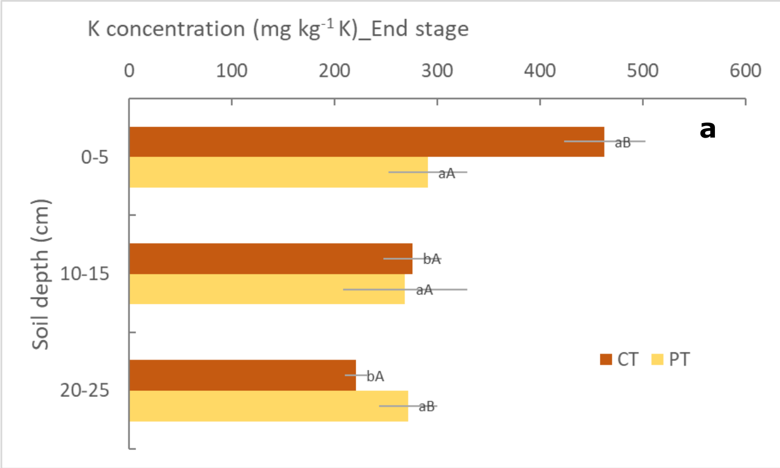
Different lowercase letters (a and b) indicate significant differences among soil depths (p-value<0.05); Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05); Different capital letters (X and Y) indicate significant differences between the growth stages (p-value<0.05).

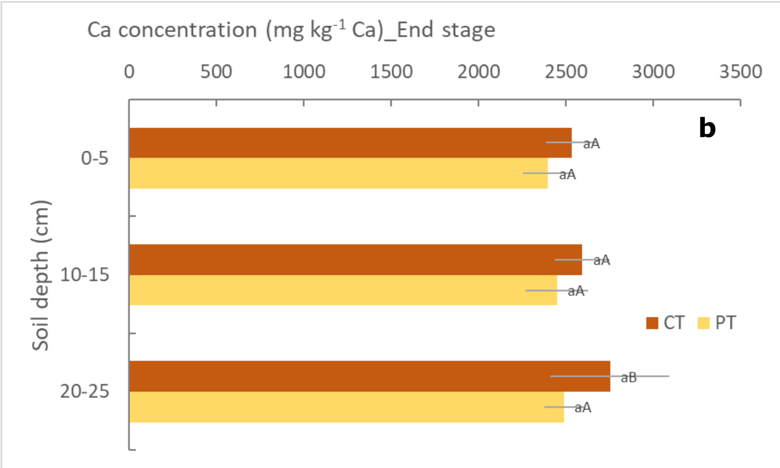
Figure 16. a and c: NH_4-N concentration (mg kg^{-1}) and NO_3-N concentration (mg kg^{-1}) in the initial stage (V3); b and d; NH_4-N and NO_3-N concentration in the end-stage (VT).



Different lowercase letters (a and b) indicate significant differences among soil depths (p-value<0.05);
Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05)

Figure 17. Available phosphorus (mg kg^{-1} P) by $CaCl_2$ (a) and Bray extraction (b) and the total P in the CT and PT at 0-25 cm depth for the growth stages V3.





Different lowercase letters (a and b) indicate significant differences among soil depths (p-value<0.05); Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05).

Figure 18. Available-K (a) and Ca concentrations (b) (mg kg^{-1}) in the CT and PT at 0-25 cm depth for the growth stages VT.

From the data, we can see that generally, in the initial stage, the soil nutrient concentration was lower in the CT than in PT; otherwise, the soil nutrient concentration was higher in the CT than in PT at the end of growth stage suggesting the soil nutrient mineralization that was proved by the ratio of MBC to TOC of both CT and PT that in the range of 1-5%, implying that the role of microorganisms in the carbon and nutrient cycles was not impeded (Insam, 1990; Sparling, 1992). In the present experiment, DHA and GLU's enzymatic activities were unrelated to the NH_4-N concentration (Table 11). This situation was probably because most of the mineral N came from fertilizers. Nevertheless, differences in tillage practice significantly affected the NH_4-N and NO_3-N concentrations during the growing season (Fig. 16).

