

## Fine root contribution to the soil carbon stock of an agroforestry system in a Caatinga-Atlantic Forest transition zone

### Contribuição de raízes finas no estoque de carbono do solo de um sistema agroflorestal em zona de transição caatinga-mata atlântica

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#### ABSTRACT

The objective of this work was to evaluate the distribution of fine roots and its influence on the soil organic carbon stock, at a depth of 20 cm, in a *Grevillea robusta* and *Coffea arabica* agroforestry system. The study was conducted in an agroforestry system established 15 years ago in a transition area of Caatinga and Atlantic Forest biomes in Brazil. *G. robusta* trees representing the most frequent diameter class were selected, and three distances of these trees (0, 0.75 and 1.50 m) and two soil collection depths (0–10 and 10–20 cm) were defined. The root samples were scanned and quantified using a software program. There was a general predominance of roots with a diameter of 0.6 mm at the shortest distance from the surface layer, while there was a predominance of roots with a diameter of 0.4 mm in the 10–20 cm layer. The root carbon stock at a distance of 0.75 m was higher at a depth of 0–10 cm (0.60 Mg ha<sup>-1</sup>). The soil organic carbon stock also showed higher results in the 0–10 cm layer compared to the 10–20 cm layer, although with significant variation only in the distance of 1.5 m. There was a higher concentration of fine roots in the topsoil, probably influenced by a greater availability of water and nutrients from plant residues. The soil carbon stock is not closely related to root density or root carbon stock. The data presented in this study do not provide a definitive conclusion.

**Keywords:** *Grevillea robusta*; *Coffea arabica*; root density; root diameter; organic matter.

#### RESUMO

O objetivo deste trabalho foi avaliar a distribuição de raízes finas e sua influência no estoque de carbono orgânico total do solo, em 20 cm de profundidade, em um sistema agroflorestal de *Grevillea robusta* e *Coffea arabica*. O estudo foi realizado em um sistema agroflorestal estabelecido há 15 anos em uma área de transição dos biomas Caatinga e Mata Atlântica no Brasil. Árvores de *Grevillea robusta* mais representativas da classe de diâmetro de maior frequência foram selecionadas e definidas três distâncias de coleta destas árvores (0, 0,75 e 1,50 m) e duas profundidades do solo (0–10 e 10–20 cm). As raízes presentes nas amostras foram digitalizadas e quantificadas com auxílio de um *software*. Na menor distância da camada superficial houve predomínio de raízes com diâmetro de 0,6 mm, enquanto, em todas as distâncias da camada 10–20 cm, houve dominância de raízes com diâmetro de 0,4 mm. Na distância de 0,75 m, o estoque de carbono das raízes foi superior na profundidade de 0–10 cm (0,60 Mg ha<sup>-1</sup>). O estoque de carbono orgânico do solo também apresentou maior resultado na camada 0-10 cm em relação à camada 10–20 cm, embora com variação significativa apenas na distância de 1,5 m. Na camada superficial, ocorreu maior concentração de raízes finas, provavelmente influenciada por uma maior disponibilidade de água e nutrientes provenientes dos resíduos vegetais. O estoque de carbono do solo não está intimamente relacionado com a densidade de raízes e estoque de carbono das raízes. Os dados apresentados neste estudo não fornecem uma conclusão definitiva.

**Palavras-chave:** *Grevillea robusta*; *Coffea arabica*; densidade de raízes; diâmetro de raízes; matéria orgânica.

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## Introduction

Agroforestry systems (AFS) are defined as any land use system that implies the deliberate introduction or maintenance of two or more plant species, where at least one of these is arboreal or other perennial species and is associated with agricultural crops, pasture and/or livestock to exploit the ecological and economic interactions of the different components (NAIR; NAIR, 2014). AFS are known for contributing to mitigating greenhouse gases (STOUT; LAL; MONGER, 2016; VICENTE; GAMA-RODRIGUES; GAMA-RODRIGUES, 2016), as they provide environmentally beneficial services and economic advantages to farmers (RODRIGUES *et al.*, 2007).

