DOCTORAL (PHD) DISSERTATION

HANAA THARWAT MOHAMED IBRAHIM

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ASSESSING THE IMPACT OF EARTHWORMS ON CROP PRODUCTIVITY AND SOIL HEALTH UNDER DIFFERENT TILLAGE PRACTICES

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HANAA THARWAT MOHAMED IBRAHIM

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Name of PhD School	Doctoral School of Environmental Sciences
Discipline:	Environmental Sciences
Head:	Csákiné Dr. Michéli Erika, CMHAS MATE, Institute of Environmental Sciences, Department of Soil Science
Supervisor(s):	Dr. Barbara Simon, PhD MATE, Institute of Environmental Sciences, Department of Soil Science Dr. Györgyi Gelybó, PhD MATE, Institute of Environmental Sciences, Department of Water Management and Climate Adaptation

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Approval of Head of Doctoral School	Approval of Supervisor(s)

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LIST OF ABBREVATION

$BaCl_2$	Barium chloride
BD	Bulk density
C	Carbon
CO_2	Carbon dioxide
CT	Conventional tillage
CTL	Control
DP	Deep ploughing
DT	Deep tillage
EWF	Earthworm fenced area
EC	Electrical conductivity
FAO	Food and Agriculture Organization
HCl	Hydrochloric acid
IUSS	International Union of Soil Science
JETF	Józsefmajor Experimental and Training Farm
Mg CO_2 /50 g/10 day	milligram of carbon dioxide per 50 gram of soil per 10 days
NT	No tillage
NP	Normal ploughing
P_2O_5	Phosphorus pentoxide
P	Ploughing
KCl	Potassium chloride
$K_2Cr_2O_7$	Potassium dichromate
K_2O	Potassium Oxide
RT	Reduced tillage
SC	Shallow Cultivation
SMR	Soil microbial respiration
SMC	Soil moisture content
SOC	Soil organic carbon
H_2SO_4	Sulfuric acid
WRB	World Reference Base for Soil Resources

1. INTRODUCTION

1.1. Background and rationale

Soil health holds paramount importance due to its implications for both planetary and human health. The vitality of soil lies in its multifunctionality, as it supports critical processes such as biomass production, carbon regulation, biodiversity habitat, nutrient cycling, and water cycling (Shahane and Shivay, 2021). It assumes a pivotal role in sustaining plant and animal production, augmenting water, and air quality, controlling nutrient availability, accumulating soil carbon, supporting biodiversity, and mitigating erosion (Peter *et al*., 2023). The healthiness of soil is closely intertwined with the well-being and productivity of crops, as well as the promotion of sustainable agriculture (Misbah *et al*., 2023). Moreover, it is intricately linked to human and animal nutrition, as it is responsible for providing 98.8% of our food and sustaining our nutritional needs (Handayani *et al*., 2022). Furthermore, a healthy soil has a harmonious combination of physical, chemical, and biological properties that promote the growth of diverse and productive plant communities (Lal, 2020), as well as the potential to significantly contribute to climate change mitigation and environmental integrity (Horwath, 2022).

A healthy soil system interacts with biotic and abiotic factors, influencing the capacity to support diverse life forms and exchange matter and energy with the environment (Peter *et al*., 2023). Soil health pertains to the overall state of the soil and its capacity to sustain the growth of plants and provide essential services to the ecosystem (Doran *et al.,* 2000; Pimentel *et al.,* 2013). Recent studies (Weight and Watchers, 2022) indicated that enhancing soil health through practices like cover cropping can improve physical, chemical, and biological properties, leading to increased productivity, water and nutrient absorption, stress tolerance in plants, and higher crop quality.

Furthermore, soil biota and soil organic carbon (SOC) are key indicators of soil health, and their interplay plays a significant role in maintaining soil fertility and productivity (Koorneef *et al*., 2023). Soil biota refers to the diverse community of living organisms found in soil, including bacteria, fungi, protozoa, nematodes, arthropods, and earthworms, which play a vital role in soil functioning and can reflect changes in soil management practices (Kozhevin, 2023; Poeplau and Don, 2023). They influence soil structure through their activities such as burrowing, tunnelling, and aggregation. Earthworms, for instance, create channels in the oil, promoting aeration and water infiltration, thus improving soil structure and porosity (Bardgett and van der Putten, 2014; Wall *et* *al.,* 2015). Additionally, the stability of SOC influences various soil functions, such as nutrient provisioning and carbon sequestration (Weverka *et al.,* 2023). Moreover, SOC contributes to soil structure stability and aggregation by acting as a binding agent. Soils with higher SOC content typically exhibit improved water retention, aeration, and resistance to erosion (Lal, 2004). SOC influences climate regulation by sequestering atmospheric carbon dioxide. Practices that enhance SOC levels, such as conservation tillage and cover crops, can mitigate climate change by reducing greenhouse gas emissions and enhancing carbon storage in soils (Lehmann and Kleber, 2015).

1.2 Research problem

Soil biota is essential for maintaining and enhancing soil properties and soil health, particularly SOC and soil tillage, which in turn support plant growth, crop production, and ecosystem services. However, soil biota is often neglected or overlooked in soil management practices, and their diversity and functions are poorly understood and quantified. While there are studies that have investigated the effects of different tillage practices on soil organisms and SOC dynamics (William *et al*., 2021; Janelle and Pham, 2022; Li-Jin *et al*., 2022). Several previous research (Dominati *et al*., 2010; Barrios, 2007; Brussaard, 2012; Lavelle *et al*., 2006) have stressed the significance of soil biota, or more specifically soil invertebrates in the supply of ecosystem services. These investigations have not given earthworms much attention. Despite the potential benefits of earthworms in improving soil quality and nutrient availability, there is a notable gap in our understanding of the specific mechanisms and optimal management practices that maximize their contribution to enhancing agricultural crop yields. Various studies (Bouché, 1977; Edwards and Bohlen, 1996; Brown *et al*., 2004; Lavelle, 2001; Blouin *et al*., 2013) have shown positive correlations between earthworm presence and increased crop productivity, there is limited comprehensive research that explores the intricate interactions between earthworm species, soil types, agricultural practices, and crop varieties, which hinder the development of precise recommendations for farmers seeking to harness the full potential of earthworms for sustainable and higher-yield agriculture. There is a need for more integrated research that considers the impact of soil biota on SOC sequestration and turnover under different tillage and management practices (Juliane *et al*., 2016). Addressing this research gap is critical for promoting environmentally friendly and economically viable agricultural systems. Additionally, more studies are required to explore the interactions between earthworms and soil microorganisms in the decomposition of organic matter. Therefore, the aim of this research was to explore how earthworms indirectly influence crop development and yields as well as how they enhance soil fertility and structure through the investigation of how soil properties, such as texture, pH, organic matter content, and nutrient availability, influence earthworm populations and their activities, and how these interactions enhance crop yield.

1.3. Study objectives

The objectives of the present research were the following:

1. To assess the effects of three tillage methods (no-till - NT, shallow cultivation - SC, and ploughing - P) on soil health in a long-term continuous systematic soil tillage experiment, focusing on selected physical properties (bulk density and soil moisture content), chemical properties (pH(KCl), soil organic carbon content and stock), and biological properties (soil microbial respiration, earthworm abundance, biomass, and species composition), alongside yield assessments.
2. To evaluate the effect of enhanced earthworm presence on sunflower plant traits (height, head diameter, head weight, and stem weight) under three different tillage practices, including NT, P, and SC.

1.4. Justification of the study

Understanding the role of earthworms in agricultural ecosystems and the impact of different tillage methods is essential for achieving sustainable crop production and effective soil management. This study aims to evaluate the effects of three tillage methods no-till (NT), shallow cultivation (SC), and ploughing (P) on soil health within a long-term systematic tillage experiment. The research focuses on physical properties (bulk density and soil moisture content), chemical properties (pH(KCl), soil organic carbon content, and stock), and biological properties (soil microbial respiration and earthworm populations), alongside crop yields. Additionally, the study investigates how enhanced earthworm presence influences sunflower plant traits, including height, head diameter, head weight, and stem weight, under the same tillage practices. Earthworms are central to maintaining soil health and ecosystem functionality, making their interaction with tillage methods a crucial area of research for sustainable agriculture. By burrowing through the soil, they enhance soil structure, aeration, and nutrient cycling (Blouin *et al*., 2013). Investigating their impact on crop yield is essential because understanding how earthworm activity affects crop yield can guide sustainable agricultural practices (Bertrand *et al*., 2015). If earthworms significantly enhance yield, promoting their presence could be beneficial. Earthworms contribute to soil fertility by breaking down organic matter and improving nutrient availability. Their activities can indirectly influence crop productivity (Bertrand *et al*., 2015). If earthworms enhance crop yield, integrating them into farming practices could reduce reliance on chemical fertilizers and pesticides. Earthworms alter soil properties in several ways: their burrowing creates channels, improving water infiltration and root penetration. Investigating this impact helps us to understand soil resilience and stability. Earthworms consume organic matter, mix it with soil, and excrete nutrient-rich casts. This process affects soil organic carbon content, nutrient availability, and microbial activity. They stimulate microbial communities, influencing soil respiration rates. Investigating this link provides insights into soil health and carbon cycling. Investigating NT impact is crucial because it minimizes soil disturbance, preserves organic matter, and reduces erosion. It may enhance soil structure and microbial diversity. Shallow cultivation balances soil aeration and weed control. Assessing its effects on bulk density, moisture content, and pH helps determine its suitability for sustainable agriculture. Ploughing disrupts soil structure but can bury weed seeds and pathogens. Investigating its impact on soil properties informs trade-offs between weed control and soil health. This study aims to bridge the gap between earthworm ecology, soil properties, and crop productivity. By understanding these relationships, we can make informed decisions for sustainable agriculture and ecosystem management.

2. LITERATURE REVIEW

2.1 Importance of soil biota

Soil biota is a broad expression used by soil scientists to describe all soil organisms that live and communicate in the soil environment (Ramesh and Chandra, 2021; Yazi *et al*., 2023). They play a vital role in the soil and represent a large function of global biodiversity and the global ecosystem (FAO, 2005; Petchey and Gaston, 2006). According to Ritz *et al*. (2004), soil biota is the biological engine of the earth, driving and modifying physical, chemical, biological, and ecological processes in worldwide soils. Soil biota is classified into three types: macro, mesobiota, and microbiota/fauna. Bacteria and fungi are the most common microbes found in agricultural soil and grassland environments (Riesenfeld *et al*., 2004; De Vries *et al*., 2006). Soil macro and mesofauna are biota groupings that are significant in soil medium. Earthworms, termites, arthropods, millipeds, ants (macrobiota), protozoa, and nematodes (microbiota) are examples of these species (Coleman, 2001). Macrofauna are essential for moving soil particles, changing soil structure, and increasing soil moisture (Ritz *et al*., 2004). Soil biota also has a critical function in the regulation of greenhouse gases. They are accountable for the capture and storage of carbon (C) in the soil, thereby mitigating the level of carbon-dioxide (CO_2) present in the atmosphere (Fortuna, 2012). The role of soil organisms is pivotal in ensuring the long-term sustainability of both natural and managed ecosystems, as they assume the responsibility for a wide range of services provided by these ecosystems (Barrios, 2007). Additionally, soil organisms hold great significance in the agricultural industry as they actively contribute to the enhancement of soil fertility and facilitate the essential nutrient cycling processes that are indispensable for the optimal growth of crops (Fortuna, 2012).

The enhancement of soil health is achieved through the participation of soil biota, encompassing bacteria and other organisms, in the intricate workings of soil C. These interactions assume a pivotal position in the facilitation of nutrient cycling, the decomposition of organic substances, and the safeguarding against diseases within the soil ecosystem (Amit and Shahane, 2023). Microorganisms, specifically, exert a substantial influence on the improvement of soil quality by facilitating the decomposition of organic matter, the transfer of nutrients, and the enhancement of soil structure (Gougoulias *et al*., 2014). They demonstrate a prompt responsiveness to changes within the soil ecosystem and consequently, can serve as indicators of soil health (Yanlong *et al*., 2023). As a result, it has been determined that the roles fulfilled by soil biota in the modification and enhancement of soil qualities and characteristics are of utmost significance (Lupswayi *et al*., 1998; Miyazawa *et al*., 2000). The recognition of these soil biota processes as fundamental activities employed as biological indicators of soil health has also been brought to light (Pankhurst *et al*., 1997; Kibblewhite *et al*., 2008). Additionally, soil carbon dynamics, such as the sequestration of C in the soil, are influenced by soil biota. Microbes help decompose plant matter, release CO_2, and sequester carbon in the soil, which can mitigate the effects of climate change (Xu *et al*., 2020). The presence of soil biota also affects the composition and activities of microbial communities, such as the abundance of bacteria and fungi, and the activities of C related hydrolase enzymes (Meetei *et al*., 2022). The role of soil biota for performing crucial jobs in the soil varies, according to Coleman (2001), they control the dynamics of soil organic matter, soil C sequestration, and greenhouse gas emission as the primary driving factors behind nutrient cycling. Additionally, they alter soil physical composition and water flow patterns, boost plant health, enhance the amount and effectiveness of nutrient absorption, and preserve soil quality (Denef *et al*., 2001; FAO, 2005; Wang *et al*., 2008; Dominguez *et al*., 2014; Castro-Huerta *et al*., 2015). A study by Bardgett and van der Putten (2014) demonstrated that soil biota, particularly earthworms and mycorrhizal fungi, enhance soil structure, nutrient cycling, and organic matter decomposition, thereby contributing to improved soil health. Recent research by Hartmann *et al*. (2017) emphasized the intricate interactions between plant roots, soil biota, and soil health, highlighting that fostering diverse and functional soil biota is essential for sustainable land management. According to Pulleman *et al*. (2012), earthworms are the biological indicator of the soil ecosystem because they show how fertile and healthy the soil is for growing crops. A high earthworm population denotes a high richness of microorganisms in the soil. The number of earthworms in the soil affects the health of the soil and shows the microorganisms like bacteria, fungi, viruses, and other creatures in the soil (Lakzayi *et al*., 2015).

2.2 Importance of earthworms

Earthworms are recognized as ecosystem engineers because they play a critical role in soil ecology by digesting and cycling nutrients, enhancing soil structure, and serving as a food source for other species (Martin, 1982; Edwards, 2004; Pérès *et al*., 2010; Blouin *et al*., 2013; Capowiez *et al*., 2015; Frazao *et al*., 2019). There are over 6,000 known species of earthworms, and they are found all over the world, from the tropics to the polar regions, and are essential to soil health and the decomposition process of organic matter (Edwards and Bohlen, 1996). They are found in all soil types, from deserts to woods to grasslands, although they prefer soil that is wet, well-drained, and rich in organic matter, such as dead leaves or compost (Lavelle and Spain, 2001). Through their feeding, burrowing, and casting activities, they modify soil porosity, soil aggregate stability, soil organic matter, nutrient availability, water infiltration, soil aeration, and soil texture (Syers and Springett, 1984; Pulleman *et al*., 2003; Bossuyt *et al*., 2006; Hedde *et al*., 2013; Van Groenigen *et al*., 2014; Andriuzzi *et al*., 2015; Lemtiri *et al*., 2014). According to their feeding habits, earthworms are divided into three different groups in the ecosystem: endogeic, epigeic, and anecic (Bouché, 1977; Thakuria *et al*., 2010).

Endogeic earthworms: They dwell in tunnels that are less obvious from the surface and are often smaller in size than other forms of earthworms. These worms consume the soil itself, dissolving organic debris and drawing nutrients from the soil's mineral composition. They may be found in a wide range of soils, although loams and clays with high mineral content are where they are most often found (Edwards and Bohlen, 1996; Lavelle and Spain, 2001). Since they aid in aeration and increase porosity, they are crucial for preserving soil structure and fertility (Bonkowski and Roy, 2012). They also aid in the decomposition and recycling of organic materials, which promotes the soil's nutrient cycling (Zimmermann *et al*., 2007). Moreover, endogeic earthworms are crucial for the physical and chemical activities that take place in the soil, including the transfer of nutrients and water (Hendrix, 2010). The *Aporrectodea caliginosa* and *Aporrectodea rosea* (Figure 1), which may be found all over the globe, especially in Europe and North America, an example of an endogeic earthworm. They are usually 5 to 8 cm long, they do not have pigments, and they feed on the mineral and organic content of the soil and create horizontal burrows through the soil to move around and feed (Blouin *et al*., 2013).

A) B)

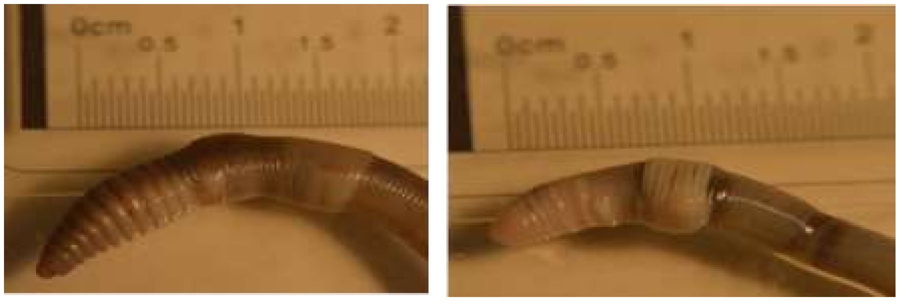


Figure 1. Endogeic earthworms, A) Aporrectodea caliginosa, B) Aporrectodea rosea

(Photo by Dr. Barbara Simon)

Epigeic earthworms: Epigeic earthworms have a short lifetime (a few months). They are smaller than other earthworms that dwell in the very topsoil and occupy the detritus-sphere while eating partly decomposed debris (Lavelle and Spain, 2001). They are often seen in forests, where they are essential to the breakdown of leaves and other plant components, they consume and decompose the litter, releasing nutrients that are subsequently accessible for plants and other species to absorb (Blakemore, 2012). *Eisenia fetida* and *Eisenia veneta* (Figure 2) are two popular species of epigeic earthworms that are often employed in vermicomposting systems. They are used in the vermicomposting process to turn organic waste into compost that is rich in nutrients (Lee, 1985; Edwards, 2004).

A) B)

Figure 2. Epigeic earthworms, A) Eisenia fetida, B) Eisenia veneta
(Source: A: Ravi Kumar et al. (2022), B: naturalhistorymuseum.blog)

Anecic earthworms: these earthworms feed on detritus on the topsoil layer, and construct permanent, vertical burrows that can extend several meters deep into the soil, they are known for their vertical burrowing behaviour and are typically found in grasslands and forest ecosystems (Lavelle and Spain, 2001). They are also known as deep-burrowing earthworms, as they create long, permanent burrows in the soil. These earthworms live in permanent tunnels that they make in the soil and come to the surface only to feed (Blakemore, 2014). Common habitats for anecic earthworms include meadows, woodlands, and agricultural fields (Lavelle, 1988; Lee and Foster, 1991; Blakemore, 2000; Edwards, 2004). Anecic earthworms have distinctive physical characteristics that set them apart from other types of earthworms. They typically have a dark red or brown coloration (Figure 3) and they are distinguished by their long, cylindrical bodies, with some species growing up to 30 cm in length (Hendrix and Bohlen, 2002; James and Davidson, 2012). Their bodies are divided into several rings and have a pointed head and a flat tail, which helps them to burrow deep into the soil (Bird and Bird, 1991; Ratner and Miller, 1959).

