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Root growth and crop performance of soybean under chemical, physical, and biological changes after subsoiling

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Abstract

Chemical, physical and biological soil attributes can facilitate soybean root growth in greater volume and depth in the soil, which can minimize yield reduction caused by water deficit. Soil management can contribute positively or negatively to these soil attributes. The aim of this work was to evaluate the root growth and crop performance of soybean, in response to chemical, physical and biological changes after subsoiling at different depths. At the R5 phenological stage, trenches were made for sampling and soil collection for chemical, physical and biological analysis and root growth was carried out. At V5, V7, R2 and R5 stages, plants were collected to evaluate height, leaf area and dry mass. At V5, stage number and dry mass of the nodules were evaluated. Subsoiling increased pH and Ca, and decreased Al in the soil, resulted in higher relative density and did not affect in mechanical penetration resistance compared to non-subsoiled soil. Basal respiration and soybean nodulation were higher in the subsoiled soil. Up to 15 cm depth, there were 87.91% of the total root dry mass and 78.79% of the total root volume. Initial and final plant growth were the same in subsoiled and non-subsoiled soil. Number of nodules in the subsoiled soil was 28% higher than in the non-subsoiled soil. Under these study conditions, subsoiling provides lower root growth but benefits grain yield.

1 | INTRODUCTION

Soybean [*Glycine max* (L.) Merril] yield has been constantly increasing due to research and genetic improvement advances. With this knowledge about this crop, it is possible to guarantee vigorous plants with high productive potential. However, although techniques and information have improved, unfavorable environmental conditions may limit yield.

Abbreviations: FC, field capacity; OM, organic matter; PR, penetration resistance; PWP, permanent wilting point; RD, relative density; TP, total porosity.

Yield is sensitive to environmental conditions, especially temperature and availability of light and water (Mundstock & Thomas, 2005). Among these conditions, water deficit is the most limiting, with the greatest negative impact on production (Casagrande et al., 2001). Under stress conditions, plants trigger various changes in biochemistry, physiology and morphology (Souza, Catuchi, Bertolli, & Soratto, 2013). One reaction of the plant to water deficit is the stomatal closure, which results in a lower rate of CO_2 assimilation (Pirasteh-Anosheh, Saed-Moucheshi, Pakniyat, & Pessarakli, 2016), due to the water potential reduction in the cells (Silva, Magalhães Filho, Sales, Pires, & Machado, 2018). Water deficit can modify photosynthetic pigments and gas exchange, impairing the plant growth and productive potential (Anjum et al., 2011). Negative impacts on yield of up to 50% can be achieved on soybean plants under stress conditions (Lisar, Motafakkerazad, Hossain, & Rahman, 2012).

Roots absorb the water required for plants full functioning (Lazarovitch, Vanderborght, Jin, & Van Genuchten, 2018). Therefore providing adequate conditions for root growth in volume and depth in the soil can be an alternative to reduce stress caused by water deficit (Gewin, 2010). This is because having a higher amount of roots means higher soil volume exploited, that is, more roots in contact to the soil solution (Balbinot et al., 2018), increasing nutrient uptake (Goss, Miller, Bailey, & Grant, 1993).

The root phenotype plasticity study in the growing environment is important because this characteristic can have a great impact on crop yield (Postma, Schurr, & Fiorani, 2014). The soil-plant-atmosphere interaction can be understood when assessing the plants root system, and may contribute to higher yield by improving management practices that aimed to increase soil exploration by roots for water and nutrients (Bordin et al., 2008; Fan, McConkey, Wang, & Janzen, 2016).

Soil chemical, physical and biological attributes can negatively affect root growth. Chemical constraints are due to the lack of essential elements and/or presence of toxic elements, while physical restraint is mainly due to higher soil density, which increases penetration resistance and modifies soil porosity (Doussan, Pagès, & Pierret, 2003). In the absence of soil microbiological organisms, the process of organic waste decomposition and nutrient cycling may be compromised (Hungria, Franchini, Brandão-Junior, Kaschuk, & Souza, 2009).

In addition to the interference in root growth, pH can change the nutrient absorption capacity, which may lead to mineral deficiency or toxicity by metals such as aluminum (Brown, Koenig, Huggins, Harsh, & Rossi, 2008; Gentili, Ambrosini, Montagnani, Caronni, & Citterio, 2018). Calcium may favor cell elongation and expansion, increasing root growth (Fageria & Moreira, 2011). Soil organic matter is a natural fertilizer with biological properties not found in inorganic fertilizers (Zandonadi & Busato, 2012) that affect root architecture and nutrient uptake (Herder, Isterdael, Beeckman, & Smet, 2010). Phosphorus provides plant energy, signaling and stimulating root hairs development (Sousa, Gomes, Souza, & Vasconcelos, 2010). Soil physical components are usually a consequence of soil density, because higher soil density results in compacted soil, which increases root penetration resistance (Bengough, Mckenzie, Hallett, & Valentine, 2011), reduces the soil volume explored by the roots, and the contact with the soil solution (Valentine et al., 2012). It also reduces the soil porosity, which consequently increases cryptopores and decreases soil macropores and micropores (Veiga, Reinert, Reichert, & Kaiser, 2008).

Core Ideas

- The highest volume and mass of soybean roots are in the first centimeters of the soil.
- The growth of soybean roots presents a positive correlation with soil porosity and respiration.
- Subsoiling improved soil chemical attributes.

When soil compaction is observed, management operations to break the compacted layer are necessary. This interference commonly occurs through scarifiers or subsoilers provided with cutting discs in front of the stems to prevent straw incorporation into the soil (Seki, Seki, Jasper, Silva, & Benez, 2015). Subsoiling is indicated for mechanical soil decompaction in the sub-surface layer (Macedo, Monteiro, & Santos, 2016), which generates cracks and provides surface roughness, reducing superficial water runoff (Secco & Reinert, 1997) without soil revolving, considering the minimum cultivation principle (Macedo et al., 2016).

In southern Brazil, there is a common presence of Latosols that are generally correlated with Oxisols (American soil taxonomy) and Ferralsols (WRB system). Latosols comprise soils at advanced weathering stages, with consequent concentration of 1:1 clay minerals and oxides (Schaefer, Fabris, & Ker, 2016). The soil compaction is a common and constant problem on most farms that use no-till system and have this type of soil. In no-tillage, there is absence of soil tillage and heavy machine traffic over time, favoring the natural reconsolidation of soil particles (Reichert, Suzuki, Reinert, Horn, & Hakansson, 2009). Thus, subsoiling could be required to decrease soil density (Marasca, Lemos, Silva, Guerra, & Lanças, 2015; Souza, Souza, Cooper, & Tormena, 2015). In Red Latosol, with clayey texture the subsoiling positively affected rice yield (Pinheiro, Stone, & Barrigossi, 2016), in red-yellow latosol with sandy texture, there was a decrease in PR values caused by the subsoiling equipment's rod action (Gonçalves, Lopes, Cavalieri-Polizeli, Fiedler, & Stahl, 2019). The subsoiling in clay soil, improves the physical properties of soil, reduces root density in surface soil, and increases deep root growth, which alleviates root crowding in the upper soil layer and enhances water and nutrient absorption in the deeper soil layer of maize plants (Sun et al., 2017).

The hypothesis is that subsoiling improves soil physical structure and consequently modifies soil chemical and biological aspects, benefiting root growth in greater soil volume and depth. Thus, in order to understand root growth in soil alterations, the objective of this work was to evaluate the root growth and crop performance of soybean in response to soil chemical, physical and biological changes at different depths under subsoiling.

2 | MATERIALS AND METHODS

2.1 | Site description

The soybean cultivar used was 53i54. The experiment was conducted on a farm in the 2017/2018 harvest, in Coxilha, Rio Grande do Sul, Brazil (28°07′ S, 52°17′ W; 721 m altitude). The soil was classified as humid dystrophic Red Latosol (Streck et al., 2008), with 11% sand, 21% silt and 68% clay, more than 2 meters depth. The climate is temperate, with harsh winter and high summer temperatures. The average annual temperature is 17.5°C and the average annual rainfall is 1,787.8 mm.