Interspecific competition for water and nutrients between intercropped crops is a variable that interferes in the productivity of AFS. Tree roots can extend into the crop lines in the active crop competition zone, which occurs up to just over 1 m apart, but mainly in the surface layers (THEVATHASAN; GORDON, 2004). Thus, the choice of deeper-rooted tree species in AFS may reduce vertical competition due to their expansion by layers not exploited by crops, promoting better use of the soil profile (PADOVAN *et al.*, 2015; BORDEN; THOMAS; ISAAC, 2017). On the other hand, even for deep-rooted species, it is observed that a large volume of roots is present in the surface layers, absorbing nutrients from the mineralization of plant residues. According to Defrenet *et al.* (2016), even though species in AFS exploit different soil niches, coffee roots often dominate the fine root population of the system in surface layers. It is due to higher root biomass in coffee AFS (MEIRELES *et al.*, 2019).

The accumulation of organic carbon in the soil in AFS results from added and decomposing plant residues (from shoots and roots) coming from the different species that compose the system (CHATTERJEE *et al.*, 2018). It is estimated that the soil organic carbon (SOC) stock in AFS differs across regions of the world and at different soil depths, ranging from 30-300 Mg C ha<sup>-1</sup> (AGEVI *et al.*, 2017).

Roots in general, and in particular fine roots, constitute an important carbon input into the soil (LIAO *et al.*, 2014). The degree of fine root carbon (FRC) contribution to SOC depends on land use and management system, which determines the root architecture, cycling rate, root exudates and colonization by mycorrhizae (HERTEL; HARTEVELD; LEUSCHNER, 2009; POLLIERER *et al.*, 2012). In addition, root distribution is influenced by edaphic characteristics. For example, Addo-Danso *et al.* (2020) reported a positive relationship between base saturation and specific root length and specific root area. Fine roots also show greater length in tropical sites where available phosphorus in the soil is low. Le Bissonnais *et al.* (2018) evaluated the effect of land use gradients (monocultures to AFS and forest) on soil aggregate stability, which was higher in surface layers than deeper layers. Aggregate stability was the main driver of SOC, cation exchange capacity and root traits.

The quantification of root biomass and its distribution in the soil may help to understand the relationship between root dynamics and SOC stock. Although many studies on this topic have already been performed with tree species, most of them are based on quantifying the total

root system biomass, and the number of studies measuring the density of fine roots in different diameter classes is limited. In this work, fine roots were found in the surface layer and directly related to the higher concentration of organic matter and nutrients (WITSCHORECK; SCHUMACHER; CALDEIRA, 2003). Chatterjee *et al.* (2020) also reported that the SOC stock in coffee AFS was increased only to a depth of 10 cm after 17 years of establishment due to shade-pruned *Erythrina spp.*

Given the above, the present study aimed to evaluate the distribution of fine roots and its influence on the SOC stock, at a depth of 20 cm, in a *Grevillea robusta* and *Coffea arabica* agroforestry system. Our study is based on the fact that fine root turnover is the dominant form of below-ground carbon input (UPSON; BURGESS, 2013), with a cycle of less than one year (FREITAS; BARROSO; CARNEIRO, 2008).

## Materials and methods

### Study site characterization

The study was carried out in an AFS formed by the *G. robusta* A. Cunn. ex. R. Br and *C. arabica* plants planted 15 years ago, spaced 3.5 m (between trees) × 1.5 m (between trees and coffee plants) × 2.5 m (between coffee plants). The area is located in Lucaia District, Planalto municipality, Bahia State (coordinates UTM X: 334277 and Y: 8368812). The AFS was located in an area with pasture naturally formed with predominance of genus *Brachiaria* grass. Other species were not present in the AFS.

The region has an average altitude of 943 m and a tropical altitude climate (Cwb type according to the Köppen classification), with an average temperature of 19.2°C and rainfall of 641 mm year<sup>-1</sup> (CLIMATE-DATA, 2012). The study area is located in a transition section between the Caatinga and Atlantic Forest biomes and has soil classified as dystrophic yellow latosol (EMBRAPA, 2013). The chemical characteristics are presented in Table 1.

