



**University of Natural Resources and  
Life Sciences**

Department of Forest and Soil Science  
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# MASTER THESIS

## „Biological drivers of soil aggregate stability under different tillage intensities”

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## ABSTRACT

Soil tillage systems are in the focus of sustainable agricultural practices. Two different systems are often compared, conventional and conservation tillage. The mechanical disturbance during tillage has detrimental effects on soil organisms, like earthworms and fungal hyphae and hence reduces soil aggregate stability (SAS). Conservation tillage practices can reduce soil erosion and, hence, mitigate soil organic carbon (SOC) losses.

This study aims to determine the effects of long-term soil management on SOC concentration, SAS as well as fungal biomarkers and microbial necromass and potential relationships therein in bulk and rhizosphere soil and earthworm casts.

To elucidate tillage effects an experimental field that was subject to four different tillage intensities, (i) conventional tillage (CT), (ii) reduced tillage (RT), (iii) minimum tillage (MT) and (iv) no-tillage (NT) since 2006, was sampled summer 2019. On each of the four replicate plots earthworm enriched sub-plots were set up by installing fences and 15 *Lumbricus terrestris* were added. Soil samples were taken from bulk and rhizosphere soil of each plot and from earthworm sub-plots.

SAS analysis was negatively related with tillage intensity. Further, conservation tillage plots (NT and MT) showed highest amounts of SOC and DOC as well as ergosterol, glomalin and amino sugars. The highest values of the biological parameters were observed in MT, while SAS was highest in NT. This indicates stimulation of microbial growth by minor soil disturbance. Short term application of earthworms did not affect SOC but showed high DOC concentration. We conclude that conservation tillage practices such as MT are beneficial for SOC likely via soil biological parameters compared to CT.

Keywords: soil tillage systems, soil aggregate stability, carbon sequestration, earthworms

## KURZFASSUNG

Bodenbearbeitungssysteme stehen im Fokus nachhaltiger landwirtschaftlicher Praktiken. Oft werden zwei verschiedene Systeme wie die konventionelle und konservierende Bodenbearbeitung verglichen. Die mechanische Störung durch die Bodenbearbeitung wirkt sich nachteilig auf Bodenorganismen wie Regenwürmer und Pilzhyphen aus und verringert somit die Bodenaggregatstabilität (SAS). Konservierende Bodenbearbeitungspraktiken können Bodenerosionen verringern und somit Verluste an organischem Kohlenstoff (SOC) im Boden ausgleichen.

Diese Studie zielt darauf ab, die Auswirkungen von langfristiger Bodenbewirtschaftung auf die SOC-Konzentration, SAS sowie Pilzbiomarker und mikrobielle Nekromasse und mögliche Beziehungen in Massen-Boden und Rhizosphären-Boden und Regenwurm-Würfen zu bestimmen.

Zur Bestimmung der Bodenbearbeitungseffekte wurde ein Versuchsfeld untersucht, das seit 2006 vier verschiedenen Bodenbearbeitungsintensitäten unterworfen ist und im Sommer 2019 beprobt wurde: (i) konventionelle Bodenbearbeitung (CT), (ii) reduzierte Bodenbearbeitung (RT), (iii) minimale Bodenbearbeitung (MT) und (iv) keine Bodenbearbeitung (NT). Auf jedem der vier Replikatparzellen wurden mit Regenwürmern angereicherte Teilparzellen durch Installation von Zäunen angelegt und 15 *Lumbricus terrestris* Regenwürmer hinzugefügt. Bodenproben wurden aus Massen-Boden und Rhizosphären-Boden jeder Parzelle und aus Regenwurm-Teilparzellen entnommen.

Die SAS-Analyse war negativ mit der Bodenbearbeitungsintensität verbunden. Darüber hinaus zeigten konservierende Bodenbearbeitungsflächen (NT und MT) die höchsten Mengen an SOC und DOC sowie Ergosterol, Glomalin und Aminosackern. Die höchsten Werte der biologischen Parameter wurden bei MT beobachtet, während SAS bei NT am höchsten war. Dies weist auf eine Stimulierung des mikrobiellen Wachstums durch geringfügige Bodenbearbeitung hin. Die kurzfristige Anwendung von Regenwürmern hatte keinen Einfluss auf den SOC, zeigte jedoch eine hohe DOC-Konzentration. Wir schließen daraus, dass konservierende Bodenbearbeitungspraktiken wie MT für SOC womöglich über bodenbiologische Parameter von Vorteil sind, als im Vergleich zu CT.

## STATUTORY DECLARATION

I, André Wittmann, declare that I have written my master thesis on my own, independently, and I have not used any other sources than the ones declared as well as I have marked all literature quoted literally or by content. I also declare that I have not submitted this master thesis in any form as an examination paper in my home country, Austria, or abroad and that this master thesis is fully consistent with the master thesis assessed by the assessor.

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Date

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Signature

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# 1 INTRODUCTION

## 1.1 SOIL TILLAGE

Soil tillage as an agricultural management practice, describes the mechanical disturbance of the soil for beneficial soil condition to grow field crops. It is done on the one hand to prepare a clean seedbed, free of weeds, for next sowing, on the other hand for burying of crop residues of the previous crop, which acts as a natural fertilizer (Stiles, 2017). However, the turnaround of the soil also increases the risk of soil erosion and losses of soil organic C, especially, in hilly areas (Sherer et al., 2017). Soil erosion after precipitation can have dramatically consequences.

Badalíková, 2010, revealed that the soil moisture needs to be considered before soil cultivation. Soil cultivation has existed for a very long time in history, starting with human labor to machine-assisted work with tractors since the early 1900s (Macmillan et al., 2003). Machine based soil cultivation can be distinguished between conventional tillage as the typical ploughing, reduced and minimum tillage with a cultivator and disc harrow or no-tillage (direct seeding) method (Bista et al., 2017). Direct seeding systems leave the surface nearly complete covered with crop residues, while conventional tillage maintain about 30 % of left-over plant material on the surface. Further, no-tillage reduces the risk of erosion (e.g. wind and water erosion) by 95 % compared to ploughing (Vlăduț et al., 2015).

Certainly, the readiness of soil tillage is widely spread all over Europe. No-till systems can reach up to 10 % more yield on average, on arable land on silty-clay soils (Schlatter et al., 2014). Finland and France are pioneers in no-till practices; data is based on a survey from 2007-2008 where around 200.000 ha was managed with no-till (Derpsch et al., 2009). However, the disadvantages of no-tillage practices must also be taken into account. The University of Nebraska revealed that the increased input of large quantities of herbicides for weed removal as well as a slower soil warming rate, which can delay the shoot state of crops compared to conventional tillage fields. These can be questionable drawbacks for no-tillage agriculture (Anderson, 2018, Jasa, 2010).



## 1.2 SOIL AGGREGATE STABILITY (SAS)

Soil aggregate stability is explained by the fact that a cluster of soil particles is formed physically to shape the soil structure (Lynch et al., 1985). Amétketa, 1999, considered that soil aggregate stability is an essential driver, which affects a sustainable agricultural production.

High soil aggregate stability is beneficial for the pore system in the soil that provide air/oxygen for microbial processes and also plant available dissolved nutrients inside these pores. These pores serve as a habitat for soil flora & fauna and represent hot spots, where processes like mineralization, decomposition of organic matter, water infiltration and root penetration take place. These processes are vital to provide nutrients for plant growth (Gupta et al., 2008).

A negative influence on the soil structure can be induced by soil compaction. Reduced aggregate stability can also be caused by soil compaction, which is often a result of the usage of heavy machinery for soil tillage. It can further enhance soil surface runoff due to heavy precipitation (Are et al., 2018). Especially fine textured soils (i.e. clay-loamy soils) with low organic matter content, less soluble salts, high sodicity and a low exchangeable Ca:Mg ratio are prone to compaction (Hall, 2020).

Several soil tillage intensities can have diverse influences on the soil structure. While conventional tillage completely destroys soil aggregates, minimal or reduced tillage can correlate positive with stable aggregates in soil (Zhou et al., 2020). In fact, conventional tillage has more impact on the soil structure by turning over (ploughing destroys aggregates and organic matter), however, it is suggested to have less effect on the C storage potential in contrast to less soil management, (i.e. Paul et al., 2013).

## 1.3 SOIL ORGANIC CARBON (SOC)

Soils are the largest terrestrial C reservoir with 2.500 Pg, 1.550 Pg for soil organic C and around 950 Pg for soil inorganic C, C stored down to a depth of 1 m (Wani et al., 2016). Soil organic matter (SOM) contains C, and it is crucial importance to understand the stability of SOM and the processes how C can be sequestered (Lal, 2007). Soil organic C

can help to strengthen the role of soils to mitigate climate change effects (Amanullah et al., 2017). Analysis of 67 long-term agricultural experiments revealed that, on average, a change from tillage to no-till management can reduce C losses up to 43 – 71 g C /m<sup>2</sup> per year<sup>-1</sup>. Therefore, C can be sequestered or at least prevented from losses, if the soil is not left exposed and covered with catch crops (West et al., 2002).

Sustainable management practices in agricultural production are more important than ever. It is needed for several reasons. Losses of soil C can be accelerated, due to changing climate (e.g. more severe droughts during summer seasons), limited resources (e.g. phosphorus), incorrect soil management and global warming, increasing population, soil sealing and land consumption (Roston, 2017, White et al., 2012, Kassam et al., 2013). C and nitrogen contained in soil organic matter are essential for microbial growth and energy production (Lin et al., 2019). Hence, a well-balanced ratio of nitrogen and C is necessary for building stable soil organic C (Plant Natural Research Center, 2020).

In general, aboveground (from litter) and belowground (from root exudates) C inputs can be described the main drivers of SOC and leached DOC accumulation in soils. Belowground entry of C from plant root in rhizosphere soils are 190 % more efficient than aboveground entry from e.g. precipitation events and rhizosphere soils are enhanced with 340 % more stabilized C compared to bulk soil with 170 % (Sokol et al., 2019).

## 1.4 EARTHWORMS (*LUMBRICUS TERRESTRIS*)

The described earthworm species in this study is also known as the “common earthworm”, the *Lumbricus Terrestris* (Family: *Lumbricidae*), which belongs to the biggest species of earthworms with a mean length of 9 – 30 cm in Austria (Christian et al., 1999). This species is native in Middle Europe and parts of South-Eastern Europe (Blakemore, 2006).

The digging of tunnels up to a depth of three meters counted among to its usual way of life. Also, earthworms largely feed on half-rotted plant material, which is dragged into the earthworm channel and is digested there (Werner et al., 1990). The result finds itself on the surface again, also known as earthworm casts. This has positive impacts on the soil

quality and helps to increase the amount and mixing of soil organic material and to produce large term stable humus (Brady et al., 2009).

Earthworm activity in soil, promotes an improved soil aggregate stability because of macro aggregate formation, and also water holding capacity and can sustain plant growth even under severe drought periods (Blanchart, 1992).

Earthworms intensively plough the soil and promote cultivation by the formation of macropores and hence are important for soil structure. These earthworm channels can be very useful to plant roots for an easy root expansion (Nodirovna et al., 2019). According to a study of Klavivko et al., 1997, earthworm's abundance was low on more precise ploughing arable land compared to no-till operations in Indiana and Illinois, because of the destruction of the macropores with soil tillage activities. Earthworms increase soil aeration and water drainage in heavy rainfall events and hence water holding capacity due to the pores. Higher soil aeration improves the rate of decomposition of plant material because of microbial activity in soil, which can be abundant in and around the earthworm holes (Sims, 1981).

Hence earthworms are common for its use as a biomarker for a vital soil life, especially in organic crop farming (Persaud, 2019).

## 1.5 SOIL MICROBIAL BIOMASS

Soil microbial biomass plays an important role in terrestrial ecosystems (Araújo et al., 2010). It consists of bacteria and fungi and latter can be the determining factor for nutrient cycling in soil (Khan et al., 2016) (Ritz, 2005). Microorganisms, the living biomass in organic matter, can be responsible for soil quality with their functions of decomposition and mineralization of plant material and hence the recycling of nutrients, which are important for crop growth (Brookes, 2001) (Singh et al., 2018).

### 1.5.1 AMINO SUGARS

Amino sugars are part of the cell walls of microorganisms and are measured in soil to determine microbial necromass. These are summarized as mannosamine, muramic acid, galactosamine and glucosamine. The importance of microbial biomass in soil is widely known. Roberts et al., 2007, revealed in their study that microbial necromass contains a

considerable amount of organic C and plays a substantial role in building stable humus. While the microbial priming effect (higher decomposition rate of SOM after fresh organic matter input) is well investigated, the role of dead microorganisms and their contribution to stable SOC is still in its infancy. A recent concept, the so-called microbial C pump, was recently described by Liang et al., 2017. The authors suggest that when the proportion of necromass outbalances the priming effect, stable SOC is build up, (entombing effect). Studies revealed that soil microbial necromass as well as soil fungal dedicated to amino sugar content in soil (Glaser et al., 2006, Joergensen, 2018).

### 1.5.2 ERGOSTEROL

Ergosterol is a useful biomarker for detection of soil fungal biomass. Higher fungi concentrations lead to a higher decomposition rate in leaf litter (Gessner, 2020). In a study of Montgomery et al., 2000, undisturbed land showed higher fungal biomass than cultivated soils. Djajakirana et al., 1996, investigated a correlation between ergosterol and soil organic C and microbial biomass C in their study. Ergosterol, is an ingredient of cell-membranes of saprotrophic fungi, plays an important role as a biomarker for fungal activity in soils.

### 1.5.3 GLOMALIN RELATED SOIL PROTEIN (GRSP)

Glomalin related soil protein (GRSP) is a protein generally overserved in Arbuscular mycorrhizal fungi (AMF), which was first described by an US soil scientist (Wright et al., 1996). It is said that GRSP is located in their cell walls and it gives the soil it's tilth. Due to the fact it is a mycorrhizal fungus, the fungi help the plant to extend its roots and produces the GRSP, while the plant provides C for growth (Nichols et al., 2002). It is not fully discovered yet, but it is expected that the GRSP (Glycoprotein) is responsible for the storage of soil C because of its function of gluing the soil particles together, means a link between GRSP and SAS (Khursheed, 2016). Singh et al., 2012, revealed that the GRSP content in soil varies with different management practices with increased atmospheric CO<sub>2</sub> and GRSP contents in soil layers. GRSP is suggested to contribute more to SOC storage in deeper soil layers in comparison to near surface layers (Wang et al., 2017).