2.2 | Experimental design

The experimental design was in randomized blocks with five replications, with different depths and presence of subsoiling. Each plot was 15 meters long for 15 seeding lines, with the plots following the seeding line one after the other.

The sowing was performed on 17 Oct. 2017. Seeds were inoculated with *Bradyrhizobium japonicum*, treated with insecticides and fungicides. The base fertilization was 15 kg ha^{-1} of N, 87 kg ha^{-1} of P_2O_5 and 21 kg ha^{-1} of K_2O. Due to the high solubility of KCl and possible damage to seed or seedling development, most of the potassium was applied before sowing soybean in an amount of 200 kg ha^{-1} KCl. During the soybean cycle, rainfall was 360.8 mm (Figure 1A).

The subsoiling was carried out in 2016 with the Terrus subsoiler. The subsoiler had 4 stems that reached 40 cm depth in the ground and were spaced 60 cm apart. At the time of subsoiling, the soil was in conditions of adequate humidity, and 15 days before subsoiling there were no precipitations (Figure 1B). In this area, there was a perception of physical problems, due to constant traffic of big machinery. As it is a tillage with no-tillage system, only at sowing the cutting disc can reach up to 15 cm depth, so it was observed that the roots concentrated in the first 15 cm depth, with an angle close to 90° due to the soil physical impediment to roots penetrate, that is why it was subsoiled up to 40 cm depth trying create conditions for the roots to deepen.

Due to soil characteristics in the experiment region, providing adequate conditions for root growth up to 40 cm depth is already considered ideal, since it is a clay soil that presents great impediment to root growth and deeper subsoiling demands a lot of tractor power and does not portray the region reality.

Before subsoiling, a dose of calcitic lime 2,000 kg ha^{-1} with a total neutralizing power (PRNT) of 88%, was applied. Before subsoiling, this area was cultivated with corn (*Zea mays* L.), and after was sown black oats (*Avena strigosa* Schreb.), and for the summer crop, soybean. In 2017 black oats were sown again, and then the soybean evaluated in this experiment. The subsoiled and non-subsoiled areas received the same management and were located side by side.

Soybean phenological stages was determined by Fehr and Caviness (1977) scale. Five and seven nodes on the main stem with fully developed leaves beginning with the unifoliolate nodes, comprises V5 and V7 stages, respectively. The R2 stage is when there is an open flower at one of the two uppermost nodes on the main stem with a fully developed leaf. The R5 stage is when the seed is 3 mm long in a pod at one of the four uppermost nodes on the main stem with a fully developed leaf.

2.3 | Soil chemical attributes

At the R5 phenological stage, trenches were made for sampling and soil collection for chemical, physical and biological analysis and root growth, with one trench per repetition, totaling 10 trenches. To evaluate the chemical attributes, soil in the sowing line up to 60 cm depth, in layers of 5 cm, was collected, totaling 12 samples per trench. Hydrogenionic potential (pH, H_2O), aluminum (Al, $cmol_c$ dm^{-3}), calcium (Ca, $cmol_c$ dm^{-3}), organic matter (OM, %) and phosphorus (P, mg dm^{-3}) were determined. Considering that root growth starts in the sowing line, all the samples were performed in this site. The pH was determined in water 1: 1, P by the Mehlich I method, OM by wet digestion, and exchangeable Ca and Al with KCl 1 mol L^{-1}.

2.4 | Soil physical attributes

Soil samples with preserved structure were collected, using cylinders with 5 cm of diameter and height. Division of the soil density by the maximum soil density determined relative soil density. Relative soil density was evaluated because it considers the maximum soil density that is dependent on soil texture. The maximum soil density was determined as the function of the clay content (Marcolin & Klein, 2011), and soil clay content was determined by the pipette method (Embrapa, 1997). Total porosity was determined by the equation proposed by Embrapa (1997). The pores were classified into macropores (> 0.05 mm), micropores (0.05–0.0002 mm) and cryptopores (< 0.0002 mm) and were determined by increasing tensions in porous plate funnels. At 6 kPa (60 cm) tension the macropores were determined (Embrapa, 1997), and the cryptopores (1500 kPa) were determined by the equation that considers the soil clay content (Klein, Baseggio, Madalosso, & Marcolin, 2010). The micropores were

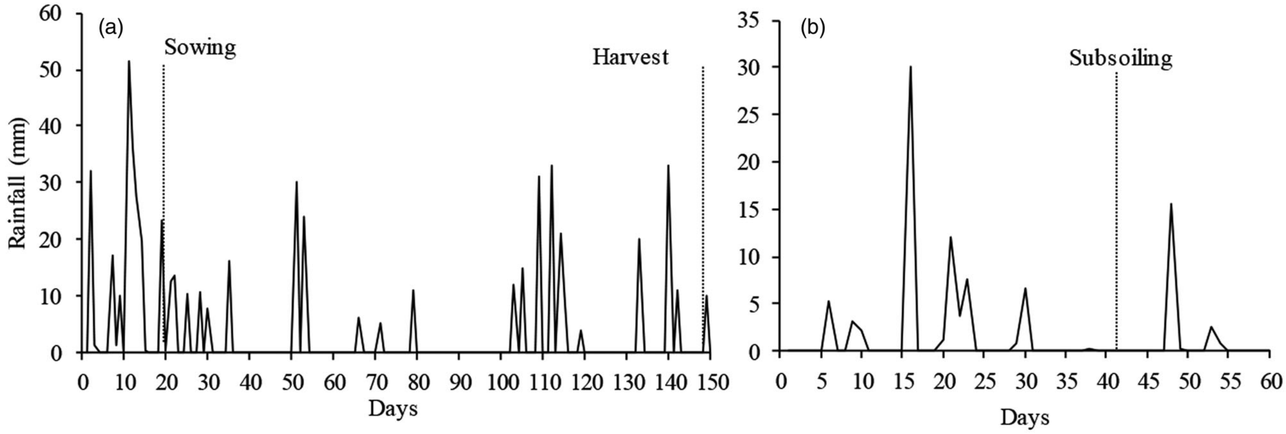


FIGURE 1 Rainfall during soybean cycle, corresponded from 1 Oct. 2017 to 28 Feb. 2018 (a) and near the moment of subsoiling, corresponded from 1 May 2016 to 30 June 2016 (b). Data collected on the experiment site

determined by the difference between the 6 and 1500 kPa tensions. The penetrometers used in the field presented operational problems related to the difficulty of maintaining a constant penetration rate (Moraes, Silva, Zwirtes, Anderson, & Carlesso, 2014) and the variation of soil water content in the various layers (Chancellor, 1977). Due to the difficulties encountered in determining soil mechanical resistance to field penetration, this should preferably be determined in the laboratory using an electronic penetrometer. Soil mechanical penetration resistance was determined in the laboratory using an electronic penetrometer (Marconi MA-933) with a constant velocity of 0.17 mm s^{-1}, equipped with a 200-N cell and a cone with 4 mm of base diameter and half-angle of 30°, and the data were collected in every second of penetration. Soil moisture at the moment of penetration was determined soon after the penetration resistance evaluation, with variation among the samples. The cylinder with the soil was weighed at the moment of penetration and after the dry soil. The moisture under tension of 6 kPa corresponds to the field capacity and the humidity under 1500 kPa corresponds to the permanent wilting point, so the difference between both is the amount of water available to the plants.

2.5 | Soil biological attributes

Microbial biomass and soil basal respiration were evaluated as soil biological indicators, using the colorimetry methodology developed by Bartlett and Ross (1988). Microbial biomass analysis consists of fumigated and non-fumigated samples in the chloroform presence and potassium permanganate as an oxidizing agent. For basal respiration, 50 g of sieved soil was used, incubated in glass flasks sealed to absorb CO_2 that the soil released, and had inside it a beaker with 10 ml of NaOH solution (1 mol L^{-1}). Five days after incubation in dark and under 25 ± 2°C of temperature, the beaker was removed and CO_2 contained in the NaOH was precipitated with addition of 2 ml barium chloride ($BaCl_2$), and excess of NaOH was titled with HCl solution (0.5 mol L^{-1}), and phenolphthalein as indicator.