Table 11. Pearson's correlations (r) among soil properties

	NH_4-N	NO_3-N	P-Bray	P-$CaCl_2$	P-total	K_2O	CaO
DHA	-0.06	-0.05	0.25	0.46*	0.51*	0.81**	-0.03
GLU	-0.03	-0.10	-0.17	0.51*	0.01	0.17	0.42*
PHOS	-0.28	-0.37	0.21	-0.10	0.36	0.03	0.45*

**, ** Significant at the 0.05 and 0.01 level (2-tailed), respectively*
SWC= Soil water content; POXC= Permanganate oxidizable carbon, DHA= Dehydrogenase activity, GLU= β-glucosidase activity; PHOS= Phosphatase activity

The evolution of the mineral N-form is also influenced by microbial activity and plant nutrient uptake. In the CT soils, the concentration of each mineral N-form was generally lowest at the V3 stage (Fig. 16a, b) due to the immobilization associated with higher C content. Because of the higher C/N ratio, the N content of mineral fertilizers is initially used by the microbes in CT soils (Wood & Edwards, 1992; van den Bossche et al., 2009).

The higher mineral N concentration in the deeper layers of PT soils is a consequence of the deeper inversion tillage (Pandey et al., 2010). However, the N-immobilization process ceases by the VT stage, and the organic N content of the microbes begins to mineralize. It was found that mineral N concentrations were already higher in the VT phase in CT plots compared to PT. This may be related to the significantly higher POXC concentration in CT than in PT. The sufficient quantity of POXC and the availability of soil moisture stimulated the GLU in the 0-5 cm layer in CT, leading to enhanced NH_4-N concentration higher than in the deeper layer.

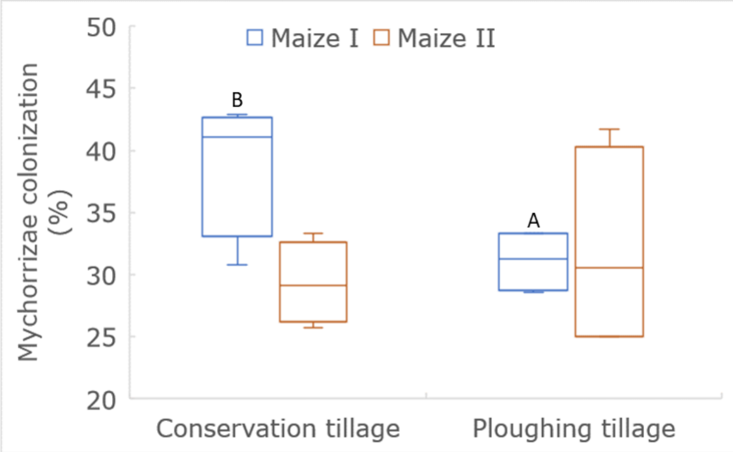
During phosphorus mineralization in the soil, PHOS is involved in catalyzing the hydrolytic reactions of phosphate groups (mono or diesters), which eventually release inorganic P into the soil solution (Nèble et al., 2007; Criquet & Braud, 2008). CT markedly affected the concentration of available Bray-P at the 0-5 cm depth (Fig. 16a), but it had no impact on $CaCl_2$-soluble P (Fig. 16b) or total P (Fig. 17c).

Nevertheless, the low PHOS in CT and PT was associated with higher soil P content in the whole profile. The application of phosphorus fertilizer before planting and the presence of mineralized phosphorus led to a sufficient amount of phosphorus being available for the maize plants. In numerous studies, soil PHOS was found to be more inversely proportional to soil inorganic P content. Specifically, an increase in inorganic P in the soil inhibits PHOS (Olander & Vitousek, 2000; Gianfreda et al., 2005).

PHOS was more active at 0-5 cm than at deeper depths (Appendix 3) and slightly positively correlated to the total P concentration (Table. 13). PHOS was strongly related to the growth stage and P uptake of maize roots. PHOS is related to the presence of roots and microorganisms. A more developed root system in V7 and VT produces more PHOS than V3 (initial stage) (Appendix 3). In addition, P uptake by the maize roots leads to the depletion of P in the soil solution; therefore, PHOS rises rapidly to maintain the equilibrium of P in the soil system (Hayes et al., 1999; Machado & Furlani, 2004).