## 1.6 OBJECTIVES AND AIM OF THE STUDY

The aim of the study was to investigate long-term effects of different soil tillage intensities on soil aggregate stability and particle size distribution as well as the potential on soil organic C and biomarker aggregate stability under the influence of earthworms on arable land of various tillage intensities on an experimental field in Hollabrunn, Lower Austria, Austria.

Under this framework, not only soil structure related parameters like soil aggregate stability and particle size distribution of sand, silt and clay were determined, but also some other physical-chemical as well as microbiological parameters were taken into account. These analyses were conducted from bulk and rhizosphere soil from the experimental site.

Specifically, we tested the following hypotheses:

*H1: Soil aggregate stability declines with tillage intensity, due to physical disruption/destruction of aggregates and is related with higher SOC and DOC concentrations.*

*H2: Reduced soil tillage increases the C sequestration potential by a larger proportion of soil fungal abundance (which is related with higher microbial necromass).*

*H3: Earthworm casts are suggested to show higher concentration of DOC (and therefore stable SOC) followed by rhizosphere and bulk soils in conservation tillage compared to conventional tillage.*

## 2 MATERIAL AND METHODS

### 2.1 EXPERIMENTAL SITE AND SETUP

The experimental field (0.80 ha) is located in Hollabrunn, in the political district with the same name, in the region of the Weinviertel, north east of Austria (48.33.45.34 N; 16.3.53.72 E). This arable land is 238 m above sea level and part of an ongoing field trial with varying tillage intensities, which was established in 2006. The climate is Mediterranean with mean annual precipitation of around 617 mm and a mean annual temperature of 9.7°C (ZAMG, 2020).

The soil type, according to the World Reference Base (WRB) and the Austrian EBOD soil reference base, is a chernozem (CH) from loess with a pronounced accumulation of humus in the topsoil and according to the particle size scale it's a "Ts" (sandy silt) soil. The parent material is gravel. Loess is very common source material in the Weinviertel region, and it's very suitable for arable farming because of its carbonate rich properties (Wessely et al., 2006).

The sand content is 22.8 %, silt content 54.6 % and clay content 21.6 %. The pH-value (H<sub>2</sub>O) is 7.8 and soil is rich in carbonate with 9.9 %.





Figure 1: Experimental field at the Agricultural School of Hollabrunn, Lower Austria, AT. (Photo modified from Google Earth, 2020)

Table 1: Plot scheme on the experimental field

<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>2</b>	<b>1</b>	<b>4</b>	<b>3</b>	<b>4</b>	<b>3</b>	<b>2</b>	<b>1</b>
101	102	103	104	105	106	107	108	109	110	111	112

The plot scheme explains how the several tillage intensities were arrayed on the field.

- 1) Conventional Tillage (CT) – plough
- 2) Reduced Tillage (RT) – cultivator (“Grubber”)
- 3) Minimum Tillage (MT) – disc harrow
- 4) Non-Tillage (NT) – direct seeding on the preceding crop

In 2019, the plot scheme, was continued with the same order of the several tillage intensities. The treatments were investigated in 4 replicate plots: Conventional Tillage (CT), Reduced Tillage (RT), Minimal Tillage (MT) and Non-Tillage (NT), which were arranged on the field from west to east in different arrangement (see: Table 1).

*Zea Mays* was sown in spring 2019 and harvested in autumn 2019. The preceding crop in 2018 was a catch crop mix and before a legume crop (grain pea) was cultivated on the field.

The dimensions of the tillage plots are 6 x 100 m (w, l) and the size of earthworm plots are 20 x 20 x 200 x 300 cm (w, d, w, l) (see Figure 2). The earthworm fences were installed in April 2019 with a black colored plastic board, which was placed into the soil. Inside these fences 15 *Lumbricus Terrestris* per plot were released.



Figure 2: Installed earthworm fences (in the foreground) on the site (April 2019).

In the background there is a warning tape for the tractor.



## 2.2 SOIL SAMPLING AND MANAGEMENT

The soil sampling was conducted in July 2019 at the end of the shoot state from bulk and rhizosphere soil on the tillage plots. During sampling, the weather conditions were sunny and warm with 23°C (73°F) and the soil had rather dry conditions (Figure 3). For every plots, the soil showed a mean water content of 2.3 %.



Figure 3: Soil sampling in July 2019 with a spade.

On every plot, bulk soil was sampled with a spade from 0-10 cm depth inside the plant rows, rhizosphere soil was collected directly from the area of the rhizosphere of the maize crop, by shaking off the soil from the roots, and the visible earthworm casts, together with some soil material, were collected with a spoon from the topsoil inside each fence. A total ~1 kg bulk and rhizosphere soil were taken per tillage treatment. Unfortunately, earthworm cast material was limited due to dry conditions. Plot 107 was not able to sample for earthworm casts because we suggested a less population of earthworms inside this plot.

Soils samples were air-dried and stored at room temperature for several days and homogenized by sieving for < 2 mm, prior to further analysis.

A total of 24 samples from bulk and rhizosphere soil and 11 samples from earthworm cast material were taken from the field for laboratory analysis.

## 2.3 LABORATORY ANALYSIS

### 2.3.1 SOIL-CHEMICAL PARAMETERS

#### 2.3.1.1 GRAVIMETRIC WATER CONTENT

Five g of air-dried soil was put into a porcelain cup and dried in a laboratory oven (Mettler GmbH + Co. KG, Germany) at 105°C overnight. The dry weight was determined and calculated in % DM (dry matter) according to ÖNORM B 4410 (Austrian Standards, 2009).

#### 2.3.1.2 ELECTRICAL CONDUCTIVITY (EC)

Electrical conductivity was measured according to ÖNORM L 1099 (Austrian Standards, 2015). Briefly 5 g of sieved soil and 50 ml distilled water were combined in a plastic beaker. The samples were shaken overnight with a head over head shaker. After shaking, the samples were filtered through folded filters. EC measurements were conducted with a glass electrode from a WTW Multi 3420 device (WTW GmbH, Germany).

#### 2.3.1.3 PH-VALUE

The soil pH-value classifies the soils in acidity and alkalinity and is an indication for nutrient availability of the crop (Queensland Government, 2013). The determination of the pH-value was conducted according to ÖNORM L 1083 (Austrian Standards, 1999). Briefly, 5 g of soil blended with 25 ml of distilled water and interacted together over night and measured. The measurement was done with a pH-meter WTW Labor-pH-meter inoLab Multi 9620 and a pH-electrode SenTix 980 (both WTW GmbH, Germany) in suspension.

#### 2.3.1.4 SOIL AGGREGATE STABILITY

Soil aggregate stability was determined via wet sieving method after ÖNORM L 1072 (Austrian Standards, 2004). 80 ml of distilled water was added to steel beakers and 4 g of sieved soil from <2 mm was put into a 250 µm sieve. After 5 min of operation, while the sieve is constantly emerged and submerged, the content was fully stirred. The remaining content of the sample was put into a porcelain bowl and was put into a drying oven overnight at 105°C (Memmert GmbH + Co. KG, Germany). After drying the samples were suspended in 25 ml tetrasodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7 \times 10\text{H}_2\text{O}$ ) and dispersed for 2 hours. Finally, the samples were thoroughly rinsed to remove impurities (biomass) The remains were put back into the porcelain cup and dried overnight at 105°C.

The determination of the SAS in % was done with the following formula.

$$SAS (\%) = \frac{m_{A,S} - m_s}{m_p - m_s}$$

$m_{A,S}$  = mass of stable aggregates and sand in g

$m_s$  = mass of sand in g

$m_p$  = mass of probe (sample)

The analysis was done in 3 technical replicates and the results are the means of the values.

#### 2.3.1.5 DISSOLVED ORGANIC CARBON (DOC)

The DOC content was determined for soil samples by using 10 g of sieved soil and extracting it with 100 ml distilled water and a magnetic stirrer in a plastic beaker. The stirrer was responsible for the dispersion of the soil in the beaker. The sonication was done with an ultrasonic homogenizer Bandelin Sonopuls HD 2200 with a booster horn SH 213G (Bandelin electronic GmbH, Germany). The probe was put into the soil water suspension and operated for 2 min with 1 cm immersion depth and a 2 µm probe amplitude. After sonication, the sample was filtered through a folded filter (0.45 µm Munktell Ahlstrom) and additional syringe filtration (0.45 µm nylon membrane filter, both VWR International, USA), to remove particles that interfere with absorbance measurements.

The solutions were used for DOC measurements using a UV-VIS diode array spectrophotometer (Agilent 8453, Agilent Technologies, USA) equipped with Flow Injection Analysis (FIA) and a 1 cm quartz cuvette. Absorbance was read at a wavelength of 254 nm. The absorbance reads were transformed into DOC concentration according to the equation from Brandstetter et al., (1996).

$$DOC (mg L^{-1}) = 0.449 * A_{254nm} (m^{-1}) + 1.0$$

*A = Amplitude*

#### 2.3.1.6 PARTICLE SIZE DISTRIBUTION (PSD)

The particle size distribution is describing the distribution of different particle sizes in the soil. According to the ÖNORM L 1061-2 (fine pores) (Austrian Standards, 2001), the measurement of sand content was done with 10 g of soil which was added to 25 ml tetrasodium pyrophosphate ( $Na_4P_2O_7 * 10H_2O$ ) for destroying soil aggregates. After 7 hours of suspension of the soil in a 100 ml plastic beaker the samples were shaken overnight with a head overhead shaker. The samples were then passed through a wet vibration sieving tower (Fritsch analysette 3 PRO, Fritsch GmbH, Germany) and washed for 2 min. (or as long as there were no impurities coming out of the drain hose) with an amplitude of  $0.2 \text{ mm min}^{-1}$ . Results are given as mass fractions of 630  $\mu\text{m}$  (coarse sand), 250  $\mu\text{m}$  (medium sand) and 63  $\mu\text{m}$  (fine sand), calculated from initial weight and final weight of the porcelain bowls. The silt and clay fractions were estimated with the finger method (Government of Western Australia, 2019).

#### 2.3.1.7 TOTAL CARBON (TC)

For total elemental analyses of C and N, oven dry soil was ground in a ball mill, Retsch MM 200 (Retsch GmbH, Germany), for homogenization. An amount of  $\sim 2 \text{ mg}$  was weighed with a Sartorius fine weight scale (Sartorius AG, Germany) and put into tin cups. These cups were put into a Thermo scientific Flash smart CHNS/O Elementar Analyzer (Thermo Fisher Scientific, Inc., USA) equipped with an autosampler and carrier gas He as well as two quartz pipes with  $1030^\circ\text{C}$  and  $650^\circ\text{C}$  for incineration. The oven temperature was  $40^\circ\text{C}$ , carrier flow  $120 \text{ ml min}^{-1}$ , oxygen  $110 \text{ ml min}^{-1}$  and reference flow  $100 \text{ ml min}^{-1}$ . The C in the samples was combusted and measured with a thermal conductivity detector. Reference material was used for calibration, and values calculated in  $\%_{\text{w/w}}$ .



#### 2.3.1.8 CARBONATE CONTENT – (C<sub>INORG</sub>)

The amount of carbonate (CaCO<sub>3</sub>), which is given in mass %, was measured with a Scheibler apparatus, according to ÖNORM L1084 (Austrian Standards, 1989). Briefly, 1g of air-dried soil was put in a glass beaker and carefully 10 ml of 6 M hydrochloric acid (HCl) were added by shaking the beaker. The glass beaker is connected with a column that is filled with liquid to determine that volume that is replaced C dioxide (CO<sub>2</sub>). After 10-15 min of continued shaking of the beaker, the volume of C dioxide (CO<sub>2</sub>) was evaluated volumetrically (volume of CO<sub>2</sub> outgassed) in ml. The conversion for CaCO<sub>3</sub> was done by multiplication of voluminal CO<sub>2</sub> with air pressure and 1.204 divided by 273.3 added with °C room temperature multiplied with mass weight soil.

#### 2.3.1.9 TOTAL ORGANIC CARBON (TOC)

For the calculation of the TOC, the amount of the inorganic C content (C<sub>inorg</sub>) in % was subtracted from the total C (TC) content measurement. The result was the TOC content in mass %-w/w.

#### 2.3.1.10 SOIL ORGANIC MATTER (HUMUS)

The soil organic matter content was calculated by multiplying the total organic C (TOC) in % with the factor of 1.72 according to Scheffer/Schachtschabel (Amelung et al., 2016). The SOM content is given in %.

### 2.3.2 MICROBIOLOGICAL PARAMETERS

#### 2.3.2.1 ERGOSTEROL

The fungal biomarker ergosterol was extracted according to Gong et al., 2001 with minor modifications. Briefly, 3 g of soil was combined with 30 ml of methanol and shaken overnight with a head overhead shaker. In order to better mix the samples, they were ultrasonicated. After this process the soil-methanol extract was filtered with 0.45 µm Munktell Ahlstrom folded filters and centrifuged for 10 min with a Heraeus Multifuge 3s (Thermo Fisher Scientific Inc., USA) on the following day.

0.5 ml of filtrate from the previous day was transferred into HPLC vials and subsequently analyzed with an Agilent 1100 series HPLC device connected with a UV wavelength detector (VWD) G1314A, set at wavelength 282 nm. The chromatographic separation was

conducted with a C18 column in reversed phase mode. The mobile phase was methanol with 5 % H<sub>2</sub>O. The ergosterol concentrations ranging from 0.78 to 25 ppm served as a standard. The retention time for ergosterol was set for 6 min and 50 sec.

#### 2.3.2.2 GLOMALIN RELATED SOIL PROTEIN (GRSP)

The analysis of the Glomalin Related Soil Protein (GRSP) requires a glomalin extraction and protein determination according to the Bradford assay. It is a rapid method for determination of the protein in the solution with a Coomassie Brilliant Blue G-250 (Bradford Reagent) dye (Bradford, 1976). The absorbance of the dye together with the (protein) sample can be measured spectrophotometric at a calibrated wavelength of 595 nm (Ninfa et al., 2010).