Anecic earthworms have a special digestive mechanism that makes it possible for them to effectively consume plant matter. They dredge this material into their burrows to eat it, feeding on leaves and other plant waste that fall to the top of the soil (Darwin, 1881). One of the most important roles of anecic earthworms in the ecosystem is their ability to improve soil structure and nutrient cycling (Blouin *et al*., 2013). Their burrowing activities help to increase soil porosity, which allows for better water infiltration and aeration and create channels for roots to grow and for soil organisms to move through, which helps to promote healthy soil ecosystems (Lavelle, 1988; Edwards, 2004; Hendrix and Bohlen, 2002; Jouquet *et al*., 2019). Anecic earthworms have also been shown to play an important role in C cycling (Six and Paustian, 2014). As they consume organic matter in the soil and incorporate it into their burrows, they help to sequester C in the soil (Mando *et al*., 2011). This has led to increased interest in the use of anecic earthworms for C sequestration and soil health improvement (Bouché, 1977; Lal, 2008; Lal, 2015).

Lumbricus terrestris is an anecic earthworm (Figure 3), which means it is a deep burrowing earthworm that creates vertical burrows in the soil. These earthworms are found in a broad variety of settings and are recognized by their great size and characteristic vertical burrows (Lavelle and Spain, 2001; Blakemore, 2015). They are also recognized as having a significant impact on the cycling of nutrients and soil health (Hendrix and Bohlen, 2002). *Lumbricus terrestris*, sometimes known as the common earthworm, is an annelid worm species belonging to the *Lumbricidae* family. It is one of the most well-known and extensively dispersed species of earthworms, found in Europe, Asia, North America, and Australia, among other regions (Ratner and Miller, 1959). The cylindrical bodies of common earthworms are split into many pieces known as segments; with an average length of 20 to 25 cm when stretched, it is the largest species of earthworm found in nature, the body is coated with tiny bristles, or setae, which aid the worm's movement through the soil, and they have a brownish or reddish-brown upper-side and a paler underside (Edwards and Bohlen, 1996; Blakemore, 2019).

Figure 3. Anecic earthworm Lumbricus terrestris (Source: Barbara Simon)

The interactions among anecic, epigeic, and endogeic earthworms

Epigeic earthworms, such as *Eisenia fetida*, primarily inhabit the soil surface and consume decomposing organic matter. They do not significantly alter soil structure but contribute to organic matter fragmentation and microbial activity. *Lumbricus terrestris*, as a deep-burrowing anecic species, interacts with epigeic earthworms by redistributing organic material. It pulls surface litter into its burrows, which may reduce the available food source for epigeic species, potentially leading to competitive interactions (Bottinelli *et al*., 2015). However, this action also enhances microbial colonization, indirectly benefiting epigeic species by increasing the bioavailability of nutrients (Brown *et al*., 2000). Additionally, epigeic and anecic earthworms influence each other's activity indirectly through microbial-mediated processes. The organic material that epigeic earthworms fragment and partially digest undergoes further microbial decomposition, creating nutrient-rich residues that can be transported into deeper soil layers by *Lumbricus terrestris*.

This interaction contributes to soil fertility and the stabilization of soil organic carbon (SOC) (Bertrand *et al*., 2015). However, the presence of *Lumbricus terrestris* may also suppress epigeic species by altering the microenvironment, such as changes in moisture and aeration in upper soil layers, making conditions less favourable for their survival (Shipitalo and Butt, 1999).

Endogeic earthworms, such as *Aporrectodea caliginosa*, dwell in the mineral soil and create horizontal burrows while feeding on soil organic matter. Their interaction with *Lumbricus terrestris* is often complementary rather than competitive. The deep vertical burrows *of Lumbricus terrestris* enhance soil aeration and water infiltration, creating favourable conditions for endogeic species (Blouin *et al*., 2013). Additionally, the mixing of organic material from surface layers into deeper soil layers promotes microbial activity, which supports endogeic earthworm populations (Shipitalo and Butt, 1999). In some cases, facilitative interactions have been observed, where the activity of *Lumbricus terrestris* improves the habitat conditions for endogeic species, enhancing soil biological functions (Marhan and Scheu, 2006). Research suggests that endogeic earthworms may also benefit *Lumbricus terrestris* by increasing soil aggregation and stabilizing burrow structures, preventing collapse and maintaining aeration pathways. This mutual reinforcement of soil architecture enhances the long-term persistence of both species in agricultural and natural ecosystems (Blouin *et al*., 2013). However, in highly competitive environments with limited organic inputs, *Lumbricus terrestris* may outcompete endogeic species by monopolizing deeper soil layers with its burrowing activity (Bottinelli *et al*., 2015).

The interactions between *Lumbricus terrestris* and epigeic/endogeic earthworms play a crucial role in maintaining and enhancing soil health. The presence of multiple functional groups within an earthworm community significantly influences nutrient cycling, soil structure stability, and plant growth (Brown *et al*., 2004; Edwards, 2004). Anecic earthworms such as *L. terrestris* create deep vertical burrows, which improve soil aeration, enhance root penetration, and facilitate water infiltration (Bouché, 1977; Capowiez *et al*., 2014). Endogeic earthworms, which predominantly inhabit the soil matrix and ingest soil rich in organic matter, contribute to the homogenization of soil aggregates, improving soil porosity and water retention (Blouin *et al*., 2013; Lavelle *et al*., 2001).

These functional interactions are complemented by epigeic species, which rapidly decompose surface litter, releasing nutrients that support microbial activity and plant growth (Curry and Schmidt, 2007). Empirical studies have demonstrated that earthworm diversity enhances soil ecosystem services. For instance, Bertrand *et al*. (2015) reported that the combined activity of anecic and endogeic earthworms significantly improved organic matter distribution and soil aggregate stability, ultimately benefiting plant root development and microbial diversity. Additionally, research by van Groenigen *et al*. (2014) emphasized that earthworm-driven bioturbation plays a key role in increasing soil organic carbon sequestration, which is essential for soil fertility and climate change mitigation. Despite these benefits, shifts in earthworm community composition due to agricultural practices or climate change could alter these interactions, potentially leading to a decline in soil ecosystem services (Lubbers *et al*., 2017). Intensive tillage disrupts earthworm habitats by mechanically destroying burrows and exposing them to predation, while the use of chemical fertilizers and pesticides has been shown to negatively affect earthworm abundance and diversity (Pelosi *et al*., 2014; Briones and Schmidt, 2017). A long-term study by Chan (2001) found that conventional ploughing systems significantly reduced earthworm biomass compared to conservation tillage practices, indicating the detrimental effects of intensive soil disturbance.

To optimize the benefits of these interactions, sustainable soil management practices should be adopted. Reduced tillage, cover cropping, and organic amendments promote diverse earthworm communities, allowing their natural interactions to enhance soil structure and fertility (Six *et al*., 2004; Fonte *et al*., 2009). Conservation tillage, which integrates minimal soil disturbance, permanent organic soil cover, and crop diversification, has been shown to foster earthworm activity, contributing to improved nutrient cycling and soil resilience (Hendrix *et al*., 2008). Furthermore, studies by Schmidt *et al*. (2021) highlight that integrating organic farming practices, such as compost application and residue retention, can enhance earthworm-mediated soil processes, ultimately leading to increased agricultural productivity.

2.3 Effects of earthworms on soil properties

The maintenance of soil productivity and health is largely attributable to earthworms (Edwards, 2004). Because of the substantial influence they have on soil properties (physical, chemical, and biological) (Lavelle *et al*., 2006: Edwards and Bohlen, 1996).

2.3.1 Physical properties

According to several studies (Edwards, 1998; Lee, 1985; Lee and Foster, 1991; Lavelle and Spain, 2001; Nico *et al.,* 1991), earthworms improve soil structure by tunnelling into it and making channels that let air and water flow more. This increases soil porosity, which encourages improved plant nutrient uptake and root growth (Edwards, 2004). Earthworms also play a role in enhancing soil physical properties like bulk density (BD), hydraulic conductivity, and aggregate stability (Capowiez *et al*., 2012). They can mix soil layers and alter soil structure through their feeding, burrowing, and casting activities (Jonatan *et al*., 2023). This mixing of soil layers by earthworms leads to changes in soil density profiles, which can be used to estimate bioturbation rates in the field (Mingan *et al*., 2022). Earthworms burrow through the soil, creating channels and pores. These channels allow better soil aeration and water infiltration, reducing soil compaction and consequently lowering bulk density (Edwards and Bohlen, 1996). When they ingest organic matter, earthworms create castings (excrement), which are rich in nutrients and aid in bringing soil particles together to form stable aggregates that withstand erosion (Brown *et al*., 2000). Moreover, soil aggregates have lower BD compared to individual soil particles (Six *et al*., 2004). Their activities, such as burrowing and casting, increase soil water infiltration and storage, leading to higher soil water content (Li *et al*., 2020). According to research conducted by Ganault *et al.* (2022), earthworms improve the soil water holding ability by adjusting soil temperature and reducing soil water evaporation, leading to decrease in surface soil water content loss but an increase in subsoil water content loss. The canals that earthworms make boost the soil ability to retain water, which can be crucial in dry or arid conditions (Hendrix *et al*., 2002), and this decreases soil erosion and improves soil water retention (Edwards, 2004; Blouin *et al*., 2013).

2.3.2 Chemical properties

Earthworms affect the chemical characteristics of soil in a variety of ways, which helps to increase soil quality and fertility (Edwards, 2004; Liu *et al*., 2021). Earthworms consume soil particles and excrete them as nutrient-rich castings, which increases soil fertility (Edwards, 2004; Lal, 1995; Hendrix *et al*., 2002). They also break down organic materials and release nutrients like nitrogen, phosphorus, and potassium in a form that is easily absorbed by plants (Edwards and Bohlen, 1996). The process of nitrogen mineralization in residues is accelerated by earthworm activity. According to Rizhiya *et al*. (2007) and Cortez *et al*. (2000), this process increases the availability of inorganic nitrogen from plant material for both plants and microbes. It's crucial to remember, however, that an increase in earthworm populations might result in a rise in nitrous oxide (N_2O) emissions from agricultural soils. Earthworm activity accounts for about half of the *in situ* N_2O emissions in certain soils (Drake and Horn, 2006). According to a recent study, earthworms may create up to 3×108 kg of N_2O yearly worldwide (Drake and Horn, 2006).

Numerous biotic and abiotic variables, including earthworms, have an impact on the dynamics of soil C (Wolters, 2000). Due to their function as ecosystem engineers, earthworms are essential for soil C sequestration. According to research (Bossuyt *et al*., 2005), the presence of earthworms has a beneficial effect on the soil organic carbon (SOC) concentration. Burrows made by earthworms in the soil improve soil aeration and water penetration. In turn, this encourages microbial activity and the breakdown of organic materials, which raises SOC (Lal, 1997). Earthworms consume organic matter, partly digest it, and then excrete castings that are rich in nutrients on top of the soil. The integration of these casts into the soil enhances SOC since they are high in organic C (Edwards and Bohlen, 1996). The impact of various earthworm species on SOC varies. For instance, anecic earthworms dig deep tunnels and deposit organic material on the surface, but endogeic earthworms prefer to ingest and blend more organic materials into the mineral soil (Curry, 2004). According to Cambardella and Elliott (1992) soil type has a significant impact on how earthworms affect SOC. Sandy soils may give different results than clay soils when it comes to earthworm activity. Evidence suggests that earthworms significantly influence soil structure and the distribution of organic matter through their casting activities (Van Groenigen *et al*., 2019). However, a study has focused on the critical role of the earthworm cast itself, despite the fact that earthworm casts have been identified as potentially beneficial for long-term C protection (Vidal *et al*., 2019).

2.3.3 Biological properties

By producing an energy-rich mucus that activates microorganisms through a priming effect (Jenkinson, 1966) and signal molecules that have hormone-like effects and influence plant gene expression, earthworms are regarded as important ecological mediators that have the ability to affect soil functions and microbial activities (Binet *et al*., 1998; Lavelle *et al*., 2016; Puga-Freitas and Blouin, 2015). The soil can breathe and the gas flows freely because of the pathways that earthworms have dug into it. These results enhanced soil structure, which promotes greater plant growth (Edwards, 2004). Earthworms increase soil microbial activity by breaking down organic materials and raising oxygen levels. This increases the number of helpful microorganisms, such as bacteria and fungus, which aid in the decomposition of organic matter and the release of nutrients (Domínguez and Edwards, 2011). They have a significant impact on soil microbial communities since they are one of the most important fauna groups in soils in terms of population and biomass (Blouin *et al*., 2013). Earthworms enhance microbial respiration in the soil by increasing the availability of organic matter and nutrients (Edwards, 2004). As earthworms consume organic materials like dead plant matter and excrete nutrient-rich castings, they facilitate the decomposition process, providing a food source for soil microbes (Brown *et al*., 2000). This increased availability of organic matter stimulates microbial activity and respiration, leading to improved nutrient cycling and soil fertility (Zhang *et al*., 2023; Medina-Sauza *et al*., 2019). The burrowing action of earthworms creates channels for root penetration, allowing roots to explore deeper soil layers for water and nutrients (Edwards and Bohlen, 1996). Additionally, the nutrients released through earthworm castings provide a readily available food source for plant roots, stimulating root growth and development (Blouin *et al*., 2013).

2.4 Effects of *Lumbricus terrestris* on soils

The species *Lumbricus terrestris* plays a fundamental role in maintaining the health and productivity of terrestrial ecosystems, particularly in agricultural and natural soil environments. As a deep-burrowing anecic earthworm, *L. terrestris* significantly contributes to soil bioturbation, which enhances soil aeration, water infiltration, and organic matter decomposition. Their function as decomposers and soil builders is vital in preserving soil fertility and structure by incorporating organic residues into the soil profile and stimulating microbial activity, which in turn supports plant growth and the sustenance of numerous other organisms. This earthworm species plays an integral role in food webs, serving as a primary prey item for a wide range of predators, including birds, small mammals, amphibians, and other invertebrates (Blouin *et al*., 2013). Consequently, their abundance and activity can have cascading effects on trophic interactions and ecosystem stability. One of the key biochemical contributions of *Lumbricus terrestris* to soil health is its ability to regulate soil pH. These earthworms excrete alkaline substances, such as calcium carbonate and bicarbonate, through their skin and gut, which can neutralize acidic soils and create a more favourable environment for plant growth (Jiménez *et al*., 1996; Lavelle and Spain, 2001). This function is particularly crucial in areas where acidification due to anthropogenic activities, such as excessive fertilizer application and acid rain, threatens soil productivity.

By maintaining a balanced pH, *L. terrestris* facilitates nutrient availability, particularly for plants that require specific pH conditions to access essential elements such as nitrogen, phosphorus, and potassium (Edwards and Bohlen, 1996; Brown et al., 2000; Zimmermann et al., 2019).

In addition to its chemical contributions, *L. terrestris* enhances soil physical properties, promoting improved structure and porosity. The species' burrowing activity creates deep vertical channels that not only allow water to penetrate more efficiently but also reduce the risk of surface runoff and erosion, which is particularly beneficial in agricultural fields. Research by Monzón-Verona *et al*. (2017) suggests that the presence of *L. terrestris* can mitigate soil compaction, a major problem in modern intensive farming systems, by increasing pore spaces and facilitating root penetration. This can lead to improved root architecture, enabling crops to access deeper soil layers for water and nutrients, ultimately resulting in better growth and higher yields. However, the impact of *L. terrestris* on soil microporosity and soil fauna distribution may vary depending on soil type, as observed in studies by Nuutinen *et al*. (2017), where *L. terrestris* settlement in clay soils increased the spatial patchiness of soil fauna but did not significantly alter their overall field-scale abundance. Beyond soil structure and nutrient cycling, *Lumbricus terrestris* also plays an important role in plant disease suppression, particularly in agroecosystems. In maize farming, this species exhibits a species-specific bioregulatory effect by effectively suppressing certain Fusarium species, which are known to cause plant diseases such as root rot and ear rot (Christine *et al*., 2021). The extent of this bioregulatory performance, however, depends on the specific Fusarium species involved, which means that *L. terrestris* can function as both an ecosystem service provider and a potential disservice, depending on the agricultural context. Understanding these dynamics is crucial for integrated pest and soil health management in sustainable farming practices.

Moreover, recent research has highlighted *Lumbricus terrestris* as a potential bioindicator for assessing soil contamination, particularly in relation to microplastics. Carolina *et al*. (2020) found that *L. terrestris* can ingest and bioaccumulate microplastic particles, making them a useful organism for monitoring pollution in terrestrial environments. The ability of these earthworms to interact with pollutants and influence their distribution within soil layers suggests that they could serve as early indicators of environmental degradation caused by plastic pollution. This opens up new avenues for using earthworms in environmental risk assessment and soil remediation strategies.

2.5 Effects of earthworms on plants

Earthworms are widely recognized as beneficial soil organisms, primarily due to their ability to enhance soil fertility and improve plant development. This perception is supported by extensive research highlighting their contributions to soil health and agricultural productivity (Lee, 1985; Edwards and Bohlen, 1996). The presence of earthworms in the soil can significantly benefit plant growth by improving nutrient availability, particularly essential macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Tomati and Galli, 1992; Yoshitake *et al*., 2014). These nutrients are released through the digestion of organic matter by earthworms, which subsequently enhances soil fertility and promotes plant vigour. Beyond nutrient enrichment, earthworms influence various aspects of plant growth and soil structure. Their burrowing activities create networks of channels that facilitate root penetration, enhance soil aeration, and increase water infiltration, all of which are critical factors for robust plant development (Blossey and Hunt-Joshi, 2003). The improved soil porosity resulting from earthworm activity allows plant roots to access oxygen more effectively, reducing the risk of root suffocation and improving overall plant health. Research has demonstrated that earthworm presence can directly contribute to increased plant biomass, enhanced root proliferation, and greater nutrient uptake efficiency (Scheu, 2003; Liu *et al*., 2017; Hendrix *et al*., 2008). For example, Brown *et al*. (2004) found that maize yields were significantly higher in plots with active earthworm populations compared to those without, underscoring their role in agricultural productivity. Similarly, studies on sunflower and barley have indicated that earthworm activity positively influences growth parameters and stress resilience by optimizing nutrient cycling and soil microbial interactions (Koprna *et al*., 2016; Fricano *et al*., 2021). In addition to their direct effects on plant growth, earthworms can indirectly contribute to agricultural sustainability by reducing pest populations and inhibiting plant diseases. Some studies suggest that earthworms consume harmful soil-dwelling pests such as slugs, snails, and insect larvae, thereby reducing plant damage and minimizing the need for chemical pest control (Edwards and Bohlen, 1996). Furthermore, their influence on soil microbial communities fosters the proliferation of beneficial microorganisms that can suppress soilborne plant pathogens, enhancing plant resilience against diseases (Lavelle and Spain, 2001; Brown and Fragoso, 1999). Earthworm casts, which are rich in organic matter and nutrients, further contribute to soil fertility by improving its physical and chemical properties. These casts contain high concentrations of plant-available nitrogen and phosphorus, which are crucial for healthy crop growth. Additionally, the decomposition of organic residues facilitated by earthworm activity accelerates carbon sequestration, thereby promoting long-term soil health and sustainability (Wall *et al*., 2012).

