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

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DOCTORAL (Ph.D.) DISSERTATION

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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. LITERATURE REVIEW

2.1 The importance of tillage practices in agricultural production and soil degradation

Soil tillage is a common activity in agricultural production that aims to provide a favorable soil environment for crop growth and yield. When soil is cultivated, soil aggregates are broken up, and soil is aerated. Specifically, farmers do tillage for many reasons, such as seedbed preparation, controlling weeds, incorporating manure or fertilizer spread on the soil surface, mixing crop residue into the soil, leveling the soil, and activating pesticides (USDA, 2018). In Hungary, tillage is a primary method with a long-term tradition, and it has been begun since the 15th century. The agricultural revolution in the 19th century involved machinery in soil ploughing. The intensive tillage, which is fully mechanized, improves the yield significantly in a relatively short time (Birkás et al., 2017).

2.1.1 Types and characterization of tillage practice

Intensive tillage, also known as conventional tillage (PT), involves deep ploughing to prepare the soil for planting crops. PT consists of two stages: primary and secondary tilling techniques. Primary tillage is the first stage in preparing the soil when the top layer is loosened and broken up. This cultivation method commonly employs a plough, harrow, or cultivator. Ploughing is the predominant form of primary tillage. Ploughing involves overturning the uppermost layer of soil, which promotes soil aeration and enhances drainage. Ploughing has the dual purpose of aerating compacted soils and eradicating weeds and grasses. Moldboard and disc plough are the predominant form of plough employed for initial soil cultivation.

Secondary tillage refers to any further soil manipulation that occurs after the original planting and establishment of the crop. Secondary tillage encompasses many activities, such as cultivating and harrowing. Secondary tillage maintains soil looseness and aeration, facilitating water infiltration, drainage, and root growth. Additionally, it contributes to weed management, disintegrates soil clumps, and facilitates the infiltration of nutrients into the soil. The main differences between primary and secondary tillage, including the equipment, are shown in table 1 (Tractor Junction, 2022).

Moldboard ploughing is the most common type of PT. This method involves soil inverting by a plough, creating big clods. This procedure facilitates the fragmentation of densely packed soil, enhances the aeration of the land, and promotes better drainage. After moldboard ploughing, intense tillage might involve using disc harrows, cultivators, subsoilers, chisel ploughing, and rotary hoes to break down the soil into smaller particles. This process improves the structure of

the seedbed, making it easier for fertilizer and herbicide application before planting. There are several types of moldboards commonly used in soil cultivation, including helical, semi-digger, and general-purpose moldboard (Nassir, 2018). These types are characterized by the size of the equipment, as follows: landslide length, overall length of bottom, shared culling width perpendicular to the direction of travel, shared wing angle, and lateral directional moldboard tail angle.

Table 1. The main differences between primary and secondary tillage (according to Tractor Junction, 2022)

Indicators	Primary tillage	Secondary tillage
Tools	Plough (moldboard, disc plough)	Such as cultivator, harrow, planks and roller, and disc harrow.
Working	To open and loose the soil	To control weeds, prepare seedbeds, take the soil to a fine tilth, and break clods.
Tilling time	Till the land after the last harvesting	Till the land after primary tilling
Tilling depth	Deep Tilling (around 15 to 90 cm)	Only the upper surface tilling (about 15 cm)
Tilling purposes	For burying of weeds and crop residues	For preparing the land for sowing or planting
Tilling results	Provides a rough surface finish	Providing a fine finish

Deep ripping (deep tillage) is the other technique in primary tillage that is usually used to improve the soil, which is dense in subsurface horizons (Bateman & Chanasyk, 2001). This method involves disturbing the soil below the normal cultivation layer. Deep ripping employs a ripper with three vertical tines 50 cm apart, each 2.5 cm thick and sharpened at the leading edge, with a hardened tip about 3.5 cm wide and 10 cm high. For research purposes, these rippers have four rows of tines (tine group) that rip at 10, 20, 30, and 40 cm depth. Commercial rippers usually have two rows of tines that rip at 20 and 30 cm or 30 and 50 cm depth. This spacing was considered adequate for effective loosening (Ellington, 1986).

Having learned the effect of PT on the soil, the attention of soil tillage today is shifting to create and maintain harmony between soil protection and cropping technologies. In other words, soil tillage is intended to improve the soil's physical and biological condition and preserve it in a way and to a depth that matches the cropping and protection task. CT is the answer to the above concept. CT harmonizes soil protection with the demands of the crop, soil, and climate (Birkás et al., 2017; Bogunović et al., 2019). In some previous publications, CT is defined as any tillage system where at least 30% leaves the litter on the soil's surface. The litter then becomes the residue covering the area between crop harvest and planting. CT has the seedbed preparation process that requires the presence of residue mulch and an improvement in surface roughness as the main criteria. CT is also closely related to reducing tillage intensity so that the disturbance of soil aggregate will decrease significantly.

Without ruling out the presence of a minimum 30% of crop residue after harvesting, the experts in general grouped the conservation tillage (CT) into four main groups: no-tillage, reduced tillage, stubble or much tillage, and ridge tillage (Busari et al., 2015; Carter, 2005). No-tillage refers to a method of land cultivation that includes little or no disturbance to the soil surface, with the sole disturbance occurring during planting. On the other hand, minimum or reduced tillage refers to a practice that entails a reduced amount of soil disturbance, specifically by ploughing utilizing primary tillage equipment. Mulch tillage is preparing or tilling the soil to maximize the coverage of plant wastes or other materials on the surface. Ridge tillage is a farming technique where crops are planted in rows on top of or along the sides of ridges constructed at the beginning of the cropping season.

2.1.2 Impact of intensive ploughing on crop yield and soil properties

PT has a positive impact on crop yield according to a meta-analysis of 1.530 observations in 67 experimental sites worldwide by (Schneider et al., 2017) indicated that deep tillage practice (in 60% of data) increased yield. In other investigations, deep tillage improves the soil's physical properties, specifically soil bulk density and root penetration reduction (Li et al., 2022). Deep tillage might be an alternative method for making crops more resilient to climate change and mitigating the yield losses caused by droughts (Alcántara et al., 2016).

On the other hand, numerous studies suggested that intensive cultivation has undoubtedly deteriorated the physical properties of most European soils since many decades ago (Saini and Grant, 1980). The moldboard ploughing in tillage operation may distract the pore continuity and aggregate stability, resulting in sediment mobilization, erosion, and surface hardening. This effect frequently exposes aggregates to physical disruption (Al-Kaisi et al., 2014). This physical damage exposes the protected organic matter (OM), enhances the accessibility of OM to microorganisms, increases the oxidation, and then accelerates its decomposition. Furthermore, the OM will be lost into the atmosphere as carbon dioxide (Naresh et al., 2017; Buragienė et al., 2019).

PT also potentially led to soil compaction. The utilization of heavy machinery, including wheel loads (Arvidsson and Keller, 2007), traffic frequency of machinery (Botta et al., 2008), and size and pressure of machinery tires (Afzali et al., 2014) is giving more stress to the soil which frequently causes of the soil compaction in agricultural soil (Bergamin et al., 2015). Soil compaction exerts a negative effect on soil's physical properties. Kuht and Reintam (2004) and Tenu et al. (2012) recorded the increase in soil bulk density of the plough layer by 0.11–0.26 g cm^{-3}. The breakdown of soil aggregate by ploughing activity in the upper soil or surface layer resulted in a high infiltration rate, and the large number of soil particles transported into the subsoil

layer caused soil compaction. Soil compaction inhibits the plant root from reaching the deeper layer and lowers the soil infiltration rate.

Moldboard ploughing practice increases the soil porosity at 0-30 cm depth by 8.2 to 28% (Taser and Metinoglu, 2005) and at 0-18 cm depth by 17-40% (Lipiec et al., 2006) in Fluvisol soil. The changes in total porosity are related to alterations in pore size distribution. The implementation of PT reduces bulk density, and it negatively medium-strength significantly correlated with the number of meso-pores (0.2–30 μm) and macro-pores (>30 μm) (r= -0.53 and r=-0.54 respectively). The composition of soil pores impacts the pore size distribution (Romaneckas et al., 2022). Pore size distribution and the continuity of pores or pathways drive soil water infiltration and retention (Hillel, 1998). A study by Lipiec et al. (2006) suggested that the three hours of cumulative infiltration in PT increases by 36-62% compared with reduced and no-tillage treatment (CT). Amami et al. (2021) also reported that the mean infiltration of moldboard ploughing (PT) is greater (5.6 cm h^{-1}) than reduced and no tillage (CT) by 4.6 and 2.5 cm h^{-1}. He also mentioned that the infiltration rate in no-tillage was smaller by 34.1% compared with PT. Generally, a high infiltration rate is favorable for plants and the environment. The inappropriate management of a high infiltration rate can lead to the risk of leaching of NH_4-N, NO_3-N (or susceptible soil nutrients), pesticides, and the loss of P from high P-content soil (Ceferino et al., 2021). The reduced tillage and the abundance of crop residue on the soil surface of CT controls the infiltration rate. In dense clay or compacted soil, the organic matter binds with the soil particles, resulting in stable aggregates and increasing porosity and infiltration.

2.1.3. The effect of conservation tillage on soil properties and crop yield

CT practice affects the soil's physical properties. Rashidi and Keshavarzpour (2007) reported that a higher bulk density of 1.50 g cm^{-3} was observed for the minimum tillage (MT) compared to CT by 1.41g cm^{-3}. A higher soil penetration resistance of 1105 kPa was obtained for the MT treatment than for CT (560 kPa). According to Martínez et al. (2011), CT practice minimized the soil water reduction in the dry season of two years of study other than PT by (23 to 31% and 40% consecutively).

CT practice induces soil water storage as well, which is a function of soil pore space and pore-size distribution, which are governed by soil texture and structure. The minimum soil disturbance and the increase of soil organic matter and soil cover by crop residue promote the soil water-holding capacity. The increase of soil water content by 12.4 to 16.6% was observed in CT compared to PT by Sharma et al. (2011). Another experiment by Bekele et al. (2022) indicated that no-tillage has 30-41.4%, one-time tillage has 14-57%, and two-time tillage has 8-46.6% soil

moisture content advantage over PT methods, which shows the ability of CT methods in conserving soil moisture.

The paradigms of soil tillage are not only to create good soil physics but also to maintain the soil organic carbon; CT practice led to changes in the soil carbon dynamics compared to PT (López-Garrido et al., 2014). Many studies observed that CT practices significantly influence the total soil organic carbon content (Naresh et al., 2015; Song et al., 2019). Yadav et al. (2017) recorded that microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) are higher in CT than PT. The MBC value in PT varied range from 130 to 135 (at 0-15 cm depth) and from 123 to 124 mg kg^{-1} soil (at 15-30 cm depth). Meanwhile, in the CT, the MBC value ranged from 141 to 152 and from 29 to 138 mg kg^{-1} at 0-15 cm and 15-30 cm depth, respectively.

In addition to soil organic carbon, CT application contributes to the accumulation of nutrients on the soil surface (Abdollahi and Munkholm, 2014; Song et al., 2019). A 20-30% increase of potentially mineralizable nitrogen in CT was also proved in a three-year study by Gajda and Przewłoka (2012). However, the increase of soil organic carbon by the CT is not usually accompanied by the improvement of soil nutrients. A three-year study in Argentina by Sokolowski et al. (2020) showed the increase of soil organic carbon and aggregate stability in CT plots, but the total N and exchangeable P in 0-20 cm depth were not significantly different with a PT. Higher organic matter in soil under CT appeared in the three years experiment in South Africa by (Haruna and Nkongolo, 2019), but throughout the study, the interaction effects of management practices (tillage) on soil nutrients were difficult to predict.

Another study by Cooper et al. (2020) in the UK revealed a different pattern over five years. The result could not prove the significant accumulation of soil organic carbon, nitrate, phosphorus, available potassium, and magnesium on the soil surface depth under either direct drill or shallow-inversion tillage methods (CT). No significant effect of CT (fall strip-till with shanks, spring strip-till with colters, and shallow vertical till) on C and N compared to the PT (standard chisel plough) also suggested in a four-year study by (Daigh et al., 2019).

CT can stimulate the conductivity of the soil environment. This circumstance supports the biological activities in the soil. The previous investigations in European soil demonstrated that the population of earthworms (Rasmussen, 1999; Dekemati et al., 2019) Nematode (Amossé et al., 2016; Bongiorno et al., 2019) are significantly affected by CT. Organic matter is a food source for soil macro-microorganisms. Hence, tillage without the addition of organic matter will decrease the soil quality. The tillage system controlled the microorganism communities. Based on his study, the soil bacterial and archaeal communities were dominated by the phyla Proteobacteria (22.77%) and *Acidobacteria* (17.43%). Fungal communities were dominated by the phyla Ascomycota (60.01%) and Basidiomycota (13.91%). These results confirm the previous finding by Sun et al.

(2018), who reported that tillage treatment strongly affected microbial communities' structure and distribution by soil depth.

Soil microbial activities are associated with the activity of soil enzymes. CT significantly increased substrate utilization (including amino acids, carboxylic acids, polymers, phenolic compounds, and carbohydrates) of >0.25 and <0.25 mm aggregates, which is very important for soil microbe activities (Guo et al., 2016). These activities can be estimated by measuring the activity of dehydrogenase. Some previous studies revealed the higher activity of dehydrogenase up to 34.3% under reduced tillage compared to conventional tillage (Sharma et al., 2011; Zhong & Zeng, 2020).

The presence of crop residue providing more substrate available and minimum soil disturbing by CT resulted in the high activity of β-glucosidase (Liu et al., 2023), reflecting a larger microbial capability to metabolize carbohydrates in CT (Acosta-Martínez et al., 2007; León et al., 2017). β-glucosidase is affected as well by the duration of CT application. The activity of β-glucosidase is not always higher in the CT, especially after the introduction of CT (Melero et al., 2009; Tian et al., 2020). The increase of β-glucosidase activity in CT varies depending on the duration of application. Mangalassery et al. (2015) suggested that the average β-glucosidase activity in CT of Gleysol, Stagnosol, and Leptosol soil is 28% higher than in PT. The investigation by Chen et al. (2019) of 11 years in Cambisol suggested an increase of β-glucosidase activity by 62%. Similarly, Liu et al. (2023) documented the more active β-glucosidase activity in CT, resulting in 32.13% higher soil organic carbon stock than PT in temperate Cambisol soil under 13 years of experiment.

CT results in a different microenvironment characteristic that contributes to different soil enzyme activity. For instance, CT resulted in the accumulation of SOC on the soil surface other than the deeper layer, which induced higher β-glucosidase activity on surface depth (Mina et al., 2008; Liu et al., 2023). The CT will preserve more precipitation water, modulating the β-glucosidase activity (Copec et al., 2015; Wen et al., 2023).

The implementation of CT has been reported to induce phosphatase activity (Lemanowicz et al., 2016; Yang et al., 2016). The abundance of crop residue in CT increased microbial biomass. Microbes could simultaneously immobilize more available P when receiving a carbon source from plant residue (Zhang et al., 2012). Reduced tillage affects labile and total soil P stock correlated with P dynamic linking to phosphatase activity (Margenot et al., 2017). The type of plant residue in CT also influences the phosphatase activity. A three-year study by Yang et al. (2016) suggested that corn straw mulch increased soil phosphatase by 19%–173%, and grass mulching increased the rate of phosphatase by 31%–196% compared to PT.

Minimum soil disruption in the CT brings positive effects on arbuscular mycorrhizal fungi propagules, including spore number, colonized root, and species richness (Alguacil et al., 2008).

Arbuscular mycorrhizal hyphae have been positively correlated with soil aggregate stability (Kabir and Koide, 2002). CT is a plough less tillage that can reduce aggregate breaking; therefore, the hyphal network remains intact, and the density of active hyphae is larger than soil under PT (Cornejo et al., 2009; de la Cruz-Ortiz et al., 2020).

Mycorrhiza produces glomalin, a glycoprotein that is strongly related to soil aggregation and soil structure improvement (Leifheit et al., 2014; Morris et al., 2019). CT protects the soil aggregate and preserves the mycorrhizal colonization, which in turn affects the glomalin concentration. The more abundant of arbuscular mycorrhizal mycelium may lead to more glomalin production in the CT. Wright et al. (2007) reported that the total-glomalin-related soil protein (T-GRSP) and easily extractable-glomalin-related soil protein (EE-GRSP) concentration produced in CT soil is more than two times (8.16 mg g^{-1} and 2.03 mg g^{-1}, respectively) compared to those produced in PT (3.96 mg g^{-1} and 1.16 mg g^{-1} respectively). Otherwise, the hyphal network disruption due to tillage operations, reduced glomalin production, and reduced aggregate stability (Kabir, 2005).

