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

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1. Introduction

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, 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).

1.1 Research Objectives

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.

2. Materials and methods

2.1 Study area

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 1). The topography is level. According to the World Soil Reference Base (IUSS Working Group WRB, 2015), the soil type is Endocalcic Chernozem (Loamic). 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).

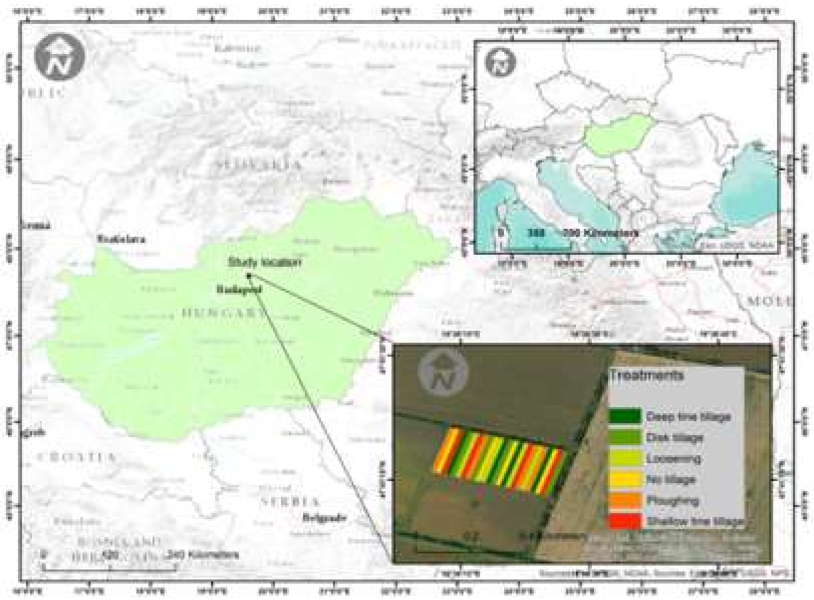

Figure 1. Location of the study area (Józsefmajor-Hatvan, Central Hungary).

(Source: Dekemati et al., 2020)

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 2 and 3. 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.

