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## MIXED AND MONOSPECIFIC STANDS OF EUCALYPTUS AND BLACK-WATTLE. II – FINE ROOT BIOMASS DENSITY

**ABSTRACT:** The aim of this study was to evaluate fine root biomass density (FRBD) in mixed and monospecific stands of *Eucalyptus grandis* x *E. urophylla* and *Acacia mearnsii* (black wattle) in Bagé-RS (Southern Brazil). An experimental trial was installed with three treatments: 100% *Eucalyptus* (100E); 100% *Acacia mearnsii* (100A); 50% *Eucalyptus* + 50% *Acacia mearnsii* (50E:50A). The trial was carried using a randomized block design with three replicates. The fine root (≤ 2.0mm) biomass density was determined 8 and 18 months after planting the trees. Soil samples were collected, with a cylindrical extractor auger (d = 7.0 cm), from four depths (0 - 5, 5 - 10, 10 - 20 and 20 - 30 cm) at each sampling point. After 8 months, the FRBD distribution was the same in both species and in all soil layers, reaching the maximum projection at 125 cm from the tree trunk. After 18 months, the root biomass density was higher in the monospecific black wattle stand than in the monospecific eucalyptus stand and the mixed stand. The fine root biomass density was highest in the 5 - 10 cm layer close to the trunk, for the planting row spacing, the planting line and the diagonals between two planting lines. Knowledge about fine root growth and distribution in soil at initial stages of stand development may help in decision-making for intensive forestry, thus ensuring more efficient use of soil resources.

Histórico:
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Palavras chave:
*Eucalyptus grandis* x *E. urophylla*
*Acacia mearnsii*
Sistema Radicular
Interação entre Plantas

## PLANTIOS MONOESPECÍFICOS E MISTOS DE EUCALIPTO E ACÁCIA-NEGRA. II – DENSIDADE DE BIOMASSA DE RAÍZES FINAS

**RESUMO:** O objetivo deste estudo foi avaliar a densidade da biomassa de raízes finas (DBRF) em povoamentos monoespecíficos e misto de *Eucalyptus grandis* x *E. urophylla* na região sul do Brasil. Para isso, instalou-se área experimental no município de Bagé-RS, a qual foi constituída por três tratamentos: 100E (100% de eucalipto); 100A (100% de *Acacia mearnsii*); 50E:50A (50% de eucalipto + 50% de *Acacia mearnsii*) em delineamento de blocos ao acaso, com três repetições. Determinou-se a densidade de biomassa de raízes finas (DBRF ≤ 2,0mm) aos 8 e 18 meses após o plantio. A amostragem foi realizada com tubo extrator de formato cilíndrico com 7,0 cm de diâmetro. Para cada ponto de amostragem, coletaram-se amostras em quatro profundidades: 0 – 5; 5 – 10; 10 – 20 e 20 – 30 cm. Verificou-se que a densidade de biomassa de raízes finas (DBRF) aos 8 meses de idade, nos diferentes sistemas de cultivo, possui o mesmo comportamento para a ocupação das diferentes camadas do solo, atingindo uma projeção máxima de 125 cm de distância em relação ao tronco da árvore. Já, aos 18 meses após o plantio, verificamos que no cultivo monoespecífico de acácia-negra a densidade de raízes foi superior em relação ao monocultivo e plantio misto de eucalipto. A maior densidade de biomassa de raízes finas encontra-se na camada de 5 a 10 cm de profundidade, nas proximidades do tronco da árvore e na linha de plantio, seguida pela diagonal e entrelinha de plantio. O entendimento do crescimento e distribuição da biomassa radicular nos estágios iniciais de crescimento de povoamentos florestais pode ajudar na decisão de práticas silviculturais a serem adotadas a fim de assegurar uma utilização mais eficiente dos recursos do solo.