For the determination of the Easy Extractable Glomalin (EEG) 1 g of soil (from 1-2 mm) was amended with 8 ml of citrate buffer (20 mM, C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub> x 2H<sub>2</sub>O 5.882 g l<sup>-1</sup> H<sub>2</sub>O) with pH 7 to a 15 ml vial for centrifugation and autoclaving. After autoclaving the soil suspension process at 121°C for 30 min in a Centroclav high-pressure sterilizer CV II/1600 (Kelomat GmbH, Austria) the samples were cooled down for a couple of min and centrifuged at 3,800 rpm at 10°C (equals: 5.327 x g) for 15 min with a table centrifugation device Hettich Rotanta 460 R (Andreas Hettich GmbH & Co. KG, Germany). The supernatant was collected in an additional 15 ml vial for later analysis of EEG with the Bradford protein assay, see below.

The left-over pellet from the EEG vial was then blended with 8 ml citrate (50 mM, C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub> x 2 H<sub>2</sub>O 14.705 g l<sup>-1</sup> H<sub>2</sub>O) with pH 8 and homogenized as much as possible for the extraction of the Total Glomalin (TG). For example, a Vortex machine (VWR International, USA) as well as an ultrasonic bath device Elmasonic S 100H (Elma Schmidbauer GmbH, Germany) was used to stir the soil particles in the vial. After a complete homogenization, the vial was autoclaved again for 60 min at 121°C in the Centroclav, mentioned above. The samples were again centrifuged at 3.800 rpm for 15 min and the supernatant was put into a 50 ml vial. The weight was noted. This procedure was repeated once, and the supernatants were combined to get a pale red colored suspension. The red color is an indication of Glomalin, which was determined subsequently with the Bradford assay.

The determination of the GRSP and therefore the concentration of proteins was done with the Bradford assay. Standards were also necessary for the analysis, therefore Bovine serum albumin (BSA) (12 mg per 12 ml A.d., Sigma A3, 156-5 g) was chosen. An exact concentration of the standards was necessary because of the tendency to absorb water of the proteins (Bradford, 1976).

The standards (10, 20, 30, 40, 75, 100 ppm) as well as 20 µl of suspension were pipetted double in a clear 96-well plate next to each other and filled up with 20 µl distilled water, done with an Eppendorf single-chancel microliter pipette (Eppendorf Austria GmbH, Austria). 200 µl of Bradford reagent was added into the well plate and the samples were measured for the protein concentration, bound by the dye, immediately at 595 nm with a spectrophotometric plate reader Enspire Multimode 2300 (Perkin Elmer Inc., USA).

#### 2.3.2.3 AMINO SUGARS

This method for the determination of the soil amino sugars: glucosamine ( $C_6H_{13}NO_5$ ), galactosamine ( $C_6H_{13}NO_5$ ), mannosamine ( $C_6H_{13}NO_5$ ) and muramic acid ( $C_9H_{17}NO_7$ ) is strongly related to a study conducted by Keiblinger et al., 2018. A study where an optimized HPLC method was used for the determination of amino sugars and muramic acid from soil samples from a field trail in Marchfeld, Austria.

The extraction of amino sugars is related to Appuhn et al., 2004 and Indorf et al., 2011. 500 mg of sieved soil was mixed with 10 ml 6 M HCL and boiled at 105°C for 6 hours. 0.5 ml of concentrate was dried at 40°C and evaporated with an additional 0.5 ml H<sub>2</sub>O and finally centrifuged with an additional 1 ml H<sub>2</sub>O. The final supernatant was frozen for -18°C and saved up separation.

Amino sugars are separated using a HPLC and detected with a fluorescence detector. In particular, for the separation, mobile phase A contains 5 mM Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (sodium citrate) from 13.436 g and 5 mM C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub> (sodium acetate) from 354 mg with a pH-value of 5.3. The solution was filtered with a 0.45 µm nylon membrane filter and 15.24 ml is withdrawn from it and 7.62 ml C<sub>4</sub>H<sub>8</sub>O Tetrahydrofuran (THF) as well as 7.62 ml CH<sub>3</sub>OH (Methanol) is added to the solution. The desired solution equates proportional to 90 : 8.5

: 0.75 : 0.75 %. The mobile phase B contains 500 ml Millipore H<sub>2</sub>O and 500 ml Methanol, which correlates to a ratio of 50:50 %.

The detection requires a pre-column derivatization procedure, for that a sample or standard volume of 2.0 µl was mixed with 20 µl of C<sub>8</sub>H<sub>6</sub>O<sub>2</sub> (Ortho-Phthaldialdehyde [OPA]) reagent and 5 µl of 10 mM H<sub>3</sub>BO<sub>3</sub> (Sodium Borate) buffer with pH 8.2, according to a related amino sugar determination from a study from Appuhn et al., 2004. The whole derivatization was an automated procedure using an G4226A autosampler connected with an HPLC (Agilent 1290 infinity series). The injection volume was 0.50 µl and. The binary pump G4220A had a flow of 1.5 ml min<sup>-1</sup> with a stop time set for 21 min.

A Zorbax Eclipse Plus C<sub>18</sub> RR 4.6x100 mm was used for chromatographic separation. The fluorescence spectroscopic detection (FLD) G1321A was done with an excitation wavelength of 330 nm and an emission wavelength of 445 nm with an PMT gain factor of 17. The temperature in the thermostatic column compartment G1316A was set to 50°C.

The calibration curve consisted of glucosamine, galactosamine, mannosamine and muramic acid and came up with 0.625, 1.25, 2.5, 5.0 and 10.0 ppm

## 2.4 STATISTICAL EVALUATION & PREPARATION OF DATA

The program Sigma Plot was used for the statistical evaluation and graphical illustration. Tukey's HSD, multiple comparison test (post-hoc test) was used for the comparison of the means as well as two-way ANOVA for the differences between the treatments (tillage intensities) and soil type (bulk, rhizosphere and earthworm cast). The raw data was transferred into MS Excel and individual calculation was done separately.



## 3 RESULTS

### 3.1 SOIL AGGREGATE STABILITY

Soil aggregate stability showed higher stability with reduction of tillage intensity. There was a strongly significant effect of increased tillage treatment on aggregate stability. No significant interactions occurred between treatment and soil type as well as between the two soil types bulk and rhizosphere soil ( $p > 0.05$ ). Further, there was no significant difference between MT and NT, but the SAS of MT and NT were about double that of CT. The total means of the soil type for bulk soil (33.5 %) and for rhizosphere soil (34.5 %) showed no significant differences as well as MT (42.2 %) and NT (43.3 %), but there is a significant difference between the means of CT (19.4 %) and NT (43.3 %) and for bulk as well as rhizosphere soil. However, no difference between bulk and rhizosphere soil was determined regardless of tillage.

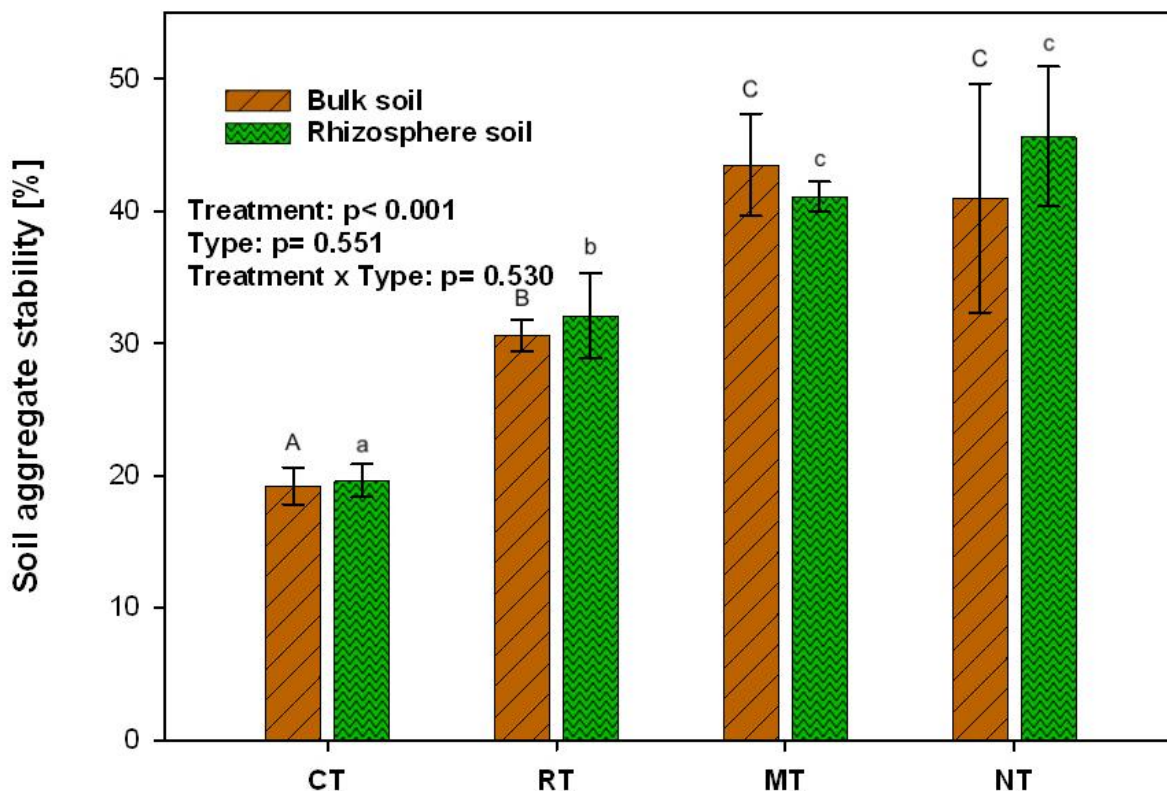


Figure 4: Soil aggregate stability in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and sampling positions were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

## 3.2 SOIL ORGANIC CARBON

The highest SOC content was found in the MT tillage plots with an amount of 1.6 % each in bulk soil rhizosphere soil, compared to the lowest organic C with 1.2 % and 1.3 %, found in CT plots, see Figure 5.

With regard to the results of the two-way ANOVA the treatment effect (tillage) was most prominent, with a trend for higher SOC content in rhizosphere soil compared to bulk soil albeit not significantly. The post-hoc analysis showed no significant differences between the several tillage intensities. However, there was found a trend to higher concentrations of SOC in rhizosphere soil in RT and NT plots (see \* in Figure 5).

Figure 6 shows that there is a relationship between SAS and SOC with a linear regression coefficient ( $R^2$ ) of 0.86 (86 %) and outliers in SOC content among all treatments.

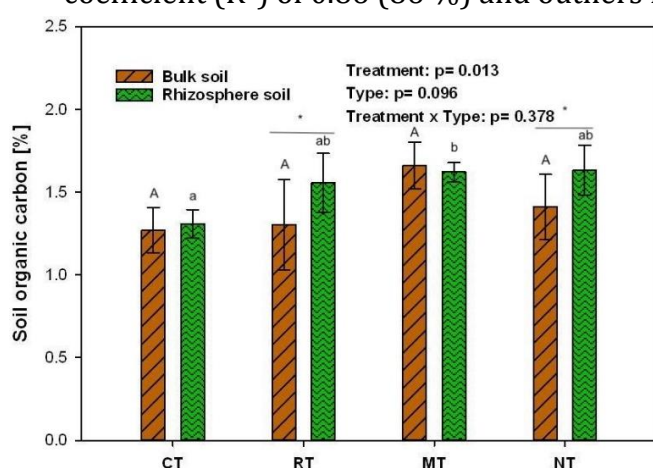


Figure 5: Soil organic C in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicate trend for unassimilated concentrations in bulk rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

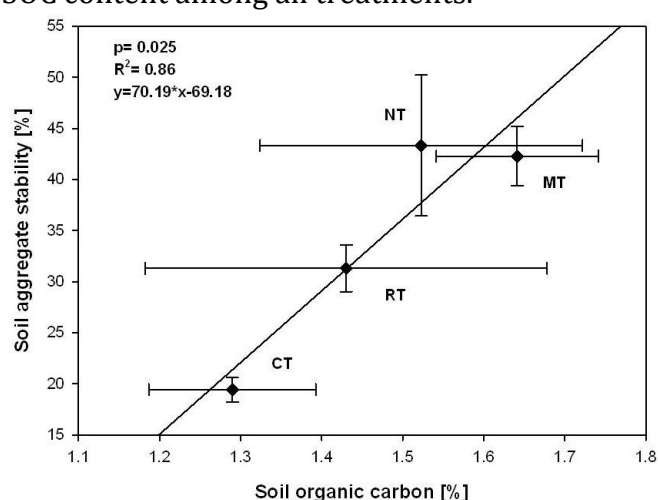


Figure 6: Linear regression of mean values of bulk and rhizosphere soils of soil aggregate stability and soil organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

### 3.3 DISSOLVED ORGANIC CARBON

There is no significant effect of the interaction between treatment and type ( $p = 0.209$ ). There is a significant difference in the DOC content between MT plots and CT plots. Highest with a mean DOC content of  $217 \text{ mg kg}^{-1}$  in MT (bulk and rhizosphere soil) and  $144 \text{ mg kg}^{-1}$  in CT plots respectively. There is a significant difference between these two treatments in regard to DOC. Therefore, MT plots showed the highest DOC concentrations with values about 50 % higher than conventional tillage. The NT plots showed a trend to higher DOC concentration in rhizosphere soil compared to bulk soil, see Figure 7.

Figure 8 shows that ( $R^2 = 0.87$ ) 87 % of the variability in the dataset of SAS is explained by the regression line of the DOC content.