2.6 | Root growth

Roots were collected with an iron structure with dimensions of 45 cm by 9.25 cm by 5 cm in length, width and depth, respectively. These dimensions were determined according to row spacing (45 cm) and plant density of 240,000 plants, which represents 9.25 cm spacing between plants. The roots were collected up to 45 cm depth, in 5 cm layers, totaling nine samples per trench. From 45 cm depth, the roots presence was practically nonexistent. Trenches were made transversal of the sowing line, so root collections were made from half of one interline to half of the other interline (45 cm). Soil separation from the roots was done by washing with running water using a 0.7 mm mesh sieve. In the washing some soil remained in the sieve and then the roots were removed from the sieve, with tweezers. After this, roots were analyzed in WinRhizo Software, determining volume, surface area, diameter and root length. These roots were dried at 65°C for dry mass determination. Root volume and dry mass were transformed to hectare, considering soil volume collected from 0.00208 m^2.

2.7 | Vegetative growth and grain yield

In V5, V7, R2 e R5 stages, 10 plants were collected, totaling 50 plants per treatment. Plant height, leaf area and dry mass per plant were evaluated. In V5 stage plants were collected with roots to determine the number and dry mass of the nodules per plant. Eight lines of 5 m per replicate were harvested and determined mass of thousand grains (g) and grain yield (kg ha^{-1}), with the moisture content corrected to 13%.

2.8 | Statistical analysis

Shoot growth, nodulation and yield means were compared by the Tukey test, and root growth, chemical, physical and biological attributes were compared by the Scott-Knott test.

After normality test of means, data were submitted to analysis of variance (ANOVA). Due to the number of treatments, the shoot growth, nodulation and yield means were compared by the Tukey test for subsoiled and non-subsoiled soil, and root growth, chemical, physical and biological attributes were compared by the Scott-Knott test for different depths, subsoiled and non-subsoiled soil. The purpose of the Scott-Knott method is to separate treatment averages into homogeneous groups, thus minimizing sum of squares within, and maximizing it among groups without overlapping them. And when the number of treatments is small, there is no need to separate by groups, as there is an ease in interpreting the results.

For both tests, .05 of error probability were considered. Variables that presented differences between subsoiling treatments were analyzed using Pearson's correlation coefficient between soil attributes and root growth variables, at $p \leq .05$ and a principal component analysis was performed to identify which soil attributes influenced root growth.

3 | RESULTS

3.1 | Chemical attributes

Subsoiling improved soil chemical attributes (Figure 2), increased hydrogenionic potential (pH) and calcium (Ca) and decreased aluminum (Al). In the 12 layers evaluated, pH was equal in 11 to 60 cm of depth, presenting 4.86 of mean, while the layers 0–5 and 6–10 cm differed from each other, and from the others, with the highest soil pH values (Figure 2A).

Al content increased with increasing soil depth (Figure 2B). The lowest amount of Al was found in the 0–5 cm (0.13 $cmol_c$ dm^{-3}) layer, followed by 6–15 cm, with an average 0.44 $cmol_c$ dm^{-3}, and from 16 cm depth the Al stabilized with an average of 0.80 $cmol_c$ dm^{-3}. The amount of Al decreased approximately 40% in subsoiled compared to non-subsoiled soil.

Calcium decreased with increasing soil depth, stabilizing from 31 cm depth (Figure 2C). From 0–10 cm depth, the average Ca was 7.42 $cmol_c$ dm^{-3}, from 11–30 cm deep the average was 4.49 $cmol_c$ dm^{-3} and in the other depths the average Ca was 2.85 $cmol_c$ dm^{-3}. The subsoiled soil presented higher amount of calcium, with the difference 0.67 $cmol_c$ dm^{-3} compared to the non-subsoiled soil.

The OM behavior was similar to Ca at different depths, decreasing with increasing soil depth, and the subsoiled soil presented lower OM than non-subsoiled soil (Figure 2D). From 0–5 cm depth the amount of OM was 4.24%, while in the last layer evaluated, the concentration of OM was 1.59%. Thus, a gradient of OM is observed in the soil profile, and 34.66% of OM was in the 0–15 cm depth layer.

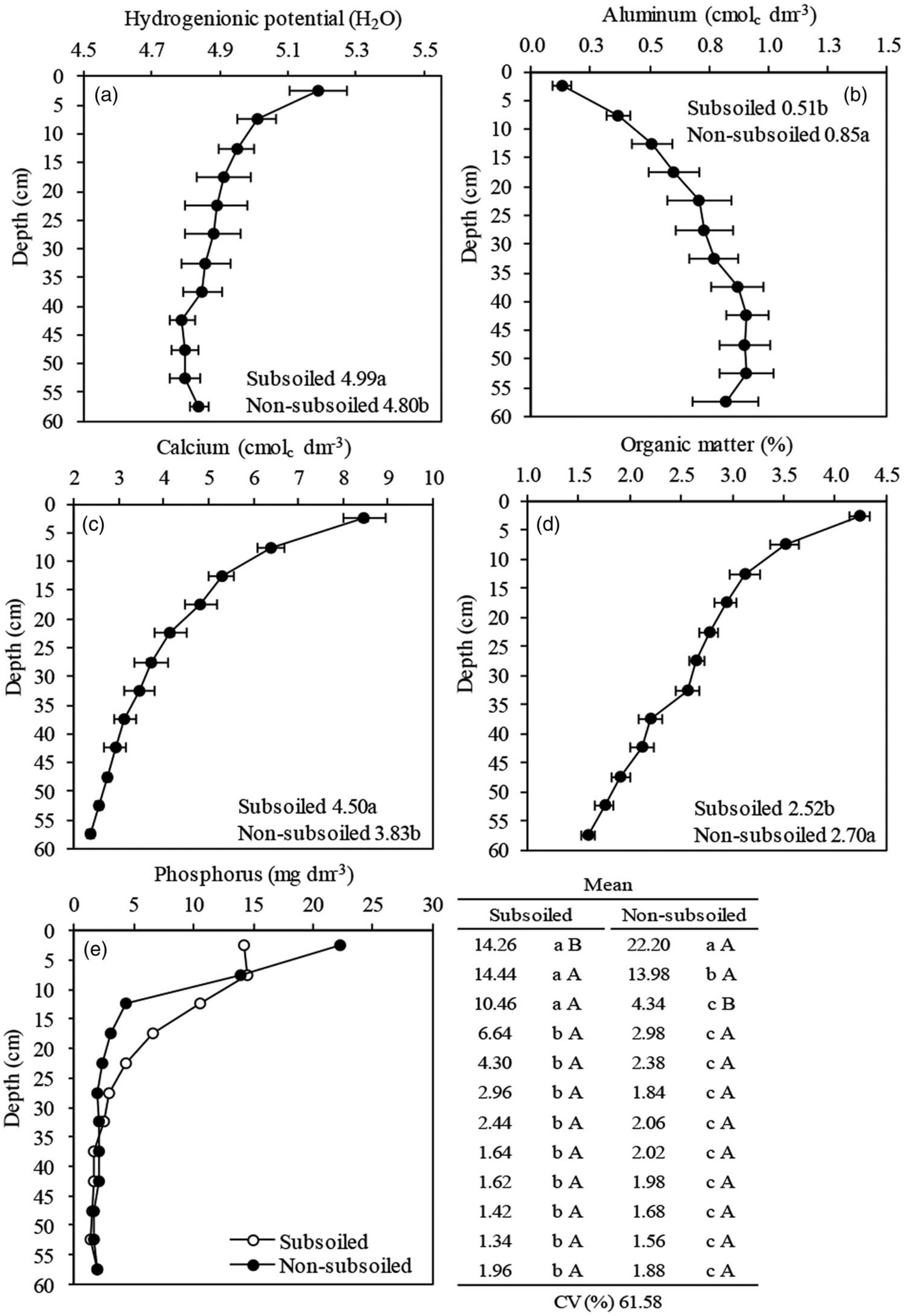
Phosphorus was the only chemical attribute that showed interaction between soil management and layers (Figure 2E). In the 0–15 cm similar P amounts was observed, with an average of 13.05 mg dm^{-3}, while in the other layers the average was 2.70 mg dm^{-3}. In subsoiled soil, the 0–5 cm layer was higher than the 6–10 cm, which was higher from the others. Subsoiled soil showed approximately 38% of P amount in the 0–5 cm layer. However, subsoiled soil presented a lower amount of P in the 0–5 cm, but increased the amount of this attribute in the 10–15 cm layer.

