

Evaluation of the root system of Vetiver grass (*Chrysopogon zizanioides* L. Roberty) using different sampling method

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Research Article

Keywords: Plant Roots, Root length, Evaluation methodologies, Use-Effectiveness

Posted Date: June 29th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1777934/v1>

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Version of Record: A version of this preprint was published at Environmental Systems Research on September 2nd, 2022. See the published version at <https://doi.org/10.1186/s40068-022-00262-8>.

Abstract

Background: The objective of this work was to evaluate the root system of Vetiver grass through different methodologies. The trench method evaluated 50 images in 10 x 10 cm squares in its vertical face. The volumetric ring method uses a metallic ring with a volume of 50 cm³ (diameter $\cong$ 4.6 cm and height $\cong$ 3.0 cm) to collect samples in the same trench's vertical face. A hand auger hole method worked with soil samples and roots removed from the surface up to 0.50 m depth. The monolith method comprises a 0.50 m wide, 0.50 m long, and 1 m deep monolith. A spatial correlation between root volume and root area was observed in the kriging maps by the volumetric ring method.

Results: The root area values for both the volumetric ring method and the hand auger hole method were similar, up to 0.10 to 0.20 m. Among the analyzed variables, the root area showed the best correlation coefficient among the analyzed root methods, especially those that use the limited spatial distribution by its sampling collection, such as the volumetric ring method and the hand auger hole method ($r = 0.526$).

Conclusions: The studied methods can be separated into two groups: those that provide more detailed information about the behavior and distribution of the root system, such as trench and monolith methods. The trench method requires turning the soil. Both require greater spatial coverage, and therefore have greater precision, and the methods that better describe the morphological characteristics of the features, such as the volumetric ring method.

Introduction

The natural soil cohesion resulting from interactions between the vegetation and the soil mineral particles (silt, sand, and clay) improves the shear strength and contributes to erosion control in a direct relationship between the physical bonds formed in the contact areas of these particles and chemical bonds or cementation between them (Harichane et al. 2011; Khezri et al. 2015; Akay et al. 2018). Erosion can be defined by the process of soil loss caused by agents such as water, wind, or anthropic actions promoting the breaking of the soil structure, resulting in losses of its most fertile layers (Rubira et al. 2012; Caldas et al. 2016; Momoli and Cooper 2016; Guerra and Jorge 2017).

The soil cover promoted by cultivated plants in agroecosystems, or that inhabit slopes and riparian areas (Cammeraat et al. 2005; Holanda et al. 2020; Dorairaj and Osman 2021) and through its root system contributes to the increase in soil shear strength. It is necessary to understand vegetation's role in increasing the soil resistance to erosion, particularly in Vetiver grass (*Chrysopogon zizanioides* L. Roberty). The plant is one of the most used and studied species to protect slopes (Chong and Chu 2007; Araújo Filho et al. 2015; Gnansounou et al. 2017; Niu and Nan 2017; Santos et al. 2018; Mondal and Patel 2020).

The vetiver grass has biotechnical characteristics that promote an efficient soil cover (Holanda et al. 2012, 2017; Hamidifar et al. 2018), such as dense and deep rooting. They grow and reach up to 1.80-meter depth in 6 months (Eab et al. 2015), at an average growth rate of approximately 30 cm/month⁻¹.

The length of the stem combined with the inflorescence varies from 1.95 to 2.0 meters (Banerjee et al. 2019). Because of these characteristics, Vetiver grass is recognized for its ability to conserve soil and stabilize slopes (Mickovski et al. 2005; Mickovski and Beek 2009; Donjadee and Tingsanchali 2013; Amiri et al. 2019).

The root system of Vetiver grass can grow in extreme environments, not only in relation to temperature, but tolerance to long periods of drought and flooding, common in riverbanks, and tolerance to saline soils, contaminated with heavy metal or soil low fertility or with high pH (Barbosa and de Lima 2013; Teixeira et al. 2015; Bernardino et al. 2016; Amiri et al. 2017; Aloui et al. 2018; Itusha et al. 2019). This species is present in the most varied climates, mainly tropical and subtropical, adapted to different altitudes and climatic conditions (Truong 1999). It is widely used in the recovery of degraded areas, emphasizing erosion control, since its root system has many cylindrical fibers capable of presenting an average tensile strength of 310 MPa (Teerawattanasuk et al. 2014; Holanda et al. 2020).

Although the methodologies for deep roots evaluation have strongly improved and the number of studies addressing this subject has increased in the recent decades, the studies on the roots confined to the upper horizons of the soil are the most frequent (Tracy et al. 2010; Maeght et al. 2013; Freschet and Roumet 2017). The most common methods used on the structure and root distribution in the soil depth have not changed substantially: the excavation techniques (trench) and auger sampling are still the preferred methods (Vasconcelos et al. 2003a; Marcuzzo et al. 2012; Zhang et al. 2019a; Yamase et al. 2021). According to Hosoya et al. (2016), with the technology evolution, new methodologies have been proposed, providing lower costs, greater precision, and shorter analysis time, such as the methods for digital image processing (PDI), with emphasis on the Safira software (Fibers and Roots Analysis System) (Crestana et al. 1994). Comparative studies are required in relation to the methodologies, the so-called traditional or digitals, for better data accuracy. The objective of this work was to evaluate the root system of Vetiver grass using different methodologies to compare them and make a recommendation based on the obtained results.

Materials And Methods

Experimental Area

The experimental area was located in the Campus of the Universidade Federal de Sergipe (UTM Coordinates 10 ° 55'46.7 "S and 37 ° 06'12.7" W) in the municipality of São Cristóvão, in Sergipe state, in northeastern Brazil, in soil classified as Entisol (Santos et al. 2018), where Vetiver grass plants (*Chrysopogon zizanioides* L. Roberty) are grown, spaced 0.3 m between the lines and 0.2 m between plants in the line. A trench 5 x 1 x 1 was dug parallel to the planting line of Vetiver grass, where different methodologies for root collection were applied. The vertical wall of the trench was near to 0.05 m of the plants with exposed roots, randomly chosen, representing the set of plants from the experimental area (Bergamin et al. 2010). The methods of the trench (Vasconcelos et al. 2003b), the volumetric ring (Claessen et al. 1997), the hand auger hole, and the monolith (Böhm 1979) were used.

Trench method

The collection and the subsequent data analysis using the trench method were performed according to Carvalho et al. (De Carvalho et al. 2020). The trench method allows both the roots quantification and the assessment of roots distribution in the soil. After the trench excavation, careful scarification was performed on its front face as a preparation to be photographed and sampling, thus avoiding losses. The trench presented front dimensions of 1.0 x 1.0 meters. It was not possible to go deeper due to the more superficial water table in the soil profile. With a water pump with a 20 m long hose, the slope was subjected to a water jet that allowed cleaning the soil that covered the roots in the trench. After that, the roots were painted using yellow spray paint to highlight their contrast to the soil color to be analyzed by SAFIRA software. New scarification was necessary to remove paint excess with a steel spatula, which also covered the soil behind the roots to leave the yellow painted roots contrasting with the soil (Fig. 1). The pictures were taken in 0.1 x 0.1 m squares using a wooden framed grid of 1.0 x 1.0 m dimensions with 100 squares, adapting the methodology described by Teixeira et al. (2017). In each profile, 50 pictures were taken using a digital camera and then digitized with a spatial resolution of 512 x 512 pixels and 256 shades of gray per pixel.