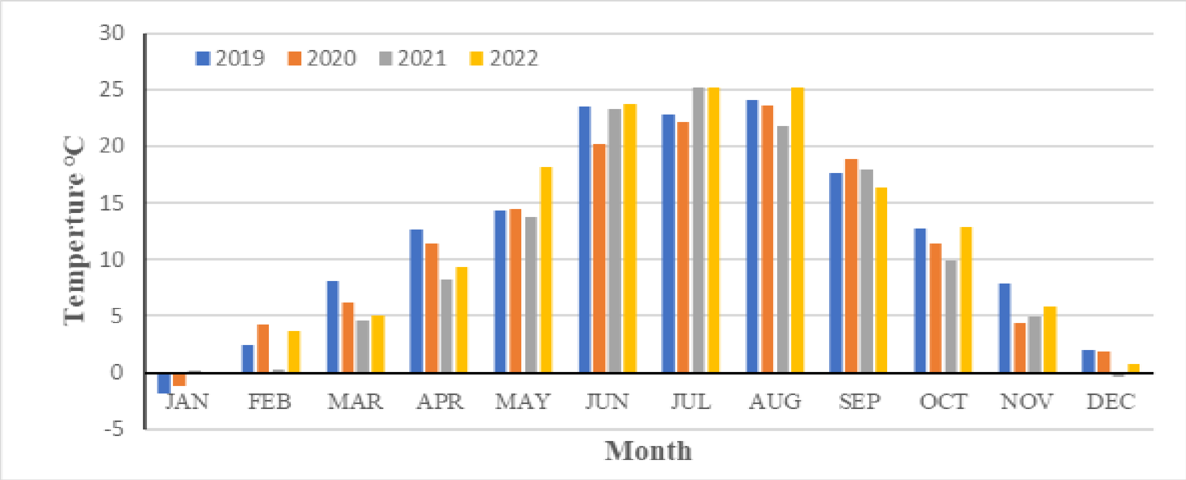

Figure 2. Monthly temperature data (2019- 2022). Sourced from NASA

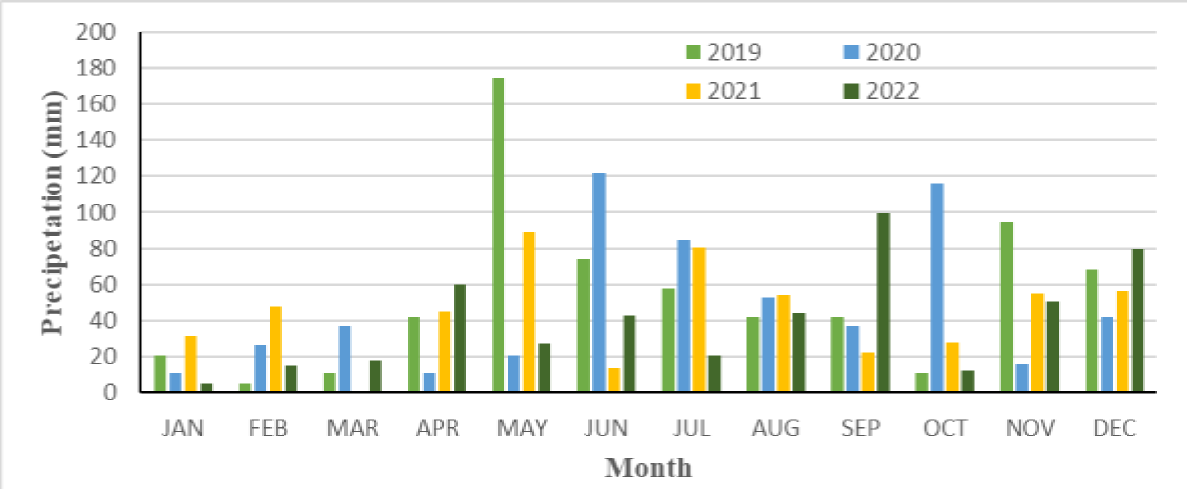

Figure 3. Monthly precipitation data (2019- 2022). Sourced from NASA

2.2 Experimental design

The experiment was conducted between 2019 and 2022. The 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. Each plot measures 13 × 180 m, while the total area of all treatments is 5.5 hectares. For our research, we focused on three treatments that represent increasing degree of soil disturbance: no-till (NT), shallow cultivation (SC, 20 cm), and ploughing (P, 30 cm).

2.3 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.

2.3.1 Soil properties analyses

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 the four spatial 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.

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 cm, 10-20 cm, 20-30 cm and 30-40 cm) 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. 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. The colour of the mixture was measured by a UNICAM Photometer (UV2 043506) (UNICAM, Montreal, Canada) (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. 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 makes 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.

Earthworm extraction was carried out on the from the big field 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}) the same way. The earthworm species were determined according to Csuzdi and Zicsi (2003).

2.3.2 Earthworm enhancement

Preparation of the earthworm enhancement fences

As an integral part of the experimental setup, small fences (2.5 × 3 m area) 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). One specific earthworm species (*Lumbricus terrestris*) was introduced within these fenced areas, accompanied by the distribution of straw as an organic amendment. 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.

Three rows of sunflower could fit it one fence. *Lumbricus terrestris* earthworm species was ordered from Canada. These earthworms were carefully packaged and delivered in a labelled box. 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. The chopped straw (10-15cm), which was created from the leftover stalk after wheat grains are 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 / 7.5 m^2, 10-15 cm length) was spread over the soil in the fence area to cover the earthworms and help them acclimate to their new environment, as well as in the control area.

Measurement of plant parameters

The sunflower 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 analyzer by dry combustion method (CNHS elemental analyzer). The thousand kernel weight (g) was also measured with an equipment seed counter at the Department of Crop Production.

2.4 Statistical analyses

All statistical analyses were conducted using R (version 4.2.2, R Core Team 2021). Data were analysed to determine statistical significance between treatments. For the field data, normality was assessed using Q-Q plots before conducting an ANOVA. Tukey's post-hoc HSD test was applied for multiple comparisons of treatment means. For the earthworm enhancement data, linear models were employed to compare treatment effects, with statistical significance identified at $p < 0.01$. A two-way ANOVA was used to evaluate the main effects and interactions between tillage and earthworm treatments. Tukey's HSD test was applied for post-hoc comparisons where significant effects were detected.

3. Results and discussion

3.1Effect of tillage on selected soil properties

3.1.1 Physical analyses

The soil bulk density values that were measured are shown in Figure 4. 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.