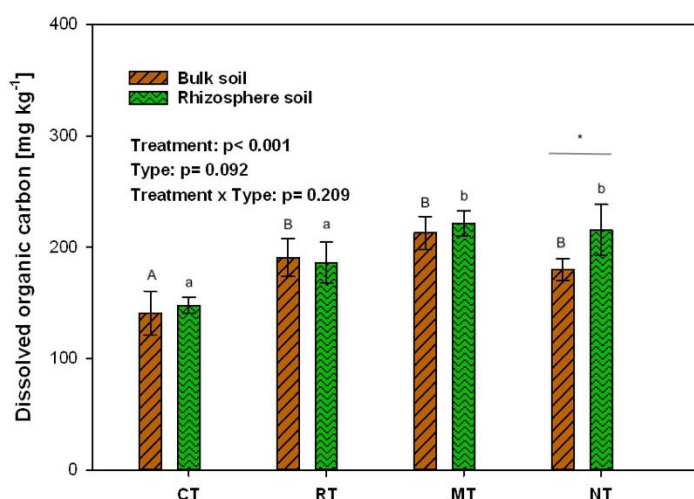


Figure 7: Dissolved organic C in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicates trend for unassimilated concentrations in bulk and rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

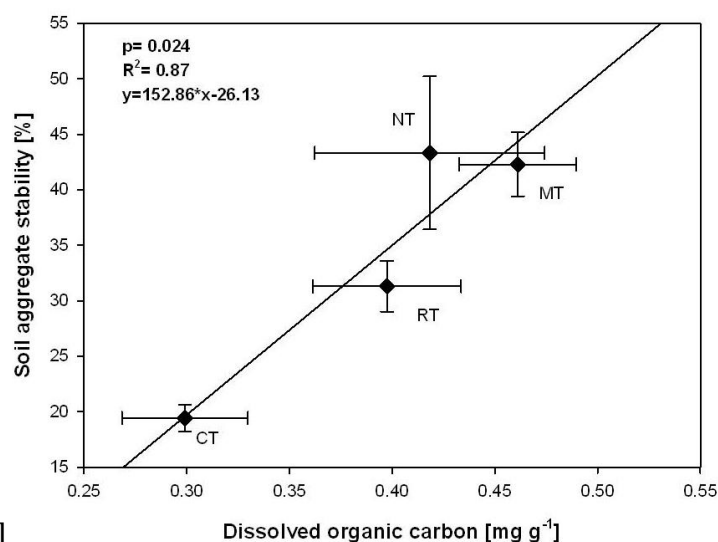


Figure 8: Linear regression of mean values of bulk and rhizosphere soils of soil aggregate stability and dissolved organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

### 3.4 ERGOSTEROL

The results of the ergosterol analysis showed that the treatment effect was highly significant ( $p < 0.001$ ). CT showed significantly lowest ergosterol concentrations, while highest concentrations of ergosterol were observed for MT (and RT). The Tukey's HSD test showed that MT plots were significantly different to CT and NT plots, see Figure 9.

Highest ergosterol concentration was found in MT plots albeit there was no significant difference between bulk and rhizosphere soil. However, again, there is a trend to higher concentrations in rhizosphere soil compared to bulk soil. Please see \* in Figure 9. There were found large variabilities in ergosterol contents among all treatments, especially in rhizosphere soils in RT and NT plots.

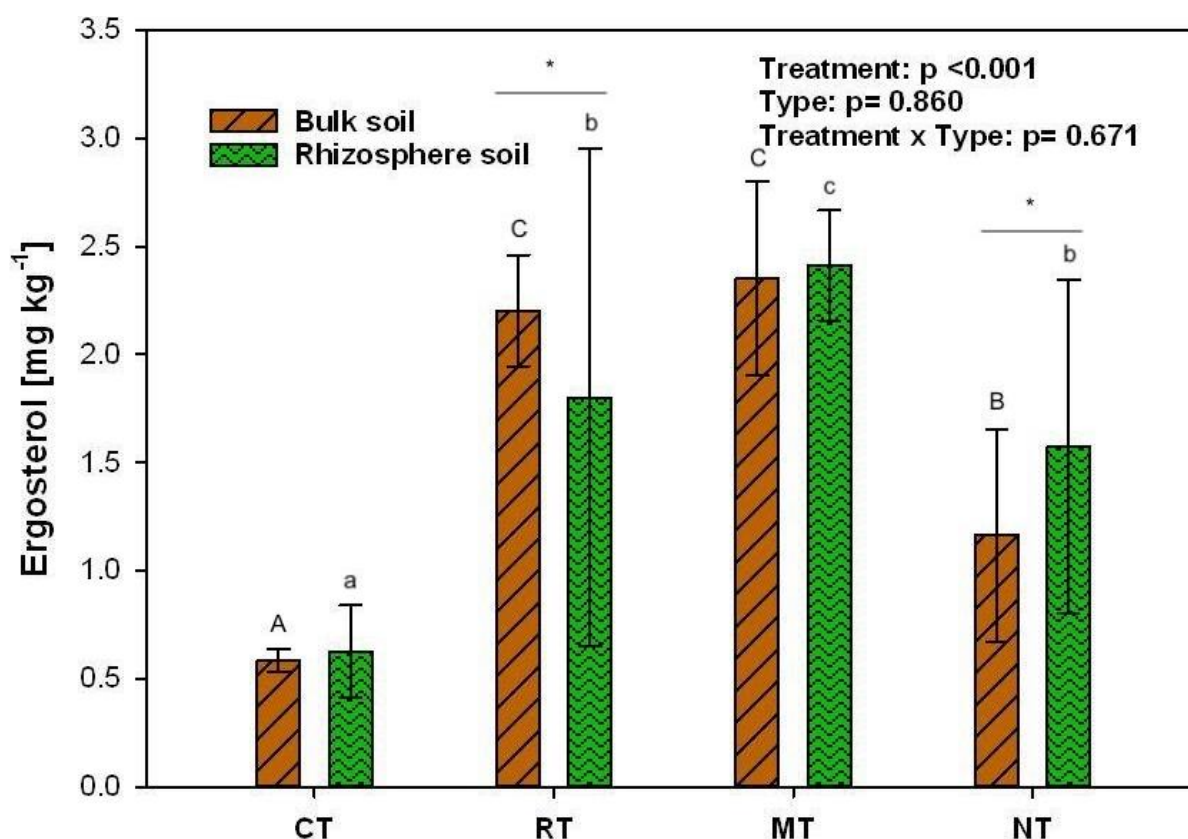


Figure 9: Ergosterol in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicate trends to unassimilated concentrations between bulk and rhizosphere soils as well as higher variability in rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

Figure 10 and 11 show the relation of ergosterol with SOC and DOC, respectively. In Figure 11, there is a higher significant difference between the RT and NT compared to CT than in Figure 10. Both Figures show significant interactions between ergosterol and DOC & SOC ( $p < 0.05$ ). Further there are similar analysis results noticeable in both figures, but SOC has a large variability especially in NT and RT plots, see Figure 10.

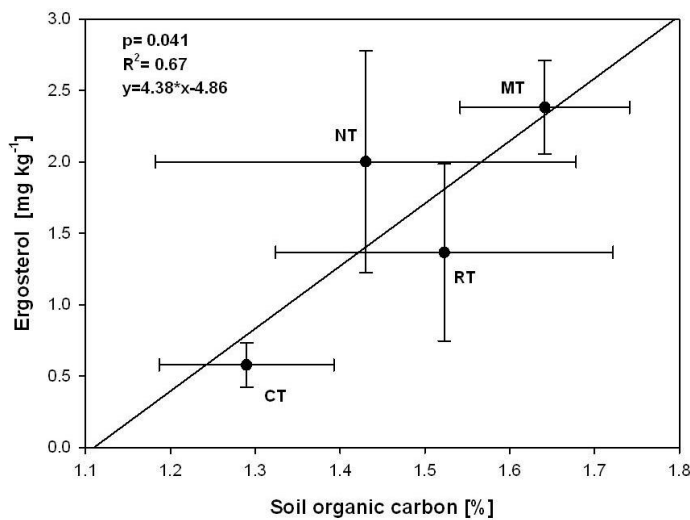


Figure 10: Linear regression of mean values of bulk and rhizosphere soils of ergosterol and soil organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

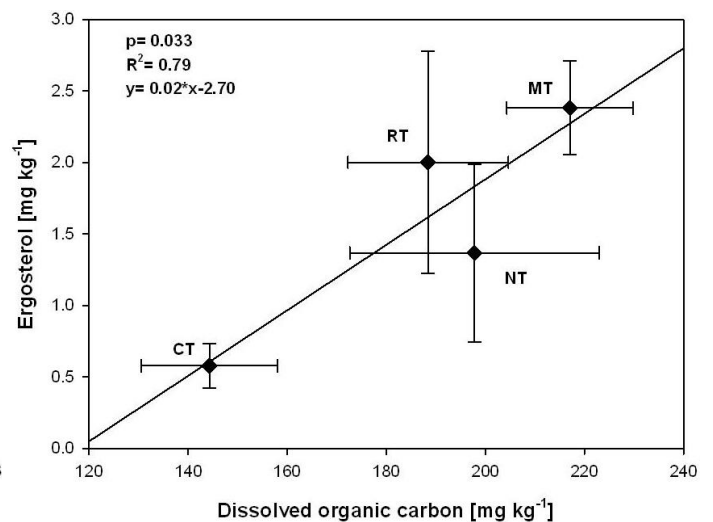


Figure 11: Linear regression of mean values of bulk and rhizosphere soils of ergosterol and dissolved organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

### 3.5 GLOMALIN RELATED SOIL PROTEIN (GRSP)

The data of the GRSP analysis, accordingly to the two-way ANOVA showed no significant tillage effect of GRSP among the tillage intensities ( $p > 0.05$ ) as well as no effect of the interaction of treatment and soil type ( $p = 0.867$ ), see Figure 12. The content was highest in MT plots, albeit, no significant different was found between the two sampling positions bulk and rhizosphere soil.

Figure 12 shows a higher GRSP concentration in MT compared to CT with  $1.9 \text{ mg g}^{-1}$  to  $1.5 \text{ mg g}^{-1}$ , however NT plots with  $1.7 \text{ mg g}^{-1}$  showed even lower GRSP concentrations than MT plots.

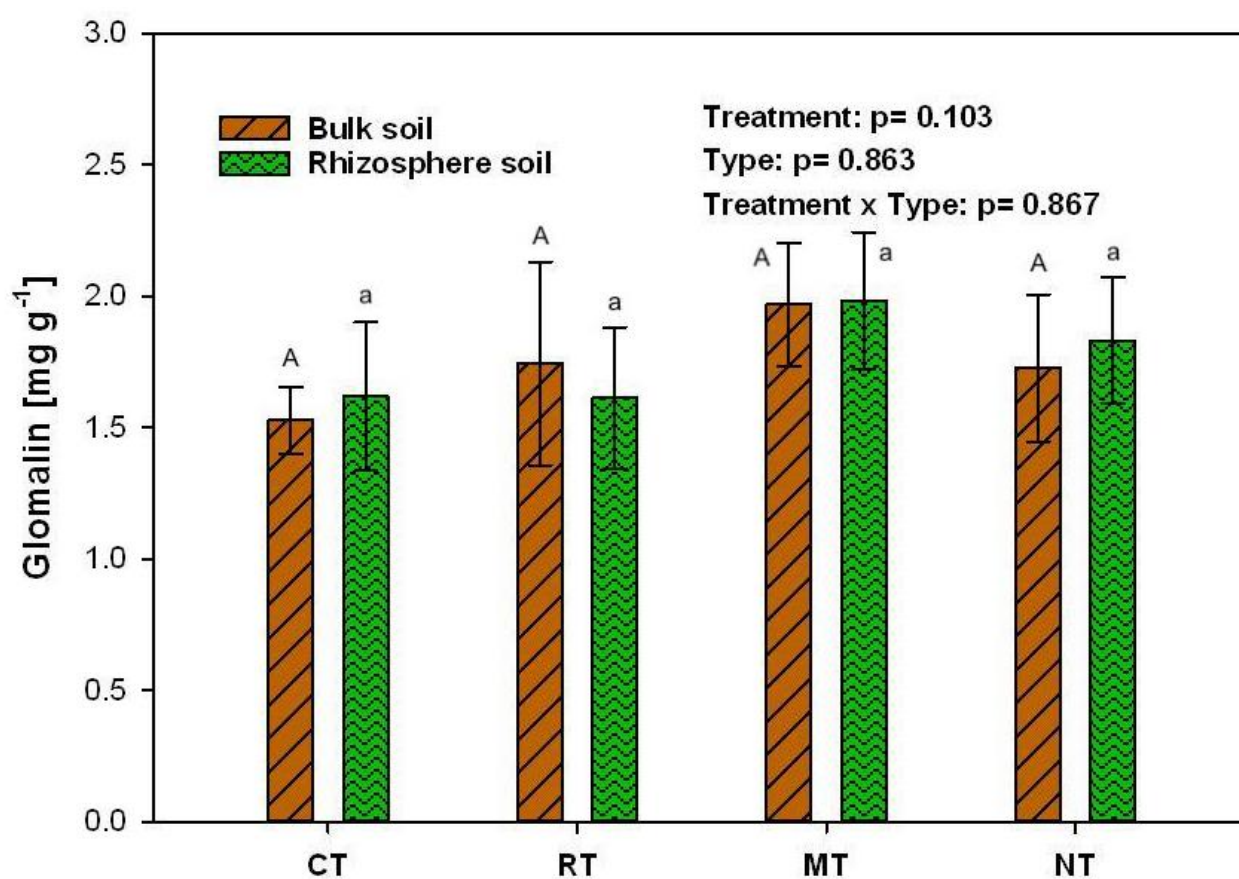


Figure 10: Glomalin in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.



Correlation analysis for SOC and GRSP data resulted in a linear regression with 96 % ( $R^2 = 0.96$ ) and  $p < 0.05$  of SOC fits to the regression of glomalin and a correlation coefficient of 0.98 implies that there is very strong relation between both soil parameters. There is a large variability noticeable in RT plots, see Figure 14.