CT application induces crop yield, and the result can diversify depending on many factors, such as soil and climate conditions, the duration of CT application, and crop species (Van den Putte et al., 2010; Parvin et al., 2014). A ten-year investigation by Madarasz et al. (2016) in Hungarian Luvisol soil indicated that the crop yield of CT was somewhat better than PT. He also reported that CT implementation did not influence the yield in the initial three years of the experiment. The higher crop yield of CT was stable in the fourth year of investigation; however, it was significant only in the last several years. According to a meta-analysis in 21 European countries by Achankeng & Cornelis (2023), the crop yield under CT (ridge and strip tillage) led to a 5% increase over PT. Mainly, the CT method decreased yield by 8% under no-tillage and 18% under ridge tillage; otherwise, strip tillage showed a 7% gain. The availability of soil nutrition is one important factor that drives plant growth and production. CT application is unhesitating and improves the carbon and nutrient cycle in the soil. However, the contribution to the increase of soil nutrients is still unclear. As a result, the crop yield under CT practice is still inconsistent in many places.

2.2 Concept and measurement of soil health

2.2.1 Defining and measuring soil health in science

Soil is a precious, scarce, and non-renewable natural resource essential for human life. The process of forming a one-centimeter topsoil takes hundreds of years; however, the topsoil can be easily destroyed in only a few years. The increase in the world population stimulated the over-exploitation of soil to produce more food. Intensive agriculture contributes to the over-exploitation

of the soil. The intensification of agriculture has also led to the degradation and exhaustion of soil and land. Land degradation has become a global concern because it can cause crop productivity to decline and the economy to decline, which risks food security and farmers' livelihoods (Bhattacharyya et al., 2015; Mirzabaev et al., 2023).

Soil health is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans (Doran and Zeiss, 2000). Soil health is also frequently described as a dynamic, life-sustaining condition promoting soil organisms, nutrient cycling, and physical properties that require providing a fundamental necessity of life—food, fiber, fuel, and shelter—while conserving water and air quality as well (Karlen et al., 2019). The term "soil health" was introduced in 2000. Numerous soil scientists consider this concept more holistic than soil quality and better encompasses the soil's biological attributes (Powlson, 2020). The terms soil health and soil quality are sometimes used interchangeably. According to experts, soil quality mainly pertains to the soil's ability to fulfill specific human requirements, such as supporting the development of any crop. On the other hand, soil health primarily pertains to the soil's ongoing ability to sustain plant growth and preserve its functions (Bünemann et al., 2018).

Management of soil health is crucial for ensuring sustainable agricultural production and maintenance of soil biodiversity, including microbial diversity. The physical and chemical environment influences the microbial activity and functional diversity of soils, hence impacting their functions. These factors function as significant indicators of soil health. Ideal indicators of soil health are those soil properties that exhibit rapid change in response to natural or anthropogenic processes.

Bulk density, water-holding capacity, and soil aggregate stability have been identified as optimal physical indicators. Well-established chemical indicators include pH, EC, organic carbon, and soil nutrient status. However, the physical and chemical properties generally have a slow response compared with the microbiological and biochemical properties. Bulk density has been considered a good indicator of soil health due to its vital functions, such as aeration, infiltration, rooting depth, compaction, soil porosity, plant nutrient availability, and soil microorganism activities. Furthermore, bulk density is also related to the water holding capacity that is associated with infiltration, soil available water, and distribution. Aggregate stability is involved in maintaining important ecosystem functions in soil, including organic carbon accumulation, infiltration capacity, movement and storage of water, root, and microbial community activity, and soil erosion resistance, which makes aggregate stability useful as an indicator of soil health.

Soil pH is important in determining the solubility of various compounds, ion bonding, and the presence of diverse microbes. Soil EC is an indicator that quantifies the concentration of salt and describes the cycling of nitrates and biological processes. Soil organic carbon is a crucial factor in

evaluating soil health since it impacts significant functional activities in soil, such as nutrient storage, particularly nitrogen, water retention capacity, aggregate stability, and microbial activity.

2.2.2 Microbial indicators of soil health

Soil health varies depending on the situation and location. Soil characteristics, soil use, and environmental circumstances have been frequently selected as soil health indicators. Quantifying soil health is challenging due to the many interconnections between soil measures and the varying temporal dynamics of the parameters. Soil health indicators are often determined using multivariate and principal component analysis (Nandan et al., 2019; Juhos et al., 2023). Soil health is integrated with soil physical, chemical, and biological properties (table 2) (Raghavendra et al., 2020).

Table 2. Soil health indicators, according to Raghavendra et al. (2020)

Soil health indicators	Rationale for selection
Bulk density	Plant root penetration porosity and adjust the analysis to a volumetric basis.
Soil aggregate stability	Soil structure, erosion resistance, and crop emergence are early indicators of soil management effect.
Water holding capacity/ infiltration	Drought tolerance, leaching, and erosion potential.
Soil acidity/pH	Nutrient availability, pesticide adsorption, and mobility, process models.
EC (electric conductivity)	Defines crop growth, soil structure, and water infiltration; presently lacking in most process models.
CEC (cation exchange capacity)	CEC represents the total amount of exchangeable cations that soil can absorb.
Soil organic carbon/ organic matter	Defines soil fertility and soil structure, pesticide and water retention, and use in process models.
Soil nutrients status	Availability of crops, leaching potential, mineralization/ immobilization rates, process modeling, capacity to support plant growth, and environmental quality indicators.
Suspected pollutants	Plant quality and human and animal health.
Soil respiration, microbial biomass	Biological activity, process modeling, estimate of biomass activity, early warning of management effect on organic matter.
Soil enzymes (dehydrogenase, β-glucosidase, Acid and alkaline phosphatase)	Electron transferences in the respiratory chain in living cells, C oxidation, organic phosphorus cycling, source and/or drain of C and nutrients, microbial mineralization of organic carbon.
Mycorrhiza	Nutrient mobilization, soil aggregation.
Trichoderma	Residue decomposition.
Lipid profiling	Diversity and biomass.
Earthworm	Indicate relative change in soil structure, nutrient recycling, regulation of soil water, aeration, and provision of soil water and aeration, and provision of drainage.
Glomalin	positively related to soil edaphic factors, associated with AM fungal infections and useful for monitoring desertification, soil, and land degradation by anthropogenic activities.

All indicators in Table 2 are not always selected to evaluate soil health; however, the selection of indicators is more determined by the purpose. For instance, soil enzymes and labile carbon concentration are frequently considered the leading indicators in soil health assessment in

agricultural activities (Yang et al., 2021; Liptzin et al., 2022), mycorrhiza for reclamation of stressed ecosystems and drought tolerance (Augé et al., 2015; Begum et al., 2019), and glomalin in mitigation of land degradation problems (Singh et al., 2022).

Dehydrogenase

Dehydrogenase appears in all viable microbial cells, not in stabilized soil complexes. This enzyme is categorized as an oxidoreductase, a class of enzyme that catalyzes the electrons transfer from one molecule, the reductant, also called the electron donor, to another, the oxidant, also called the electron acceptor. In a soil environment, dehydrogenase plays a significant role in the soil organic matter oxidation by transferring hydrogen from organic substrates to inorganic acceptors (Zhang et al., 2010) (Fig. 1). Dehydrogenase can be used as a source of information about the population of living microorganisms in the soil and the total microbiological activity of the soil (Makoi and Ndakidemi, 2008). Soil dehydrogenase indicates the performance of a group of intracellular enzymes in living soil microbes. These enzymes measure the metabolic reactions involved in the oxidative energy transfer of soil microorganisms (Kujur and Kumar Patel, 2014).

$$\underset{\text{Substrate}}{\mathbf{RH_2}} \xrightarrow{\textit{Dehydrogenase}} \underset{\text{Oxidized substrate}}{\mathbf{R + 2H^+ + 2e^-}}$$

Figure 1. Soil dehydrogenase activity

Lenhard was the first person to use the 2,3,5-triphenyl tetrazolium chloride method to measure DHA and look at the activity of microbes in the soil. This was published in 1956 (Benefield et al., 1977). Until recently, monitoring dehydrogenase, a respiratory enzyme and integral part of all soil microorganisms, was still frequently used to measure soil's biological activity at a given time.

The dehydrogenase is affected by many factors, both environmental and anthropogenic. Dehydrogenase is relatively decreased in low-water-content soil (Wolińska and Bennicelli, 2010). Borowik and Wyszkowska (2016) reported that dehydrogenase is highest in soils with a moisture content of 20% to 40% of the maximum water capacity. Adak et al. (2014) also suggested that the dehydrogenase linearly increases in the soil with 13 to 21% of soil water content. Additionally, the investigation (Stêpniewski et al., 2000; Brzeziñska et al., 2001) showed that the dehydrogenase activity was high in the soil that was overflowing with water (flooded). Trevor (1984) reported a high positive correlation between dehydrogenase and substrate concentration, incubation temperature, and soil pH.

Aeration is also an essential factor driving the activity of soil microorganisms. Soil aeration is closely linked to the redox potential (E_h) and oxygen diffusion rate (ODR). The investigation by Brzezinska et al. (1998) on the soils of Luvisol and Phaeozem indicated that soil dehydrogenase

is highly negatively correlated (r=-0.81) with the redox potential. Similarly, a laboratory experiment with Mollisol soil by Perotti (2015) also showed a strongly negative correlation between soil dehydrogenase and E_h (r=-0.99). The E_h of soil is influenced by an electron acceptor (oxygen or another oxidizing agent) and the pH. In soil with good drainage, the E_h ranges from +400 to +700 mV, while in waterlogged soils, the E_h decreases from +400 to -300 mV.

ODR is the transport of oxygen gas in the soil that is essential for supplying the roots (Feng et al., 2002). In general, ODR is closely related to E_h. According to Husson (2013), O_2 does not exist in the soil when the E_h value is under +350 mV. At the same soil porosity, oxygen diffusion is less in humid soil than in dried soil because of a higher percentage of air-filled pores (Feng et al., 2002). In other conditions, when most of the soil pores are waterlogged, the oxygen diffusion will approach zero (Wu et al., 2003), and the Eh values in such a situation may be as low as -300 mV.

Soil acidity or pH governs the ionization of functional groups of organic molecules and the forming of substrates and enzymes (Wolinska and Stepniewsk, 2012). The acidity suppressed dehydrogenase and tended to increase with the increment of soil pH. Dehydrogenase was associated with the pH (range from 3.73 to 5.03) in different soil parent material and tree species experiments (Błońska et al., 2016). Another study by Cooper and Warman (1997) suggested that soil dehydrogenase was also connected to the pH (range from 6.0 to 6.50) under different soil amendment additions.

Organic matter is essential in the soil as the source of microorganisms' energy and enzyme production (Sinsabaugh et al., 2008). Many studies have revealed that the quantity and quality of organic matter affect dehydrogenase activity. For example, Veres et al. (2013) reported that decreased litter production in oak forests reduced soil dehydrogenase. Conversely, more organic matter will maintain the larger and more active microbial biomass and higher dehydrogenase in the soil (Chodak and Niklińska, 2010; Bonanomi et al., 2011). According to Pramanik et al. (2010) and Adak et al. (2014), the dehydrogenase is more affected by quality and quantity, showing higher dehydrogenase on easily decomposable organic matter, vermicompost, compared to farmyard manure and paddy straw mulch.

β-glucosidase activity

β-glucosidase plays a vital role in C-cycle and soil management practices, including tillage practice (Mariscal-Sancho et al., 2018). β-glucosidase is involved in the degradation of cellulose in soils and can potentially monitor biological soil quality (Turner et al., 2002). Soil β-glucosidase is produced mainly by saprotrophic microorganisms such as bacteria and fungi, but it is also present in root exudates and the gut of soil fauna (Lammirato, 2011; Veena et al., 2011). The

principal role of β-glucosidase in cellulolytic microorganisms is to catalyze the hydrolysis of cellobiose and cello-oligosaccharides, producing glucose during bioconversion (Singh et al., 2016). β-glucosidase acts in the last phase of the cellulose degradation process by hydrolyzing the cellobiose residue (Gil-Sotres et al., 2005). These reactions produce glucose as the final product, an important C energy source for the growth and activity of soil microorganisms (Merino et al., 2016). The diagram of cellulose decomposition (Sylvia et al., 2005) is shown in Fig. 2.

β-glucosidase was determined to involve P-nitrophenol glucosidase (PNP-G). A colorimetric technique measures the PNP released by glucosidase (Hayano, 1973; Sinsabaugh et al., 1999). The presence of β-glucosidase converts the substrate to PNP, which can be easily measured in the supernatant spectrophotometrically (Strahsburger et al., 2017).

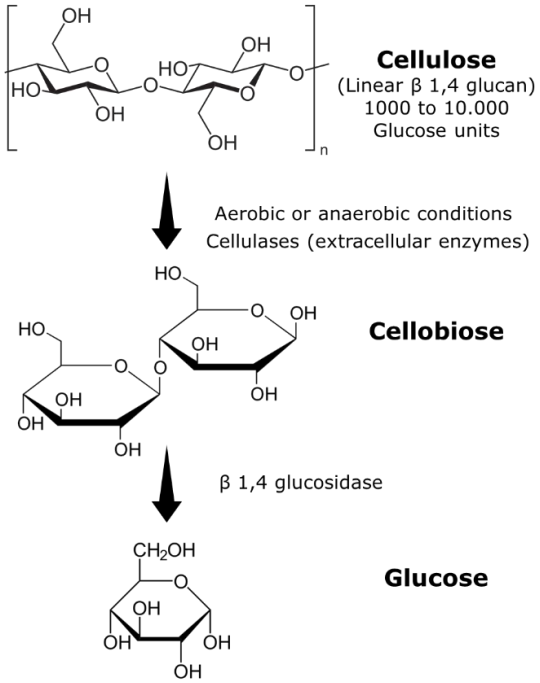


Figure 2. Decomposition of cellulose (Sylvia et al., 2005)

β-glucosidase is strongly correlated to soil moisture (Steinweg et al., 2012). In a laboratory experiment, it was reported that β-glucosidase is significantly higher in the soil under 50% of regular precipitation treatment compared to the standard (100%) and excess precipitation (150%). Another study by Borowik and Wyszkowska (2016) indicated that during 16 weeks of incubation, the β-glucosidase is more active in treatments with 20 and 40% water holding capacity than in dry soil and 60%. The soil zymography study by Zhang et al. (2023) suggested that the spatial distribution of β-glucosidase was strongly related to soil moisture content and root hairs. Root hairs and optimal water content increased the hotspot area of β-glucosidase. The hotspot area was higher under optimal soil water content (70% of water holding capacity) than in drought conditions (30% of water holding capacity).

In general, the activities of soil enzymes increase concurrently with the incline of temperature up to the optimum catalytic value (Voroney et al., 2007). Zhang et al. (2011) reported that the temperature ranges of 10-30 °C affected the kinetic parameters of soil β-glucosidase. Otherwise, Kotroczó et al. (2022) stated that temperature does not play a primary role in the development of β-glucosidase.

Besides soil temperature, β-glucosidase was also sensitive to soil pH changes (Kotroczó et al., 2022). According to Neesa et al. (2020); Wade et al. (2020), β-glucosidase showed a consistent pattern with more significant activity in moderately acidic soil to neutral pH and lower activity at alkaline pH. On the other hand, the study of Turner et al. (2002) suggested the absence of a correlation between soil pH and β-glucosidase.

Phosphatase

Phosphatases are the extracellular enzymes that catalyze the hydrolysis of phospho-ester bonds in organic P-containing substrates, releasing inorganic P in the form of orthophosphates that soil biota and plants can use (Dotaniya et al., 2018) (Fig. 3).

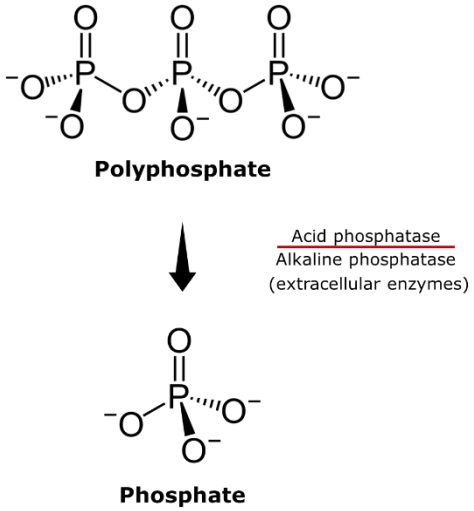


Figure 3. Decomposition of phosphate (Dataniva et al., 2018)

According to George et al. (2008), fungi and bacteria in the soil, as well as plant-root exudates, produce phosphatase. Phosphatase is an important indicator and describes soil organic P mineralization. Based on the optimum pH, phosphatase is principally categorized into acid and alkaline (Dick et al., 2000). Acid phosphatase is mainly found in acid soils with 4-6 pH ranges, while alkaline phosphatase dominates in alkaline soils with a pH of 9-11 (Dodor and Tabatabai, 2003).