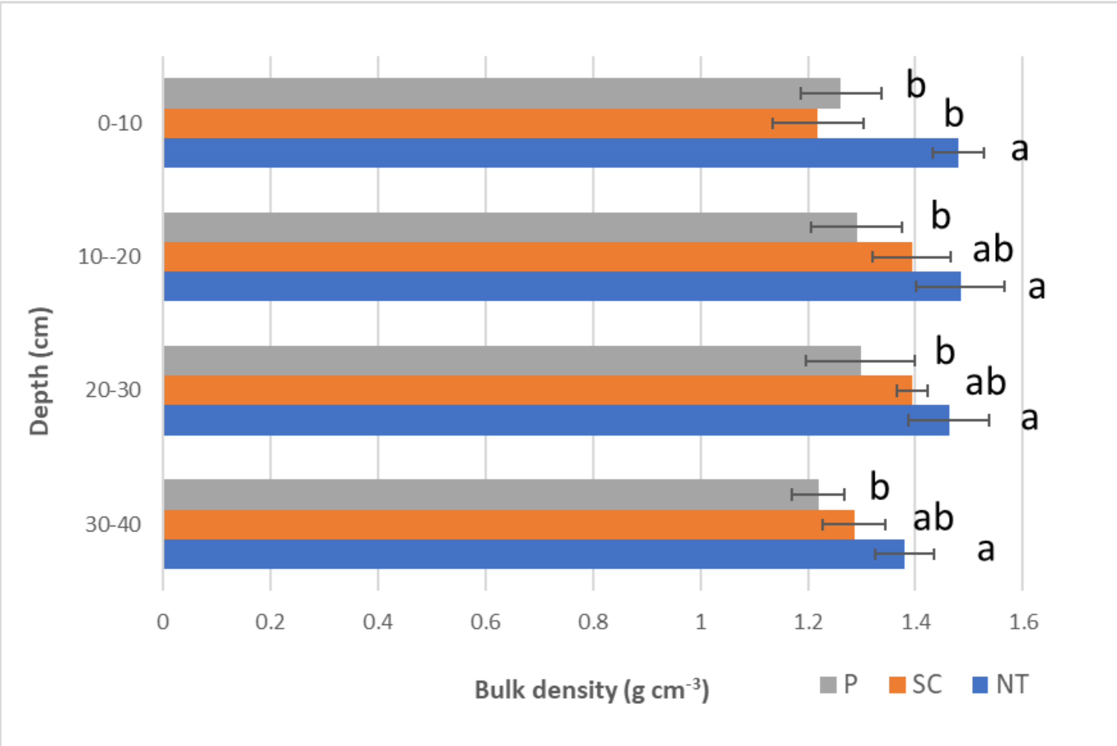

Figure 4. The soil bulk density values (Autumn, 2020) (P - ploughing, SC - shallow cultivation, NT - no till). The same letters beside the bars designate no statistical difference.

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.

3.1.2. Chemical analyses

Figure 5 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.

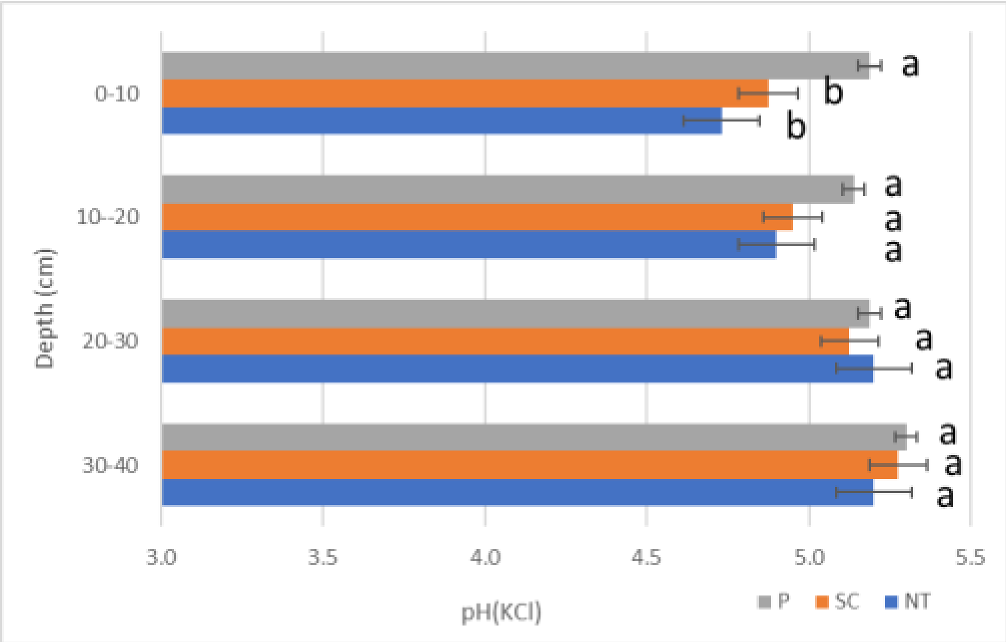

Figure 5. The soil pH (KCl) values (Autumn, 2020), (P - ploughing, SC - shallow cultivation, NT - no till). The same letters beside the bars designate no statistical difference.

Soil organic carbon varied significantly across tillage treatments (Figure 6), with the highest values in the top layer (0–10 cm) under no-tillage (NT, 2.5%), followed by shallow cultivation (SC, 2.4%) and ploughing (P, 2.0%). In the bottom layer (30–40 cm), P had the highest SOC (2.0%) compared to NT (1.7%) and SC (1.6%). NT and SC showed a gradual SOC decrease with depth, while P exhibited uniform SOC distribution due to soil mixing and enhanced microbial activity. These trends align with findings from long-term studies, which observed similar patterns in tillage systems. Comparative studies, such as those by Dekemati *et al*. (2019) and Ernst and Emmerling (2009), confirm these patterns but highlight variability based on soil type and tillage intensity. P treatments generally showed less SOC stratification, while NT and SC preserved higher SOC in the topsoil.

These findings underscore the influence of tillage on SOC distribution, with NT and SC better conserving organic carbon in surface layers than P, which promotes uniformity at the cost of topsoil enrichment.