Volumetric ring method

Samples (soil + root) were collected from the front face of the trench in each grid with a volumetric ring performing a volume of 50 mm³ (Fig. 2a). This non-destructive method collects soil samples of known internal volume (Teixeira et al. 2017).

After the soil samples (soil + root) collection, they were packed in plastic bags and then taken to dry at room temperature. Next, the roots were separated with tweezers and washed in a sieve using running water, and placed in the forced circulation oven for drying for 24 hours at 60°C. This procedure has been adapted from the method proposed by Fante Junior et al. (1999).

The images resulting from the pictures of the collected roots with the volumetric ring were digitized and subsequently processed by the SAFIRA software, which provided data on volume, surface root area, roots length, and diameter. The Fiber and Root Analysis System (SAFIRA) is the SIARCS® Software (Integrated System for Root and Ground Cover Analysis) (Jorge and Rodrigues 2008).

This software uses distance transformation through the thresholded and skeletalized images of the roots to determine the length, volume, area, and diameter of the roots. The length of the roots was calculated by Eq. 1:

$$C = H / L \quad (1)$$

Where A is the area and L is the width.

The width is obtained by means of Eq. 2:

$$L = (P - \sqrt{P^2 - 16}) / 4 \quad (2)$$

It was assigned a P root perimeter value by selecting the edge of the roots in the image and measuring the distances between the central points of each pixel belonging to the edge. The sum of all Distances (D) of the border pixels results in the perimeter and the sum of P_1 to P_2 in a Cartesian plane is obtained by Eq. (3):

$$D = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \quad (3)$$

In the construction of the Heatmaps, the kriging interpolation method was used and later smoothed with the Splane transform, once it estimates values of the point to be plotted using a mathematical function that minimizes the curvature of the surface resulting in a smooth surface and closer to the expected in this work (Marcuzzo et al. 2012). Kriging uses a non-biased and minimal variance linear interpolator, which ensures the best estimate of non-sampled data (Freddi et al. 2006).

Hand auger hole method

The hand auger hole sampling consists of taking soil samples that contain the roots with no need to dig trenches. The used hand auger hole must have a known volume to have a sampling standardization (Ratuchne et al. 2016). Samples were taken from the soil surface up to 0.50 m depth using a hand auger hole of 0.05 m in diameter and 0.10 m in height. Four deformed soil samples were collected per layer of 0.10 m, near Vetiver plants, separated by 0.50 m in the line. The vetiver grass line was 3.0 meters apart between the three sampling sites, considering that there is a common root occupation zone for plants from two neighboring lines. The separation of the roots from the soil samples and drying were carried out as specified for the volumetric ring method.

Monolith method

This sampling method has the advantage of the accuracy analysis, considering the vertical and horizontal distribution of the root system and obtaining the root mass by size class (Böhm 1979; Lopes et al. 2010). The monolith sampling was carried out at a depth of 1m, leveling the surface and wall with a straight spade. The block was divided into ten layers of 0.10 m, performing ten different samples.

The roots were cleaned by washing with a water jet, using a 1.0 mm mesh sieve in order to minimize the loss of the finer roots (Böhm 1979). After washing, the roots were packed in paper bags and dried in an oven with forced air circulation, at 65°C, for 72 hours.

Experimental design

To analyze roots length and diameter, the average of each quadrant used the SPSS Software (SPSS Corp 2017). Initially, the Anderson-Darling statistical test was applied to identify whether the set of measures had a normal distribution, considering the significance level of 0.05 (5%). Logarithmization to perform

the paired comparisons (Post hoc) and Duncan test with *p-value* with significance at 0.05 (5%) were performed to better predict the results. Pearson's correlation analyzes among the methods was performed using the results of the analyzed variables.

The *Spline* method was used to elaborate the *heatmaps* of the root system of Vetiver grass using the data collected by Safira, described by (Yamamoto and Landim 2015), as an interpolation method that suggests values by a mathematical function, which minimizes the curvature of the surface, resulting in a smooth surface that passes exactly through the sampled point.

Results

Evaluation by the trench method

The trench method allowed to build descriptive *heatmaps* of the root system by interpolation using the average data obtained by the Integrated System for Root Analysis and Soil Coverage (SIARCS), which Safira Software processed through the equation of smoothing *Spline*, expressed by the number of nodes (points where the segments connect), the order and the degree of the polynomial as described by Silva et al. (2019). The obtained images provide different values for each root collection depth.

A maximum value for Root Volume of 85.887 mm³ in the initial squares of the analyzed trench was observed, precisely among the squares identified as H, J, and I up to 0.10 m in depth (Fig. 4A). As an indirect method, which considers the exposure of the root for the collection of pictures, even considering the cleaning of the front face of the photographed trench, soil particles adhered to the roots may have brought inaccuracies to the result, amplifying it.

There is an outstanding root volume in the D and E squares, in the shallow layers of the soil at a depth of 0.00 m to 0.30 m, as also observed by Carvalho et al. (De Carvalho et al. 2020) studying *Paspalum millegrana* Schrad, a species of poaceae family that also presents biotechnical characteristics (Holanda et al. 2017). These authors applied the same methodology, registering a higher concentration of roots also in the first 0.50 m of soil.

It is not expected from any species to present a uniformity of the root system behavior in the soil depth. Even in a deep sandy soil, a greater concentration of roots is observed up to 0.50 m depth. This suggests that the greatest reinforcement promoted by the root system occurs in the shallow layers where the root density is the highest (Figures. 1 and 3). This behavior is also shown in other variables such as the root area that in 0.20 m depth reached a maximum values of 462.723 mm² in quadrant G and H. An average for this depth was 105.305 mm²as, presenting statistic differences among the studied depths for this variable (Table 1). It is also noticed an outstanding expression of the values of root area from column C to column J, reaching a depth of 0.40m. A spatial correlation between the Root Volume (A) and the Root Area (Fig. 4A and B) was also observed in the kriging maps.

Table 1

Average values of root volume, root area, root length, and root diameter per sampling point using the volumetric ring method.

