Effects of no-till on root architecture and root-soil interactions in a three-year crop rotation

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ABSTRACT

No-till (NT) is widely recommended for a series of environmental advantages such as reduction of soil erosion, mitigation of phosphorus pollution, sequestration of carbon from the atmosphere and increase of water retention in soils. However, experimental evidence to date shows conflicting results with respect to the effects of NT on soil physical parameters and root development of plants. A three-year field study (2014, 2015, and 2016) was conducted to assess the effects of NT *vs* CT on root growth in maize, soybean, and winter wheat, on a silty clay loam soil in the northern Italy. Root length density (RLD), diameter class length (DCL), root dry weight (RDW) and roots composition (C and N) in the top 60 cm of soil were characterized. The total amount of roots C (TRC) was calculated by multiplying RDW by roots C content. Relationships among roots, soil bulk density (BD) and penetration resistance (PR) were investigated using the non-parametric Spearman rank coefficient. RLD was significantly increased under NT compared to CT at the top soil layer (0-5 cm) in maize (6.37 cm cm^{-3} versus 2.03 mg cm^{-3}) and winter wheat (5.38 cm cm^{-3} versus 2.90 cm cm^{-3}), while it was lower in NT than in CT in the deeper soil (5-15 cm) only in maize (3.19 cm cm^{-3} versus 4.53 cm cm^{-3}). RDW was increased under NT compared to CT at the same soil layer in maize (3.86 mg cm^{-3} versus 0.50 mg cm^{-3}), soybean (4.33 mg cm^{-3} versus 0.43 mg cm^{-3}), and winter wheat (0.96 mg cm^{-3} versus 0.38 mg cm^{-3}). A mixed impact of tillage occurred on C:N ratio. NT reduced roots C:N ratio of maize (-9%), increased C:N ratio of soybean (+14%), and did not affect C:N ratio of winter wheat. This was mainly related to the effect of NT on roots coarser than 2 mm, which decreased average roots N content. Soil BD and PR decreased during time under NT. A negative correlation between root traits (RLD, RDW) and soil physical parameters (BD, PR) was found in this study under NT while no correlation occurred for CT. This corroborates the hypothesis that roots are a major driver of soil physical condition and suggests that stability of continuous biopores is much more relevant to affect root growth than the total amount of pores.

1. INTRODUCTION

Conservation Agriculture (CA) can be defined as a sustainable management approach of agro-ecosystems for improved and sustained productivity, conserving the environment and increasing at the same time soil fertility (FAO, 2011). CA and, in particular, no-tillage (NT) lead to a series of advantages by saving resources and input (Lal, 2008). Reduction of runoff and erosion, mitigation of phosphorus pollution, enhancement of soil organic carbon (SOC), increased soil water retention (Lal, 2004; Soane et al., 2012) are some of the main outcomes of NT practices. Although NT is recognized as one of the most sustainable soil management systems (Reicosky and Saxton, 2007; Tabaglio et al., 2009) reaching up to 70% of the total cultivated area in South America (Holland, 2004; Derpsch and Friederich, 2009), it is not widespread in Europe (Basch et al., 2008) where a decrease in crop yield during its establishment has been reported (Brouder and Gomez-Macpherson, 2014; Pittelkow et al., 2015). A series of studies reported that adoption of NT decreased soil quality, increasing soil compaction and bulk density (BD), with negative effects on roots growth and development in a large number of crops (Quin et al., 2006; Guan et al., 2014).

A well-established and deep root system is essential for the absorption of nutrients (Doussan et al., 2006), and water (Gaiser et al., 2012; Mckenzie et al., 2009). Size and distribution of roots are strongly influenced by the physical properties of the soil. BD and aggregate stability (AS) affect the relationship between filled and void spaces (Ball et al., 2005), determining the aeration degree of the soil (Vogel, 2000) and root growth as a consequence. At the same time, it is widely accepted that roots play a major role in macro-aggregate stabilization (Denef et al., 2007) and that their C/N ratio has a great influence on microbial activity (Rasse et al., 2005) and soil priming effect (Graaff et al., 2014, 2013). In addition, fine roots, having a diameter lower than 2 mm, are very dynamic and play a key role in the ecosystem C cycling (Finér et al., 2007). When NT is implemented oxygen concentration in the subsoil is limited, and, as a result, mineralization of organic matter is reduced and accumulation of organic carbon increases (Kong et al., 2005; Lützow et al., 2006).

Under conventional soil management, tillage practices affect, crop growth, nutrient uptake and soil properties (Spedding et al., 2004). However, tillage operations only disturb the structure of the arable topsoil. The structure of subsoil under conventional tillage, as of the whole soil profile under NT soil management, is considerably influenced by roots development and turn-over: during growth, they exceed a pressure which generates a reorganization of the soil pore network (Kolb et al., 2012). After crop harvest and root decomposition, dug channels remain empty in the soil, forming biopores (Jones et al., 2004). Soil burrowing animals, such as anecic earthworms, can influence soil structure. When tillage intensity is reduced, the populations of anecic earthworms can be promoted (Curry et al., 2002; Peigné et al., 2009), which in turn can contribute to the formation of biopores (Ehlers, 1975; Joschko et al., 1989). Conversely, a series of studies report that topsoil under NT is usually cooler and moister (Dwyer et al., 1996; Muñoz-Romero et al., 2012), characterized by a higher BD (Munkholm et al., 2012; Soane et al., 2012), that causes high penetration resistance (PR), than under conventional tillage (CT) (Chassot et al., 2001). Under NT, these negative features can sometimes cause a soil structure stratification, which can limit root penetration and promote a lateral and superficial root development (Qin et al., 2006).

Root spatial distribution, quantified by the root length density (RLD) index, has been investigated in a number of studies but with controversial results. Taking into account the whole soil profile, some studies found that at flowering RLD was higher under NT than under CT (Hilfiker and Lowery, 1988; Baligar et al., 1998; Holanda et al., 1998), while others found the opposite situation (Karunatilake et al., 2000; Sheng et al., 2012), and still others found that RLD was similar under NT and CT (Hughes et al., 1992; Dal Ferro et al., 2014). To our knowledge few studies report on the effect of tillage vs NT on root dry weight (RDW) and no one on carbon and nitrogen content of roots. The objective of this work was to study how (i) the main traits of root architecture and (ii) its composition (C and N) are affected by soil management (CT *vs* NT) in the top 60 cm of soil under maize, soybean, and winter wheat. The relationships among roots, soil compaction and bulk density were also investigated.

2. MATERIALS AND METHODS

2.1 Experiment and treatments

The field experiment was carried out at the CERZOO experimental research station in Piacenza (45°00'21.6'' N, 9°42'27.1'' E; 68 m above sea level), Po valley, northern Italy. Soil was silty clay loam; *fine, mixed, mesic, Udertic Haplustalf* (Table 1). The climate is temperate; mean annual temperature and precipitation are 12.2 °C and 890 mm respectively. Climatic data were collected from a meteorological station positioned in the experimental field (Table S1).

The experimental design was a randomized complete block (RCB) with four blocks and two treatments: conventional tillage (CT) and no-tillage (NT). The single plot size was 1430 m^2 (65 m x 22 m). The experiment was established in 2010 to compare: (i) CT, which included an autumn plowing (35 cm) and two passages of rotating harrow in spring (15-20 cm) to prepare the seedbed, and (ii) NT, consisting of direct sowing on untilled soil using a double-disk opener planter for seed deposition.

Crop sequence was a three-year crop rotation, which included winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and soybean (*Glycine max* L.). All plots had been subjected to conventional tillage before starting the experiment. Crop residues were incorporated into the soil in the CT plots, while they were left on the soil surface in NT plots. During non-growing seasons, a cover crop of hairy vetch (*Vicia villosa* L.) was sown in NT plots right after harvesting the previous main crop. Two weeks before sowing of the following main crop, hairy vetch was terminated by spraying 3 L ha^{-1} of Roundup Platinum (Glyphosate 79.5%).

In 2014, plots were cropped with maize, fertilized at a rate of 250 kg N ha^{-1}. Sowing was carried out on the 24th of April, with the a maize hybrid FAO maturity group 600 (SNH 9609), and harvest took place on 24th of September after physiological maturity when kernel humidity was 22%. Weeds were controlled at four leaves visible stage by spraying 1.2 L ha^{-1} Ghibli (Nicosulfuron 4.2%) and 1.3 L ha^{-1} Calaris (Terbuthylazine 29.3%; Mesotrione 6.2%). In 2015 the main crop was soybean with a maturity group 1- (BAHIA), which was planted on 8th of May and harvested at the beginning of October (1st) . No fertilizer was applied during soybean growing season, while weeds were suppressed by using 2 L ha^{-1} Stratos (Cicloxidim 21%) and 5 g ha^{-1} Harmony (tifensulfuron-methil 75%). Durum winter wheat (Monastir) was sown after soybean harvest on 19th of November and it harvested on 8th of July. A rate of 170 kg N ha^{-1} was applied at the end of February. Both maize and soybean were irrigated to prevent water stress, while winter wheat was cropped under rain fed condition. Planting density was measured in all plots at anthesis: (i) maize had 7.7 and 7.0 plants m^{-2}, (ii) soybean had 35.4 and 32.2 plants m^{-2} , (iii) and wheat had 439 and 405 spikes m^{-2} for CT and NT respectively. Inter-row distance was: 0.7 m for maize, 0.35 m for soybean, and 0.15 m for winter wheat.