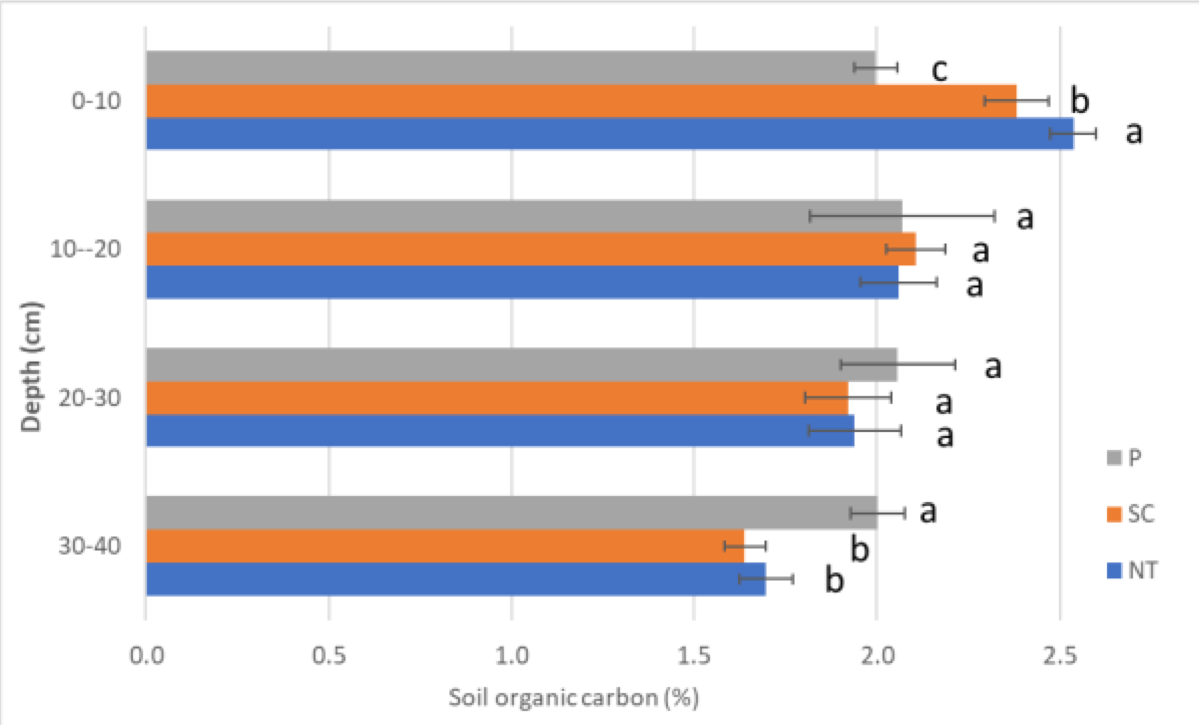

Figure 6. The soil organic carbon values (Autumn, 2020). (P – ploughing, SC – shallow cultivation, NT – no-till). The same letters beside the bars designate no statistical difference.

The SOC stock values varied significantly among the tillage treatments, particularly in the top layer (0–10 cm). NT exhibited the highest stock (37.6 t ha^{-1}), followed by SC (29.0 t ha^{-1}) and P (25.2 t ha^{-1}). In the lower layer (30–40 cm), P recorded the highest SOC stock, with no significant differences observed in the middle layers (10–20 cm, 20–30 cm). These findings align with studies highlighting NT ability to enhance SOC stock in the topsoil due to reduced soil disturbance and conservation practices, as reported in Chernozem soils in Russia (Kostyukevich *et al.*, 2021) and Ukraine (Bondarenko *et al.*, 2019). Other studies, such as those by Moussadek *et al.* (2014) and Biernat *et al.* (2017), similarly emphasize the benefits of NT in improving SOC storage in surface layers compared to ploughing. Regional differences in SOC stock values were noted, influenced by soil type, climate, and tillage intensity. For instance, studies in tropical soils (Pinheiro *et al.*, 2015) reported lower SOC stock due to higher temperatures and precipitation, while measurements in Hungary (Jakab *et al.*, 2023) showed variations due to bulk density heterogeneity and seasonal timing. The correlation between SOC content and SOC stock highlights the importance of considering bulk density alongside SOC concentrations for more precise assessments, as recommended by Gál *et al.* (2007). These findings reinforce the advantages of NT in maintaining higher SOC stocks, particularly in the topsoil, while also highlighting the impact of local conditions on SOC distribution.