Samples depth ^a —m—	Analyzed variables ^b							
	Root volume		Root area		Root length		Root diameter	
	—mm ³ —		—mm ⁻² —		—m—		—mm—	
	Mean	Log ¹⁰ *	Mean	Log ¹⁰	Mean	Log ¹⁰	Mean	Log ¹⁰
0.00–0.10	20.864	0.721 a	105.305	0.601 a	0.451	0.698 a	0.251	-0.544 e
0.10–0.20	7.170	0.713 a	39.162	0.486 e	0.216	0.676 b	0.302	-0,530 d
0.20–0.30	9.015	0.675 b	42.594	0.586 ab	0.197	0,609 d	0.314	-0,515 c
0.30–0.40	4.470	0.677 b	22.193	0.566 bc	0.139	0.633 c	0.337	-0,491 a
0.40–0.50	4.230	0.664 c	21.943	0.602 a	0.114	0.643 c	0.335	-0,494 a
0.50–0.60	3.964	0.663 c	20.136	0.591 ab	0.126	0.566 e	0.225	-0.541 a
0.60–0.70	1.170	0.669 bc	9.890	0.545 cd	0.149	0.480 g	0.301	-0,530 d
0.70–0.80	1.247	0.673 bc	9.405	0.528 de	0.091	0.480 f	0.316	-0,511 c
0.80–0.90	1.278	0.687 b	8.833	0.513 e	0.089	0.458 f	0.327	-0,500 b
0.90–1.00	2.817	0.677 b	15.875	0.551 cd	0.104	0.610 d	0.334	-0,489 a
f-value	7.032	18.206	8.0112	16.434	378.100	165.404	5.993	109.216
Deviation error	112.563		514.015		22.202		0.113	
df	30735	2955	30736	2956	30736	2956	30736	2956
Valid samples	30736	29557	30737	29557	30737	29557	30737	29557
*Means with logarithmic transformation followed by the same letter do not differ by Duncan's test at 5% probability; ^a number of observations (n) = 30737.								

The results of the root length allowed the identification of the grids that presented higher values in the shallow layers of the soil (Fig. 4). The root length showed maximum values from the F grid to the I column to the 0.20 m depth. Remarkable values for root length are also observed in the first 0.50 m depth plotted in the heatmaps, showing statistics differences downward (Table 1), although slightly different to the trench's deeper values. This soil has a weak structure, which breaks down into simple grains, and has a predominance of macropores, which is favorable to root growth of vetiver grass and reach greater depths. If the soil has a highly expansive clayey or loam-clay texture, the root will be confined to the cracks, resulting from the saturation and drying processes (2016), then the Vetiver grass behavior will be different.

When the *heatmaps* of Fig. 5 were verified, Vetiver grass presented larger root diameters for the depth of 0.30–0.50 m of 0.445 mm. However, a relatively uniform distribution of roots of medium diameters was observed, the so-called secondary roots. There is also a very uniform distribution of fine roots, tertiary roots, of great importance to improve soil cohesion and consequently the resistance to erosion. The variation in tensile strength is directly related to the increase in diameter and directly linked to the root structure; then, thinner roots have high cellulose content. Consequently, they are more robust and important to increase shear strength from soil (Machado et al. 2015). Vetiver roots can reach a resistance of 48.16 MPa for roots with 0.233–0.907 mm in diameter (Zhang et al. 2019b).

The observed root diameter values are distributed throughout the map, showing that these roots under the studied conditions behave almost homogeneously, forming a dense network for the entire trench, in agreement with Gomes et al. (2020). This is an important characteristic of Vetiver grass used as a phytoremediation agent in polluted soil by heavy metals because of its good capacity for minerals bioaccumulation and probably because of its higher root density. This very well-organized root architecture in primary, secondary, and tertiary roots (Noorasyikin and Zainab 2016) presents varied diameters, but with a predominance of (thin) tertiary roots, increasing nutrients' absorption (Lim et al. 2004; Sharma 2016; Gnansounou et al. 2017; Batista et al. 2017). Also, the significant average diameter with good surface area coverage helps to increase the maximum tensile strength compared to other species.

Table 1 Average values of root volume, root area, root length, and root diameter per sampling point using the volumetric ring method.

Evaluation by the volumetric ring method

The data associated with the variables root volume and root length related to the analysis of the volumetric ring method were logarithmized, thus making it possible to obtain the significant differences related to these characteristics and their similarities, showing that they are correlated.

In this method, the highest values of root volume (0.771 mm³ and 0.725 mm³) were registered in the layers between 0.60 to 0.80 m depth. (Table 2), reaching a root area of 6.587 mm². Once the greater the root volume in a limited space such as the volumetric ring, the greater was the area occupied by these

roots is, in agreement with the results of Carvalho *et al.* (De Carvalho et al. 2020) working with Paspalum grass. Two third of the root volume was present in the initial 0.80 m depth showing a difference in the samples in 0.00–0.10 m, 0.10–0.20 m, and 0.20–0.30 depths, although there was a massive presence of roots in volume and area up to the last evaluated squares at one-meter depth.

Table 2
Average values of root volume, root area, root length, and root diameter per sampling point using the volumetric ring method.

Samples depth ^a —m—	Analyzed variables ^b							
	Root volume		Root area		Root length		Root diameter	
	—mm ³ —		—mm ⁻² —		—m—		—mm—	
	Mean	Log ¹⁰ *	Mean	Log ¹⁰	Mean	Log ¹⁰	Mean	Log ¹⁰
0.00–0.10	0.640	– 0.439ab	5.131	0.588a	0.014	0.096c	0.373	– 0.759de
0.10–0.20	0.512	– 0.578b	4.489	0.497abc	0.014	0.111c	0.327	-0.509ab
0.20–0.30	0.607	– 0.528ab	5.062	0.599ab	0.015	0.115c	0.338	-0.494a
0.30–0.40	0.467	– 0.603bcd	5.269	0.504abc	0.017	0.175abc	0.303	-0.558ab
0.40–0.50	0.439	– 0.617bcd	4.407	0.442abc	0.018	0.300abc	0.295	-0.549ab
0.50–0.60	0.467	– 0.648cde	4.898	0.503abc	0.020	0.250a	0.266	-0.590bc
0.60–0.70	0.771	– 0.505a	6.587	0.588a	0.019	0.198abc	0.314	-0.565abc
0.70–0.80	0.725	– 0.443a	5.874	0.562a	0.019	0.200ab	0.315	-0.759e
0.80–0.90	0.367	– 0.759e	4.090	0.415c	0.019	0.194abc	0.249	-0.655cd
0.90–1.00	0.435	– 0.696de	4.320	0.442bc	0.016	0.1446bc	0,277	-0.585abc
f-value	1.651	6.795	1.986	3.219	6.061	7.479	10.876	7.378
Deviation error	3.55	0.45	15.20	0.52	1.82	0.33	0.20	0.53
df	12.65	0.67	230.76	0.27	3.30	0.11	0.04	0.29
Valid samples	3352	3336	3352	3336	3352	3336	3352	3336
*Means with logarithmic transformation followed by the same letter do not differ by Duncan's test at 5% probability; ^a number of observations (n) = 3353.								

The comparison between the root volume data in the volumetric ring and the trench method showed that the most significant volumes in both methods were between 0.30 and 0.40 m depth. It is important to

highlight that the results are much more concentrated in the volumetric ring method, considering the limited ring volume. Root Volume and Root Area presented a high correlation, expressed by the correlation coefficient of 0.844 ($\alpha^2 = 0.05$).

Table 2 Average values of root volume, root area, root length, and root diameter per sampling point using the volumetric ring method.

The maximum root length was 0.174 m in 0.50 to 0.60 m depth of the trench, with no significant difference among the observed squares. The trench method presented a maximum root length of 0.299 m up to 0.50 m depth and the volumetric ring method. An important characteristic of Vetiver grass is to grow at the root length without compromising its absorption capacity (Bernardino et al. 2016).