2.5.1 Effects on sunflower (*Helianthus annuus*)

The sunflower is the world's fourth largest oil-seed crop, and its seeds are used as food and its dried stalk as fuel. It is a well-known flowering plant that belongs to the *Asteraceae* family (Harter *et al.*, 2004; Muller *et al*., 2011). Sunflowers are often used in crop rotation systems to break pest cycles. They are resistant to many pests and diseases that commonly affect other crops, reducing the need for chemical pesticides. By including sunflowers in crop rotations, farmers can help manage pest populations and minimize the reliance on synthetic inputs, promoting more sustainable agricultural practices (Altieri *et al*., 2017). Sunflower can also exploit residual water left in the subsoil by previous crops (Fereres *et al*., 1993; Cabelguenne and Debaeke, 1998). However, sunflower extracts more water from the profile than other crops and leaves less water in the soil for the next crop which can be detrimental to yield in dry conditions (Anderson *et al*., 1999). Sunflowers could improve soil quality through a process called phytoremediation. They have the capacity to extract heavy metals and toxins from contaminated soil, reducing soil pollution. Sunflowers are known to accumulate lead, arsenic, uranium, and other harmful substances, thereby cleaning up contaminated areas and making the soil safer for other crops to grow (Pilon-Smits, 2005).

Earthworms dramatically increased sunflower growth and yield by improving soil qualities such as water-holding capacity, organic matter content, and soil structure, as well as increasing soil aggregation and aeration, which resulted in increased sunflower growth (Dube *et al*., 2016). Mishra *et al*. (2018) discovered that earthworms increased soil microbial activity, allowing sunflowers to absorb more nutrients and grow larger. They also discovered that earthworms increased soil enzyme activity and microbial biomass carbon and nitrogen, improving nutrient availability for sunflower plants (Huerta *et al*., 2007). According to a study by Gao *et al*. (2020) earthworms improved sunflowers' photosynthetic efficiency, allowing them to grow and produce more. The authors found that by raising the chlorophyll content and photosynthetic rate, earthworms enhanced sunflower plant growth and yield (Edwards and Bohlen, 1996: Brown, 1995; Singh *et al*., 2011; Ansari *et al*., 2017; Zou *et al*., 2021). They have assumed that using earthworms could be an effective and long-lasting method for increasing the yield and quality of sunflower crops.

Earthworms can also cause damage to sunflowers by feeding on their roots, which can result in reduced plant growth and yield. This damage is usually more significant when the number of earthworms is high, and the soil is moist (Kavitha *et al*., 2012; Arancon *et al*., 2003).

2.5.2 Effects on oat (*Avena sativa)*

Oats hold significant importance as a cereal crop in Hungary due to their nutritional value, adaptability to various climates, and contribution to the agricultural economy (Herzog and Kormos, 2023). It can thrive in a wide range of soils and climatic conditions, including colder regions of Hungary. Their resilience to poor soil quality makes them suitable for diverse agricultural zones within the country. Compared to other cereals like wheat or barley, oats require fewer inputs such as fertilizers and pesticides, making them a more sustainable crop option (Peterson, 2001). Oats are often used in crop rotation systems, helping to improve soil health and reduce the prevalence of pests and diseases. They are especially effective in preventing soil erosion due to their dense root system. Their inclusion in rotations with legumes or other cereals can enhance soil fertility, improving the yield of subsequent crops (Crews and Peoples, 2004). Earthworms have a significant positive impact on the growth of cereal crops, including oats.

A study by Fonte (2023) demonstrates that earthworms contribute to 6.5% of global grain production by enhancing soil structure and fertility, thereby benefiting crops like oats. The study emphasizes the role of earthworms in sustainable farming practices such as no-till agriculture. According to a study by Bedano *et al*. (2019) highlights the contribution of earthworms to ecosystem processes in no-till farming systems, improving soil health and supporting higher yields in cereal crops, including oats. Earthworm activity in the soil can lead to improved nitrogen cycling, which is crucial for cereal crops like oats. A study by Liu *et al.* (2020) has shown that the presence of earthworms can enhance the productivity of crops, by boosting soil fertility and root proliferation. Other studies on cover crops found that oats, due to their high carbon-to-nitrogen ratio, were particularly favoured by earthworms, improving their population and activities in the soil ecosystem (Hobbs and Schuman, 2000).

2.6 The vegetation phases of the sunflower (*Helianthus annuus*)

Sunflower growth and development can be divided into vegetative and reproductive phases (Contreras *et al*., 2016). The vegetative phase consists of different growth stages that play a crucial role in determining the plant's overall productivity, including the development of the root system, leaves, and stem, which provide the necessary support and photosynthetic capacity for reproductive growth (Boyer,1982). The vegetative development of sunflower follows a specific sequence of stages, commonly classified using the Fehr and Caviness (1977) system or the newer BBCH scale. These stages are essential for understanding the crop's management and optimizing agronomic practices.

Germination and emergence (VE Stage)

The germination phase begins when the seed absorbs water (imbibition) and activates enzymatic processes, leading to radicle emergence (Schneiter and Miller, 1981). Under optimal soil temperature (7–10°C) and moisture conditions, the hypocotyl elongates, pulling the cotyledons above the soil surface within 4–10 days (Dewey and Murray, 2019). Emergence success is influenced by soil type, depth of sowing, and seed vigour (Seiler, 2007).

Cotyledon stage (VC Stage)

Once the cotyledons fully expand, the plant begins photosynthesis to support further development (Schneiter and Miller, 1981). The initial root system starts branching, anchoring the plant and facilitating nutrient uptake (Connor and Hall, 1997). Early establishment is critical, as environmental stress at this stage can affect subsequent vegetative growth.

First to fifth true leaf stages (V1 to V5 Stages)

The first true leaf appears (V1) within 7–14 days after emergence, followed by sequential leaf development (Connor and Hall, 1997). By V5 (five leaf pairs), sunflower exhibits increased leaf expansion, with leaves arranged in an alternating pattern along the stem (Seiler, 2007). During these stages, stem elongation begins, and root growth intensifies, promoting nutrient uptake (Hall *et al*., 1990).

Vegetative expansion (V6 to Vn Stages)

Sunflowers continue to produce leaves until the initiation of the reproductive phase (R1 stage). Stem thickening occurs, and internodes elongate significantly, determining the final plant height (Schneiter and Miller, 1981). By V12–V14, the maximum photosynthetic activity is reached, supporting later reproductive growth (Dewey and Murray, 2019). Tillage, fertilization, and irrigation practices influence leaf area index (LAI) and biomass accumulation (Andrade *et al*., 1993).

Transition to reproductive growth (Final vegetative stage, Vn)

The vegetative phase ends when the terminal bud differentiates into a floral bud (R1 stage) (Schneiter and Miller, 1981). The total number of leaves is determined before this stage, impacting the plant's ability to support seed filling (Seiler, 2007). Environmental conditions such as temperature, photoperiod, and nutrient availability play crucial roles in regulating vegetative-to-reproductive transition (Andrade *et al*., 1993). During the vegetative phase, sunflowers require sufficient nitrogen, phosphorus, and potassium to support leaf development and root expansion (Hall *et al*., 1990). The establishment of deep root systems enhances drought resilience and water uptake efficiency (Connor and Hall, 1997). Early vegetative growth is vulnerable to insect pests (e.g., cutworms, aphids) and fungal pathogens (e.g., downy mildew) (Seiler, 2007). Proper vegetative development ensures optimal biomass production and photosynthetic efficiency, directly impacting final grain yield and oil content (Andrade *et al.,* 1993).

2.7 Effects of soil tillage on earthworms

Soil tillage may have positive or negative effects on earthworm populations depending on the level, frequency, and timing of soil cultivation (Lee *et al*., 1985; Tullberg, 2007; Lal *et al*., 2001). Many studies have been conducted to evaluate the impact of soil tillage on earthworm populations in various agricultural systems and regions (Edwards and Bohlen, 1996; Six *et al*., 2006; Lumbreras *et al*., 2014; Pérès *et al*., 2015; Ponge *et al*., 2016) (Table 1).

Table 1. Effects of tillage practices on earthworm populations

Tillage Practice	Positive Effects	Negative Effects
Conservation Tillage	Minimal soil disturbance, maintaining earthworm habitat structure (Blanco-Canqui and Lal, 2007; Six *et al*., 1999), increased organic matter from crop residues benefits earthworms (Franzluebbers *et al*., 2000; Singh and Gupta, 1977). Improved soil aeration due to reduced disturbance (Reicosky and Allmaras, 1987), favorable crop rotations enrich soil and support earthworm abundance (Malhi and Gill,1982; Tonitto *et al*., 2006)	Direct injury due to deep ploughing (Russell, 1956), and altered habitat (soil temperature, moisture, organic matter availability) (Russell, 1956). Decreased soil health due to excessive disturbance (Mosier *et al*., 2021), and annual ploughing leads to systematic decline in populations (Peigné *et al*., 2009).
No-tillage (NT)	Preservation of soil structure and habitats, higher earthworm populations, increased organic matter content, and improved soil structure promotes activity (Blanco-Canqui *et al*., 2006; Gregorich *et al.,* 1994; Six *et al*., 2002).	Soil compaction, poor aeration, and more weeds and pests.
Reduced tillage (RT)	Preservation of some soil structure and habitats, moderate earthworm populations, maintained organic matter content, and reduced soil compaction (Li *et al*., 2020).	Moderate reduction in earthworm populations compared to conventional tillage, and disruption to soil structure and habitats (Lagerlöf *et al*., 2012)
Conventional Tillage	Breaks soil compaction, decreases the weed density, loosens soil structure, and homogenizes soil.	Conventional tillage practices are harmful to earthworms (Blouin *et al*., 2013).

2.7.1 Positive effects

Tillage practices that mitigate soil compaction or integrate crop residues as a nourishment for earthworms have the potential to sustain or augment earthworm populations (Wuest *et al*., 2005; Metzke *et al*., 2007). In the context of no tillage (NT) systems, the presence of crop residues on the soil surface serves several beneficial functions such as maintaining cool and moist soil conditions, enhancing soil structure, and providing a source of nourishment for earthworms (House and Parmelee, 1985; Wardle, 1995; Chan, 2001). Consequently, the long-term implementation of NT practices is expected to create advantageous circumstances for earthworms through the improvement of various soil physical properties including soil moisture content (SMC), porosity, bulk density (BD), and the availability of food resources (Kladivko, 2001). Many studies have recorded elevated populations of earthworms in NT and reduced tillage (RT) systems in comparison to those observed in conventional tillage (CT) systems (Edwards and Lofty, 1977; Rovira *et al*., 1987; Chan and Heenan, 1993). Similarly, UK studies discovered that RT methods supported more earthworms and diverse earthworm ecosystems than CT systems (Baker *et al*., 2016). In contrast to CT, Morugán-Coronado *et al*. (2018) found that RT practices had a positive effect on earthworm populations in Spain. Garrido-Becerra *et al*. (2020) discovered in Spain that RT practices benefited epigeic and anecic earthworms while having no effect on endogeic earthworms. Ernst *et al*. (2009) reported that the presence of anecic and epigeic species may lead to reduced competition, less compaction in the upper 10–20 cm of the soil, and an increase in organic matter within the soil.

According to Deibert *et al*. (1991), they found that employing spring sweep tillage, involving shallow cultivation (SC) and mixing the upper 7.5-10 cm soil layer, led to a greater population of earthworms compared to spring plough tillage (P), which involved tilling to a depth of 20 cm. Gerard and Hay (1979) conducted a long-term tillage experiment in England where they compared different ploughing methods, including deep ploughing (DP) (30-35 cm, furrows 45 cm apart), normal ploughing (NP) (15-20 cm, furrows 22.5 cm apart), tined cultivation (12-30 cm deep, tines 22.5 cm apart, two to three passes), and NT. They observed that the number of earthworms was lowest with DP, increased progressively through tined and normal cultivation, and reached the highest numbers with NT. The authors attributed the larger population of earthworms under NT to several factors. Firstly, they mentioned that reduced mechanical damage during P and harrowing under NT contributed to the higher numbers. Additionally, the authors noted that the higher soil water content and the presence of a litter layer in the spring, resulting from the absence of soil disturbance, further supported the increased earthworm population. These factors together encouraged longer periods of feeding and cocoon production among the earthworms. Studies suggest that SC may be compatible with healthy earthworm populations, but it's worth noting that other factors, such as soil type, climate, and management practices, can also influence earthworm populations (Görres and Perumpral, 2000; Birkhofer *et al*., 2008; Pérès *et al*., 2013; Zhang *et al*., 2019).

2.7.2 Negative effects

Tillage has an impact on earthworms found in agricultural fields. This impact is not limited to mechanical harm and predation caused by P activities. Additionally, earthworms are affected by alterations in their habitat, such as changes in soil structure, organic matter content, and the distribution of organic matter and moisture (Capowiez *et al*., 2009). Frequent tillage harms earthworms and exposes them to predators, while deep tillage (DT) ruins earthworm tunnels, forcing earthworms to spend energy constructing burrows in unstructured soils rather than reproducing (Edwards and Lofty, 1982; Clapperton *et al*., 1997; Chan, 2001). In addition, the effect of tillage may differ depending on species or functional group. Compared to soils treated with RT (rotary or disc harrow), anecic earthworms are comparatively less common in soils treated with CT (Capowiez *et al*., 2009). This may have to do with the larger size of anecic earthworms making them more susceptible to mechanical harm (Ernst *et al*., 2009). According to a study conducted by Kladivko (2001), tillage is predicted to have a negative impact on populations of anecic earthworms, such as *Lumbricus terrestris* which travel through vertical burrows to feed and reproduce at the soil surface, and epigeic earthworms, such as *L. rubellus*, whose habitat is disrupted when the litter layer is chopped and incorporated. Heavy soil tillage has a negative impact on *Lumbricus terrestris* populations because P can disrupt their habitat by reducing soil structure and organic matter and increasing soil compaction, which can impair their ability to migrate through the soil (Blouin *et al*., 2013).

Excessive tillage can disrupt the organic matter decomposition process by reducing earthworm populations, which can lead to a decrease in nutrient cycling and soil fertility (Briones and Schmidt, 2017). According to Sánchez-Moreno *et al*. (2012), excessive soil tillage can have a negative impact on earthworm populations, resulting in decreased soil health and productivity. In

Chinese wheat fields, Hu *et al*. (2017) found that tillage intensity significantly reduced the biomass and abundance of earthworms. Research conducted in Spain found that tillage reduced earthworm populations by 30 to 40% when compared to NT regimes (Blanco-Moure *et al*., 2014). According to a study conducted in the United States by Blanco-Canqui *et al*. (2018), NT and RT strategies boost surface-dwelling earthworm populations while harming deep-burrowing earthworm populations. Earthworm populations have been shown to be affected by cultivation time and degree (Hendrix and Bohlen, 2002). According to a study conducted in France by Pelosi *et al*. (2019), early autumn tilling was more damaging to earthworm populations than late autumn tilling. It was also shown that earthworm populations in clay soils were more susceptible to cultivation than those in sandy soils.

2.8 Effect of soil tillage on soil organic carbon

Tillage affects SOC through its influence on soil aeration, aggregate stability, residue incorporation, and microbial activity. Conventional tillage, such as mouldboard ploughing, disturbs soil aggregates, exposing organic matter to microbial decomposition and accelerating CO_2 release (Six *et al*., 2000). Conversely, reduced tillage and NT systems help maintain soil structure, enhance aggregate stability, and reduce organic matter decomposition, thereby promoting SOC retention (Lal, 2004). NT practices enhance SOC storage by minimizing soil disturbance and preserving crop residues on the surface. This practice fosters carbon input through plant residues and root biomass while reducing SOC mineralization rates (Kern and Johnson, 1993). SC provides an intermediate effect by incorporating residues while maintaining some soil stability, whereas deep P leads to significant SOC losses by exposing deeper soil layers to oxidation (Plaza-Bonilla *et al*., 2013).

Long-term tillage studies suggest that conservation tillage systems, including NT and SC, result in higher SOC concentrations in the topsoil compared to conventional ploughing. A meta-analysis by Angers and Eriksen-Hamel (2008) demonstrated that NT increased SOC levels by 10–30% in the top 10 cm of soil over 10–20 years. However, some studies indicate that NT might redistribute SOC within the soil profile, leading to deeper storage rather than overall SOC gains (Powlson *et al*., 2014).

Additionally, long-term NT systems enhance SOC stability by increasing the proportion of microaggregates that protect organic matter from microbial decomposition (Six *et al*., 2002).

However, the effectiveness of NT in SOC sequestration depends on factors such as soil type, climate, cropping system, and residue management (West and Post, 2002).

SOC interacts with various soil physical, chemical, and biological properties that are influenced by tillage. Bulk density is often higher under NT due to reduced soil disturbance, which can affect root penetration and water infiltration (Blanco-Canqui and Lal, 2008). On the other hand, NT generally improves soil moisture retention, benefiting microbial activity and organic matter decomposition rates (Franzluebbers, 2002). Soil microbial respiration, an indicator of microbial activity and carbon cycling, is often higher in NT systems due to increased organic matter availability and stable microhabitats (Mangalassery *et al*., 2015). Earthworm abundance and diversity also increase under NT, contributing to SOC stabilization through bioturbation and the formation of stable soil aggregates (Lubbers *et al*., 2013).

3. MATERIALS AND METHODS

3.1 Site description

The Józsefmajor Experimental and Training Farm (JETF) of GAK Ltd. (Agricultural Centre Gödöllő) (47° 41’ 31.7" N, 19° 36’ 36.1" E, 110 m a.s.l.) established the long-term tillage experiment in 2002 (Figure 4). The topography is level. According to the World Soil Reference Base (IUSS Working Group WRB, 2015), the soil type is Endocalcic Chernozem. The humus concentration is 3.12%.

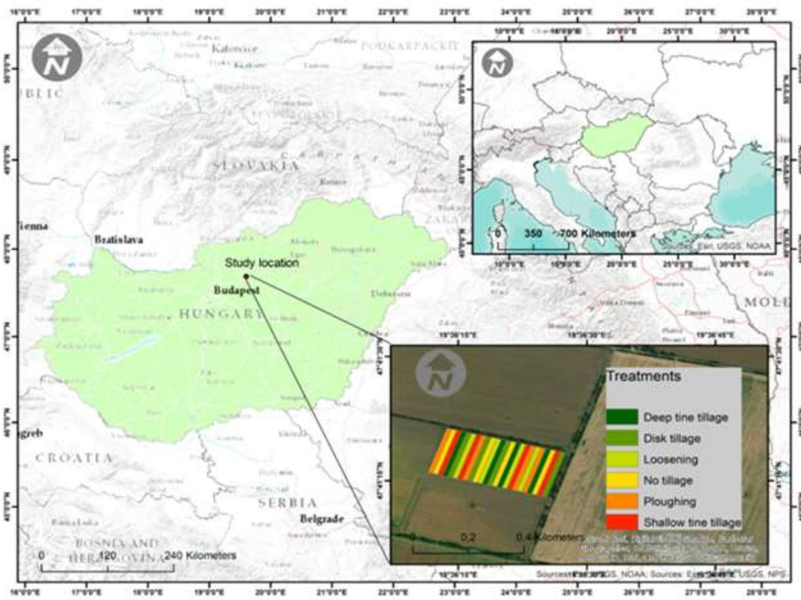


Figure 4. Location of the study area (Józsefmajor-Hatvan, Central Hungary).

