



Effects of tillage and straw treatments on earthworm abundance

Study in two Swedish long-term field trials

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Abstract

Earthworms have several functions in the soil, for instance redistribution of organic matter and enhancing plant available nutrients. Earthworms are divided into three ecological groups, with different functions in the soil. The groups are: anecic, endogeic and epigeic. Studies has shown that tillage practices effect the earthworms, where often reduced tillage increase earthworm abundance in comparison to inversion tillage. However, the ecological groups of earthworms respond different to tillage method, where endogeic earthworms are less effected, in comparison to the anecic earthworms, which are more sensitive to an intensive tillage.

The aim of this study was to compare tillage treatments (conventional tillage, reduced tillage, and no-tillage) and straw treatments (incorporated straw or removed straw) regarding the earthworm abundance, and their relationship to soil properties. Five treatments were included in this study; conventional tillage with straw removed or incorporated, reduced tillage with straw removed or incorporated and no-tillage. Earthworms and soil properties (aggregate stability, basal respiration, penetration resistance and carbon, nitrogen, and phosphorus content) were measured in two long-term field trials near Lanna experimental station, in Lidköping, Sweden.

The results showed that the abundance of earthworms in total and juveniles where highest in the no-tillage treatments, and conventional tillage showed the lowest abundance for earthworms in total and juveniles. Also, earthworm biomass where highest in the no-tillage treatments. The abundance of adult and endogeic earthworms was highest in reduced tillage with straw incorporated, between the four other treatments there was no difference for the abundance. We did not see any effect of straw treatment in conventional tillage and no-tillage, however, in reduced tillage the straw treatments influence the abundance of adult and endogeic earthworms. Our study indicates that there is no difference between no-tillage and conventional tillage regarding the endogeic and adult abundance, but regarding the total abundance no-tillage is more favourable. Among the soil properties, turbidity/aggregate stability and total soil carbon was for instance correlated with earthworm biomass, and abundance of total earthworms and juveniles.

Keywords: anecic, conventional tillage, endogeic, no-tillage, reduced tillage

Table of contents

List of tables	7
List of figures.....	8
1. Introduction	9
1.1 Earthworms and soil properties	9
1.2 Ecological groups of earthworms.....	10
1.3 Tillage and earthworms.....	10
1.4 Organic matter and earthworms	11
1.5 Aim and research questions	12
2. Materials and methods	13
2.1 Location and treatment description.....	13
2.2 Earthworm sampling	14
2.3 Soil moisture	15
2.4 Water holding capacity.....	16
2.5 C, N and P measurements.....	16
2.6 Basal respiration measurements	17
2.7 Aggregate stability	17
2.8 Penetrometer measurements	19
2.9 Statistical analysis.....	20
3. Results	22
3.1 Earthworms in total and distribution of species and ecological groups	22
3.2 Tillage and straw treatments and earthworm presence.....	22
3.3 Effects and interaction between tillage and straw treatments	27
3.4 Correlation between soil properties and earthworms	30
4. Discussion	31
4.1 Effects of tillage and straw on earthworm presence	31
4.1.1 Tillage management	31
4.1.2 Straw treatment	33
4.2 Soil properties	34
4.2.1 Penetration resistance	34
4.2.2 Total carbon, nitrogen, and phosphorus content.....	35
4.2.3 Basal respiration	36

4.2.4 Aggregate stability	36
5. Conclusion.....	38
References	39
Populärvetenskaplig sammanfattning	43
Acknowledgements.....	45
Appendix 1	46
Appendix 2	47
Appendix 3	49
Appendix 4	50

List of tables

Table 1. Description of the management for the field trials.....	14
Table 2. Treatments in the study.....	20
Table 3. Treatments and presence of earthworms	23
Table 4. Effects of tillage and straw treatment.....	27
Table 5. Effects of single treatments on earthworm presence.....	29
Table 6. Correlation between earthworm presence and soil properties.	30

List of figures

Figure 1. Sampling of earthworms.	15
Figure 2. Turbidity measurement.	18
Figure 3. Turbidity measurement.	19
Figure 4. Penetrometer measurement.	20
Figure 5. Earthworm biomass and the response to treatments.	23
Figure 6. Abundance of earthworms and the response to treatments.	24
Figure 7. Abundance of juvenile earthworms and the response to treatments.	25
Figure 8. Abundance of adult earthworms and the response to treatments.	25
Figure 9. Abundance of endogeic earthworms and the response to treatments.	26
Figure 10. Abundance of anecic earthworms and the response to treatments.	26

1. Introduction

1.1 Earthworms and soil properties

Earthworms have several functions in the soil, as they distribute and decompose crop residues from the surface to deeper layers, creating burrows, and the cast contains plant available nutrients (Edwards and Arancon 2022). Earthworms also have an important role for the soil fertility (Bhadauria & Saxena 2009). Soil fertility is correlated with soil organic matter and a decrease in soil organic matter will cause the fertility to decrease as well (Pulleman et al. 2005). Soil organic matter can be protected by the earthworm activity, because the earthworms create a stable microaggregate structure in the soil, and a stable structure tends to protect the organic matter against decomposition (Pulleman et al. 2005). The storage of carbon have been shown to increase in water-stable aggregates >1mm with earthworm presence (Ketterings et al. 1997). In addition, a larger earthworm population can increase soil respiration, especially when organic fertilizers are applied, which may decrease the carbon storage in the soil (Schindler Wessells et al. 1997). The increased respiration is a result of increased microbial activity or a higher respiration by the earthworms (Schindler Wessells et al. 1997). Regarding soil nutrients, a meta-analysis done by van Groenigen et al. (2014) showed that the presence of earthworms seems to correlate with crop yield which can be increased by 25 %, which may be due to an increase in nitrogen mineralization. Another study showed that plant available nitrogen can be increased by earthworm activities, especially when inorganic fertilizer is applied (Bohlen & Edwards 1995). Moreover, earthworms casts have an important role, as it contains a higher amount of nutrients in comparison with the bulk soil, both macro- and micronutrients (Tomati & Galli 1995).

Earthworms also have an important role in improving the soil structure (Chan 2001). The soil structure can be enhanced by for instance the earthworm cast and the creation of burrows (Chan 2001). Earlier research have also shown that the amount of water-stable aggregates with >1 mm in diameter that can be increased by earthworm presence (Ketterings et al. 1997). Also, it has been showed that plant roots combined with earthworms in a soil increased the aggregate stability

compared to a soil with no plant roots, while presence of earthworms but without plant roots did not increase the aggregate stability (Fonte et al. 2012).

1.2 Ecological groups of earthworms

Earthworm species have different functions in the soil, through their movement patterns and food intake, therefore they can be divided into three ecological groups; anecic, endogeic and epigeic (Chan 2001). Epigeic earthworms have a fast growth rate and reproduction and can be found on the soil surface (Chan 2001), where they mostly feed on crop residues (Asshoff et al. 2010). *Lumbricus rubellus* and *Lumbricus festivus* are examples of epigeic earthworms (Sherlock 2012). Anecic earthworms feed on the organic matter at the soil surface and mix it in the mineral soil, where they often can be found (Chan 2001). The anecic earthworms create vertical burrows that are permanent and due to their movement and feeding behaviour, they translocate organic matter to the deeper soil layers and mineral soil are moved to the upper layers (Asshoff et al. 2010). For instance, *Lumbricus terrestris*, *Lumbricus castaneus* and *Aporrectodea longa* are examples of anecic earthworms (Sherlock 2012). Finally, endogeic earthworms mostly feed on mineral soil with humus content (Chan 2001) and create horizontal burrows that are not permanent (Asshoff et al. 2010). Some common endogeic species are *Allolobophora cholortica*, *Aporrectodea rosea*, *Aporrectodea caliginosa*. (Sherlock 2012) and *Aporrectodea tuberculata* (Hale 2013). In Sweden, earthworm species like *L. castaneus*, *L. terrestris*, *A. longa*, *A. cholortica*, *A. caliginosa*, *A. rosea* and *A. tuberculata* can be found (Torppa & Taylor 2022).

1.3 Tillage and earthworms

In this study, three tillage systems will be analysed, which are no-tillage, reduced tillage, and conventional tillage. No-tillage practices can be classified by direct drilling without any tillage before seeding (Lal 2013). Conventional tillage means that the soil is inverted and the tillage is often intensive (Jaleta et al. 2019). Reduced tillage is often classified where the intensity of tillage is reduced (Jaleta et al. 2019).

From previous research, there are several examples of how earthworms are affected by different tillage methods, and evidence that ecological groups respond differently to these practises. Epigeic and anecic species seem to be more negatively affected by more intensive tillage in comparison to the endogeic species (Briones & Schmidt 2017), while endogeic species seems to be less impacted by conventional tillage (Wyss & Glasstetter 1992; Briones & Schmidt 2017). Torppa & Taylor (2022) showed that the abundance of the endogeic earthworms was not varying between tillage methods (conventional, reduced and no-tillage), while the

anecic earthworm abundance was higher in the no-tillage practices (Torppa & Taylor 2022). In addition, according to Briones & Schmidt (2017), earthworm populations are decreasing with more intensive tillage. Tillage methods such as direct drilling have been shown to be more friendly for earthworms, in comparison to conventional tillage (Briones & Schmidt 2017). Results from a field trial showed that direct drilling practices favoured a higher amount of earthworms in comparison to conventional tillage in 8 of 14 sites, but the results also showed the opposite in some sites, where there was no difference or the amount of earthworms was higher in the conventional tilled plots (Kladivko et al. 1997). Another field trial experiment, showed that no-tillage practises had a larger earthworm population compared to reduced and conventional tillage (Eriksen-Hamel et al. 2009). In contrast, there is also studies showing no difference in total earthworm abundance between different tillage systems when they compared reduced and conventional tillage and no-till (Torppa & Taylor 2022). However, the earthworm community can have the capacity to be adapted to different tilled systems (Briones & Schmidt 2017). In Sweden, Boström (1995) showed that earthworms can recover one year after intensive tillage, where a rotary cultivator and a plough was used. In another study, Carter (1988) compared direct drilling and ploughing and showed that during the first year the earthworm population was higher in the direct drilling treatment, but after three years there was no significant difference between direct drilling and ploughing.