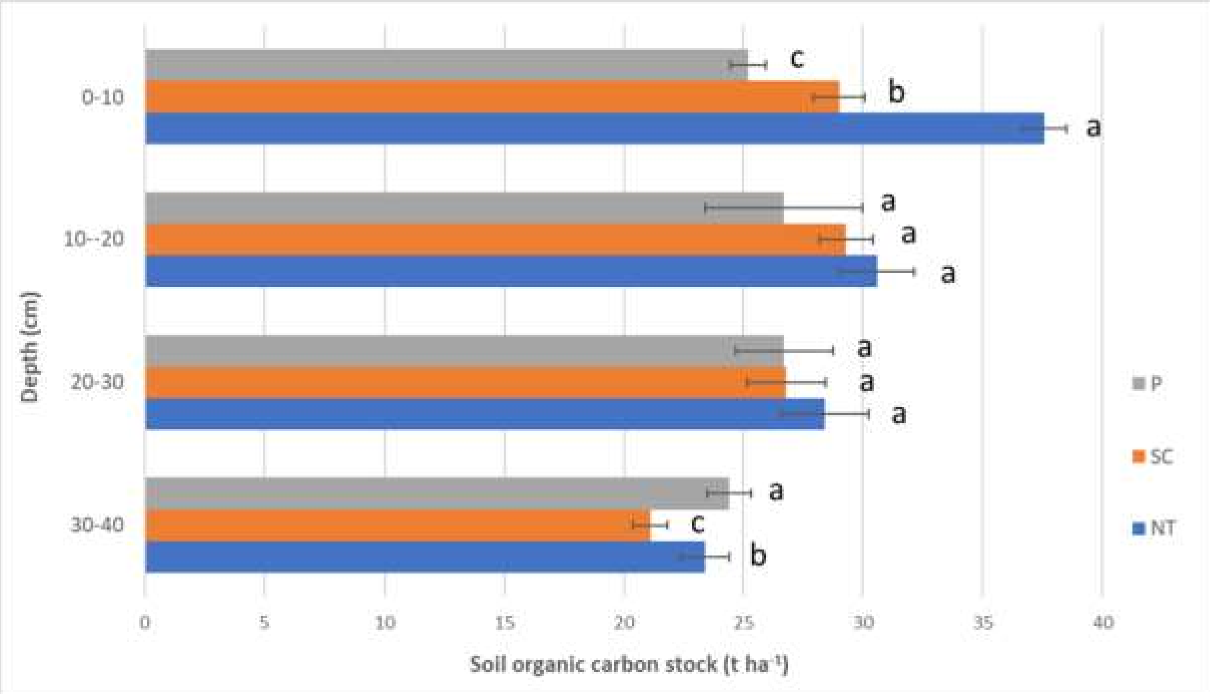

Figure 7. The soil organic carbon stock values (Autumn, 2020). (P – ploughing, SC – shallow cultivation, NT – no-till). The same letters beside the bars designate no statistical difference.

3.1.3 Biological analyses

Soil microbial respiration values (Figure 8) were significantly higher in NT (22.8 mg CO_2/50 g/10 days) than P (10.03 mg CO_2/50 g/10 days), with SC (19.25 mg CO_2/50 g/10 days) also exceeding P (NT = SC > P). This reflects greater microbial activity in NT and SC compared to P, consistent with studies (Jha *et al.*, 2022; Mirzavand *et al.*, 2020). Similar trends were observed in prior in situ measurements at the site (Gelybó *et al.*, 2022). Both autotrophs and heterotrophs contributed to respiration, influenced by environmental factors like rainfall and soil moisture. Future research should explore microbial biomass, diversity, and respiration partitioning for deeper insights.

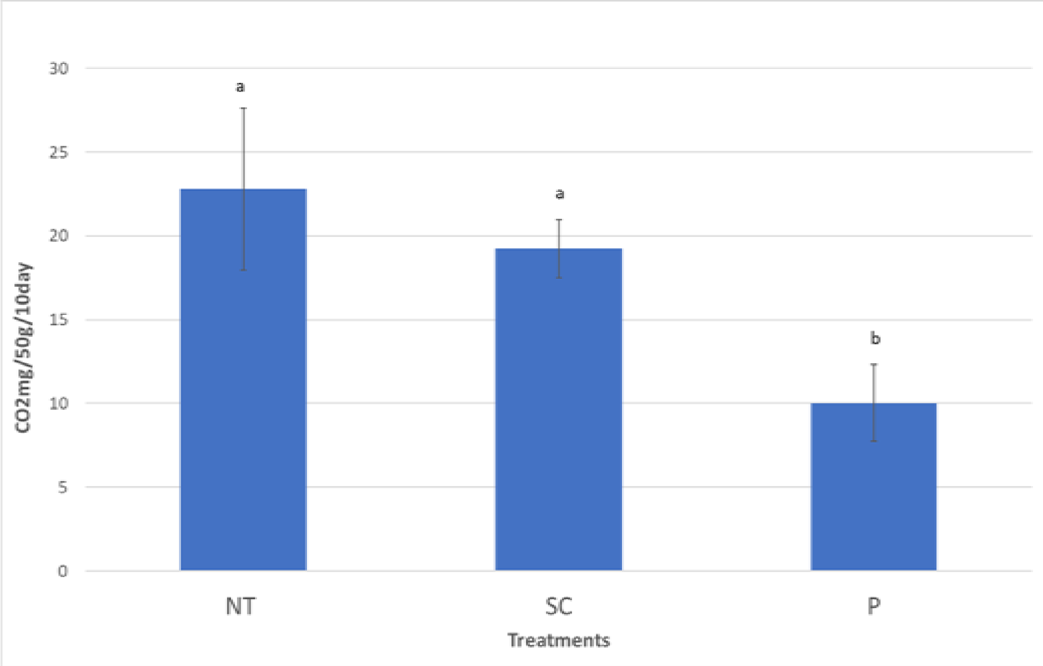

Figure 8. The soil microbial respiration values (Autumn, 2020). (P – ploughing, SC – shallow cultivation, NT – no till). The same letters beside the bars designate no statistical difference