3.2 | Physical attributes

Subsoiling resulted in a higher relative soil density, which decreased the soil pore space (Figure 3A, B). Layer of 0–5 cm had the lowest density, with 0.67 of mean, followed by the 6–15 cm one, with 0.79 of mean. Relative density was stabilized from 16 cm of depth, with 0.91 of mean for the layers.

Soil porosity presented a negative relation with subsoiled soil, presenting lower mean in comparison to the non-subsoiled soil (Figure 3B). Total porosity was the inverse of the relative density, and the 0–5 cm layer presented the highest porosity, with 0.63 cm^3 cm^{-3} of mean, followed by the 6–15 cm, with 0.58 cm^3 cm^{-3} of mean. From 16 cm of depth, total porosity stabilized, with 0.54 cm^3 cm^{-3} of mean for the layers.

More soil macropores and micropores were found in the non-subsoiled soil, while the cryptopores did not differ between subsoiled and non-subsoiled soil (Figure 3C, D, E). The amount of macropores was higher in the first soil layers and from 21 cm depth they stabilized (Figure 3C). The micropores were superior in the non-subsoiled soil, but did not differ between the layers (Figure 3D). Since water available to plants is the amount of water stored in the micropores, it can be observed that no difference was found between the layers for the amount of water available to plants, with an average of 0.14 m^3 m^{-3} (Figure 3D). Subsoiling impaired the available water storage to the plants, reducing 0.01 m^3 m^{-3}, which



Mean			
Subsoiled		Non-subsoiled	
14.26	a B	22.20	a A
14.44	a A	13.98	b A
10.46	a A	4.34	c B
6.64	b A	2.98	c A
4.30	b A	2.38	c A
2.96	b A	1.84	c A
2.44	b A	2.06	c A
1.64	b A	2.02	c A
1.62	b A	1.98	c A
1.42	b A	1.68	c A
1.34	b A	1.56	c A
1.96	b A	1.88	c A
CV (%) 61.58			

FIGURE 2 Chemical attributes in subsoiled and non-subsoiled soils, in the different soil layers.
Hydrogenionic potential (a), aluminum (b), calcium (c), organic matter (d) and phosphorus (e) of subsoiled and non-subsoiled soil, in different layers, in the soybean R5 stage. Means compared by the Scott-Knott test ($p \leq .05$). Horizontal bars represent ± standard error of the mean. Means followed by the same lowercase letter in the column did not differ from each other in soil layers and means followed by the same capital letter in the row did not differ from each other for subsoiled and non-subsoiled soils

represents 10 L less water per cubic meter of soil. Cryptopores was the inverse from the macropores, with higher amounts in the deeper soil layers. Layer of 0–5 cm presented 0.19 m^3 m^{-3} of mean, while the 6–15 cm layer did not differ and had 0.225 m^3 $m^{-3}$of mean, while the 16–40 cm layer had 0.264 m^3 m^{-3} of mean and the others had 0.287 m^3 m^{-3} of mean (Figure 3E). The mean of all layers for each pore class was 0.16, 0.14 and 0.26 m^3 m^{-3} for macropores, micropores and cryptopores, respectively, and the cryptopores corresponded to approximately 46% of the total soil pores.

The permanent wilting point was different between the soils, presenting means of 0.25 and 0.26 m^3 m^{-3} for subsoiled

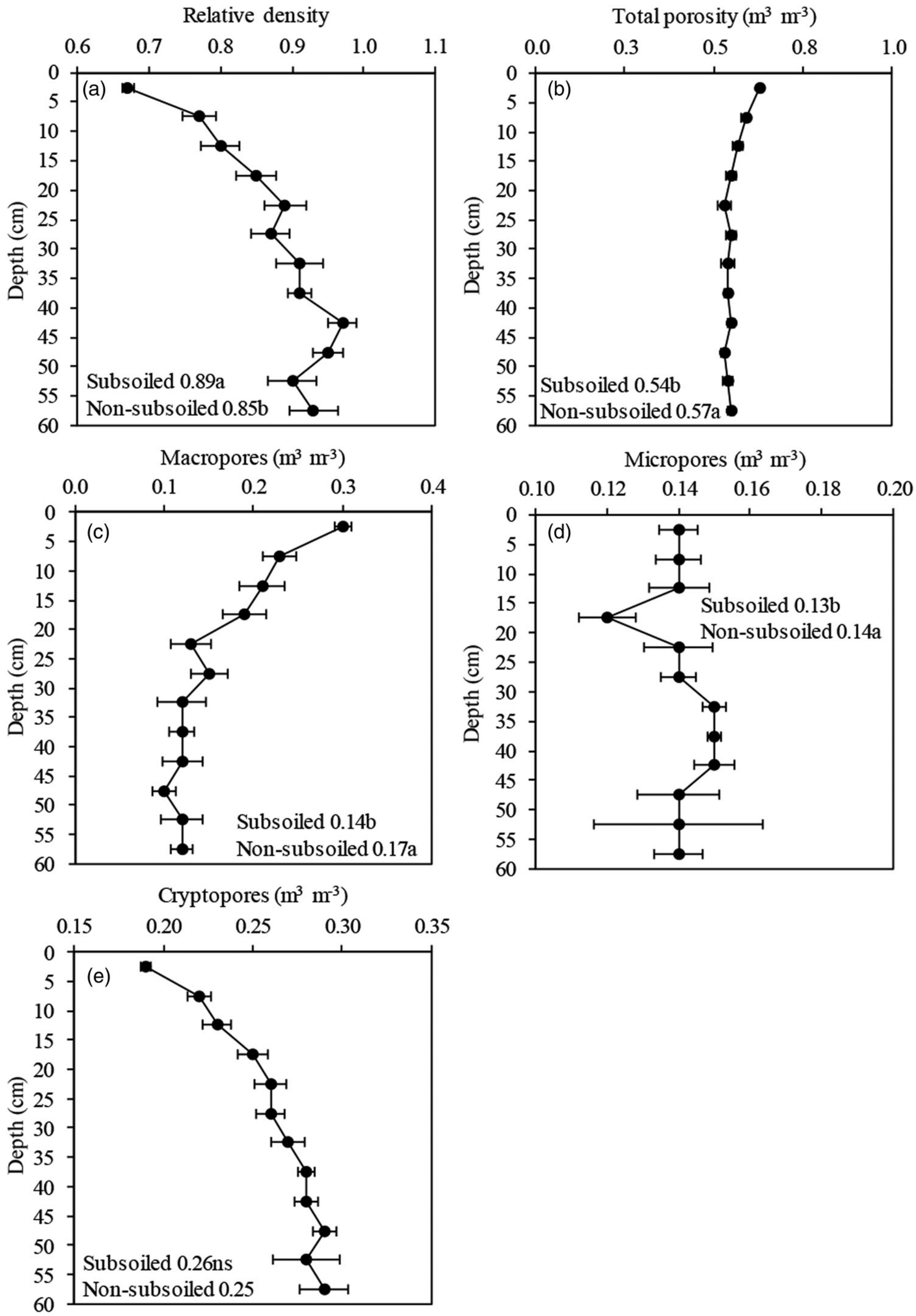


FIGURE 3 Physical attributes in subsoiled and non-subsoiled soils, in the different soil layers. Relative density (a), total porosity (b), macropores (c), micropores (d) and cryptopores (e) of subsoiled and non-subsoiled soils, in different layers, in the soybean R5 stage. Means compared by the Scott-Knott test ($p \leq .05$). Horizontal bars represent ± standard error of the mean

and non-subsoiled soil, respectively, while field capacity did not differ between the managements (Figure 4). Subsoiled soil did not differ from the non-subsoiled soil in the mechanical penetration resistance (Figure 5). But differed between the layers, where in 21–60 cm the resistance was the same, with mean of 3.33 MPa, which represents approximately twice of the resistance in the first 20 cm of depth.