The impact on earthworms by different tillage methods mostly depends on the mechanical damage, caused by for instance a plough. Earthworms are also affected by changes in the physical and biological conditions in the soil, due to changes in temperature and the placement of crop residues when tillage is used (Chan 2001).

1.4 Organic matter and earthworms

Crop residue input can have an effect on the abundance of earthworms, but the placement of crop residues have been shown to be less important than the amount of crop residues (Frazão et al. 2019). A study in Sweden, conducted in a crop rotation with wheat, legumes and oil seed rape, showed that the total earthworm abundance increased in a more diverse crop rotation when they compared it to a crop rotation consisting only of wheat and barley (Torppa & Taylor 2022). Food supply may support the earthworm population. For instance, more earthworms were found in fields where wheat was direct drilled in a lay of white clover compared to direct drilled wheat and wheat drilled on ploughed soil (Schmidt et al. 2003). Meanwhile, no effect on the earthworms was found between direct-drilled wheat and conventional tilled wheat (Schmidt et al. 2003). In contrast, a study by Eriksen-Hamel et al. (2009) showed that the earthworm population was not affected by the input of residues. Also, a comparison between burned straw and chopped and

spread straw showed that the total earthworm amount is not affected by straw treatment, but the number of *L. terrestris* was for instance higher in the treatment with spread straw (Barnes & Ellis 1979). In addition, Nuutinen (1992) found that leaving the straw may have a positive effect on earthworm biomass, especially on *L. terrestris*. Earthworms feed on organic matter (Chan 2001) and it seems that earthworms prefer organic materials with a high amount of nitrogen (Ketterings et al. 1997). Another study showed that organic fertilizers, such as sewage sludge, have a positive effect on earthworm abundance compared to inorganic fertilizers, where sludge high in nitrogen seems to be favourable (Emmerling & Paulsch 2001).

1.5 Aim and research questions

This study aimed to compare different tillage methods (no-tillage, reduced tillage, and conventional tillage) and crop residue treatments (incorporation or removal of crop residues) and how it affects the presence of earthworms. The presence of earthworms was also connected to different soil properties. As earthworms have an important role in soil functions (Edwards & Arancon 2022) it is important to study how earthworms are affected by tillage practices. The following research questions were addressed in this study:

- How are earthworm abundance and biomass affected by different tillage methods (no-tillage, reduced tillage, and conventional tillage)?
- How are earthworm abundance and biomass affected by different crop residue treatments (incorporation or removal of crop residues)?
- How are the ecological groups of earthworms affected by different tillage systems and crop residue treatments?
- Is there any correlation between the presence of earthworms and soil properties (basal respiration, aggregate stability, penetration resistance and amount of carbon, nitrogen, and phosphorus in the soil)?

2. Materials and methods

2.1 Location and treatment description

The long-term field experiments used in this study, R2-4010 and R2-4017, are located at two different fields near Lanna experimental station, in Lidköping, Sweden. The soil of the two fields is classified as silty clay. All sample collection and measurements in field was conducted at the beginning of October 2022 and the fields were not tilled after the harvest at that time. The field experiment R2-4010 started in 1974 and has six different treatments with four blocks/replicates and in this study, four of the treatments were sampled (A1, A2, C1 and C2). The field experiment has three different tillage management methods in combination with two different straw treatments (Table 1). The whole experiment, if possible, is being stubble cultivated with different equipment after the harvest. In the last years a Väderstad Carrier has been used for stubble cultivation and the seed bed preparation was conducted by harrowing. The sowing machine was a Väderstad Rapid. The crop rotation was dominated by cereals, mostly oat and winter wheat and the crop of 2022 was spring barley.

The field experiment R2-4017 started in 1982 and has 12 different treatments in two blocks/replicates and in this study, six of them were sampled (A3, A4, C1, C2, C3 and C4). In this field experiment there are three different tillage treatments in combination with four different straw/tillage treatments (Table 1). The stubble cultivation, in the tilled plots, is conducted with different equipment, and the last year a Väderstad Carrier has been used. The sowing machine was a Väderstad Rapid. Treatments A3, A4, C3 and C4 are being stubble cultivated after the harvest and in treatments C1 and C2 there is no cultivation after harvest. The seed bed preparation in the tilled plots is conducted by harrowing. The crop rotation is dominated by cereals, mostly oat and winter wheat and the crop of 2022 was oat. Treatments C1 and C2 are direct drilled, without any tillage. Treatments C3 and C4 are similar to treatments C1 and C2 in R2-4010 because they are stubble cultivated after the harvest. As well, treatments A3 and A4 are similar to treatment A1 and A2 in R2-4010 because they are stubble cultivated after the harvest and then ploughed.

In the following part, the treatment names Ct-Sr, Ct-Si, Rt-Sr, Rt-Si and no-tillage will be used. Ct respectively Rt is conventional tillage (ploughing)

respectively reduced tillage (stubble cultivation), Sr respectively Si is straw removed respectively straw incorporated and no-tillage is direct drilling.

Table 1. A description of the management for R2-4010 and R2-4017, where the letters represent the tillage system and the numbers the straw system.

Field trial	Treatment	Management
R2-4010	A	Ploughing every year
R2-4010	B	Ploughing some years
R2-4010	C	No ploughing
R2-4010	1	Straw removed
R2-4010	2	Straw incorporated
R2-4017	A	Ploughing
R2-4017	B	Direct drilling, ploughing some years
R2-4017	C	Direct drilling
R2-4017	1	Straw incorporated
R2-4017	2	Straw removed
R2-4017	3	Straw removed and stubble cultivation
R2-4017	4	Straw incorporated and stubble cultivation

2.2 Earthworm sampling

The earthworm sampling was conducted by digging a 30 * 30 cm hole, with a depth of 20 cm. For each plot, there were three replicates, and the holes were distributed in a line from edge to edge, in the middle of the plot. The soil that was dug up was placed in a plastic bag and was hand sorted to find earthworms (Figure 1). In the soil pit after the soil was removed, 2 L of a mustard mix was poured to get the earthworms up from the deeper layers. The mustard mix was prepared by using 10 g of mustard powder (Coleman's Mustard) per litre of water. The mustard mix was left to infiltrate for 20 minutes, after that it was searched for earthworms (Figure 1). All earthworms from the same plot were collected in a jar. Later, the earthworms were cleaned with water and the biomass (of all the worms found per replicate/plot) was measured. For species identification, earthworms were preserved in jars in ethanol (80%). In the lab, the earthworms were counted and divided into juveniles and adults. The adult species were determined using different earthworm keys (Andersen 1997; Sherlock 2012; Hale 2013) and loupe, WILD MZ8 (Leica, Germany). The number of earthworms was then calculated to obtain the density/abundance of earthworms (individuals./m²).



Figure 1. The left picture shows how the earthworm measurements were conducted and the right picture shows the hole after the mustard mix was poured, with some earthworms coming up from the deeper soil layer.

2.3 Soil moisture

The soil was collected using a soil probe, which was hammered down in the soil to a 40 cm depth. The soil from the soil probe was divided into four depths, 0 to 10, 10 to 20, 20 to 30 and 30 to 40 centimetres. The soil measurements were taken close to the earthworm holes and two replicates per plot were conducted. The soil was collected in airtight plastic bags to avoid evaporation. The bags were stored in a cold room at 3 °C. When measuring the soil moisture in the lab, the plastic bags were kneaded to get the water from the inside of the bags to the soil. After that, the soil was placed in aluminium containers and the wet weight of the soil was recorded. All aluminium containers were placed in a dry oven with a temperature of 105 °C for 24 h. After 24 h, the soil dry weight was recorded (including the aluminium container). The soil dry weight was corrected by subtracting the weight of the aluminium container. And the soil moisture was calculated as follows:

$$W_1 - W_2 = W_3$$

$$(W_3 \div W_1) \times 100 = \% \text{ water}$$

Where W_1 respectively W_2 is the weight of wet soil respectively dry soil and W_3 is the weight of water.

2.4 Water holding capacity

In the lab the water holding capacity (WHC) was measured for each plot, and two replicates per plot were used. In total, 60 samples were prepared, 56 with 0-2 mm sieved soil and 4 controls without soil. For this, the filter paper was folded and put into a funnel that was placed in a bottle. Then, between 5 to 10 g of soil was added to each filter and deionized water was poured, even for the controls, until the soil was completely covered with water. At the bottom of each funnel, Parafilm was used to prevent water from leaching. After preparing all samples and adding the water, Parafilm was put on the funnel to avoid evaporation. After approximately 2 hours, the Parafilm from the bottom of the funnels were removed. After being drained over a night, the wet soil including the filter paper was weighed, as well as the controls. The weight of the dry soil, wet soil and wet filter paper was used to calculate the water-holding capacity.

