

Article – Agronomy/Soil Science **Autumn Cover Crops Increase Deep Root Growth of Soybean in no-Tillage System**

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HIGHLIGHTS

- Cover crops under contour farming significantly improved soybean root growth in subsoil after two years.
- Minirhizotron technic enables root study of annual crops in the field, but high root data variability demands special care with plant stand close to the tubes after sowing, and high replication number.

Abstract: Root growth is crucial in crop production and is influenced by soil attributes, but also affects soil chemical, physical and biological quality, along with management and conservation practices. In this paper, soybean root growth data from a field study at Guarapuava, Paraná State, Brazil are examined. In 2019, at a farm with over 35 years of no-tillage system (NT) adoption, three management sets were established in 1,1 ha macroplots: 1) farmer's NT standard (FNT), without terraces, with downhill farming and with autumn fallow; 2) autumn cover crops and contour farming added to FNT, as best management practices (BMP); 3) terraces and contour farming (TC) added to FNT. Soybean was sowed in November 2020 and 2021, and minirhizotron tubes (1 m long, 7 cm wide) were installed into the soil just after soybean emerged in 2020. Root length, area, volume, and diameter were evaluated with the help of a root scanner. Data were submitted to descriptive statistics. The added conservation practices improved root length, area and volume, significantly for BMP in the subsoil (40-60 cm) compared to FNT standard. Minirhizotron enabled the study of roots in the field, but data variability was high, so that for annual crops with pivoting roots like soybean, root observation points (6 per plot) must be increased, and crop stand close to the observation points is a critical font of error.

Keywords: Glycine max; Root Length; Green Manure; Contour Farming; Terrace.

INTRODUCTION

Largest soybean (*Glycine max* L.) exporter, Brazil is also the largest producer, with 154,5 million t [1]. Brazilian farmers manage soybean fields mainly under no-tillage (NT), which was introduced in Parana State in the 1970's, then was adopted in the south states, and expanded north across the Cerrados biome along with soybean cultivation. Also participating in integrated production systems, with forage and forest species [2], soybean and NT dominate the national agricultural sector [3] due to the profitability of the crop, and the celerity and economy in field operations with NT, which also controls water erosion by not disturbing the soil, so that the straw in the surface protects the soil from the impact of raindrops, and soil structure develops [4].

However, quality of mulch and of soil attributes under NT are not sufficiently achieved just by the absence of soil tillage and by the cultivation of cash crops [5]. NT was designed as a conservation management system, with a set of practices that guarantee its functionality over time, and the partial adoption of the practices commonly generates problems, such as vertical stratification of soil fertility and subsurface soil compaction [6]. Besides incomplete adoption of the system, there are cases in which conservation practices are discontinued after years of NT adoption, such as the removal of terraces, based in the belief that NT is efficient to control water erosion, what has brought back runoff problems [7].

Terraces and contour farming create physical barriers to runoff, reducing its speed and erosive potential [8,9], but have been abandoned to enable mechanization regardless of contour, even downhill and in favor of runoff, aiming at greater distances between maneuvers and machinery efficiency [10]. Practices such as crop rotation and cover crops, which promote the chemical, physical and biological quality of the soil are less used [11–13], but rotating crops with different root systems, in terms of nutrient absorption and spatial distribution in the soil, is important to improve nutrient cycling and increase biological activity [14]. Cultivation of proper cover crops may help in soil decompaction process, the biopores left by their roots may favor the root growth and the yield of succeeding cash crops, and also reduce surface runoff by improving water infiltration [15,16].

Plant roots are affected by soil properties and reciprocally influence soil quality, so that root growth may be considered an indirect measure of soil conditions to crop production [16]. Roots are source of organic material for the soil and, therefore, affect soil structure, aeration and biological activity [17]. The study of roots, however, is considered difficult, time consuming, and the accuracy of the results is usually not very great, which discourages this kind of research. Field study methods for plant roots can be expensive (rhizotrons) and are usually destructive (excavation), incompatible with long-term field trials [18].

Minirhizotron is an adapted technique that allows the study of roots in a non-destructive, relatively low cost and convenient way, consisting of transparent acrylic tubes inserted into the soil prior to root growth, and adapted scanners to capture root images from inside the tubes, which are later processed using image software [19]. There are few results with this method in field experimentation in tropical and subtropical conditions, but it can be a tool for evaluating root growth and infer about soil quality and management practices for crops like soybean. The aim of this paper was to evaluate soybean root growth under NT system, as carried out by a farmer and with additional soil and water conservation practices.