2.2 Samples collection

Maize root sampling was carried out at anthesis (Qin et al., 2006) on July 25th 2014, with a self-constructed “Shelby” tube sampler of known volume (7 cm diameter and 120 cm length) that was pressed with the hydraulic arm of a digger to 0.6 m depth (Amaducci et al., 2008) at three positions on the perpendicular to the crop row in each plot: at 0 cm (on the row, i.e. close to the base of the sampled plant but not including the maize stalk), at 35 cm (mid-row) and at 17.5 cm (between row and mid-row).

Also in soybean and winter wheat soil cores were taken at anthesis (on July 30th 2015 for soybean and on May 18th 2016 for winter wheat) but, due to their narrow inter-row distance, from only two sampling positions: 0 cm and 17.5 cm (mid-row,) for soybean, and 0 cm and 8.75 cm (mid-row) for wheat. After extraction, each soil core was divided into five different portions, relative to five soil depths: 0-5 cm, 5-15 cm; 15-30 cm; 30-45 cm; 45-60 cm. The total number of sub-samples was 120 for maize, 80 for soybean, and 80 for winter wheat.

2.3 Measurement of soil physical properties

Soil compaction was evaluated *in-situ* by measuring penetration resistance (PR) at the field capacity. Measurements were made after soil sampling, using a standard soil cone penetrometer (Soil Compaction Meter SC 900, Spectrum Technologies, Inc., Plainfield, IL), with a 1.25 cm x 2.45 cm cone. Data were recorded every 2.5 cm down to a depth of 45 cm (Tabaglio et al., 2009). For each plot 15 measurements were performed. Soil gravimetric moisture content was determinate by using the oven dry method (drying sub-samples at 105 °C for 48 h). Bulk density (BD) at each soil depth was calculated dividing the oven-dry weight of each soil portion by its volume.

2.4 Root characterization

Soil samples were stored at -20 °C until root separation and analysis were carried out. After defrosting, samples were kept in a solution of oxalic acid (2%) for 2 h, in order to facilitate the separation of roots from soil, and then they were washed in a hydraulic sieving-centrifugation device (Dal Ferro et al., 2014; Chimento and Amaducci, 2015). Cleaned roots were recovered from the water using a 2 mm mesh sieve (Cahoon and Morton, 1961). Finally, in order to prevent mold contamination, roots were hand-cleaned from organic particles and immersed in 10% (*v*/*v*) ethyl alcohol solution (Monti and Zatta, 2009).

Roots were scanned and the images were acquired using the TWAIN interface at 600 dpi with the scanner Epson Expression 10000xl, equipped with a double light source to avoid roots overlapping (Chimento and Amaducci, 2015). Determination of Root Length Density (RLD, cm cm^{-3}) and root diameter were performed with the software winRHIZO *Reg* 2012. After, Root Dry Weight (RDW, mg cm^{-3}) was gravimetrically determined by drying roots at 60 °C until constant weight. About 1 g of dry material per each sample was then weighed and analyzed by Dumas combustion method with an elemental analyzer varioMax C:N for carbon (C) and nitrogen (N) determination (VarioMax C:NS, Elementar, Germany). The Diameter Class Length (DCL, mm cm^{-3}) was calculated for very fine (0.0-

0.5 mm), fine (0.5-2.0 mm) and coarse (> 2 mm) diameters for the whole soil profile (Zobel and Weisel, 2010).

2.5 Statistical analysis

Data were subjected to analysis of variance (ANOVA) with the mixed effect model using the "nlme" package (Pinheiro et al., 2015) of RStudio3.3.3. When normal distribution was not confirmed using the Sharpiro-Wilk test, data were log transformed before analysis. Distance and depth were included in the mixed effect model as fixed factors while block effect was considered as random. Mean values were separated using the "Post-Hoc Interaction Analysis" package (De Rosario-Martinez et al., 2015) (α=0.05). Multivariate correlation analysis was used to assess the relationship between root and soil parameters (i.e. RLD, soil bulk density and penetration resistance). The correlations were assessed using the non-parametric Spearman rank coefficient (ρ). A P-value of 0.05 was used as the threshold for statistical significance.

3. RESULTS

3.1 Root length density (RLD), root dry weight (RDW), and roots C and N content

In maize, on average, mean RLD and mean RDW were not affected by tillage systems (Table 2), even though RLD tended to be higher (P = 0.0573) in NT than in CT (Table 3). Roots C content was never affected by tillage systems or by any other factor (Table 2) and it was on average 39.6%. On the other hand, roots N content was higher in NT than in CT (+14.5%), which turned into a significantly lower roots C:N ratio under NT (-8.7%) than under CT (Table 3). The effect of tillage system on roots traits increased in the following year, with soybean, (Table 2) when NT significantly increased both RLD and RDW compared to CT (Table 3). In detail, RLD was 63.5% higher under NT than under CT, and RDW was four times larger in NT than in CT (Table 3). Tillage system did not affect soybean roots C and N content (Table 2), while roots C:N ratio was higher under NT than under CT (+13.9%) (Table 3). The positive effect of NT *vs* CT on root development in soybean was confirmed the following year, when a significant increase of both RLD (2.64 versus 1.91 cm cm^{-3}) and RDW (0.33 versus 0.17 mg cm^{-3}) occurred in winter wheat. Roots C and N content, as well as roots C:N ratio in winter wheat were not affected by tillage system (Table 3).

RLD, RDW, root N content and C:N ratio were significantly affected by soil depth (De) both for maize, soybean, and winter wheat. In particular, RLD, RDW, and roots N content decreased along the soil profile. Conversely, roots C:N ratio increased as a consequence of root N content decreasing along the soil profile (Figure S1).

Distance from the row (Di) played a minor role on root traits. In maize, moving from the row (0 cm) to the inter-row position (35 cm), RLD, RDW and C:N ratio decreased, while roots N content increased (Figure S2). Increasing Di led to lower RDW also for soybean (P = 0.0023), while RLD was not affected (Table 2). No significant effects of Di was found in winter wheat for any of the studied parameters.

The interaction between tillage and De (T × De) was significant for RLD and RDW in maize and winter wheat (Table 2). In soybean only RDW was significantly affected by the interaction T × De, not RLD (Table 2). In in the top 5 centimeters of soil, RLD and RDW of maize, were respectively 2 and 6 times higher under NT than under CT(Figure 1). In contrast, in the 5-15 cm layer, the same root traits were higher under CT than under NT, although differences were significant for RLD only (+47.7%). In soybean, RLD tended to have higher values in NT than in CT across different De (Figure 1). On the other hand, RDW of soybean was higher in NT than in CT the top 5 centimeters, while no differences were found from 5 to 60 cm (Figure 1). Generally, those conditions were statistically sufficient to cause differences between NT and CT in soybean (Table 2). The same pattern was found in winter wheat, where NT led to larger values than CT for both RLD (+85%) and RDW (+153%), in the top 5 centimeters of soil (Figure 1). Although the positive effect on RLD and RDW was only significant in the first centimeters of soil, NT tended to increase RLD also in the 5-15 cm layer (Figure 1). As roots C content was never changed by tillage or by any other factor, differences between NT and CT in terms of total amount of root carbon (TRC) left into the soil retraced RDW results for all crops (Figure S3).

Roots N content and roots C:N ratio were significantly affected by the interaction T × De only in maize (Table 2). In particular, root N content was statistically higher in NT than in CT from 5 to 45 cm De. Conversely, C:N ratio was higher under CT than under NT in the 5-15 and 15-30 cm layers (Figure 2).

Interaction between tillage and distance from the row (T × Di) did not lead to any changes for all the considered parameters (Table 2). Interaction between soil depth and distance from the row (De × Di) played a minor role (Table 2) and only for maize, where RLD, RDW and roots C:N ratio were affected because of the large significant impact of Di ($P < 0.0001$) on those parameters previously reported (Table 2).

The interaction T × De × Di was significant for RDW in maize (Table 2). In detail, RDW was higher in NT than in CT (10.75 versus 0.87 mg cm^{-3}) in the top soil layer (0-5 cm) and on the row (0 cm Di) (Table S2). In soybean and winter wheat, T × De × Di was also significant for RLD because of major differences between CT and NT in the top soil layer, where RLD in soybean was higher in NT than in CT at 17.5 cm Di (3.23 versus 1.10 mg cm^{-3}), while in winter wheat RLD increased under NT (6.04 versus 2.42 mg cm^{-3}) at 0 cm Di (Table S2).

3.2 Diameter class length (DCL)

Results of diameter class length (DCL) indicated that across soil depth, and for different crops, the large majority (from 96 to 99%) of roots had a diameter lower than 2 mm (Table 4). Among these, very fine roots (0.0-0.5 mm) were more frequent (56-83%) than fine ones (0.5-2.0 mm) (16-43%). This root distribution among diameter classes was affected by tillage systems in each crop (Table 4). Statistical differences in DCL occurred in the top soil layers (0-5; 5-15 cm), while no differences in DCL was found between 15 and 60 cm.