The WHC was calculated according to the following formula:

$$\begin{aligned}W_1 - W_2 &= W_3 \\W_3 - W_4 &= W_5 \\W_5 \div W_3 &= WHC\end{aligned}$$

Where W_1 is the wet weight of soil and filter paper and W_2 is the average weight of four wet filter papers. W_3 respectively W_4 is the weight of wet soil respectively dry soil and W_5 is the weight of water. WHC is 100 % water-holding capacity. An average of the two replicates was taken to establish an approximation of WHC. The measurements of WHC were conducted to obtain information on an estimate of how much water to add to the soil used for the basal respiration measurements. For the incubation of the basal respiration samples deionized water was added to the soil to obtain 60 % of the WHC.

2.5 C, N and P measurements

For the carbon, nitrogen and phosphorus sampling a soil probe was hammered 20 centimetres down in the soil. Three replicates per plot were done and the samplings were conducted in field in connection to the earthworm sampling. The soil was collected in bags. At SLU Ultuna, the soil was air dried at a temperature of 34 °C in aluminium containers. After approximately 5 days, the dry soil was sieved to 0-2 mm particle size. The samples were sent to The Soil and Plant Laboratory at SLU to analyse total carbon (C), total nitrogen (N) and total phosphorus (P) content in the soil. The carbon and nitrogen of the soil sampled were measured by dry combustion on a TruMac CN (LECO Corp, USA) and the phosphorus was determined by using an ICP-AVIO 200 (PerkinElmer, USA).

2.6 Basal respiration measurements

In this study, for soil basal respiration measurements a respirometer (Respicond 96) was used. The respirometer is an automatic system that measures produced CO₂ every hour and consists of 95 jars, placed in a water bath with a temperature of 20 °C. Each lid has a conductivity cell with two platinum electrodes. In the conductivity cell, 10 ml of 0.3 M potassium hydroxide (KOH) was placed. The KOH captures produced CO₂, which leads to hydroxide ions being consumed and carbonate ions being produced. When carbonate ions are produced the conductivity decreases and the respirometer measures the changes in conductivity which is a measure of respiration (Nordgren 1988).

For the basal respiration measurements, the soil samples were taken from approximately 20 centimetres depth and soil from the whole depth was collected and placed in labelled plastic bags. Three replicates per plot were used and the samplings were conducted in field in connection to the earthworm sampling. At the lab, the bags were air dried at a temperature of 34 °C and after approximately 7 days the soil was sieved to 2 mm. Before measuring the basal respiration, the samples were pre-incubated for 7 days in a growth chamber, SED-41 (Percival, USA) at a temperature of 20 °C. Three replicates per plot were prepared, having a total of 84 samples and three controls, without any soil. In each jar, 20 g of soil was added, as well as deionized water to obtain approximately 60 % WHC. After the water was added, each bottle was covered with Parafilm to prevent evapotranspiration and placed in the growth chamber. One week after the pre-incubation the samples were placed in the respirometer. Before the start of the basal respiration measurements, 0.3 M KOH solution was prepared and added to the respirometer cells. After all jars were placed in the respirometer, the measurement started and the mg CO₂/h was measured for 7 days, but only the last four days of data were used due to the higher influence of CO₂ from the air during the first days. The mean value of the three controls was subtracted from the mean value for the last four days, and then calculated to obtain mg CO₂/h per 100 g soil, this measure was later used in the statistical analysis.

2.7 Aggregate stability

For the aggregate stability/turbidity measurements, the soil was collected between 0-20 centimetres depth with a spade. Soil close to the spade was not collected, due to the risk of destroyed aggregates. Also, the first soil layer, including plant residues, was not collected. Three replicates per plot were conducted and the samplings were conducted in field in connection to the earthworm sampling. The soil was collected and stored in a cold room at 3 °C. At the lab, the fresh soil from the jars was sieved by hand, by using two sizes of sieves, 1 and 8 mm and the soil

fraction between 1 and 8 mm was used in the measurement. In addition, “rainwater” was prepared by using deionized water mixed with a small amount of tap water, with an electrical conductivity between 5 and 10 $\mu\text{S}/\text{cm}$ which was measured by a conductivity meter, Cond 3310 (WTW GmbH, Germany). Approximately 7 g soil ($\pm 0,05$ g) of the sieved soil was placed in six containers with a strainer at the bottom, of the wet sieving apparatus (Royal Eijelkamp Company, Netherlands), (Figure 2). Then on six metallic containers, 70 ml of rainwater was poured, and the containers were placed in the wet sieving machine. The containers with soil were placed above the containers with rainwater. When the rainwater and soil were prepared, the wet sieving machine was turned on and it was running for 6 minutes, during that time the soil was “wetted”. After 6 minutes the plate was raised above the water and the containers with soil were laid on an angle so the water would drain from the soil, into the containers with rainwater. All containers with rainwater were poured into 250 ml jars. From the initial 6 samples, the filtrated water from 2 samples was placed in a jar, creating 3 subsamples per plot. All the jars were filled with additional rainwater to reach the 250 ml level and stored in a cold room at 3 $^{\circ}\text{C}$.



Figure 2. The picture shows the wet sieving apparatus with the containers containing soil above the metal containers containing rainwater.

The turbidity was measured using a turbidimeter TL2300, (HACH, USA), (Figure 3). The turbidimeter was turned on and the jars were taken out from the cold room an hour before measuring. Before each measurement, the jars were shaken and approximately 30 ml water from the jar was pipetted and poured into a glass

cylinder. The glass cylinder was placed in the turbidimeter, and the first turbidity was measured (Tub 1), where for instance clay and smaller aggregates are shown in the measurement (Sheldrick & Wang, 1993 see Bölscher et al. 2021). All jars were placed on a table and left undisturbed for 4.5 hours. After 4.5 hours, the second measurement was conducted (Tub 2), following the procedure previously explained, where particles of clay are shown in the measurement (Sheldrick & Wang, 1993 see Bölscher et al. 2021). For the statistical analysis one mean value was used from the replicates.



Figure 3. A picture of the turbidimeter, where the glass cylinder was placed below the black lid on the turbidimeter. The samples were in the bottles to the left.

2.8 Penetrometer measurements

The penetration resistance was measured using an electronic penetrometer, of the brand Eijelkamp (Royal Eijelkamp Company, Netherlands), the cone at the penetrometer had an angle of 60° and the base area was 1 cm^2 . The penetrometer was pressed down by hand vertically 40 centimetres into the soil (Figure 4) at five locations in each plot by the same person during all measurements. The measurements were conducted in field in connection to the earthworm sampling. The data of the penetration resistance was registered in the penetrometer and was later downloaded to a computer. A mean value for the profile 1-10 cm (PR 1-10) and 11-20 cm (PR 11-20) were calculated and used for the analysis.



Figure 4. The penetrometer was pushed down vertically in the soil.

2.9 Statistical analysis

For the statistical analysis, treatments from R2-4010 and R2-4017 that had similar management were grouped into one treatment (Table 2). The no-tillage treatments C1 and C2 in R2-4017 are used because they are not disturbed by tillage. The conventional tilled treatments (ploughing) are named by Ct, the reduced tilled treatments (stubble cultivation) by Rt, and when the straw is removed or incorporated the treatment is named by Sr respectively Si. Only in table 4 and 5 the no-tillage treatments are separated by straw treatment.

Table 2. A description over how the treatments in respectively field experiment (R2-4010 & R2-4017) was grouped together into one treatment.

Treatment	R2-4010	R2-4017	Description
Ct-Sr	A1	A3	Conventional tillage, straw removed
Ct-Si	A2	A4	Conventional tillage, straw incorporated
Rt-Sr	C1	C3	Reduced tillage, straw removed
Rt-Si	C2	C4	Reduced tillage, straw incorporated
No-tillage		C1 & C2	No tillage

For the statistical analysis, RStudio (R version 4.2.2) was used, and the normality test was conducted by using Shapiro-Wilk test. The data from basal respiration measurements were not normal distributed, and therefore were Log-transformed. All data was analysed by a one-way ANOVA and the significant data was analysed by a Post-hoc LSD test. When analysing the effects of tillage and straw treatment

on earthworm presence, a two-way ANOVA was used, followed with a Post-hoc test. No-tillage was also separated by straw treatment, incorporated, or removed when analysing the effects of tillage and straw treatment (Table 4 and 5). However, for this thesis, the no-tillage results in Table 5 are not discussed in depth as there are not enough replicates to provide an appropriate conclusion. Pearson correlation test was used to analyse the correlations between the biomass and abundance of earthworms in total, adults, juveniles and endogeic to the penetration resistance, turbidity, basal respiration and carbon, nitrogen, and phosphorous content. No separate statistical analysis was performed on anecic and epigeic earthworms because the data and transformed version of the data (rot/cubrot) were not normally distributed.