A large amount of phosphorus in the soil is organically bound; meanwhile, plants only uptake inorganic phosphorus. Therefore, the mineralization of organic P becomes crucial and can have a

vital influence on plant nutrition (Nannipieri et al., 2011). This mechanism is linked to phosphatase and can simultaneously indicate inorganic phosphorus availability for plants and microorganisms (Piotrowska-Długosz and Charzyński, 2015).

Phosphatase is a typically mechanistic response to soil P deficiency in the plant-soil system. When the amount of P in the soil is insufficient, plant roots and microorganisms enhance the secretion of phosphatase into the rhizosphere to hydrolyze organic P, increasing solubilization and remobilization of phosphate, affecting the ability of the plant to overcome phosphorus-stressed conditions (Janes-Bassett et al., 2022).

Phosphatase determination has a similar principle to β-glucosidase determination. The colorimetric procedure (spectrophotometer) is used to estimate p-nitrophenol (PNP) that is hydrolyzed from p-nitrophenol phosphatase (PNPP) (Tabatabai and Bremner, 1969; Sinsabaugh et al., 1999).

It is generally known that soil acidity (pH) drives the soil phosphatase. Dick et al. (2000) stated that alkaline phosphatase is more dominant than acid phosphatase when the soil pH increases. Several studies have indicated the correlation between soil water content and phosphatase, as demonstrated by Sardans et al. (2006). Huang et al. (2011) observed that acid phosphatase exhibited more activity during the rainy season compared to the dry season, indicating a preference for higher water conditions. In a separate study conducted by Margalef et al. (2017), it was shown that drought decreases the levels of acid and alkaline phosphatase. Brandt et al. (2011) reported an elevation in phosphatase levels because of drought conditions.

Phosphatase is also inhibited by the availability of inorganic P, which can be caused by the addition of P (Olander and Vitousek, 2000; Janes-Bassett et al., 2022). The root-fungi symbiotic mycorrhiza is another factor that influences the phosphatase. The study of Joner and Jakobsen, (1995) indicated that mycorrhiza decreases the alkaline phosphatase in the soil without organic matter treatments and vice versa. Qi et al. (2022) reported that phosphatase is linked to mycorrhiza (colonization, spore number) and the P- availability in the soil.

Labile carbon

Soil organic matter is a constituent fraction of soil. The Food Agriculture Organization defines soil organic matter as any material produced originally by living organisms (plant or animal) that is returned to the soil and goes through decomposition. In other words, soil organic matter includes all organic matter (living and non-living) present in the soil. Non-living organic matter consists of two main distinct fractions, labile-C and stable-C, which vary in size, turnover time, and composition in the soil (Baldock and Skjemstad, 2000; Enchilik et al., 2023)

Wambsganss et al. (2017) and Duddigan et al. (2019) classified soil organic carbon into three main groups (pools) (labile-C, oxidizable-C, and stable-C) shown in Fig. 4.

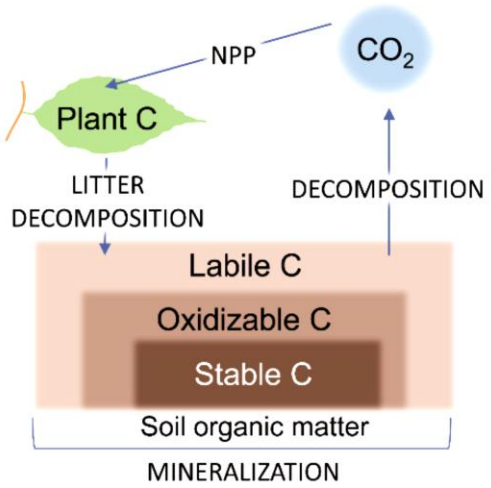


Figure 4. Diagram of the carbon cycle and fractionation of soil organic matter (Enchilik et al., 2023)

The labile-C fraction, including bioavailable and easily decomposable organic compounds, undergoes a faster turnover (Lorenz et al., 2021). The soil microorganism communities are important in plant residue decomposition (Kotroczó et al., 2020). The decomposition process will influence the nutrient dynamics in the soil. According to many scientists, there are five labile carbon fractions, i.e., particulate organic matter carbon (POMC), which consists mainly of partially decomposed organic residues (Haynes, 2005); dissolved organic carbon (DOC) that represents the organic carbon in the soil solution; hydrophilic DOC (Hy-DOC), represents the more bioavailable part of the DOC (Bolan et al., 2011), primarily consisted of plant root and microbial exudates, products of hydrolysis and leachates from organic matter (Leinemann et al., 2018); Permanganate oxidizable carbon also referred to as active carbon, representing the microbially available carbon energy sources, that is microorganism food. It comprises several easily decomposable substrates (i.e., polysaccharides, a fraction of microbial biomass); cold water extractable organic carbon (CWEOC) and hot water extractable carbon (HWEC) are mainly present in the soil solution or loosely bound to soil minerals and is prone to short-term seasonal variation (Schulten et al., 1995; Malobane et al., 2020).

Chandrika et al. (2016) and Malobane et al. (2020) observed that the CWEOC, HWEOC, POMC, and POXC under CT were considerably higher than those in PT. The investigation of Badagliacca et al. (2020) suggested that POXC is significantly related to total inorganic (TIC) and TOC, textural fractions, temperature, and precipitation-related indexes. Permanganate oxidizable carbon is also correlated with various soil textures, chemistries, particle sizes, and management

systems (Nunes et al., 2020; Wade et al., 2020). Another study by Hurisso et al. (2018) also demonstrated a positive correlation between Permanganate oxidizable carbon and soil pH. Otherwise, a weak relationship between permanganate oxidizable carbon and pH and EC is reported by (Gasch et al., 2020).

Mycorrhiza

Mycorrhiza is a symbiotic association between plant roots and fungi (Fig. 5). Arbuscular mycorrhizal fungi are perhaps the most common of these root symbionts. Arbuscular mycorrhizal fungi are an extraordinary symbiosis of plants formed by ~80% of terrestrial plants and by obligate symbiotic fungi of the phylum Glomeromycota. Glomeromycotan fungi are usually known as arbuscular mycorrhizal fungi (Schüßler et al., 2006).

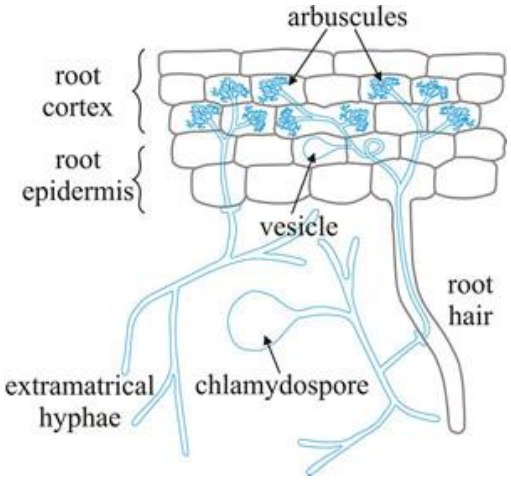


Figure 5. Diagram of the arbuscular endomycorrhiza (Moore David et al., 2011).

Mycorrhiza is considered as soil health indicator due to its effects on soil structure and ecology, specifically in soil formation, soil aggregation, soil fertility, nutrient availability, and bio-geo-cycling (Purin and Rillig, 2007). The hyphae structure of arbuscular mycorrhizal fungi and the secretions of glomalin, a protein related to the mycorrhizae, are much involved in soil aggregation and soil structure improvement (Purin and Rillig, 2007; Leifheit et al., 2014; Morris et al., 2019)

Allen et al. (2003) described seven types of mycorrhizae, i.e., arbuscular, ecto, ectendo-, arbutoid, monotropoid, ericoid, orchidaceous mycorrhizae. Arbuscular and ectomycorrhiza are the most abundant and widespread in the soil. Arbuscular mycorrhizae fungi or endomycorrhiza are defined as obligate symbiotic biotrophs; therefore, arbuscular mycorrhizal fungi cannot grow without a host plant supplying them with carbohydrates, glucose, and sucrose (Harrison, 2005). Arbuscular mycorrhizal fungi extent of the plant contact area with soil. Smith and Read (1997) reported that arbuscular mycorrhizal fungi enhance root absorption area up to 47-fold. The

symbiosis boosts plant growth and increases abiotic stress tolerance, including salinity, drought, high and low-temperature stress, land mining degradation, and heavy metal toxicity (Wahab et al., 2023).

Several abiotic factors influence mycorrhizal activity, such as soil pH, fertility, and climatic conditions. Soil pH ranging from 4.5 to 7.5 does not impact symbiotic activity between arbuscular mycorrhizal fungi in plant roots (Bücking and Kafle, 2015). Mycorrhizal fungi vary in pH tolerance, reflected by the different arbuscular mycorrhizal fungi species in different soil acidity levels. For instance, *Acaulosporaceae* frequently occurs in low pH soils, whereas other species, e.g., *Glomeraceae*, prefer alkaline and neutral substrates. Soil pH is related to the germination of mycorrhiza spores (Bainard et al., 2014; Salih Alkobaisy, 2023).

Numerous studies identified that the role of arbuscular mycorrhizal fungi is associated with soil fertility that stimulates plant growth (Sheikh-Assadi et al., 2023). Arbuscular mycorrhizal fungi absorb macro and micronutrients, like N, P, K, Cu, and Zn, and translocate them to the plant in which they are symbiotic (Rui et al., 2022; Sheikh-Assadi et al., 2023). Arbuscular mycorrhizal fungi show the most remarkable effect in improving P-uptake when their plant is in P deficiency (Abbott et al., 1984; Grant et al., 2005). Likewise, the high N content in the soil suppresses mycorrhiza activity (Maaroufi et al., 2019; Xu et al., 2022).

The activity of arbuscular mycorrhizal fungi is very dependent on climatic conditions (Jerbi et al., 2020; Salih Alkobaisy, 2023). Temperature, humidity, and light are three elements of climate affecting arbuscular mycorrhizal fungi activity. The investigation of Parke et al. (1983) suggested the temperature response of mycorrhiza resembled a bell-shaped pattern; the value was between 7.5 and 35 °C with the optimum at 18–25 °C. Extremely low or high temperatures will reduce arbuscular mycorrhizal fungi colonization in the soil. Soil humidity is strongly related to precipitation. Low precipitation decreases soil humidity and increased oxygen concentrations, resulting in the germination spore and growth of arbuscular mycorrhizal fungi (Kilpeläinen et al., 2020).

Light is linked to the energy for photosynthesis. Konvalinková and Jansa (2016) stated that arbuscular mycorrhizal fungi depend on the supply of C for the photosynthesis of their host plant for growth and metabolism. Previous studies reported that the decreases considerably reduce root arbuscular mycorrhizal fungi colonization in light shading (Schreiner and Pinkerton, 2008; Konvalinková et al., 2015).

Glomalin

The term “glomalin” is described as a heat-stable glycoprotein produced by the hyphae and spores of arbuscular mycorrhizal fungi, which we classify as Glomeromycot (Wright and Upadhyaya, 1998; Vlček and Pohanka, 2020; Rillig and Steinberg, 2002). The total glomalin concentration in soil is closely related to the total soil organic matter content (Wright et al., 2007; Zbíral et al., 2017). (Fokom et al., 2012) reported that glomalin accounts for a relative proportion of 2-15 mg g^{-1} soil organic carbon and approximately contributed 5-10% of total SOC. Glomalin is stable, long residence time in the soil, and is less sensitive to environmental changes (Rillig et al., 2001). Due to this characteristic, glomalin is frequently used as a soil health indicator (Zbíral et al., 2017; Šarapatka et al., 2019)

In many studies, glomalin has been linked consistently to soil and plant health, including soil aggregation (Fokom et al., 2012; Rillig and Mummey, 2006), soil carbon storage (Preger et al., 2007; Rotter et al., 2017), improving plant growth under abiotic stress condition (Santander et al., 2017), soil nutrient content and distribution (Lovelock et al., 2004) heavy metal chelation (González-Chávez et al., 2004; Gao et al., 2017) and plant productivity (Jansa et al., 2020).

A citrate buffer solution involving an autoclave has been the accepted protocol to extract two fractions of glomalin, i.e., easily extractable glomalin-related soil protein (EE-GRSP) and total glomalin (TG- GRSP) from the soil (Janos et al., 2008; Wright and Upadhyaya, 1998) EEG is a newly produced glomalin deposited into the soil and more active fraction, on the other hand, TG is the total amount of glomalin extracted from the soil (Wright and Upadhyaya, 1998; Meng et al., 2021).

Glomalin production is controlled by a balance between arbuscular mycorrhizal fungi production and microbial decomposition, dependent on arbuscular mycorrhizal fungi assimilation from host plants (Violi et al., 2007). Several investigations have also indicated a positive correlation between glomalin concentration and soil organic carbon content (Gispert et al., 2018), suggesting glomalin's potential contribution to C storage so it can act as a sensitive index for measuring the soil carbon pool. Glomalin production is also affected by the arbuscular mycorrhizal fungi species. In this case, the arbuscular mycorrhizal fungi species *Scutellospora heterogama* produced more glomalin than *Glomus intraradices* (Violi et al., 2007). Wu et al. (2015) reported that soil enzymes like arbuscular mycorrhizal fungi, catalase, and peroxidase activity positively correlate with glomalin production. Soil enzymes have a key role in controlling the biochemical transformation of soil and sustaining the cycles of soil carbon, nitrogen, and phosphorus. As a result, they impact the alteration of glomalin.

Climatic conditions, including temperature, precipitation, and CO_2 concentration, are fundamental parameters for the production and decomposition of glomalin. High temperature

decreases glomalin concentration (Wang et al., 2020). It can be explained that the increase in temperature will stimulate organic matter decomposition, while glomalin becomes susceptible to being decomposed by microbes (decrease the concentration); in other words, warming declines the immunoreactive of glomalin and aggregate stability due to higher microbial activities decomposing glomalin (Rillig et al., 2003). Otherwise, according to (Miller et al., 1995), the low temperature will slow the decomposition of the hyphal of arbuscular mycorrhizal fungi; therefore, it does not hinder glomalin production. A study by Wang et al. (2022) suggested that the combination of low temperature and rainfall reduces the allocation of C to arbuscular mycorrhizal fungi by the plant host, inhibiting arbuscular mycorrhizal fungi hyphal growth and decreasing the arbuscular mycorrhizal fungi biomass and glomalin production. The CO_2 concentration in the atmosphere drives the glomalin concentration as well. The elevation of atmospheric CO_2 levels will stimulate arbuscular mycorrhizal fungi hyphal development, which in turn enhances glomalin production (Vodnik et al., 2008; Jia et al., 2016).

Soil health parameters affected by spatial and temporal

Plenty of studies have reported that soil health parameters are affected as well by location (spatial) and time (temporal) (Glina et al., 2021; Abay et al., 2022). Soil depth is another type of spatial (vertical) variability that induces microbial processes and carbon and nutrient cycle (Minick et al., 2022). Stone et al. (2014) and Erdel and Şimşek (2023) reported that the activity of soil enzymes decreases along with the increase of depth in a soil profile associated with substrate availability and microbial biomass abundance. Those two experiments also showed that decreased soil enzyme activity and increased soil depth are due to reduced root growth and lower organic matter inputs from the root residue in lower depths. On the contrary, many investigations indicated an extensive activity of soil enzymes in the deeper soil layers concerning the pedogenic process (Dove et al., 2020; Marinari et al., 2021).

Soil enzyme activities also responded differently to temporal variability related to soil enzyme's climate sensitivities (Wallenstein et al., 2009). For instance, soil enzyme activities decrease in summer and winter with decreasing moisture and temperature. Apart from that, (Abay et al., 2022; Nannipieri et al., 2011) observed the variation of soil enzyme activity in arid and semi-arid forest ecosystems induced by the existence of canopy gaps in the growing season that controlled the physicochemical properties. He also documented that soil temperature and water content significantly affected the soil enzyme activities.