### Fine root and soil collection

We selected six *G. robusta* trees in the most frequent diameter measurements at a height of 1.3 m (DBH) (class center = 27.95 cm) to perform the root and soil collection. The DBH measurement distribution for trees considering an amplitude of 6.14 cm is shown in Figure 1. The selection was carried out in a total area of 1.5 ha with 132 *G. robusta* tree/ha and 3530 *C. arabica* plants/ha.

Soil sampling was performed on the *G. robusta* planting line at 0, 0.75 and 1.50 m from the trunk of each selected tree at depths of 0–10

**Table 1 – Chemical attributes of soil under a *Grevillea robusta* and *Coffea arabica* agroforestry system.**

pH	P	K	Ca	Mg	H + Al
H <sub>2</sub> O	mg/dm <sup>3</sup>	-----cmolc/dm <sup>3</sup> -----			
6.2	41.5	0.5	3.8	2.6	2.7

and 10–20 cm (Figure 2). Two undisturbed soil samples to evaluate fine roots and soil density and one disturbed sample for SOC determination were taken at each distance and depth, making a total of 108 samples. The disturbed samples were taken using a Dutch auger and the undisturbed samples using a cylindrical ring auger. It was not possible to identify the origin of the roots as to whether they came from trees or coffee.

**Soil density**

Soil density was determined by the volumetric ring method (EMBRAPA, 2017) in samples with preserved structure and known volume (7 cm in height and 7 cm in diameter, totaling 269.3 cm<sup>3</sup> volume).

**Fine root mass and diameter**

The soil samples were placed in plastic containers and then washed with running water and collected on a 0.25 mm sieve to remove the soil mass to determine the mass and diameter of fine roots (KUMAR; UDAWATTA; ANDERSON, 2010). After washing, all roots were manually clamped and arranged on white-bottomed acrylic slides.

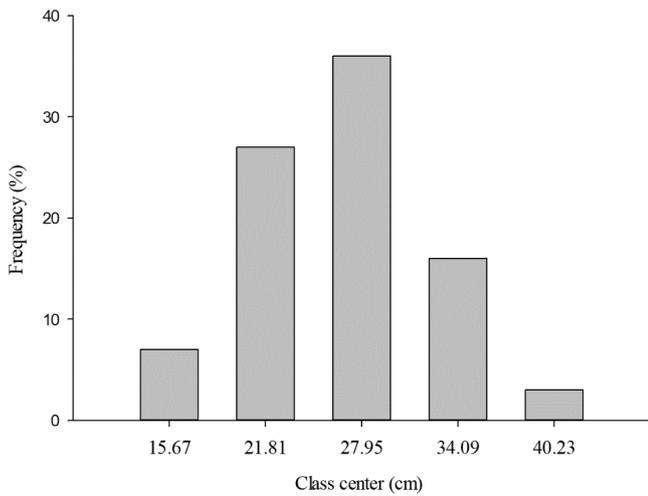


Figure 1 – Frequency histogram of the diameter classes at a height of 1.3 m from the soil of *Grevillea robusta* in an agroforestry system with *Coffea arabica*.

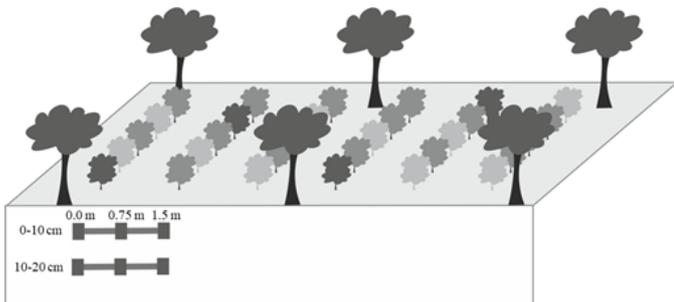


Figure 2 – Representation of soil and fine root sampling points in a *Grevillea robusta* and *Coffea arabica* agroforestry system.

The roots of each sample were scanned (Figure 3) and were distributed in ten root diameter classes (0.45, 0.63, 0.81, 1.0, 1.18, 1.34, 1.52, 1.70, 1.87, 2.05 mm) with the aid of SAFIRA® software (JORGE; RODRIGUES, 2008) for the different studied distances and depths, according to the method described by Costa *et al.* (2014).