3. Results

3.1 Earthworms in total and distribution of species and ecological groups

In this study, 1001 earthworms were found and 316 of them were adults. Of the adults, seven different species were identified and those belonged to the three ecological groups. In the anecic group, 24 earthworms were found, belonging to one species, *L. terrestris* (Linnaeus 1758). In comparison, for the endogeic group, 286 earthworms were found, *A. caliginosa* (Savigny 1826) was the most common species in the group with a total of 221 individuals. The other species from the endogeic group were *A. tuberculata* (Eisen 1874) *A. rosea* (Savigny 1826) and *A. chlorotica* (Savigny 1826). Only six earthworms, belonging to the epigeic group were found and the species in that group were *L. Rubellus* (Hoffmesiter 1845) and *L. festivus* (Savigny 1826).

3.2 Tillage and straw treatments and earthworm presence

The effects of tillage and straw treatments on the presence of earthworms are shown in Table 3. For all earthworm measurements, there were significant ($P < 0.05$) differences between treatments of earthworm biomass, abundance of the total earthworm presence (ind./m²), abundance of adult and juvenile (ind./m²) and for endogeic earthworm abundance (ind./m²) (Table 3).

Table 3. Treatments (Ct-Sr, Ct-Si, Rt-Sr, Rt-Si and no-tillage) and earthworm presence. The measurements include: the biomass of earthworms (g), total earthworm abundance (individuals per m²), adult and juvenile abundance (individuals per m²), and endogeic earthworm abundance (individuals per m²)

Measurement	Df	F-value	P-value
Earthworm biomass (g)	4	18.356	<0.050
Earthworm abundance (ind./m ²)	4	24.092	<0.050
Adult abundance (ind./m ²)	4	13.503	<0.050
Juvenile abundance (ind./m ²)	4	27.498	<0.050
Endogeic abundance (ind./m ²)	4	12.581	<0.050

The average biomass varied from approximately 5 to 40 grams and no-tillage has the highest biomass of earthworms (Figure 5). The results show a significant difference in biomass between no-tillage and treatments Ct-Sr, Ct-Si, Rt-Sr and Rt-Si. Treatment Rt-Si has the second highest earthworm biomass and treatment Ct-Sr and Ct-Si did not differ from each other in biomass. The mean abundance of earthworms in total varied from approximately 60 ind./m² to 260 ind./m² (Figure 6) where no-tillage has the significantly highest abundance of earthworms. Treatments Ct-Sr and Ct-Si did not differ from each other in abundance, and they have the lowest abundance in comparison to the other treatments. Treatment Rt-Si has the second highest abundance.

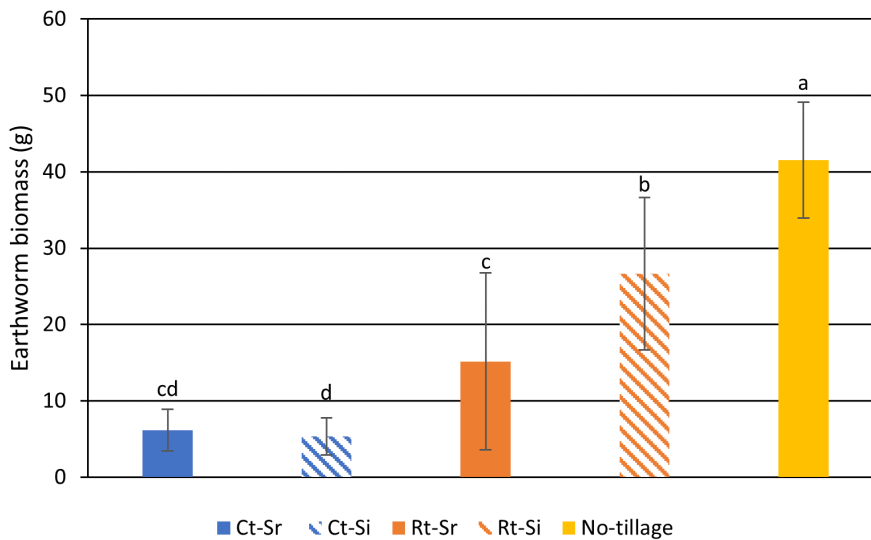


Figure 5. Mean value over the earthworm biomass in the five different treatments, where Ct is conventional tillage, Rt is reduced tillage, Sr is straw removed and Si is straw incorporated. The error bars show the standard deviation and bars with different letters have statistical differences. $P < 0.05$.

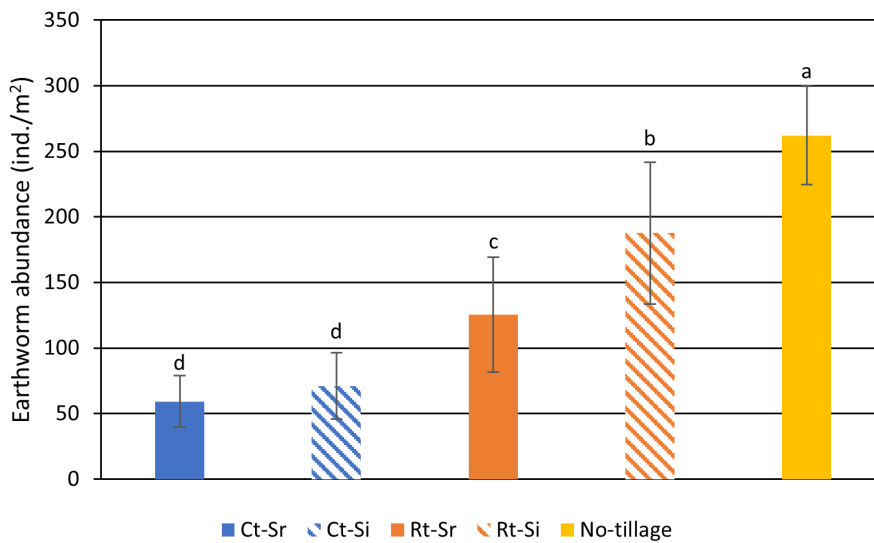


Figure 6. Mean abundance of earthworms in the five different treatments, where Ct is conventional tillage, Rt is reduced tillage, Sr is straw removed and Si is straw incorporated. The error bars show the standard deviation and bars with different letters have statistical differences. $P < 0.05$.

The abundance of juveniles varied from approximately 40 ind./m² to 220 ind./m² (Figure 7), where no-tillage has the highest abundance, while treatments Ct-Sr and Ct-Si have the lowest abundance. There is no significant difference in abundance between treatments Rt-Sr and Rt-Si, but the treatments have a significant difference in abundance between treatments Ct-Sr and Ct-Si and no-tillage. The adult abundance varied from approximately 20 ind./m² to 80 ind./m² (Figure 8), and in comparison, to e.g., total earthworm abundance no-tillage did not have the highest abundance. Treatment Ct-Sr, Ct-Si, Rt-Sr and no-tillage have no significant difference from each other in abundance, whereas treatment Rt-Si has the statistically highest abundance.

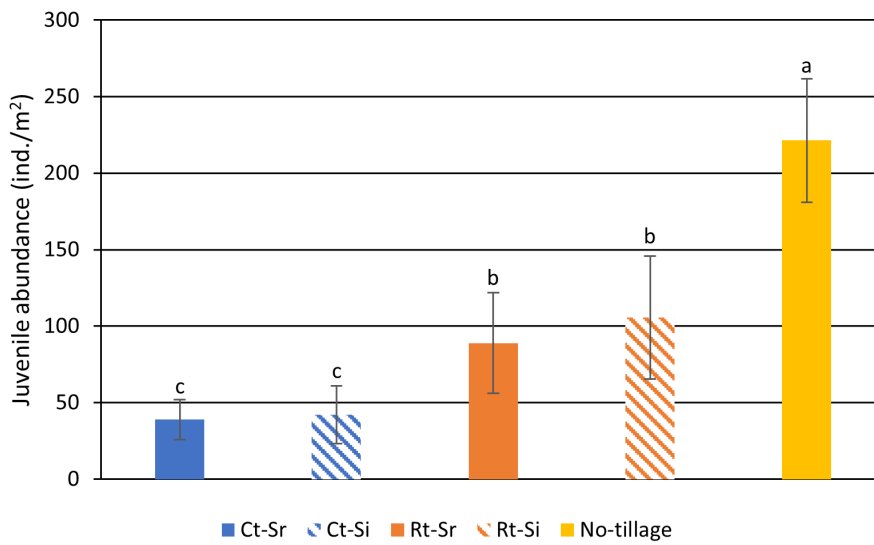


Figure 7. Mean abundance of juveniles (individuals/m²) in the five different treatments, where Ct is conventional tillage, Rt is reduced tillage, Sr is straw removed and Si is straw incorporated. The error bars show the standard deviation and bars with different letters have statistical differences. $P < 0.05$.

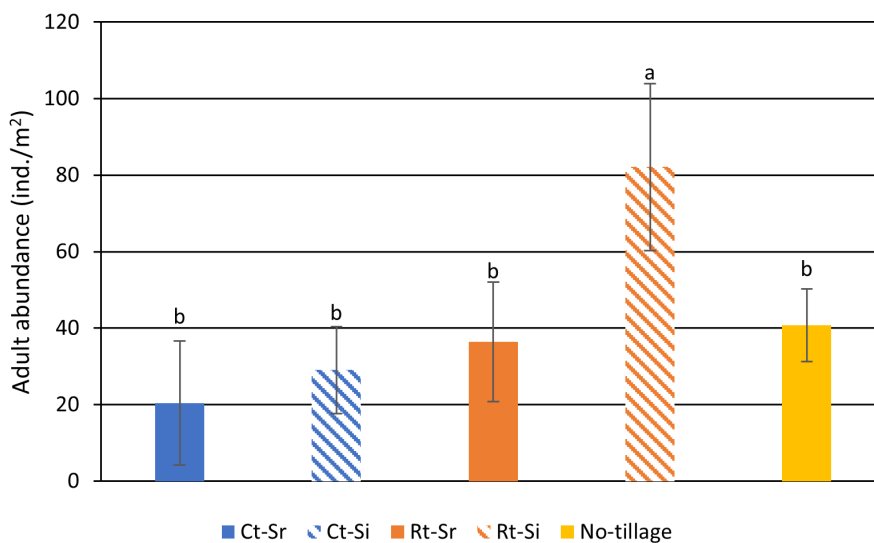


Figure 8. Mean abundance of adult earthworms (individuals/m²) in the five different treatments, where Ct is conventional tillage, Rt is reduced tillage, Sr is straw removed and Si is straw incorporated. The error bars show the standard deviation and bars with different letters have statistical differences. $P < 0.05$.