(Source: Dekemati et al., 2020)

The soil has a clay loam texture, with 37% sand, 27% silt, and 36% clay (Tharwat *et al*., 2024) and a structure ranging from granular to blocky (Kovács and Tóth, 2008). Its pH varies from slightly acidic to neutral (Somogyi and Máté, 2015). The soil also hosts diverse microbial communities, including bacteria, fungi, and actinomycetes (Tóth and Dér, 2010).

The climate observed at the experimental farm can be classified as continental. The average annual temperature is 10.3°C and during the vegetation period it is 15°C (New *et al*., 2002). The annual mean precipitation (between 1961 and 1990; data derived from the climatic dataset of the Climatic Research Unit) is 560 mm, with 395 mm occurring during the vegetative season (Popova *et al*., 2018). The 2019–2022 research period at the JET Farm is covered by the data (annual temperature and precipitation) shown in Figures 5 and 6. It is observed that the years 2019 and 2020 exhibited higher levels of precipitation, measuring 643 mm and 575 mm respectively, in comparison to 2021 and 2022, where precipitation levels were recorded at 523 mm and 475 mm. There was no irrigation done in the experimental area.

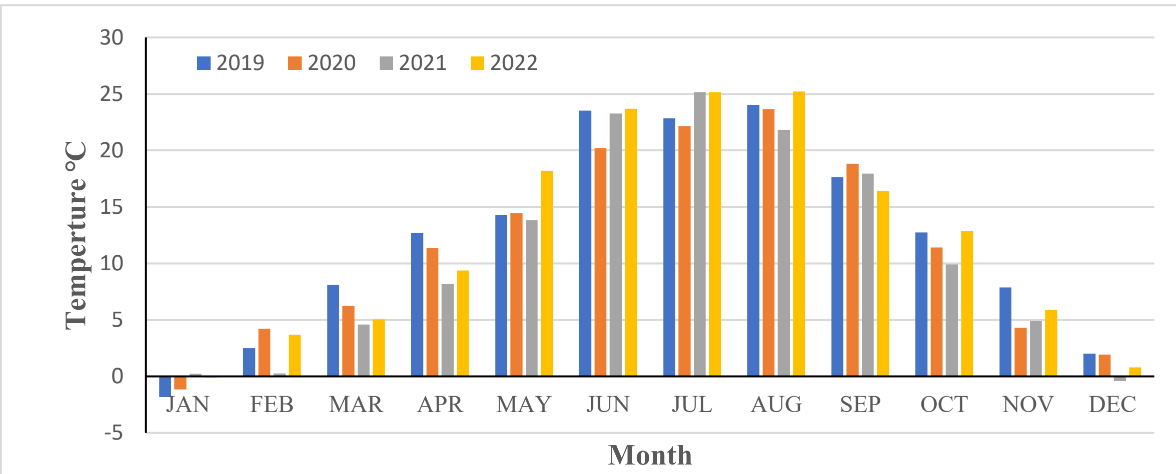


Figure 5. Monthly temperature data (2019- 2022). Sourced from NASA

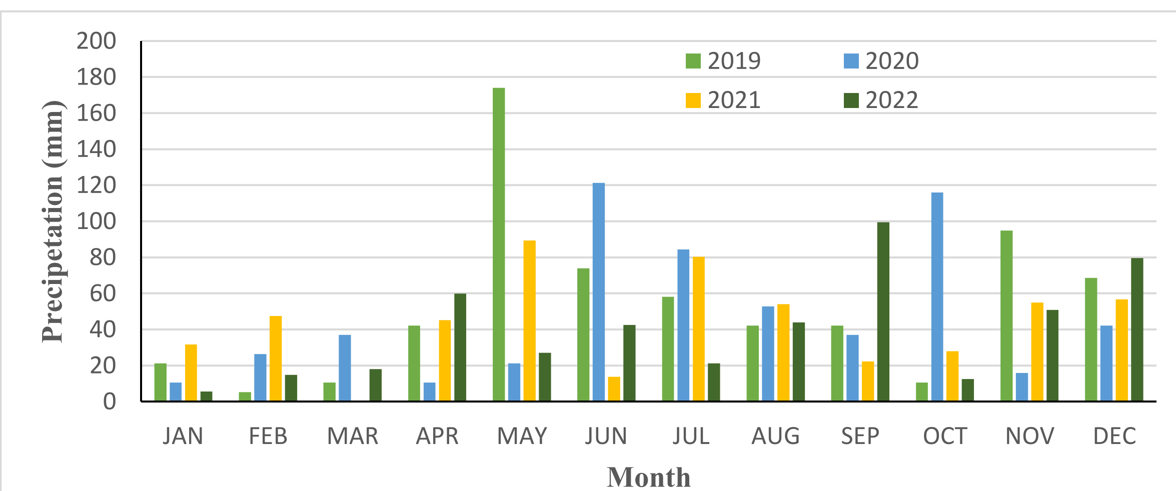
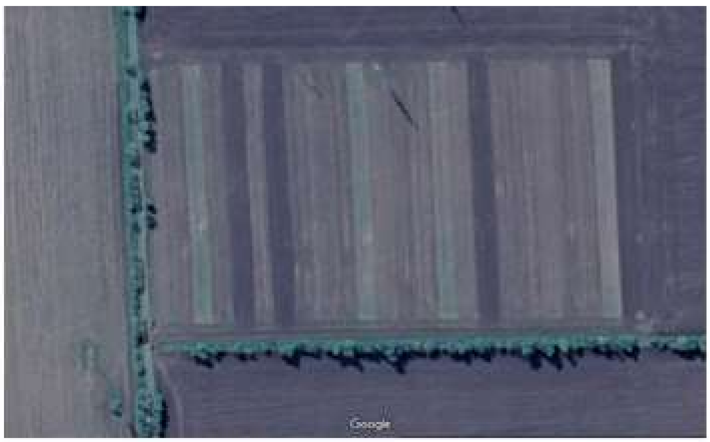


Figure 6. Monthly precipitation data (2019- 2022). Sourced from NASA

Experimental design

The current experiment was conducted between 2019 and 2022 (Table 2). This long-term experiment follows a randomized block design with four replicates, consisting of six tillage treatments: mouldboard ploughing, deep and shallow tine cultivation, disk tillage, loosening, and no-till (Figure 7). Each plot measures 13 × 180 m, while the total area of all treatments is 5.5 hectares. For our research, we focused on only three treatments that represent increasing degree of soil disturbance: no-till (NT), shallow cultivation (SC, 20 cm), and ploughing (P, 30 cm).



1st Replicate						2nd Replicate						3rd Replicate						4th Replicate					
D	SC	NT	DC	P	L	P	SC	L	DC	NT	D	DC	L	NT	D	P	SC	L	D	DC	SC	NT	P

Figure 7. Józsefmajor Experimental and Training Farm's long-term experiment layout (disking (D), shallow cultivation (SC), no-till (NT), deep cultivation (DC), ploughing (P), and loosening(L). (Source: Dekemati et al., 2019)

Table 2. Outlines the types of crops considered during the study period, along with a comprehensive list of significant management activities associated with each crop.

	2019–2020 **Winter oat** ***Avena sativa***	**2020–2021** **Spring sunflower** ***Helianthus annuus***
Tillage	2 October 2019	20 April 2021
Seedbed preparation	3 October 2019	23 April 2021
Seeding	9 October 2019	23 April 2021
Variety	Mv Hópehely	Syngenta One Star
Seeding rate	175 kg ha^{-1}	56,000 seeds/ha^{-1}
Fertilizers	Top dressing, 60 kg ha^{-1} CAN, N:27 (20 February 2020)	120 kg/ha^{-1} CAN, N:27 (NH_4NO_3 + $CaMg(CO_3)_2$) 120 kg/ha^{-1} CAN N:27 (31 March 2021)
Crop protection	Sekator OD 0.15 L ha^{-1} (10 April 2020) Tango Star 1 L ha^{-1} (10 April 2020) Decis Mega 0.15 L ha^{-1} (16 May 2020)	Fozát 480, 6 l/ha^{-1}, (2021 March 31) Pulsar 40SL, 1.2 l/ha^{-1} + Silvet Star 0.1 l/ha^{-1} (2021 June 01) Pictor 0.5 l/ha^{-1}, (25 June 2021)
Harvesting	15 July 2020	16 September 2021
Growing period (day)	279	145

During the period of crop harvesting, the remnants of the crop were chopped and distributed in one pass, after which the soil was left undisturbed until the primary process of soil preparation, with the intention of preserving the moisture content in the soil. To maintain uniformity across the various treatments, fertilizers such as nitrogen (in two doses totalling 100 kg N ha^{-1}), phosphorus (in the form of P_2O_5, 100 kg ha^{-1}), and potassium (in the form of K_2O, 50 kg ha^{-1}) were applied.

3.2 Methodological framework of the study

The methodological framework of this study is designed to systematically investigate soil ecology, employing a structured approach to enhance earthworm populations and analyse soil properties. It integrates experimental design with practical fieldwork and precise laboratory techniques to provide a comprehensive understanding of soil dynamics and ecological interactions.

3.2.1 Soil properties analyses

Soil sampling

The soil sampling was conducted at JETF to determine the physical, chemical, and biological parameters for the three soil tillage treatments (NT, SC, and P), three samples were collected from each plot in four replicates (n=12). Soil samples were randomly collected using a hand trowel across the field, as well as monthly and after harvest from the fence and control areas. The soil profile at the study site, shown in Figure 8, illustrates the distinct horizons and soil structure where sampling was conducted.

Figure 8. Soil profile at the Józsefmajor (JETF), showing the soil horizons present at the study site (Source: Michéli, 2024)

Soil physical analyses

Using an Eijkelkamp undisturbed soil sampler, **soil bulk density** samples were randomly obtained from each of the three treatments (NT, SC, and P) in four repetitions, ranging in depth from 0 to 40 cm at intervals of 10 cm. The bulk density was computed by dividing the soil sample's weight (in grams) in the cylinder after it had been oven-dried to 105 °C by the cylinder's volume (100 cm^3).

The **soil moisture content** was assessed from the bulk density samples through the utilization of the gravimetric moisture determination method at a temperature of 105°C for a duration of 24 hours, as outlined by Buzás (1993). This procedure was replicated four times to ensure accuracy. The moisture content was then determined by subtracting the weight of the soil sample that had been subjected to oven-drying (expressed in grams) from the weight of the wet soil (also expressed in grams). Subsequently, this value was divided by the weight of the oven-dried soil (expressed in grams) and multiplied by 100 (Buzás, 1993).

Soil chemical analyses

In September 2020, composite soil samples of at least 9-10 random subsamples were collected from the soil (0-10, 10-20, 20-30 and 30-40cm) under the three studied treatments (NT, SC, and P) for chemical analysis. Using a digital pH meter (HACH-LANGE, HQ411D) (Hach Lange GmbH, Vesenaz, Switzerland), the **pH (KCl)** of the samples was measured potentiometrically by using a 1:2.5 soil to 1M KCl ratio (Buzás, 1988). The **electrical conductivity (EC)** was assessed by measuring the electrical resistance of a 1:5 soil-water suspension using a conductivity cell (Figure 8). For the analysis of **soil organic carbon** (SOC), the soil samples were ground and passed through a 0.2 mm mesh, after which 0.200-0.2020 g of soil was measured. The content of soil organic carbon (%) (SOC) was determined through wet oxidation with a mixture of 5% $K_2Cr_2O_7$ + cc. H_2SO_4 at a ratio of 1:2 (Figure 9). The colour of the mixture was measured by a UNICAM Photometer (UV2 043506) (UNICAM, Montreal, Canada) (Ellert *et al*., 1995).

Figure 9. Measurement of EC and SOC (Photo by Hanaa Tharwat)

The **SOC stock** values (t ha^{-1}) were calculated by multiplying the bulk density (expressed in kg m^{-3}) with the relevant 10 cm layer (0.1 m) soil slice of a one-hectare (10,000 m^2) area in order to obtain the weight of the soil slice. Subsequently, the SOC stock value was calculated (in tonnes per hectare) by considering the percentage of the SOC content of the 0.1 m deep, one-hectare soil slice (Ellert *et al*., 1995).

Soil biological analyses

Soil microbial respiration (SMR): with a few minor modifications, the analysis of SMR complied with ISO 16072:2002(E) and Cheng *et al*. (2013) guidelines. To modify the moisture level, 10 ml of deionized water was added to 50 g of new soil in an airtight container. The samples were incubated for 10 days in the dark at room temperature (22°C) with a conical holding 10 ml of 1.0 M NaOH (Figure 10). After 10 days, the conical was removed, and the trapped CO_2 was precipitated by adding 1 ml of $BaCl_2$ to the NaOH solution. Phenolphthalein was added in two or three drops; it made the solution pink. Then, until the solution became colourless, it was titrated against 0.5M HCl. The determination was conducted in triplicates. Additionally, controls (triplicate flasks without soil) were made.

Figure 10. Soil samples in incubation jar (Photo by Hanaa Tharwat)

Earthworm extraction was carried out from the big field (Figure 11) using the manual sorting technique indicated in ISO 23611-1 (2006) to determine the abundance, biomass and species under the three different treatments. Using a spade, 25×25×25 cm soil blocks were collected from each treatment (NT, SC and P) in four replicates. The sampling locations within the treatments were selected randomly. The excavated soil was laid out on a plastic sheet, and earthworms were carefully looked for. The earthworms were then put in plastic bottles holding 70% ethanol. Later in the laboratory, the earthworms were washed with tap water to remove the remaining soil particles from their bodies before being transferred to 4% formalin for fixation and then stored in 70% ethanol for species identification. First, the number of earthworms was counted and represented as individuals per sample to determine the overall abundance of earthworms. Second, to calculate the average number of earthworms per square meter (ind m^{-2}), the number of earthworms in each sample was multiplied by a factor of 16. Another estimate was made of the total biomass (g m^{-2}) in the same way. The earthworm species were determined according to Csuzdi and Zicsi (2003).

Figure 11. Earthworm sampling by hand-sorting technique from the study area (Photo by Dr. Barbara Simon)

3.2.2 Earthworm enhancement

Preparation of the earthworm enhancement fences

As an integral part of the experimental setup, small fences (2.5×3m area) (Figure 12) were designed and implemented following the sunflower seeding within the three selected tillage plots (NT, SC, P) in 2021. The goal was to study the effects of earthworms on crop productivity and soil quality, repeating a previous experiment carried out by Dr. Pia Euteneuer from the University of Natural Resources and Life Sciences (Vienna, Austria). By using the same methodology, this study ensures consistency and reliability in the results, allowing for direct comparisons between the findings of this study and Dr. Euteneuer's research. One specific earthworm species (*Lumbricus terrestris*) was introduced within these fenced areas, accompanied by the distribution of straw as an organic amendment. *Lumbricus terrestris* was selected for the earthworm fence (EWF) test due to its specific ecological traits that make it highly beneficial for soil health and plant growth. These species are characterized by their deep burrowing behaviour, which results in the creation of permanent vertical burrows. These burrows enhance the movement of water through the soil, improving water infiltration, aeration, and root penetration, which can significantly contribute to improved sunflower growth. The ability of *Lumbricus terrestris* to create these structures promotes better soil structure by facilitating the exchange of gases and water within the soil profile. Furthermore, anecic earthworms contribute to nutrient cycling by transporting organic materials from the surface into deeper soil layers. This process not only helps in breaking down organic matter but also makes nutrients more accessible to plants, promoting enhanced soil fertility. The continuous cycling of nutrients supports the overall health of the soil and increases its capacity to support plant growth, making it particularly beneficial for crops like sunflowers, which require sufficient nutrient availability for optimal development.

A control area of the same size (2.5×3m) was also designated in each treatment with straw on the top, thus one *earthworm fenced area* (EWF) plus one *control area* (CTL) per treatment, all together 24 small areas to consider. The fence's placement in the research area was identified for the three soil tillage treatments (NT, SC, and P), and the corners were marked with stakes. The distance between each fence post was measured and marked, ensuring that the spacing was uniform. The holes for the fence poles were dug 50 cm deep using a post hole digger, the fence poles were installed in the holes, a level was used to ensure their straightness (10 poles 50 cm in length), and the holes were filled with soil and packed securely around the posts. The fence rails were attached to the fence posts using screws, and the fence poles were hammered into the ground 20 cm into the soil and 20 cm above the ground, and any space around the fence poles was filled with soil and compacted securely. The frame was covered with 50-cm-wide, 280 g m^{-2} heavy-duty polyethylene, and the foil was turned over the frame and squeezed between two 50-cm poles to improve wind resistance and prevent worms from escaping. Shorter poles were then screwed against the inner poles to prevent the foil from rupturing during a storm. The fence posts and panels have been surrounded with damp earth to help settle the soil.

Figure 12. The process of constructing the fence in the study area (Photo by Dr. Barbara Simon)

Three rows of sunflowers could fit in one fence. *Lumbricus terrestris* earthworm species was ordered from Canada. These earthworms were carefully packaged and delivered in a labelled box (Figure 13). The earthworms were distributed equally on the soil surface within the fenced area (about 100 earthworm individuals per fence), during late afternoon after sunset. To ensure both the earthworms and the fence well-being, meticulous planning, cutting, digging, and monitoring are required during both the construction of the fence and the addition of earthworms to the soil.

Figure 13. Labelled box containing Lumbricus terrestris earthworms
(Photo by Dr. Barbara Simon)

The chopped straw (10-15cm), which was created from the leftover stalk after wheat grains were harvested, was added to the soil to feed earthworms. The addition of straw to soil can provide numerous benefits that support earthworm populations, including increased organic matter, improved soil structure, enhanced microbial activity, and moisture retention (Chu *et al*., 2022). A thin layer of straw (1.25 kg/7.5m^2, 10-15 cm length) was spread (Figure 14) over the soil in the fenced area to cover the earthworms and help them acclimate to their new environment, as well as in the control area.

Figure 14. Spread straw in the fence (front) and control area (back)

(Photo by Dr. Barbara Simon)

Measurement of plant parameters

The sunflower parameter (plant height (cm)), and soil moisture content (%) were measured on six occasions (24 June; 8 and 22 July; 4 and 26 August and 8 September 2021) during the vegetation period. The diameter of sunflower heads (cm), the weight of heads (g) and stems (g) were measured after harvest (8 September 2021). The carbon and nitrogen content of the sunflower seeds and stems were also measured with the Carbon-Nitrogen analyser by dry combustion method (CNHS elemental analyser). The thousand kernel weight (g) was also measured with an equipment seed counter at the Department of Crop Production.

Sunflower plants were counted at the seedling establishment stage in each plot (2.5×3 m) for only EWF and CTL areas under the three tillage treatments (NT, SC, P). The recorded plant numbers were used to determine treatment effects. The mean and standard deviation of sunflower counts were calculated for each treatment.

3.3. Statistical analyses

Statistical analyses for the data originating from the big field data

All statistical analyses and the determination of the statistical significance of the differences between the treatments were conducted using the R (4.2.2) Statistical Program (R Core Team 2021). To determine if there were any significant changes, an ANOVA was used, and Q-Q plots were employed to test for normalcy beforehand. Tukey's post-hoc HSD test was used for multiple comparisons of the treatment means.