In maize, the significant increase of RLD at the soil surface (0-5 cm) under NT was due to a general increase of all the diameter classes (Table 4). In particular, very fine, fine, and coarse diameter roots were higher in NT than in CT by 208%, 216%, and 771%, respectively. On the other hand, at the 5-15 cm soil depth CT increased RLD value by significantly increasing only very fine roots (+57%) as reported in Table 4. CT tended to increase coarse roots in maize in each soil layer between 5 and 60 cm, rather than fine and very fine ones (Table 4), however, due to a large variability within replicates, tillage effect was not statistically significant. The effect of NT on root architecture traits of soybean was limited to coarse roots (Table 4), which were higher under NT than under CT at the 0-5 cm soil depth (0.0864 versus 0.0267 cm cm^{-3}). Conversely, in the top 5 centimeters, NT increased the amount of very fine and fine roots of winter wheat, compared to CT, by 71% and 128%, respectively (Table 4). In the 5-15 cm soil layer only fine roots were larger in NT than in CT (+108%).

3.3 Soil physical properties

During the 3-year experiment, soil bulk density (BD) was generally higher under NT than under CT (Table 3). In 2014, BD (P = 0.0046) was 9% higher in NT thn in CT (1.51 kg dm^{-3} versus 1.39 kg dm^{-3}). No difference between NT and CT (1.40 kg dm^{-3} versus 1.43 kg dm^{-3}) were found in July 2015, while BD was again 8% higher under NT than under CT in 2016 (1.37 kg dm^{-3} versus 1.27 kg dm^{-3}) (Table 3). During the trial, BD progressively declined under NT from 1.51 kg dm^{-3} to 1.40 kg dm^{-3} and 1.37 kg dm^{-3}, in 2016, 2015 and 2014 respectively (Table 3).

De significantly affected BD in all years (Table 2) with the lowest BD values in the top soil layers (data not shown). Nevertheless, the interaction T × De did not affect BD in 2014 (P = 0.8394). In 2015 and 2016, T × De was significant, in particular, BD under NT was 25% and 28% lower than under CT in 2015 (P = 0.0003) and in 2016 (P = 0.0094), respectively (Figure 3). In fact, BD under NT showed a progressive decline in the top soil layer, starting from 1.43 kg dm^{-3} in 2014, down to 1.01 kg dm^{-3} in 2016 (Figure 3). In the 5-15 cm layer no differences occurred between CT and NT over the 3-year experiment, while NT generally increased BD in the deeper soil (Figure 3).

Spearman rank coefficient (ρ) showed a negative correlation between root traits (RLD, RDW) and BD under NT (Table 5). In 2014 and 2015, no significant correlations under CT were found, although the relationship between RLD/RDW and BD tended to be positive (Table 5). In 2016, RLD/RDW of winter wheat and BD under CT were positively correlated (Table 5).

Penetration resistance (PR) did not differ between tillage systems both in 2014 (P = 0.1876) and in 2015 (P = 0.7998), while PR was statistically lower in NT than in CT in 2016 (P < 0.0001) (Table 3). De significantly affected PR in all years (Table 2) as the lowest PR values were found in the top soil layer (data not shown). In 2014, PR was significantly higher in NT than in CT from 5 to 10 cm De, while no differences could be reported in the deeper soil layers (Figure 4). In 2015, PR was again higher under NT than under CT at the 5-10 cm De. However CT had higher PR than NT at around 35 and 20 cm depth, which correspond to the soles of plough and harrow, (Figure 4). In 2016, no difference of PR between CT and NT were found in the top 15 centimeters of soil, while higher PR under CT than under NT was found in the soil profile between 15 and 45 cm (Figure 4).

The ρ test showed that RLD and RDW of maize were negatively correlated to PR under NT. No correlation under CT was found (Table 5). No relationship between soybean RLD/RDW and PR were found (Table 5), while, NT caused, again, a negative relationship between RLD/RDW of winter wheat and PR (Table 5).

4. DISCUSSION

4.1 Tillage effects on RLD and RDW

Development and spatial distribution of roots along the soil profile are important drivers of nutrients and water uptake by crops, and therefore of plant growth and yield (Guan et al., 2014). Tillage practices are fundamental components of soil management systems, that can affect root distribution and root traits (Chassot et al., 2001; Ji et al., 2013; Li et al., 2017a). Generally, roots distribution is concentrated in the top centimeters of soil and close to the row because of the greater availability of nutrients (Lynch, 2011), and environmental constraints in the deeper soil such as soil resistance (Buczko et al., 2009). Root length density (RLD) and root dry weight (RDW) are valuable parameters for characterizing root systems (Amato and Ritchie, 2002; Monti and Zatta, 2009; Chimento and Amaducci, 2015).

In the present study, RLD and RDW were higher at the soil surface than at deeper soil layers (Table 2). These results are in compliance with studies carried out by Guan et al. (2014) on maize, by Gao et al. (2010) on soybean and by Qin et al. (2004) on winter wheat. In maize, RLD and RDW were higher at 0 cm distance from the row compared to the inter-row, which corroborates earlier studies (Mengel and Barber, 1974; Quin et al., 2006). On the other hand, in both soybean and winter wheat no statistical differences related to lateral distribution of root system were found (Table 2). This indicates that the vertical distribution of roots is more affected by soil conditions than their horizontal distribution (Liedgens and Richner, 2001), especially with a reduced inter-row distance.

Average root length and root biomass in maize were not affected by tillage system (Table 2), which is in apparent agreement with early research from Hughes *et al.* (1992). Considering the distribution of these root traits along the soil profile it appears that RLD and RDW were larger under NT than under CT in the top 5 centimetres, while the opposite was true in the 5-15 cm soil layer (Figure 1).

Recent studies report that CT increased root development of maize (Guan et al., 2014; Li et al., 2017a) because of higher root density in soil layers deeper than 5 cm. Besides controversial literature results, due to different experimental conditions (i.e. soil texture, irrigation,…), it seems that the top 5 cm could be indicated as a critical threshold for roots development differences between CT and NT (Martinez et al., 2008; Dal Ferro et al., 2014).

NT positively affected root development of soybean (Table 3). This corroborates previous findings by Li et al. (2017b), which reported an increase in soybean root biomass up to 60 cm depth under NT compared to CT in the long-term. It has been shown that soybean root growth is increased as a result of crop rotation and cover crops root growth, which leaves biopores (Calonego and Rosolem, 2010). Under NT, different root systems and channels formed by decaying roots create continuous porosity and link the soil surface to deeper layers, resulting in greater root colonization at depth (Ehlers et al., 1983; Williams and Weil, 2004). However, soybean roots usually accumulates in the top soil layers under NT (Li et al., 2017b), which is consistent with results in the present study (Figure 1). The great difference of roots density between the two tillage systems was mainly due to the establishment of more favourable conditions under NT than under CT in the top 5 centimeters of soil after transition (Lal, 2004).

Higher RLD and RDW of winter wheat in NT than in CT at the 0-5 cm soil depth retraced what found for soybean. Similar results were reported by Martinez et al. (2008), which observed a higher RLD under NT compared to CT in the topsoil, and by Huang et al. (2012), which showed how NT increased also winter wheat RDW at 0-10 cm soil depth. Higher root development in the topsoil for NT than for CT may be attributed to continuous and progressive residue accumulation on the soil surface as well as cover crops use. This increases soil aggregate stability, water holding capacity (De Vita et al., 2007; Bottinelli et al., 2017) and soil organic carbon content, which in turn stimulate nutrient release and root growth (Martinez et al., 2008). In the present study, RLD of winter wheat under NT tended to be higher than under CT also at 5-15 and 15-30 soil layers, which suggests an improvement of soil conditions also in the subsoil after 6 years of NT adoption.

4.2 Tillage effects on roots carbon and nitrogen

To infer on the effect of tillage systems on roots C sequestration, the total amount of roots C (TRC) was calculated by multiplying RDW by roots C content, and since the latter was never affected by any of the experimental factors, TRC pattern was similar to that of RDW (Figure S3). Therefore, TRC was higher under NT than under CT for soybean (0.40 mg cm^{-3} versus 0.10 mg cm^{-3}) and winter wheat (0.12 mg cm^{-3} versus 0.06 mg cm^{-3}), while no statistical differences between NT and CT were found in maize (Table 2) even though TRC was statistically higher in NT than in CT in the top soil layer ($P < 0.0001$) (Figure S3). TRC is a relevant C input of the soil that can affect SOC (Dalal et al., 2005; Kong and Six, 2010). Dalal et al. (2011) found a significant positive correlation between SOC and estimated cumulative root dry matter over 40 years of wheat cropping in the first 30 cm of soil, while the results of the present study suggest that the effect of NT on SOC is limited to the top layer (0-5 cm) for silty clay loam soils. However, it has been established that roots plays a major role to sequester and stabilize C in the first layers, where otherwise it could be potentially lost (Rasse et al., 2005). Furthermore, roots C has a higher residence time into the soil compared to leaves and stems C, as root tissue is more durable and recalcitrant to mineralization (Barber, 1979).