The abundance of endogeic earthworms varied from approximately 20 ind./m² to 80 ind./m² (Figure 9). It follows the same trend as the abundance of adults, where treatment Rt-Si has the highest abundance, while treatment Ct-Sr, Ct-Si, Rt-Sr and no-tillage did not differ from each other and showing a lower abundance than treatment Rt-Si.

For the abundance of anecic earthworms (Figure 10) no statistical analyses were conducted, as the number of individuals was too small to conduct any analyses.

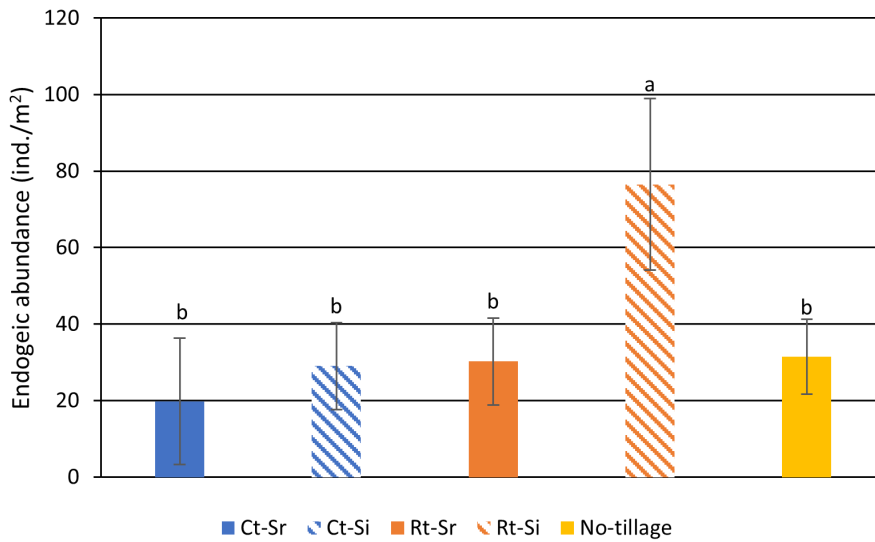


Figure 9. Mean abundance of endogeic earthworms (individuals/m²) in the five different treatments, where Ct is conventional tillage, Rt is reduced tillage, Sr is straw removed and Si is straw incorporated. The error bars show the standard deviation and bars with different letters have statistical differences. $P < 0.05$.

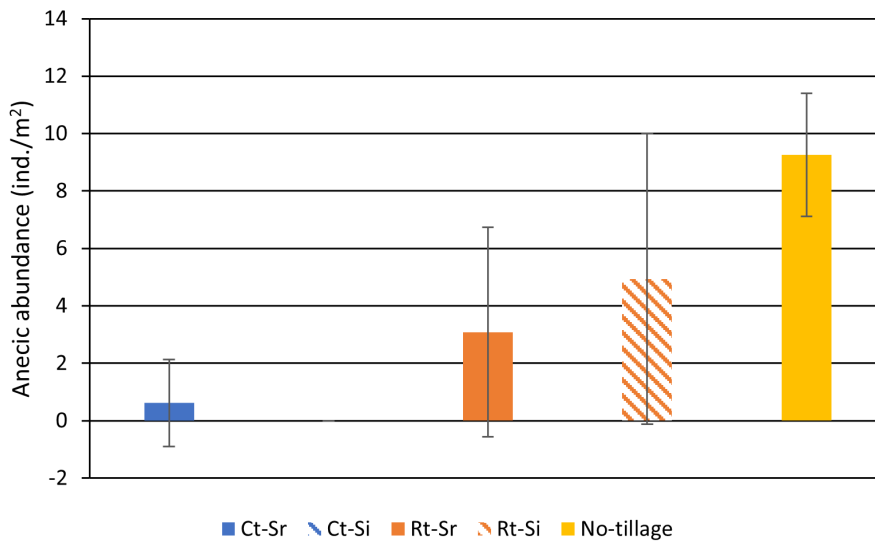


Figure 10. Mean abundance of anecic earthworms (individuals/m²) in the five different treatments, where Ct is conventional tillage, Rt is reduced tillage, Sr is straw removed and Si is straw incorporated. The error bars show the standard deviation. No statistical analysis was performed.

3.3 Effects and interaction between tillage and straw treatments

Our results show differences between treatments on earthworm biomass and abundance. To further understand these effects, we analysed the individual and interaction effects of tillage and straw management on these parameters (Table 4). Our results show that tillage and straw treatment have significant effects on adult abundance and endogeic abundance ($P < 0.05$) and there is also an interaction effect ($P < 0.05$). In contrast, the earthworm biomass, earthworm abundance and juvenile abundance show only effects of the tillage treatment ($P < 0.05$), but no effect of straw treatment.

Table 4. The results from the two-way ANOVA analysis of the effects of tillage and straw individually and their interaction. Significant effects are displayed in bold.

Measurement	F-value	P-value
Earthworm biomass		
Straw	2.006	0.171
Tillage	31.769	<0.050
Tillage:Straw	2.326	0.121
Earthworm abundance		
Straw	3.381	0.080
Tillage	32.936	<0.050
Tillage:Straw	1.434	0.260
Adult abundance		
Straw	13.119	<0.050
Tillage	11.459	<0.050
Tillage:Straw	4.646	<0.050
Juvenile abundance		
Straw	0.281	0.601
Tillage	43.952	<0.050
Tillage:Straw	0.309	0.738
Endogeic abundance		
Straw	14.238	<0.050
Tillage	9.014	<0.050
Tillage:Straw	5.471	<0.050

Moreover, we were able to identify which treatment combinations had significant effects on the earthworm parameters quantified (Table 5). For the earthworm biomass and earthworm abundance the tillage treatment had a significant effect when the straw was incorporated in the combinations, no-tillage – conventional tillage and reduced tillage – conventional tillage ($P < 0.05$). The tillage treatment also had an effect in the combination reduced tillage – no tillage and no-tillage –

conventional tillage when the straw is removed ($P < 0.05$). There is no effect of straw treatment for earthworm biomass and abundance. For adult abundance and endogeic abundance, the straw treatment had an effect in reduced tillage, also there is an effect of tillage treatment when the straw is incorporated in the combinations reduced tillage – conventional tillage and reduced tillage – no-tillage ($P < 0.05$). In addition, for juvenile abundance there is no effect of straw treatment. For juvenile abundance there is an effect of tillage treatment both when straw is removed and incorporated in the combinations, no-tillage – conventional tillage and reduced tillage – no-tillage ($P < 0.05$).

Table 5. Effects of single treatments on earthworm presence, where reduced is reduced tillage, conventional is conventional tillage, directdrill is no tillage, I is straw incorporated and R is straw removed. Significant effects are displayed in bold.

Measurement	P-value
Earthworm biomass	
Conventional:R-Conventional:I	0.999
No-tillage:I- Conventional:I	<0.050
Reduced:I- Conventional:I	<0.050
No-tillage:R- Conventional:R	<0.050
Reduced:R- Conventional:R	0.401
No-tillage:R- No-tillage:I	0.999
Reduced:I- No-tillage:I	0.499
Reduced:R – No-tillage:R	<0.050
Reduced:R - Reduced:I	0.100
Earthworm abundance	
Conventional:R-Conventional:I	0.891
No-tillage:I- Conventional:I	<0.050
Reduced:I- Conventional:I	<0.050
No-tillage:R- Conventional:R	<0.050
Reduced:R- Conventional:R	0.124
No-tillage:R- No-tillage:I	0.999
Reduced:I- No-tillage:I	0.503
Reduced:R – No-tillage:R	<0.050
Reduced:R - Reduced:I	0.151
Adult abundance	
Conventional:R-Conventional:I	0.711
No-tillage:I- Conventional:I	0.995
Reduced:I- Conventional:I	<0.050
No-tillage:R- Conventional:R	0.687
Reduced:R- Conventional:R	0.585
No-tillage:R- No-tillage:I	1.000
Reduced:I- No-tillage:I	<0.050
Reduced:R – No-tillage:R	0.999
Reduced:R - Reduced:I	<0.050
Juvenile abundance	
Conventional:R-Conventional:I	0.985
No-tillage:I- Conventional:I	<0.050
Reduced:I- Conventional:I	0.085
No-tillage:R- Conventional:R	<0.050
Reduced:R- Conventional:R	0.133
No-tillage:R- No-tillage:I	0.998
Reduced:I- No-tillage:I	<0.050
Reduced:R – No-tillage:R	<0.050
Reduced:R - Reduced:I	0.953
Endogeic abundance	
Conventional:R-Conventional:I	0.670
No-tillage:I- Conventional:I	0.999
Reduced:I- Conventional:I	<0.050
No-tillage:R- Conventional:R	0.906
Reduced:R- Conventional:R	0.870
No-tillage:R- No-tillage:I	1.000
Reduced:I- No-tillage:I	<0.050
Reduced:R – No-tillage:R	0.999
Reduced:R - Reduced:I	<0.050