In the growing season, the growth stage, as a kind of temporal variation, is fascinating to discuss, even though obtaining the single effect of root activity from the effect of the plant growth stages is difficult. Regardless of many interactions with plant growth stages, (Deng et al., 2019)

revealed the variation of soil enzyme activity of different vegetative growth periods of *Caragana korshinskii* Kom in Loess Plateau, China. Likewise, Zhang et al. (2005) demonstrated the alteration of soil enzyme activities under the various growth stages of the spruce plant (Picea spp.) forest in the Eastern Qinghai-Tibet Plateau. Nugroho et al. (2023) also reported that the corn growth stages significantly drive the soil enzyme activity that is linked to the nutrient supply in Hungarian Luvisol.

III. MATERIALS AND METHODS

3.1 Experimental background

3.1.1 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).

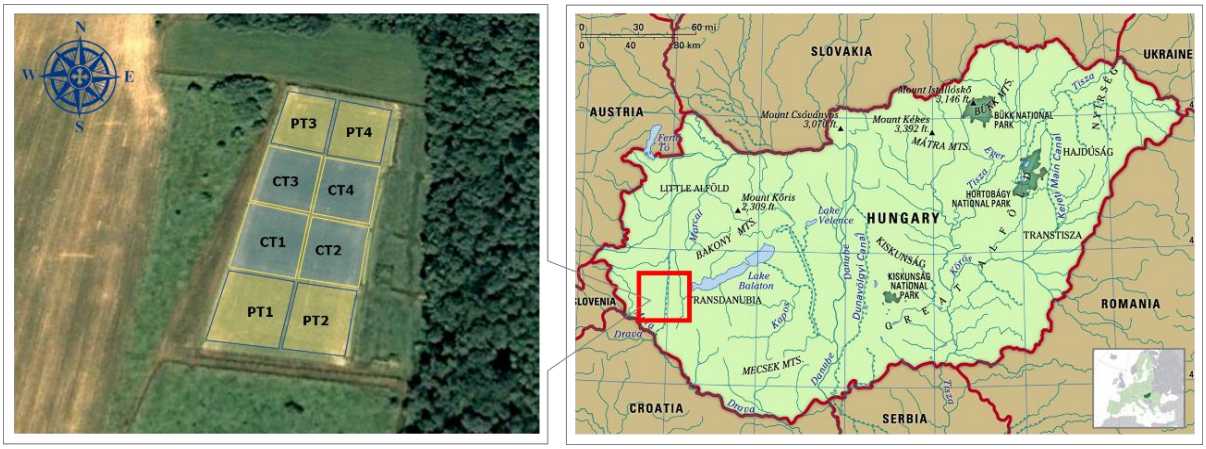


Figure 6. Research location and the layout of the field experiment. CT: conservation tillage, PT: ploughing tillage

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.

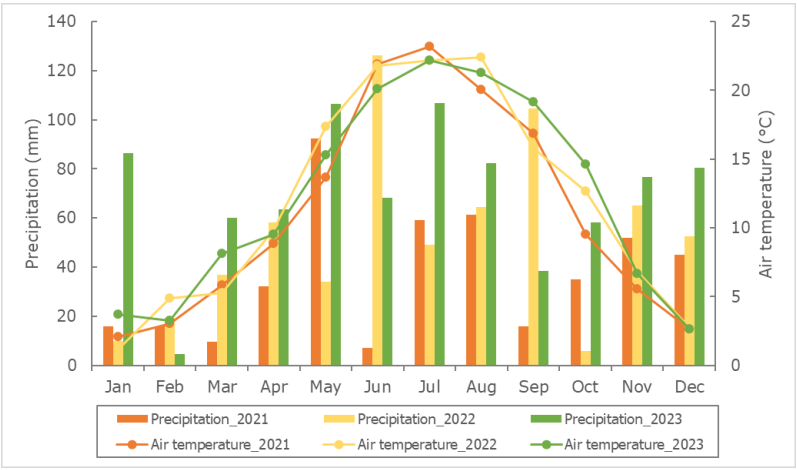


Figure 7. Monthly precipitation and air temperature (2021-2023) at the experimental site (Hungarian Meteorological Service, Sármellék station).

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	pH (H_2O)	pH (KCl)	BD	Clay	Silt	Sand	SOC-PT	SOC-CT
(cm)	-	-	(g cm^{-3})	----------%----------			----------%----------	
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). The details of agronomical operations in the CT and PT plots are summarized in Table 4.

3.1.2 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.

Row 1	Row 2	Row 3	Row 4
M1	M2	M0	M2
M2	M1	M1	M0
M0	M1	M2	M0

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).

3.2 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 4. The agronomical operations in the experimental field

Date	Agronomical operation
12/03/2021	Seed bed preparation-harrowing (in PT plot)
12/04/2021	Maize sowing, 55.000 seeds ha^{-1} (in CT and PT plot)
31/11/2021	Harvesting (in CT and PT plot)
16/11/2021	Chopping of maize residue (in CT and PT plot)
20/11/2021	Fertilization: 27 kg N 0.8 ha^{-1}, 20 kg P_2O_5 0,8 ha^{-1}, and 40 kg K_2O 0,8 ha^{-1} followed by discing (in CT and PT plot)
25/11/2021	*Moldboard* ploughing and harrowing (in PT plot)
12/03/2022	Ring float by *crosskill roller* (in PT plot)
14/04/2022	Seed bed preparation-harrowing (in PT plot)
19/04/2022	Sunflower sowing, 56.000 plant ha^{-1} + fertilization 81 kg N ha^{-1} + soil disinfector application 15 kg ha^{-1} (in CT and PT plot)
21/04/2022	Mixed herbicide application 9.5 l ha^{-1} (in CT and PT plot)
27/05/2022	Herbicide application for *Sorghum halepense* 1.5 l ha^{-1} (in CT and PT plot)
17/06/2022	Foliar fertilizer application 5 l ha^{-1} + fungicide application 1 l ha^{-1} (in CT and PT plot)
23/09/2022	Harvesting (in CT and PT plot)
24/09/2022	Sunflower stubble chopping and discing. Basic fertilization (LAT N 27) at a dose of 100 kg ha^{-1}, TARNOGRAN PK (Ca Mg S) 12-23 (6-4-10) FOSFOR-POTASSIUM at a dose of 200 kg ha^{-1}. (in CT and PT plot)
24/09/2022	Cover crop: Sowing dose 50 kg/ha, cover crop mix: DÉMÉTÉR BIOSYSTEM Tillage Mix ATTILA PK (in CT plot)
02/01/2023	*Moldboard* ploughing and harrowing (in PT plot)
09/03/2023	*Discing* (in PT plot) + roller.
05/04/2023	Seedbed preparation. (in PT plot)
05/05/2023	Chopping of cover crops. (in CT plot)
23/05/2023	Sowing of maize (VADERSTAD TEMPO) - Maize seed Hybrid SY UNITOP, sowing 72000 seeds ha^{-1} + soil disinfection FORCE 1,5 G at 15 kg ha^{-1} + 300 kg ha^{-1} of 27% GENEZIS pesticide in a row. Sowing depth 5 cm. Maize was very late in going into the soil due to unfavourable weather conditions, so that even a very early hybrid was not in harvestable condition by mid-September, which largely determined the development of the following cover crop mixture until the onset of cold weather. (in CT and PT plot)
01/06/2023	Weed control. Pesticide: MILAGRO 040 SC 4 L ha-1, CALARIS PRO dosage: 1.5 L ha^{-1}, EUCAROL dosage: 0.5 L ha^{-1}, FIX PRO dosage: 0.1 L ha^{-1}. in CT and PT plot
03/10/2023	Harvesting (in CT and PT plot)

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.

3.2.1 Physicochemical parameters

Bulk density (BD)

BD was measured using the ring/cylinder method. A cylinder of known diameter and height is inserted in the soil. A soil sample that has exactly the internal dimension of the cylinder is collected and dried. The bulk density is equal to the ratio of the soil sample's dry mass divided by its volume and is expressed in grams per cubic centimeter (g cm^{-3}) (Blake & Hartge, 1986).

Total organic carbon (TOC) and permanganate oxidizable carbon (POXC)

Combustion at 900 °C was used to determine the TOC using a Shimadzu TOC-L device equipped with an SSM 5000A solid sample combustion (Jakab et al., 2016, 2019). POXC concentration was examined by the permanganate oxidation method by Weil et al. (2003). A 10 ml of 0.02 M $KMnO_4$ solution was added to a 1 g air-dried soil sample. The soil mixture was then shaken at 125 rpm for 5 minutes. A 200 µl of soil solution and 10 ml of distilled water was added afterward and centrifuged at 3000 rpm for 10 minutes for separating the supernatant and filtrate. A spectrophotometer at 565 wavelength was employed to measure the POXC concentration, defining as the carbon (C) that can be oxidized by $KMnO_4$. To determine the sample $KMnO_4$ concentration, the sample absorbance was compared with a standard curve that ranged from 0.005 to 0.02 mol L^{-1} $KMnO_4$. Based on the standard curve, the concentration was calculated as follows:

$$KMnO_4,\ mol\ L^{-1} = 0.0395x - 7E\text{-}05,\ R^2 = 0.9992;\ where,\ x\ represents\ absorbance$$

Sample POXC was calculated as follows:

$$POXC\ (mg\ kg^{-1}) = (0.02 - KMnO_4\ mol\ L^{-1}) \times 9000\ mg\ C\ mol^{-1} \times 10$$

Ammonium (NH_4-N) and nitrate (NO_3-N) concentration

NH_4-N and NO_3-N concentrations were measured by the salicylate method (Kempers & Zweers, 1986). Soil extract was made by putting 50 ml of 1 M KCl or 0.01 M $CaCl_2$ solution into 10 g of air-dried soil, shaken for 1 hour, and filtered (Houba et al., 2000). Pipette 5 ml soil extract into a beaker glass and react with reagent #1 (mixture of NaOH + $C_3Cl_2N_3NaO_3$) and reagent #2 (mixture of $C_7H_5NaO_3$ + $Na_3C_6H_5O_7$ + Na_2[Fe $(CN)_5NO$], leave it for 30 minutes. Soil NH_4-N concentration was quantified using a spectrophotometer at 655 nm wavelength (absorbance mode). To determine the sample NH_4-N concentration, the sample absorbance was compared with a standard curve that ranged from 0.00 to 2.00 mg L^{-1} NH_4 – N.

NO_3-N concentration was determined by adding 1 ml $C_7H_5NaO_3$ to a 5 ml soil extract in a beaker glass (Houba et al., 2000), which was then put in a sand bath to evaporate the liquid phase until the precipitate appeared in the bottom of beaker glass, then cooled. Furthermore, the precipitate was dissolved in 1 ml of H_2SO_4 concentrate. A 25 ml distilled water and 5 ml 10 M NaOH were poured into the beaker glass; the suspension was then transferred to a 50 ml flask. Another 19 ml distilled water was added for dilution. Twenty minutes later, nitrate concentration was measured from the substrate using a spectrophotometer at 410 nm wavelength (absorbance mode). To determine the NO_3-N concentration of the sample, the absorbance of the sample was compared with a standard curve that ranged from 0.00 to 80.00 mg L^{-1} NO_3-N.

Easily available P, potential available P, and total P concentration

Easily available P, potential available P, and total P were estimated using different extraction and preparation methods. For easily available P determination, 10 g of air-dried soil and 50 ml of 0.01 M $CaCl_2$ solution were shaken for 1 hour and filtered using filter paper. A 5 ml of soil extract was pipetted into the beaker glass, then reacted with 7.5 ml of $(NH_4)_2MoO_4$ and 0.5 ml of $SnCl_2$ solutions and left for 30 minutes (Houba et al., 2000). A spectrophotometer at 438 nm wavelength (transmission mode) was employed to measure the subtraction. To determine the easily available P concentration, the sample transmissions were compared with a standard curve ranging from 0.00 to 20.00 mg L^{-1} P_2O_5.

Potential available P was examined by reacting to the 2.5 g air-dried soil with P-Bray solution (0.03 M NH_4F + 0.1 M HCl) (Bray & Kurtz, 1945). Soil extract was obtained by filtering the soil mixture after 1 hour of shaking. After 30 minutes, the extract was measured spectrophotometrically with a similar technique in measuring easily available P. To determine the potential available P concentration of samples, the sample transmissions were compared with a standard curve that ranged from 0.00 to 100.00 mg L^{-1} P_2O_5.

The total organic and inorganic phosphorus contents in soil are called total phosphorus. 1 g soil ash that was prepared by ignition soil at 550 °C for 1 hour was used. A 50 mL of 1N H_2SO_4 was added to 1 g soil ash in the plastic bottle, shaken for 16 hours, and filtrated. A 10 ml complexing agent, the vanadate-molybdenum reagent, was added and left for 30 minutes (Pardo et al., 2003). Furthermore, the substrate was measured at 400 nm wavelength (absorbance mode, vanadate-molybdenum reagent as the blank). To determine the potential total P concentration of sample, the sample absorbances were compared with a standard curve that ranged from 0.00 to 300.00 mg L^{-1} P.

Exchangeable potassium and calcium concentration

Cation bases were examined using the ammonium acetate solution method. A 2.5 g air-dried soil was incorporated with 16.5 ml of 1 N $C_2H_7NO_2$ and shaken for 5 minutes. The mixture then was centrifuged at 2500 rpm for 10 minutes. The liquid supernatant was decanted 3-4 times to a 50 ml volumetric flask; distilled water was then added to make up the volume to 50 ml. The potassium and calcium concentrations were measured by a flame photometer. Meanwhile, magnesium concentration was identified by an atomic absorption spectrophotometer (AAS).

3.2.2 Monitoring of the biological properties

Dehydrogenase activity (DHA)

DHA was assayed by the optimizing method of Veres et al. (2013). Three test tubes with 1 g of fresh soil were prepared for this analysis. A soil in one test tube was reacted with 1 ml 2,3,5 triphenyl tetrazolium chloride solution (TTC), and the other two tubes with 1 ml of 0.1 M Tris buffer solution (without TTC), vortexed and incubated for 24 hours at 30 °C. The blank samples, without soil, were also prepared for every three soil samples (nine test tubes) to eliminate the spontaneous decomposition of the substrate. At the end of incubation, 4 ml Methanol was added to each test tube to stop the enzymatic reaction and put back in the incubator for 2 hours. Furthermore, the soil suspension (6 ml) was centrifuged at 2500 rpm for 10 minutes, and the clear supernatant (pink-colored) was then measured by a spectrophotometer at a wavelength of 546 nm. DHA can be expressed as a rate of formation of TPF (a pink-colored compound triphenyl formazan) from the reduction of TTC. The equation is given below:

$$DHA\ (TPF\ \mu g\ g^{-1}\ dry\ soil) = TPF\ (\mu g\ ml^{-1}) \times V/dwt \times m$$

Where:

dwt: dry weight of 1g of wet soil

m: mass of measured wet soil (g)

A: the volume of the solution added to the soil during the test

We prepared a standard curve in advance, which we saved in the photometer and used for subsequent measurements. From TPF standard solution (Reanal), 0, 0.5, 1.0, 2.0, 3.0, and 4.0 ml were pipetted into volumetric flasks (50 ml), and then 8.3 ml of Tris buffer (pH 7.6) was added. It was made up to 50 ml with ethanol to give the following concentrations: 0, 5, 10, 20, 30, and 40 µg TPF ml $^{-1}$.

β-glucosidase activity (GLU)

GLU was assayed by a protocol proposed by Sinsabaugh et al. (1999). The principle of this method is converting an artificial substrate p-nitrophenol glucosidase (PNP-G) into a colored product (p-nitrophenol) that is easily detected by a spectrophotometer. 1 ml soil suspension was pipetted from soil-water suspension (1: 20) to the three test tubes. 1 ml of 10 mM PNP-G solution was reacted with the solution of the two test tubes, whilst one other tube was reacted with 1 ml of 0.5 M Na-acetate buffer. The blank samples, without soil, were also prepared for every three soil samples (nine test tubes) for the values correction. All test tubes were then stored in the incubator for 2 hours at 30 °C temperature. For terminating the reaction, 0.5 ml of 0.5 M Tris-hydroxymethyl (aminomethane) solution (pH 12, adjusted by NaOH) was added in all test tubes, followed by the

addition of 2 ml of 0.5 M $CaCl_2$ then vortexed. The mixture was then centrifuged at 2500 rpm for 10 minutes. A 410 nm wavelength spectrophotometer was used to measure the color density of the supernatant. The amount of p-nitrophenol production can indicate GLU. The equation is given below:

$$p\text{-}Nitrophenol\ (\mu g\ g^{-1}\ dwt\ h^{-1}) = \frac{C \times v}{dwt \times SW \times t}$$

Where:

C: measured concentration of p-Nitrophenol (µg g^{-1})

dwt: dry weight of 1g moist soil

v: total volume of the suspension in ml

SW: the weight os the soil samples used (1g)

t: incubation time in hours

We prepared a standard curve in advance, which we saved in the photometer and used for subsequent measurements. For the standard curve, the dilution series was prepared from a pNP standard (SIGMA) stock solution with a concentration of 10 µmol/ml. 0.5 ml of 0.5 M $CaCl_2$ and 2.0 ml of 0.5 M NaOH were added to the stock solution diluted with Na-acetate buffer, resulting in a solution of 4.5 ml, which is the same as the samples with its volume. The activity of the enzyme was expressed in units of µmol pNP g^{-1} h^{-1}, based on dry soil.