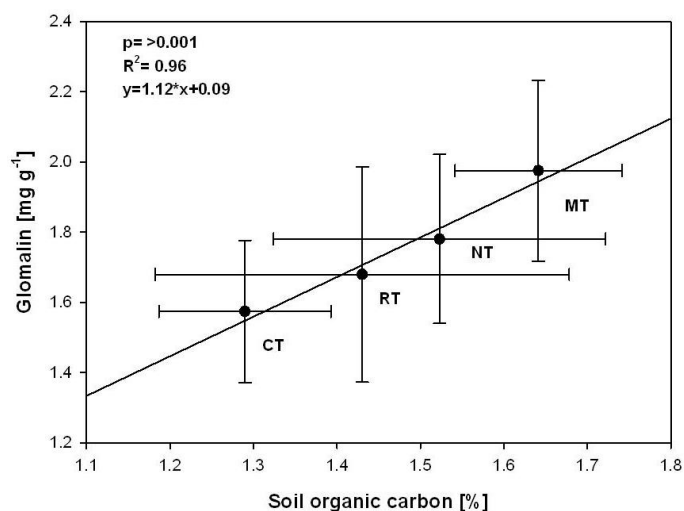


Figure 13: Linear regression of mean values of bulk and rhizosphere soils of glomalin and soil organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

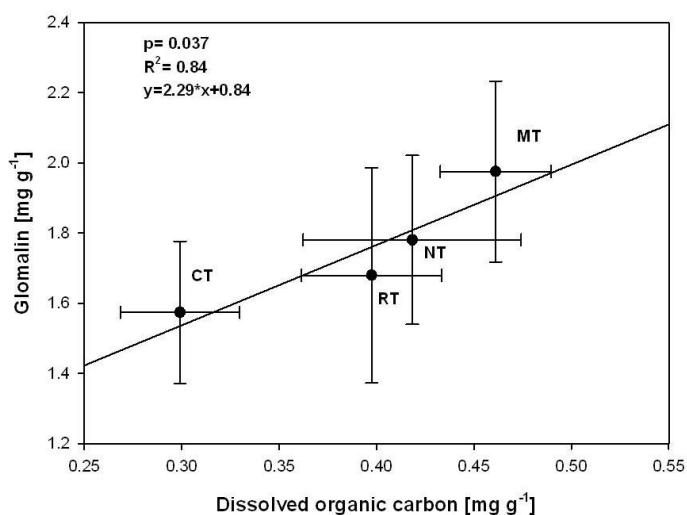


Figure 14: Linear regression of mean values of bulk and rhizosphere soils of glomalin and dissolved organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

### 3.6 AMINO SUGARS

There were no significant interactions found between the treatments (tillage intensities) and the sampling positions (type) ( $p = 0.122$ ) but the treatment effect of total amino sugar concentration in soil was significant ( $p < 0.05$ ). In total, highest amino sugar concentration was found in MT plots with  $2059 \text{ mg kg}^{-1}$ , followed by NT plots with  $1867 \text{ mg kg}^{-1}$  and RT with  $1856 \text{ mg kg}^{-1}$  in contrast to lowest amount in CT plots with  $1506 \text{ mg kg}^{-1}$ .

A significant difference was found in rhizosphere soil in MT & NT compared to CT tillage treatments, see lower-case letters in Figure 15.

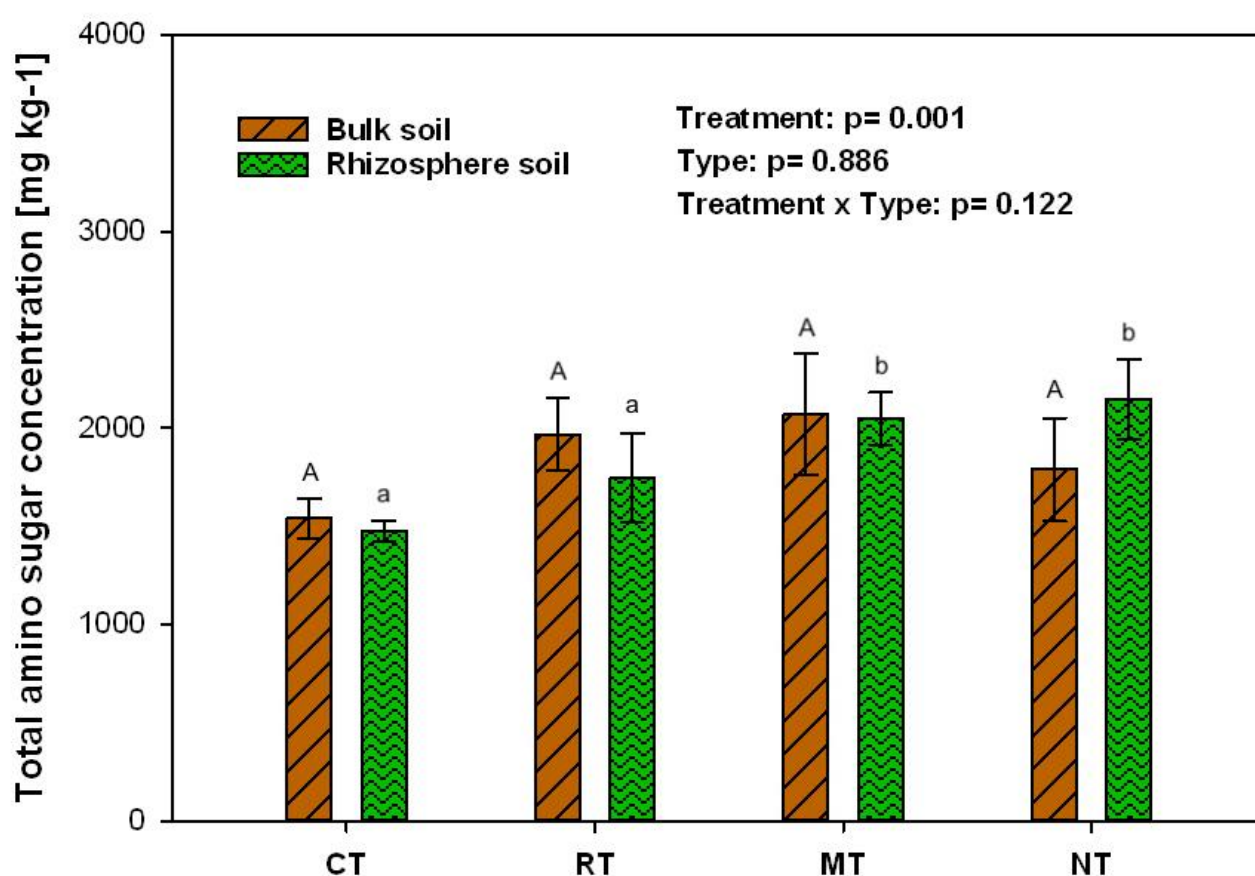


Figure 11: Total amino sugar concentration in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.



The analysis of the mannosamine showed that highest concentration was found in bulk soil in MT plots, see Figure 16. Although no significant difference was found, there was found a large variability in MT plots in bulk soil as well as a noticeable trend of higher concentrations in bulk compared to rhizosphere soil.

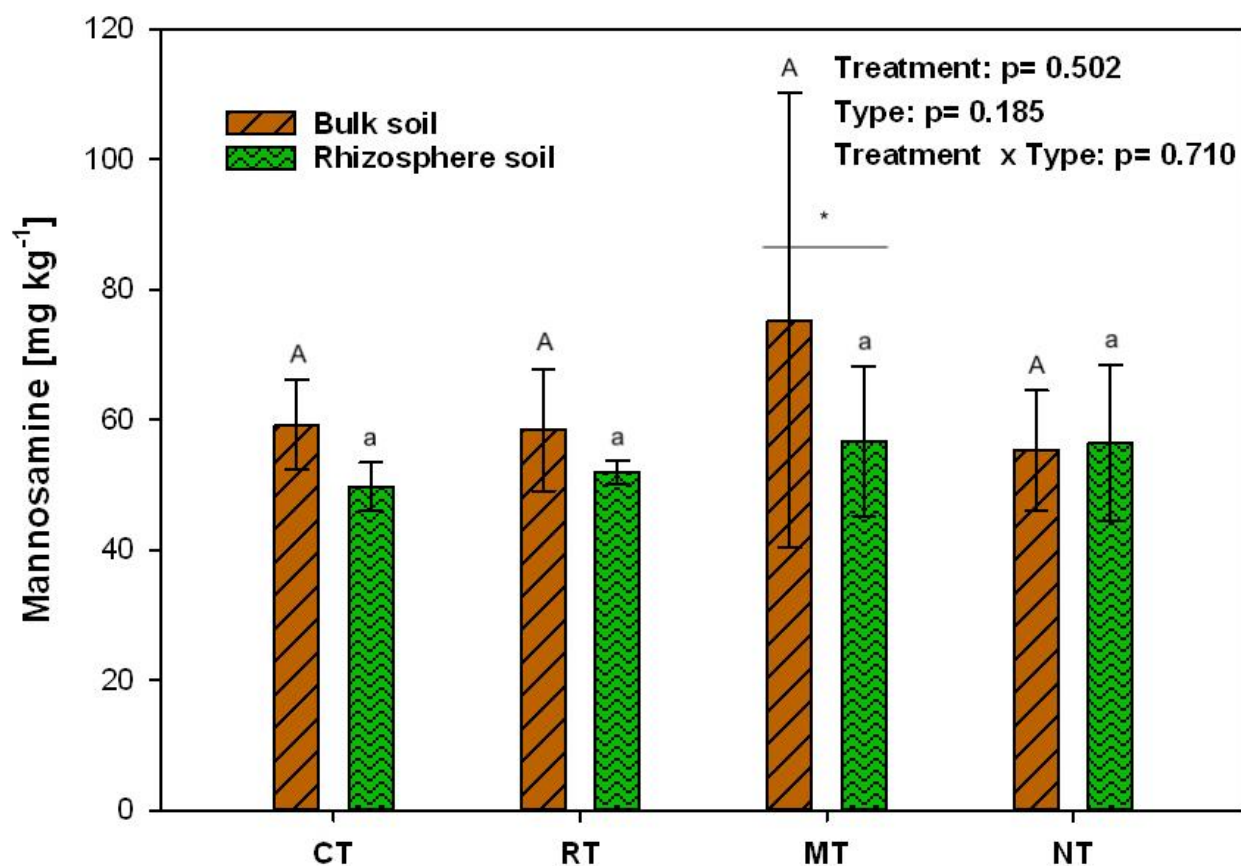


Figure 12: Mannosamine in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicate trends to unassimilated concentrations between bulk and rhizosphere soils as well as higher variability in bulk soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

Figure 17 shows muramic acid concentration with significant effect of treatment. The intensively managed soil (CT) showed lowest concentrations. The two-way ANOVA showed that there is no significant interaction effect between treatment (tillage intensities) and sampling position (type). Whereas, it is almost significant ( $p= 0.065$ ). A trend to higher concentrations in rhizosphere soil was found in NT plots, see \* in Figure 17.

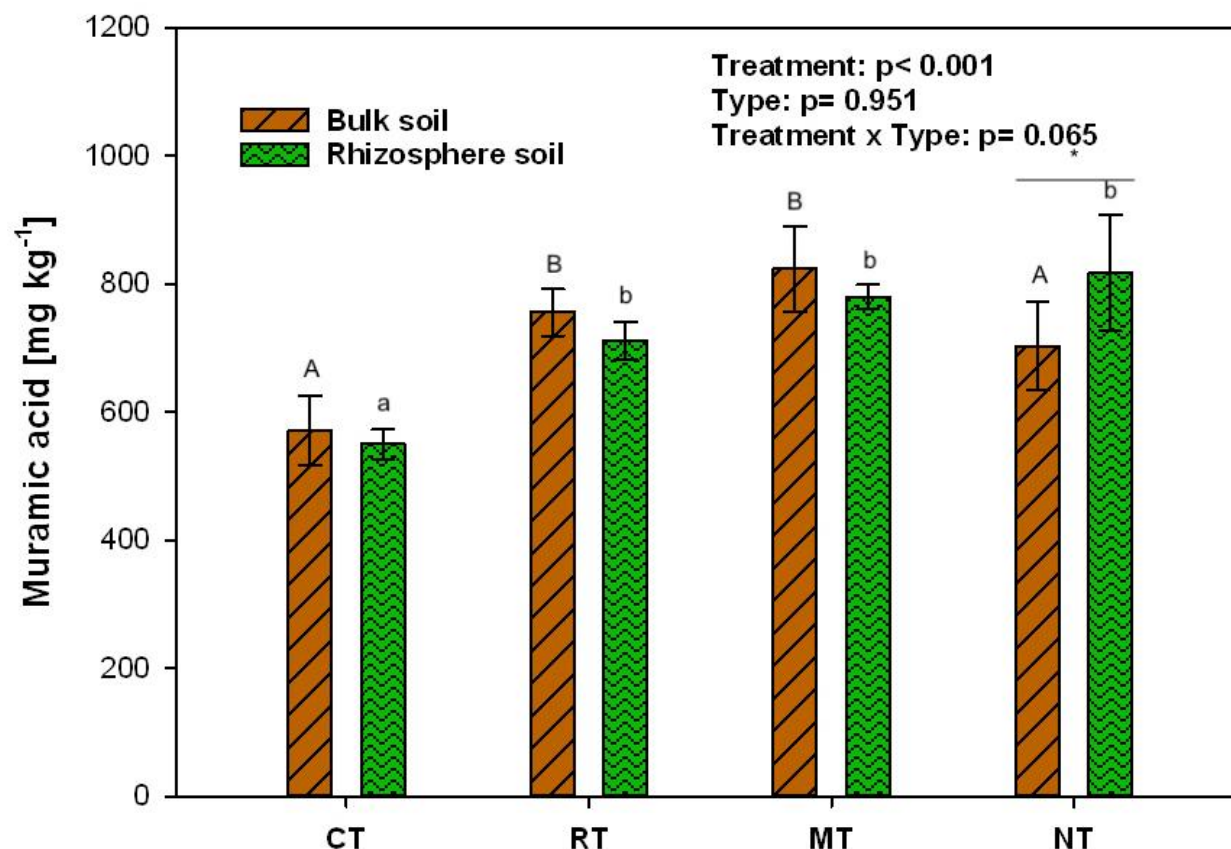


Figure 13: Muramic acid in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicate trends to unassimilated concentrations between bulk and rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

Figure 18 shows the analysis of the galactosamine within the amino sugar detection. There is a slightly significant tillage treatment effect noticeable ( $p = 0.048$ ). In NT plots, there is again a trend to higher concentrations in rhizosphere soil. The data show relatively similar results to muramic acid and there is again a significant interaction between rhizosphere soil in NT plots compared to CT plots.