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## INTRODUCTION

Tree root systems represent one of the main components of forest ecosystems and perform vital processes such as adsorption of water and soil nutrients, support of the arboreal structure and storage of nutrients and carbon (QUING-CHENG, 2002; FORRESTER et al., 2006; AL AFAS et al., 2008; TANG et al., 2013; HUANG et al., 2014). Knowledge about tree root systems (biomass, distribution and soil interactions), especially the fine roots, is fundamental for decision-making regarding soil preparation and fertilization practices (e.g. site and time of application). In addition to such practical applications, knowledge about the root system configuration is essential for understanding basic ecophysiological processes, especially those related to mineral nutrition and tree water balance (MELLO et al., 1998; LACLAU et al., 2013).

Studies of mixed planting of Eucalyptus and leguminous tree have demonstrated increased above-ground biomass of all species involved (VIERA et al., 2013). This has been demonstrated in mixed *Eucalyptus globulus* and *Acacia mearnsii* plantations (KHANNA, 1997; BAUHUS et al., 2000; FORRESTER et al., 2004; FORRESTER et al., 2005; FORRESTER et al., 2006), *Eucalyptus saligna* and *Albizia falcataria* (BINKLEY et al., 2000), *Eucalyptus saligna* and *Falcataria molucna* (BINKLEY et al., 2003), *Eucalyptus grandis* and *Acacia mangium* (LACLAU et al., 2008), as well as eucalyptus and native leguminous tree species (COELHO et al., 2007).

However, few studies have considered the underground biomass in these systems. Huang et al. (2014) suggested that inclusion of *Acacia mangiun* in *Eucalyptus urophylla* plantations could enhance soil carbon sequestration and increase soil microbial community diversity and abundance. Forrester et al. (2013) indicated that the inclusion of nitrogen-fixing trees in eucalypt plantations may increase soil carbon stocks as a result of increased productivity. The fine root biomass distribution in mixed plantations of eucalyptus and nitrogen-fixing trees are greatly affected by intraspecific and interspecific interactions (LACLAU et al., 2013).

Root growth may occur in association or independently of the growth of aerial parts. The factors that determine root growth are complex and include nutritional status, oxygen availability in soil, growth hormones, carbohydrate supply (GONÇALVES; MELLO, 2004) and physical soil structure (TANG et al., 2013). Young stands are strongly influenced by the type of soil tillage, method of fertilizer application, type and frequency of weeding and row spacing, among other factors (SCHENK, 2006; ZHOU et al., 2007). In the present study, we evaluated the soil occupied by fine root biomass density in mixed and monospecific stands of *Eucalyptus grandis* x *E. urophylla* and *Acacia mearnsii* in Southern Brazil.

## MATERIAL AND METHODS

### Site description

The study was conducted at an experimental site near Bagé (Rio Grande do Sul, Brazil) (coordinates, 31°14’43” S and 54°04’55” W) at an elevation of 242 m above sea level. According to the Köppen system, the climate is classified as subtropical wet, with an average annual rainfall of 1,364 mm, average annual temperature of 17.5 °C, mean maximum temperature of 23.5 °C and mean minimum temperature of 12.3 °C. A climate chart corresponding to the study period in Bagé city is shown in Figure 1.

The soil in the experimental area is classified as Eutric Nitosol (FAO classification), which is characterized by natural low fertility, strong acidity and high aluminum saturation. The soil chemical and physical attributes are summarized in Table 1. Soil fertility and soil physical attributes were characterized in three samples collected from each depth (see below) before establishment of the trial. Five subsamples were collected at each depth and combined to make each replicate sample. The following were also measured at each depth: pH ($H_2O$), with a pHmeter; phosphorus and organic matter (potassium dichromate), with a spectrophotometer; potassium, with a flame photometer; calcium and magnesium by atomic absorption spectroscopy; and exchangeable aluminum by titration, according to the methodology described by Tedesco et al. (1995). All analyses were performed in the Laboratory of Forest Ecology (Federal University of Santa Maria, Brazil). The soil is characterized by a sandy loam texture and intermediate density.