Both roots N content and roots C:N ratio varied along the soil profile (Table 2). Roots N content decreased, while C:N ratio increased moving down the soil profile (Figure S1), which confirms previous findings on maize (Dietzel et al., 2017). Different tillage systems led to statistical differences in roots C:N ratio, but effects were mixed (Table 2): NT reduced roots C:N ratio of maize, increased C:N ratio of soybean, and did not affect C:N ratio of winter wheat (Table 3). It has been shown that roots C:N ratio increases with increasing roots diameter as coarse roots contains less N compared to fine and very fine roots. Previous results reported a C:N ratio for coarse root of 79:1, while for root diameters lower than 2 mm C:N ratio was 43:1 (Gordon and Jackson, 2000). Effects of NT on coarse roots density (Table 4) could be helpful to explain variations in roots C:N ratio (Figure 2). In maize, NT increased coarse roots as well as the other diameter classes in the top soil layer (0-5 cm), while CT had a similar increasing tendency on coarse roots from 5 to 30 cm and from 45 to 60 cm depth (Table 4). Following this pattern, roots C:N ratio was higher under CT than under NT in the same soil layers. Conversely, in the top soil layer (0-5 cm) maize roots tended to have a higher C:N ratio in NT than in CT (Figure 2). In soybean, NT significantly increased the amount of coarse roots in the top 5 cm layer (Table 4). As a consequence, roots C:N ratio was higher in NT than in CT (Table 3). Coarser taproots of soybean under NT compared to CT in the topsoil were the result of higher soil compaction in NT than in CT from 5 to 10 cm depth (Figure 4). Earlier results reported that a great mechanical resistance of soil may contribute to increase roots diameter (Cannell and Haves, 1994), which corroborates findings in the present study. Changes in C:N ratio of winter wheat roots did not occur, which confirmed that this parameter is affected by tillage only when the amount of coarse roots was modified and thus the roots N content.

4.3 Tillage and roots effects on soil physical parameters

Root growth and decomposition, together with earthworms activity, enhance aggregate stability and soil porosity (Six at al., 2000), which leads to progressively decrease soil bulk density (BD) in the long-term (Nawaz et al., 2017). Under NT, these biopores are not periodically disrupt by tillage and represent a favourable environment for root growth in the top soil layer (Williams and Weil, 2004). Organic matter within biopores, which derives from root exudates or dead roots decomposition, plays a major role in root development, as it serves as a source and reserve of nutrients (Calonego and Rosolem, 2010). Conversely, total porosity resulting from tillage is artificial and short lived as it is promoted by mechanical implements which destroy macro-pore continuity and destabilize soil structure (Busscher et al., 2002).

However, many studies reported that during the conversion from plow to NT, soil BD tends to increase (Munkholm et al., 2003; Alvarez and Steinbach 2009; Soane et al., 2012; Palm et al., 2014), as a consequence of a transient compaction, which should disappear with time (Vogeler et al., 2009). This is consistent with results presented in this study, which showed higher BD under NT than under CT along the soil profile in 2014 and a gradual reduction in BD under NT after that (Table 3), as a result of the 30% decrease in the top soil layer (Figure 3).

As for soil BD, penetration resistance (PR) decreased under NT during the experiment time (Table 3). In 2014 and 2015, CT showed a linear increase of PR until 10 cm depth, while NT had an exponential increase of PR in the same soil layer (Figure 4). This pattern was consistent with previous studies comparing tilled and NT soils (Tebrügge and Düring, 1999; Ferreras et al., 2000; Lampurlanés and Cantero-Martínez, 2003; Singh and Malhi, 2006; Tabaglio et al., 2009). However, the difference of PR between CT and NT in the topsoil did not occur in 2016 (Figure 4). This suggested that the improvement of soil physical conditions started to affect not only the top 5 cm, but also deeper soil layers. Franzen et al. (1994) observed significantly lower PR under NT than under CT down to 10 cm depth due to the effect of mulching. This was in agreement with results presented in this study, which also reported clear limitations for soil compaction under CT due to soles of ploughed and harrowed in 2015, and lower PR in NT than in CT in 2016 (Figure 4).

It has been shown that roots of cover crops may help to decrease soil BD and PR under NT (Williams and Weil, 2004; Osunbitan et al., 2005; Chen and Weil, 2010) and the importance of roots (both of cover and main crops) as actors of a process dubbed “bio-drilling” is well known (Cresswell and Kirkegaard, 1995). The negative correlation between root traits (RLD, RDW) and soil physical parameters (BD, PR) found in this study under NT (Table 5) corroborates earlier results (Dal Ferro et al., 2014) and reinforces the hypothesis that roots are a very relevant driver of soil physical condition after tillage interruption (Logsdon and Karlen, 2004). On the other hand, root traits played a minor role for modifications of soil physical parameters under CT as soil porosity is mainly influenced by tillage (Table 5). This poor overall relationship under CT suggests that the total amount of pores is not the major factor affecting root growth in soils, while stability of continuous biopores is much more relevant.

5. CONCLUSIONS

Roots distribution is usually concentrated in the top centimeters of soil because of the greater availability of nutrients and environmental constraints such as soil resistance. Root growth of field crops can be influenced via the tillage system as a consequence of altered soil properties.

NT effect on roots development was evident on all crops (maize, soybean and winter wheat) in the top soil layer (0-5 cm) where it increased RLD and RDW, compared to CT. CT rather increased RLD and RDW compared to NT in the deeper soil (5-15 cm) only in maize. TRC suggests that the positive effect of NT on SOC is limited to the top layer (0-5 cm) for silty clay loam soils.

NT had a mixed impact on roots N content and on C:N ratio. This was mainly dependent on the effect of tillage on the percentage of roots coarser than 2 mm, which decreased average roots N content.

Both soil BD and PR decreased during time under NT. The significant correlation between root traits (RLD, RDW) and soil physical parameters (BD, PR) under NT corroborates the hypothesis that roots are a very relevant driver of soil physical condition. Last but not least, stability of continuous biopores is much more relevant than the total amount of pores to affect root growth.

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Figures and Tables

Table 1. Soil physical and chemical properties of the topsoil (0-30 cm depth) at the beginning of the experiment.

Parameter	Unit	Value
Sand (2 - 0.05 mm)	g kg^{-1}	122
Silt (0.05 - 0.002 mm)	g kg^{-1}	462
Clay (< 0.002 mm)	g kg^{-1}	417
pH (KCl 1 M)		5.4
$CaCO_3$ (volumetric)	g kg^{-1}	2
Organic Matter (Walkley and Black)	g kg^{-1}	21
total N (Kjeldahl)	g kg^{-1}	1.2
available P (Na bicarbonate 0.5 M, pH 8.5)	mg kg^{-1}	31.9
exchangeable K (Ba chloride, pH 8.1)	mg kg^{-1}	294
C.E.C. (Ba chloride, pH 8.1)	$cmol^{+}$ kg^{-1}	29.7

Table 2. Analysis of variance of root length density (RLD), root dry weight (RDW), roots C and N content, roots C:N ratio, bulk density (BD) of soil and penetration resistance (PR) as affected by tillage, soil depth, and distance from the row.

Year	Crop	Source of variation	RLD	RDW	C	N	C:N ratio	BD	PR
			P-value						
2014	Maize	tillage (T)	0.0573	0.4909	0.5425	**0.0001**	**< 0.0001**	**0.0046**	0.1876
		soil depth (De)	**< 0.0001**	**< 0.0001**	0.0648	**0.0079**	**< 0.0001**	**0.0114**	**< 0.0001**
		distance from the row (Di)	**< 0.0001**	**< 0.0001**	0.1784	**0.0046**	**< 0.0001**	0.1362	NA
		T x De	**< 0.0001**	**< 0.0001**	0.5001	**0.0065**	**0.0048**	0.8394	**0.0067**
		T x Di	0.0743	0.3148	0.3098	0.4339	0.2366	0.7315	NA
		Di x De	**0.0046**	**0.0004**	0.3094	0.0855	**0.0471**	0.0830	NA
		T x De x Di	0.1716	**0.0426**	0.8942	0.0904	**0.0062**	0.0655	NA
2015	Soybean	tillage (T)	**0.0002**	**< 0.0001**	0.2194	0.0963	**0.0398**	0.7342	0.7998
		soil depth (De)	**0.0001**	**< 0.0001**	0.3064	**0.0382**	**0.0067**	**0.0001**	**< 0.0001**
		distance from the row (Di)	0.4325	**0.0023**	0.5470	0.3716	0.0946	0.4519	NA
		T x De	0.4985	**0.0013**	0.2641	0.7238	0.3462	**0.0003**	**0.0008**
		T x Di	0.8163	0.6476	0.4782	0.9097	0.5421	0.9076	NA
		Di x De	0.8099	0.0641	0.3152	0.3699	0.0916	0.7113	NA
		T x De x Di	**0.0479**	0.9155	0.3103	0.8799	0.6714	0.8316	NA
2016	Winter Wheat	tillage (T)	**0.0386**	**0.0040**	0.1112	0.1591	0.2441	**0.0062**	**< 0.0001**
		soil depth (De)	**< 0.0001**	**< 0.0001**	0.0784	**0.0063**	**0.0073**	**0.0218**	**< 0.0001**
		distance from the row (Di)	0.4594	0.5492	0.1684	0.3059	0.3879	0.3800	NA
		T x De	**0.0054**	**0.0028**	0.0893	0.9994	0.9782	**0.0194**	**< 0.0001**
		T x Di	0.0830	0.1416	0.3198	0.9762	0.8672	0.1310	NA
		Di x De	0.9306	0.7394	0.8048	0.5820	0.4988	0.6483	NA
		T x De x Di	**0.0134**	0.4065	0.5505	0.8649	0.4906	0.6443	NA