For the root diameter, an uniformity was observed in the depth range from 0.00 to 0.50 m with a maximum value of 0.351 mm at the initial depth to 0.269 mm at a depth of 0.50 m. This homogeneity can also be observed in the root area that presented a maximum value of 4.011 mm^{-2} , going up to 2.904 mm^{-2} at a depth of 0.50 m. Observing the Deviation error and the valid samples for both the mean values and the logarithmic values of the root diameter (Table 3), less deviation and more reliability were noticed in this method in the analysis of root diameters compared to the other analyzed variables. Hamidifar et al. (2018), working with root diameter of vetiver grass at different depths using the sectioned analysis method at a depth of 0.10 m, similar to the ring method, observed greater values for smaller depths when observing the samples in low plant density.

Table 3

Average values of root volume, root area, root length and root diameter per sampling point, using the hand auger hole method.

Samples depth ^a —m—	Analyzed variables ^b							
	Root volume		Root area		Root length		Root diameter	
	—mm ³ —		—mm ⁻² —		—m—		—mm—	
	Mean	Log ¹⁰	Mean	Log ¹⁰	Mean	Log ¹⁰	Mean	Log ¹⁰
0.00–0.10	1.529	-0.618c	13.509	0.576b	0.034	0.380a	0.232	-0.637c
0.10–0.20	2.267	-0.399ab	17.109	0.711ab	0.035	0.342ab	0.280	-0.565b
0.20–0.30	1.551	-0.412ab	12.569	0.711ab	0.031	0.368a	0.288	-0.5601b
0.30–0.40	1.045	-0.571bc	14.430	0.568a	0.029	0.266b	0.264	-0.585b
0.40–0.50	1.910	-0.248a	13.509	0.811a	0.034	0.300a	0.334	-0.503a
f-value	1.605	5.968	2.293	3.812	2.791	2.481	17.867	22.267
Deviation error	15.03	0.78	65.78	0.781	5.25	0.44	0.14	0.17
df	226.05	0.610	4327.82	0.610	27.56	0.195	0.22	0.03
Valid samples	1525	1525	1525	1525	1525	1525	1525	1525
*Means with logarithmic transformation followed by the same letter do not differ by Duncan's test at 5% probability; ^a number of observations (n) = 1526.								

Evaluation by the hand auger hole method

Root volume gradually decreases with the depth increase as is shown in the first depths ranging from 0.00 to 0.30 m, about 45.72% of the total observed volume, with approximately 18.41% concentrated in the upper layer (0.0–0.10 m), and 27.30% in the layer right below (0.10–0.20 m). A significant difference in root volume was observed with a maximum value of 2.267 mm³ in the 0.00 to 0.20 m depth, similar to what was observed by Le et al. (2017). Again, it seems that was created a favorable condition to form a dense network in the superficial layers. Considering a direct sampling method and the volumetric ring method, similar values were noted in the shallow layers between these two methodologies. On the root area, the maximum value was 17.109 mm⁻² in 0.10–0.20 m, and in comparison, with the volumetric ring method, the presented values are more significant.

Table 3. Average values of root volume, root area, root length and root diameter per sampling point, using the hand auger hole method.

The root length presented more significant values in the first two layers, 0.034 mm² and 0.035 mm², at a depth of 0.0 to 0.20 m. The behavior of the Root Diameter by the hand auger hole method shows similarities in relation to the trench method and related to the volumetric ring method, with the highest value of 0.334 mm in the subsurface layers such as 0.40 to 0.50 (m).

Evaluation by the monolith method

In all parameters of the monolith method, the data presented significant differences all over the soil depth. According to Lopes et al.(2010) and Maeght et al. (2013), the monolith method presents advantages such as the possibility of greater precision in evaluating the initial soil records, requiring the others to use digital tools. Therefore, to implement this method, it is necessary to join horizontal of the collected data, sectioning only in layers, which may cause the most expressive values in all factors observed in this method. As a disadvantage, part of the fine roots is omitted in the final evaluation because it is a destructive method, in addition to the greater volume of managed soil.

In this method, it is important to highlight the larger soil evaluated area, that is, 1 m² in every 0.10 m layer (Table 4), turning this the one which presented the most considerable amount of observations (71.750), which results from a larger collection of samples compared to the other methods. Therefore, the results provide more outstanding values when compared to the volumetric ring and hand auger hole methods, both with a smaller analyzed soil volume. In the monolith, relative uniformity of the root volume was observed when comparing the superficial and subsurface layers.

Table 4

Root Volume, Root Area, Root Length, and Root Diameter of Vetiver roots using the monolith method.

Samples depth ^a —m—	Analyzed variables ^b							
	Root volume		Root area		Root length		Root diameter	
	—mm ³ —		—mm ⁻² —		—m—		—mm—	
	Mean	Log ¹⁰ *	Mean	Log ¹⁰ *	Mean	Log ¹⁰ *	Mean	Log ¹⁰ *
0.00–0.10	1.465	-0.630e	10.806	0.529e	0.017	0.308c	0.212	-0.604e
0.10–0.20	1.348	-0.590e	11.336	0.575e	0.018	0.339c	0.247	-0.610e
0.20–0.30	5.470	-0.042c	32.247	0.979c	0.026	0.433b	0.354	-0.482c
0.30–0.40	1.455	-0.339d	11.544	0.746d	0.023	0.368c	0.303	-0.530d
0.40–0.50	4.149	0.072c	25.366	1.070ab	0.035	0.505a	0.373	-0.459c
0.50–0.60	6.127	0.249b	32.422	1.178b	0.032	0.479a	0.473	-0.384c
0.60–0.70	9.126	0.483a	45.904	1.348a	0.039	0.500a	0.529	-0.320a
0.70–0.80	3.051	0.264b	18.424	1.140b	0.022	0.346c	0.504	-0.327a
f-value	12.599	436.307	19.062	310.713	38.195	60.069	18.645	753.635
Deviation error	88.39	1.00	229.26	0.80	7.70	0.44	0.10	0.20
df	781.20	1.01	5256.28	0.64	59.43	0.19	0.08	0.041
Valid samples	71749	70759	71727	70663	93175	92933	71683	70718
*Means with logarithmic transformation followed by the same letter do not differ by Duncan's test at 5% probability; ^a number of observations (n) = 93.176.								

Table 4. Root Volume, Root Area, Root Length, and Root Diameter of Vetiver roots using the monolith method.

In evaluating root volume, root area, and root length using the monolith method, significant differences were observed mostly in the deeper layers ranging from 0.40 to 0.80 m. It was noticed that in 0.60–0.70 m depth, higher values were identified in all variables. In greater quantity than the secondary ones, the presence of adventitious roots impacted the values for root area 0.40 to 0.70 m depth. This behavior agreed with the findings of Hamidifar (2018), as the growing capacity in lateral extension by secondary roots near this depth, thus providing a good anchor for aggregate soil particulates, making this system of deep roots more resistant to erosion.

On the root diameter, higher values were presented in the deepest layers of the trench. Even though the Vetiver grass presented a heterogeneous behavior in all analyzed variables by monolith methods, this heterogeneity was not enough to provide strong differences among the values showing that the root system properly occupies the soil. It is important to highlight that the spatial representativeness of this methodology is its best characteristic for each sampled point.