Phosphatase activity (PHOS)

The determination PHOS employed a similar protocol in GLU determination (Sinsabaugh et al., 1999), which is based on the amount of p-nitrophenol (PNP) that is converted from p-nitrophenyl-phosphate (PNP-PO_4). In PHOS measurement, 0.5 mM PNP-PO_4 was used instead of PNP-β solution in GLU determination. All test tubes were then incubated for 1 hour at 30 °C temperature. After 1 hour, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added to stop the reaction and vortexed. PHOS was determined spectrophotometrically at 410 nm wavelength. PHOS is reflected by the formation of PNP. The preparation of the equation and the standard curve is the same as that used for the GLU.

Glomalin concentration

Easily extracted glomalin-related soil proteins (EE-GRSP) were measured using the Bicinchoninic acid method (BCA) proposed by Stoscheck (1990). Soil extract was prepared by reacting 1 g air-dried soil with 4 ml of 20 mM (pH=7) citrate buffer in the autoclavable test tubes. The soil mixture was then placed in an autoclave at 121°C, 30 minutes. The samples then were cooled and centrifuged at 5000 rpm for 15 minutes. The supernatant was transferred later to sealed glass tubes and stored at ± 4 °C. For glomalin measurement, 20 µl of the soil extract was

incorporated with 1 ml of the SWR (standard working solution) reagent (shake the reagent beforehand), vortexed, and incubated at 60 °C for 30 minutes. Glomalin concentration was assessed spectrophotometrically at 562 nm wavelength. The absorbance values were substituted into the equation of the standard straight line:

$$y = 2.4407x - 0.4028, \text{ where: } x = \text{absorbance}$$

We prepared a standard curve in advance. BSA has been usually used as a standard for glomalin assays (Gadkar & Rillig, 2006; Rosier et al., 2006) from BSA 0.2, 0.4, and 0.6; concentration solutions of 0.8 and 1.0 mg/ml were prepared and, after the above procedure, photometered at 562 nm.

Mycorrhiza colonization assay

The colonization of arbuscular mycorrhiza fungi (AMF) was observed on the maize root of CT and PT. After the field sampling, fine roots were prepared following the modified method of Phillips & Hayman (1970). After careful washing with tap water, the roots were softened in 7% KOH solution for 24 h, washed in water, acidified in 5% lactic acid in water for 1–24 h, and stained with 0.01% aniline blue in 5% lactic acid for 24 h at room temperature. The stained roots were stored in Lactoglycerol until they were used for slide preparation. Parameters of AMF colonization were evaluated microscopically using thirty 1 cm root fragments per sample and calculated as percentages: frequency and arbuscular content of mycorrhization of root fragments (F%).

Total fungi

Total fungi were determined by the most probable number (MPN) method (Libisch et al., 2010), which measures the presence or absence of fungi propagated in a dilution series (10^{-1} to 10^{-9}) of *Sabouraud* nutrient broth. The MPN value was determined by counting the key number, which was then interpreted using the Hoskins table to infer the population of soil fungi.

3.3 Data analysis

We employed two statistical software for the pot and field experiment data. 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 Statistics for Windows software version 29.0 (IBM Corp., 2019). The significant level of *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 tested, i.e., normality, homogeneity of variance, and sphericity. In case the value of Greenhouse Geisser (ε) in the sphericity test is > 0.6, the MANOVA should be used instead of R-MANOVA. Bonferroni's method 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 the 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 that will be used for Tukey's test or Games Howell's test. For the PCA, the Kaiser-Meyer-Olkin (KMO) check was performed to assess the appropriateness of the model after the assumption test.

R software (Core Team, 2018), was employed in a Random Forest-based approach. First, the dataset was reviewed, and the Box-Cox methods treated and transformed the outliers (Box & Cox, 1964). The random forest (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' (Breiman et al., 2006), 'caret', "caret' (Kuhn, 2020), and 'rfPermutate' "rfPermutate' (Archer, 2013). Moreover, the plots were generated using the 'ggplot2''ggplot2' package (Wickham, 2016). 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 (Díaz-Uriarte & Alvarez de Andrés, 2006). 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 (Gregorutti et al., 2015). The significance of the important metrics was assessed by the 'rfPermute''rfPermute' package (Archer, 2013), estimating the null distribution of important metrics for each predictor variable and the observed *p-value*.

IV. RESULTS AND DISCUSSION

4.1 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.

4.2 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.

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)

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).

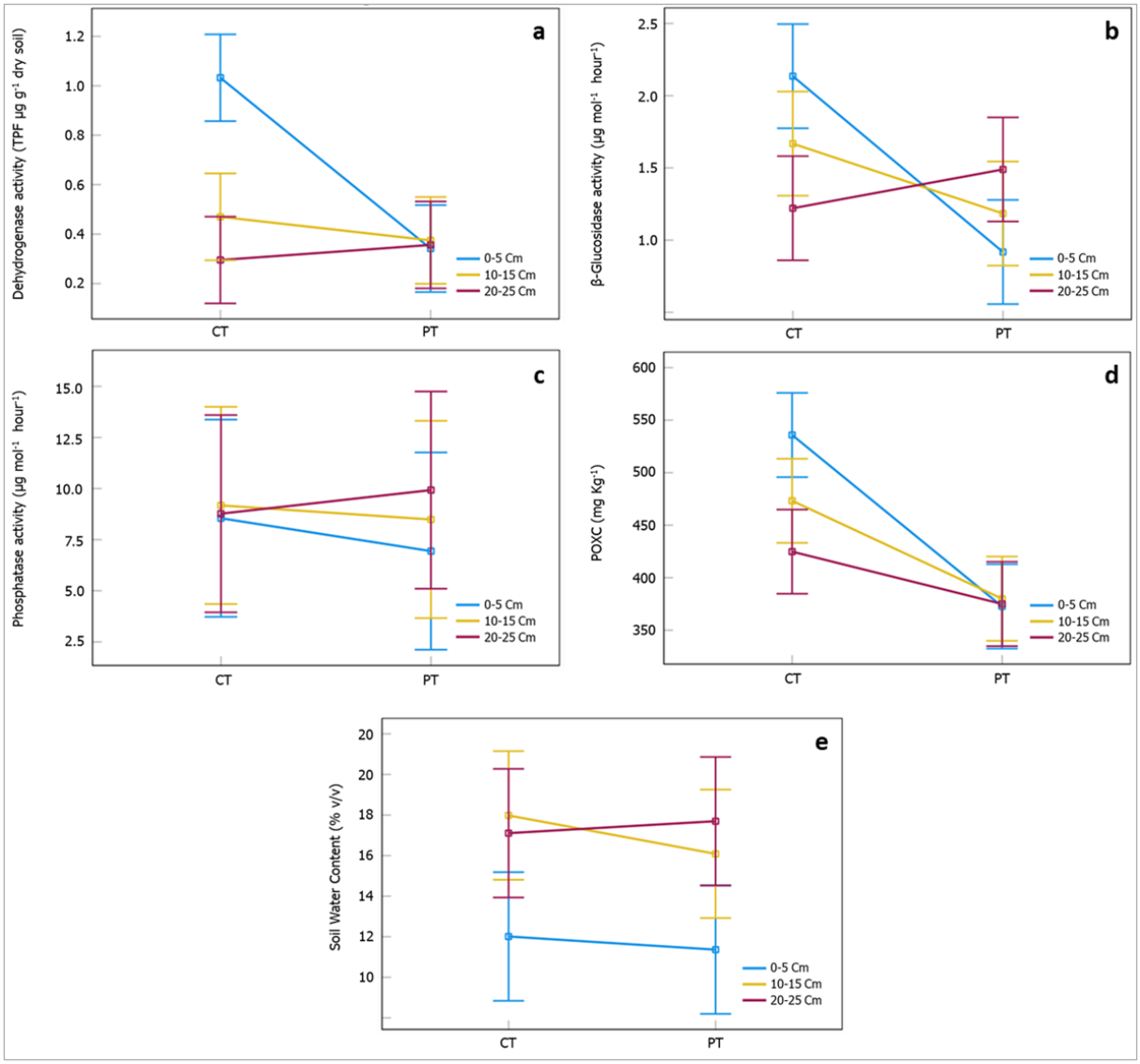


Figure 10. Interaction diagram showing the combined effect of soil depth (0-5; 10-15, and 20-25 cm) and tillage system (CT and PT) on the soil enzymes, POXC, and SWC.

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.

4.3 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}

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

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.

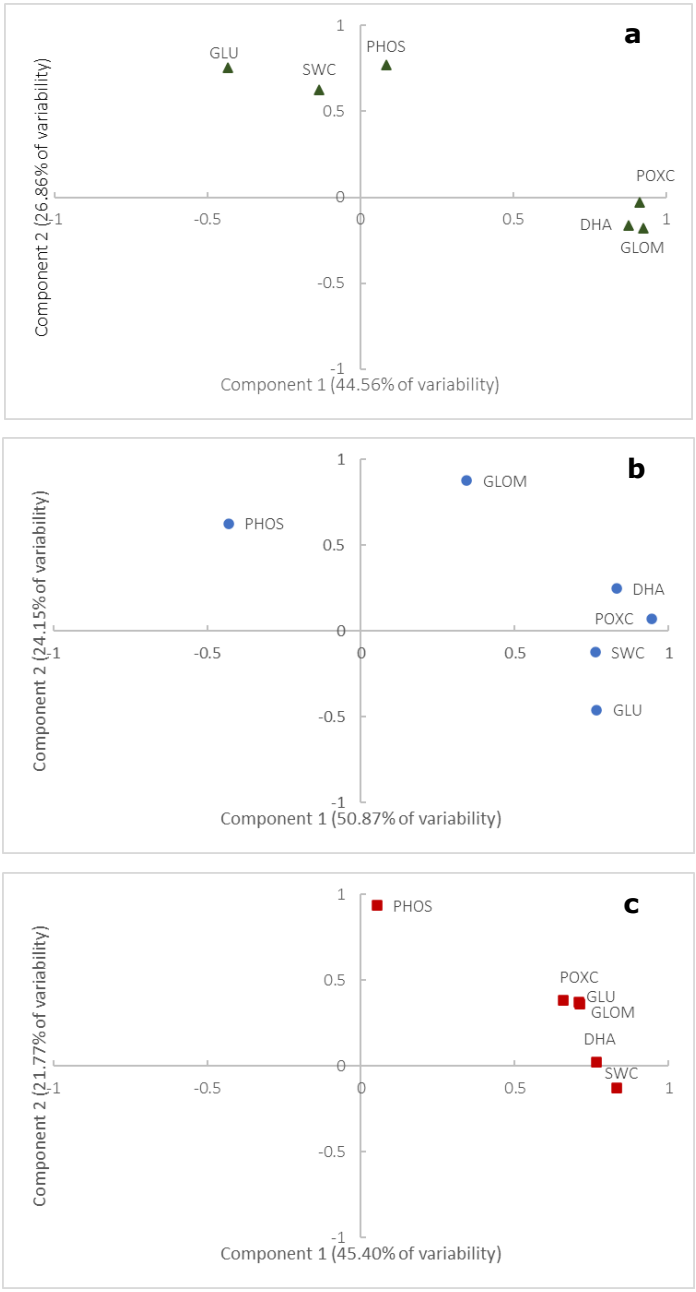


Figure 11. Principal Components Analysis in 0-25 cm depth of (a) maize I, (b) sunflower, and (c) maize II. *DHA= Dehydrogenase activity, GLU= β-glucosidase activity, PHOS= Phosphatase activity, POXC= permanganate oxidizable carbon, SWC= Soil water content, GLOM= Glomalin concentration.*

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.

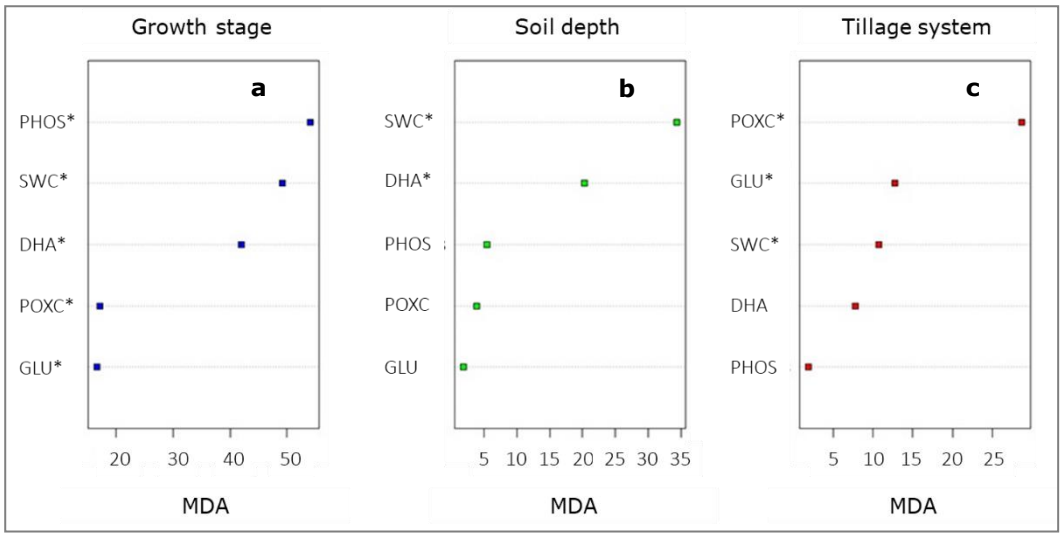


Figure 12. The overall importance of the soil parameters in differentiating the growth stage, soil depth, and tillage system was calculated as the mean decrease in accuracy (MDA) using the Random Forest classification model. '*' denotes a significantly different (p<0.05) of the soil parameters according 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

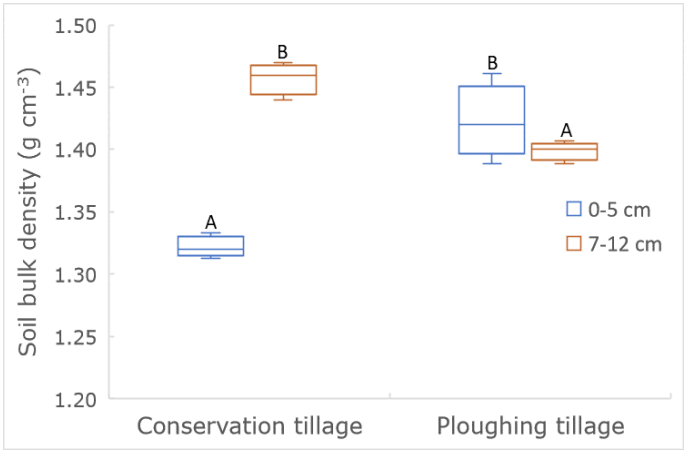
	DHA	PHOS	SWC	GLU
PHOS	0.191*			
SWC	0.216**	-0.369**		
GLU	0.186*	-0.085	0.090	
POXC	0.496**	0.018	0.099	0.188*

'*' 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).