MATERIAL AND METHODS (Style 2.1 Section)

The study is part of the project "Soil chemical indicators, root growth and yield of soil management practices under no-tillage system in Midwestern Parana State", part of the Agroresearch Network in Mesoregion-3 of Parana (PR). A representative catchment of soil use and management in Guarapuava-PR was selected in Entre Rios County, belonging to the soil map unit LBd5, under the domain of Typic Hapludox soils (Latossolos Brunos) [20]. In March 2019, after corn harvest, a farm with more than 35 years of NT system history was selected, and three macroplots measuring 65 m x 165 m (1.1 ha) each were set up in an area with homogeneous surface and slope (4%). Initial characterization of soil chemical attributes and granulometry are shown in Table 1.

Table 1. Soil chemical attributes**¹** and particle size distribution for the 0-20 cm depth layer at macroplot site.

 $1 P$ = phosphorus, and K⁺ = potassium, extracted by Mehlich-1; SOC = soil organic carbon, determined by Walkley-Black; Al^{3+} = aluminum, Ca²⁺ = calcium, and Mg²⁺ = magnesium, extracted by KCl 1 mol L⁻¹; H+Al = potential acidity, estimated by SMP buffer; particle size analysis by the pipet method, with NaOH dispersing agent.

Macroplot 1 was maintained with the farmer's NT standards (**FNT**), without terraces, with downhill farming oriented by greater distances between tractor maneuvers, using the following 4-year crop rotation: ¾ soybean + ¼ corn (*Zea mays*) in summer, autumn fallow, wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) in winters preceding soybean, and black oat (*Avena strigosa*) cover crop preceding corn. In **macroplot 2**, best management practices (**BMP**) were added to FNT standard, with autumn cover crops and contour farming. In **macroplot 3**, terraces and contour farming (**TC**) were added to the FNT standard.

Soybean was planted in November 2019 (cv. K6221), 2020 and 2021 (cv. BMX Zeus), and the roots were evaluated in 2020 and 2021 crop seasons, when row width was 40 cm, seeding rates were 16 and 15 seeds m⁻¹, and fertilizations used 250 and 370 kg ha⁻¹ of 00-25-25 (NPK), respectively. The seeds were treated (insecticides and fungicides) and inoculated (*B. japonicum*) for biological nitrogen fixation. In the 2020/2021 crop season, soybean was preceded by wheat in winter, and BMP plot was previously planted with forage turnip (*Raphanus sativus*) in autumn. In the 2021/2022 crop season, soybean was preceded by barley in winter, and BMP plot was previously planted with a mix of black oat, rye (*Secale cereale*), common vetch (*Vicia sativa*) and hairy vetch (*Vicia villosa*) in autumn.

Precipitation data were collected during the soybean crop seasons (Figure 1), by an automatic meteorological station at the experimental site.

Figur*e* **1***.* Monthly precipitation (mm) between November and March during the soybean crop seasons of 2020/2021 and 2021/2022*,* in Entre Rios County, Guarapuava, Parana State, Brazil.

After soybean emergence in 2020, an adapted probe was used to install minirhizotron's transparent acrylic tubes (1 m long, 7 cm in diameter) between the crop rows, perpendicularly to the soil surface. Duplicate tubes were installed in the upper thirds of the slope, and also in the middle and in the lower thirds, totaling 6 tubes per plot. At R1-R2 stages (flowering) of soybean [21], root images on the surface of the tube walls were captured in the 0-20, 20-40 and 40-60 cm depth layers, using a root scanner (CI-600, CID Bioscience, USA). In 2021, soybean was planted with the tubes already installed, and seeder was operated so that the tubes remained between the seeding rows. Image capture followed the same procedure as for 2020.

The images were analyzed with the RootSnap (CID Bioscience, USA) software, and the root length, root area and root volume were totalized by image, i.e. by soil depth layer on each tube, while root diameter was averaged by image, to obtain the mean root diameter by soil depth layer on each tube. The results of the root growth attributes were grouped by soil depth layer in each macroplot, with six observations per layer (six tubes) for each macroplot. Data were submitted to descriptive statistical analysis using SISVAR software [22], and the confidence interval calculated for 95% reliability was used to compare the means of root attributes between the three sets of soil and water management and conservation practices.

RESULTS

Root length presented a decrease pattern from 0-20 cm down to 40-60 cm depth in the soil (Figure 1). No effects of conservation practices were observed in the 2020/2021 crop season, when root length reached a maximum of 354,66 cm in the 0-20 cm soil layer of TC plot (Figure 2a), and data variability was very high (large confidence intervals of the means).

The variability was lower in the 2021/2022 crop season, when root length dropped to a maximum of 60 cm in the 0-20 cm layer of TC plot (Figure 2b). In this second season, root length presented an increase pattern with BMP and TC plots in comparison to FNT for all three soil layers evaluated, and BMP root length was significantly higher in comparison to FNT in the 40-60 cm layer, while TC was intermediary and showed no difference in comparison to the other plots.