On the values of the coefficients of variation of root volumes in different methods (Fig. 6a), it is observed that the monolith method presented shorter values, varying from a minimum value of 2.29% in 0.40 to 0.50 m depth and the maximum of 34% for 0.20 to 0.30 m depth. The monolith method also showed lower values for root diameter (12%, 15% and 19%) in deeper depths (Fig. 6b). Still, on the root diameter the volumetric ring method was the one with the highest coefficients of variation. On the root volume data, the Trench method presented the highest values of coefficient of variation as well as a greater variation between the sampling depths in the comparison to the other studied methods. Probably this can be explained by the root heterogeneity observed in the photos, overestimating the obtained values (Vasconcelos et al. 2003b). Then, the lower the coefficient of variation, the greater the precision of the data, indicating that the soil profile methods were more adequate to detect differences between the depths and analyzed variables. Also was observed that the volumetric ring method was the one that showed the least variation between the different samples depth, and the greatest variation between the studied methods occurred in the depth range of 0.00 m to 0.50 m, reducing as the depth progresses.

On the root area, root diameter and root length, the Trench Method was the one presenting the lowest coefficients of variation (Figs. 6b, 6c and 6d). Vepraskas and Hoyt (1988) found lower coefficient of variation values in the data from the methods which obtained their data from direct images, in comparison to other methods (core-break), showing good accuracy of the results and saving time in the collection in the comparison to the invasive methods.

Discussion

The evaluation of the root length by Safira software using the trench method provides an understanding of higher values of root concentration up to the depth of 0.50 m, suggesting a greater reinforcement promoted by the root system in the shallow layers where the root density is the largest, featuring a biotechnical effect.

The highest values of root volume obtained by the trench and the volumetric ring methods were found in the superficial layers of 0.30 and 0.40 m in depth, similar behavior between the two methods in relation to the greatest lengths of the roots up to the depth of 0.50m.

The greater roots area and volume gives the monolith method greater accuracy related to the studied variables in comparison with the volumetric ring and hand auger hole methods, which consider a smaller analyzed volume. This particularity of these methods indicates that their accuracy is dependent on the sampling location.

The observed root volume values showed a strong significant correlation between the trench and the volumetric ring method ($r = 0.914$) and between the volumetric ring and the Auger hole methods ($r = 0.763$). A moderate correlation between the volumetric ring method and the hand auger hole ($r = 0.514$) was also observed (Table 5). The correlations of root volume by the monolith method were negative in comparison to the other methods.

Table 5

Linear correlation coefficient (r) between Root volume, Surface area, Root area, Root length, root diameter and different methods.

Root volume ¹ —mm ³ —	Trench	Volumetric ring	Hand Auger hole	Monolith
Trench		0.914*	0.514	-0.110
Volumetric ring	0.914*		0.763	-0.252
Hand Auger hole	0.514	0.763		-0.467
Monolith	-0.110	-0.252	-0.467	
Root area ¹ —mm ⁻² —				
Trench		-0.254	0.228	0.042
Volumetric ring	-0.254		0.526	0.026
Hand Auger hole	0.228	0.526		-0.107
Monolith	0.042	0.026	-0.107	
Root length ¹ —m—				
Trench		-0.216	0.540	0.318
Volumetric ring	-0.216		0.277	0.124
Hand Auger hole	0.540	0.277		0.356
Monolith	0.318	0.124	0.356	
Root diameter ¹ —mm—				
Trench		0.074	0.449	-0.027
Volumetric ring	0.074		-0.692	-0.502
Hand Auger hole	0.449	-0.692		0.308
Monolith	-0.027	-0.502	0.308	
¹ number of observations (n) = 30143, 3353, 6908 and 381; *significant at a 0.05 level; **significant at a 0.01 level.				

Table 5. Linear correlation coefficient (r) between Root volume, Surface area, Root area, Root length, root diameter and different methods.

An important reason to reach the mentioned results was the data collection procedure for each method. While the trench and volumetric ring methods consider the data collection limited to the squares, the monolith and hand auger hole considered the use of overlapping horizontal lines. Another important point to be highlighted is the negative correlations between the monolith method and the others, which may have occurred because of the disadvantage of the loss of fine roots omitted in the final evaluation because it is a destructive method as mentioned above, thus limiting a better analysis.

The root area showed a moderate but positive correlation between the volumetric ring and hand auger hole methods ($r = 0.526$) presented in Table 4. An essential factor to be observed is that both methods work with limited areas, interfering in the results considering dense root systems such as vetiver grass.

The trench and hand auger hole presented the best correlation in the root area, even though that was moderate but very similar to what happened to root volume. For these two methods, the deviation error was relatively low, even obtaining higher mean values than the other methods, showing that both methods are applicable without major damage in the evaluation of the root length of the vetiver. The diameter of the root did not show a strong correlation between the methods, except between the methods of the trench and manual auger, quite similar to the correlation between these methods in relation to the root length.

Among the analyzed variables, the root area showed the best correlation coefficient among the analyzed root methods, especially related to those that use the limited spatial distribution by its sampling collection, such as the volumetric ring method and the Auger hole hand method ($r = 0.526$). A probable factor is the limited area of collection relative to the object's diameter. Poaceas, which have fasciculated roots, have a significantly greater amount of secondary roots and adventitia than pivoting roots (Chen et al. 2007), thus occupying a larger surface area and greater aggregation capacity of the soil.

Conclusion

The particularities of each evaluated method show intrinsic advantages and disadvantages to each one. As a direct method, the monolith method seems to be more suitable for more quantitative analysis considering the greater volume of studied soil, providing a better evaluation of each soil layer, and fit to digital tools, despite the need for soil volume disturbance.

The studied methods can be separated into two groups: those that provide more detailed information about the behavior and distribution of the root system, such as trench and monolith methods. The trench method requires turning the soil. Both require greater spatial coverage, and therefore have greater precision, and the methods that better describe the morphological characteristics of the features, such as the volumetric ring method. The group represented by the volumetric ring and the hand auger hole methods, even though they are faster, easier to operate, do not require specialized equipment, once they are expeditious collecting smaller samples (consequently less representativeness), but present high

feasibility. For better representativeness, it is recommended to increase the collected and analyzed sample number, decreasing their variability, and increasing their reliability.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Some of the data used for this study can be accessed from osf.io/ay26j/?view_only=06c37cd0ccba4616afdc7f91a2cacf7a. Other support data can be obtained from the corresponding author up on request.

Competing interests

The authors declare no competing interests.

Funding

There was no funding.