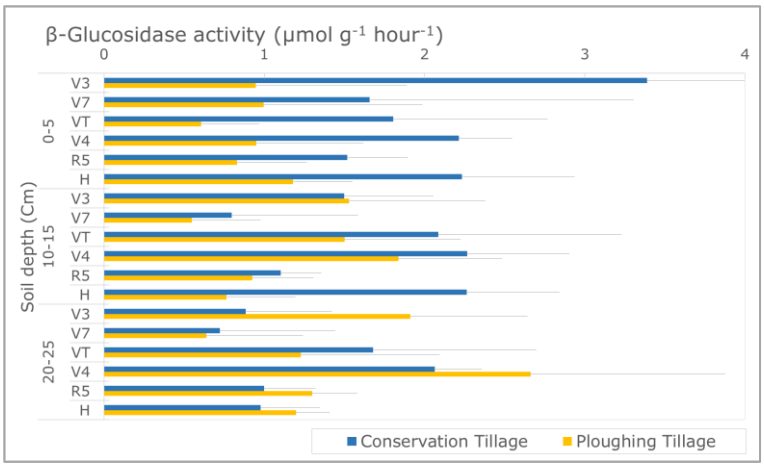
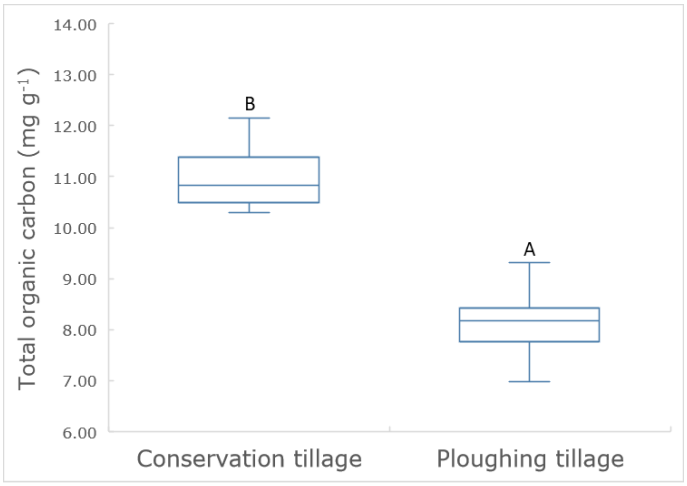


Figure 14. The dynamic of GLU is at 0-5, 10-15, and 0-25 Cm in CT and PT practice.

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).

4.4 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).

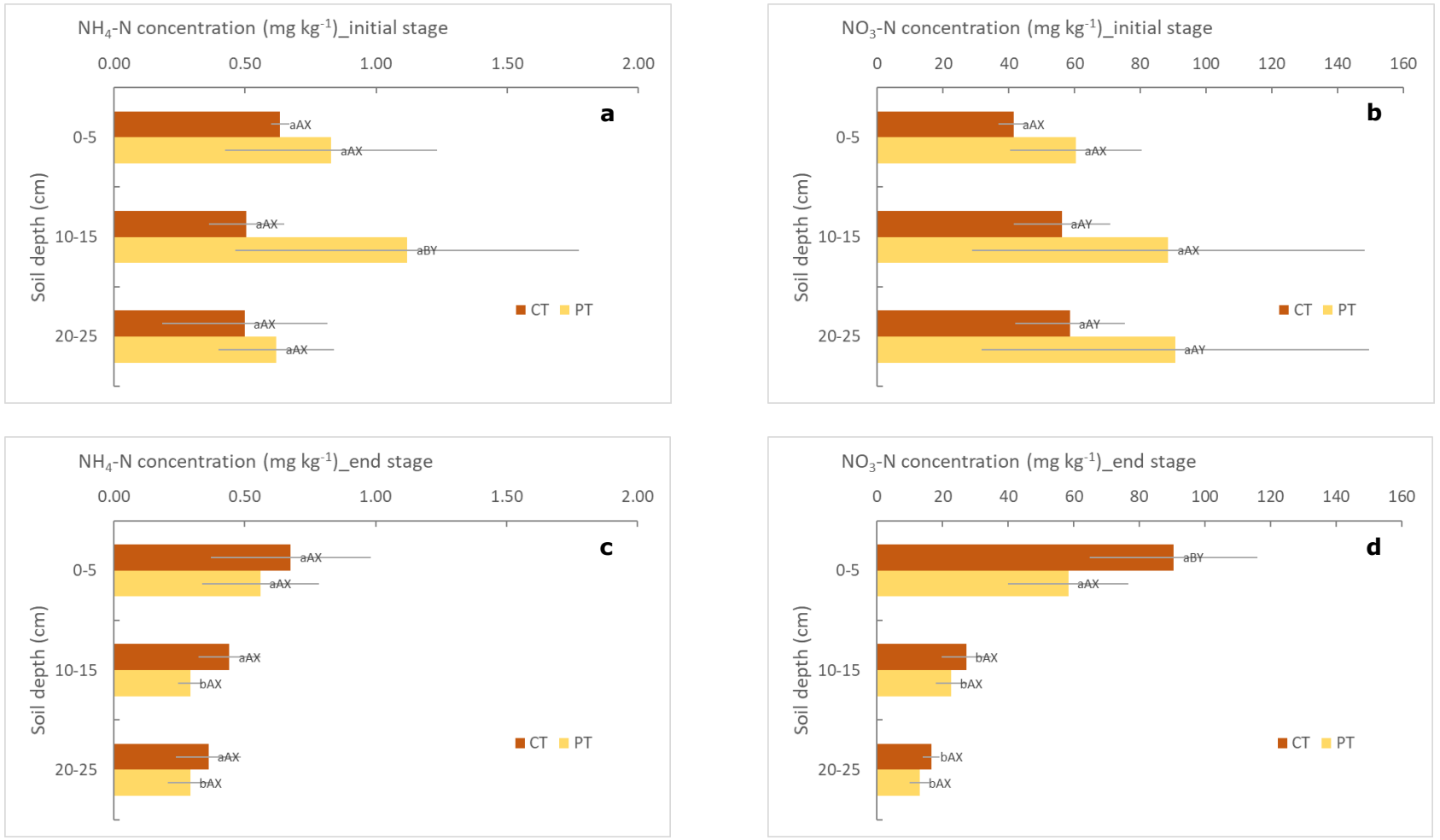


Different capital letters (A and B) indicate significant differences between the tillage systems (p-value<0.05)

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).

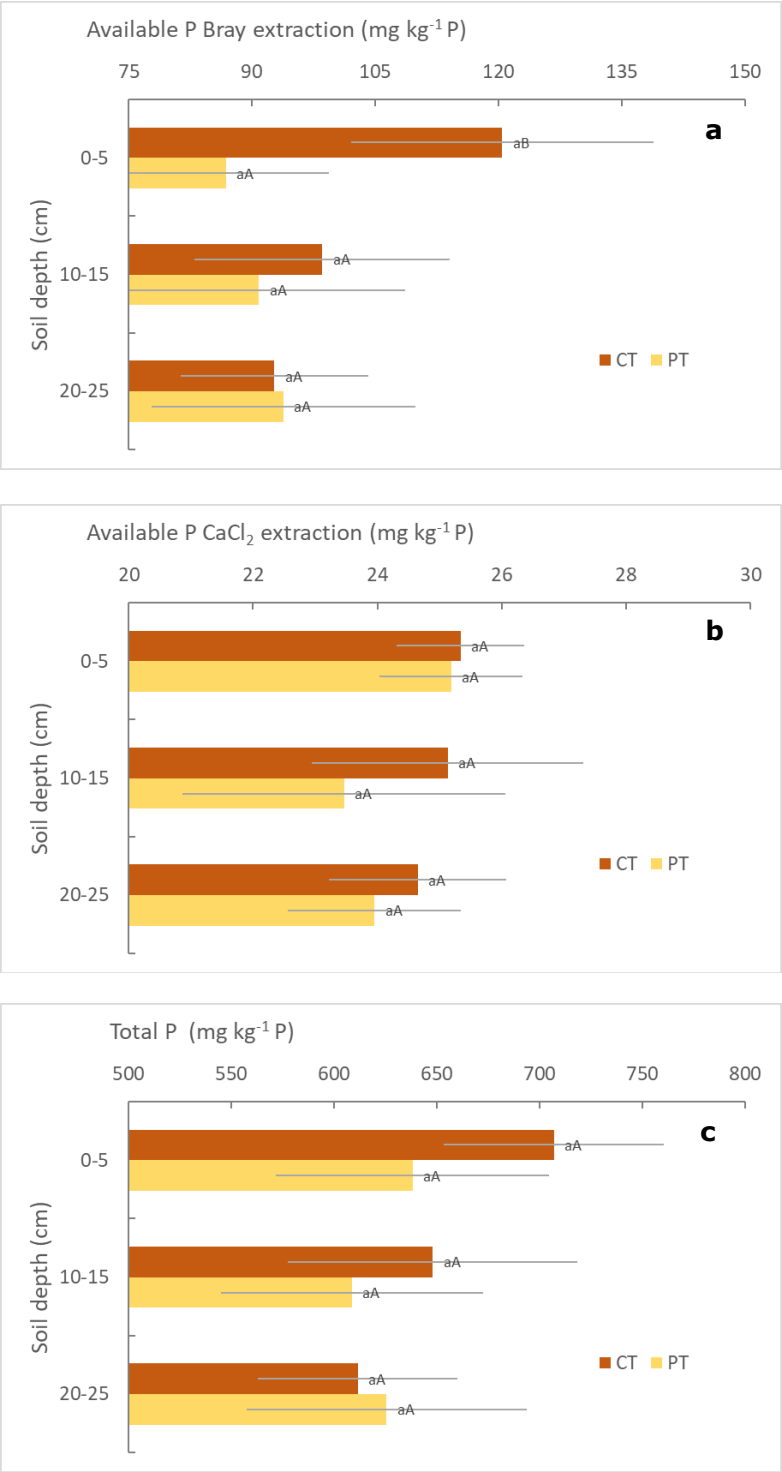
High TOC concentration induces microbial biomass activity and soil nutrient mineralization due to the sufficient energy source. The process of decomposition and mineralization responds to the presence of crop residues in the soil, and it is also linked to the soil enzyme activity associated with plant 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).

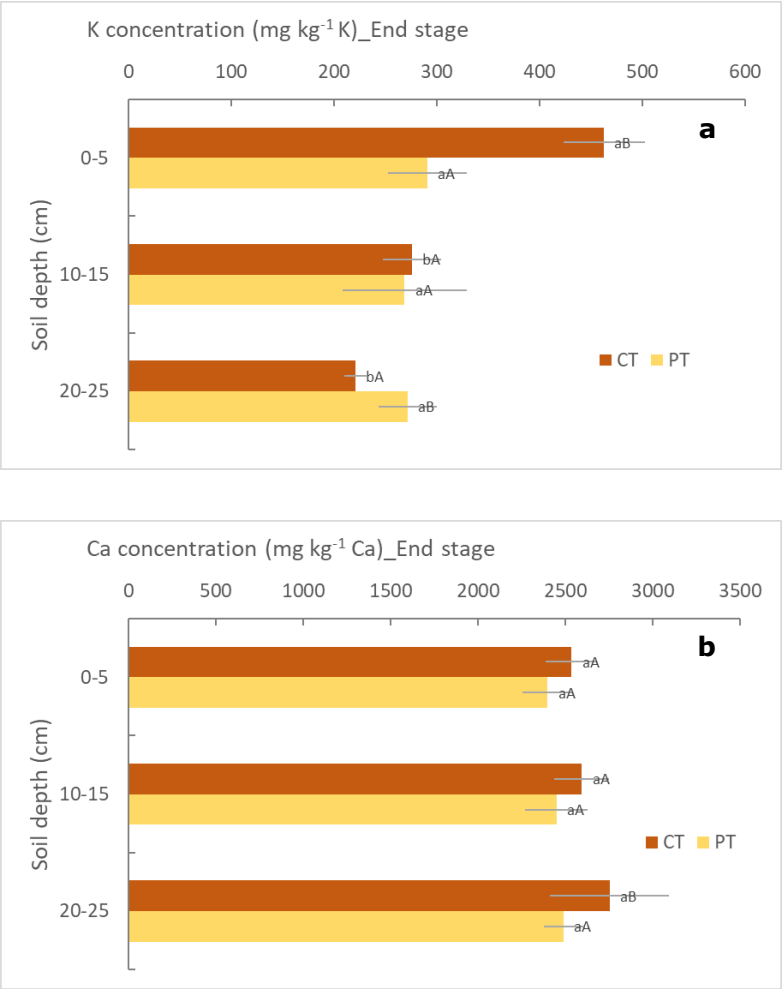
Figure 16. a and c: NH_4-N concentration (mg kg^{-1}) and NO_3-N concentration (mg kg^{-1}) in the initial stage (V3);

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).

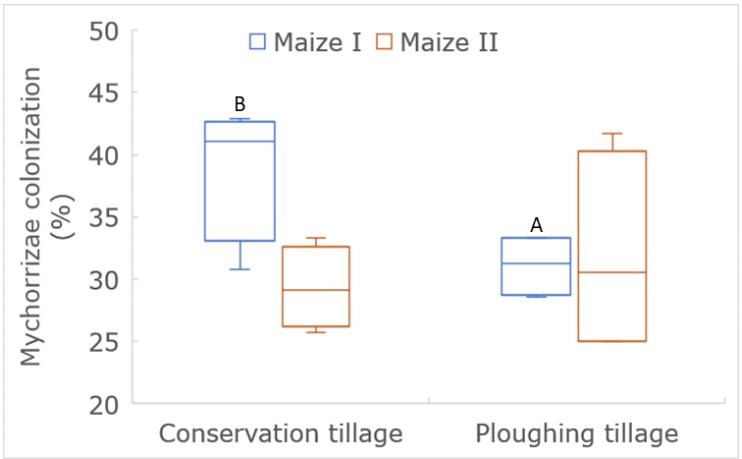
Nevertheless, the low PHOS in CT and PT was associated with higher soil P content in the whole profile. The application of phosphorus fertilizer before planting and the presence of mineralized phosphorus led to a sufficient amount of phosphorus being available for the maize plants. In numerous studies, 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.

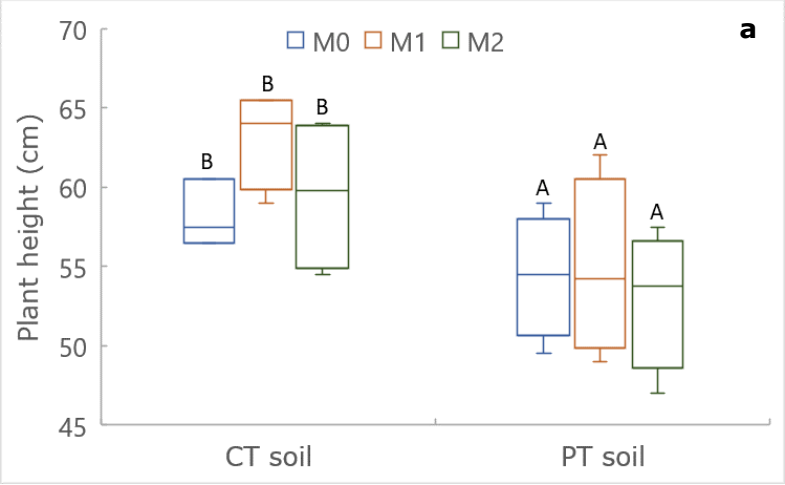
The minimization of erosion and the intensity of soil biological activity and nutrient mineralization processes are particularly important when fewer nutrients are applied than removed from the crop area. In this study, the nutrient balance (N, P, and K) was also calculated based on the nutrient removal 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.



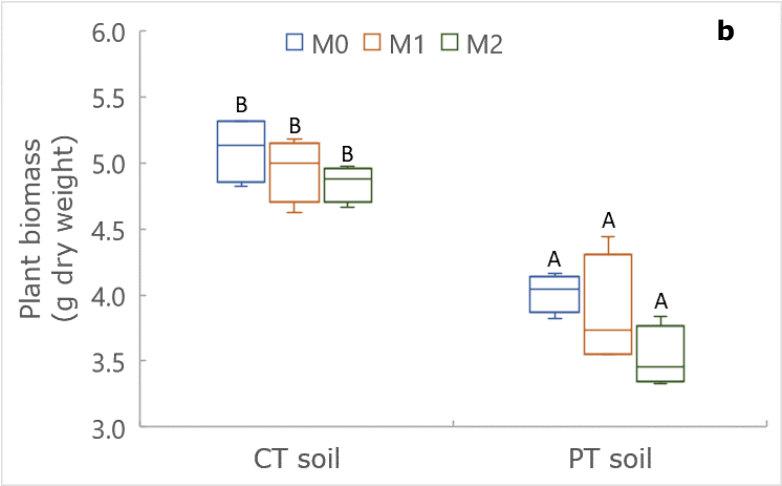


Figure 20. Plant height (a) and dry biomass (b) weight of maize of pot experiment. M= molasses concentration, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2)

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.