Earthworm abundance and biomass were significantly higher in NT (189.3 ind m⁻²; 41.26 g m⁻²) and SC (125.3 ind m⁻²; 36.95 g m⁻²) compared to P (48 ind m⁻²; 7.4 g m⁻²), as shown in Figure 9. NT supported three earthworm species (*A. rosea*, *A. georgii*, *A. caliginosa*), SC supported two (*A. rosea*, *A. caliginosa*), and P supported only one (A. rosea), all belonging to the endogeic morphotype. The higher abundance and diversity in NT and SC align with studies showing reduced tillage favours earthworm populations due to less mechanical disturbance and improved soil conditions.

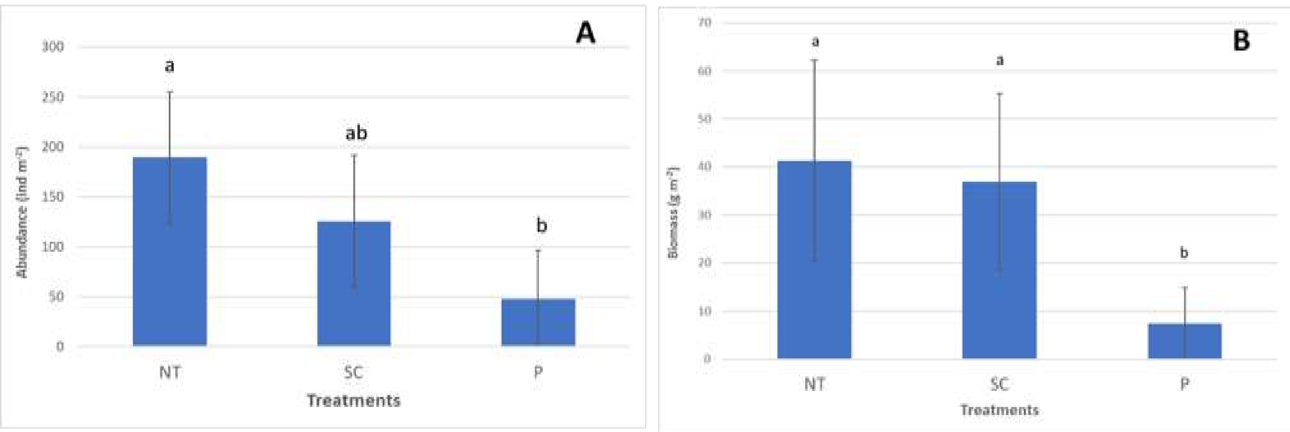

Figure 9. (A) The earthworm abundance values (Autumn, 2020). (B) The earthworm biomass values (Autumn, 2020). (P — ploughing, SC — shallow cultivation, NT — no-till). The same letters above the bars designate no statistical difference.

Low pH (4.7–5.3) likely limited species diversity, favouring acid-tolerant species like *A. rosea* and *A. caliginosa*. Sampling methods, such as hand-sorting, may have underestimated anecic species. Winter oat yields were highest under SC (8.11 t ha^{-1}), followed by NT (7.82 t ha^{-1}) and P (6.82 t ha^{-1}). SC superior yield is attributed to reduced soil compaction and improved moisture retention, while NT's mulch layer enhanced biological activity and water availability. Lower yields in P reflect increased disturbance and reduced organic matter. Climatic variability, including erratic rainfall, also plays a critical role in crop productivity, emphasizing the need to adapt tillage practices to optimize soil health and yield under changing environmental conditions.

3.2 Effects of earthworm enhancement and tillage on plant traits

3.2.1 Head diameter of sunflowers

The interaction between tillage practices and earthworm enhancement significantly influenced sunflower head diameter (Figure 10). Shallow cultivation (SC) with an earthworm fence (EWF) resulted in the highest median head diameter, demonstrating that minimal soil disturbance optimizes conditions for earthworm activity, which enhances soil health and plant growth. Conversely, ploughing (P) showed reduced head diameter under EWF, likely due to soil structure disruption, which limits the benefits of earthworm activity. In no-tillage (NT) systems, the presence of earthworms did not significantly increase head diameter, as NT practices already improve soil structure and organic matter.