3.4 Correlation between soil properties and earthworms

Earthworm biomass has a negative strong correlation with turbidity 1 and 2 (Tub 1 and Tub 2) (Table 6). Whilst basal respiration (BR) has a moderate positive correlation (0.48) to earthworm biomass, and there is also a strong positive correlation between carbon (C), nitrogen (N), phosphorus (P) and penetration resistance in the 1-10 cm depth and 11-20 cm depth (PR 1-10 and PR 11-20) with earthworm biomass. Moreover, earthworm abundance has a strong negative correlation with Tub 1 and Tub 2. While C, N, PR 1-10, and PR 11-20 have a strong positive correlation with total earthworm abundance. Finally, BR and P have a positive moderate correlation with earthworm abundance. Adult abundance has a moderate negative correlation with Tub 1 and Tub 2, also there is a moderate positive correlation with BR and PR 11-20. C, N, P and PR 1-10 have no correlation with adult abundance. For the endogeic abundance, there is only a moderate positive correlation with BR and PR 11-20. In comparison, juvenile abundance has a strong negative correlation with Tub 1 and Tub 2. For C, N, P, PR 1-10, and PR 11-20 there is a strong positive correlation with juvenile abundance. BR has a moderate positive correlation with juvenile abundance. How the soil properties differ between the treatments are shown in appendix.

Table 6. Results from the pearson correlation test with correlation coefficient (*r*) and *P*-values, from analysing biomass and abundance of earthworms and abundance of adult, juvenile and endogeic earthworms and how it is correlated between different soil parameters. Significant correlations are displayed in bold.

Earthworm parameter	Tub 1	Tub 2	BR	C	N	P	PR 1-10	PR 11-20
Earthworm biomass								
<i>r</i>	-0.780	-0.784	0.482	0.756	0.757	0.708	0.784	0.809
<i>P</i> -value	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Earthworm abundance								
<i>r</i>	-0.784	-0.796	0.609	0.748	0.759	0.648	0.725	0.800
<i>P</i> -value	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Adult abundance								
<i>r</i>	-0.391	-0.419	0.586	0.245	0.252	0.108	0.199	0.562
<i>P</i> -value	<0.050	<0.050	<0.050	0.208	0.196	0.586	0.310	<0.050
Juvenile abundance								
<i>r</i>	-0.785	-0.787	0.496	0.799	0.810	0.735	0.791	0.734
<i>P</i> -value	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Endogeic abundance								
<i>r</i>	-0.324	-0.340	0.561	0.167	0.172	0.020	0.106	0.487
<i>P</i> -value	0.092	0.076	<0.050	0.395	0.382	0.918	0.591	<0.050

4. Discussion

This study aimed to analyse the effect of tillage and straw treatments on earthworm presence and connect the presence of earthworms with different soil properties. No-tillage benefitted the biomass of earthworms and earthworm and juvenile abundance (Figure 5, 6 and 7), while reduced tillage with straw incorporated favoured adults and endogeic earthworm abundance more (Figure 8 and 9). We were able to see differences between conventional tillage, reduced tillage and no-tillage, and that are expected due a higher disturbance of the soil is disadvantageous for earthworms (Briones & Schmidt 2017). There was also an interaction effect of tillage and straw treatment for adult and endogeic abundance (Table 4). There was only an effect of straw management in reduced tillage, for the earthworm parameters adult and endogeic abundance (Table 5). As expected, earthworm biomass and earthworm and juvenile abundance correlated positively with total carbon content and soil respiration. Perhaps a bit surprising, earthworms also correlated positively with resistance to soil penetration. While adults and endogeic earthworm abundance did not show any correlation with carbon, nitrogen and phosphorus content (Table 6).

4.1 Effects of tillage and straw on earthworm presence

4.1.1 Tillage management

Earthworm biomass and abundance showed a similar trend as juvenile abundance when analysing the response to tillage treatments. That may be due to most of the earthworms found being juveniles. No-tillage treatment had the statistically highest abundance of earthworms in total and juveniles and the highest earthworm biomass (Figure 5, 6 and 7). These results are similar to other studies, for example Briones & Schmidt (2017) found that no-tillage practices will increase the abundance of earthworms in comparison to ploughed soils. The same study also found that non-inversion tillage does not affect the abundance of earthworms in comparison to ploughing (Briones & Schmidt 2017), which contradicts the results from this study. Our results show that reduced tillage increase the earthworm abundance and juvenile abundance, as well as earthworm biomass when straw is incorporated in comparison to conventional tillage (Figure 5, 6 and 7). However, in the study by

Briones & Schmidt (2017) reduced tillage implements tillage systems as shallow ploughing for instance, and in our study no inversion tillage was used in reduced tillage, which may explain the differences between the studies.

The adult abundance and endogeic earthworm abundance showed similar trends. This is related to the number of adult earthworms collected were in majority of endogeic species. For adult and endogeic abundance, reduced tillage with straw incorporated (Rt-Si) had a larger abundance than the other treatments (Figure 8 and 9). There was no significant difference between treatment Ct-Sr, Ct-Si, Rt-Sr and no-tillage according to the adult and endogeic abundance (Figure 8 and 9). Briones & Schmidt (2017) showed that endogeic earthworms were affected by tillage treatment, where there was a higher abundance when tillage is reduced. However, the abundance of anecic and epigeic species seems to increase at a higher rate when the tillage is reduced in comparison to endogeic species (Briones & Schmidt 2017). Also, Nuutinen (1992) found that *A. caliginosa* is less affected by tillage treatments.

According to our results, anecic earthworms seem to be more affected by tillage than endogeic species and the highest abundance of anecic earthworms was found in no-tillage and the lowest in conventional tillage (Figure 10). There is important to notice that no statistical analysis (e.g., ANOVA analysis) was done on anecic earthworms due to the data was not normally distributed. However, anecic earthworms have previously been shown to be more sensitive to intensive tillage (Briones & Schmidt 2017). Similar to our study, Wyss & Glasstetter (1992) found that endogeic species are less impacted in ploughed soil in comparison to the anecic species, which may be due to the smaller size of endogeic species. The anecic species showed a higher abundance than endogeic species in minimum tilled plots, and a lower abundance in ploughed plots than endogeic species, which may be due to the destruction of their vertical and permanent burrows by ploughing (Wyss & Glasstetter 1992). Also, Torppa & Taylor (2022) found that there were more anecic earthworms in no-tilled soils than in conventional tillage. The anecic earthworms may also be affected negatively by ploughing due to the crop residues is placed in a depth of approximately 20 cm, and they mostly feed on the soil surface (Chan 2001).

According to our study, juveniles seem to be more impacted by different tillage methods than adults. This can be explained by the high number of endogeic earthworms found and according to Wyss & Glasstetter (1992), the endogeic earthworms are less impacted by tillage and, most of the adult earthworms found in our study were endogeic. According to Bertrand et al. (2015), adult earthworms seem to be less affected by tillage methods in comparison to juvenile earthworms, which corresponds to our results. However, the result from our study contradicts the results from Emmerling (2001), where the abundance of adult and juvenile earthworms were decreased in ploughed soils, in comparison to non-inversion tillage. However, in the study by Emmerling (2001), the reduced tillage systems

differ from the reduced tillage systems in our study, due to in our study the last years a Carrier was used for stubble cultivation which has a quite shallow tillage and in the study by Emmerling (2001) the tillage depth was 30 cm, which may lead to different results. In studies where the impact of tillage on earthworms are examined, the tillage methods may differ, therefore it is important to describe how and when the tillage is conducted, as the type of tillage conducted has an impact on earthworms (Chan 2001). However, the results from our study demonstrates that tillage have effects on earthworms which can be explained by mechanical damage, changes in biological or physical conditions (Chan 2001) and also their exposure to predators (Briones & Schmidt 2017).

4.1.2 Straw treatment

For the adult abundance and endogeic abundance the incorporation of straw had a positive effect on earthworms, but only in combination with reduced tillage, while for the earthworm biomass and earthworm and juvenile abundance the straw treatment had no significant effect (Table 5). Our results for adult and endogeic earthworms contradict the findings of Eriksen-Hamel et al. (2009), where the earthworms were not affected by residue input in conventional, reduced and no-tillage. However, in the study by Eriksen-Hamel et al. (2009), there was no description of how straw treatment affects the earthworm population in different tillage methods, only how they were affected in general. In our study, there were differences in straw treatment between the tillage methods, where for instance straw treatment in conventional tillage does not have any effect on earthworm abundance while in reduced tillage straw treatment has an effect on adult abundance for instance (Table 5). The differences among studies may also occur due to different amount of crop residues, which is not specified in either our study or by Eriksen-Hamel et al. (2009). High removal of corn stover reduces the earthworm abundance in comparison to a lower rate of removal in no-tilled soils (Blanco-Canqui & Lal 2007b), which is similar to our results for earthworm abundance and adult and endogeic abundance in reduced tilled soils. But it is important to notice that in their study the earthworm species were not specified, and they only used corn residues (Blanco-Canqui & Lal 2007b). Also, a higher residue mulch rate from wheat can increase the earthworm population in no-tilled soils without any crops (Blanco-Canqui & Lal 2007a). Nevertheless, comparisons of the studies above to the results of our study are not easy to make, due to the different types of crop residues used in the field trials. However, it seems that crop residues may influence earthworms in non-conventional tilled soils. Why the straw treatment did not show any effect in conventional tillage in our study may be due to the residues in ploughed soils are not well mixed in the soil in comparison to reduced tillage. After ploughing, the straw mostly is placed deeper in the soil on the plough depth which may leads to less straw available for the earthworms.