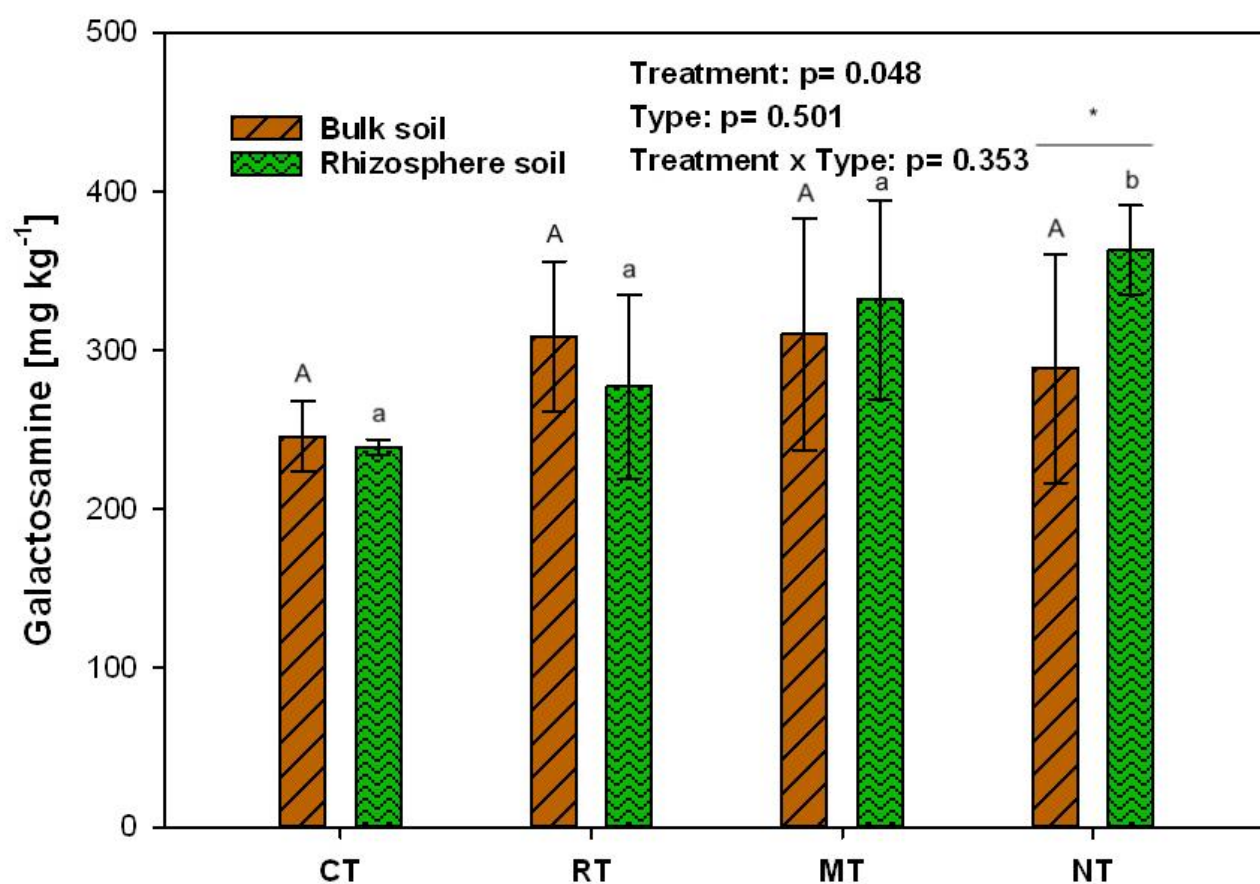


Figure 14: Galactosamine in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicate trends to unassimilated concentrations between bulk and rhizosphere soils as well as higher variability in rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

The analysis of the concentration of glucosamine shows a significant treatment effect in soils ( $p = 0.011$ ). The significant interaction between rhizosphere soils in NT compared to CT plots is noticeable again and the \* mark the unassimilated concentrations in either bulk and rhizosphere soils in RT and NT plots, see Figure 19.

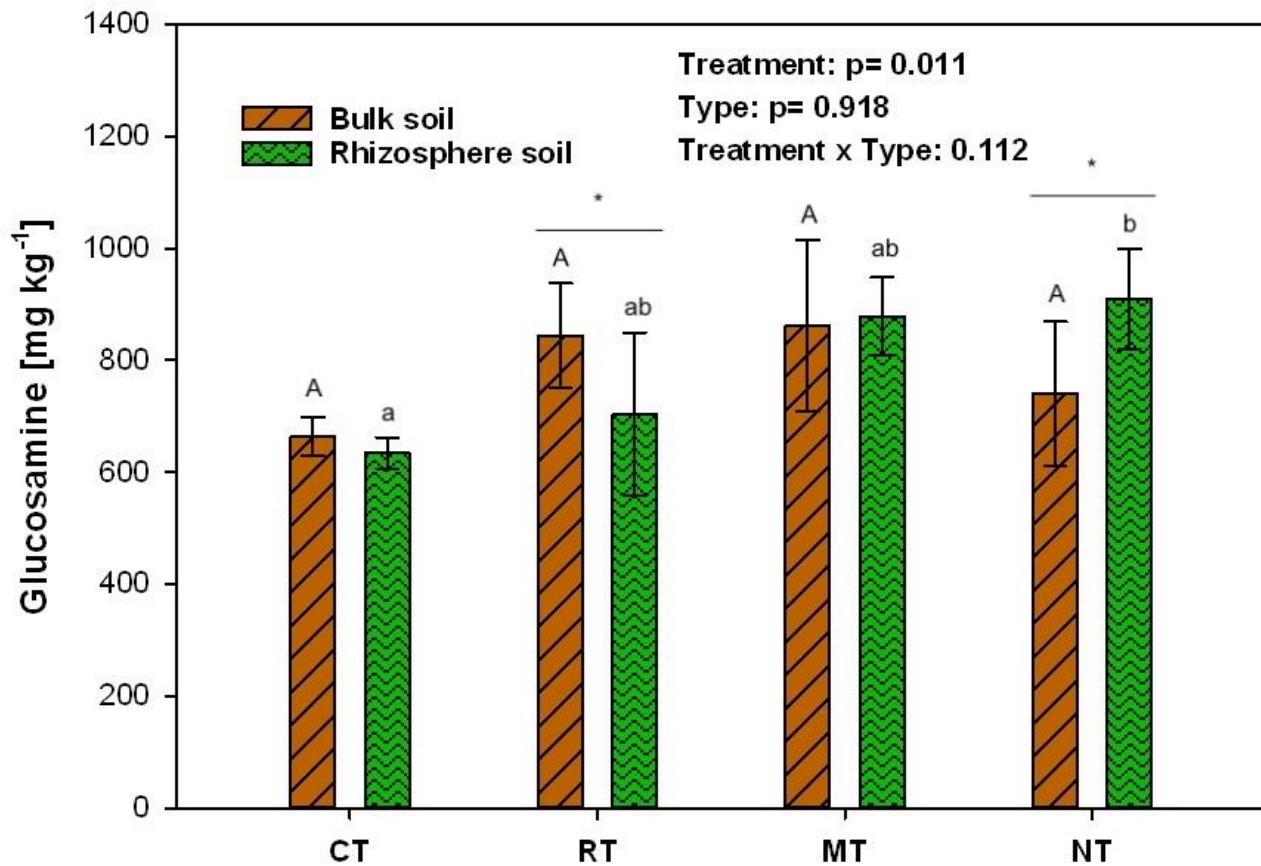


Figure 15: Glucosamine in different soil tillage intensities (treatment) in bulk and rhizosphere soil (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk and lower-case letters in rhizosphere soil. \* indicate trends to unassimilated concentrations between bulk and rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

Figure 20 and 21 show the relation between total amino sugar concentration and the dissolved and soil organic C. Both Figures show a relatively high relation of the amino sugar concentration to the C parameters. Especially, 98 % of the data in Figure 21 fits to the regression line. Therefore, the correlation coefficient of 0.99 points out that there is a very strong relation of the DOC to the amino sugars in the soil.

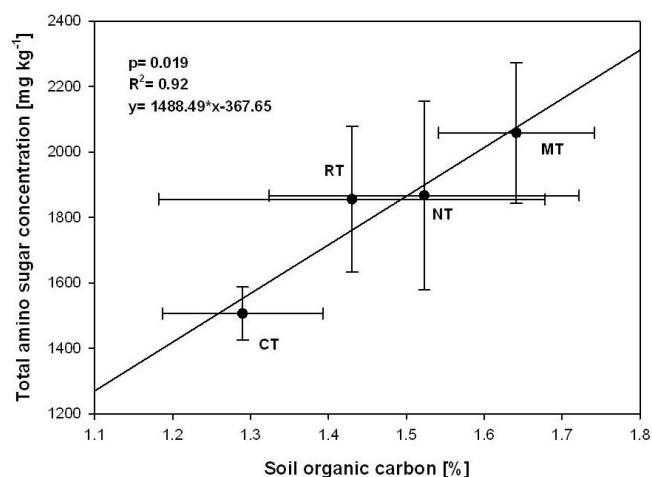


Figure 16: Linear regression of mean values of bulk and rhizosphere soils of amino sugars and soil organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

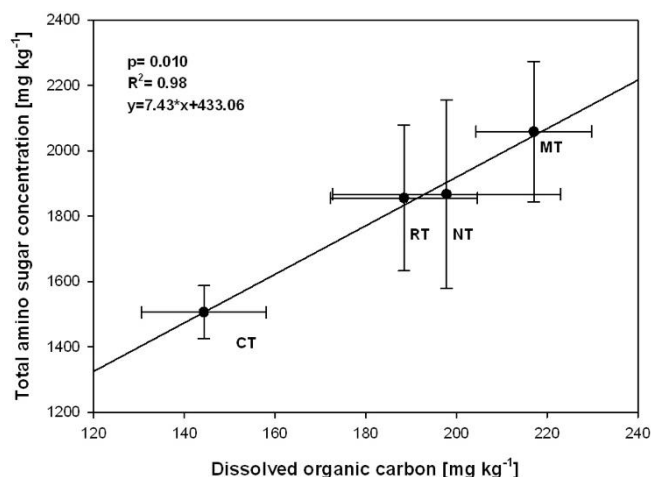


Figure 17: Linear regression of mean values of bulk and rhizosphere soils of amino sugars and dissolved organic C. The coefficient is shown as  $R^2$ . The error bars indicate standard errors.

### 3.7 EARTHWORM CAST

Figure 22 shows the comparisons of the samples of bulk and rhizosphere soil with earthworm casts in terms of soil organic C. Besides the fact to unassimilated concentrations in bulk and rhizosphere soil in RT and NT plots, no significant differences were found.

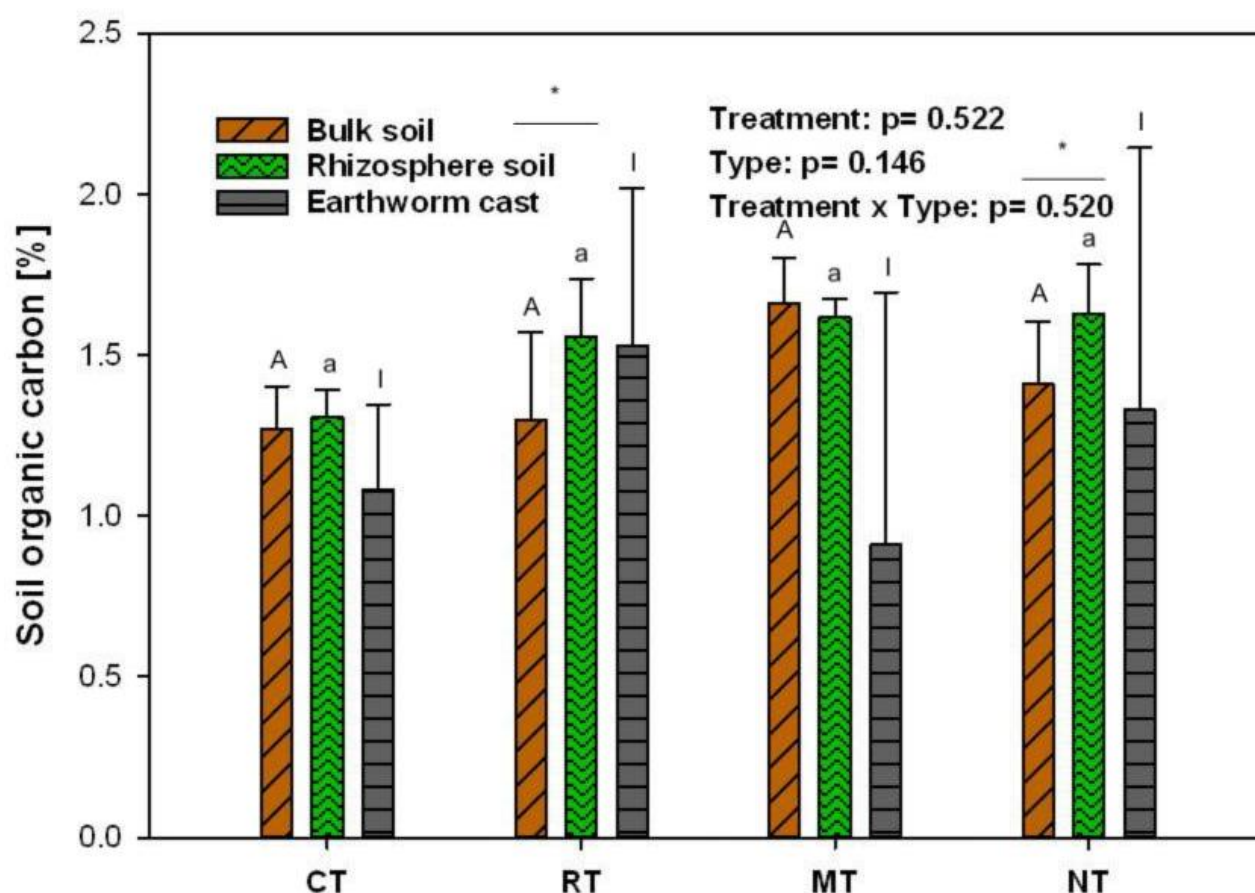


Figure 18: Soil organic C content in different soil tillage intensities (treatment) in bulk and rhizosphere soil, as well as in earthworm casts (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk, lower-case letters in rhizosphere soil and roman numerals in earthworm cast. \* indicate trends to unassimilated concentrations between bulk and rhizosphere soils as well as higher variability in rhizosphere soils. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.