Statistical analyses for the data originating from earthworm enhancement data

Linear models were used in R to identify and compare the control effects of treatments. At $p < 0.01$ statistical significance was found. Specifically, two-way ANOVA was conducted as a linear model to assess the main effects and interactions of tillage and earthworm treatments. Tukey's HSD test was applied for post hoc comparisons where significant effects were found.

4. RESULTS AND DISCUSSION

4.1 Effect of tillage on selected soil properties

In this study, we investigated the impact of three different tillage (no-till - NT, ploughing - P, and shallow cultivation - SC) on selected soil physical, chemical and biological parameters in a long-term soil tillage experiment in JETF.

4.1.1 Physical analysis

The **soil bulk density** values that were measured are shown in Figure 15. Comparing NT to P and SC, NT demonstrated a significant difference in the first layer (0–10 cm). There was a significant difference between NT (1.48 g cm^{-3}) and P (1.26 g cm^{-3}) and SC (1.22 g cm^{-3}). Only NT was greater than P for the following layers (10–20, 20–30, and 30–40 cm); however, P did not significantly differ from SC. The measurement of **soil moisture content** was also conducted as a background parameter using the bulk density samples, to determine whether there were any significant variations among the treatments in the four different layers. The distribution of moisture content was found to be uniform, except for higher moisture values observed in the case of the P treatment at a depth of 30–40 cm.

Soil bulk density findings indicate that tillage methods had a significant effect on the upper layer (0-10 cm) in the NT treatment (Figure 15). This resulted in the highest bulk density value (NT = 1.48 g cm^{-3}) when compared to the other two treatments (SC = 1.22; P = 1.26 g cm^{-3}). In the layers below, only NT showed a significant difference from the P treatment (NT > P). Gál *et al*. (2007) also discovered significantly higher bulk density values at depths of 0-30 cm for NT compared to P. In fact, they observed a 10% increase in bulk density between 0 and 5 cm, a 15% increase between 5 and 15 cm, and a 17% increase in bulk density values at depths of 15 and 30 cm under NT when compared to the P treatment. Moussadek *et al*. (2014) found greater bulk density values in Vertisol and Cambisol under NT compared to P, while in the case of Luvisol, they also found higher bulk density for NT, except for the top layer (0-5 cm).

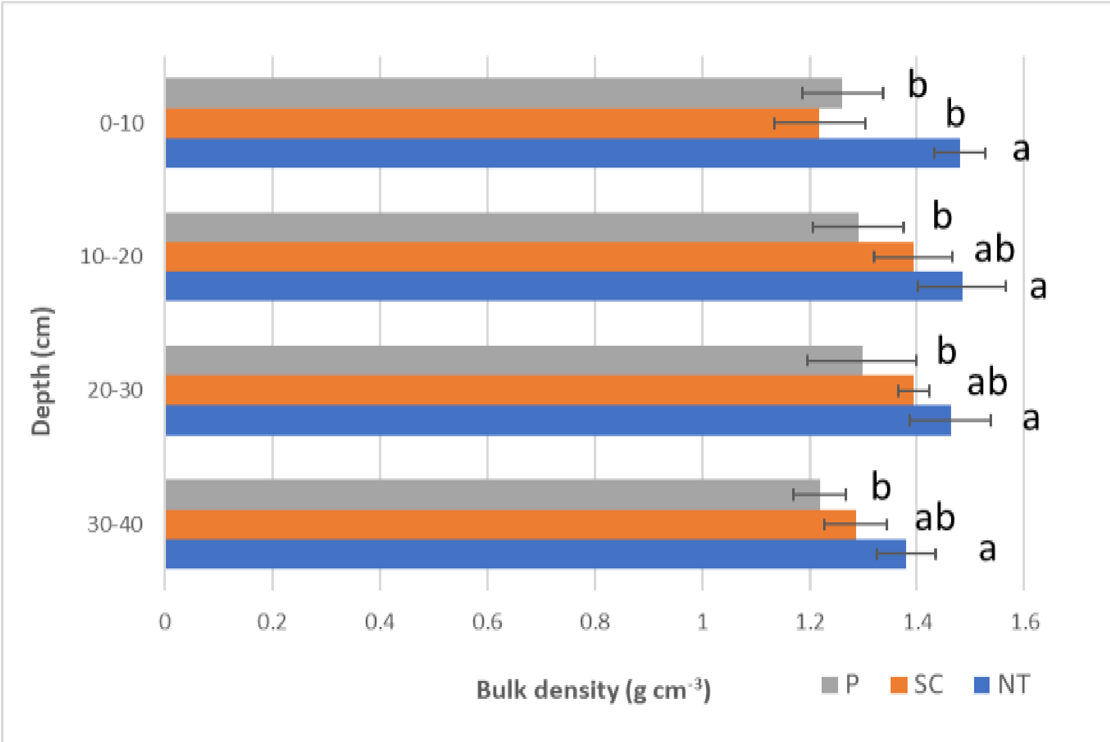


Figure 15. The soil bulk density values (Autumn, 2020) (P - ploughing, SC - shallow cultivation, NT - no till). The same letters beside the bars designate no statistical difference.

4.1.2 Chemical analyses

pH (KCl)

Figure 16 shows the values for the pH (KCl) of the soil. In P, SC, and NT, the pH (KCl) readings ranged from 5.1 to 5.3, 4.9 to 5.3, and 4.7 to 5.2. The top layer (0–10 cm), which had the greatest values for P (P > SC = NT), was the only layer where a significant difference was seen; the other layers did not show any significant differences. In the 0–10 cm layer, P had the highest pH (KCl) value at 5.2, followed by SC at 4.7 and NT at 4.9.

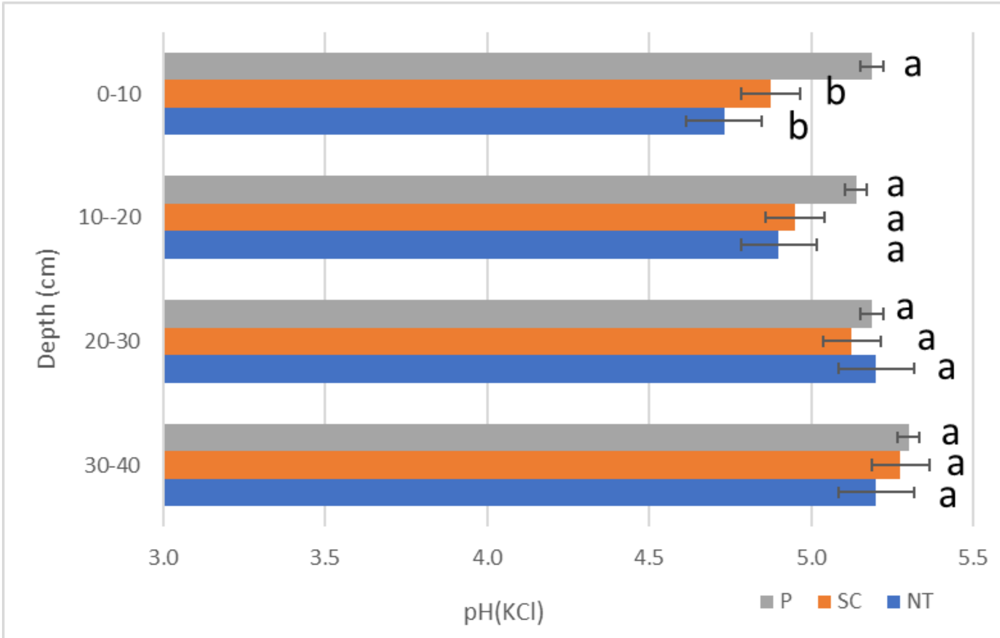


Figure 16. The soil pH (KCl) values (Autumn, 2020), (P - ploughing, SC - shallow cultivation, NT - no till). The same letters beside the bars designate no statistical difference.

Soil organic carbon

The values of SOC are displayed in Figure 17. These values ranged from 1.7% to 2.5% in NT, 1.6% to 2.4% in SC, and 2.0% to 2.1% in P. Notably, there were significant disparities observed in only two layers, specifically the top layer (0-10 cm) and the bottom layer (30-40 cm). The highest value was recorded in the case of NT (2.5%), followed by SC (2.4%), and finally the P treatment (2.0%) in the top layer. In the lowest examined layer, the P treatment (2.0%) exhibited a significantly higher value compared to the other two treatments (NT = 1.7%; SC = 1.6%). The measurements we conducted revealed significant variations in soil organic carbon (SOC) values among the three tillage treatments in both the top layer (0–10 cm) (NT > SC > P) and the lowest examined layer (30–40 cm) (P > NT = SC) (Figure 16). The NT and SC treatments exhibited a gradual decrease in SOC values with increasing depth, whereas the P treatment displayed a relatively uniform distribution of SOC throughout the examined depths (Figure 16). These findings are consistent with the results reported by Gál *et al*. (2007), West and Post (2002), Luo *et al.* (2010), Blanco-Canqui and Lal. (2008), who observed a gradual decline in SOC in their tillage experiment that spanned 28 years in the NT treatment (0–5 cm: 3.5; 5–15 cm: 2.6; 15–30 cm: 2.3%; 30–50 cm: 1.1%). Conversely, the SOC distribution in the P treatment within the 0–30 cm depth range exhibited a high degree of homogeneity (0–5 cm: 2.39; 5–15 cm: 2.41; and 15–30 cm: 2.45%; 30–50 cm: 1.5%).

This can be attributed to the thorough mixing and turning effect of the P tillage in the topsoil, which leads to increased microbial activity and subsequent decomposition of soil organic matter (Gál *et al*., 2007; Karlen *et al*., 1994; Drijber *et al*., 2000).

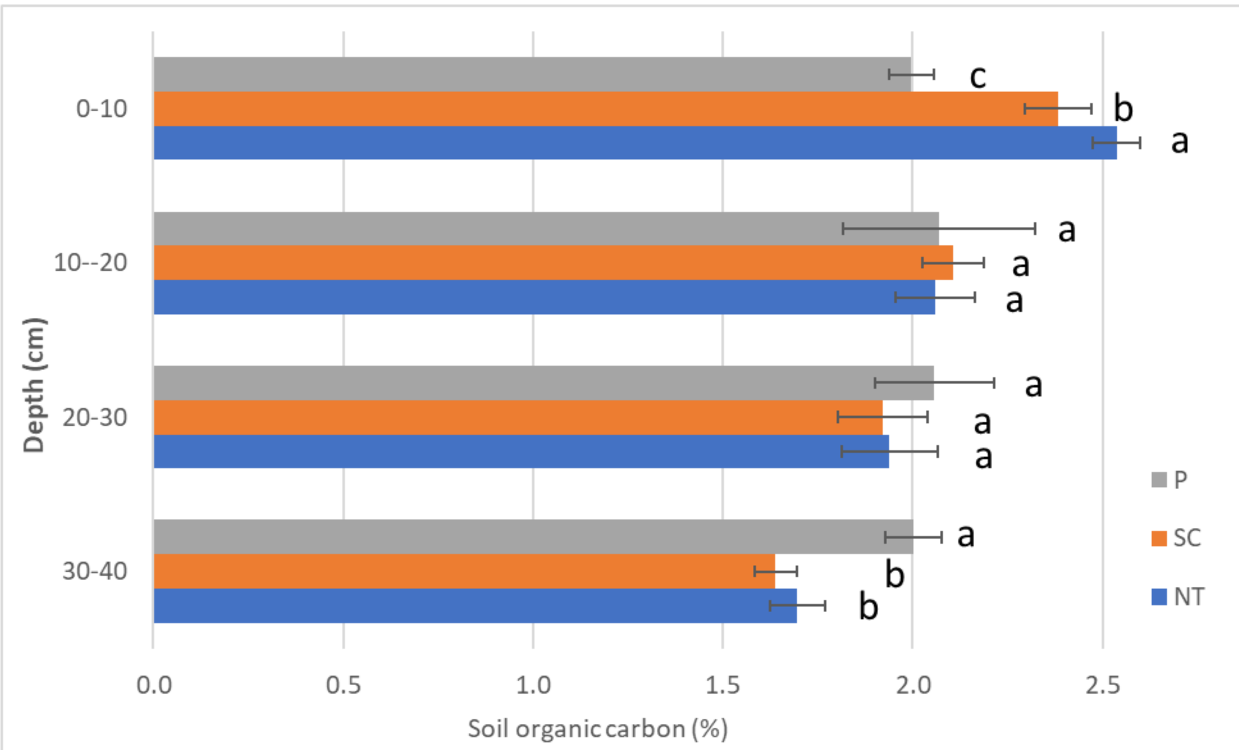


Figure 17. The soil organic carbon values (Autumn, 2020). (P – ploughing, SC – shallow cultivation, NT – no-till). The same letters beside the bars designate no statistical difference.

The SOC values observed on soil samples collected from the long-term tillage experiment conducted at Józsefmajor in 2015 exhibited slightly different patterns in the P treatment (Dekemati *et al*., 2019). The SOC values showed a gradual decrease with increasing depth: 1.8% (0–10 cm), 1.7% (10–20 cm), 1.6% (20–30 cm), and 1.5% (30–40 cm). In the NT treatment, the SOC values displayed a more pronounced decrease with depth: 2.3%, 1.8%, 1.6%, and 1.4%, respectively. Similarly, in the SC treatment, the SOC values showed the following trend: 2.06%, 2.03%, 1.8%, and 1.4% at depths of 0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm, respectively. In comparison, (Ernst and Emmerling, 2009) reported lower SOC values in an Eutric Cambisol with silt loam (topsoil) and clay loam (subsoil) in their experimental site in Welschbillig, Southern Eifel, Germany. For the P treatment at a depth of 25 cm, they found SOC values of 1.56%, 1.52%, and 0.87% at depths of 0–10 cm, 10–20 cm, and 20–30 cm, respectively. For the cultivation treatment at a depth of 15 cm, they found SOC values of 1.79%, 1.21%, and 0.75%. Furthermore, for the NT treatment, they obtained SOC values of 1.75%, 1.14%, and 0.66% after ten years of tillage operation. Their experiment also showed a decreasing trend in SOC values.

Soil organic carbon stock

The stock values of SOC are displayed in Figure 18. In the NT treatment, the values ranged from 23.4 to 37.6 t ha^{-1}, while in the SC treatment, they ranged from 21.1 to 29.3 t ha^{-1}. In the P treatment, the values ranged from 24.4 to 26.7 t ha^{-1}. A significant difference was observed in the upper layer (0–10 cm), with the highest values seen in NT (37.6 t ha^{-1}), followed by SC (29.0 t ha^{-1}), and then P (25.2 t ha^{-1}). The two middle layers (10–20, 20–30 cm) did not exhibit any significant difference, whereas in the lower layer (30–40 cm), the order was P > NT > SC.

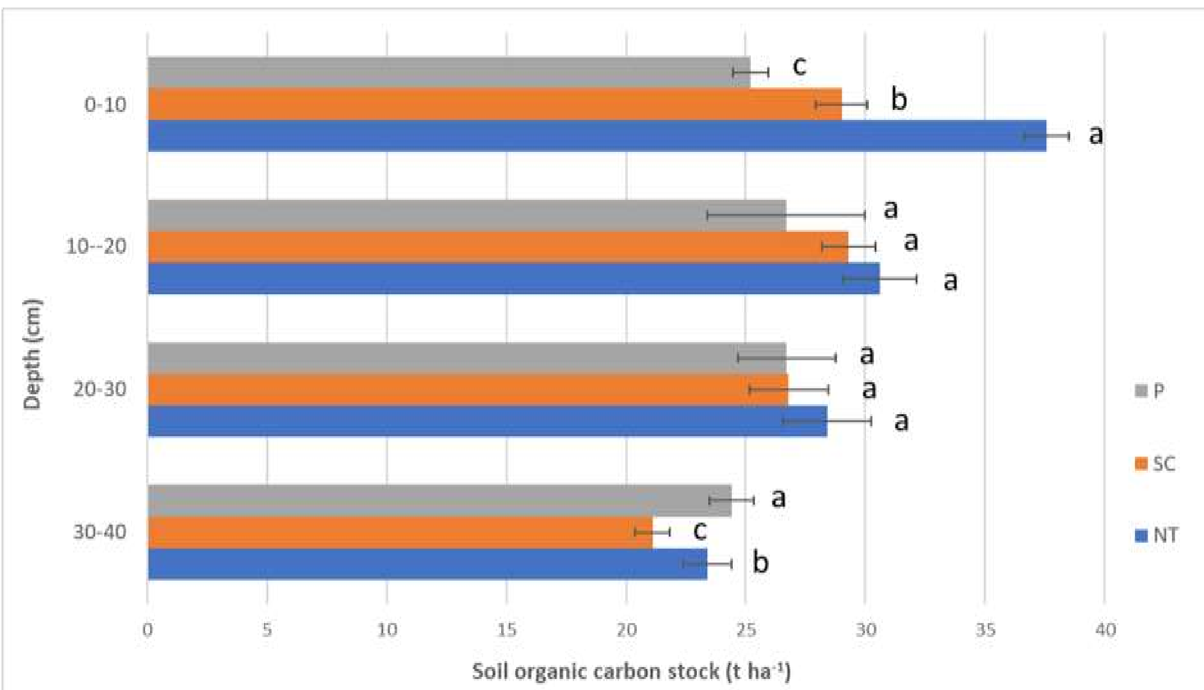


Figure 18. The soil organic carbon stock values (Autumn, 2020). (P – ploughing, SC – shallow cultivation, NT – no-till). The same letters beside the bars designate no statistical difference.