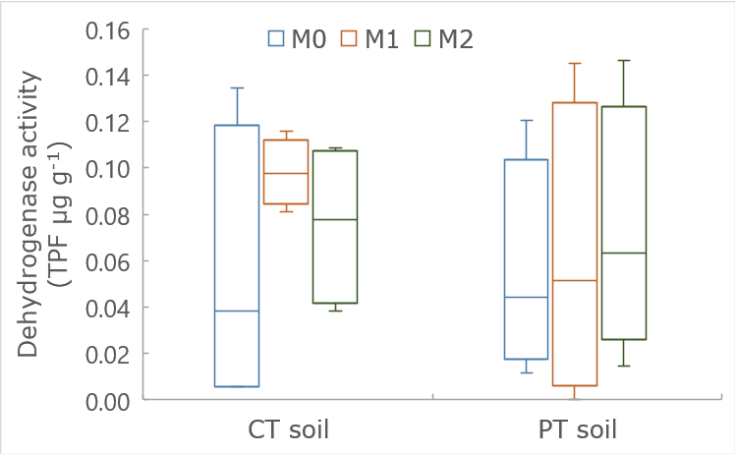


Figure 21. DHA (TPF µg g^{-1}) of CT and PT of pot experiment. M= Molasses concentrations, 0 (M0), 0.05 (M1), and 0.2 g L^{-1} of water (M2), n= 4.

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.

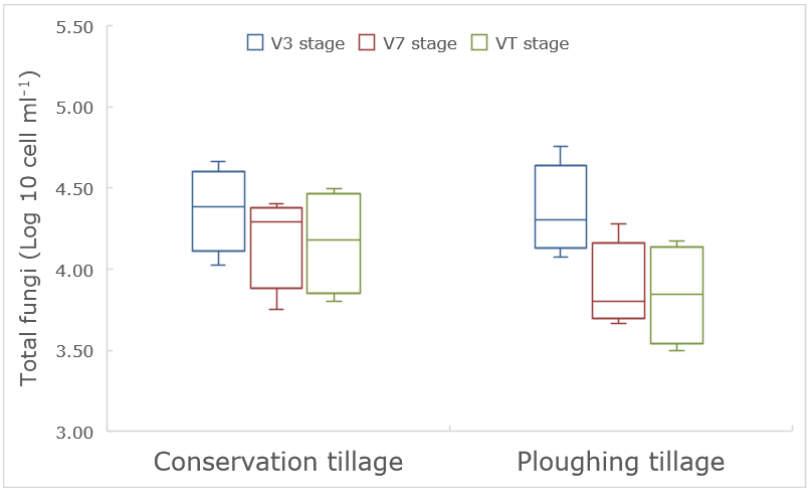
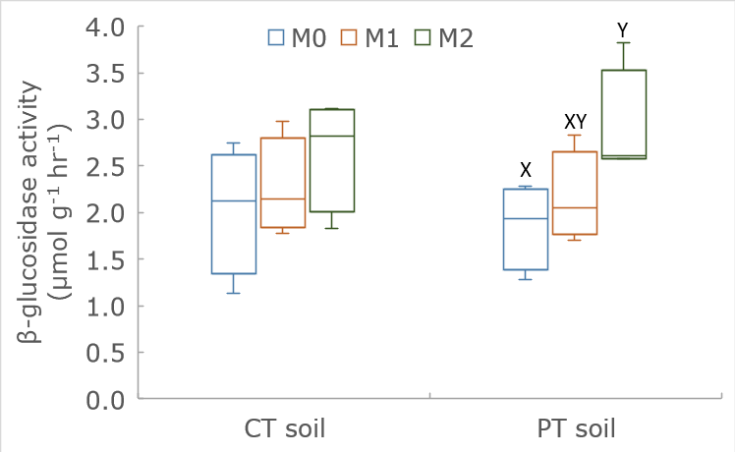


Figure 22. Total fungi at 0-5 cm depth of CT and PT for three growth stages, n= 4.

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.

V. 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.

VI. 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.

SUMMARY

The study entitled "Indicative soil biological parameters in long-term conventional and conservation tillage experiment" comprised the field and pot experiment. The field experiment was located near Szentgyörgyvár, Zala county, Southwest Hungary (N 46°44'53.32" E 17° 8'48.54" E). The altitude of the experimental field is 150 m above sea level with a 10% slope. 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. The soil is characterized as Haplic Luvisols (loamy humic) (IUSS Working Group-WRB, 2015), formed by sandy Loess with a slight acidity. The soil texture is moderately fine (5% clay, 58% silt, 37% sand). Soil acidity at 0-30 cm layer is 6.25 (pH H_2O) and 4.69 (pH KCl).

This study compared two soil tillage practices, conservation (CT) and conventional tillage (PT), of three growing seasons: maize I (2021), sunflowers (2022), and maize II (2023). Each tillage treatment had four replication plots (25 m long × 24 m wide). CT was treated by non-inversion reduced tillage (to a depth of 8–12 cm), leaving ~30 % of the previous crop residues to cover the soil surface. In addition, a cultivator machine (8–10 cm depth) was operated to control weed problems. On the other hand, PT was prepared by moldboard ploughing (to a depth of 25-30 cm), followed by harrowing and seed-bed preparation. After harvesting, the plant residues were left in both CT and PT.

Soil samples were collected three times in each growing season. Samples were taken by shovel and auger in each plot at 0-5, 10-15, and 20-25 cm of 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 for a maximum of four weeks. For soil chemical analysis, another 100 g of composite soil sample was exposed at room temperature (20 °C) until the soil reached air-dried condition.

Soil water content (SWC) (w/w %) was determined using the gravimetric method. Bulk density (BD) was measured using the method proposed by Blake and Hartge (1986). Total organic carbon was determined by the combustion method at 900 °C (Jakab et al., 2019). POXC was examined using the permanganate oxidation method (Weil et al., 2003). The optimizing method by Veres et al. (2013) was used to determine the dehydrogenase activity (DHA). The β-glucosidase activity (GLU) was assessed by the conversion method of p-nitrophenol β-glucosidase (PNP-β-G) proposed by Sinsabaugh et al. (1999). The phosphatase activity (PHOS) was determined by the conversion method of p-nitrophenyl-phosphate (PNP-PO_4) (Sinsabaugh et al., 1999). Glomalin concentration was analyzed using the BCA method (Stoscheck, 1990). Mycorrhizae colonization was calculated following Phillips and Hayman (1970). Total fungi were determined by the most

probable number (MPN) method (Libisch et al., 2010). A pot experiment with maize as the crop was carried out using the composite soil (0-20 cm) of CT and PT; a randomized design with four replications was employed. Three levels of molasses concentration, 0 g L^{-1}, 0.05 g L^{-1}, and 0.2 g $L^{-1,}$ were applied. POXC, DHA, GLU, plant height, and dry weight biomass were measured at the end of the experiment (after eight weeks).

Soil ammonium (NH_4-N) and nitrate (NO_{3^-}-N) were measured with 0.01 M $CaCl_2$ extraction (Houba et al., 2000), while the NH_4-N and NO_{3^-}-N concentrations in the soil extracts were measured with the salicylate method using a spectrophotometer (Kempers and Zweers, 1986; Yang et al., 1998). Easily available P was determined with 0.01 M $CaCl_2$ extraction (Houba et al., 2000) and potentially available P with the 0.03 M NH_4F and 0.1 M HCl extraction method (Bray and Kurtz, 1945), while the P concentration of soil extracts was measured using molybdate reagent spectrophotometrically. The total phosphorus content in the soil samples was measured after ignition using the 1N H_2SO_4 extraction method. The total P concentration was measured using a vanadate-molybdenum reagent (Pardo et al., 2003). Cation bases (K, Ca, and Mg) were determined using the 1 M ammonium acetate extraction method (Chapman, 1965), using a flame photometer to measure the potassium and calcium concentrations.

Statistical analysis was performed using IBM SPSS statistics for Windows, version 29.0 (IBM Corp., 2019) for the analysis of variance (ANOVA), repeated measures multivariate analysis of variance (R-MANOVA), principal component analysis (PCA), Boxplot, and Pearson correlations. A random Forest Analysis (RF) by the R software 4.2.1 version was employed by R software (Core Team R, 2018).

The study aimed to investigate the dynamic of soil microbiological activities and plant nutrition potential after the long-term practice of conservation tillage. We also monitored the soil biological parameters to assess the sensitivity of soil biological parameters and find the best indicator for monitoring the effect of tillage practice. In addition, we evaluated how the long-term implementation of conservation tillage affected plant development and production.

The findings indicated that CT induces the plant development parameters. The stem and flower diameter of sunflowers and the root capacity of maize were notably higher in the CT than in the PT soil. Nevertheless, CT application does not have a significant effect on maize and sunflower yield. Soil fertility and agronomical practices were also quite important in determining the success of plant production. Through the small pot experiment, we proved that the available substrate stimulated the soil biological parameters; however, the additional nutrient source (chemical or organic fertilizer) was still necessary to supply plants with nutrients.

Spatial and temporal variability impact the soil biological indicator. We obtained that the temporal variability of the soil health indicators in the PT soils was more significant than in the

CT soils, indicating a more balanced environment for biological activity in the CT soils. Our results also demonstrated the significant effect of tillage × soil depth interaction on DHA, GLU, and POXC. The PHOS was notably affected by vegetative and generative phases of cultivated plants, primarily indicating the effect of the plant roots. Environmental factors such as soil moisture, aeration, and temperature dominantly affected the DHA. Hence, DHA can be used mainly to monitor the temporal variability of soil microbiological activity in the 0-5 cm layer. As the indicators of the C-cycle and mineralization, the vertical distribution of GLU and POXC showed significant differences between CT and PT soils, showing the effect of tillage in any phenological phase of crops.

Long-term tillage practice alters the soil's biological indicators. Two main factors described the sensitivity level of soil biological indicators. DHA, POXC, and glomalin are more sensitive indicators of tillage and crop rotation. Meanwhile, GLU, SWC, and PHOS have a lower level of sensitivity. PHOS showed the lowest sensitivity compared with GLU and SWC. POXC significantly influences DHA and glomalin. On the other hand, SWC significantly controls GLU.

Tillage intensity, soil depth, and growth stages significantly influenced soil enzyme activities and the concentration of soil nutrients. Less soil disturbance resulted in a significantly larger concentration of soil carbon parameters [total organic carbon (TOC) and POXC] in the CT plots, where the GLU and DHA in the upper soil layer increased significantly compared to PT. The high amount of organic matter and the excellent resistance to erosion observed in CT also contributed to the higher concentration of available nutrients (NH_4-N, NO_3-N, K, and Ca) and total P in the surface soil layer. PHOS was highest in the mid-stage of vegetative growth and was positively correlated to the total P concentration. The changes in soil water content were negatively correlated with the change in DHA and PHOS. Overall, due to the more balanced environmental conditions, the decomposition of organic substances was more balanced and slower in CT than in PT. This implied that the mobilization of nutrients in the soil was more balanced as well and that the nutrients were released gradually.

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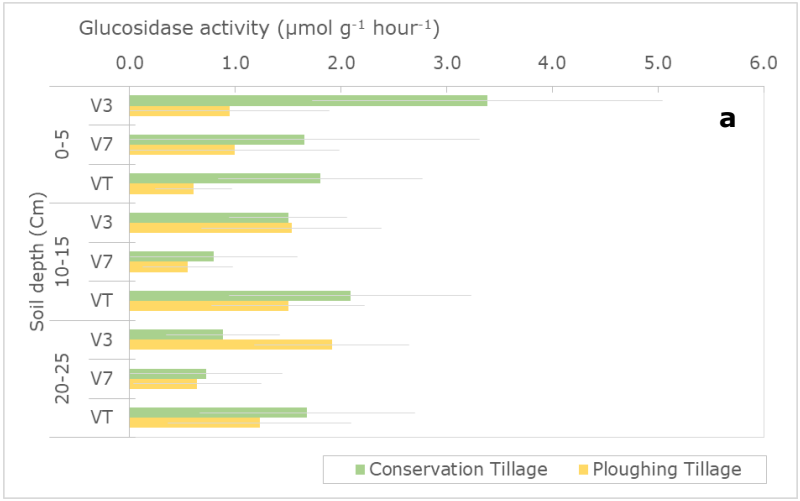
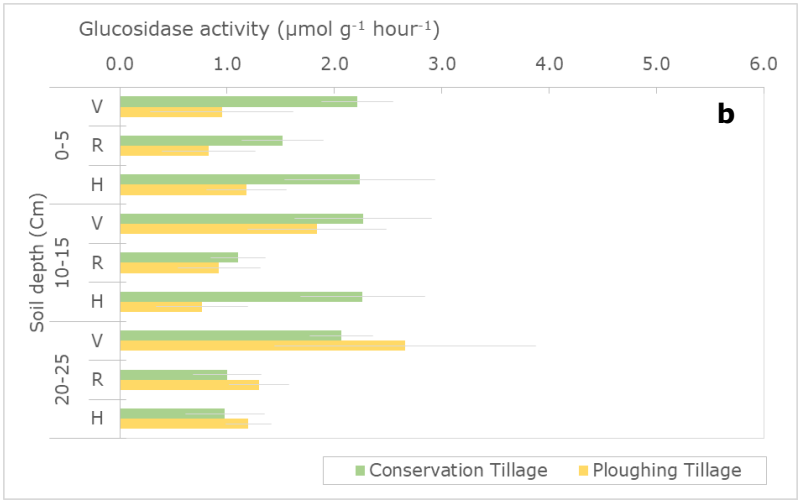
APPENDIX

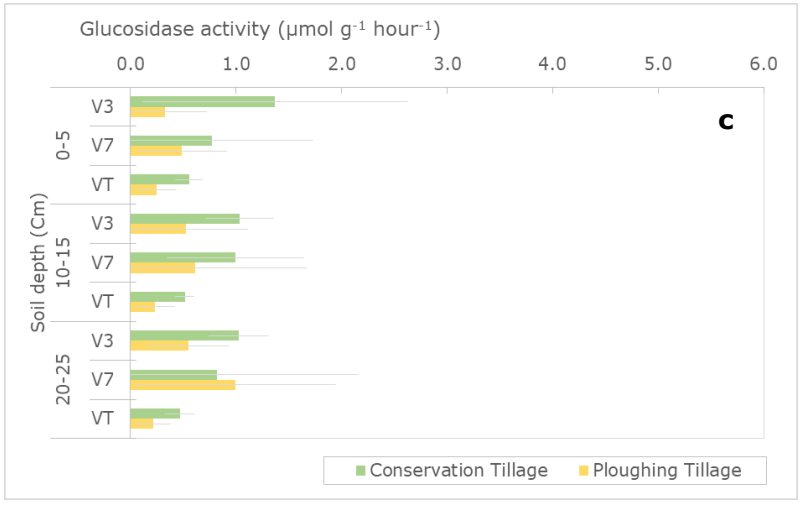
Dehydrogenase activity (DHA)

Appendix 1. DHA (TPF µg g^{-1}) in CT and PT at 0-25 cm depth for the growth stages in the growing season of 2021 (a), 2022 (b), and 2023 (c).