Whereas Figure 23 shows a significant difference in the DOC concentration in the earthworm cast samples compared to bulk and rhizosphere soil samples (see type with  $p < 0.001$ ). However, there is no tillage effect found on DOC content in soils ( $p = 0.155$ ). According to the following data, most of the DOC content in earthworm cast samples was found in MT plots with  $563 \text{ mg kg}^{-1}$ , followed by RT plots with  $483 \text{ mg kg}^{-1}$  and nearly similar concentrations with  $373 \text{ mg kg}^{-1}$  in NT plots and  $340 \text{ mg kg}^{-1}$  in CT plots. Yet, there were large variabilities found in earthworm cast samples, especially in NT plots.

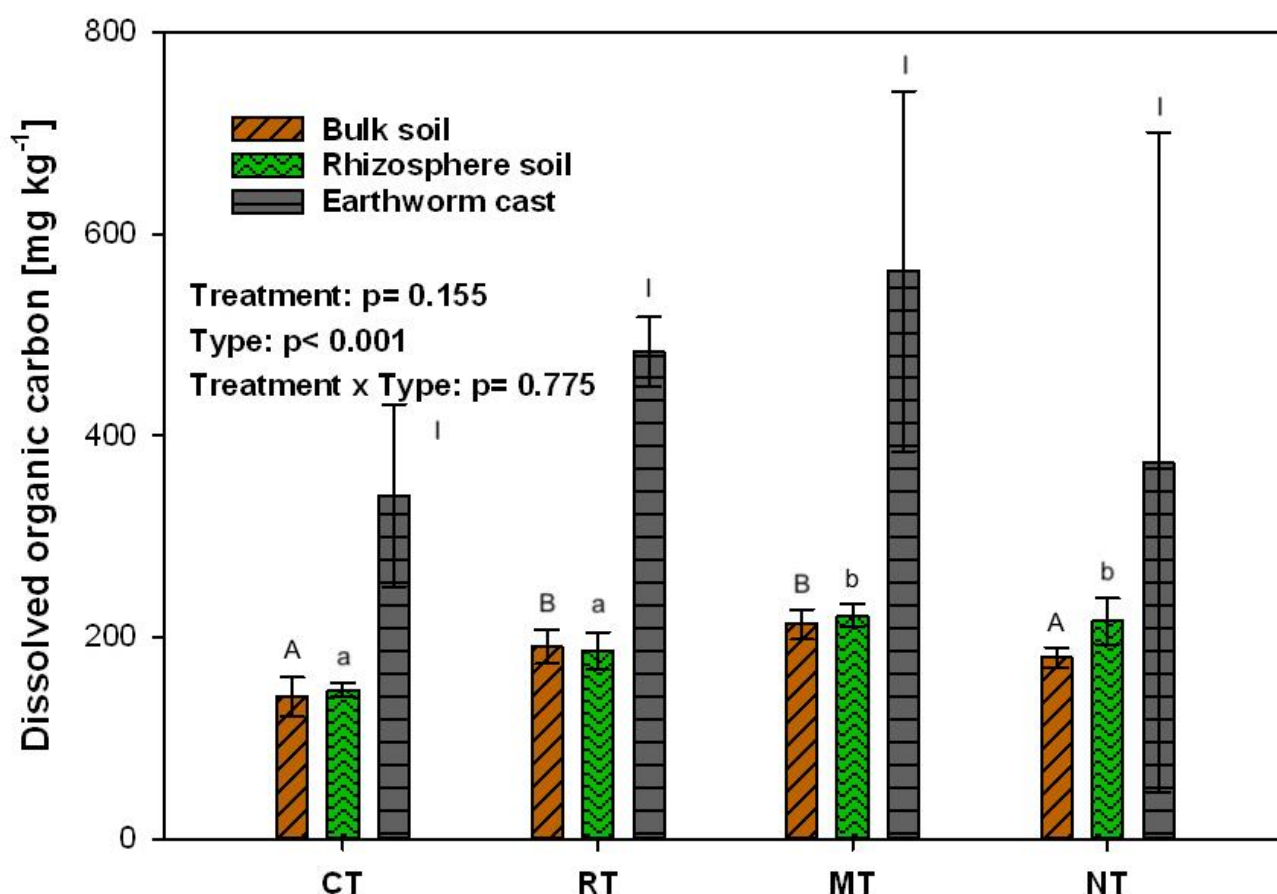


Figure 19: Dissolved organic C content in different soil tillage intensities (treatments) in bulk and rhizosphere soil, as well as in earthworm casts (type). Tillage intensities and soil types were compared with a two-way ANOVA continued with Tukey's HSD multiple range test. Error bars indicate standard error. Upper-case letters indicate significant differences in bulk, lower-case letters in rhizosphere soil and roman numerals in earthworm cast. CT = Conventional, RT = Reduced, MT = Minimum, NT = Non tillage.

### 3.8 YIELD DATA OF FIELD TRIAL 2019

The following data shows the yields from the field trial of 2019. The harvest of the grain maize (*Zea Mays*) was done on 23<sup>rd</sup> of October 2019.

There were no significant differences in terms of gross yield found between the different tillage intensities. The highest amount of grain maize yield was harvested on the NT plots (see Figure 24) with 122.7 dt/ha compared to the lowest amount of yield with 117.2 dt/ha on CT plots. The data show that there is an additional yield of 5 % possible on NT plots compared to CT plots.

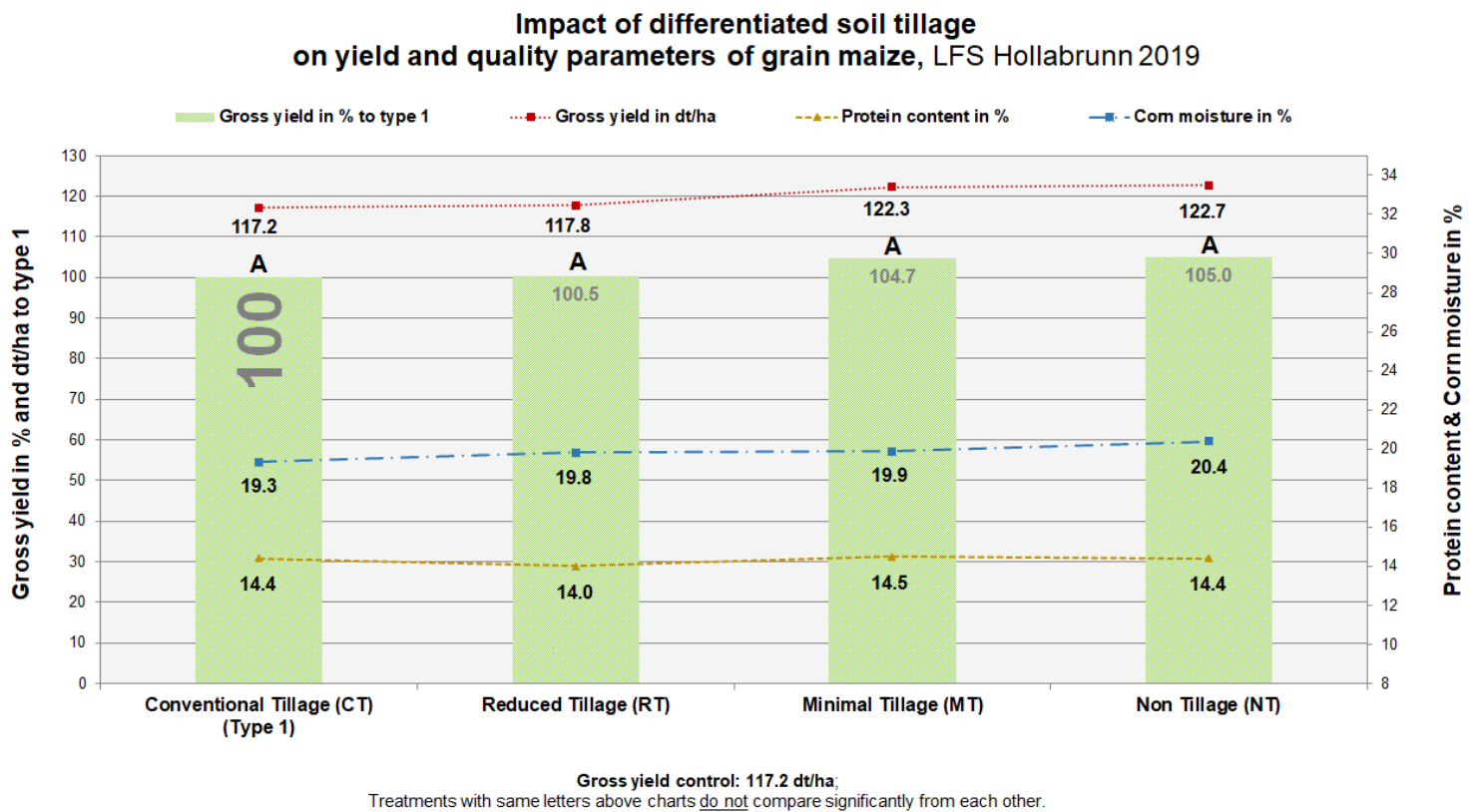


Figure 20: Impact of differentiated soil tillage on yield and quality parameters of grain maize (*Zea Mays*), LFS Hollabrunn 2019.



## 4 DISCUSSION

There have been many soil tillage experiments with different questions in different climatic regions all over the world. They all have in common, how the several intensities affect the soil structure and potential effects on crop yields.

The field trials of the Austrian Agency of Health and Food Safety (AGES) are one of the longest in Austria, which run for more than 30 years (Franko et al., 2016). Similar to the field trial of Hollabrunn, the AGES field trial in Fuchsenbigl, Marchfeld, Austria, investigates the impacts of different tillage intensities on soil parameters since 1988. The aim of the field experiment is to find out how the soil structure and, subsequently, crop yields can be affected with different machinery inputs. The advantage of the field experiment of the Agricultural School of Hollabrunn is that it also includes NT and not only different mechanical tillage treatments are compared.

In this study, the SAS was measured with the wet sieving method in bulk and rhizosphere soil. The results showed an increase from conventional tillage (CT) to non-tillage (NT), with a reduction in tillage intensity. The contents of SAS from CT (lowest) to NT (highest) plots range from 19.4 % to 43.3 %.

Kasper et al., 2019, reported similar results from the Fuchsenbigl site, which shows a similar climate as in Hollabrunn, but different soil texture (fine sandy loam). SAS analysis showed most stable aggregates in less intensive managed soils. They were being able to clearly verify that minimized tillage had far more stable aggregates than intensive tillage by 18.2 % in CT and 37.6 % in MT (minimum tillage) plots. The increase in aggregate stability was comparable in both sites, although the soil type was different. While the highest SAS could be observed for NT. Soil tillage leads to a destruction of aggregates. The soil structure is most stable in NT plots, which reduces further the risk of soil erosion events.

In the present study, lowest amounts of SOC and DOC were found in intensively tilled plots (CT). The data of this study show that NT plots had a higher SOC content than CT plots, with 1.52 % and 1.29 % respectively, see Figure 5 and 24. The highest SOC contents were

found in MT plots. The comparison of the results for SAS and SOC (Figure 4 and 5) showed significant differences between intensively versus less intensively tilled plots. The results suggest that reduced soil tillage stabilizes the organic C in soil and also ensures increased aggregate stability.

Du et al., 2015, revealed in their Chinese study similar results. No-tillage plots enhance the C sequestration potential, because of a better developed soil structure. Soils in conservation tillage plots in a study of Six et al., 2000, led also to an increased formation of microaggregates compared to intensively managed plots. The C can be better stored inside the pores, which helps the soil for an enhancing input potential of C (e.g. sequestration potential) for agricultural used soils. A higher soil organic matter contributes an increased effectiveness of the sequestration potential which leads to a better soil quality (Karim et al., 2010).

As mentioned above, higher aggregate stability could reduce erosion and hence SOC losses. This is supported by a previous study that was conducted on the same field experiment by Klik et al., 2020. The authors investigated the impacts of several tillage intensities (CT, MT and NT) on potential soil erosion processes. With a reduction of tillage practices, a decrease of surface runoff on silty clay loam soils up to 55 % (3.6 to 5.3 t ha<sup>-1</sup>) in MT and 60 % (1.9 to 3.0 t ha<sup>-1</sup>) in NT plots, was observed. These lower erosion rates (equals higher SAS rates) correlate with reduced SOC losses up to 86 % for MT and 89 % for NT plots. Higher SAS and therefore higher soil organic C pools together with cover crops can be supportive for decreased surface runoff.

The loss of C in agriculture is often related with intensive tillage, by its negative impacts on e.g. soil structure (soil erosion). Conservation methods like NT combat these negative influences and can enhance soil fertility (Kern et al., 1993). Haddaway et al., 2017, summarized in their review the results of 351 studies and conclude that reduced tillage methods promote soil fertility and yield rates of these soils because of an increased accumulation of SOC in the upper soil.

Higher soil cover in conservation tillage plots could result in increased DOC concentrations. Root exudates could provide higher inputs and eventually result in

increasing SOC levels via direct sorption pathway as suggested by Sokol et al., 2019. Our results showed that about 30 % more DOC was found in the conservation (means of MT+NT) compared to conventional (means of CT+RT) tillage plots. MT plots showed highest amounts with 217 mg kg<sup>-1</sup> (mean of bulk and rhizosphere soil) compared to CT plots with 144 mg kg<sup>-1</sup>.

These results support the first hypothesis.

Our study shows highest content in ergosterol analysis in MT plots, there is still a significant difference in concentrations between CT and NT plots (Figure 9). Moderately tilled soils (RT & MT) showed highest ergosterol concentrations in our study. Similar results were reported by Karlen et al., 1994, who observed enhance the ergosterol content with less intensive tillage. In particular, the authors mentioned significantly more ergosterol in NT plots compared to CT plots. In the present study, NT did not show the highest ergosterol concentrations, which indicates a potential stimulation of fungal growth by moderate tillage/disturbance. CT plots showed lowest concentrations in soils. This suggests that CT, may severely destroys fungal hyphae via the mechanical forces of ploughing in the intensively tillage treatment. However, the data suggest that moderate soil tillage is not detrimental to saprotrophic fungal biomass in soils.

The results of our study show significant differences in GRSPs between CT and MT plots and highest GRSP concentrations in less intensive tillage plots (see comparison of Figures 4 and 12). Similarly, Van Groenigen et al., 2010, reported that GRSP was enhanced in RT plots in the 0-5 cm layer on plots with winter wheat grown in Ireland. The plots also showed higher biomass of saprophytic fungi, which is comparable to our results in ergosterol analysis, where higher concentrations of fungal biomass found in less intensive managed soils.