It is important to notice that the results in our study may be affected by that the four replicates from the no-tillage treatment are only from one field trial (R2-4017), while for the other treatments (Ct-Si, Ct-Sr, Rt-Si and Rt-Sr) the treatments come from two different field trials (R2-4010 and R2-4017). The two field trials are located in two different fields, approximately 300 meters between the trials, which may have a different abundance of earthworms and soil properties, such as penetration resistance and carbon content. Otherwise, treatments Ct-Si, Ct-Sr, Rt-Si and Rt-Sr may be more comparable due to for each treatment, it was two replicates from R2-4017 and four replicates from R2-4010.

4.2 Soil properties

4.2.1 Penetration resistance

In our study, there was no significant correlation between penetration resistance at a depth between 1-10 cm to endogeic abundance (Table 6). However, at a depth of 11-20 cm, there was a significant positive correlation between penetration resistance and endogeic abundance. The positive correlation indicates that we could expect a higher abundance of earthworms in soils with higher penetration resistance at that depth. Adult abundance also had a significant positive correlation with penetration resistance in the depth 11-20, but not in the depth 1-10 cm. Earthworm biomass, earthworm abundance and juvenile abundance showed a significant positive correlation with penetration resistance in the depth 1-10 cm and 11-20 cm. According to Wyss & Glasstetter (1992) endogeic species are affected negatively by a more compacted soil in minimal-tilled plots, but it is unclear whether the tillage systems itself or the higher compaction that affected the endogeic species negatively. In that study they also found other endogeic species that is not presented in our study (Wyss & Glasstetter 1992), which may be an explanation of the differences between the studies. A correlation with penetration resistance and endogeic abundance at a depth between 11-20 cm is quite surprising due to a higher bulk density reduces endogeic burrowing activities (Capowiez et al. 2021) and the abundance of earthworms decreases with an increase in bulk density (Beylich et al. 2010). In the paper from Beylich et al. (2010), there was no description of how different earthworm species were affected which make the studies hard to compare. Crittenden et al. (2014) also showed that endogeic species are negatively affected by a higher penetration resistance. The positive correlations between earthworm abundance and penetration resistance in our study are not expected. However, penetration resistance is highly influenced on soil water content and during or before sampling it was raining, which may affect the penetration result. But, when comparing the soil moisture content with the penetration resistance, it seems that the soil moisture content is not affecting the penetration result. According to

Arrázola-Vásquez et al. (2022), the burrowing rates of *A. caliginosa* and *A. longa* will decrease with a higher penetration resistance. But regarding *A. caliginosa* the decreases was higher in comparison to *A. longa* (Arrázola-Vásquez et al. 2022). However, the burrowing rates and earthworm abundance may not correlate but the burrowing rates may indicate how earthworms are affected by penetration resistance. However, this correlations in our study may indicate that no-tillage or reduced tillage create other soil properties that are important for the earthworms and that there are several factors involved to create favourable conditions for earthworms. Even if there was positive correlation between earthworm abundance and penetration resistance, the resistance is may not too high to affect the earthworm abundance. In another study there was a high earthworm abundance in no-tillage treatments, while it also was a high penetration resistance (Dekemati et al. 2019) which is similar to our results. In Appendix 1, the average values of penetration resistance in the different treatments are shown, where no-tillage has the highest penetration resistance. In the depth 1-10 cm the average value varied between 0.95 and 1.62 MPascal and in the depth 11-20, it varied between 1.76 and 2.84 MPascal.

4.2.2 Total carbon, nitrogen, and phosphorus content

Adult and endogeic abundance had no significant correlation with C, N and P while earthworm biomass, earthworm abundance and juvenile abundance showed a significant positive relationship to the same parameters (Table 6). Adult and endogeic abundance may show the same trend, as most of the adult earthworms collected were endogeic species. No-tillage had the highest total C content, followed by treatment Rt-Si and Rt-Sr, and treatments Ct-Si and Ct-Sr had the lowest total C content. However, there are no significant differences in C content between treatments Rt-Sr & Rt-Si, Rt-Sr & Ct-Si and Ct-Si & Ct-Sr (Appendix 2). Ernst & Emmerling (2009) could not find any significant difference in soil organic carbon at 0 to 30 cm depth when comparing direct sowing with ploughing, but they could find that the anecic species *A. longa* seems to prefer a high SOC content. Hendrix et al. (1992) found a positive correlation between earthworms and soil organic carbon (0-5 cm depth) and that no-tilled plots had a higher soil organic carbon content than ploughed soils, which is similar to our results. If the different carbon content in the treatments depends on the tillage method or earthworm abundance is difficult to conclude. A meta-analysis showed that soil carbon in 0-60 cm depth does not change between conventional and no-tillage (Luo et al. 2010). In comparison, the carbon content will increase at 0-10 cm depth with no-tillage compared to conventional tillage (Luo et al. 2010). The carbon measurement in this study was taken at 0-20 cm depth, so the tillage method probably influenced the carbon content. However, soil organic matter may be protected by earthworm activity (Pulleman et al. 2005) and Bossuyt et al. (2005) showed that the soil carbon

can be protected by earthworms presence and compared with bulk soil, and also earthworm casts can increase the soil organic carbon (Zhang & Schrader 1993).

In the no-tilled treatment in our study, the carbon content was higher, which may explain the higher abundance of anecic species in these plots. Torppa & Taylor (2022) found a higher soil organic carbon content in no-tilled plots near the soil surface, and it can be explained by the anecic feeding preferences on organic carbon at the surface (Chan 2001). However, in our study, the carbon samples were taken at a depth from 0 to 20 cm, and it is hard to say if the soil surface contains more carbon as it is an average carbon content for the whole profile.

According to the nitrogen and phosphorus content, earthworm cast contains a higher level of nitrogen and phosphorus in comparison to bulk soil (Van Groenigen et al. 2019) which may be an explanation of the correlations shown in Table 6.

4.2.3 Basal respiration

In our study, we found a positive relationship between all earthworm parameters and basal respiration (Table 6). Multiple studies show contradictory results regarding the influence of earthworms on soil respiration. For instance, Schindler Wessells et al. (1997), showed that the presence of earthworms leads to higher soil respiration, and at the same time, the soil respiration depends on types of fertilizer used (e.g. inorganic fertilizer vs. manure) and the season. In addition, the soil respiration rate may depend on a higher microbial activity due to earthworm presence or by the direct respiration from the earthworms (Schindler Wessells et al. 1997). For instance, it has been reported that microbial respiration can increase by approximately 15 % when *L. rubellus* is present (Haimi & Huhta 1990). While Bossuyt et al. (2005) found that the presence of earthworms does not have any effect on soil respiration. The presence of earthworms affect the microbial biomass, where the microbial biomass can be decreased by the presence of endogeic earthworms (Scheu et al. 2002). In contrast, the casts of anecic earthworms may create favourable conditions for the microbial biomass (Medina-Sauza et al. 2019). However, according to Medina-Sauza et al. (2019) the effects of anecic and endogeic presence on microbial biomass are varying, where the presence of earthworms both can have a positive or negative influence on the microbial biomass. In Appendix 3 the average value of basal respiration is shown for each treatment.

4.2.4 Aggregate stability

Endogeic abundance does not show any significant correlation with turbidity (1 and 2) (Table 6). Whilst earthworm abundance, juvenile abundance, adult abundance, and earthworm biomass show a significantly negative correlation to turbidity. It is known that low turbidity means a low clay concentration (Etana et al. 2009) and a

high aggregate stability (Ulén et al. 2012). Therefore, the negative correlations imply a higher presence of earthworms (abundance or biomass) with an increase in aggregate stability. Our results are in agreement with other studies, which reported that earthworm presence may result in higher water-stable aggregates (Ketterings et al. 1997). However, we should also consider that higher aggregate stability might also be an effect of the tillage method rather than earthworm activity. The no-tillage treatment had the lowest turbidity (1 and 2), followed by both the reduced tillage treatment, while both the conventional treatments have the lowest turbidity (1 and 2), data shown in Appendix 4. Kasper et al. (2009) reported that minimum tillage can increase the level of stable aggregates in comparison with conventional and reduced tillage. This may be the case in our study, where no-tillage has a lower turbidity than the reduced and conventional tilled treatments.

The earthworm biomass and the abundance of earthworms and juveniles, have a significant correlation to all soil parameters (Table 6). However, it is unclear whether the presence of earthworms is affecting the soil properties, or if the relationships only is a result of different tillage and straw treatments. It is also important to notice when analysing the results in Appendix, that the no-tillage treatment is only from one field trial, while the other treatments (Ct-Si, Ct-Sr, Rt-Si and Rt-Sr) are from two different field trials.