3.3 | Biological attributes

Microbial biomass did not differ between the subsoiling and non-subsoiling managements; however it was different among the layers (Figure 6A). It was observed that the upper layers concentrate the greater microbial biomass. In the first 5 cm depth it concentrated 23.65%, 6–10 cm concentrated

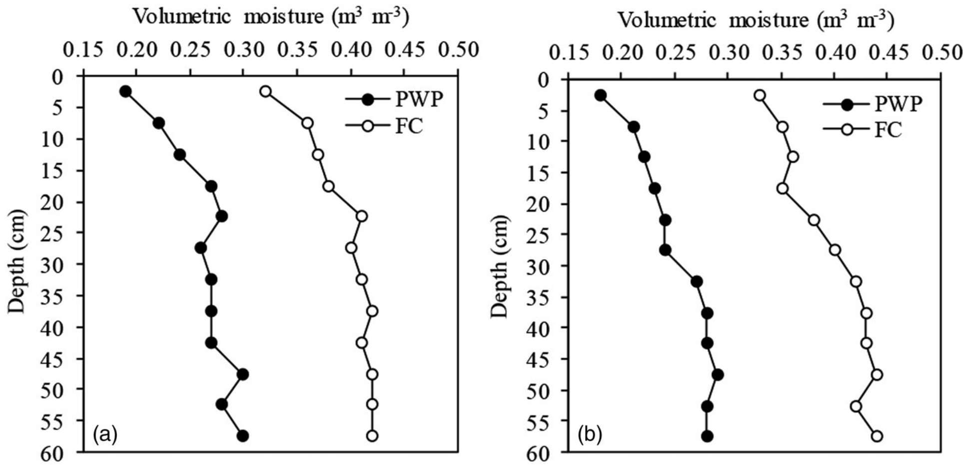
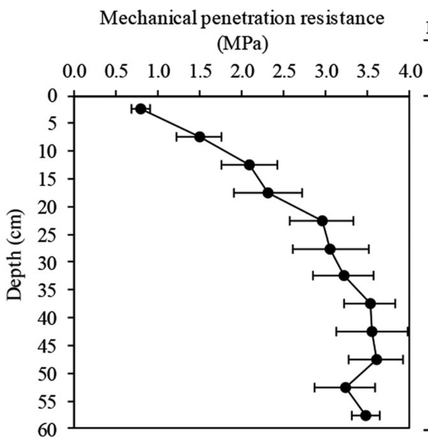


FIGURE 4 Volumetric moisture of permanent wilting point and of field capacity in subsoiled and non-subsoiled soils, in the different soil layers. Volumetric moisture of subsoiled soil (a) and non-subsoiled soil (b) in different layers, in the soybean R5 stage. Means compared by the Scott-Knott test ($p \leq .05$)



Mean moisture (m^3 m^{-3})	
Subsoiled 0.34ns	
Non-subsoiled 0.34	
0.26	c
0.30	b
0.32	b
0.31	b
0.35	a
0.31	b
0.36	a
0.37	a
0.36	a
0.36	a
0.37	a
0.37	a
CV (%) 12.82	

FIGURE 5 Mechanical penetration resistance in subsoiled and non-subsoiled soils, in the different soil layers. The table to the right of the graph corresponds to the mean of the subsoiled and non-subsoiled soil, in the different layers for penetration resistance (PR) and volumetric moisture at the moment of the penetration. Means compared by the Scott-Knott test ($p \leq .05$). Horizontal bars represent ± standard error of the mean. Means followed by the same lowercase letter in the column did not differ from each other

16.41% and 11–20 cm concentrated 22.18%, totaling in the first 20 cm depth 62.25% of microbial biomass. Basal respiration of microorganisms decreases with increasing soil depth, showing approximately 55% of total respiration in the first 20 cm depth (Figure 6B). Subsoiling increased the basal respiration in 5.88 mg kg^{-1}.

3.4 | Root growth

Subsoiling did not modify root dry mass (Figure 7A). The sum of the all layers totaled 921.37 kg ha^{-1}, and 619.65 kg ha^{-1} of this total corresponds to the 0–5 cm of depth, that means that 67% of the total root dry mass is in the soil superficial layer. Root volume was similar to the dry mass, with approximately 48% in the 0–5 cm layer (Figure 7B). When added, up to 15 cm of depth, 87.91% of total dry mass and 78.79% of total volume were found. Subsoiling reduced root volume by 0.19 m^3 ha^{-1}, which represents 41% less root volume. Root surface area per plant was lower in subsoiled soil, with 35.67 cm^{-2} of difference (Figure 7C). In the first 15 cm of depth, roots presented approximately 62% of the surface area. Root diameter per plant showed no difference between subsoiled and non-subsoiled soil (Figure 7D). With the soil depth increase, the root diameter decreased. Root mean diameter from 11 cm was smaller in 2.25 times compared to the 0–5 cm layer. Root length from 11 to 25 cm depth was higher in non-subsoiled compared to subsoiled soil (Figure 7E). The total roots length in the sum of depths was 69.28 m in non-subsoiled soil and 51.40 m in subsoiled soil.

3.5 | Vegetative growth and grain yield

An equal initial and final plant development was observed in subsoiled and non-subsoiled soil (Figure 8). In R2 stage, plant height was 3.92 cm (8.46%) higher, and leaf area per plant was 164.76 cm^2 higher in the area of the subsoiled soil (Figure 8A, B). In V7 stage, plants of the subsoiled soil presented 17.4% higher plant dry mass (Figure 8C).

Subsoiling benefited the nodules number per plant, and consequently the nodules dry mass per plant (Figure 9). Nodules number in the subsoiled soil was 28% higher than in the non-subsoiled soil (Figure 9A) and this resulted in an increase of approximately 23% in the nodules mass per plant (Figure 9B). Subsoiling did not change the mass of a

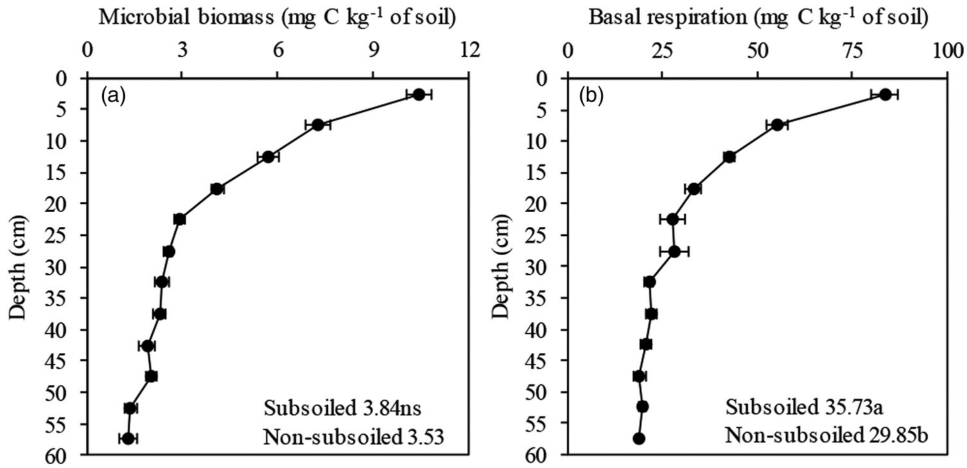


FIGURE 6 Biological attributes in subsoiled and non-subsoiled soils, in the different soil layers. Microbial biomass (a) and basal respiration (b) of the subsoiled and non-subsoiled soil, in the different layers, in the R5 soybean stage. Means compared by the Scott-Knott test ($p \leq .05$). Horizontal bars represent ± standard error of the mean

thousand grains (Figure 10A), but altered grain yield (Figure 10B). Grain production was 169 kg ha^{-1} higher in the subsoiling area.