There was a strong relationship found between GRSP and SOC with a correlation coefficient of 0.96 (see Figure 14) as well as significant higher SOC content in MT compared to CT with a correlation coefficient of 0.86 of SAS and SOC. Our GRSP results are comparable to a study conducted in Chile, who found a significant positive correlation with water stable aggregates (Curaqueo et al., 2010). In addition, the latter, also observed,

a larger proportion of GRSP and water stable aggregates, in less tilled soils compared to intensively tilled soils on a wheat-corn rotation field in Mediterranean climate conditions. In addition, they reported a 44 % increase SOC content on NT compared to CT plots within 6 years. Due to growth of mycorrhiza fungi, plants bind more CO<sub>2</sub> through photosynthesis. As a result, the bound carbon is made available to soil and microorganisms. Mycorrhiza fungi would thus contribute to accumulation of SOC concentrations in soil, whereby they also have an influence on necromass. A higher content of necromass, could sustainably increase amino sugar concentrations (Ortas et al., 2017) (Wang et al., 2016).

Less intensive soil tillage can promote the accumulation of aboveground organic crop residues which are essential for building up soil organic matter, which is in line with a higher amino sugar concentration (Li et al., 2019). In an American tillage trial of Simpson et al., 2004, the total amino sugar concentration was significantly higher in NT compared to CT, which is related to a greater accumulation of glucosamine, galactosamine and muramic acid. The amino sugar concentrations of our analysis show analog results. The comparison of the means of total amino sugars (mannosamine, muramic acid, galactosamine and glucosamine) show highest concentrations in MT plots significantly different to CT plots (Figure 15). Glucosamine comes from fungal necromass, while mannosamine is reported to be a biomarker for bacterial necromass (Joergensen, 2018).

Analysis showed a correlation of total amino sugar concentration to ergosterol concentration in soils, with highest amounts in MT plots. In particular, for the individual amino sugar mentioned above, similar results were observed (Figure 9 as well as 16, 17, 18 and 19).

Also, DOC and SOC were strongly related with amino sugar content. The strong relation of microbial necromass with SOC is suggested to be related with an increased disturbance of the soil (CT), C and amino sugar show analog decreased results (Figures 20 and 21). Both data on ergosterol and GRSP together with amino sugars are important parameters to evaluate microbial pathways for increasing SOC via the so-called “entombing effect” (Liang, 2020). As microbes can either release CO<sub>2</sub> into the atmosphere via decomposition (and this can be accelerated via the priming effect), this would lead to a decline in SOC. However, active growth of microbes in soil can contribute to build up new stable SOC via

necromass that is formed after microbes die. The microbial pathway is suggested to account for a large proportion of stable SOC formation (Sokol et al., 2018).

Our results suggest that decreased mechanical disturbance via reduced soil tillage can enhance the C sequestration potential (due to better SOC and DOC concentrations) and promotes soil fungal abundance and microbial necromass, which support the second hypothesis.

An additional investigation of this study was on the impacts of earthworms. We questioned ourselves, if there is an effect of earthworm activity (in particular: cast) on soil fertility and does this improve SOC levels. The analysis of DOC and SOC in ecast samples showed high error bars and variances. There is no statistical evidence for tillage effects on SOC in earthworm cast samples (Figure 22). No significant differences could be related to the high variation observed for SOC. The high variation could be related to a heterogenic SOC distribution for the analysis, as not pure ecast was analyzed, rather soil+ecast that differed among plots. Another option for no significant effects of earthworms on SOC content could be the short observation period, as earthworm fences were only installed with corn seedling, while effects are observed over decades.

Overall, lowest SOC and DOC contents were found in CT plots (Figure 5 and 7). Figure 22 and 23 show considerable high amounts of C, especially the DOC content was significantly higher in the earthworm cast compared to bulk and rhizosphere soil. Also, DOC concentrations varied a lot in these earthworm cast samples, as reported for SOC. Figure 23 compared bulk and rhizosphere soils, but higher concentrations in earthworm cast samples. The variation in DOC and SOC further supports the heterogeneity of the samples.

Zhang et al., 2013, revealed in their study that earthworms contribute more to a C stabilization than a conversion, which indicates that earthworms can stabilize C in their casts. We also expect that earthworms would be beneficial for SOC formation in the longer term. A possible indication that casts contribute to an accumulation of SOC in soils could be the stabilization of DOC in the soil matrix.

Soils can stabilize C in micro aggregates for hundreds of years, if the soil is remained stable (Utomo, 2014). Conventional tillage practices reduce the SOC content due to mechanical fractionation and destruction of the aggregates. The trapped C can easily be released and mineralized into the atmosphere (Luo et al., 2006) or lost via erosion.

Our results revealed that earthworms can possibly facilitate higher C content in soil by higher DOC concentrations. By binding, DOC can be converted into stable SOC compounds in soils (Sokol, et. al., 2019).

This supports our third hypothesis.

## 5 CONCLUSION

On the one hand, intensively cultivated land is less prone for weeds and diseases because of a clean seedbed, which is left for the following seed. On the other hand, this kind of management can have negative influences on the SAS. In this respect, less intensive tillage plots had the best prerequisites for a well-developed soil structure because of existing structures that provide the potential to build and protect stable C and prevent from SOC losses.

The increased formation of microaggregates in conservation tillage plots are supportive for a higher accumulation of C inside the pores, which increases the soil C sequestration potential. Further, a more stable soil structure has also positive effects on the earthworm population. Conversely, earthworms enrich the soil with their casts and contribute to a higher C concentration. Earthworms can enhance biological conversion of crop residues in the soil, which increases the soil fertility in general.

Higher gross yields can be gained on conservation tillage plots (see Figure 24 “Yield data of field trial 2019” in the appendix: NT: 122.7 dt/ha & MT: 122.3 dt/ha) compared to conventional tillage plots (RT: 117.8 dt/ha & CT: 117.2 dt/ha). That’s a surplus of +5 % on NT plots compared to CT plots. Higher soil organic matter contents (see Figure 26) can increase C sequestration potential (see Figure 5 and 7). Biological indicators like ergosterol, glomalin and amino sugars are also highest in conservation tillage plots and enhanced microbial activity, particularly fungi, may contributed to stabilize C in soils.

Hence, agricultural practitioners should consider moderate tillage as potential weed control with regard to saprotrophic fungal abundance in their soils, with the drawback of still reducing aggregate stability.

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## 9 APPENDIX

### 9.1 PARTICLE SIZE DISTRIBUTION

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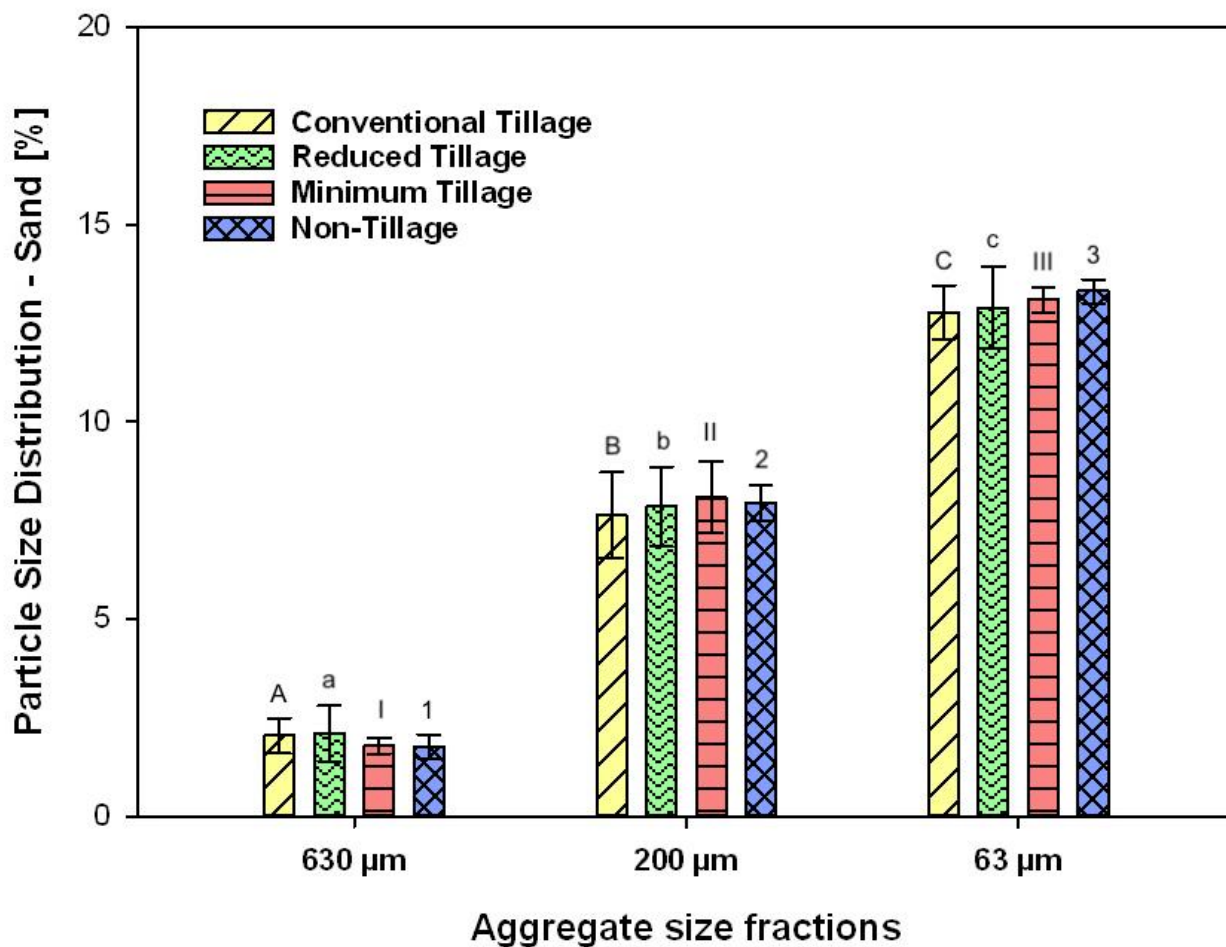


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## 9.2 SOIL ORGANIC MATTER

The calculation of the organic matter (mean values of bulk and rhizosphere soil) of the experimental field implies that there is most humus on MT plots with 2.8 % followed by NT (2.6 %), RT (2.4 %) and lowest on CT plots with 2.2 %.

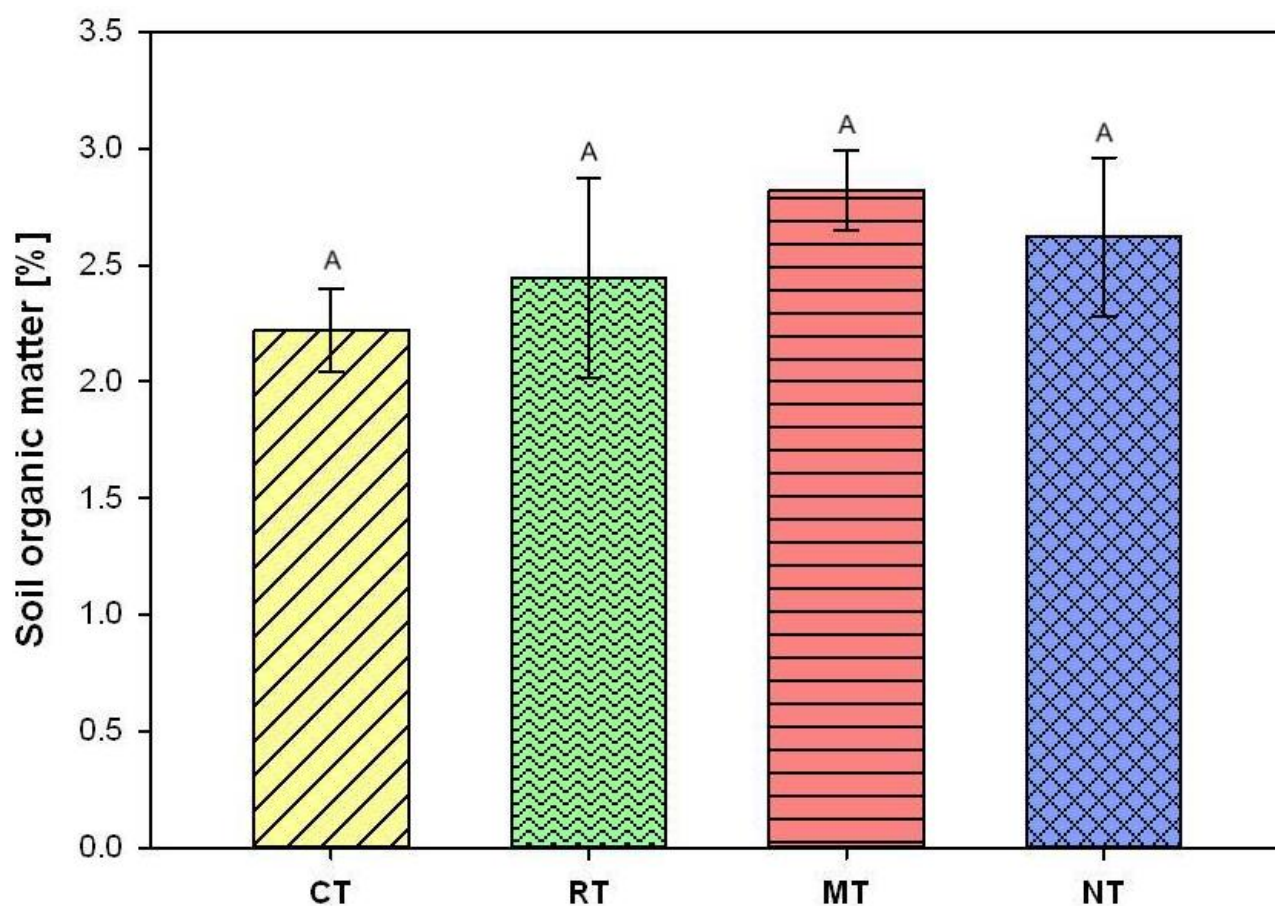


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