Author contributions

FSRH and AP conceptualized and designed the research. RJSJ, PAOS and KMAA conducted the experiments. LDVS, RNAF, LRS and VRASS drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

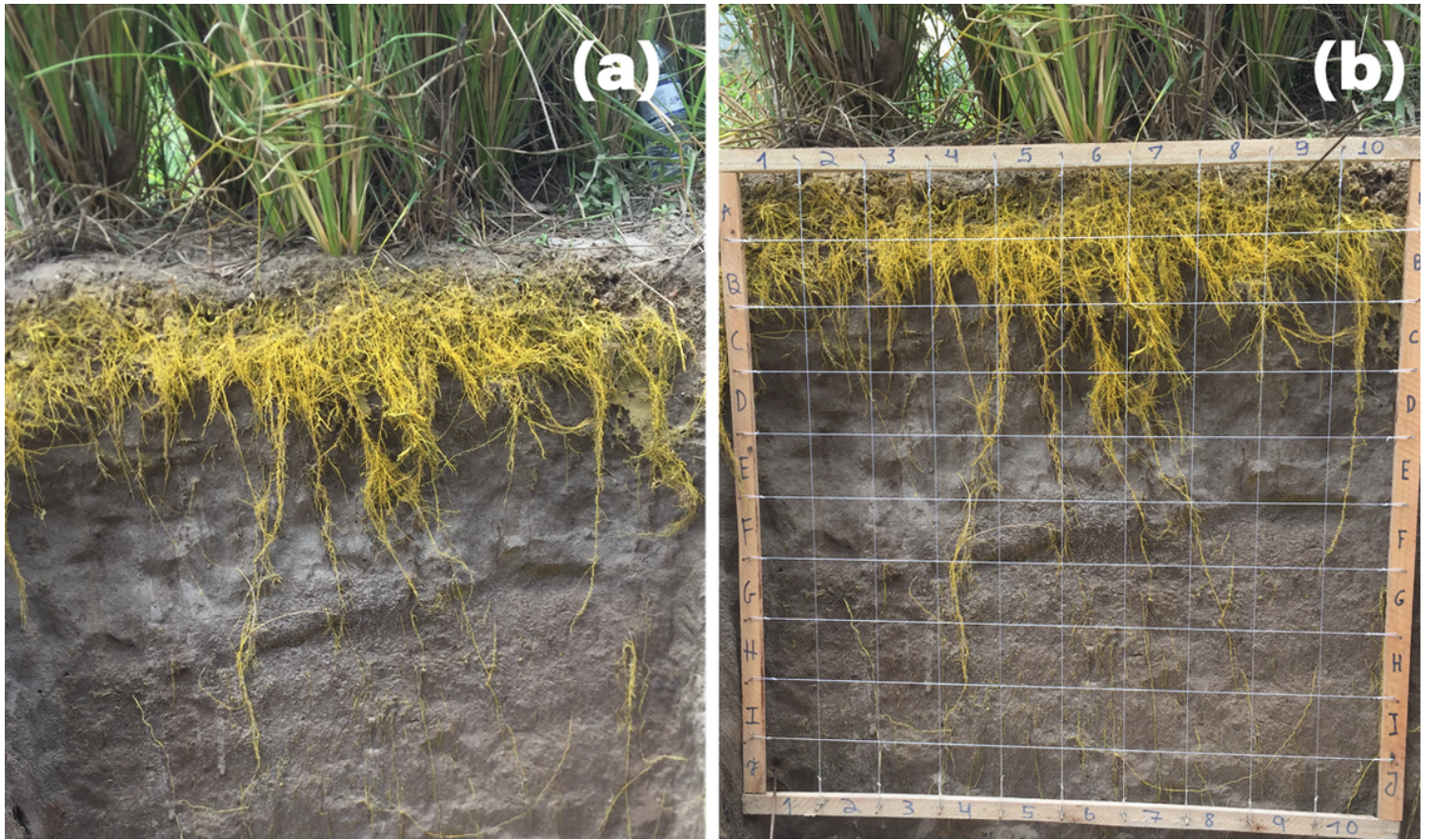


Figure 1

Front face of the trench after the yellow paint spray (A), Face of the trench showing grids for collecting pictures of the root system (B).

Source: The authors (2021).

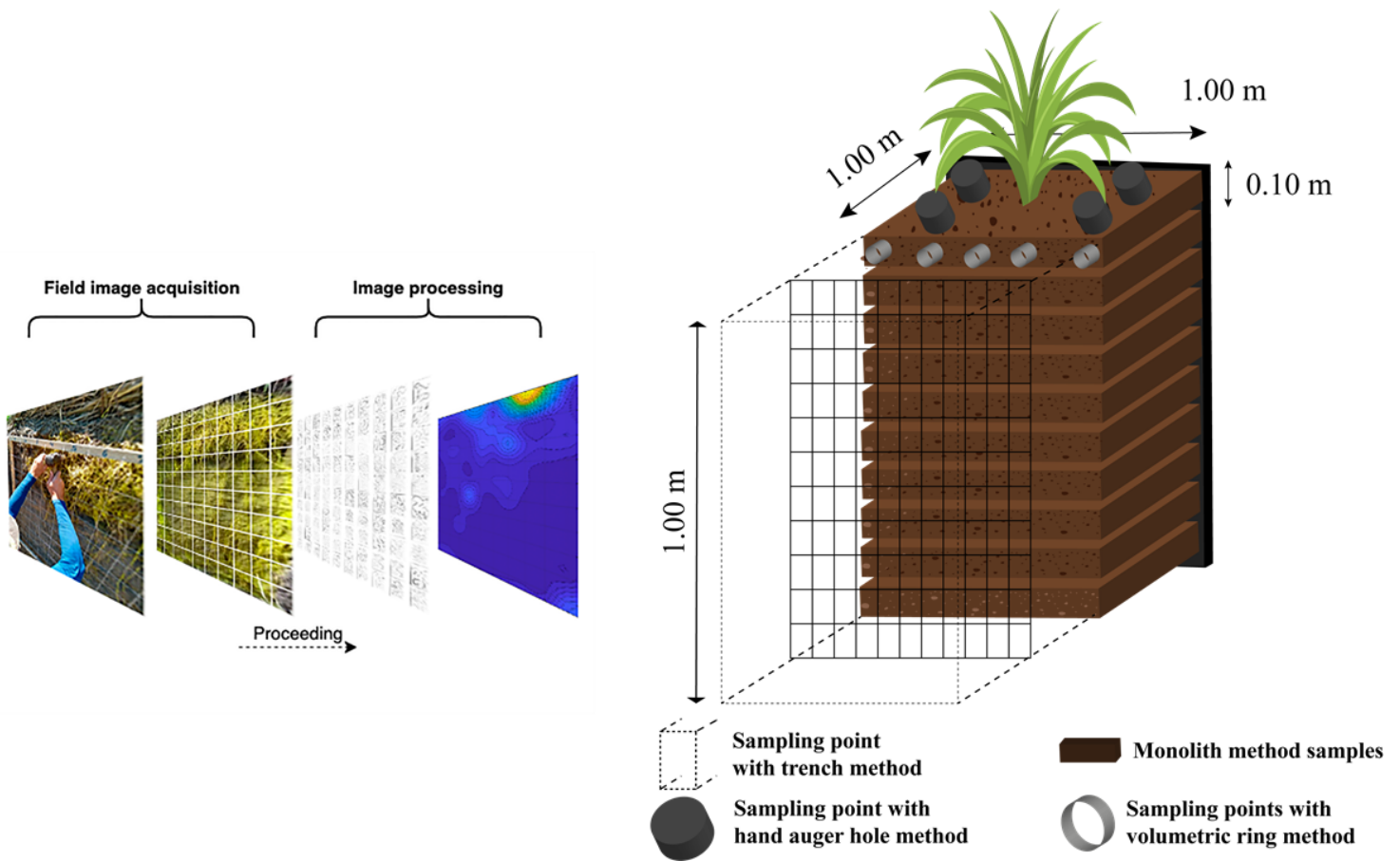


Figure 2

(a) Roots and soil collection with the volumetric ring showing the insertion of the rings in the soil on the vertical face of the trench for sample collection and subsequent image processing; (b) Systematization of adopted methods

Source: The authors (2021).