### Experiment design, sampling and statistic analysis

The experimental design consisted of random blocks with 3 treatments and 3 replicates. The following types of stands were considered: 100% eucalypts (100E); 100% black wattle (100A); and 50% eucalyptus + 50%

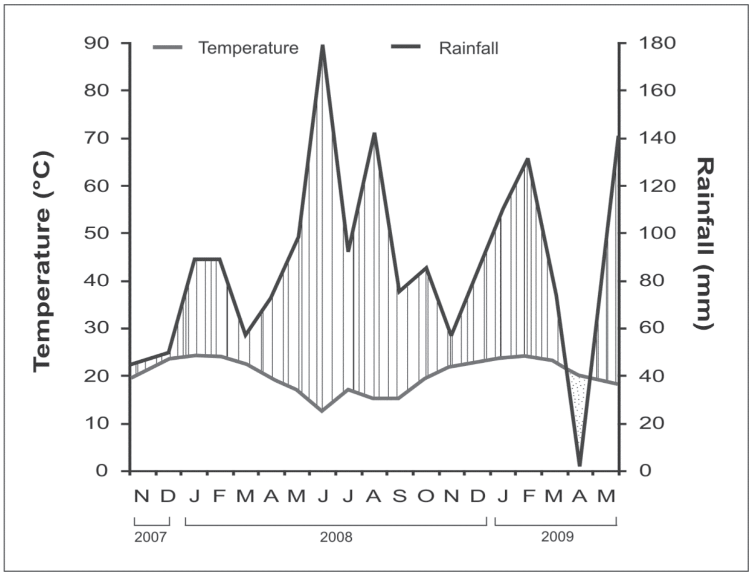


**FIGURE 1** Temperatures and rainfall verified monthly during the study period (18 months).
**FIGURA 1** Temperatura e pluviosidade mensal durante o período de estudo (18 meses).

black wattle (50E:50A). In the mixed stand, one tree of each species was evaluated. The trees were planted in November 2007 at 4.0 m x 1.5 m spacing, after 50 cm-depth single-stem subsoiling in the planting row. Chemical fertilizer (06:30:06 N - $P_2O_5$ - $K_2O$ + 7% of Ca + 6% of S + 0.1% of B + 0.5% of Cu) was added to the soil, at an application rate of 300 kg·ha$^{-1}$, in the planting row.

We sampled the fine root biomass (≤ 2.0 mm) 8 and 18 months after planting the the monospecific and mixed eucalyptus and *Acacia mearnsii* stands. Selection of trees for root sampling was based on the mean diameter at breast height (dbh) at 8 months (100E = 4.43 cm; 100A = 3.58 cm; 50E:50A = 4.41 and 3.42 cm for eucalyptus and black wattle, respectively) and at 18 months (100E = 7.61 cm; 100A = 7.52 cm; 50E:50A = 7.56 and 7.61 cm for eucalypt and black-wattle, respectively). The sampling points were systematically distributed by allocation of eight transects (2 for the planting spacing, 2 for the line and 4 for the diagonal) from the centre of the tree trunk in relation to mean soil level (Figure 2). All sampling points were identified with ordinal numbers (1 to 38). The collecting points were marked (in a clockwise direction) at radially arranged points equidistant (25 cm) from the tree trunk, to include the useful area of each tree considered (4.0 m x 1.5 m). The sampling method was adapted from that described

**TABELA 1** Propriedades químicas e físicas do solo na área experimental.
**TABLE 1** Soil chemical and physical proprieties in the experiment area.

| Depth (cm) | O.M. dag kg$^{-1}$ | pH ($H_2O$) | Al | H + Al | $CEC_{effect}$ | $CEC_{pH 7}$ | Ca | Mg | P | K |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | cmol$_c$ dm$^{-3}$ | | | | | | mg·dm$^{-3}$ | |
| 0-5 | 20 | 4.8 | 0.9 | 6.5 | 2.8 | 8.4 | 1.0 | 0.7 | 6.7 | 80.3 |
| 5-10 | 18 | 4.8 | 1.0 | 7.8 | 2.9 | 9.6 | 1.0 | 0.7 | 3.6 | 54.6 |
| 10-20 | 14 | 4.7 | 1.3 | 7.8 | 2.7 | 9.3 | 0.8 | 0.6 | 2.0 | 36.6 |
| 20-30 | 11 | 4.7 | 1.5 | 8.5 | 3.0 | 10.0 | 0.8 | 0.6 | 1.2 | 31.0 |