After scanning, the root samples were placed in aluminum containers, which were then put in a forced-air oven at 65°C for 72 hours. The samples were subsequently weighed on an analytical balance accurate to 0.001g to determine dry mass.

**Fine root density**

Root dry mass was used to determine soil root density by means of Equation 1:

$$D = m/v \tag{1}$$

in which:

D = density in g cm<sup>-3</sup>;

m = root mass in g;

v = ring volume (269.4 cm<sup>3</sup>);

**Fine root and soil organic carbon stock**

Root (after oven drying) and soil (after air drying and 2.0 mm sieving) samples were macerated in a mortar. Next, 0.02 g of roots and 0.2 g of soil subsamples were removed and submitted to chemical analysis to determine carbon content, using the wet oxidation method with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in acid medium and titration with ammonium ferrous sulfate (EMBRAPA, 2017).

SOC was calculated on the basis of carbon content and soil density according to Equation 2:

$$SOC = T_{SOC} (g\ 100\ g^{-1}) \times Ds \times Slt \tag{2}$$

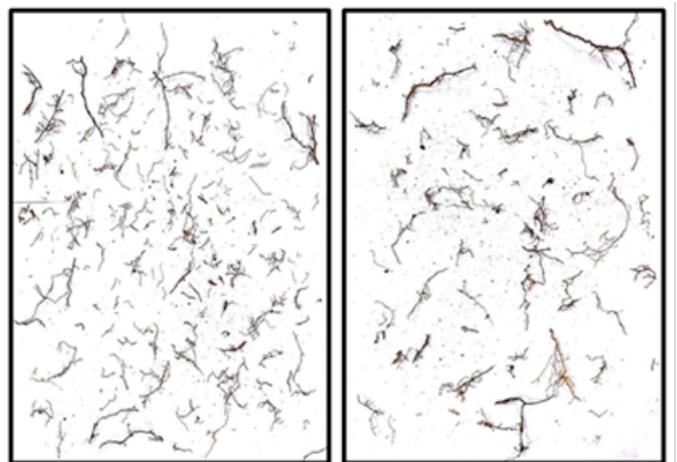


Figure 3 – Scanned images of samples utilized to determine root diameter of *Grevillea robusta* and *Coffea arabica*.

in which:

SOC = soil organic carbon stock in  $\text{Mg ha}^{-1}$ ;

$T_{\text{SOC}}$  = total soil organic carbon content;

$D_s$  = soil density ( $\text{g cm}^{-3}$ );

Slt = soil layer thickness (cm).

FRC was calculated according to Equation 3:

$$\text{FRC} = C_{\text{FRC}} (\text{g } 100\text{g}^{-1}) \times \text{Dr} \times \text{Slt} \quad (3)$$

in which:

FRC = fine root carbon stock in  $\text{Mg ha}^{-1}$ ;

$C_{\text{FRC}}$  = fine root organic carbon content;

Dr = root density ( $\text{g cm}^{-3}$ );

Slt = soil layer thickness (cm).

### Statistical analysis

The root density, SOC stock and FRC values met the parametric criteria and were then submitted to analysis of variance (ANOVA) according to a  $3 \times 2$  factorial scheme with 6 replications (3 distances and 2 depths). Student's t-test at 5% significance was adopted to compare means between distances and depths. The analyses were performed using STATISTICA® v.10.0 software (StatSoft Inc., 1984–2011).

A descriptive frequency analysis was performed for mean root diameter values using the SIGMAPLOT® v.12.0 software program (Systat Software inc.) and the contour maps were produced using the Surfer® v.8.0 program, considering a vertical Cartesian plane formed by the spatial distribution of the soil layers and distances of the *G. robusta* trees.

## Results

### Fine root diameter

Root diameters ranged from 0.4 to 1.6 mm at the depth of 0–10 cm and from 0.45 to 1.34 mm at the depth of 10–20 cm (Figure 4). Roots in classes of 0.63 mm were more frequent at distances of 0 and 0.75 m and 0.45 mm at 1.5 m.

Overall, there was a predominance of roots with a diameter of 0.6 mm in the distances near the trees at the 0–10 cm depth, while there was a predominance of roots with a diameter of 0.4 mm at the distance of 1.5 m at the 0–10 cm depth, and in all distances of the 10–20 cm depth, which represented 60 to 80% of the total roots.