β-glucosidase activity (GLU)

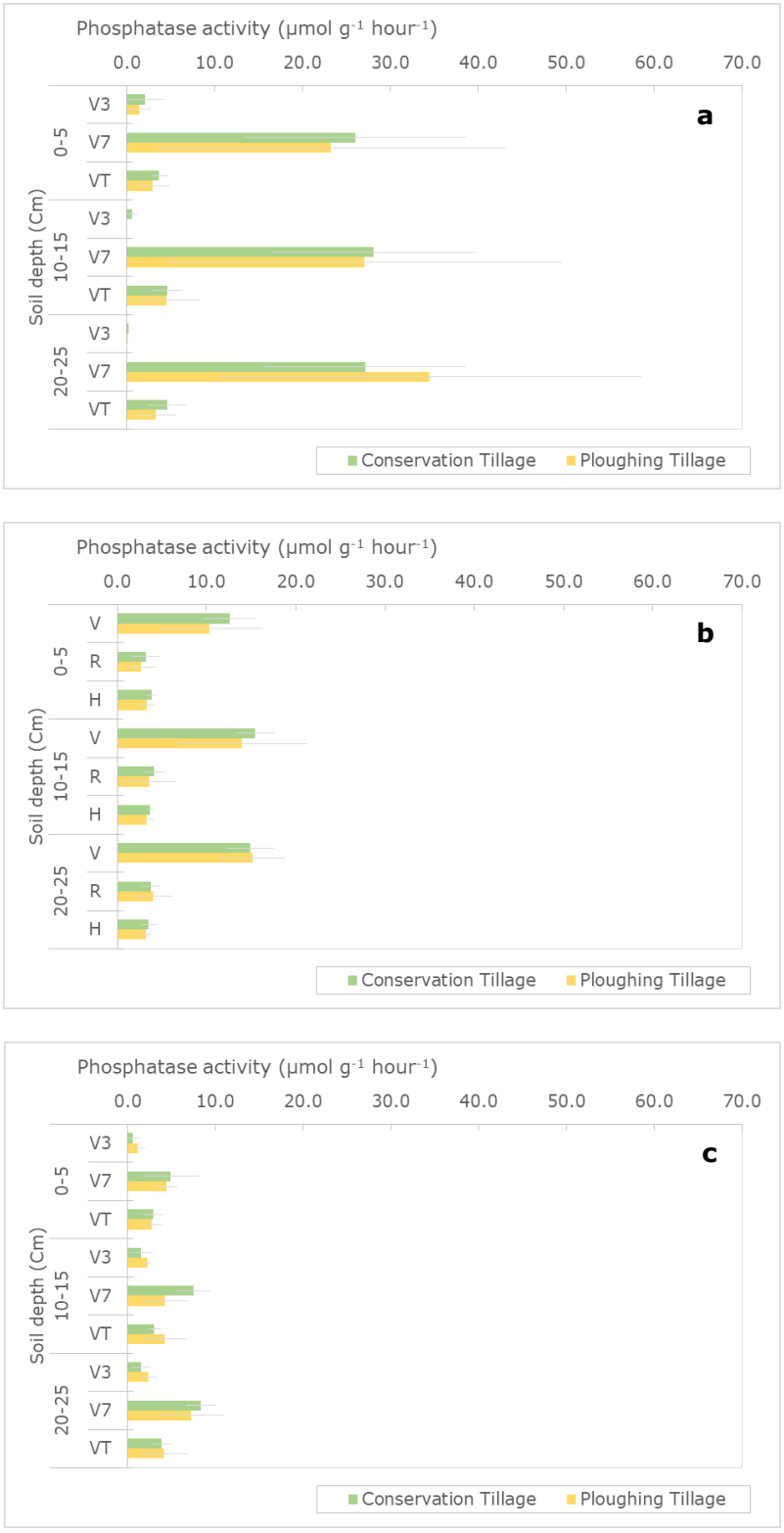






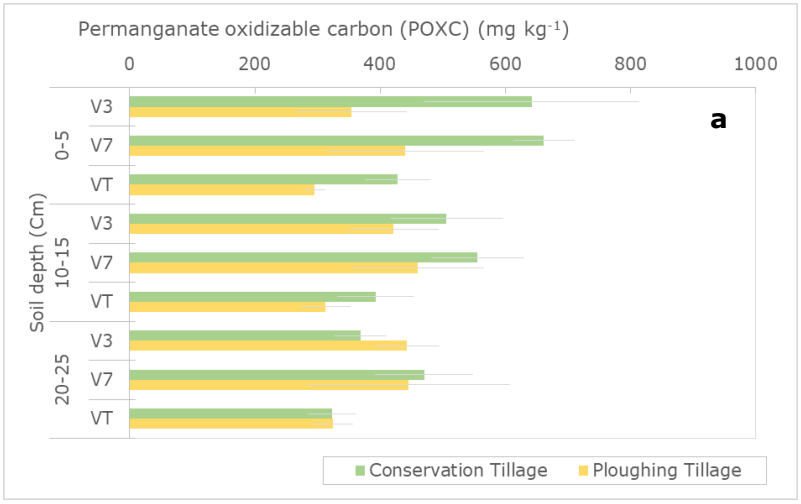
Appendix 2. GLU (µmol g^{-1} $hour^{-1}$) in CT and PT at 0-25 cm depth for the growth stages in the growing season of 2021 (a), 2022 (b), and 2023 (c).

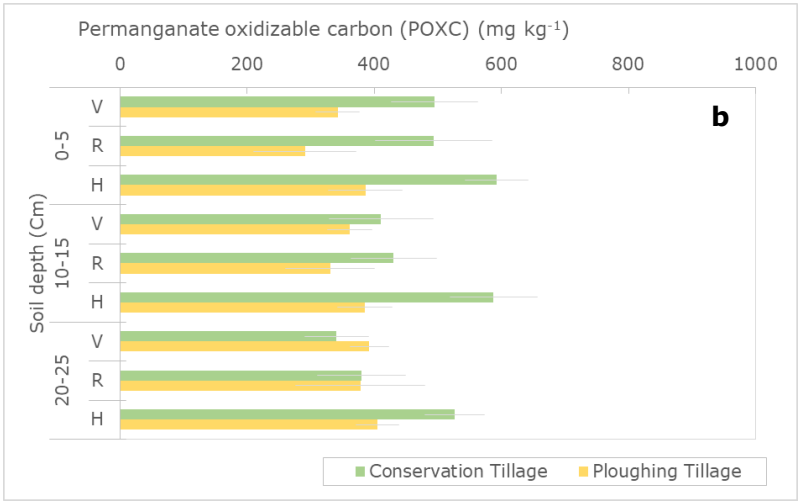
Phosphatase activity (PHOS)

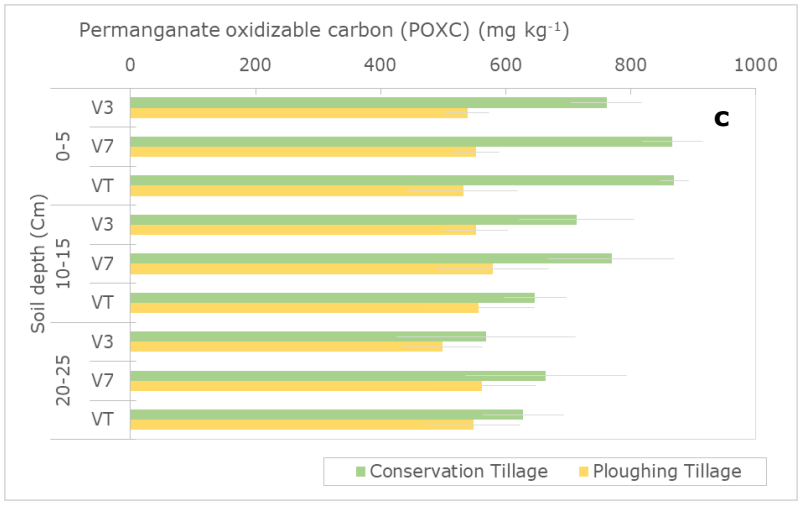


Appendix 3. PHOS (µmol g^{-1} $hour^{-1}$) in CT and PT at 0-25 cm depth for the growth stages in the growing season of 2021 (a), 2022 (b), and 2023 (c).

Permanganate oxidizable carbon (POXC)

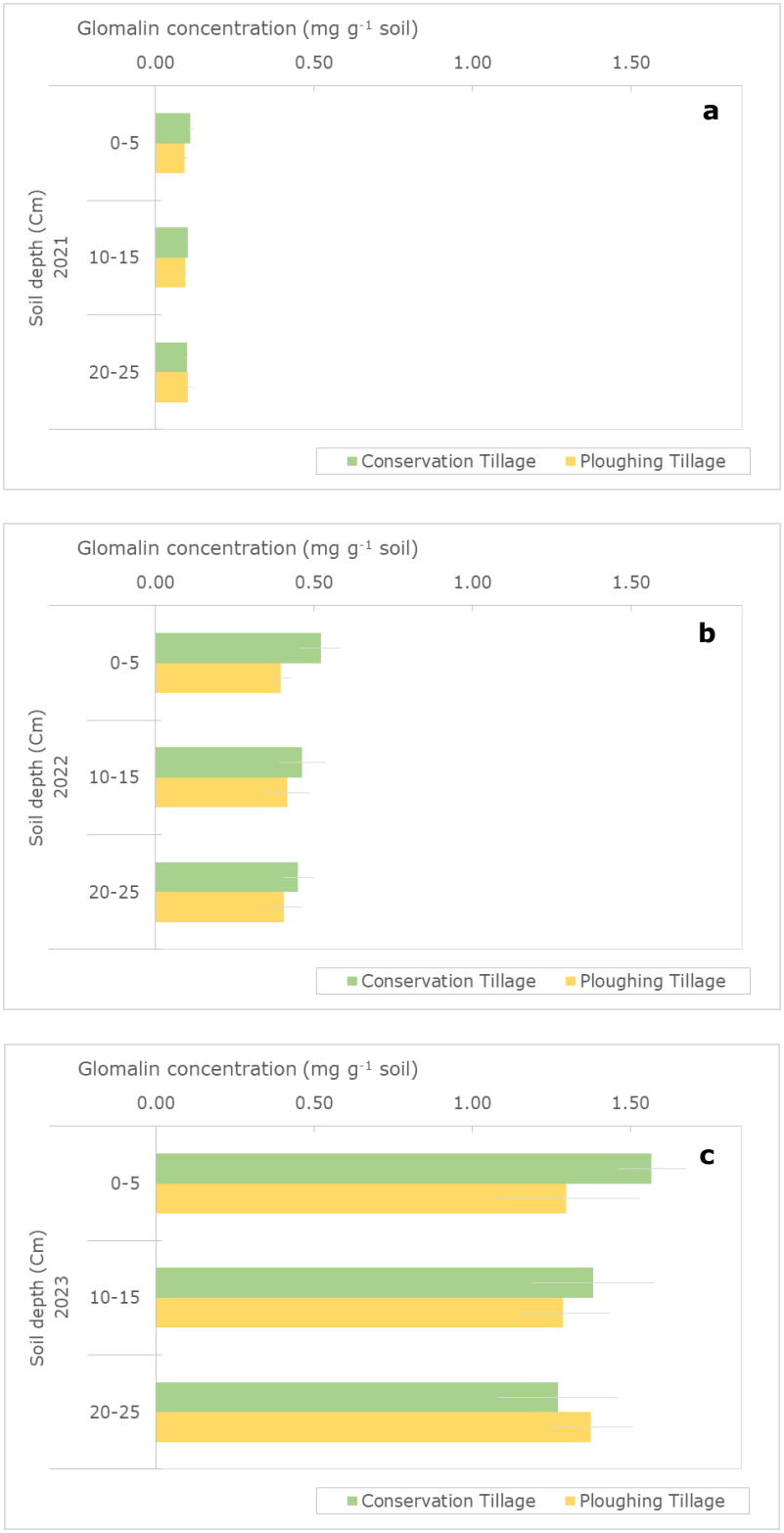






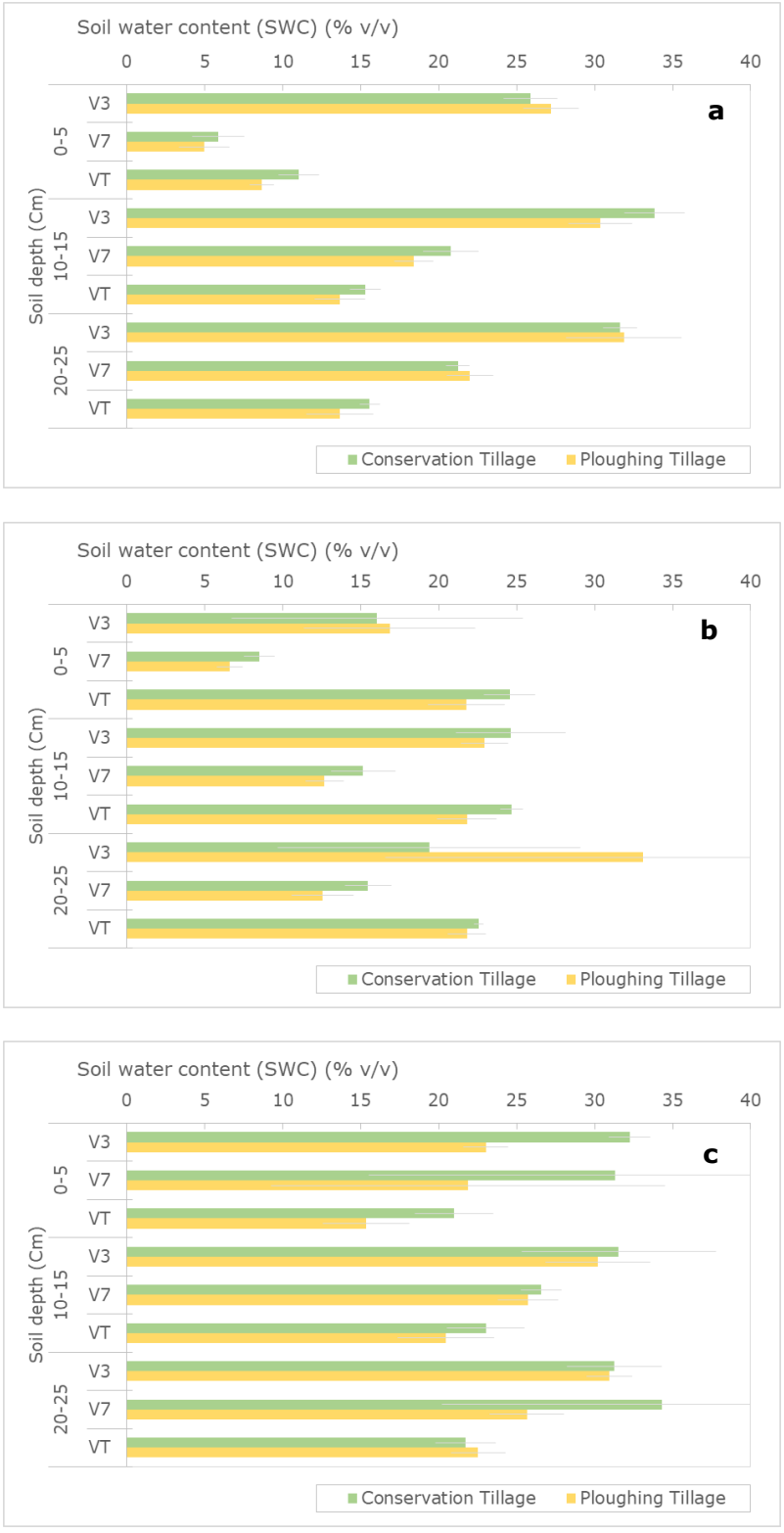
Appendix 4. Permanganate oxidizable carbon (mg kg^{-1}) in CT and PT at 0-25 cm depth for the growth stages in the growing season of 2021 (a), 2022 (b), and 2023 (c).

Glomalin concentration (GLOM)



Appendix 5. Glomalin concentration (mg g^{-1}) in CT and PT at 0-25 cm depth for the growth stages in the growing season of 2021 (a), 2022 (b), and 2023 (c).

Soil water content (SWC)



Appendix 6. Soil water content (% v/v) in CT and PT at 0-25 cm depth for the growth stages in the growing season of 2021 (a), 2022 (b), and 2023 (c).

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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)**].
3. Kotroczó, Zs., Fekete, I., Juhos, K., Prettl, N., **Nugroho, P.A.**, Várbíró, G., Biró, B., & Kocsis, T. (2023). Characterization of Luvisols Based on Wide-Scale Biological Properties in a Long-Term Organic Matter Experiment. Biology 2023, 12, 909. https://doi.org/10.3390/biology12070909 [**Scopus Q1, impact factor 4.2 (Web of science**)].
4. **Nugroho, P.A.**, Prettl, N., Kotroczó, Zs., Juhos, K., (2023). The effect of molasses application on soil biological indicators and maize growth of different tillage soil: a pot experiment. Journal of Environmental Geography, 16 (1–4), 119–124. DOI: 10.14232/jengeo-2023-44670 (Peer reviewed).
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6. **Nugroho, P.A.**, Prettl, N., Kotroczó, Zs., Juhos, K., (2022). Comparison of some soil enzymatic activities in luvisol of conservation and conventional tillage in a model experiment. Journal of Central European Green Innovation, Talajbiológia különszám, 10 (3): 3–12. DOI: 10.33038/jcegi.3558 (Peer reviewed).
7. Prettl, N., Biró, B., **Nugroho, P.A.**, Juhos, K. (2022). Labile carbon as an indicator of soil biological activity in the application of microbial inoculants and soil conditioner. Journal of Central European Green Innovation, Talajbiológia különszám, 10 (3): 13–25. (2022) DOI: 10.33038/jcegi.3559 (Peer reviewed).
8. **Nugroho, P.A.**, Kotroczó, Z., Prettl, N., Kovács, F., Madarász, B., & Juhos, K., (2024). Vertical and temporal variability of soil biological indicators in a long-term tillage experiment (under reviewed in Q1 article).

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, România, 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, România, 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)

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