Regarding the SOC stock, we discovered that under NT, the values were only significantly higher in the top layer (10 cm) (37.6 t ha^{-1}) when compared to SC (29.0 t ha^{-1}) and P (25.2 t ha^{-1}) (Figure 16); all SOC stock values in the lower layers were lower than the SOC stock in the top layer, though there were no significant differences between them. Similar values to our data were observed at a depth of 5–15 cm under NT (36.4 t ha^{-1}) and P (27.9 t ha^{-1}) in a poorly drained Chalmers silty clay loam soil (Typic Haplaquoll), according to Gál *et al*. (2007). A study in Southern Russia, which investigated tillage practices in Chernozem soils, highlighted that conservation tillage practices (including NT) help in the accumulation of SOC in the top 0–30 cm layer compared to conventional ploughing (Kostyukevich *et al.,* 2021). A long-term study on Chernozem soils in Ukraine showed that under reduced tillage, including NT, there was an increase in SOC in the 0–10 cm layer, with similar trends observed in the 10–20 cm layer. This study suggests that NT is beneficial for maintaining SOC stock, particularly in the topsoil layers (Bondarenko *et al*., 2019). Interestingly, the study conducted by Ernst and Emmerling in 2009 discovered that the highest values of soil organic carbon (SOC) stock were observed in the case of P, NT, and cultivation within the 0-30 cm depth range. However, there was no significant difference between these three treatments. Similarly, Moussadek *et al*. (2014) observed comparable trends in SOC stock when comparing three different soil types (Vertisol, Cambisol, and Luvisol) under NT and P treatments. The authors found significant differences in SOC stock for Vertisol and Cambisol. Additionally, research by Biernat *et al*. (2017) found that no-tillage (NT) practices on Chernozem soils enhanced SOC storage compared to conventional tillage practices, particularly in the surface layers. Their study concluded that the reduction in tillage intensity led to an increase in SOC accumulation. NT treatment resulted in the highest total SOC stock values at the 0-30 cm depth range, with Vertisol having 31.89 Mg ha^{-1} and Cambisol NT having 30.76 Mg ha^{-1}, compared to P treatment with 28.79 Mg ha^{-1} and 28.49 Mg ha^{-1}, respectively. The SOC stock values for the NT (0–30 cm) sites had an average of 29.35 Mg ha^{-1}, and this value was significantly different from the value for P (27.35 Mg ha^{-1}). However, it is crucial to consider that these measurements were taken in a Mediterranean climate with an annual precipitation of 450 mm in Merchouch Plateau, Morocco. In this region, the measured SOC values (Vertisol:1.22; Cambisol: 1.17; Luvisol: 0.7% at 0–15 cm depth) were also lower compared to our sites. Other researchers have also reported lower SOC stock values compared to ours. For instance, in a study conducted by Pinheiro *et al*., (2015), significantly greater SOC stock values were found in 1998 under NT (19.7 Mg ha^{-1} or 21.7 t ha^{-1}) compared to conventional tillage (disk ploughing + light disk harrowing) (16.6 Mg ha^{-1} or 18.3 t ha^{-1}) at 0–10 cm depth in tropical Dystrophic Red Latosol (Typic Haplortox) in Rio de Janeiro State, Brazil. The lower SOC stock values in their study can be attributed to the different tropical climate with higher average temperature (21 ◦C) and higher average precipitation (1200 mm) (Jakab *et al*., 2023). On the contrary, Jakab *et al*., 2023 found significantly lower SOC stock values for NT (0–10 cm: 2.89; 30–40 cm: 2.35 t ha^{-1}) and for P (0–10 cm: 2.31; 30–40 cm: 1.91 t ha^{-1}) in the same long-term tillage experiment in Józsefmajor, Hungary. This difference could be attributed to variations in bulk density values measured at different random locations and times of the year. Our measurements were conducted in September 2020, before the autumn tillage operations, while their measurements were completed in June 2019 in the stubble after harvest (Jakab *et al*., 2023). The large size of the plots in Józsefmajor (13 × 180 m) could lead to significant differences in bulk density due to the high heterogeneity of the soil. The correlation between the SOC content (Figure 17) and SOC stock values (Figure 18) is quite similar; however, there is a significant difference in the top layer (0–10 cm) among the treatments in our study. This can be attributed to the fact that SOC stock is determined by considering both the bulk density and the SOC content, thereby magnifying the differences among the tillage treatments. In their study, Gál *et al*. (2007) extended the calculation of SOC stock to a depth of 100 cm. They observed greater statistical variations between NT and P when they expressed SOC stock in terms of mass (t ha^{-1}) rather than concentrations (SOC%). Hence, they recommend the measurement of bulk density alongside SOC% for enhanced accuracy and precision.

4.1.3 Biological analysis

Soil microbial respiration

The soil microbial respiration values are presented in Figure 19. It was observed that the values were significantly greater in NT (22.8 mg CO_2 /50 g/10 day) compared to P treatment (10.03 mg CO_2 /50 g/10 day), whereas the SC treatment (19.25 mg CO_2 /50 g/10 day) exhibited significantly greater values than the P treatment (NT = SC > P).

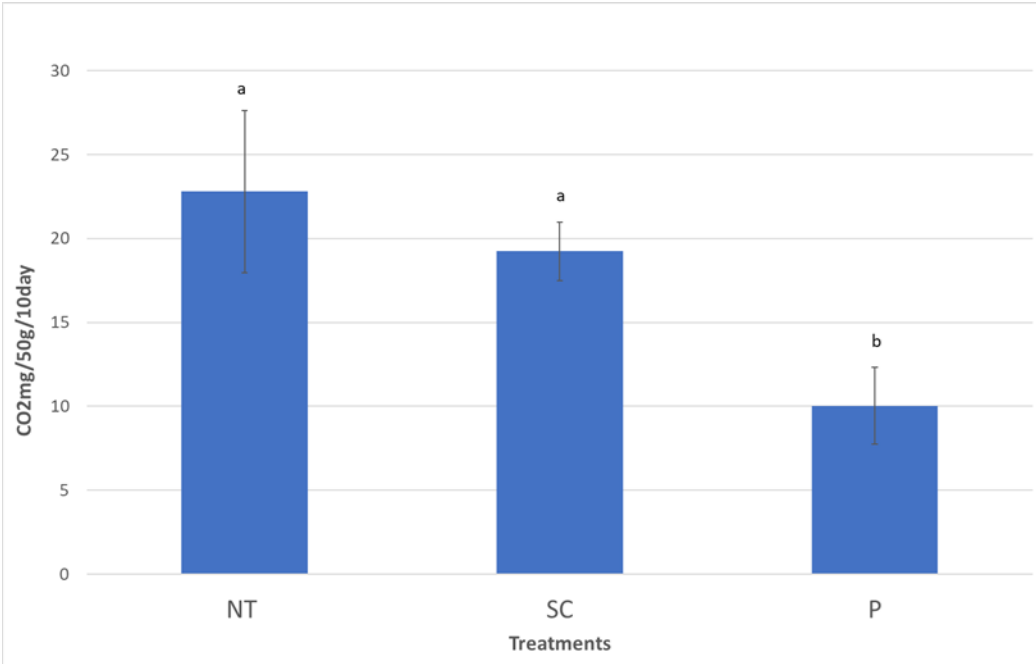


Figure 19. The soil microbial respiration values (Autumn, 2020). (P – ploughing, SC – shallow cultivation, NT – no till). The same letters beside the bars designate no statistical difference.

Soil microbial respiration was utilized to assess microbial activity in the soils of the three treatments. Our results are consistent with the literature, which states that soil microbial respiration is often greater in NT compared to reduced tillage (SC in our instance) or P (Jha *et al*., 2022; Mirzavand *et al*., 2020). A previous investigation at the same location yielded similar results for in situ soil respiration measurements (Gelybó *et al*., 2022). In this investigation, both autotrophs and heterotrophs contributed to the observed respiration. Future research could enhance our understanding of the long-term impact of tillage by investigating microbial biomass, microbial diversity, and the allocation between autotrophic and heterotrophic respiration. For instance, a study conducted by Du *et al*. (2020) revealed that soil autotrophic respiration is reduced in P compared to NT, whereas heterotrophic respiration is higher in P. However, this relationship is influenced by changes in rainfall and soil moisture. These environmental factors, along with the varying response of soil microbes to temperature and moisture changes, impact greenhouse gas emissions. Nonetheless, soil microbial respiration serves as a valuable indicator of overall microbial activity.

Earthworm abundance, biomass, species

The earthworm abundance values on the big field under the three treatments can be found in Figure 20 A. The earthworm abundance values were 189.3 in NT; 125.3 in SC; and 48 ind m^{-2} in the P treatment. Significantly greater earthworm abundance values were found in NT compared to P. The earthworm biomass values can be seen in Figure 20 B. The biomass values were 41.26 in NT; 36.95 in SC, while the value was 7.4 g m^{-2} in the P treatment. Significantly greater biomass values were found in the case of NT and SC compared to the P treatment. As for the composition of earthworm species, three species were found in the case of NT (*Aporrectodea rosea, Aporrectodea georgii, Aporrectodea caliginosa*), while two species were found in SC (*Aporrectodea rosea, Aporrectodea caliginosa*) and only one species was found in P (*Aporrectodea rosea)*. All species belong to the endogeic morphotype. *Aporrectodea rosea* endogeic species was found in all treatments. The average winter oat yield was the greatest in the case of SC (8.11 t ha^{-1}), followed by NT (7.82 t ha^{-1}) and then P (6.82 t ha^{-1}).

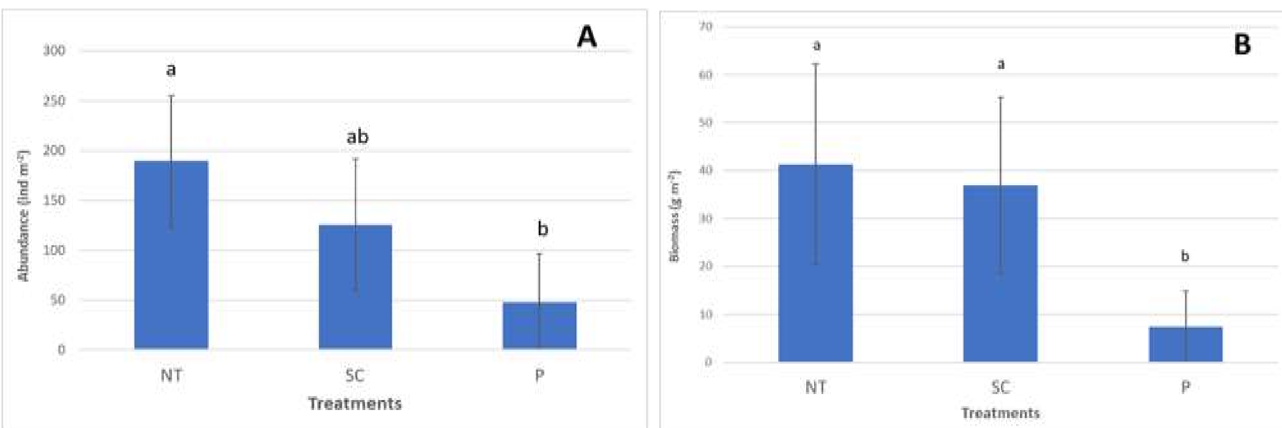


Figure 20. (A) The earthworm abundance values (Autumn, 2020). (B) The earthworm biomass values (Autumn, 2020). (P — ploughing, SC — shallow cultivation, NT — no-till). The same letters above the bars designate no statistical difference.

Significantly higher earthworm abundance was achieved in our study under the NT treatment (189.33 ind m^{-2}) and the SC treatment (125.33 ind m^{-2}) compared to the P treatment (48.1 ind m^{-2}) (Figure 20 A). In the same tillage experiment in Józsefmajor, similar trends but lower values were discovered in 2016 and 2017 (Dekemati *et al.*, 2019). In September 2016, a significantly greater earthworm abundance was found in the NT treatment (117.3 ind m^{-2}) compared to the SC treatment (37.3 ind m^{-2}) and the P treatment (21.3 ind m^{-2}). In September 2017, the highest earthworm abundance was observed in the NT treatment (90.67 ind m^{-2}), followed by the SC treatment (74.67

ind m^{-2}), and then the P treatment (42.67 ind m^{-2}) without any significant difference. Ernst and Emmerling (2009) discovered that the earthworm abundance tended to be highest in NT (157.3 ind m^{-2}), P (119.3 ind m^{-2}), and then cultivation (113.13 ind m^{-2}). However, in terms of earthworm biomass, cultivation yielded the highest value (109.8 g m^{-2}), followed by NT (103.7 g m^{-2}), and P (66.7 g m^{-2}), with no significant differences identified.

In an irrigated cropping management system (spring crops plus legumes) in Southeast France, Peigné *et al*. (2009) found statistically greater earthworm abundance and biomass in NT compared to P; however, in their other two sites (Central France cropping system, legumes; Western France cropping system), only the earthworm biomass values were significantly greater in NT compared to P. Only three species of endogeic morphotype earthworms were detected in total during our investigation in September 2020; the majority of these species were juveniles (NT: 71.8; SC: 78.7; P: 94.4%). Nine species with varying ratios among them that fall under the three morphotypes (epigeic, endogeic, and anecic) were discovered by Ernst and Emmerling (2009). In the case of P, only six species were identified, with a significantly higher number of endogeic (26.7 ind m^{-2}) in comparison to NT (nine species, with only 2.7 ind m^{-2} classified as endogeic). On the other hand, cultivation exhibited an intermediate status (eight species, primarily anecic: 25.3 ind m^{-2}), which was considerably higher than the abundance of anecic species in P. In the study conducted by Wyss *et al*. (1992), it was observed that the occurrence of ploughing operations resulted in a decrease in bulk density and an increased transportation of organic matter into deeper soil layers. Consequently, this led to an increase in the abundance of endogeic earthworms. Additionally, ploughing also enhanced the accessibility of soil organic matter in the root zone, which is particularly beneficial for these small-sized endogeic earthworms as it reduces the risk of mechanical damage caused by tillage operations. Furthermore, Ivask *et al*. (2007) found that certain endogeic earthworm species such as *A. rosea* and *A. caliginosa* exhibit a considerable tolerance towards mechanical soil tillage disturbances. The potential explanation for the absence of anecic species during the examination period of Autumn 2020 could be attributed to the utilization of the hand-sorting method for earthworm extraction. The anecic species are known to construct deep and permanent burrows, thereby presenting greater challenges in terms of sampling. Consequently, it is recommended to employ formalin or mustard solution extraction methods, as exemplified by previous studies conducted by Pinheiro *et al*. (2015), Wyss *et al*. (1992), Eisenhauer *et al.* (2008), and Gutiérrez-López *et al*. (2016).

The low pH levels of the soil may be the cause of the comparatively low number of earthworm species found in our investigation. In our instance, the pH (KCl) value in NT was 4.7 (0–10 cm), rising to 5.2 (30–40 cm) with depth; in SC, the values ranged from 4.9 (0–10 cm) to 5.3 (30–40 cm); P, on the other hand, provided somewhat higher values, ranging from 5.2 (0–10 cm) to 5.3 (30–40 cm) (Figure 15). Certain earthworm species have a pH range of 5.0 to 6.0, according to Edwards and Lofty's (1975) research at the Rothamsted Park Grass Plots; nevertheless, their populations decline below or above these pH ranges. *Aporrectodea nocturna*, *Aporrectodea rosea*, and *Aporrectodea caliginosa* are these species. Two of these species, *A. caliginosa* and *A. rosea*, are particularly prevalent on arable fields and are used in a variety of land applications. These acidic pH levels are a particularly good fit for these species. This research suggests that grain yield is impacted by tillage practices. Apart from tillage, Hungary's climatic anomalies are distinctive and important for agricultural productivity. This study suggests that tillage methods influence grain yield. However, aside from tillage, climate anomalies in Hungary play a significant and dominant role in crop production. According to Bogunovic and Kisic (2013), summer precipitation in the Moslavina region of Central Croatia noted that summer precipitation decreases while its distribution becomes more erratic. The highest grain yield observed in SC may be attributed to reduced soil compaction (evidenced by the lowest bulk density) and improved soil moisture retention. Among the three treatments, P produced the lowest yield. In contrast, NT resulted in a slightly higher yield than P, likely due to the increased mulch, which improved soil biological activity and moisture availability. Kuhn *et al*. emphasized the benefits of NT during years with average or below-average rainfall, reporting yield increases of up to 20%.

4.2 Effects of earthworm enhancement on plant traits

4.2.1 Number of sunflower plants

P had the highest sunflower count across both EWF and CTL, suggesting better establishment under this tillage. SC had the lowest variation, with consistent sunflower counts. NT showed moderate sunflower counts with some variation (Table 3). In NT, EWF had slightly higher sunflower counts compared to CTL. The presence of earthworms did not show a clear advantage as both EWF and CTL had similar sunflower numbers in P. While SC sunflower counts were nearly identical in both EWF and CTL, indicating minimal impact of earthworms under SC.

Table 3. The sunflower counts across the NT, SC, and P treatments

Tillage treatment	Earthworm Fence (EWF)	Control (CTL)
NT	14.8	13.8
SC	13.3	14.0
P	22.0	22.3

4.2.2 Height of sunflower plants

The height of the sunflowers was measured six times (24 June; 8 and 22 July; 4 and 26 August, and 8 of September 2021) during the vegetation period (Figure 21). Sunflower plant heights increase over time from 24 June to 8 September. Both earthworm fence (EWF) and control (CTL) treatments show similar trends in plant height across all dates. NT shows lower plant heights compared to P and SC, especially noticeable in the initial stages (24 June and 8 July). P shows higher plant heights compared to NT and similar or slightly higher heights compared to SC, particularly in the later stages (from 22 July onwards). This could be attributed to the lower soil temperatures observed under NT. SC shows intermediate plant heights, usually slightly lower than P but higher than NT.

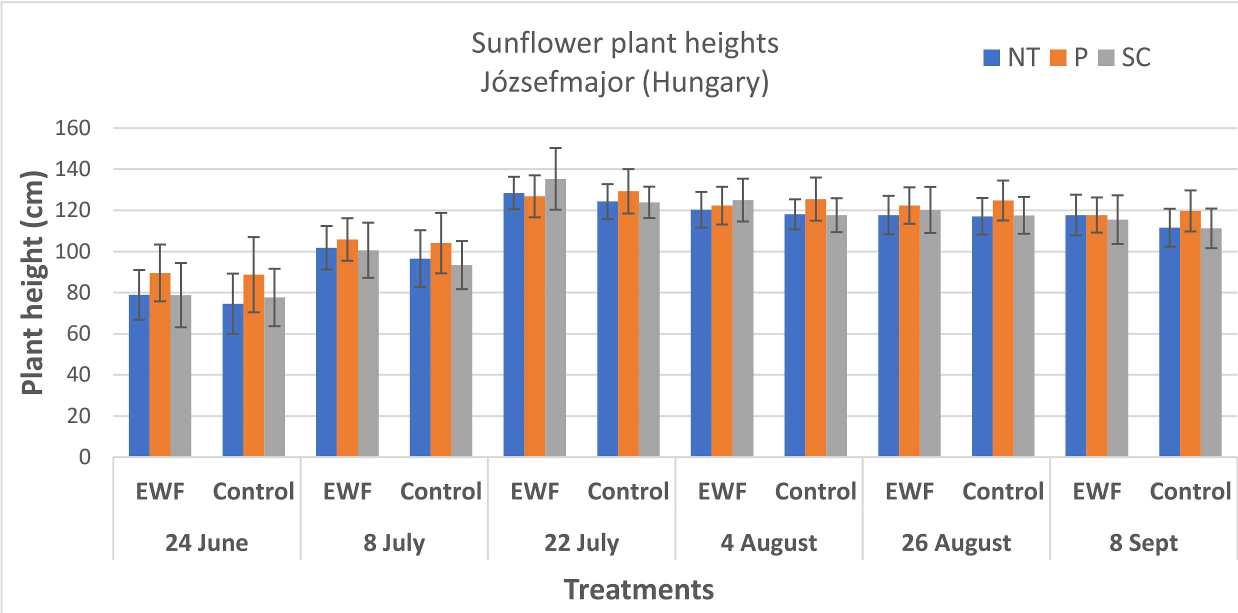


Figure 21. The height of sunflower plants during the vegetation period (2021)
(EWF – earthworm fence; NT – no-till; P – ploughing; SC – shallow cultivation)

The presence of an earthworm fence (EWF) shows a slight positive effect on plant height compared to the control (CTL), although the differences are not substantial. This indicates that while earthworms may have some beneficial effects, they are not the primary factor influencing plant height in this experiment. The presence of EWF shows a positive influence on plant height compared to CTL, especially in the initial stages (June 24, July 8). This suggests that EWF may contribute to better early growth conditions.