The continuous decrease in SWC with the plant growth stages had a tendency to stimulate PHOS ($r= -0.27$), in contrast to a previous study by Sardans and Peñuelas (2004), who stated that PHOS decreased by 31-40% when water availability was reduced to 21%. PHOS is a type of extracellular enzyme exuded mainly through plant roots. Therefore, in a particular situation, PHOS may not depend greatly on environmental factors such as SWC and substrate availability (POXC).

Tillage practice affects the mycorrhizae colonization (Fig. 19), which plays an essential role in the soil phosphorus cycle. The alterations in soil structure by changing the BD affect the mycorrhizae fungus distribution within the soil (Harris et al., 2003). Fungal hyphae were commonly found in soil with lower BD. A lower BD in this study (Fig. 13) was probably a reason for the higher mycorrhizae colonization of CT than PT in maize I. However, in the maize II, the situation was different. The higher precipitation in 2023 (832 mm) and the cover crops establishment in the growing season of 2022 have caused unpredictable weed growth and inhibited the maize performance in the 2023 growing season of the CT plot. Consequently, the maize homogeneity in several plots of CT was low due to plenty of plants growing late. Because of this, the plants allocated less photo assimilate, which reduces the growth of AMF hyphae (Chowdhury et al., 2022). The excessive use of herbicide in weed control in 2023 in the CT plot (Khursheed et al., 2019), perhaps affected colonization of mycorrhiza.



Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05)

Figure 19. Mycorrhizae colonization (%) in the CT and PT at the VT growth stages of maize in the growing season of 2021 and 2023, n=4

In relation to mycorrhizae, glomalin also has an indirect mechanism for P mineralization. Glomalin will play a role in increasing the aggregate stability so that P is not easy to lose due to leaching with runoff water. Glomalin concentration at 0-5 cm depth of CT was significantly higher than PT (Table 9), probably contributing to higher WSA.

Soil enzymes were also found to be involved in the increase of base cation in CT. There was a positive correlation between the potassium concentration and DHA and the calcium concentration and GLU (Table 11). The concentration of base cations was relatively higher near the surface layer in CT. The potassium concentration increased from 2.8 to 59.3% and the calcium concentration from 5.8 to 5.9% in the upper layer of CT (Fig. 18). This corroborated a study on a similar parent material (Loess) by Karathanasis & Wells (1990) documented the increase of potassium and calcium concentrations at the upper layer under CT from 5.4 to 61.1% and from 15.0 to 70.7%, respectively. The higher concentration of phosphorus, potassium, and calcium in CT soils may be related to the fact that the nutrients are more closely connected to the organic matter, which is more resistant to erosion (Madarász et al., 2021). Probably, due to the slower mineralization of residues, nutrient loss by leaching is also less in the case of CT tillage. Further research is needed to clarify exactly why the number of absorbable cations and phosphorus increases as a result of CT compared to PT with the same fertilization.

The minimization of erosion and the intensity of soil biological activity and nutrient mineralization processes are particularly important when fewer nutrients are applied than removed from the crop area. In this study, the nutrient balance (N, P, and K) was also calculated based on the nutrient removal by maize grain (output) against the amount of fertilizer application (input) (Karlen et al., 2015). The nutrient removal in growing season of 2021 was approximately 143 kg ha^{-1} N, 63 kg ha^{-1} P_2O_5 (= 27.5 kg ha^{-1} P) and 40 kg ha^{-1} K_2O (= 33.3 kg ha^{-1} K), while the inputs were 118.5 kg N, 37.5 kg ha^{-1} P_2O_5 (= 16.4 kg ha^{-1} P) and 37.5 kg ha^{-1} K_2O (= 31.3 kg ha^{-1} K), indicating a negative balance (output > input). The decomposition of crop residues and nutrient mobilization processes resulted in the nutrients passing into the soil solution phase. These nutrients can be accessed by the roots to meet their nutrient requirements. The difference between the two tillage methods is that mineralization and other nutrient mobilization processes are much more balanced over time in CT soils than in PT. In the case of PT, due to the inversion tillage, the mineralization of residues is initially fast, but by the end of the vegetation period, the microbial activity significantly decreases compared to CT.