Table 3. Root length density (RLD), root dry weight (RDW), root C and N content, and C:N ratio of maize, soybean and winter wheat at anthesis as influences by tillage systems. Bulk density (BD) and Penetration Resistance (PR) of soil at anthesis for each crop are also reported. Mean values ± standard deviation. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**

Main Crop	Maize (Year 2014)		Soybean (Year 2015)		Winter Wheat (Year 2016)	
Tillage system	CT	NT	CT	NT	CT	NT
RLD (cm cm^{-3})	2.24 ± 1.61	2.88 ± 3.16	1.04 ± 0.67 ***	1.70 ± 1.04 ***	1.91 ± 1.03 *	2.64 ± 1.84 *
RDW (mg cm^{-3})	0.56 ± 0.76	1.11 ± 2.96	0.25 ± 0.57 ***	1.05 ± 2.52 ***	0.17 ± 0.24 **	0.33 ± 0.42 **
C (%)	39.75 ± 3.05	39.42 ± 3.09	40.89 ± 9.69	37.46 ± 9.26	36.71 ± 1.77	35.84 ± 1.65
N (%)	1.17 ± 0.28 ***	1.34 ± 0.24 ***	4.42 ± 4.12	2.90 ± 1.71	1.03 ± 0.25	1.13 ± 0.27
C:N ratio	33.59 ± 7.36 ***	30.68 ± 7.30 ***	13.29 ± 7.85 *	15.14 ± 6.85 *	37.85 ± 9.92	33.60 ± 9.98
BD (kg dm^{-3})	1.39 ± 0.26 **	1.51 ± 0.14 **	1.43 ± 0.26	1.40 ± 0.20	1.27 ± 0.17 **	1.37 ± 0.19 **
PR (kPa)	1364 ± 759	1662 ± 617	1482 ± 774	1451 ± 423	1609 ± 747 **	1160 ± 294 **

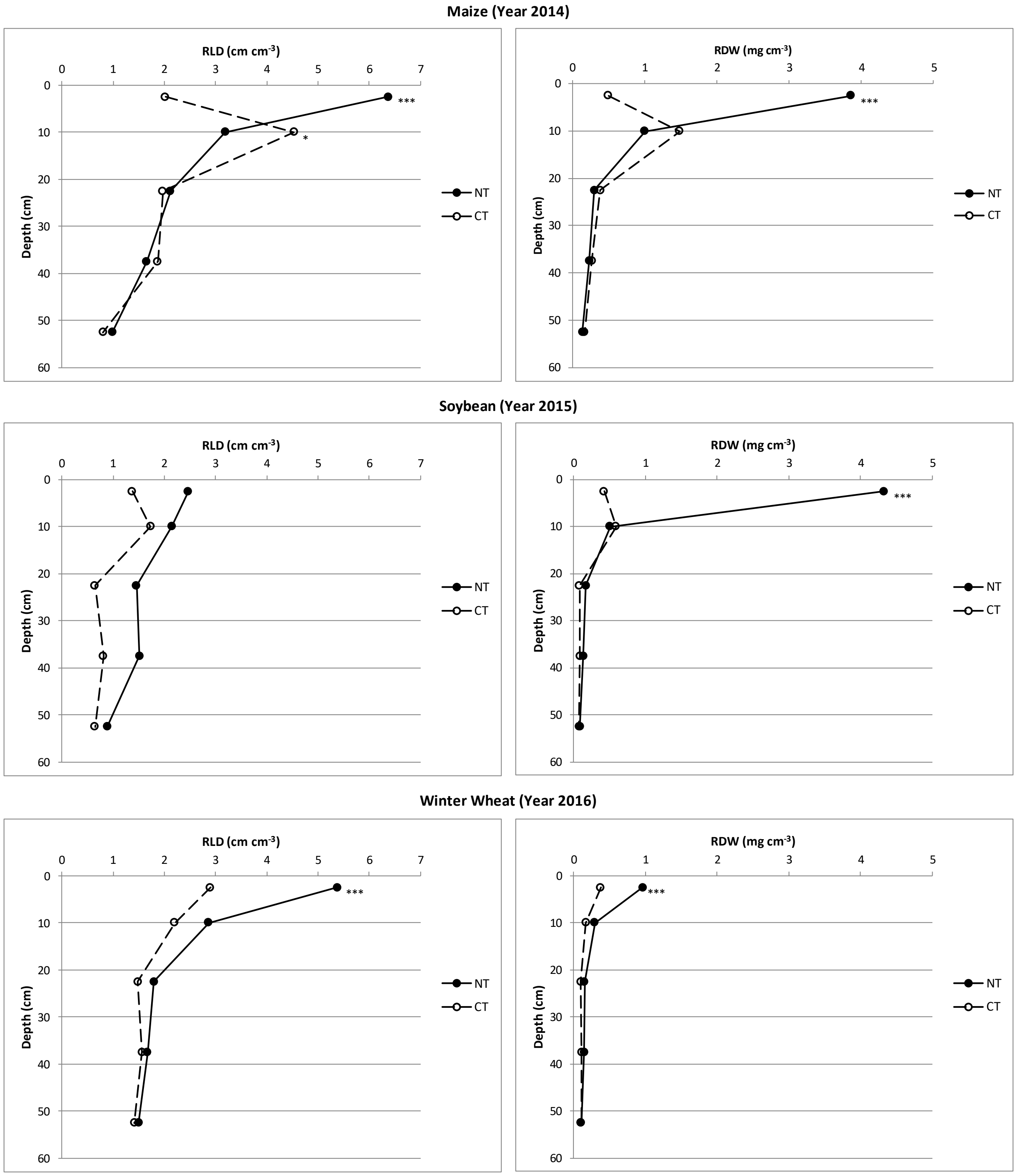

Figure 1. Root length density (RLD) and root dry weight (RDW) of maize, soybean and winter wheat as influenced by tillage system (CT: conventional tillage; NT: no-tillage) for different soil depth. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**

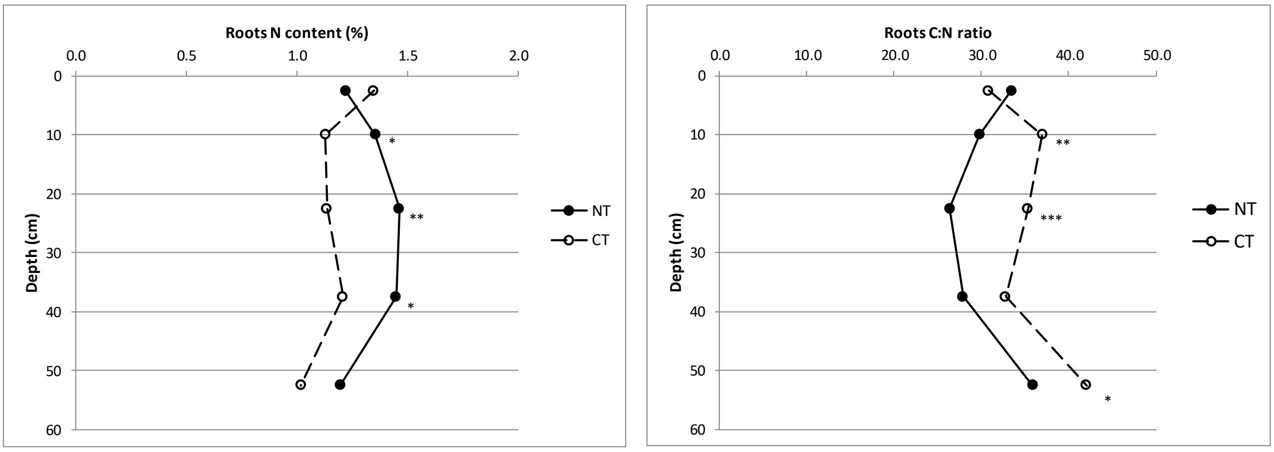

Figure 2. Root N content and roots C:N ratio of maize as influenced by tillage system (CT: conventional tillage; NT: no-tillage) for different soil depth. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**

Table 4. Diameter class length (DCL) for very fine (ø = 0.00-0.05 mm), fine (ø = 0.05-2.00 mm) and coarse (ø > 2.00 mm) roots diameters in each crop and soil depth. Letters with the same font indicate differences among tillage systems (CT: conventional tillage; NT: no-tillage) within the same crop and soil depth. Mean values ± standard deviation. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**