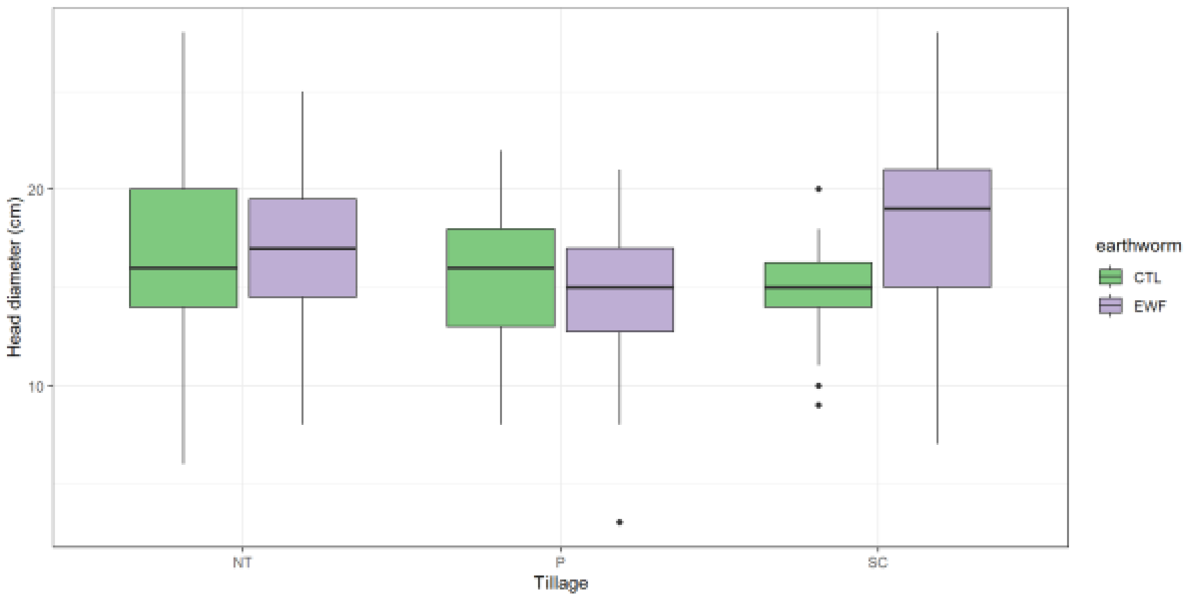

Figure 10. Head diameter of sunflower measured after harvesting in 2021

The study revealed that tillage practices play a pivotal role in modulating the impact of earthworms on sunflower growth, with SC providing the most favourable environment. The significant interaction between earthworm enhancement and tillage ($P < 0.001$) highlights the need to align biological activity with appropriate soil management strategies. SC effectively combines reduced soil disturbance with enhanced biological activity, leading to greater soil fertility and plant productivity. This corroborates findings from Chan (2001) and Six *et al.* (2004), who emphasized the synergy between minimal tillage and biological activity in improving soil quality. The results also align with previous studies indicating that earthworms contribute to nutrient cycling and soil aggregation, benefiting crop growth, particularly in conservation tillage systems. Together, these findings underscore the importance of integrating earthworm management into tillage strategies to optimize plant traits and soil health.

3.2.2 Head weight of sunflowers

The interaction between tillage practices and earthworm enhancement significantly influenced sunflower head weight (Figure 11). NT resulted in the highest variability and potential for larger head weights, with some extreme outliers in the CTL areas. However, earthworm presence under NT did not significantly increase the median head weight and even slightly reduced variability. SC provided the most favourable conditions for sunflower growth when combined with earthworm activity, leading to higher median head weights and reduced variability compared to NT.

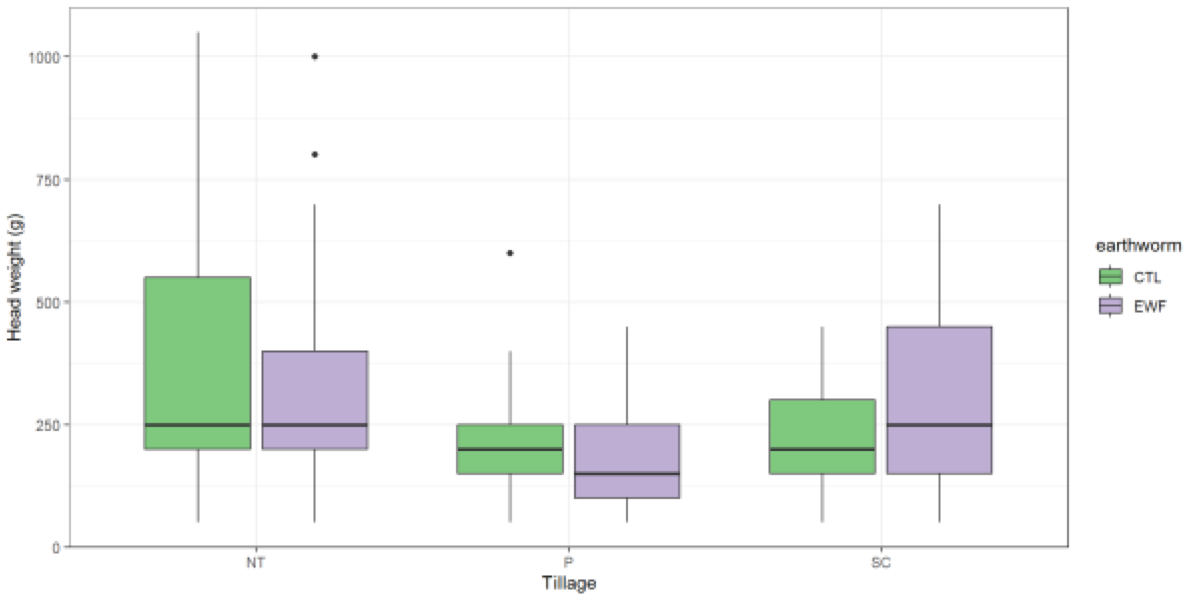

Figure 11. Head weight of sunflowers after harvest in 2021.