### Root density and fine root and soil organic carbon stocks

The interaction between distance and depth produced a significant effect for the variables of root density and SOC and FRC stocks (Table 2). Significance was only observed for depth when evaluating the isolated effect of the considered factors. The results of the distance  $\times$  depth interaction are presented in Table 3 and Figure 5.

The FRC and SOC stocks did not vary between the different distances studied. In the case of FRC, differences between depths were only found at a distance of 0.75 m, which showed the highest value in the 0–10 cm layer (Table 3). The SOC stock only showed variation between depths at a distance of 1.5 m, with higher results in the surface layer.

Higher root density was observed in the first soil layer (0–10 cm). However, there was only variation between distances at a depth of 10–20 cm (Figure 5). Higher values were observed in the distance 0 m, although only with a significant difference at the distance of 0.75 m.

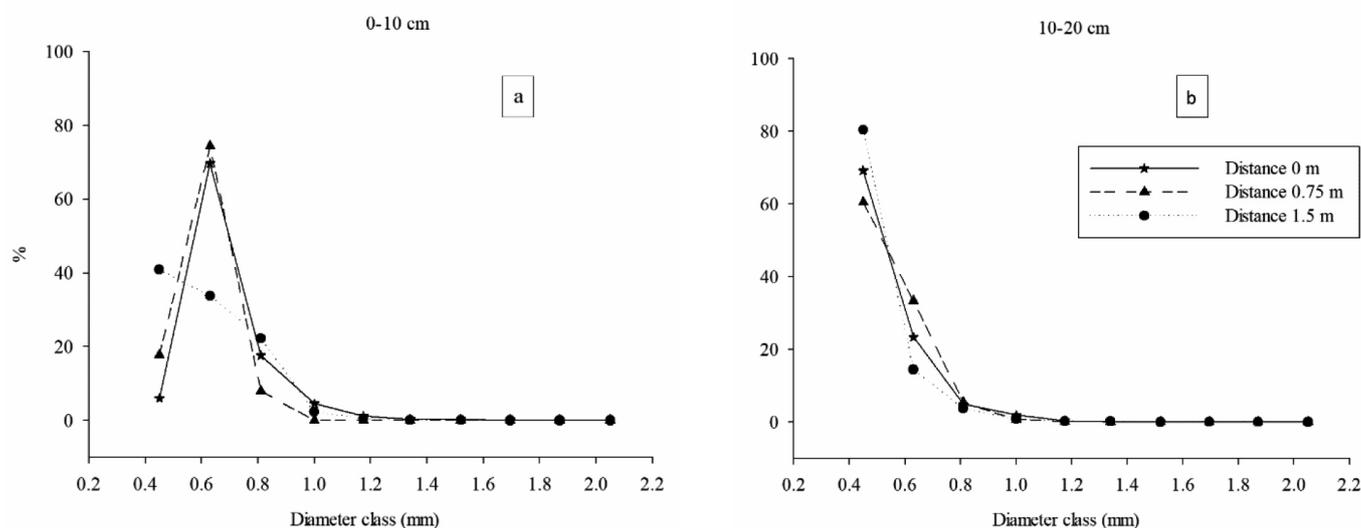


Figure 4 – Root distribution frequencies in diameter classes in the (A) 0–10 cm and (B) 10–20 cm soil layer at different distances of *Grevillea robusta* in an agroforestry system with *Coffea arabica*.

**Spatial root distribution influencing soil organic carbon stock**

The contours formed by the distribution of root density data and FRC and SOC stocks showed a decrease with increasing depth (Figure 6A, 6B and 6C). This means that higher values are present in the surface layer, with a slight displacement to positions closer to the *G. robusta* tree line. It was noted that the distance influenced the root density contours and consequently the FRC stock. Higher root concentration occurred in the 0-10 cm layer and specifically at a distance of 0 and 0.75 m, decreasing vertically and horizontally moving away from this region.