4.2.3 Head diameter of sunflowers

As Figure 22 shows, the earthworm-fenced area (EWF) in the no-tillage (NT) treatment, has a median head diameter below 20 cm, while the control area (CTL) has a median head diameter above 15 cm. In the ploughing (P) treatment the median head diameter in EWF is 15 cm and the CTL area has a median head diameter above 15 cm which is higher than the EWF. The EWF area in shallow cultivation (SC) treatment has a median head diameter below 20 cm which is higher than NT and P. The median head diameter in CTL area is 15 cm which is lower than the CTL area in NT and P.

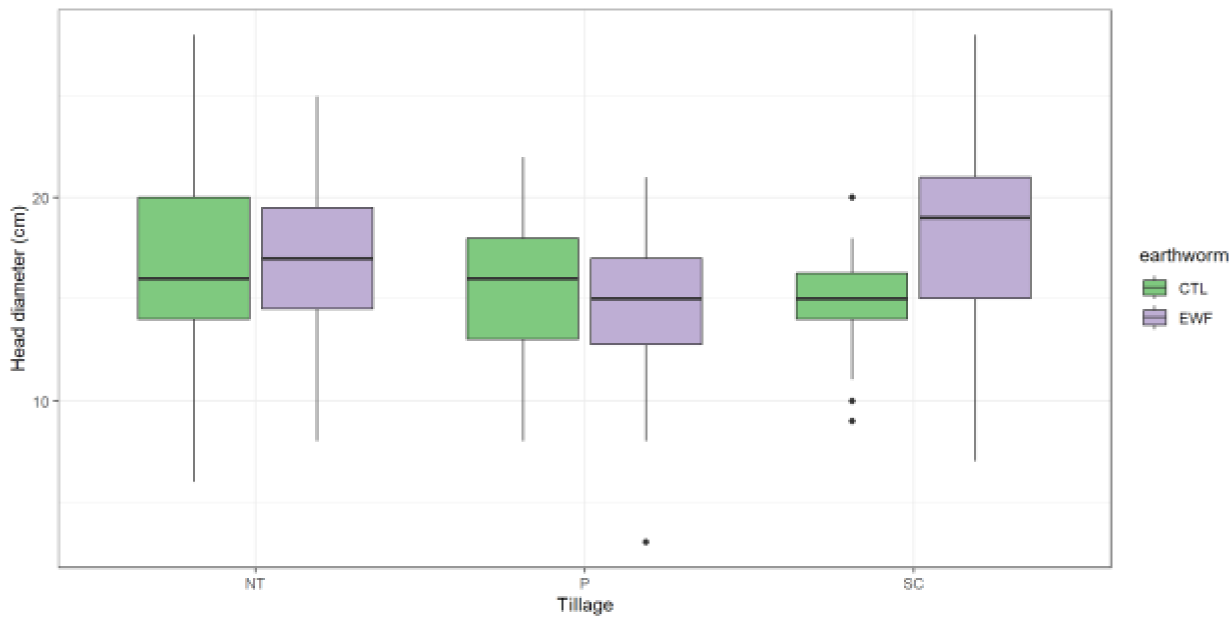


Figure 22. Head diameter of sunflower measured after harvesting in 2021

EWF in NT did not significantly change the head diameter of sunflowers. Among the treatments, SC resulted in the highest sunflower head diameter under the EWF condition. P exhibited a slightly reduced head diameter compared to NT treatments, regardless of the presence of an earthworm fence. The greatest variability in head diameter was observed in P within control areas, indicating that P might introduce environmental variability affecting sunflower growth. The positive effect of earthworms was most pronounced in SC, suggesting that SC may optimize the environment for earthworm activity, thereby enhancing sunflower growth. However, our study found that tillage significantly affected the head diameter of sunflowers ($P<0.001$) (Table 4). Additionally, the combined effect of earthworms and tillage was also significant ($P<0.001$) (Table 4), indicating that their interaction influenced the trait. However, earthworms alone did not have a significant effect on head diameter ($P=0.05$, ns) (Table 4). An earthworm fence resulted in larger head diameters than control areas, highlighting the beneficial effects of earthworms on sunflower head size.

The range observed in EWF, and CTL areas demonstrates the variability in sunflower growth, which is likely influenced by various micro-environmental factors such as soil moisture, nutrient availability, and micro-climatic conditions within the NT plots. Comparable ranges indicate that the impact of earthworm activity on the variability in head diameter under NT conditions is not significant. Similarly, the influence of earthworms on P is not prominent. This lack of effect could potentially be attributed to the disturbance of soil structure caused by P, which might counteract some of the advantages brought about by earthworms. The effect of earthworms on head diameter is more pronounced in SC, where earthworms seem to significantly increase the head diameter compared to control areas. In NT and P, the presence of earthworms does not show a substantial difference compared to control. The presence of earthworms in SC leads to a significantly larger head diameter, indicating that less intensive tillage allows earthworms to enhance soil properties effectively, promoting better plant growth. These findings corroborate research by Blouin, *et al*. (2013), demonstrating that earthworms enhance soil aggregation and nutrient availability, especially in less disturbed soil environments. Akhila and Entoori (2022) reported that earthworm activity enhances nutrient availability and root growth, particularly in less disturbed soils, corroborating the significant effects seen under SC in our study.

4.2.4 Head weight of sunflowers

The study shows that the median head weight of sunflowers in the NT treatment for both EWF and CTL areas is similar (Figure 23). The CTL area in NT exhibits a wide range of head weights, with several outliers reaching up to 1000 grams, indicating high variability and some extremely large heads in the absence of earthworms. This suggests that while the average conditions in the C area might be less conducive to uniform growth, certain plants thrive exceptionally well. Conversely, in the P condition, both EWF and CTL areas demonstrate narrow ranges and lower overall head weights (Figure 23) compared to NT and SC treatments. This suggests that the ploughed soil provides a more uniform but less optimal growth environment for sunflowers. The median head weight in the P-EWF area is lower than in the P-CTL area, though the difference is minimal, indicating that earthworms have a limited beneficial impact in ploughed soil.

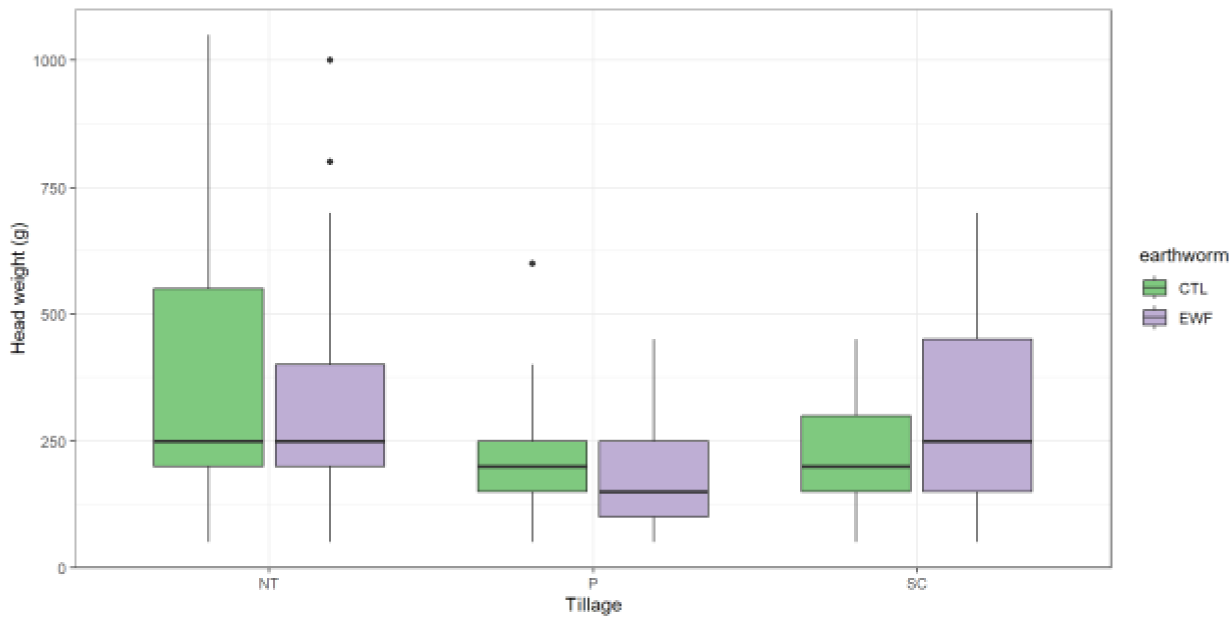


Figure 23. Head weight of sunflowers after harvest in 2021.

SC results in higher head weight in the EWF area compared to the CTL, indicating a possible beneficial effect of earthworms under this tillage treatment. In NT conditions, the presence of earthworms does not significantly increase the median head weight and may even slightly reduce it. The broader range and outliers in the control suggest that while some sunflowers achieve exceptional growth without earthworms, overall variability is higher. The presence of earthworms does not significantly alter sunflower head weights compared to the CTL area across the three tillage methods (NT, P, SC). This implies that while earthworms are known to improve soil aeration and nutrient cycling (Edwards and Bohlen, 1996; Domínguez and Edwards, 2010), their impact on sunflower head weight in this study is not statistically significant. Earthworms improve growth conditions, but their effectiveness is influenced by the tillage practice. SC maximizes the positive impact of earthworms on head weight.

4.2.5 Stem weight of sunflowers

The results presented in Figure 24 EWF and CTL areas within the NT treatment exhibit significant variability, with several outliers. Notably, the earthworm fence (EWF) displays a slightly broader range compared to other CTL areas. In P treatment, EWF and CTL areas show lower stem weights compared to NT and SC treatments. EWF areas in SC show a broader range of stem weights compared to CTL area (Figure 24), indicating that earthworms significantly enhance growth conditions leading to larger stem weights.

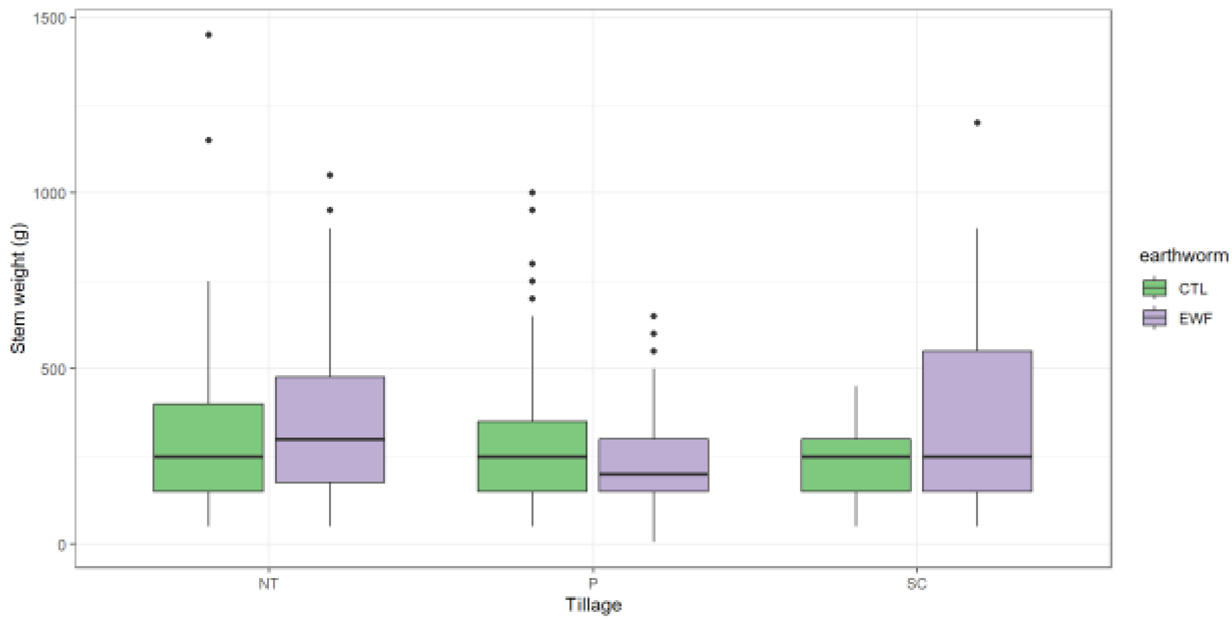


Figure 24. Stem weight of sunflower after harvest in 2021.

The direct effect of earthworms on stem weight, across the three tillage methods (NT, P, SC) is not statistically significant ($p > 0.05$) (Table 4). This suggests that earthworm presence alone does not impact stem weight. In NT conditions, the presence of earthworms slightly increases the median stem weight and contributes to greater variability, suggesting that earthworms may enhance growth conditions even with minimal soil disturbance. These findings correspond with the work of Chan (2001), who revealed that NT systems supported higher earthworm populations, which contribute to better soil structure and increased crop yields. These results emphasize how crucial it is to enhance crop output and health in agricultural operations by considering both biotic and abiotic elements. In P conditions, earthworms slightly increase the median stem weight, but the effect is minimal. The disturbance from P disrupts the beneficial effects of earthworms. These findings correspond with the work of Ernst and Emmerling (2009) find that P can disrupt soil structure, thereby affecting earthworm populations and their beneficial effects on soil health. Also study by Chan (2001), highlighting that while earthworms can improve soil structure and plant growth, the disturbance from ploughing can reduce their populations and their positive impact on the soil ecosystem.

In this study earthworms significantly increase the median stem weight in SC and contribute to greater variability and potential for higher yields, indicating that minimal soil disturbance combined with earthworm activity creates an optimal environment for sunflower growth.

Although the presence of earthworms does not provide a statistically significant effect, their interaction with tillage techniques is important. The significant interaction term indicates that NT and SC techniques promote environments favourable for earthworm activity, subsequently enhancing plant growth.

Table 4. Statistical analysis of head diameter, head weight, and stem weight of plants

	Head diameter (cm)		**Head weight (g)**		**Stem weight (g)**	
	Mean	P- value	Mean	P- value	Mean	P- value
Earthworm	52.563	0.05 ns	29756	0.2 ns	97813	0.13 ns
Tillage	168.618	$7.8\ 10^{6}$ ***	761216	$4.5\ 10^{14}$ ***	276305	$1.5\ 10^{3}$ **
Earthworm × Tillage	121.653	$1.9\ 10^{4}$ ***	116134	$6.7\ 10^{3}$ **	315720	$6.2\ 10^{4}$ ***

*Abbreviations: no significant differences between means (ns), *** Significantly different between means P<0.001, ** Significance difference between means P<0 0.01.*

The standalone effect of earthworms was not significant for head diameter, head weight, and stem weight (P-values: 0.05, 0.2, and 0.13, respectively) (Table 4). However, significant effects were observed for tillage and the interaction between earthworms and tillage across all traits, suggesting that the impact of earthworms became evident only in combination with specific tillage practices.

4.3 Effect of tillage on plant traits

4.3.1 Head diameter of sunflower

The median head diameter in NT for EWF is slightly higher compared to the control area (Figure 22). This slight difference suggests that, in NT systems, the presence of earthworms does not significantly enhance or reduce the head diameter of sunflowers. Similar median values indicate that the benefits earthworms typically provide (improved soil structure, nutrient cycling, and microbial activity) might already be achieved by the no-till practice itself. NT systems preserve soil organic matter and structure (Sapkota *et al*., 2012), which could reduce the observable additional benefits of earthworms. The median head diameter for P in EWF area is slightly lower than that for EWF NT indicating a greater variability in head diameter under NT conditions.

The median head diameter under P tillage (control) is slightly higher compared to NT tillage. However, the variability is similar between the two treatments, as indicated by the comparable height of the interquartile range (IQR) and whisker lengths. Notably, SC tillage shows the greatest variability, with both a larger IQR and several outliers. P has a different impact on head diameter (Figure 22) depending on whether an earthworm fence is present. In the earthworm fence area, ploughing slightly decreases the head diameter, while in the control area, ploughing increases the head diameter compared to SC. The median head diameter in the P treatment exhibits a considerable resemblance between EWF and CTL areas. This indicates that the act of P, as a form of tillage, could be the primary determinant affecting the growth of sunflowers, thus overshadowing the possible supplementary impacts of earthworms. A similar range in head diameter implies that P creates a consistent growth environment in both EWF and control areas (Figure 22). This consistency may stem from the homogenization of soil properties, such as nutrient distribution and soil structure, resulting from the process of P. The control areas in the p treatment have shown a slightly higher median head diameter and a slightly wider range compared to the EWF areas (Figure 22). This might suggest that while P improves overall soil conditions for plant growth, the absence of earthworms in the CTL plots could lead to more variability in sunflower head diameter. The wider range in the control areas (Figure 22) could be due to uneven soil conditions or nutrient availability that earthworms might otherwise help stabilize. NT and P show related results in both the presence and absence of earthworms, with the control areas of P having slightly higher median values (Figure 22).

Shallow cultivation in the presence of earthworms (EWF) leads to the highest median head diameter among all the treatments. SC provides a balance by reducing soil disturbance while still incorporating organic matter. The significant increase in head diameter in the presence of earthworms highlights the combined impact of minimal tillage and biological activity. Studies by Chan (2001) and Six *et al.* (2004) support these observations, demonstrating that minimal tillage combined with biological activity (like earthworms) optimizes soil health and plant growth. Birkás *et al*. (2004) highlighted the advantages of conservation tillage, such as improved soil structure, moisture retention, and reduced compaction. These benefits are reflected in the increased head diameter of sunflowers under SC. The significant joint effect of tillage methods and earthworm presence on sunflower traits ($P < 0.001$) (Table 4) indicates that the benefits of earthworms on head diameter are influenced by the specific tillage strategy employed. However, these values reflect the combined impact of both factors, not a statistical interaction, meaning the effect of one does not alter the other’s influence directly. This suggests that earthworm benefits are not uniform across all tillage practices but depend on the soil management strategy applied. Together, these factors shape plant development outcomes. A study by Mokgolo *et al*. (2024) found that in-field rainwater harvesting, a conservation tillage technique, significantly increased sunflower grain yield, head diameter, and aboveground dry matter compared to conventional tillage. This was consistent across different soil types and cropping seasons in Limpopo Province, South Africa. Chan (2001) suggests that earthworms enhance sunflower head diameter by improving soil quality through their biological activity, potentially by increasing nutrient availability and enhancing soil structure. This aligns with findings from other studies, such as van Groenigen *et al*. (2014), which reported improvements in crop yields associated with earthworm presence.

4.3.2 Head weight of sunflowers

NT conditions result in high variability in head weight, with some extreme outliers (Figure 23). The median head weight for both EWF and CTL is similar. The presence of earthworms slightly reduces the median head weight but stabilizes growth conditions, leading to fewer extreme outliers. P results in the lowest median head weights and narrow ranges for both EWF and control areas, indicating less favourable conditions for head weights. SC provides intermediate conditions with higher median head weights compared to P and less variability than NT. The head weight of sunflowers in NT treatment are larger than those in P and SC as shown in Figure 23. The head weights in SC are higher than observed in P but lower than in NT treatment for the control area (C). In P the head weights of sunflower for both EWF and CTL areas are lower and more consistent compared to NT and SC (Figure 23). As a result, the difference in sunflower head weights between the various tillage methods (NT, P, and SC) is statistically significant ($p<0.001$) (Table 4). Statistical significance suggests that the observed differences in head weights are unlikely to result from random variation and can be attributed to the tillage method employed.