Main effect			Maize (Year 2014)	Soybean (Year 2015)	W. Wheat (Year 2016)
Tillage	Root Diameter Class	Soil depth	DCL (cm cm^{-3})	DCL (cm cm^{-3})	DCL (cm cm^{-3})
CT	ø = 0.00-0.05 mm	0-5 cm	1.1722 ± 0.7193 b ***	1.7551 ± 2.5965	2.0908 ± 0.5347 b ***
		5-15 cm	2.9294 ± 1.0922 A ***	1.2294 ± 0.5603	1.8299 ± 0.9374
		15-30 cm	1.3131 ± 0.4140	0.4532 ± 0.2376	1.2128 ± 0.7670
		30-45 cm	1.2432 ± 0.4209	0.5390 ± 0.2727	1.2622 ± 0.5056
		45-60 cm	0.4512 ± 0.1664	0.3886 ± 0.1413	1.0270 ± 0.4169
	ø = 0.05-2.00 mm	0-5 cm	0.8171 ± 0.4511 *b* ***	1.1524 ± 1.8331	0.7532 ± 0.7417 *b* ***
		5-15 cm	1.4997 ± 0.7431	0.4897 ± 0.2142	0.3707 ± 0.1374 *B* *
		15-30 cm	0.6372 ± 0.1858	0.1935 ± 0.0792	0.2709 ± 0.1284
		30-45 cm	0.6158 ± 0.1383	0.2714 ± 0.1011	0.3074 ± 0.1110
		45-60 cm	0.3438 ± 0.1084	0.2619 ± 0.0668	0.3869 ± 0.1174
	ø > 2.00 mm	0-5 cm	0.0354 ± 0.0468 *B* ***	0.0267 ± 0.0685 b ***	0.0562 ± 0.1384
		5-15 cm	0.1048 ± 0.1023	0.0136 ± 0.0186	0.0026 ± 0.0046
		15-30 cm	0.0227 ± 0.0216	0.0025 ± 0.0029	0.0012 ± 0.0023
		30-45 cm	0.0100 ± 0.0100	0.0006 ± 0.0009	0.0002 ± 0.0003
		45-60 cm	0.0083 ± 0.0126	0.0004 ± 0.0006	0.0005 ± 0.0005
NT	ø = 0.00-0.05 mm	0-5 cm	3.6124 ± 2.3398 a ***	1.9621 ± 1.6020	3.5813 ± 1.4276 a ***
		5-15 cm	1.8706 ± 0.9494 B ***	1.4632 ± 0.7529	2.0904 ± 0.9111
		15-30 cm	1.4456 ± 0.5430	1.0327 ± 0.2728	1.3092 ± 0.6732
		30-45 cm	1.0561 ± 0.3404	1.0449 ± 0.3470	1.1737 ± 0.7953
		45-60 cm	0.5214 ± 0.2717	0.5739 ± 0.1636	1.0886 ± 0.5408
	ø = 0.05-2.00 mm	0-5 cm	2.5885 ± 2.3914 *a* ***	1.2153 ± 0.7852	1.7168 ± 0.7062 *a* ***
		5-15 cm	1.3114 ± 1.0398	0.6699 ± 0.2962	0.7719 ± 0.2564 *A* *
		15-30 cm	0.6626 ± 0.2026	0.4250 ± 0.0817	0.4850 ± 0.1138
		30-45 cm	0.5532 ± 0.1351	0.4709 ± 0.1578	0.4951 ± 0.1447
		45-60 cm	0.3998 ± 0.1644	0.3124 ± 0.0904	0.4128 ± 0.1286
	ø > 2.00 mm	0-5 cm	0.3083 ± 0.4653 *A* ***	0.0864 ± 0.1186 a ***	0.0796 ± 0.0803
		5-15 cm	0.0678 ± 0.0818	0.0149 ± 0.0114	0.0023 ± 0.0044
		15-30 cm	0.0091 ± 0.0116	0.0021 ± 0.0011	0.0006 ± 0.0010
		30-45 cm	0.0100 ± 0.0146	0.0028 ± 0.0047	0.0010 ± 0.0016
		45-60 cm	0.0011 ± 0.0014	0.0007 ± 0.0010	0.0009 ± 0.0012

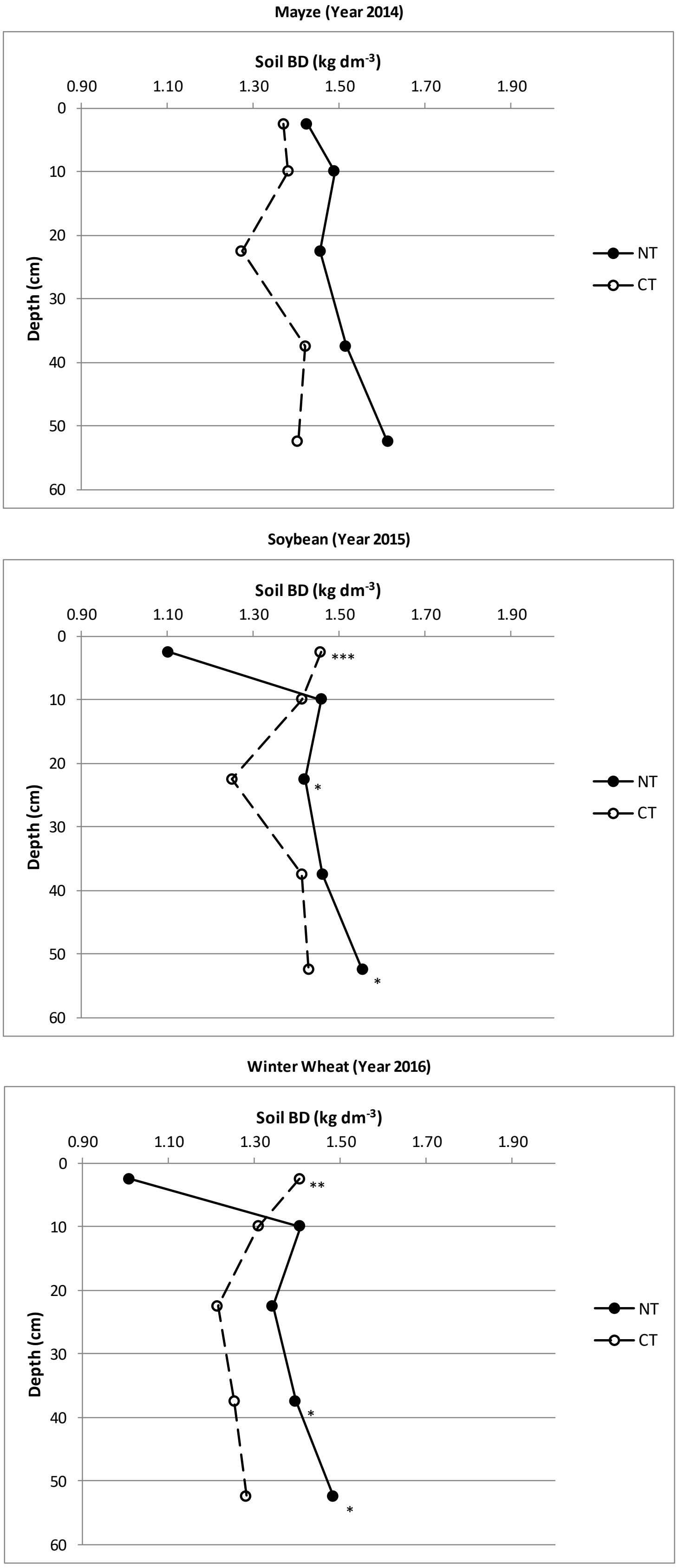

Figure 3. Soil bulk density (BD) at anthesis of maize (2014), soybean (2015), and winter wheat (2016) as a function of tillage system (CT: conventional tillage; NT: no-tillage) and soil depth.

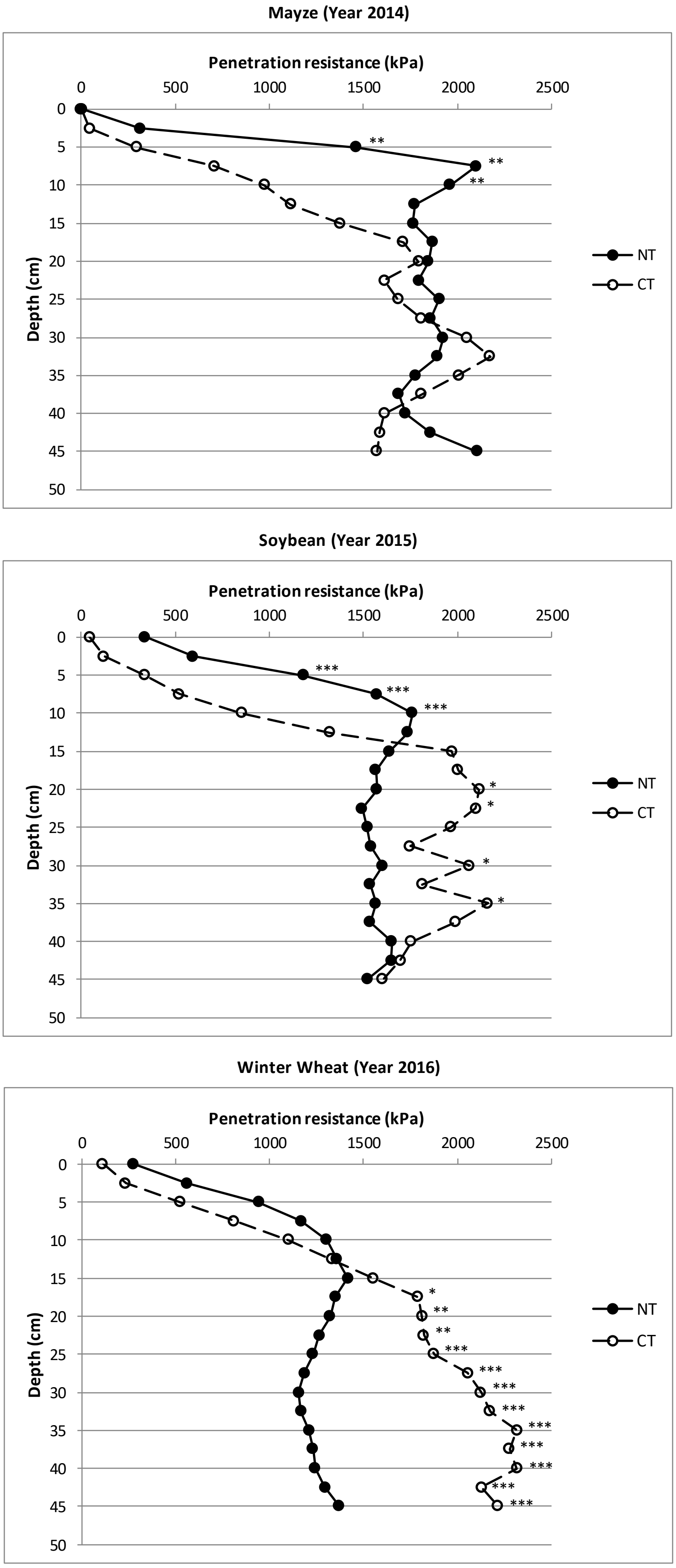

Figure 4. Soil penetration resistance at anthesis of maize (2014), soybean (2015), and winter wheat (2016) as a function of tillage system (CT: conventional tillage; NT: no-tillage) and soil depth. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**