Soil biological activity is also affected by the growth stage (Gałązka et al., 2017; Jat et al., 2020; Nevins et al., 2020), which was verified in this study. The growth stage influences the quality and quantity of root exudates, as well as microbial diversity and activity in the plant rhizosphere (Kuzyakov, 2002). The present study suggested that the three soil enzymes were more active in the middle stage (V7) than at V3 or VT (Appendix 1,2,3). This contradicts a previous observation by Gałązka et al. (2017), where DHA was higher at the end of the vegetative stage, VT, and in R1 stages.

According to Bender et al. (2013), N uptake followed a more traditional sigmoidal (s-shaped) pattern, with two-thirds of the total plant uptake acquired in the VT and R1 stages, inferring that the activity of soil enzymes is supposed was more active in these phases. However, the peak activity of soil enzymes does not always appear in the VT and R1 stages. Nevins et al. (2020) reported that the GLU was most significant during the vegetative growth stage (V6), and that soil inorganic N concentration peaked after the potential peak activity of soil GLU. This was consistent with the GLU detected in CT at the 0-5 cm depth in the current experiment. The soil NH_4-N and NO_3-N concentrations were lower in the initial stage (V3) (Fig. 16a and c), which was followed by the increase of GLU (Appendix 2). However, the opposite situation occurred in the VT stage; the NH_4-N and NO_3-N concentrations increased (Fig. 16b and d) following a decrease in the GLU (Appendix 2). Likewise, a 12-190 folds increase in PHOS was observed in the V7 stage in the 0-25 cm layer relative to V3 or VT stages (Appendix 3), which oversaw the total P concentration.

The higher TOC and lower BD resulted in the improvement of soil aggregate stability, soil macropores, and an increase in the abundance of earthworms, as details discussed in Madarász et al. (2021). Higher TOC and POXC were also related to the biomass production that was revealed from the pot experiments, in which the plant height and dry biomass in CT were notably larger than in PT (Fig. 20). High biomass production in CT is potential in soil nutrient returning.

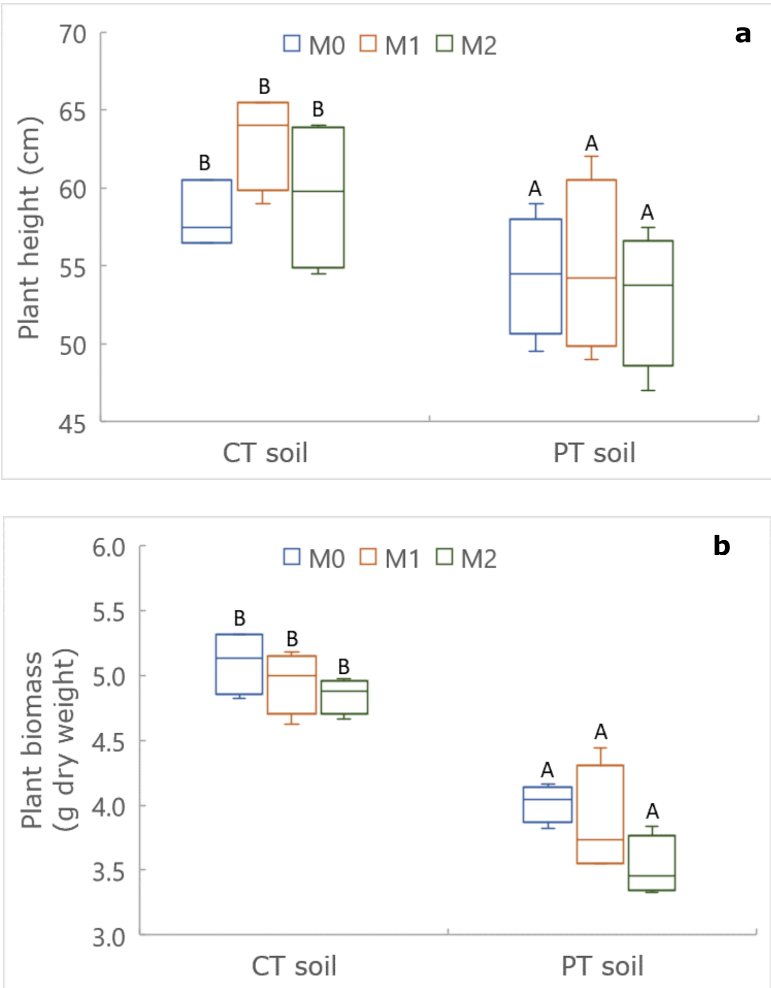


Figure 20. Plant height (a) and dry biomass (b) weight of maize of pot experiment. M= molasses concentration, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2)