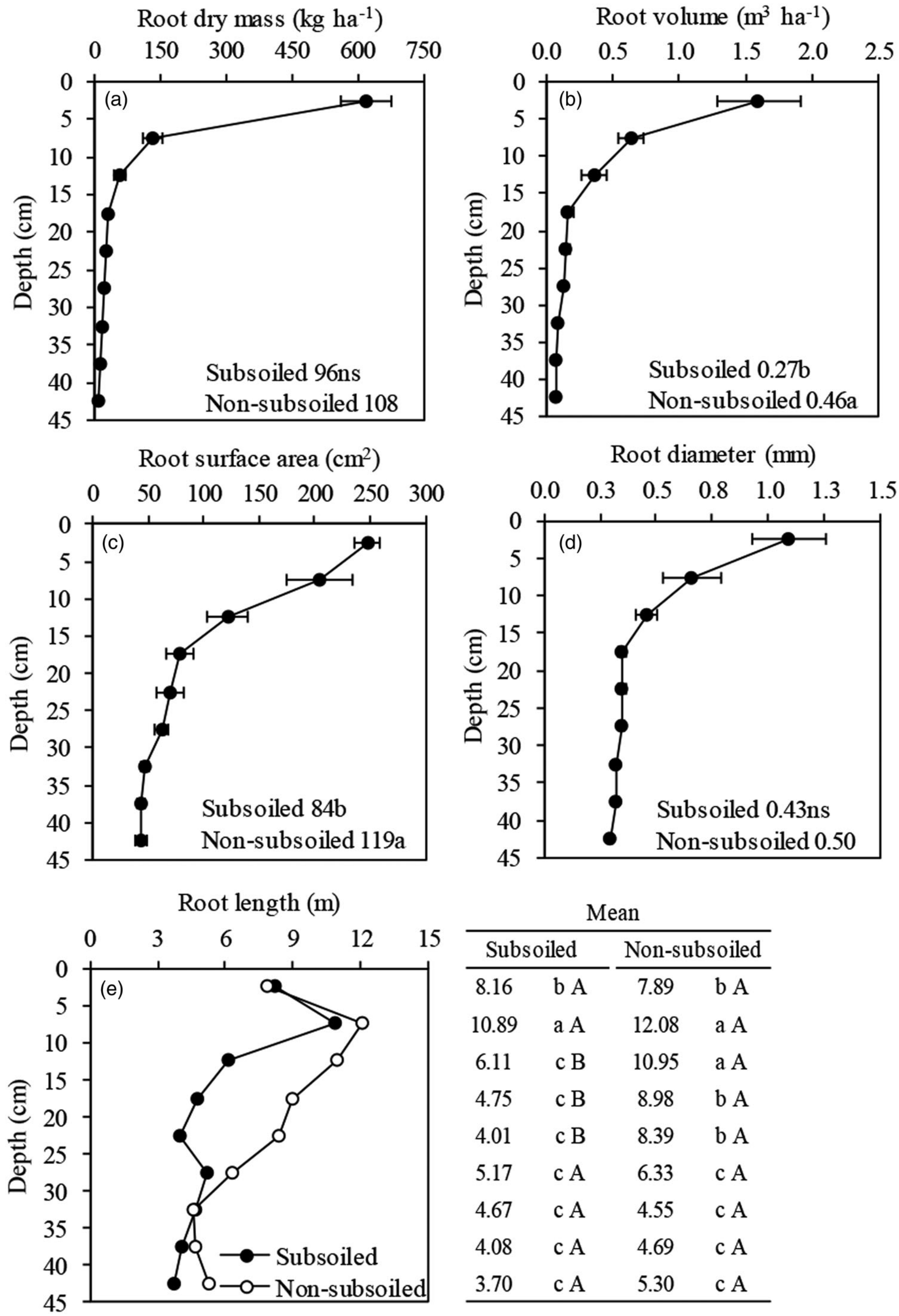
3.6 | Analysis of Pearson's correlation and a principal component

Pearson's correlation indicates that there was a positive correlation between root growth and phosphorus, organic matter, calcium, total porosity, macropores and microorganisms basal respiration (Table 1). That is, the greater presence of these components in soil depth, greater is the plant's ability to present vigorous root growth. Root growth was negatively correlated with aluminum, relative density and cryptopores.

In the principal component analysis, PC1 explains 73.48% of the data variance and PC2 explains 19.77% (Figure 11A). The variables with the highest expression in PC1 are volume, dry mass, diameter and root surface area, and soil attributes are basal respiration, carbon mass, phosphorus, calcium and organic matter. In PC2, the root length presented higher expression among the other variables analyzed. Was observed that the variables length, volume and roots diameter are very close to the unit circle, indicating that they are more representative in relation to the others, which are farther apart. This analysis also shows the influence of complementary variables on the root growth variables. The biological attributes, calcium, phosphorus, organic matter, macropores and soil pH influenced the root growth. Aluminum, cryptopores, relative density and soil mechanical penetration resistance influenced negatively the root growth. We can observe that the depths from 0 to 15 cm are concentrated in the quadrants with the highest root growth and the best chemical, physical and biological attributes of the soil (Figure 11B), while the deepest layers are in the quadrants that presented physical problems and aluminum presence. In the subsoil and non-subsoil soil treatments, the presence of non-subsoil soil in the root growth quadrants can be more observed, due to the larger number of samples that presented lower physical problems than the subsoil soil (Figure 11C).

4 | DISCUSSION

Subsoiling increased relative density, which reflected in lower total soil porosity. It affects the water availability to the plants and soil aeration, since macropores are the pores in which occur the air circulation, micropores are the ones that storage water for plants and the cryptopores retain the water that is unavailable to the plants (Klein & Libardi, 2002). Soil physical structure is considered ideal when 50% of its volume is pore (Schulte & Walsh, 1995), with 1/3 of macropores and 2/3 of micropores (Kiehl, 1979). However, determining the amount of pores needed to the fully plant roots development is complex, because it depends on soil type and crop. Freddi, Centurion, Beutler, Aratani, and Leonel (2007) observed that the amount of macropores below 0.10 m^3 m^{-3} allowed root growth of maize (*Zea mays* L.). In the soils mean, it is possible to observe that total porosity in all layers exceeded the 50%, presented at least 0.10 m^3 m^{-3} of macropores, and an amount of micropores below that was considered ideal by Kiehl (1979), with 0.14 m^3 m^{-3} of mean. Then, the pores' class that stores water to plants presented lower mean for treatments. Relative density presented a difference between the subsoiled and non-subsoiled soil, and the value of 0.89 achieved by the subsoiled soil is considered as a condition that may negatively affect plant development in Red Latosol (Klein, 2006). Root elongation decreases due to lower



Mean			
Subsoiled		Non-subsoiled	
8.16	b A	7.89	b A
10.89	a A	12.08	a A
6.11	c B	10.95	a A
4.75	c B	8.98	b A
4.01	c B	8.39	b A
5.17	c A	6.33	c A
4.67	c A	4.55	c A
4.08	c A	4.69	c A
3.70	c A	5.30	c A

FIGURE 7 Root growth in subsoiled and non-subsoiled soils, in the different soil layers. Dry root mass per hectare (a), root volume per hectare (b), surface area per plant (c), root diameter per plant (d) and root length (e) of the subsoiled and non-subsoiled soil, in the different layers, in the R5 soybean stage. Means compared by the Scott-Knott test ($p \leq .05$). Horizontal bars represent ± standard error of the mean. Means followed by the same lowercase letter in the column did not differ from each other in soil layers and means followed by the same capital letter in the row did not differ from each other for subsoiled and non-subsoiled soils

cell flow and axial cell extension, in the case of mechanical impedance, giving rise to shorter and fatter cells (Bengough et al., 2006).