| Depth (cm) | m | v | Sand (%) | | Silt (%) | Clay (%) | Density |
|---|---|---|---|---|---|---|---|
| | (%) | | (2-0.2mm)$^a$ | (0.2-0.05mm)$^b$ | (0.05-0.002mm) | (<0.002mm) | g·cm$^{-3}$ |
| 0-5 | 32.5 | 22.4 | 25 | 53 | 5 | 18 | 1.38 |
| 5-10 | 37.6 | 19.4 | 25 | 52 | 6 | 18 | 1.50 |
| 10-20 | 47.6 | 16.4 | 26 | 51 | 4 | 20 | 1.49 |
| 20-30 | 52.8 | 15.0 | 24 | 52 | 4 | 21 | 1.49 |

P and K: Mehlich-1 Extractor; Al, Ca and Mg: KCL 1 mol l$^{-1}$ Extractor; H + Al: $Ca(OAc)_2$ 0.5 mol l$^{-1}$ pH 7.0 Extractor; O.M. = Organic matter; CEC = Cation Exchange capacity; m = Aluminium saturation percentage; v = Base saturation percentage; [a] Coarse sand; [b] Fine sand.

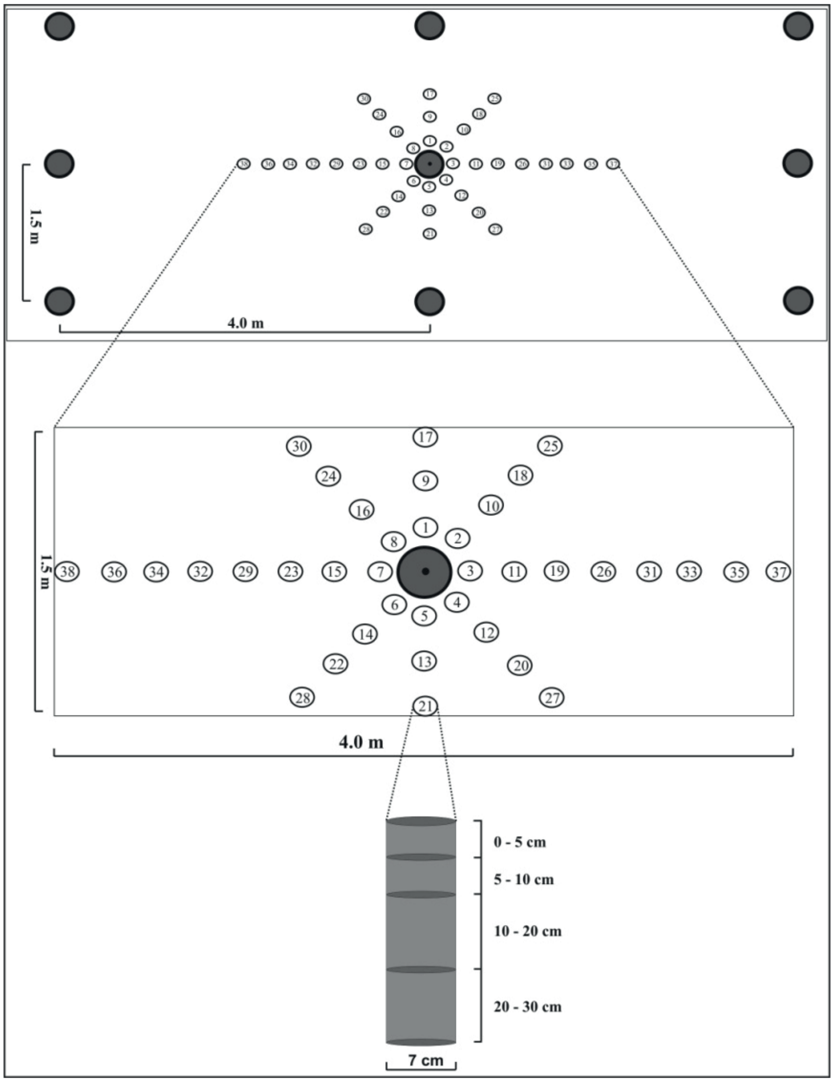


**FIGURE 2** Distribution of fine roots sample points.
**FIGURA 2** Distribuição dos pontos de amostragem de raízes finas.

by Böhm (1979). Four soil depths were considered at each sampling point (0 - 5; 5.1 - 10; 10.1 - 20 and 20.1 - 30 cm). The samples were collected with a cylindrical steel auger of inside diameter 7.0 cm.

The fine roots were separated from the soil by using water jets to wash the material through a set of sieves (2.0 and 0.34 mm meshes). All fine roots (live and dead) were placed in plastic containers with a solution of distilled water + 12% of alcohol and stored. Before analysis, the fine roots were placed on absorbent paper to remove excess moisture. Live roots were then separated from the dead roots, live roots of other plants and organic fragments, with the aid of tweezers. The live and dead roots were separated manually, on the basis of morphological characteristics, colour and flexibility. The species to which the roots belonged were easily identified, as eucalypts roots are light brown in colour and the black wattle roots are light yellow.

The roots were dried in an oven at 70 °C for 72 hours, and the dry mass was determined using an analytical balance (accuracy $10^{-4}$ g). On the basis of the fine root biomass values, cartograms can be elaborated to demonstrate the distribution of the fine roots in the soil. The cartograms illustrate the fine root biomass density distribution in the planting row spacing (points: 3, 7, 11, 