Table 5. Spearman rank correlation coefficients between root density parameters and soil physical properties in 2014 (maize), 2015 (soybean), and 2016 (winter wheat). P-values are reported.

Treatment	Variables	Bulk Density						Penetration resistance					
		Maize (2014)		Soybean (2015)		W. Wheat (2016)		Maize (2014)		Soybean (2015)		W. Wheat (2016)	
		ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
NT	RLD	**-0.4865**	**0.0065**	**-0.3814**	**0.0485**	**-0.4458**	**0.0289**	**-0.3861**	**0.0067**	-0.0385	0.8343	**-0.6594**	**0.0009**
CT	RLD	0.2003	0.1249	0.0117	0.9427	**0.4403**	**0.0045**	-0.1575	0.1249	-0.2592	0.1520	-0.2438	0.1788
NT	RDW	**-0.3578**	**0.0267**	**-0.4634**	**0.0212**	**-0.4392**	**0.0389**	**-0.4083**	**0.0040**	-0.2863	0.1122	**-0.4944**	**0.0040**
CT	RDW	0.0653	0.6203	-0.0104	0.9492	**0.3747**	**0.0179**	-0.2317	0.1131	-0.2295	0.2065	-0.2856	0.1131

Supporting information

Table S1. Mean monthly temperature and monthly precipitation at CERZOO experimental station during the study period.

Years	2014		2015		2016	
Months	Rainfall (mm)	Mean Temperature (° C)	Rainfall (mm)	Mean Temperature (° C)	Rainfall (mm)	Mean Temperature (° C)
January	154.4	6	21.6	5	23.6	4
February	121.8	8	142	5	117.2	7
March	69	12	67.4	11	60	10
April	97.2	15	62.6	15	24.6	16
May	30	19	68.4	20	79.2	18
June	66.2	23	6.6	24	49.6	22
July	75.6	23	12.6	28	5.2	26
August	48.2	23	56.2	25	33.8	25
September	19.2	20	29	20	53.2	22
October	66.2	16	60.8	14	118.8	14
November	268.4	11	55.2	9	35.6	9
December	53.8	6	7.6	5	6.6	4

Table S2. Root length density (RLD) and root dry weight (RDW) of maize, soybean and winter wheat as influenced by tillage system (CT: conventional tillage; NT: no-tillage) for different soil depths and distances from the row. Mean values ± standard deviation. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**

Main effect			Maize (Year 2014)		Soybean (Year 2015)		Winter Wheat (Year 2016)	
Tillage	Distance from the row	Soil depth	RLD (cm cm^{-3})	RDW (mg cm^{-3})	RLD (cm cm^{-3})	RDW (mg cm^{-3})	RLD (cm cm^{-3})	RDW (mg cm^{-3})
CT	0 cm (all)	0-5 cm	2.60 ± 1.55	0.87 ± 0.81 ***	1.64 ± 0.34	2.74 ± 1.08	2.42 ± 0.50 ***	0.21 ± 0.15
		5-15 cm	6.10 ± 1.33	3.02 ± 0.51	1.36 ± 0.18	1.01 ± 1.29	2.02 ± 1.36	0.20 ± 0.11
		15-30 cm	2.40 ± 0.46	0.59 ± 0.20	0.62 ± 0.33	0.09 ± 0.03	1.68 ± 1.07	0.10 ± 0.06
		30-45 cm	2.24 ± 0.22	0.39 ± 0.07	0.75 ± 0.40	0.09 ± 0.06	1.70 ± 0.72	0.12 ± 0.06
		45-60 cm	0.69 ± 0.09	0.22 ± 0.19	0.61 ± 0.24	0.07 ± 0.03	1.33 ± 0.50	0.11 ± 0.03
	8.75 cm (winter wheat)	0-5 cm	NA	NA	NA	NA	3.38 ± 1.70	0.56 ± 0.67
		5-15 cm	NA	NA	NA	NA	2.39 ± 0.84	0.15 ± 0.08
		15-30 cm	NA	NA	NA	NA	1.29 ± 0.76	0.09 ± 0.06
		30-45 cm	NA	NA	NA	NA	1.44 ± 0.54	0.10 ± 0.04
		45-60 cm	NA	NA	NA	NA	1.50 ± 0.43	0.11 ± 0.03
	17.5 cm (maize and soybean)	0-5 cm	1.71 ± 0.82	0.39 ± 0.33	1.10 ± 1.06 ***	0.12 ± 0.13	NA	NA
		5-15 cm	4.76 ± 1.80	1.11 ± 0.42	2.10 ± 0.96	0.17 ± 0.06	NA	NA
		15-30 cm	1.93 ± 0.41	0.32 ± 0.13	0.68 ± 0.32	0.08 ± 0.05	NA	NA
		30-45 cm	1.68 ± 0.23	0.19 ± 0.06	0.87 ± 0.38	0.09 ± 0.04	NA	NA
		45-60 cm	0.89 ± 0.32	0.16 ± 0.12	0.69 ± 0.19	0.09 ± 0.03	NA	NA
	35 cm (maize)	0-5 cm	1.77 ± 1.07	0.23 ± 0.15	NA	NA	NA	NA
		5-15 cm	2.74 ± 0.72	0.33 ± 0.13	NA	NA	NA	NA
		15-30 cm	1.59 ± 0.70	0.27 ± 0.14	NA	NA	NA	NA
		30-45 cm	1.68 ± 0.81	0.24 ± 0.09	NA	NA	NA	NA
		45-60 cm	0.83 ± 0.25	0.15 ± 0.03	NA	NA	NA	NA
NT	0 cm (all)	0-5 cm	13.18 ± 2.25	10.75 ± 5.68 ***	1.71 ± 1.01	7.09 ± 4.63	6.04 ± 1.82 ***	1.27 ± 0.47
		5-15 cm	5.39 ± 2.08	2.25 ± 1.56	2.31 ± 1.36	0.69 ± 0.30	2.67 ± 0.88	0.26 ± 0.07
		15-30 cm	2.41 ± 1.04	0.40 ± 0.26	1.46 ± 0.44	0.19 ± 0.10	2.04 ± 0.84	0.18 ± 0.07
		30-45 cm	1.69 ± 0.28	0.29 ± 0.06	1.68 ± 0.55	0.15 ± 0.05	1.94 ± 1.07	0.17 ± 0.10
		45-60 cm	1.25 ± 0.55	0.19 ± 0.09	0.90 ± 0.28	0.09 ± 0.02	1.67 ± 0.56	0.12 ± 0.05
	8.75 cm (winter wheat)	0-5 cm	NA	NA	NA	NA	4.72 ± 2.11	0.66 ± 0.65
		5-15 cm	NA	NA	NA	NA	3.06 ± 1.40	0.33 ± 0.19
		15-30 cm	NA	NA	NA	NA	1.55 ± 0.72	0.13 ± 0.05
		30-45 cm	NA	NA	NA	NA	1.40 ± 0.78	0.13 ± 0.06
		45-60 cm	NA	NA	NA	NA	1.34 ± 0.68	0.09 ± 0.05
	17.5 cm (maize and soybean)	0-5 cm	3.48 ± 0.92	0.55 ± 0.20	3.23 ± 1.95 ***	1.57 ± 2.12	NA	NA
		5-15 cm	2.62 ± 0.87	0.42 ± 0.21	1.99 ± 0.81	0.32 ± 0.32	NA	NA
		15-30 cm	2.17 ± 0.60	0.25 ± 0.09	1.46 ± 0.26	0.16 ± 0.04	NA	NA
		30-45 cm	1.77 ± 0.28	0.21 ± 0.05	1.36 ± 0.47	0.13 ± 0.06	NA	NA
		45-60 cm	0.93 ± 0.27	0.15 ± 0.06	0.87 ± 0.25	0.09 ± 0.04	NA	NA
	35 cm (maize)	0-5 cm	2.60 ± 1.55	0.29 ± 0.17	NA	NA	NA	NA
		5-15 cm	6.10 ± 1.33	0.35 ± 0.37	NA	NA	NA	NA
		15-30 cm	2.40 ± 0.46	0.27 ± 0.05	NA	NA	NA	NA
		30-45 cm	2.24 ± 0.22	0.22 ± 0.06	NA	NA	NA	NA
		45-60 cm	0.69 ± 0.09	0.11 ± 0.04	NA	NA	NA	NA