TOC was also associated with soil enzyme activity. A more than 50% increase in DHA was recorded in the CT soil, confirming previous studies by Roldán et al. (2005) and Szostek et al. (2022), who reported that CT notably increased DHA by 18 to 60%. The available substrate is the important factor driving DHA. We found that DHA depended on soil carbon availability (Wiatrowska et al., 2021), which agrees with the significant positive correlation (r= 0.50) detected between DHA and the POXC concentration in the present study (Table 11).

The result of the pot experiment clarifies the result of the field experiment. The application of molasses tended to increase the DHA in CT and PT (Fig. 21). The highest DHA was recorded in 0.05 mg L^{-1} of molasses application by 0.098±0.014 TPF µg g^{-1} dry soil in CT (80.76% higher than control) and 0.083±0.031 TPF µg g^{-1} dry soil in PT (50.59% higher than control). The addition of 0.2 g L^{-1} molasses concentration tended to decrease DHA in this experiment even though it was still higher than the control. We inferred that applying high molasses (M2) concentrations potentially caused the priming effects.

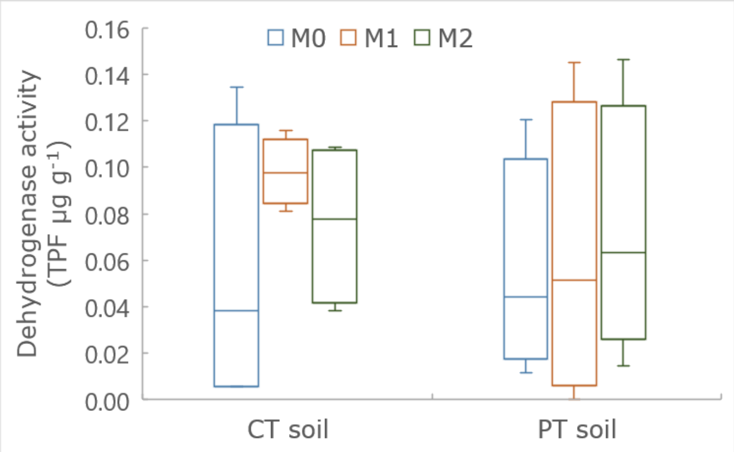


Figure 21. DHA (TPF µg g^{-1}) of CT and PT of pot experiment. M= Molasses concentrations, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2), n= 4.

The increase in DHA is linked with the microbial population growth in soil (Jha et al., 1992; Chu et al., 2007; Järvan et al., 2014). The current investigation demonstrated a somewhat higher population of fungi in CT than in PT (Fig. 22). The vast amount of soil carbon in CT boosts the growing fungi population, amplifying soil enzyme activity.

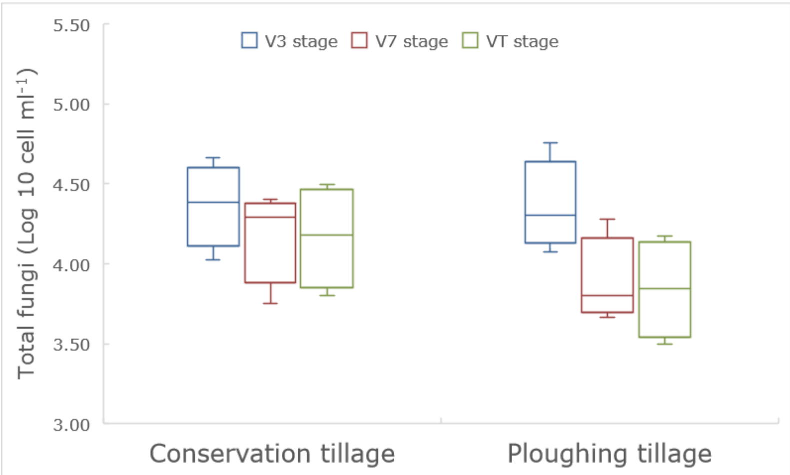
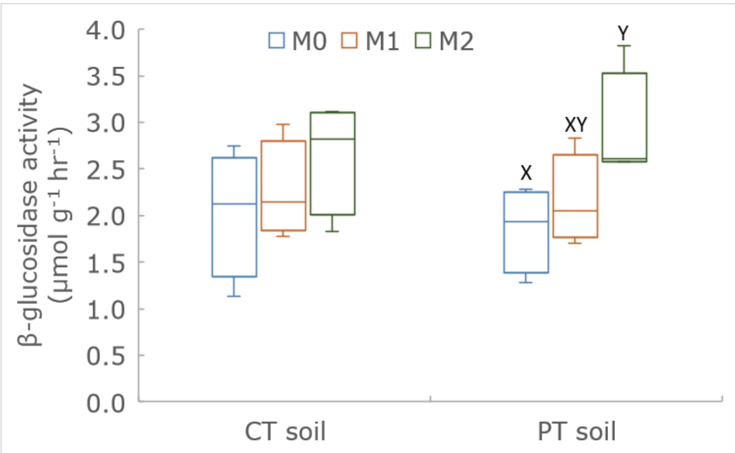