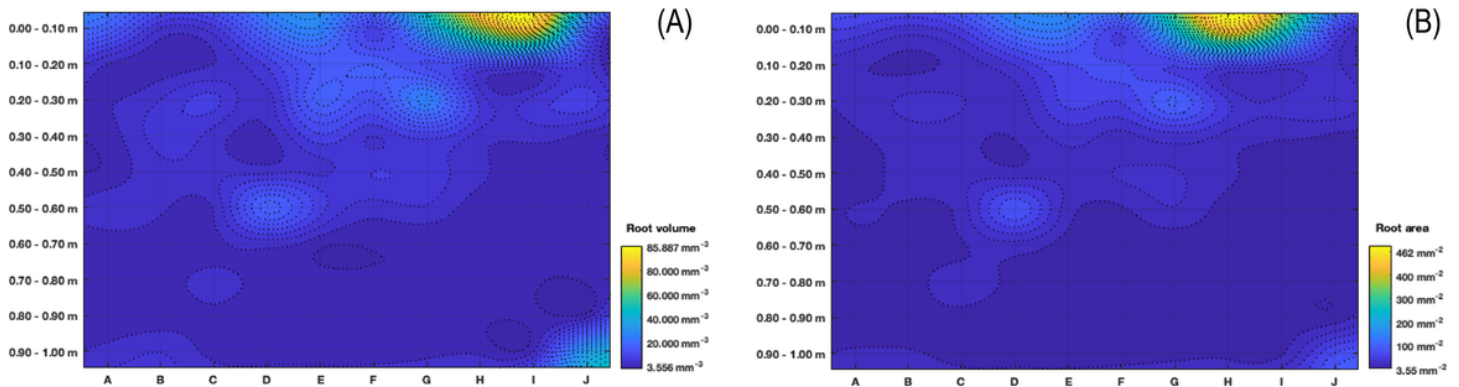


Figure 3

Heatmaps for Root Volume (A) and Root Area (B) obtained by the Trench Method

Source: The Authors (2021).

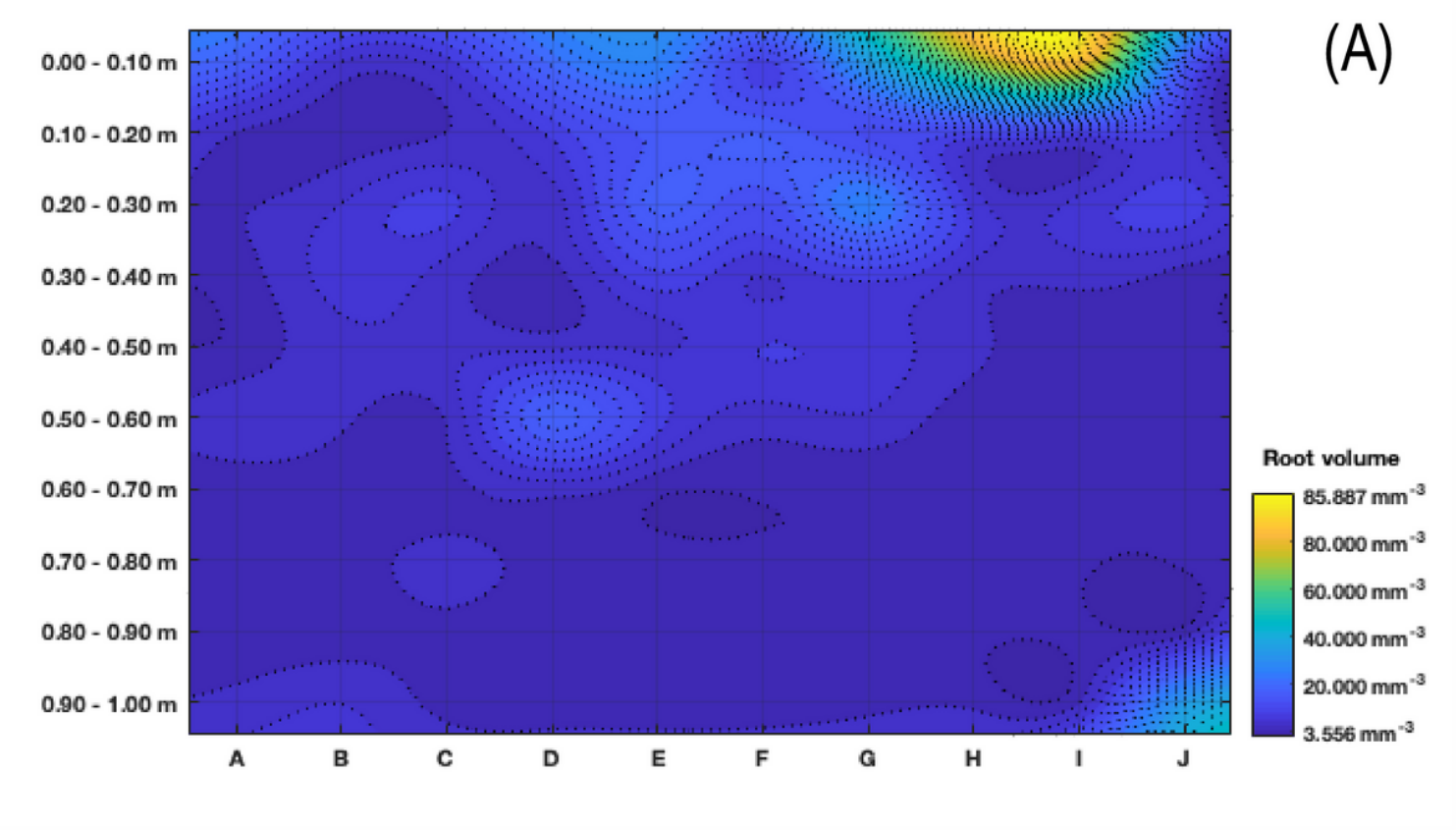


Figure 4

Heatmaps for the Root Length obtained by the Trench Method.

Source: The authors (2021).