5. Conclusion

This study aimed to compare the presence of earthworms in different tillage and straw treatment and connect the presence with soil properties. Our study confirms that the earthworm presence is affected by tillage methods, where the highest biomass and earthworm abundance are found in the no-tillage treatments. While the adult and endogeic earthworms are only affected positively by the tillage method reduced tillage, when straw is incorporated. In conventional tillage and no-tillage the straw treatment does not have any effect, however, in reduced tillage there was an effect of straw treatment according to the abundance of adult and endogeic earthworms. This study demonstrates that the effect of tillage may differ between species and ecological groups of earthworms. It seems that anecic earthworms were more affected than endogeic earthworms when comparing tillage methods. It is unclear if the earthworms are affecting soil properties such as carbon content, aggregate stability, and respiration or if the soil properties affect the earthworm presence in our field experiment. It can also be both, like a feedback cycle. However, earthworm biomass and abundance of earthworms and juveniles show a moderate or strong correlation to all soil parameters measured in this study. The findings in this study show that the type of tillage methods influence the presence of earthworms. A decrease in earthworms may lead to a lower mineral nitrogen and available phosphorus content in the soil due to the earthworm cast containing more mineral nitrogen and available phosphorus in comparison to bulk soil (Van Groenigen et al. 2019). Further research could study how crop yields corresponds to earthworm presence, because a relationship between those parameters may be an incentive for farmers to use tillage methods that benefits the earthworm population. Another way to achieve a more accurate results may be to sample earthworms over a longer period, due to the earthworm abundance may differ in the different tillage treatments depending on the year. However, the recommendation to create favourable conditions for earthworms is to implement no-tillage or reduced tillage. But other impacts of the tillage methods on agricultural management also need to be considered (e.g., plant diseases).

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Populärvetenskaplig sammanfattning

Daggmaskar har stor påverkan på markförhållandena, med avseende på bland annat markstruktur och cirkulering av organiskt material. Daggmaskar kan även ge en högre skörd, vilket kan bero på ökad kväveminerisering. Det finns olika grupper av daggmaskar; anecic, endogeic och epigeic, vilka har olika funktioner i marken. Anecic daggmaskar är relativt stora och gräver permanenta, vertikala maskgångar. De förflyttar sig från den djupare markprofilen till markytan där de hämtar föda, i form av växtrester. Växtresterna förs sedan ned i markprofilen vilket skapar en god cirkulering av växtrester genom hela profilen. Endogeic daggmaskar förekommer främst i matjorden där de intar föda i form av mineraljord berikad på organiskt material. Maskgångar av endogeic daggmaskar är horisontella och inte permanenta. Slutligen, epigeic daggmaskar befinner sig på markytan där de intar föda i form av växtrester.

Studier har visat att reducerad bearbetning eller direktsådd gynnar daggmaskar i högre grad jämfört med konventionell bearbetning som oftast inkluderar plöjning. Dock finns det skillnader mellan daggmaskgrupperna och hur de påverkas av jordbearbetning. Anecic daggmaskar påverkas oftast mer negativt av mer intensiv jordbearbetning, såsom plöjning, samtidigt som endogeic daggmaskar inte påverkas i samma utsträckning. Enligt vissa studier är det ingen skillnad i antalet endogeic daggmaskar mellan konventionell och reducerad bearbetning.

Syftet med denna studie var att undersöka hur daggmaskar påverkas i olika jordbearbetningsförsök med olika halmbehandlingar, med avseende på biomassa och antal i två långliggande fältförsök. Syftet var även att undersöka markegenskaper, som respiration och aggregatstabilitet, och att koppla dessa egenskaper med förekomsten av daggmaskar. Fem olika behandlingar undersöktes; direktsådd, reducerad bearbetning med halmen bortförd eller nedbrukad, samt konventionell bearbetning med halmen bortförd eller nedbrukad. Fältförsöken var lokaliserade på Lanna försöksstation utanför Lidköping.

Resultatet visade att biomassan av daggmaskar var högst i den direktsådda behandlingen. Antalet daggmaskar och juveniler var högst i den direktsådda behandlingen och den konventionella behandlingen hade lägst antal daggmaskar och juveniler. Antalet fullvuxna och endogeic daggmaskar var högst i den reducerade behandlingen med halmen nedbrukad, samtidigt var det ingen skillnad i antal för fullvuxna och endogeic daggmaskar mellan direktsådd och konventionell

jordbearbetning. Halmbehandling hade ingen effekt i den konventionella och direktsådda behandlingen men för reducerad bearbetning hade behandlingen med nedbrukad halm högre antal fullvuxna och endogeic daggmaskar. Resultatet visade även att det finns samband mellan aggregatstabilitet och antal daggmaskar, fullvuxna daggmaskar och juveniler, samt biomassan av daggmaskar, högre biomassa/antal gav högre aggregatstabilitet. Det är dock svårt att avgöra till vilken grad daggmaskarna i denna studie påverkar aggregatstabiliteten eller om aggregatstabiliteten främst påverkas av bearbetningsmetoderna.

Denna studie visar att vid direktsådd var antalet daggmaskar högre jämfört med reducerad och konventionell bearbetning, men att det inte finns någon skillnad för antalet endogeic daggmaskar mellan direktsådd och konventionell bearbetning. Slutsatsen är att daggmaskar påverkas av olika bearbetningsmetoder men att påverkan också skiljer sig mellan olika grupper av daggmaskar.

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Appendix 1

Table A1a. Mean value of penetration resistance in the depth 1-10 cm (PR 1-10) in the different treatments, P-value is <0.050. Mean value with different letters have statistical differences. No-tillage has the highest penetration resistance while there is no significant difference in penetration resistance between Rt-Si, Rt-Sr, Ct-Si and Ct-Sr.

Treatment	PR 1-10 (MPascal)
No-tillage	1.622a
Rt-Si	1.122b
Rt-Sr	1.120b
Ct-Si	0.973b
Ct-Sr	0.949b

Table A1b. Mean value of penetration resistance in the depth 11-20 cm (PR 11-20) in the different treatments, P-value is <0.050. Mean value with different letters have statistical differences. No-tillage has the highest penetration resistance, but there is no significant difference in penetration resistance between no-tillage and Rt-Si. Treatments Ct-Si and Ct-Sr have the lowest penetration resistance.

Treatment	PR 11-20 (MPascal)
No-tillage	2.842a
Rt-Si	2.614ab
Rt-Sr	2.352b
Ct-Si	1.934c
Ct-Sr	1.761c

Appendix 2

Table A2a. Total nitrogen content (N) in percentage, in the different treatments, P-value is <0.050. Mean value with different letters have significant differences. No-tillage has the highest N-content, followed by the reduced tillage treatments, the conventional tilled treatments have the lowest N-content.

Treatment	N (%)
No-tillage	0.233a
Rt-Si	0.188b
Rt-Sr	0.182b
Ct-Si	0.162c
Ct-Sr	0.160c

Table A2b. Mean value of total carbon content (C) in percentage, in the different treatments, P-value is <0.050. Mean value with different letters have significant differences. No-tillage has the highest carbon content, while treatment Ct-Sr has the lowest carbon content.

Treatment	C (%)
No-tillage	2.590a
Rt-Si	2.078b
Rt-Sr	2.015bc
Ct-Si	1.807cd
Ct-Sr	1.752d

Table A2c. Mean value of phosphorus content (P) in the different treatments, P-value is <0.050. Mean value with different letters have significant differences. No-tillage has the highest phosphorus content, while there is no significant difference in phosphorus content between treatments Rt-Sr, Rt-Si, Ct-Si and Ct-Sr.

Treatment	P (mg/kg)
No-tillage	587.406a
Rt-Sr	495.352b
Rt-Si	487.030b
Ct-Si	479.703b
Ct-Sr	471.739b

Appendix 3

Table A3. Mean value of Log-transformed mg CO₂/h per 100 g soil (BR) in the different treatments. P-value is <0.050. Mean value with different letters have significant differences. There is no significant difference in basal respiration between the treatments Rt-Si, Rt-Sr and no-tillage, while the treatments Ct-Si and Ct-Sr have the lowest basal respiration.

Treatments	mg CO ₂ /h
Rt-Si	-1.334a
Rt-Sr	-1.405a
No-tillage	-1.410a
Ct-Si	-1.636b
Ct-Sr	-1.694b

Appendix 4

Table A4a. Mean value of turbidity 1 (Tub 1) in the different treatments. P-value is <0.050. Mean value with different letters have statistical differences. No-tillage has the lowest turbidity, meaning that it has the highest aggregate stability. Treatments Ct-Sr and Ct-Si has the highest turbidity and the lowest aggregate stability.

Treatment	Tub 1 (NTU)
Ct-Sr	1812.444a
Ct-Si	1694.889a
Rt-Si	1094.278b
Rt-Sr	1070.722b
No-tillage	423.667c

Table A4b. Mean value of turbidity 2 (Tub 2) in the different treatments. P-value is <0.050. Mean value with different letters have statistical differences. No-tillage has the lowest turbidity, meaning that it has the highest aggregate stability. Treatments Ct-Sr and Ct-Si has the highest turbidity and the lowest aggregate stability.

Treatment	Tub 2 (NTU)
Ct-Sr	1045.500a
Ct-Si	1008.222a
Rt-Si	558.778b
Rt-Sr	535.278b
No-tillage	216.083c

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