Figure 2. Soybean root length (cm) at R1-R2 stage in the crop seasons of (a) 2020/2021 and (b) 2021/2022, captured in the soil layers of 0-20, 20-40 and 40-60 cm depth by means of the minirhizotron technique, under farmer's no-tillage (FNT) standards, and with the addition of best management practices (BMP) or terraces plus contour farming (TC), in Entre Rios County Guarapuava, Parana State, Brazil.

The decrease in the values of root length in 2021/2022 agrees with the fact that fewer roots were captured by the images in this crop season (Figure 3b), when compared to the 2020/2021 season (Figure 3a).

Figure 3. Soybean root abundance in images captured in the 0-20 cm soil depth layer, at R1-R2 stage in the crop seasons of (a) 2020/2021 and (b) 2021/2022, under farmer's no-tillage (FNT) standards, in Entre Rios County Guarapuava, Parana State, Brazil.

There was no significant effect of the management practices on soybean root surface area in none of the soil layers in the 2020/2021 crop season (Figure 4a). In agreement to the results for root length, in the 2021/2022 crop season, the FNT plot presented lower root surface area in comparison to BMP in the 40-60 cm layer, with no differences between these management sets and CT been observed. Comparing both seasons, the second presented, approximately, one tenth of the root surface area values observed in the first season (Figure 4b).

Figure 4. Soybean root surface area (mm²) at R1-R2 stage in the crop seasons of (a) 2020/2021 and (b) 2021/2022, captured in the soil layers of 0-20, 20-40 and 40-60 cm depth by means of the minirhizotron technique, under farmer's no-tillage (FNT) standards, and with addition of best management practices (BMP) or terraces plus contour farming (TC), in Entre Rios County Guarapuava, Parana State, Brazil.

Reflecting the results for root length and surface area, the soybean root volume (Figure 5) presented significant effect of managements only for the 40-60 cm layer in the 2021/2022 season. The BMP plot presented higher root volume in relation to FNT, and TC remained with intermediary root volume, not differing from BMP and TC (Figure 5b).

Figure 5. Soybean root volume (mm³) at R1-R2 stage in the crop seasons of (a) 2020/2021 and (b) 2021/2022, captured in the soil layers of 0-20, 20-40 and 40-60 cm depth by means of the minirhizotron technique, under farmer's no-tillage (FNT) standard, and with the addition of best management practices (BMP) or terraces plus contour farming (TC), in Entre Rios County Guarapuava, Parana State, Brazil.

The root diameter of soybean presented no response to management practices in none soil layers and seasons (Figure 6). Differently from other root attributes evaluated, root diameter values were similar within the crop seasons.

Figure 6. Soybean root diameter (mm) at R1-R2 stage in the crop seasons of (a) 2020/2021 and (b) 2021/2022, captured in the soil layers of 0-20, 20-40 and 40-60 cm depth by means of the minirhizotron technique, under farmer's no-tillage (FNT) standard, and with the addition of best management practices (BMP) or terraces plus contour farming (TC), in Entre Rios County Guarapuava, Parana State, Brazil.

DISCUSSION

Roots were concentrated in the 0-20 cm layer in all management sets, like in other study with soybean under no-tillage and crop rotation, which recorded higher root length density in the top 20 cm of soil [23]. This is because the root system is initiated from this layer after seed germination, so it has roots since day one of plant's life and accumulates root growth along all the crop cycle. This layer also has higher availability of nutrients and lower levels of acidity (H+AI) and toxic Al³⁺, favorable conditions for root growth, nutrient absorption and crop yield, and convenient as diagnostic layer for soil fertility tests [24]. Down through the profile, at 20-40 cm, 40-60 cm and deeper, further away from topsoil liming and fertilization effects, less nutrients are available, and the soil turns to be more acidic, normal conditions for Oxisols, like the one of the study, presenting higher Al^{3+} levels, which is toxic for roots [25].

The high magnitude of the confidence intervals of the means for length, surface area and volume of the roots (Figures 2, 4, 5) indicated greater variability in the data also close to the soil surface, according to the facts that the roots are concentrated in this layer, and that the topsoil is where roots face greater vertical and horizontal variability of chemical, physical, and biological attributes in relation to subsurface layers [26]. Also, the soybean plant presents pivoting roots, from which lateral roots originate, and in the case of the minirhizotron, they may or may not reach the walls of the acrylic tubes for image capture, thus constituting a limitation and variability font for this kind of root study, creating an intrinsic variability.