Figure 22. Total fungi at 0-5 cm depth of CT and PT for three growth stages, n= 4.

The significantly higher TOC in CT somewhat promoted higher GLU in the 0-5 cm layer at the whole growth stages. In the early growth stage in the PT, GLU increased along with the depth, which is associated with the effect of soil inversion, switching the layer with higher organic carbon to the deeper (Appendix 2). The changes in SWC (which ranged from 3.63 to 36.23 w/w %) during the growing seasons were not correlated with the GLU in this study (Table 11). This contradicted a previous study by Zhang et al. (2011) and Olatunji et al. (2022), who noted that GLU decreased significantly with the SWC reduction.

The inconsistent trend of GLU was probably caused by the imbalance of environmental factors such as humidity, temperature, and oxidative conditions in the PT soil (Eivazi et al., 2003; van den Bossche et al., 2009). The GLU correlated with DHA (r= 0.19, *p-value* < 0.05). Zhang et al. (2011) and Partey et al. (2019) stated that either GLU or DHA could be employed indirectly to identify mineralization processes in the soil.

GLU is strongly related to the decomposition of litter and SOC (Sotomayor-Ramírez et al., 2009; Choudhary et al., 2018). In general, the pattern of GLU during the growing season was inconsistent in CT and PT; although GLU tended to be greater in CT than in PT for most depths and growth stages, the differences were not significant because of the high variance. GLU was weak influenced by the POXC (Table 11), confirmed by the pot experiment that showed GLU in CT and PT increases concurrent with the increase of available substrate addition (Fig. 23). The application of 0.2 g L^{-1} molasses increased the GLU by 30.43% and 56.57% higher than the control in CT and PT, verifying the study by García-Gil et al. (2000) who reported that GLU could increase by 100% compared to the control due to adding simple organic substrates.



Different capital letters (X and Y) indicate significant differences among the concentration of Molasses (p-value<0.05)

Figure 23. GLU (µmol g^{-1} $hour^{-1}$) of CT and PT of pot experiment. M= Molasses concentrations, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2), n=4.

IV. CONCLUSIONS AND RECOMMENDATIONS

Three years of monitoring soil biological parameters have been carried out in farmland under different soil tillage systems and crop rotation. CT practice significantly improved the soil BD in the surface layer and increased the SOC carbon, resulting in the high aggregate stability revealed by our previous investigation. The aggregate stability led to significant microbial biomass, in our case, fungi population, thus promoting soil carbon mineralization indicated by higher POXC concentration in the whole depth of CT practice. POXC is a vital substrate source for the activity of microorganisms, reflected by the positive correlation between POXC concentration and DHA and GLU. We also proved through a small pot experiment that available substrate addition, molasses, tended to increase POXC concentration, DHA, and GLU. Applying available substrate also improved the plant height and biomass of maize plants. In the current study, the DHA and GLU were not directly related to N concentration; however, differences in tillage practice and growth stages significantly affected the N concentration. In the CT soils, the N concentration was lower in the beginning stage due to the immobilization associated with higher C content in crop residue. The end stage of maize terminates the N-immobilization process, and the organic N content of the microbes begins to mineralize.