SOC distribution ranged from 30 to 20 Mg ha<sup>-1</sup> in depth, and showed higher values according to distance (Figure 6C) in the surface layer at a distance of 1.5 m. On the other hand, lower SOC stock values were found at a distance of 0.75 m (25 Mg ha<sup>-1</sup>), increasing about 2 Mg ha<sup>-1</sup> close to the *G. robusta* trees. It was also possible to notice a similar distribution pattern at greater depth for all evaluated indicators.

**Discussion**

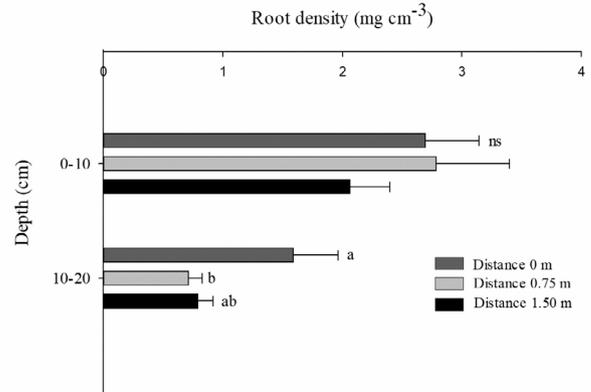
**Fine root diameter**

The predominance of roots with larger diameter in the 0–10 cm layer (Figure 4) suggests that roots close to *G. robusta* trees are associated with plant support, while the roots at increasing distance from the tree are more associated with absorption and therefore have smaller diameters. Greater amounts of subsurface roots are also associated with nutrient exploitation from plant waste mineralization (ISAAC; BORDEN, 2019). According to Mora-Garcés (2018), the distribution of fine roots generally decreases with increasing soil depth. The roots can be found in a

non-standardized grouping concentrated in cracks or animal pits, showing a large amount of short branches (ZONTA *et al.*, 2006). Fine roots in AFS can also be concentrated in locations with a large agglomeration of plant residues, absorbing nutrients directly from the litter after mineralization (THAKUR; KUMAR; KUNHAMU, 2015).

**Root density and fine root carbon and soil organic carbon stocks**

The highest FRC values observed in the surface soil layers (Table 3) were expected, as they showed higher root concentration (PADOVAN



**Figure 5 – Root density at three distances of *Grevillea robusta* trees at two soil depths. The same letters, which compare distances at the same depth, indicate no significant difference between values by the t-test at 5% probability\*.**

ns: not significant; \*bars linked to histogram correspond to standard error.

**Table 2 – Summary of variance analysis for fine root carbon (FRC) and soil organic carbon (SOC) stocks and root density in *Grevillea robusta* with *Coffea arabica* agroforestry system.**

SV	DF	Mean squares		
		FRC	SOC	Root density
Distance	2	0.08 <sup>NS</sup>	10.13 <sup>NS</sup>	1.99E <sup>-5NS</sup>
Depth	1	0.96*	263.16*	8.05 E <sup>-7*</sup>
Dist × Dep	2	0.03*	15.08*	8.58 E <sup>-7*</sup>
Error	30	4.13	41.09	0.85

SV: source of variance; DF: degrees of freedom; Dist: distance; Dep: depth; \*significant (p < 0.05) by analysis of variance; <sup>NS</sup>not significant.

**Table 3 – Fine root carbon (FRC) and soil organic carbon (SOC) stocks as a function of different distances and depths in a *Coffea arabica* and *Grevillea robusta* agroforestry system\*.**

Depth (cm)	FRC (Mg ha <sup>-1</sup> )			SOC (Mg ha <sup>-1</sup> )		
	Distance (m)					
	0	0.75	1.5	0	0.75	1.5
0–10	0.61 A (0.11)	0.60 A (0.09)	0.47 A (0.08)	26.56 A (3.03)	24.83 A (2.25)	28.36 A (1.99)
10–20	0.35 A (0.08)	0.17 B (0.03)	0.16 A (0.03)	22.36 A (4.22)	20.81 A (2.02)	20.36 B (1.00)

\*The same letters in the column indicate no significant difference between values by the t-test at 5% probability. Values in parentheses represent the standard error of the mean, n = 6.