Minirhizotron method is easier to use in terms of labor, but it may underestimate root length (density) in comparison with traditional destructive methods like excavation, exactly because of the difficulty in having roots growing exactly towards the tubes [27]. Despite of that, lower variability (coefficient of variation) for root growth attributes under no-tillage was obtained using the same method for black oat [28], which has fasciculated root system, full of thinner and more homogeneously distributed roots, that are less affected by soil compaction [29], showing that specie and other experimental conditions alter data variability for rooting.

The initial absence of effects on root attributes between management sets, in the 2020/2021 soybean season, may be due to the fact that the conservation practices were adopted in the autumn of 2019, and the time period until this soybean was planted may have been too short. Practices like cover crops, contour farming and terracing may need more time to establish significant effects on soil properties and affect cash crop performance, especially when taken into account that the soil of the area is very clayey and has high SOC content (Table 1), so that both the mineral and the organic fractions of the soil matrix result in resilient structure and buffered soil fertility conditions, which take time to be modified by management.

When the second soybean crop was cultivated in the 2021/2022 season, a longer period had passed and the adding of soil conservation practices to the farmer's standards may have accumulated effects, then starting to improve soil condition for root growth, with increases in length, surface area and volume of roots, significantly in the 40-60 cm depth layer when BMP set is compared to FNT standards. This increase of rooting attributes, although not significant, also occurred as a pattern in the 0-20 and 20-40 cm layers in BMP, and also in TC plot relatively to FNT.

Additionally, in 2021/2022 season soybean was preceded by mix of cover crops (autumn) and barley (winter), while in 2020/2021 season the crop was preceded by turnip cover crop (autumn) and barley (winter). So, besides been planted after a longer period since additional conservation practices were started, this second soybean crop came after a more diverse growth of root systems in the soil profile, which may improve soil structure with biopores that are explored by cash crops as paths of lesser resistance, and soybean roots grow through these channels after the roots of cover crops decompose, so that more access to deeper soil layers are given to a larger number of roots, turning to be an important advantage to crop yield when soil compaction or drought periods occur [30].

Although the meteorological conditions were similar and good for soybean within the seasons, with total precipitations during the cycles reaching 767 mm in 2020/2021 and 608 mm in 2021/2022, enough for soybean, November and December were drier in 2021 in comparison to 2020, what may in part explain the fact that less roots were captured in the images in the second season.

However, another difference within the crop seasons may have played a stronger effect on root abundance in the images, which is the fact that in 2020/2021 soybean was planted first and the minirhizotron tubes were installed after plant emergence, between the rows, while in 2021/2022 the crop was planted with the tubes already installed in the field, and the low speed of the tractor-seeder set close to the tubes, to avoid tube breakage, resulted in lower stand of soybean plants around the root evaluation points, another limitation and source of variability for the method considering long-term studies. Even with manual replanting after soybean emergence in the rows close to the tubes, it may have had an effect. Plant stand is a key factor for this kind of study, and keeping the same plant density within the crop seasons is challenging [16]. On the other hand, removal, and reinstallation of the tubes each season would turn the method laborious and destructive [27], discouraging its use once there exists other destructive methods that may be more efficient.

Conservation practices, which include cover crops (BMP), contour farming (BMP and TC) and terracing (TC) result in significant control of water and soil losses by runoff, keeping the soil more humid, but they may also reduce the nutrient stratification in the profile and the compaction degree of subsurface soil [31]. When the soil is moist, water may easily flow to the roots in the surface layer, but as the soil dries out water movement decreases, turning root length density (length per soil volume) fundamental for water absorption efficiency [32], so that plants may present higher root length density distributed down the soil layers in drought seasons, while in rainy Years the roots get more concentrated in the surface layer [27]. Accordingly, in the present study less difference in root growth (length, surface area and volume) was observed within the layers in the drier season of 2021/2022, while in 2020/2021 roots were more concentrated in the 0-20 cm layer (Figures 2, 4, 5).

Root diameter, unlike the other attributes, showed no tendency to decrease in depth, nor did it show the same magnitude of the confidence intervals, proving to be less affected by soil conditions, tube installation moment or crop stand. Greatly relevant in terms of root penetration into the soil, the root diameter is a determinant factor for root growth and the ability of roots to transport water and nutrients [33], especially when the soil structure is limiting. The lack of response in soybean root diameter to the different management practices studied may indicate that there were no significant differences within the macroplots in terms of soil compaction degree. A study involving managements under conventional system with tillage and under notillage system, did not observe significant responses of wheat root diameter [34].

CONCLUSION

The addition of conservation practices to the farmer's NT standard, such as the use of off-season cover crops in the autumn, contour farming and terraces can improve the growth of soybean roots in terms of length, surface area and volume, especially in depth into the soil profile. The use of the minirhizotron technique enables the study of roots, but its limitations for use with annual crops and mechanized cultivation need to be previously known, so that the technique is adapted, and data variability is controlled.

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