PHOS seems more determined by root activity and the equilibrium of P concentration: P uptake by the roots; it was evident in the beginning stage of maize, in which a high concentration of P depressed the PHOS. Conversely, in the bigger stage of maize, the PHOS increases with P uptake. Tillage practice affects the mycorrhizae colonization, which is essential in the soil phosphorus cycle. Lower BD and higher SOC stimulated the mycorrhizae colonization in our study. The concentration of K and Ca is associated with organic matter decomposition and mineralization, as suggested by the positive correlation between those cations and DHA and GLU. Higher concentrations of K and Ca in CT soils may be related to the fact that the nutrients are more closely connected to the organic matter, which is more resistant to erosion.

Soil biological indicators are also related to the variability of spatial (soil depth) and temporal (growth stages). DHA and GLU were more active in the surface layer (0-15 cm depth) than the deeper soil layers, likewise the POXC content. Tillage caused changes in environmental factors, especially SWC, aeration, and temperature, which governed the DHA. By the same approach, we proved that the growing stage was much more critical than environmental factors in describing soil biological parameter dynamics activity. For instance, the PHOS was a primary indicator of root effects that differed in the vegetative and generative phases.

The sensitivity of soil biological indicators in responding to long-term tillage activity differed. DHA, POXC, and Glomalin were very sensitive to soil tillage practice; however, PHOS has the lowest sensitivity. Meanwhile, the other indicators, GLU and SWC, were categorized between these two sensitivity levels.

CT improved the soil properties that promoted plant development. The plant height, root capacity of maize, stem, and flower diameter of sunflowers were relatively better in the CT than in the PT. However, the yield of maize and sunflower and CT practice tended to be lower than PT, especially in maize II, which was more caused by technical issues like pests and diseases attacked and unpredictable growth of weeds.

According to the results of Doctoral research, some recommendations have been formulated as follows:

1. Further research with more detailed variables, such as closer soil depth intervals (5 cm) in the deeper layer (up to 30 cm), other soil types, and different crop types, is still required to produce more rigid and accurate data so that it could be utilized to support the large-scale implementation of conservation tillage in Hungary.
2. The investigations combine the treatments of soil tillage and nutrient management, such as chemical and biofertilizer dosage, which are necessary to maintain a high yield and reduce the production costs concerning the fluctuation of chemical fertilizer prices in Europe (environmental and economy sustainability).

3. Due to limited resources and time, this current study cannot reveal in more detail the other types of soil enzymes and their role in the decomposition of plant residues; therefore, the next research should answer these questions. Apart from that, identifying the abundance of macro and mesofauna (termites, collembolas, earthworms, etc.) involved in the breakdown of organic matter and nutrient release related to soil enzyme activity is fascinating and should be carried out.

4. Lastly, observing seasonal variations of soil biological parameters under different tillage practices is necessary, which can clearly illustrate how climate factors influence soil biological indicators.

V. NEW SCIENTIFIC RESULTS

We formulated some new scientific results of Doctoral research based on our three years of field investigation and small pot experiment as follows:

1. We found that, through reduced erosion losses and improved soil structure, organic matter content, and infiltration, long-term CT contributes to the increase of available and reserve nutrients in the surface soil layers compared to the PT practice. The reduced tillage also provided favorable conditions for the decomposition of plant residues, which is also favorable from the point of view of the plant's nutrient uptake.
2. We found that long-term CT application led to more balanced environmental conditions, i.e., greater carbon parameters, more stable soil aggregates, and better SWC. Consequently, the mobilization of nutrients in the soil was more balanced as well, and the nutrients were released gradually.
3. Based on the monitoring of three growing seasons, we determined that the sensitive indicators of the microbiological effects of tillage practice are the vertical distribution of the DHA and the POXC in the investigated soil. Meanwhile, GLU and PHOS are less sensitive indicators of tillage change. In general, enzyme activities and POXC showed lower temporal variability in the case of CT compared to PT.
4. We found that the positive changes in soil biological activity and nutrient capacity that occurred over 20 years as a result of CT in the investigated area were not enough for yield increase by themselves. This is because the soil probably has little organic substrate, even for CT. From this, we can conclude that an additional nutrient source (chemical or organic fertilizer) is still necessary to supply plants with nutrients. Despite the weeding problems, however, there is still no significant yield reduction in CT plots compared to PT.