In this study, after 18 months of subsoiling, it was observed that the evaluated physical attributes did not present positive alterations in the soil and these alterations are observed in the superficial layers and not in the subsoil. The positive effects of subsoiling may be temporary and depend on soil type and management, and physical attributes may return to their original condition (Busscher, Bauer, & Frederick, 2002). One and

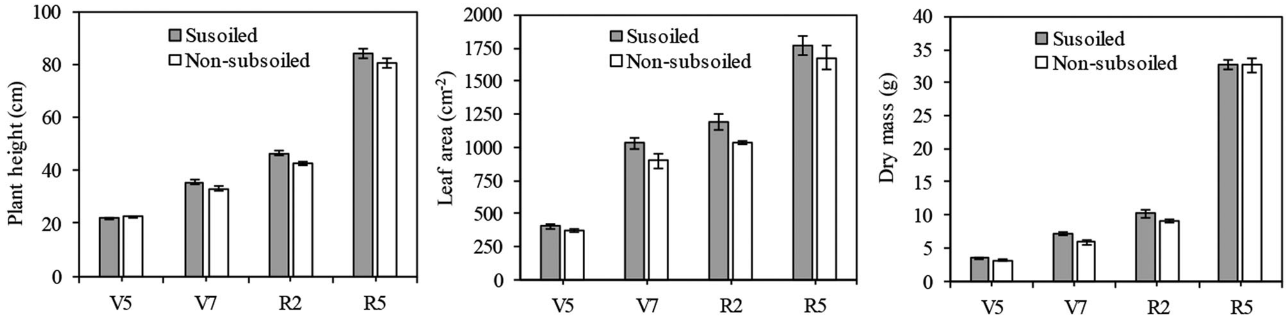
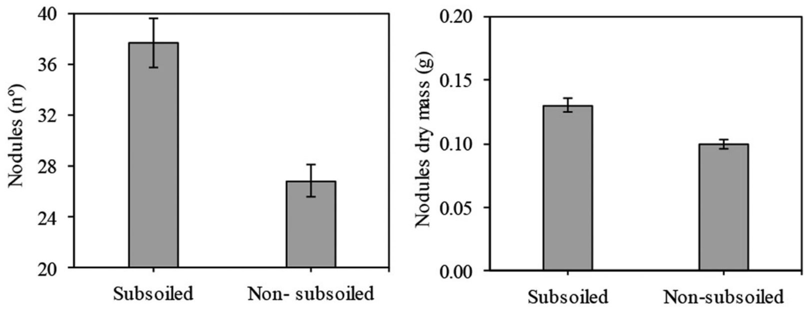


FIGURE 8 Soybean shoot growth in different phenological stages. Plant height, leaf area per plant dry mass per plant at V5, V7, R2 and R5 stages. Means compared at same phonological stage by the Tukey test ($p \leq .05$). Vertical bars represent ± standard error of the mean

FIGURE 9 Nodulation in soybean at V5 stage. Number of nodules per plant and dry mass of nodules per plant. Means compared by Tukey test ($p \leq .05$). Vertical bars represent ± standard error of the mean



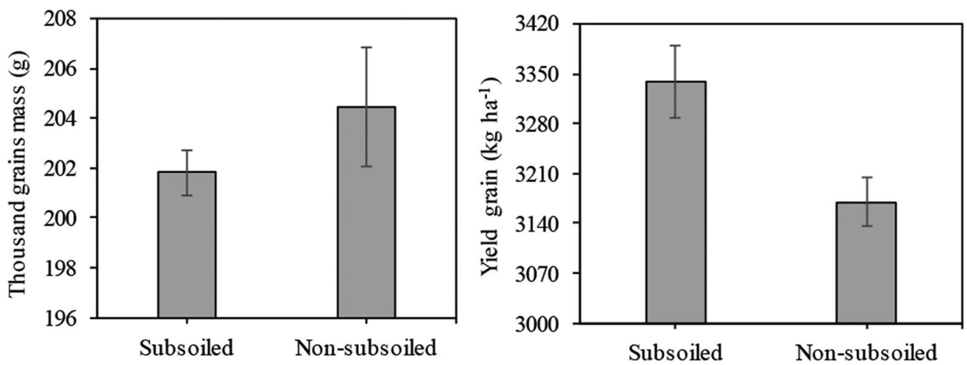


FIGURE 10 Grain yield of soybean in subsoiled and non-subsoiled soils. Mass of one thousand grains and grain yield per hectare. Means compared by the Tukey test ($p \leq .05$). Vertical bars represent ± standard error of the mean

a half years after subsoiling with conventional tine or winged tine, little difference was observed in hydraulic conductivity and air permeability in Pallic Soil in Southland, New Zealand (Drewry, Lowe, & Paton, 2000). However, there is contradictory information as to the duration of subsoiling effects. Hénin, Gras, and Monnier (1976) followed the effects of subsoiling on 22 types of soils. After a period of 2 to 3 years, only eight soils presented the operation effects, and in the other 14 soils, the researcher observed only the presence of a channel with smooth walls where the subsoiler stem passed. In soil loam, two-year results indicated that subsoiling broke up dense soil layers and improved soil properties in the tilled layer (Wang et al., 2019), while in a sandy clay loam Typical Haplustox, no positive influences of subsoiling were observed after one year of this mechanical practice (Minatel, Andrioli, Centurion, & Natale, 2006).

Subsoiled soil presented lower total porosity, and alter the pores that store water. This is explained by observation of the means of permanent wilting point (PWP) and field capacity (FC) are observed. Permanent wilting point was higher in the non-subsoiled soil, and this difference was reflected in the water available to plants. Moisture below PWP is the unavailable water to plants, because it is strongly retained by the soil matrix, and roots cannot absorb it, whereas FC is considered the amount of water after the excess drains, generally observed two days after rainfall or irrigation, when water remains almost constant at a soil depth (Kirkham, 2014).

TABLE 1 Pearson correlations between root growth and soil chemical, physical and biological attributes that presented difference between subsoiled and non-subsoiled soils

Variables	pH (H_2O)	P	Organic matter	Al	Ca	Total porosity	Relative density	Macropores	Cryptopores	Basal respiration
Root volume	ns	0.68*	0.68*	−0.34*	0.54*	0.49*	−0.54*	0.53*	−0.57*	0.67*
Root dry mass	0.35*	0.73*	0.74*	−0.45*	0.69*	0.49*	−0.55*	0.57*	−0.58*	0.80*
Root surface area	ns	0.67*	0.77*	−0.30*	0.62*	0.57*	−0.57*	0.63*	−0.69*	0.73*
Root diameter	ns	0.62*	0.61*	−0.30*	0.51*	0.43*	−0.49*	0.62*	−0.52*	0.63*
Root length	ns	0.38*	0.53*	ns	0.36*	0.50*	−0.57*	0.53*	−0.59*	0.40*

Units: P (mg dm^{-3}); Organic matter (%); Aluminum ($cmol_c$ dm^{-3}); Calcium ($cmol_c$ dm^{-3}); Total porosity (m^3 m^{-3}); Macropores (m^3 m^{-3}); Cryptopores (m^3 m^{-3}); Basal respiration (mg kg^{-1}); Root volume (m^3 ha^{-1}); Root dry mass (kg ha^{-1}); Root surface area (mm); Root diameter (mm); and Root length (m).
*Significant at the .05 probability level; ns, not significant