NT and SC cultivation practices both support higher variability and potential for larger head weights, SC combined with earthworm activity provides the most favourable conditions for sunflower growth. P, on the other hand, seems to limit the benefits of earthworms, resulting in lower and more uniform head weights. NT and SC practices benefit from earthworm activity, but the benefits are more pronounced in SC. This suggests that minimal soil disturbance in SC allows earthworms to thrive and enhance soil health, leading to better plant growth. The interaction between earthworms and tillage method is statistically significant ($p < 0.01$). This implies that the impact of earthworms on head weight is contingent upon the tillage method used. This interaction indicates that the presence of earthworms may influence outcomes differently under varying tillage practices.

The absence of a significant impact from earthworms suggests that their role in influencing sunflower head weight may be less pronounced compared to other soil and environmental factors. NT practices may create conditions favourable for sunflower growth, due to improved soil structure, moisture retention, and reduced soil disturbance. In P conditions, earthworms slightly increase the median head weight, but the overall impact is minimal. The uniformity in head weights suggests that P may limit the beneficial effects of earthworms by disrupting their habitat. The consistency in head weights suggests that P creates a uniform but less favourable growth condition for sunflowers, potentially due to increased soil erosion, reduced soil moisture, and disruption of soil biota (Montgomery, 2007). In SC, earthworms significantly increase the median head weight and contribute to greater variability and potential for higher yields. This suggests that SC combined with earthworm activity creates an optimal environment for sunflower growth.

4.3.3 Stem weight of sunflowers

NT and SC methods result in significantly higher stem weights compared to P (Figure 24). The impact of tillage on stem weight is statistically significant with a p-value < 0.01, indicating a strong correlation between tillage method and stem weight. NT conditions result in high median stem weights and variability, with some extreme outliers (Figure 24). The presence of earthworms slightly enhances these conditions. P results in the lowest median stem weights (Figure 24), indicating less favourable conditions for sunflower growth. SC results in higher median stem weights compared to P and less variability than NT, with earthworm presence further enhancing these conditions. Experimental areas with earthworm presence (EWF) exhibit higher stem weights under NT and SC tillage methods compared to CTL areas. In contrast, P results in lower stem weights, irrespective of the presence of earthworms. This observation underscores the efficacy of NT and SC methods in enhancing plant growth.

Both tillage practices NT and SC benefit from earthworm activity, with SC showing a more pronounced positive impact on stem weight. This suggests that minimal soil disturbance in SC allows earthworms to thrive and enhance soil health, leading to better plant growth (Lemtiri *et al*., 2014). P limits the benefits of earthworms, resulting in lower median stem weights and greater variability in control areas due to the disruption of soil structure and earthworm habitats. The interaction between earthworms and tillage method is highly significant (p-value < 0.001). This significant interaction indicates that the influence of earthworms on stem weight is dependent on the tillage method employed. Specifically, earthworms augment stem weight more effectively in NT and SC treatments, whereas this interaction is less evident in P treatments. Our research findings highlight the significant impact of tillage methods on stem weight and overall plant growth. The significant differences observed between NT, SC, and P methods indicate that less intensive tillage techniques, such as NT and SC, contribute to better plant growth conditions. These methods maintain soil integrity, enhance nutrient cycling, and support beneficial soil organisms, such as earthworms. This underscores the significance of considering both biological and mechanical elements in agricultural methodologies to enhance crop productivity. However, the higher stem weights observed in NT and SC methods, relative to P, may be attributed to reduced soil disturbance, improved soil structure, and enhanced nutrient availability inherent to these methods.

The presence of earthworms increases head diameter and stem weight slightly, especially noticeable in no-tillage (NT) and shallow cultivation (SC) methods. The head weight is higher in the control area for NT, indicating that earthworms may not significantly affect head weight in this tillage method. NT and SC tend to produce larger head diameters and stem weights compared to ploughing (P). Head weight is highest in NT, with substantial variability in the CTL areas. The presence of earthworms has a positive effect on head diameter and stem weight, particularly in NT and SC. Tillage practices influence all three parameters, with NT showing better results compared to P and SC.

4.4. New scientific results

1. Based on the measured soil organic carbon (SOC) content, the soil organic carbon stock was calculated in 10 cm intervals from 0–40 cm after 18 years of systematic tillage in a Endocalcic Chernozem soil. In the top layer (0–10 cm), the highest values were observed in no-till (NT) (37.6 t ha^{-1}), followed by shallow cultivation (SC) (29.0 t ha^{-1}), and then ploughing (P) (25.2 t ha^{-1}). However, in the lower layer (30–40 cm), the order changed to P > NT > SC, with P having the highest values (24.0 t ha^{-1}) in this depth range. We suggest

that the importance of measuring SOC content up to 100 cm in depth to fully capture the soil carbon dynamics.

2. I observed significantly greater earthworm populations under reduced disturbance tillage systems (NT=189.3 ind m^{-2}, and SC=125.3 ind m^{-2}) compared to P (48 ind m^{-2}), suggesting that minimal soil disruption supports earthworm habitats and biological activity. This result underscores the role of conservation tillage in fostering soil biological health, potentially contributing to improved soil structure and nutrient cycling.

3. I justified that the joint effect of earthworms and soil tillage is significant on certain measured plant traits under sunflower culture, with stem weight being significantly greater under the earthworm and tillage interaction compared to head weight and head diameter, supported by a highly significant effect ($p < 0.001$).

4. I observed that earthworm activity led to changes in soil physical properties, specifically bulk density and soil moisture content, in NT and SC treatments. While these changes did not result in direct differences in plant traits, the improved soil structure and water retention capacity suggest potential long-term benefits for crop growth and soil fertility.

5. CONCLUSIONS AND RECOMMENDATIONS

Based on our findings we concluded that our initial hypothesis (SOC content and stock would be higher under NT compared to SC and P) was partially supported. NT involves no soil disturbance, allowing surface plant residues to contribute significantly to humus formation. As expected, SOC values and stock were highest in NT, offering a promising carbon storage option for addressing climate change mitigation in the soil. While SC had lower SOC content, it still surpassed that of the P treatment. In P, the soil layer undergoes complete inversion, leading to soil aggregate disruption and increased aeration, consequently causing SOC depletion in the topsoil over time. In the lowest layers (30-40 cm), P had higher SOC levels and stock than NT and SC. This might be the outcome of rotating the soil slice down to this depth and accumulating surface stubble, which offers raw organic residue for humification at this level. Thus, we cannot generalize regarding SOC stock; we must constantly consider the specific soil depth.

Our second hypothesis, which posited that there would be a notable increase in earthworm abundance, biomass, and soil microbial respiration under NT conditions compared to (SC) and (P) treatments, was substantiated. Earthworms exhibit a preference for habitats that are left undisturbed and characterized by high levels of moisture, raw organic matter, and humus. These conditions are present in the NT treatments, despite the high bulk density resulting from minimal anthropogenic soil disturbance. The activity of earthworms in burrowing, mixing, and aerating the soil helps counterbalance this bulk density to some degree. Nevertheless, our observations revealed higher bulk density values in the surface soil layer under NT in comparison to SC and P treatments. In the case of the P treatment, a considerable decrease in earthworm abundance and biomass was noted, attributed to the extensive physical disruption and overturning of the entire soil profile, which destroys the burrow systems established by the resident earthworms. When it comes to SC, it presents a potential option in terms of the extent of soil physical disturbance that resulted in a moderate value (<1.4 g cm^{-3}) compared to NT and P treatments. Additionally, the presence of raw organic material is quite significant, creating a favourable environment for earthworms due to the abundance of food sources and a moderate level of bulk density. Therefore, according to our study findings, we determined that among the three tillage treatments (NT, SC, P), SC emerges as a viable choice for establishing a favourable soil environment for soil biota. It has the capability to maintain an adequate amount of SOC and SOC stock while also demonstrating satisfactory soil bulk density levels, making it a feasible replacement for the traditional P tillage practice.

We also determined that earthworm enhancement significantly benefits sunflower growth, particularly under SC conditions, by increasing head diameter and stem weight, though it has a limited effect on head weight. SC emerged as the most favourable tillage method, promoting optimal soil conditions and allowing earthworms to thrive. In contrast, P disrupted soil structure and reduced the beneficial impact of earthworms, leading to lower growth traits. NT methods showed variable results, with earthworms slightly improving stem weight.

5.1 Recommendations

- No-Till (NT) practices are highly recommended for maximizing soil organic carbon (SOC) content and stock, essential for climate change mitigation and improved soil health. NT's minimal soil disturbance promotes humus formation and supports higher earthworm abundance and biomass, enhancing soil structure and fertility. Despite higher bulk density in NT, earthworm activity helps mitigate this issue, making NT the most effective method for carbon storage and fostering a healthy soil ecosystem.

- SC is a viable alternative to traditional ploughing (P), offering a balance between maintaining SOC levels and providing a favourable environment for earthworms and microbial activity. SC reduces soil disturbance while preserving organic material, resulting in improved soil bulk density compared to NT and P. By minimizing soil disruption and retaining organic matter, SC supports a moderate bulk density and provides ample food sources for earthworms, making it an effective practice for sustainable soil management.

- To optimize sunflower growth, it is recommended to adopt shallow cultivation practices, enhance earthworm populations through organic amendments and reduced chemical inputs, limit ploughing, monitor soil health, and use integrated soil management strategies that combine minimal tillage with sustainable practices.

6. SUMMARY

Soil health is fundamental to planetary and human well-being, as it supports critical processes such as biomass production, carbon regulation, and biodiversity maintenance. The health of soil is intricately linked to sustainable agriculture, human nutrition, and climate change mitigation, as healthy soils ensure nutrient cycling, water retention, and carbon sequestration. Soil biota, especially earthworms, play a crucial role in maintaining soil fertility and structure by influencing soil physical, chemical, and biological properties. Despite their known benefits in improving soil quality and crop productivity, the role of earthworms has been underexplored in agricultural management practices.

This study aims to address this gap by investigating the impact of earthworm presence on sunflower plant traits (height, head diameter, head weight, and stem weight) under different tillage practices: no-tillage (NT), ploughing (P), and shallow cultivation (SC). It also seeks to evaluate the effects of these tillage methods on selected soil properties, such as bulk density, soil moisture, pH, and soil organic carbon (SOC) content. Additionally, the research will assess the overall soil health status after 18 years of continuous tillage by examining biological indicators like soil microbial respiration, earthworm abundance, biomass, and species composition, as well as crop yield. This study aims to provide insights into how earthworms influence crop development, soil fertility, and sustainable agricultural practices, contributing to enhanced productivity and environmental stewardship.

Soil microbial respiration, a measure of microbial activity, was significantly higher in NT (22.8 mg CO_2/50 g/10 days) and SC (19.25 mg CO_2/50 g/10 days) than in P (10.03 mg CO_2/50 g/10 days). These findings align with existing literature, showing that NT and SC create favourable conditions for microbial activity due to better soil structure and moisture retention. The bulk density was highest in NT (1.48 g cm^{-3}) compared to SC (1.22 g cm^{-3}) and P (1.26 g cm^{-3}) in the upper soil layer (0–10 cm), but NT also showed higher density in deeper layers. Soil organic carbon (SOC) content was greatest in NT (2.5%) in the top layer (0–10 cm), while P (2.0%) had more uniform SOC distribution across depths. SOC stock was highest in NT (37.6 t ha^{-1}) in the topsoil, followed by SC (29.0 t ha^{-1}) and P (25.2 t ha^{-1}). NT and SC treatments led to higher SOC storage in the topsoil, promoting greater soil fertility. SC recorded the highest winter oat yield (8.11 Mg

ha^{-1}), followed by NT (7.82 Mg ha^{-1}), and P (6.82 Mg ha^{-1}). The higher yields in SC and NT were due to improved soil moisture retention and biological activity.

Sunflower plants heights increased steadily over time in both the EWF and CTL treatments, with similar trends observed throughout the observation period. NT resulted in lower plant heights compared to P and SC, especially in the initial stages of growth. While earthworms showed a slight positive effect on plant height, the influence was not substantial, indicating that factors other than earthworm activity played a more significant role in determining plant height. In terms of head diameter, earthworms had a significant impact, especially in SC, where the largest sunflower head diameters were observed. SC provided an optimal environment for earthworms, leading to enhanced plant growth. In contrast, the effect of earthworms on head diameter was less pronounced in NT and P treatments, suggesting that tillage practices modulate the benefits provided by earthworm activity. This highlights the importance of minimal soil disturbance for maximizing the positive effects of earthworms. Head weight was highest in NT, where there was notable variability in the control areas. Earthworms had a limited impact on head weight, with their most noticeable effect observed in SC. In P treatments, head weights were lower and more uniform, suggesting that ploughing limits the positive influence of earthworms on sunflower growth. These findings suggest that minimal tillage practices, such as NT and SC, offer more favourable conditions for sunflower growth compared to P. For stem weight, both NT and SC resulted in significantly higher stem weights compared to P. While the presence of earthworms slightly enhanced stem growth, their influence was not statistically significant across all treatments. However, earthworms had a more pronounced effect in NT and SC, where minimal soil disturbance allowed them to improve soil conditions and support better plant growth.

7. ÖSSZEFOGLALÁS

A talaj egészsége alapvető fontosságú a bolygó és az emberi jólét szempontjából, mivel olyan kritikus folyamatokat támogat, mint a biomassza-termelés, a szénmegkötés szabályozása és a biodiverzitás fenntartása. A talaj egészsége szorosan összefügg a fenntartható mezőgazdasággal, az emberi táplálkozással és az éghajlatváltozás mérséklésével, mivel az egészséges talaj biztosítja a tápanyagok körforgását, a vízmegtartást és a szénmegkötést. A talajbiota, különösen a földigiliszták, kulcsszerepet játszanak a talaj termékenységének és szerkezetének fenntartásában azáltal, hogy befolyásolják a talaj fizikai, kémiai és biológiai tulajdonságait. Annak ellenére, hogy jól ismertek a talajminőség és a terméshozam javításában betöltött szerepük, a földigiliszták hatását a mezőgazdasági gyakorlatokban eddig alulértékelték.

Ez a tanulmány ezt a hiányosságot kívánja pótolni azáltal, hogy vizsgálja a földigiliszták jelenlétének hatását a napraforgó növényi tulajdonságaira (magasság, tányérátmérő, tányér tömege és szár tömege) különböző talajművelési rendszerekben: direktvetés (NT), szántás (P) és sekély kultivátorozás (SC). Emellett értékelni kívánja e talajművelési módszerek hatását bizonyos talajtulajdonságokra, például a térfogattömegre, a talajnedvességre, a pH-ra és a talaj szerves széntartalmára (SOC). A kutatás célja továbbá a talaj egészségi állapotának felmérése 18 év folyamatos művelés után, biológiai indikátorok, például a talaj mikrobiális respirációja, a földigiliszták mennyisége, biomasszája és fajösszetétele, valamint a terméshozam vizsgálatával. A tanulmány célja, hogy betekintést nyújtson a földigiliszták növényfejlődésre, talajtermékenységre és fenntartható mezőgazdasági gyakorlatokra gyakorolt hatásába, hozzájárulva ezzel a termelékenység növeléséhez és a környezet védelméhez.

A talaj mikrobiális légzése, amely a mikrobiális aktivitás mérőszáma, szignifikánsan magasabb volt NT (22,8 mg CO_2/50 g/10 nap) és SC (19,25 mg CO_2/50 g/10 nap) esetében, mint P (10,03 mg CO_2/50 g/10 nap) kezelésnél. Ezek az eredmények összhangban állnak a szakirodalommal, amely szerint az NT és SC kedvezőbb feltételeket teremt a mikrobiális aktivitás számára a jobb talajszerkezet és nedvességmegtartás miatt. A legmagasabb térfogattömeget NT-nél mérték (1,48 g cm^{-3}), szemben az SC (1,22 g cm^{-3}) és P (1,26 g cm^{-3}) értékeivel a felső talajrétegben (0–10 cm), de NT esetében a mélyebb rétegekben is nagyobb térfogattömeget figyeltek meg. A talaj szerves széntartalma (SOC) a legmagasabb volt NT-ben (2,5%) a felső rétegben (0–10 cm), míg P (2,0%) egyenletesebb SOC-eloszlást mutatott a mélység mentén. A szerves szén készlet NT-ben volt a legmagasabb a felső talajrétegben (37,6 t ha^{-1}), ezt követte SC (29,0 t ha^{-1}) és P (25,2 t ha^{-1}). Az NT és SC kezelések nagyobb SOC tárolást eredményeztek a felső talajrétegben, ezáltal növelve a talaj termékenységét. A legmagasabb őszi zab hozamot SC esetében mérték (8,11 Mg ha^{-1}), ezt követte NT (7,82 Mg ha^{-1}) és P (6,82 Mg ha^{-1}). Az SC és NT magasabb hozamai a jobb talajnedvesség-megtartásnak és a fokozott biológiai aktivitásnak voltak köszönhetők.

A napraforgó növénymagassága mind az a terület, amelyre gilisztát juttattunk ki (EWF), mind a kontroll (CTL) kezelésekben folyamatosan növekedett, hasonló trendeket mutatva a megfigyelési időszak alatt. NT esetében a növények magassága alacsonyabb volt, mint P és SC esetében, különösen a növekedés kezdeti szakaszában. A földigiliszták kis mértékben pozitív hatást gyakoroltak a növénymagasságra, de hatásuk nem volt jelentős, ami arra utal, hogy a növénymagasságot nagyobb mértékben más tényezők befolyásolták. A tányérátmérő tekintetében a földigiliszták jelentős hatást gyakoroltak, különösen SC esetében, ahol a legnagyobb tányérátmérőket figyelték meg. Az SC kedvező környezetet biztosított a földigiliszták számára, ami elősegítette a növények fejlődését. Ezzel szemben NT és P kezelésekben a földigiliszták tányérátmérőre gyakorolt hatása kevésbé volt kifejezett, ami arra utal, hogy a talajművelési gyakorlatok módosítják a földigiliszták tevékenységének előnyeit. Ez kiemeli a minimális talajbolygatás fontosságát a földigiliszták pozitív hatásainak maximalizálásában.

A tányér tömege NT-ben volt a legmagasabb, ahol a kontroll területeken jelentős variabilitás volt megfigyelhető. A földigiliszták korlátozott hatást gyakoroltak a tányér tömegére, leginkább SC-ben volt észlelhető a hatásuk. P kezelések esetében a tányér tömege kisebb és egyenletesebb volt, ami arra utal, hogy a szántás korlátozza a földigiliszták pozitív hatását a napraforgó növekedésére. Ezek az eredmények azt sugallják, hogy a minimális talajművelési módszerek, például NT és SC, kedvezőbb feltételeket biztosítanak a napraforgó növekedéséhez, mint P.

A szár tömege mind NT, mind SC esetében szignifikánsan nagyobb volt, mint P-ben. Bár a földigiliszták jelenléte kissé növelte a szár növekedését, hatásuk statisztikailag nem volt jelentős az összes kezelésben. Ugyanakkor NT és SC esetében a földigiliszták hatása kifejezettebb volt, mivel a minimális talajbolygatás lehetővé tette számukra, hogy javítsák a talaj állapotát és elősegítsék a jobb növekedést.

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