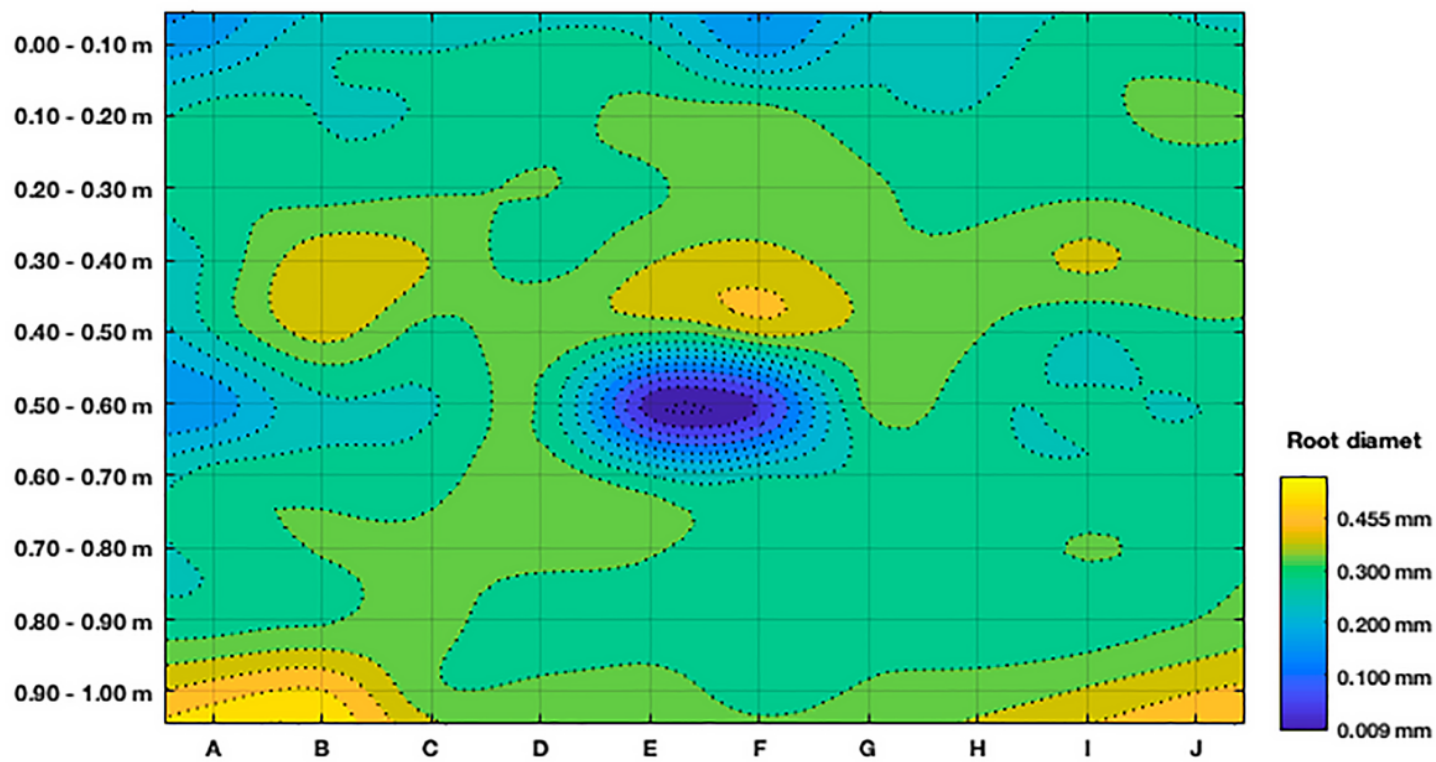


Figure 5

Heatmaps for the Root Diameter obtained by the Trench Method.

Source: The authors (2021)

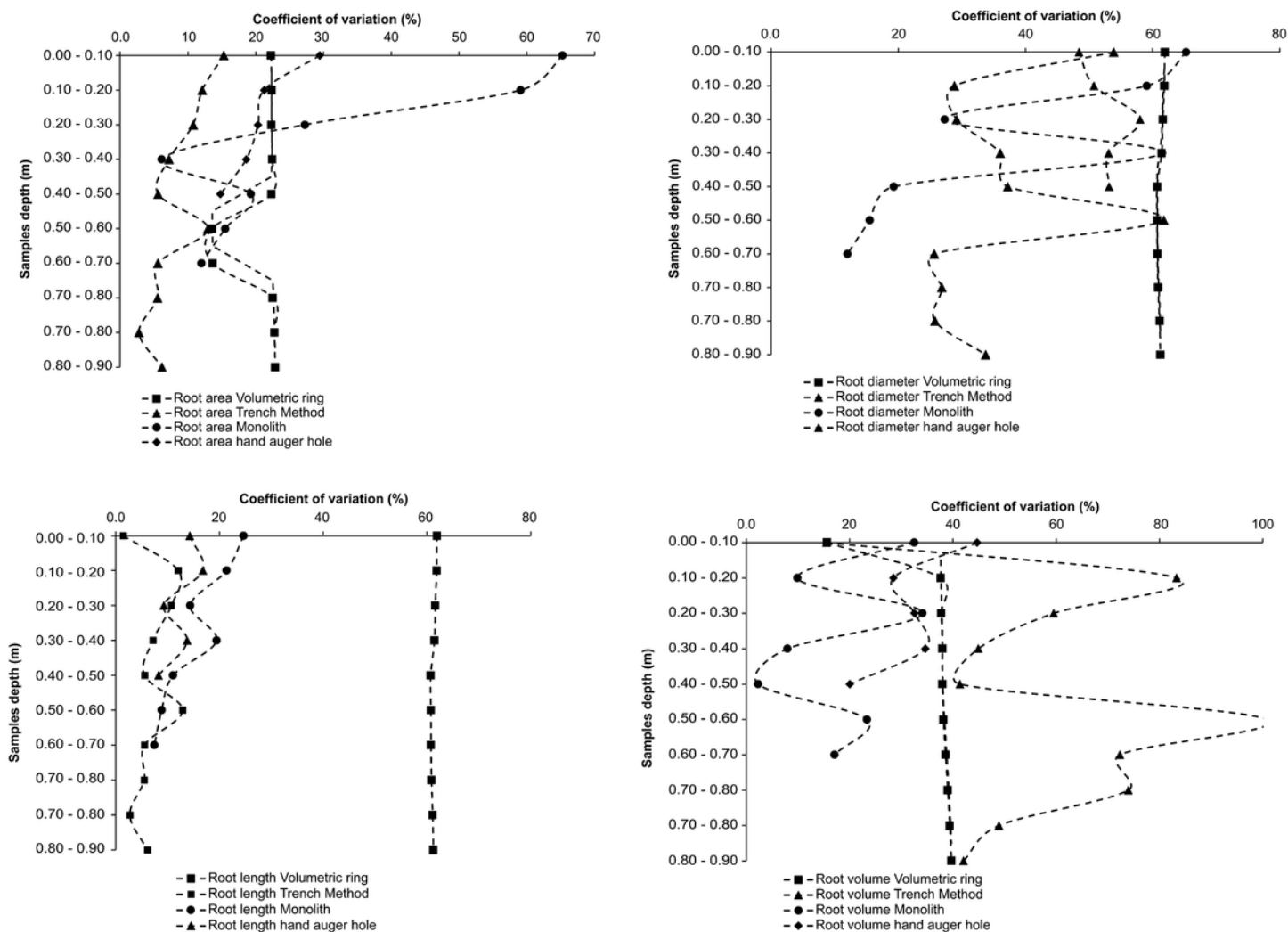


Figure 6

Coefficients of variation by sampling depths in the evaluated methods (a) root area, (b) root diameter, (c) root length and (d) root volume

Source: The Authors (2021).