In contrast, P resulted in the lowest and most consistent head weights across both EWF and CTL, indicating that soil disturbance in P limits the beneficial effects of earthworms. Earthworm impact on sunflower head weight was influenced by tillage practices, with SC maximizing their benefits. While NT supports favourable growth conditions due to improved soil structure and moisture retention, earthworms in NT had minimal additional impact, suggesting that NT practices already optimize these factors. P showed limited benefits from earthworms, likely due to habitat disruption caused by intensive soil disturbance. SC's minimal disturbance allows earthworms to thrive, enhancing soil health and promoting better plant growth. The interaction between earthworms and tillage was statistically significant ($P < 0.01$), underscoring the importance of aligning soil management strategies with biological activity to maximize crop productivity.

3.2.3 Stem weight of sunflowers

The study highlights the significant impact of tillage practices and earthworm enhancement on sunflower stem weight and growth traits (Figure 12). NT and SC create favourable conditions for plant growth, with SC showing a more pronounced positive effect of earthworm activity. NT resulted in higher median stem weights and variability, with some extreme outliers, while SC provided consistent improvement with less variability. In contrast, P produced the lowest stem weights and limited the benefits of earthworms due to habitat disruption.

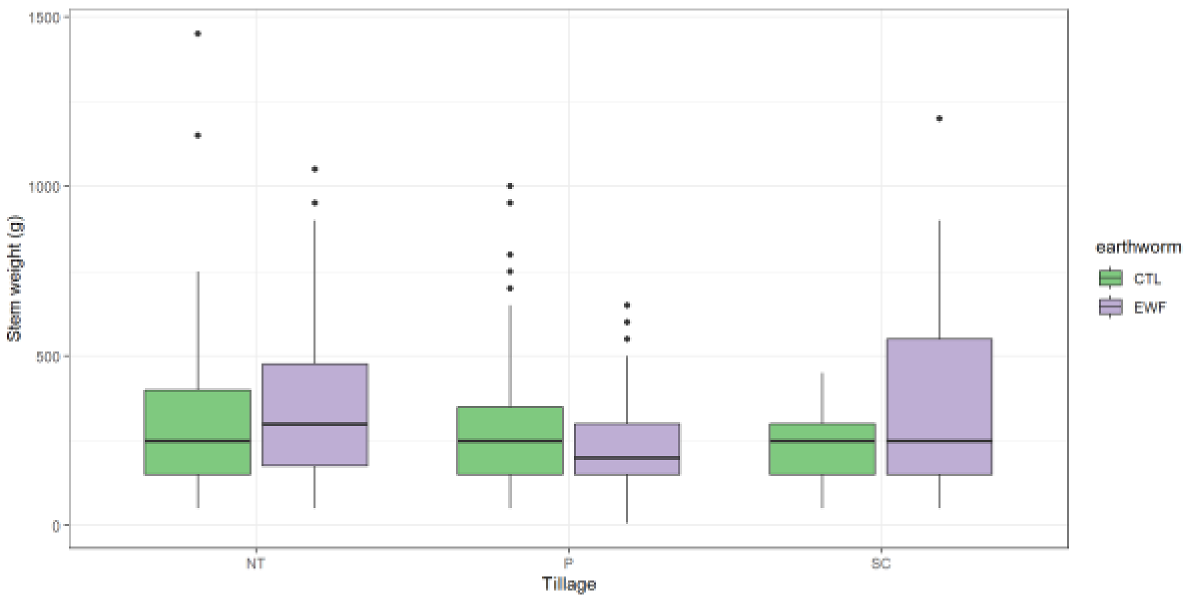

Figure 12. Stem weight of sunflower after harvest in 2021.

Although the direct effect of earthworms on stem weight was not statistically significant ($p > 0.05$), their interaction with tillage was highly significant ($p < 0.001$), emphasizing the combined role of biological and mechanical factors in enhancing crop growth. Earthworms increased stem weight and head diameter slightly, particularly under NT and SC methods, which minimize soil disturbance and support improved soil health. NT and SC practices promoted larger stem weights and head diameters compared to P, with NT producing higher variability and SC showing more consistent gains. These findings underscore the importance of adopting conservation tillage practices, such as NT and SC, which maintain soil integrity, enhance nutrient cycling, and foster beneficial soil organisms like earthworms, ultimately improving sunflower growth and productivity.

4. 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. Summary of 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.

6. Related publications

Ibrahim, **H.T.M**., Modiba, M. M., Igor, D., Simon, B., Muktar, M., Lisanwork, N. (2022): The Role of Mixed Cropping to Climate Change in Sofi District, Harari Regional State, Ethiopia. Journal of Central European Green Innovation, 75–87. doi: 10.33038/jcegi.3564.

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