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LIST OF PUBLICATIONS

Journal articles

1. **Nugroho, P. A.**, Juhos, K., Prettl, N., Madarász, B., & Kotroczó, Z. (2023). Long-term conservation tillage results in a more balanced soil microbiological activity and higher nutrient supply capacity. International Soil and Water Conservation Research. https://doi.org/10.1016/J.ISWCR.2023.03.003 [**Scopus Q1, impact factor 6.4 (Clarivate)**].
2. Juhos, K., **Nugroho, P. A.**, Jakab, G., Prettl, N., Kotroczó, Z., Szigeti, N., Szalai, Z., & Madarász, B. (2024). A comprehensive analysis of soil health indicators in a long- term conservation tillage experiment. Soil Use and Management, 40, e12942. https://doi.org/10.1111/sum.12942 [**Scopus Q1, impact factor 3.8 (Clarivate)**].
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International conference (full paper)

1. **Nugroho, P. A.**, Prettl, N., Madarasz, B., Kotroczó, Zs., Juhos, K. (2022). Soil chemical properties under 16 years conservation tillage practice in Hungary. Proceeding of 17th Carpathian Basin Conference for Environmental Sciences, April 6-9, 2022, Kolozsvár, Románia, 272-278 p. ISSN: 1842-9815.
2. Prettl, N., Kotroczó, Zs., **Nugroho, P.A.**, Pabar, S.A., Biró, B., Juhos, K. (2022). Mikrobiológiai termésnövelők és foszfor műtrágya hatása a kukorica növekedésére, két különböző talajtípuson. Proceeding of 17th Carpathian Basin Conference for Environmental Sciences, April 6-9, 2022, Kolozsvár, Románia, 160-166 p. ISSN: 1842-9815.

3. Kotroczó, Zs., Juhos, K., **Nugroho, P.A.**, Prettl, N., Várbíró, G., Fekete, I. (2022). Biológiai aktivitás és labilis szén tartalom változás egy hosszú távú szerves anyag manipulációs kísérletben. Proceeding of 17th Carpathian Basin Conference for Environmental Sciences, April 6-9, 2022, Kolozsvár, Románia, 144-152 p. ISSN: 1842-9815.

International conference (oral/poster)

1. **Nugroho, P. A.**, Juhos, K., Prettl, N., Kabalan, S., Madarász, B., & Kotroczó, Zs. (2024). Effect of long-term reduced tillage on selected soil biological indicators on luvisols in Hungary. Centennial Celebration and Congress of the International Union of Soil Sciences, May 19-21, 2024, Florence, Italy.
2. **Nugroho, P. A.**, Juhos, K., Prettl, N., Kotroczó, Zs. (2023). Soil biological indicators under sunflowers field in a long-term tillage experiment of luvisol. VIII. International Scientific Conference-Conserving Soils and Water, September 6-9, 2023, Varna, Bulgaria
3. Prettl, N., **Nugroho, P.A.**, Kotroczó, Zs., Biró, B., Juhos, K. (2023) Mycorrhiza inoculation effect with different levels in conventional tillage practice at central European climate and soils. Lippay János - Ormos Imre - Vas Károly Tudományos Ülésszak, November 16, 2023, Hungarian University of Agricultural and Life Sciences, Budapest, Hungary.
4. Prettl, N., Biró, B., **Nugroho, P.A**., Kotroczo, Zs., Kabalan, S., Juhos, K. (2023). Effect of mycorrhizal fungus inoculant in conventional crop production in the light of soil biological properties and yield. XVIII. Carpathian Basin Environmental Science Conference, May 17–19, 2023 University of Szeged, Szeged, Hungary.
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6. **Nugroho, P.A**., Juhos, K., Prettl, N., Madarasz, B. Kotroczó, Zs. (2022). Soil enzymes activity in the growing season under different tillage systems in western Hungary. Poster presentation in 22nd of World Congress of Soil Science, 31 July - 5 August, 2022. Glasgow, Scotland.
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9. Kabalan, S., Juhos, K., Prettl, N., **Nugroho, P.A**., Biró, B., (2022). Soil-dependent importance of symbiotic interactions with three cover crops. International Symposium–2022. Theme: "Biosphere & Environmental Safety", 2-6 May 2022, Obuda university, Budapest, Hungary