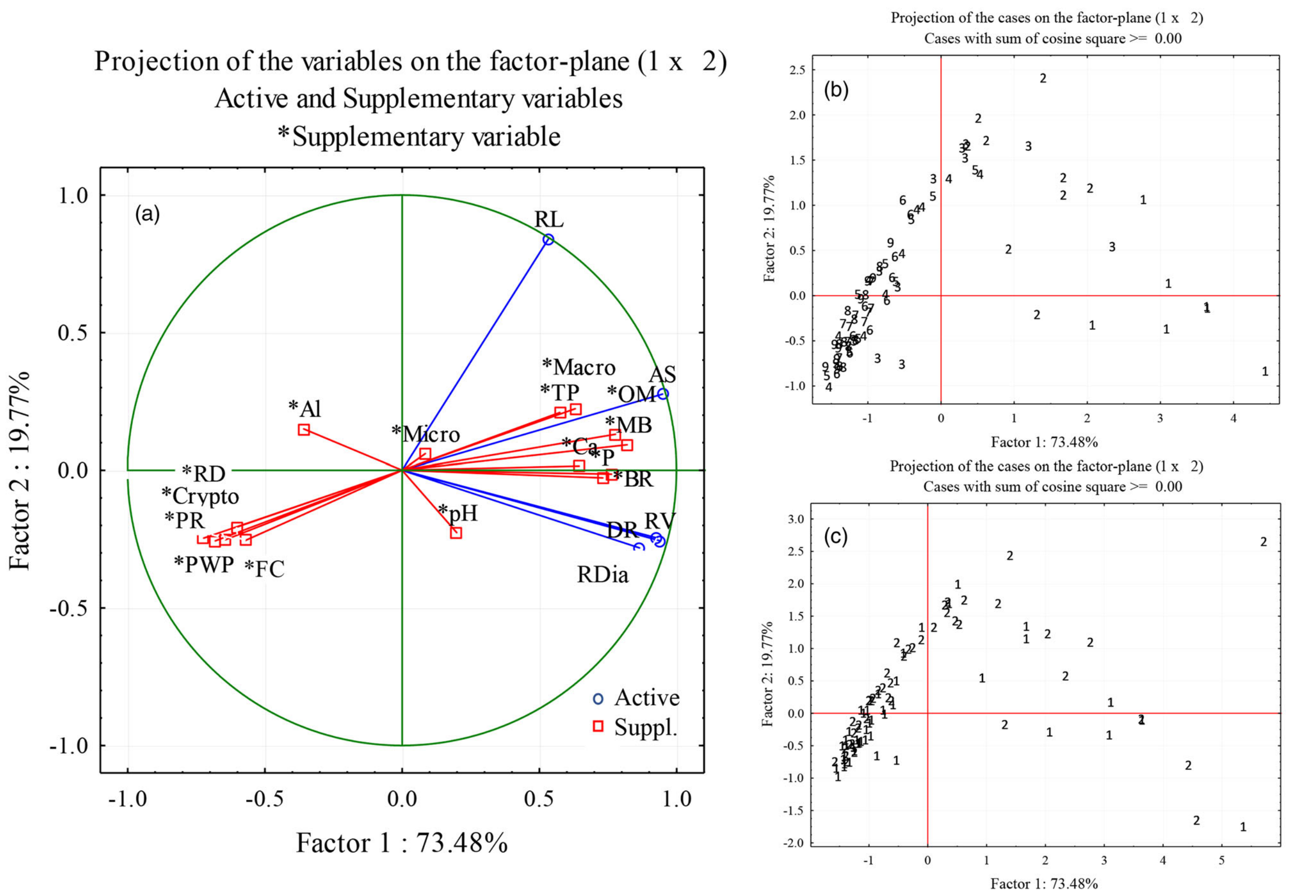


FIGURE 11 Distribution of variables and treatments by principal components. Distribution of variables (a), depths (b) and subsoiled and non-subsoiled soil (c). In (A): PR, penetration resistance (MPa); RD, relative density; Crypto, Cryptopores (m^3 m^{-3}); PWP, permanent wilting point (m^3 m^{-3}); FC, field capacity (m^3 m^{-3}); Al, aluminum ($cmol_c$ dm^{-3}); pH, hydrogenionic potential (H_2O); Micro, micropores (m^3 m^{-3}); RL, root length (m); Macro, macropores (m^3 m^{-3}); TP, total porosity (m^3 m^{-3}); AS, root surface area (mm); OM, organic matter (%); MB, microbial biomass (mg kg^{-1}); Ca, calcium ($cmol_c$ dm^{-3}); P, phosphorus (mg dm^{-3}); BR, basal respiration (mg kg^{-1}); RV, root volume (m^3 ha^{-1}); DR, root dry mass (kg ha^{-1}); RDia, root diameter (mm). In (B): 0–5 cm (1); 6–10 cm (2); 11–15 cm (3); 16–20 cm (4); 21–25 cm (5); 26–30 cm (6); 31–35 cm (7); 36–40 cm (8) and 41–45 cm (9). In (C): subsoiled soil (1) and non-subsoiled soil (2)

Soil water reduces contact between particles, reducing penetration resistance (Lomeling & Lasu, 2015). However, this was not observed in this experiment. The layers that presented higher relative density and penetration resistance contained higher clay content (data not shown) and an amount of cryptopores, which resulted in more compacted soil. Considering that from 2 MPa, the root growth may be restricted (Lomeling & Lasu, 2015; Taylor, Roberson, & Parker, 1966), subsoiled and non-subsoiled soil presented adverse physical conditions for root growth in 11–60 cm layer.

Subsoiled soil presented higher microorganisms basal respiration, which may explain the lower OM and higher nodulation. Microorganisms development in the soil is related to soil and plant conditions. Subsoiling mixes straw with soil, which improves the metabolic activities of soil microorganisms and accelerates straw decomposition (Bastian, Bouziri, Nicolardot, & Ranjar, 2009; Govaerts et al., 2007). From 7 to 28% of the carbon assimilated by soybean plants is made available to associations with symbiotic organisms, it varies according to the growing conditions of the plants and the species of symbiotic organisms (Kaschuk, Kuyper, Leffelaar, Hungria, & Giller, 2009), thus soil conditions that allow greater activity of primary plants metabolism, will benefit the symbiotic associations. Nodule formation is regulated by plant and can be inhibited by external factors, such as soil pH (Ferguson, Lin, & Gresshoff, 2013). Decomposition of organic residues in soil is performed by soil microbiota through the nutrient cycling (Jenkinson & Ladd, 1981), however, only quantifying biomass does not allow to know the actual microorganisms activity in the soil, being necessary to evaluate the metabolic functioning of the soil microorganisms community (Bowles, Acosta-Martínez, Calderón, & Jackson, 2014).

Chemical and physical attributes are involved in soil microorganisms development (Borowik & Wyszkowska, 2016; Furtak & Gajda, 2018; Wang et al., 2017). Data enable observe that microorganisms' activity was more affected positively by soil chemical, than physical attributes, and therefore basal respiration and nodulation were higher in the subsoiled soil that presented improvements in chemical structure.

Approximately 18 months after soil subsoiling, was possible to observe improvement in soil chemical attributes, however, even though those were positive changes, it did not guarantee higher root growth, probably due to the negative changes in the soil physical attributes. However, it is necessary to observe carefully that higher root dry mass, due to thicker roots is not interesting, because mass and volume of low diameter roots are necessary, since these fine roots are effectively responsible for water and nutrients absorption. Therefore, no difference was observed in subsoiled and non-subsoiled soil.

Soil cracking due to subsoiling may have benefited the percolation of limestone particles to depth, resulting in increased pH and decreased Al. The movement of limestone particles may be important to explain in part the effects on acidity and Al neutralization in subsurface soil in the subsoiled soil. Limestone located at the soil surface has reduced reaction because it presents less contact with the soil particles and less effectiveness in the deepest soil layers (Ciotta et al., 2002).

Subsoiling improved soil chemical and biological aspects, but not physical. We believe that subsoiling is an interesting alternative to try softening soil problems, but this isolated management is not the solution. Other managements need to be adopted in the field to alleviate problems, such as crop rotation, crops with aggressive root system for biological scarification. In addition, soil preparing with adequate moisture and using chemical fertilization and liming, when necessary. Soil needs to be more stratified analyzed, dividing them into more layers, for more corrected results of chemical and physical attributes.

The positive correlations between root growth variables with P, OM, Ca, TP, RD, macropores and basal respiration showed that the set of soil attributes determine the root growth. Soil microbiology is the secondary response of soil chemical and physical attributes, being a soil quality indicator. When soil provides better plants growing conditions, the plants make more photo assimilates available to soil microorganisms. Thus, soil with optimal nutrient content, absence of toxic elements, low soil density and high porosity will result in a soil rich in microorganisms and this soil type favors root growth. The grain yield was higher in the subsoiled soil than the non-subsoiled soil, evidencing the importance of the soil chemical and biological attributes for soybean grain yield.

It is notorious that soybean root growth is intense in the soil surface layer compared to deeper layers. That would not be a problem since there were no water deficit periods. Soil volume explored in depth is small and when water is restricted, the upper soil layers dry first, leaving the plants under water stress in small periods without rainfall. Greater root growth in depth does not guarantee that plant will not lose productive potential in water deficit, but ensures the mitigation of this loss. Soil chemical, physical and biological attributes, such as P and Ca content, soil density and porosity, and basal respiration of soil microorganisms affect root growth. Thus, when thinking about soil attributes to favor root growth, should be considered soil chemistry, physics and biology.

5 | CONCLUSIONS

Root growth is benefited by soil microorganisms, organic matter, pH, porosity and calcium in the soil, whereas aluminum, cryptopores volume and penetration resistance that characterize a degraded soil limit the growth of soybean roots. Under these study conditions, subsoiling provides lower root growth but benefits grain yield.

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