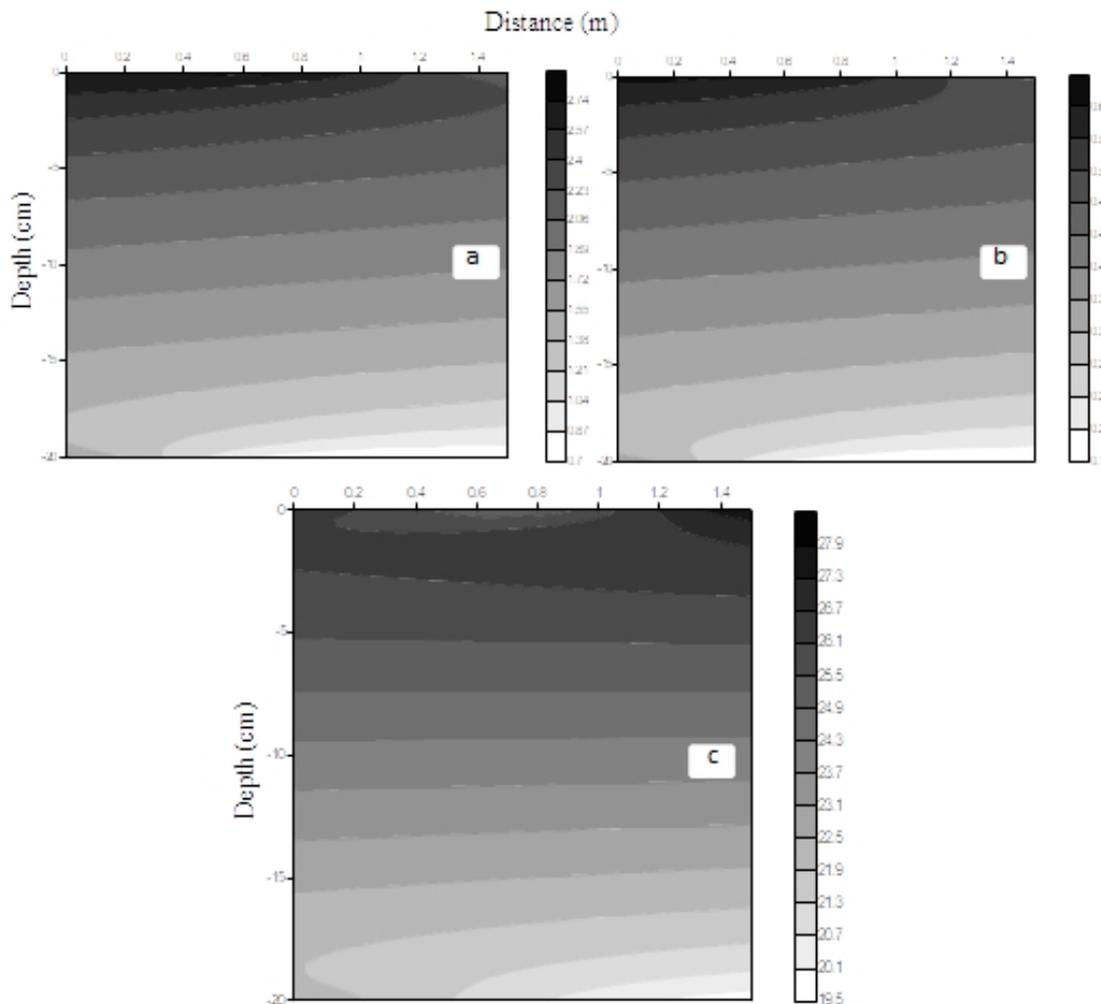
*et al.*, 2015; ALBUQUERQUE *et al.*, 2015). This results from the greater contact of the litter (leaves, branches and bark) with the soil, which promotes greater nutrient flow in the surface layer, stimulates the development of proteoid roots and the accumulation of carbon in the soil after its turnover (PULROLNIK *et al.*, 2009). Morais *et al.* (2017) found that carbon stored in fine roots is more concentrated in the topsoil (0–10 cm). The authors also observed that fine roots store 40% more carbon than thick and medium roots in this layer.