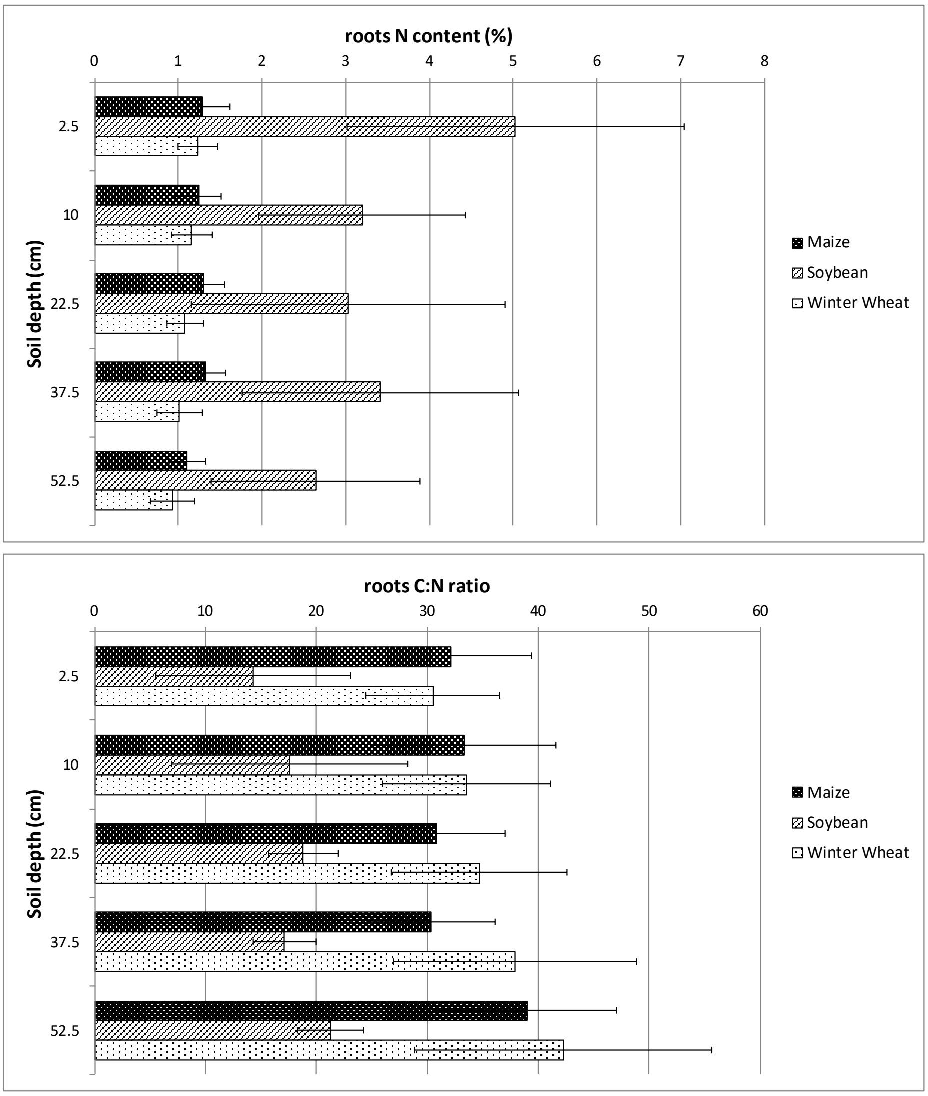

Figure S1. Roots N content (%) and roots C:N ratio of maize, as influenced by soil depth. Mean values ± standard deviation.

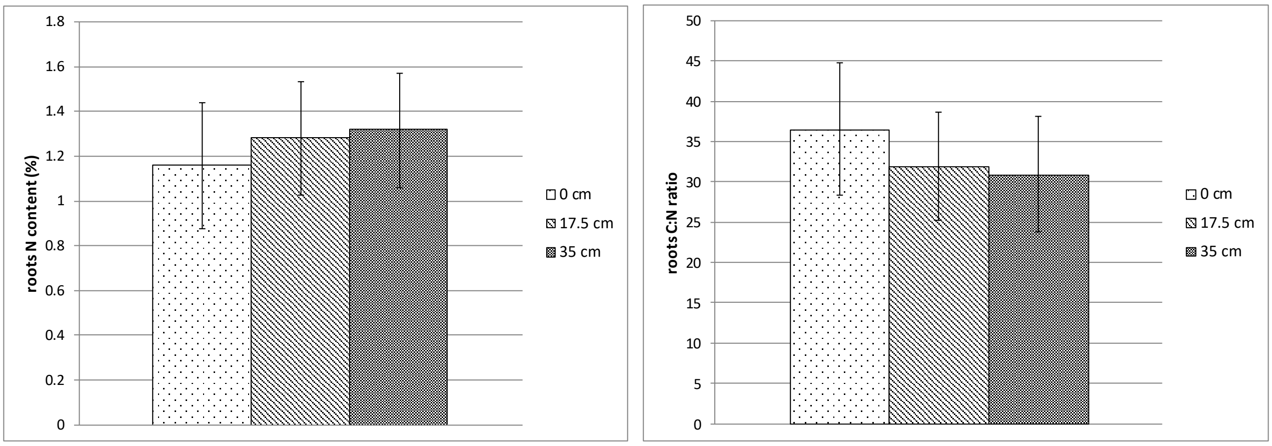

Figure S2. Roots N content (%) and roots C:N ratio of maize, as influenced by distance from the row. Mean values ± standard deviation.

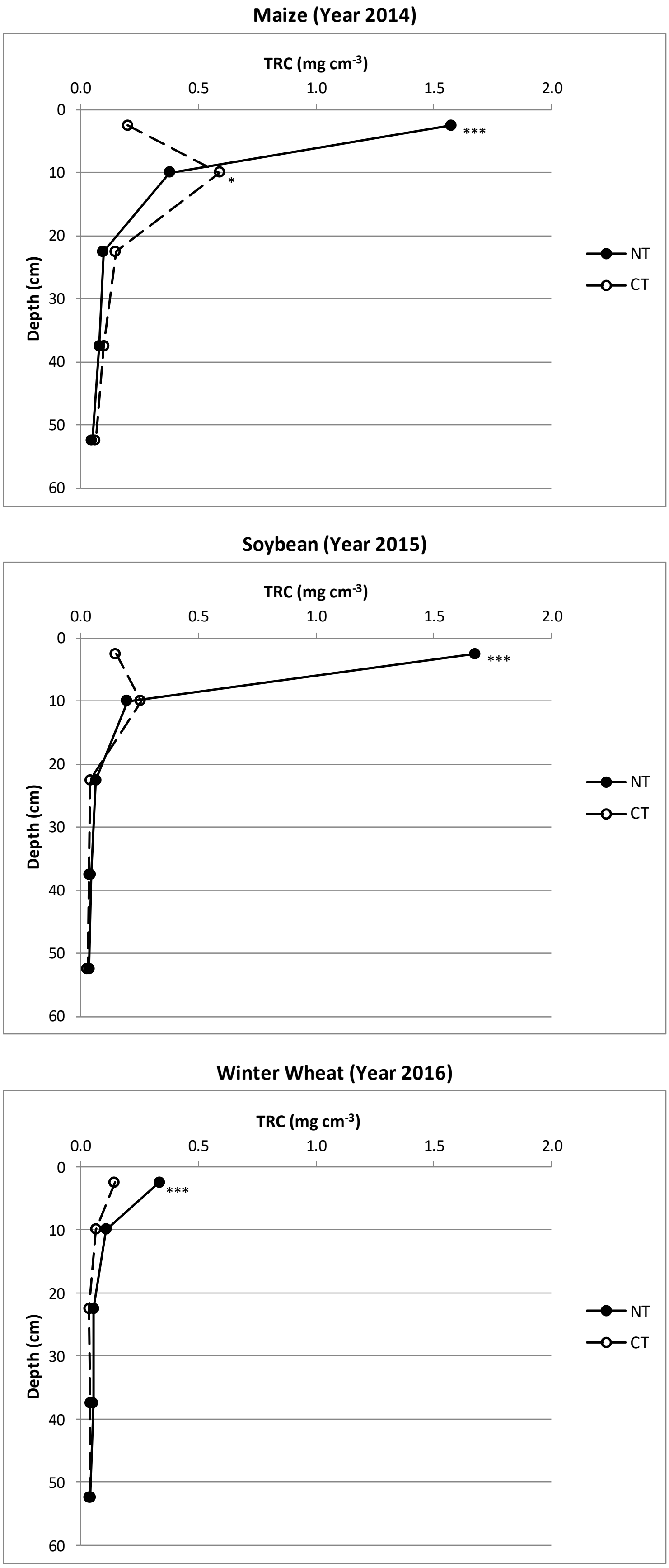

Figure S3. Total amount of root carbon (TRC) of maize, soybean and winter wheat as influenced by tillage system (CT: conventional tillage; NT: no-tillage) for different soil depth. *,,*** indicate significance at P < 0.05, 0.01, 0.001, respectively; blank is not significant.**