The absence of a difference in FRC stock at distances of 0 and 1.5 m (Table 3) suggested unevenness in *G. robusta* root development. Thus, it is likely that horizontal variability in fine root distribution is being more influenced by soil resource availability than by root architecture of the species in question. In studying the distribution of *G. robusta* roots in AFS, Smith *et al.* (1999) observed great unevenness in the distribution of fine roots and argued that the root distribution complementarity of the different components in the AFS may be com-

promised by restrictions in the availability of water and nutrients for the tree component, which results in increased competition (BALJIT; PARAMPARDEEP; GILL, 2016).

The reduction in root concentration with increasing depth (Figure 5) can be attributed to the large amount of litter found in AFSs, which contributes to the development of fine roots in the topsoil and in the organic layer itself. Similar results were obtained by Defrenet *et al.* (2016) in evaluating the biomass and root dynamics of AFSs based on coffee planted in Costa Rica, finding higher amounts of fine roots in surface soil (12% of total roots). Fine root biomass was also twofold higher in the row compared with between rows.

The litter acts as a mulch, protecting the surface soil and providing nutrients. Freitas, Barroso and Carneiro (2008) point out that the growth of fine roots ( $\leq 2$  mm) has a strong correlation with the availability of organic matter and soil moisture, being closely associated with litter, since it is a carbon source and favors water retention.



**Figure 6 – Spatial distribution (A) of root density and (B) fine root carbon (FRC) and (C) soil organic carbon (SOC) stocks at different distances of *Grevillea robusta* trees associated with *Coffea arabica*.**

### Spatial root distribution influencing soil organic carbon stock

The results obtained did not allow us to determine the origin of the evaluated roots (of *C. arabica* or *G. robusta*), which would indicate which species would predominantly be contributing to the carbon accumulation in the soil, since the roots of *C. arabica* can develop in the middle of the *G. robusta* lines and vice versa. Some mechanisms (still little known) may alter the root growth of the intercropped crop in an AFS. For example, Livsley, Gregory and Buresh (2000) reported that corn crops showed a greater amount of fine roots and root length in an AFS with *Grevillea* sp. when compared to a monoculture. A similar pattern was observed by Duan *et al.* (2019) for oat roots, which were influenced by the presence of walnut, which caused increased root length and decreased root diameter.

Regardless of the soil carbon origin, considering the components present in the AFS, it was observed that the FRC stock had little influence on the SOC stock. On the one hand, the SOC stock did not follow a similar distribution as FRC stock in the 0–10 cm layer, on the other hand there was a high correlation between SOC stock, root density and FRC stock in the 10–20 cm layer (Figure 6). This indicated that the carbon accumulation in the 0–10 cm layer depended on more litter contribution than only the carbon originating from the fine root turnover, as in natural systems of Brazilian biomes (OZÓRIO *et al.*, 2019). The influence of the FRC stock in the 10–20 cm layer was high due to a decreasing carbon incorporation rate from the surface to the deeper layer (CHATTERJEE *et al.*, 2020). AFS are known to have complex re-

lations between species which results in a heterogeneous environment, especially in the soil-plant transition.

AFSs also help to maintain the natural physical properties of the soil, especially because soil tillage is usually only done in pre-planting (FALCÃO *et al.*, 2020). The conservationist character of AFS assists in natural root turnover, without harming the soil carbon accumulation.

### Conclusion

There is a higher concentration of fine roots in the topsoil which decreases with increasing depth. The root density shows a homogeneous horizontal distribution from the base of *G. robusta*, probably being more influenced by litter and edaphic characteristics than by the fine roots. The SOC stock is not closely related to root density or root carbon stock. The data presented in this study do not provide a definitive conclusion. Thus, more investigations focusing on identifying the fine root origin of the different species in the AFS are necessary, including deeper layers (up to 100 cm) and evaluating other edaphic characteristics.

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### Contribution of authors:

Monroe, P.H.M.: Writing – original draft, Formal analysis, Investigation, Methodology. Barreto-Garcia, P.A.B.: Supervision, Methodology, Writing – review & editing. Lima, M.C.D.: Formal analysis, Writing – review & editing. Santos, R.K.A.: Formal analysis, Writing – review & editing. Oliveira, E.P.: Formal analysis, Writing – review & editing. Silva, S.R.: Formal analysis, Writing – review & editing. Gama, D.C.: Formal analysis, Writing – review.

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