15, 19, 23, 26, 29, 31, 32, 33, 34, 35, 36, 37 and 38), lines (points: 1, 5, 9, 13, 17 and 21), diagonals (points: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 25, 27, 28 and 30) and at each depth (0-5 cm, 5-10 cm, 10-20 cm and 20-30cm) (Figure 2) based on the average measurements in three replicate samples from each type of stand. Bartlett's test of homogeneity of variance and Lilliefors test of error normality were applied to the data. Data that did not comply with the required assumptions were subjected to square root and/or the inverse square root transformation. ANOVA was applied according to the following general model (I). where Y is the root density, $\mu$ is the mean value, Ti is the treatment effect, Pj is the soil depth effect, Dk(Ti) is the direction effect, dl(Dk) is the distance effect and Ti·Pj is the interaction between the treatment and soil depth. Differences were considered significant at P< 0.05. Tukey's test was used to compare the means.

$$Y_{ijkl} = \mu + T_i + P_j + D_k(T_i) + dl(D_k) + T_i \cdot P_j + \varepsilon_{ijkl} \qquad [1]$$

## RESULTS AND DISCUSSION

In the 8-month-old stands, there was no difference ($p > 0.05$) in fine root biomass density ($g \cdot dm^{-3}$), at any depth or distance from the tree trunks, between the monospecific and the mixed eucalyptus and black wattle stands (Figure 3). The initial biomass development (up to 8 months after planting) of eucalyptus and black wattle is the same as regards occupation of the different soil layers. Eighteen months after planting, significant differences between types of stand occurred up to a maximum distance of 100 cm.

In the monospecific black wattle stand, the fine root density was higher in the superficial layer (0 to 20 cm) than in the other layers, and was higher ($p < 0.05$) than in the monospecific eucalyptus and mixed stands up 100 cm from the tree trunk in the upper 10 cm of soil, and up to 75 cm in the 10 to 20 cm soil layer. Some differences ($p < 0.05$) between the monospecific black wattle and mixed stands were observed at distances of 75 and 100 cm from the tree trunk in the upper 10 cm and at a distance of 75 cm from the tree trunk in the 10 - 20 cm soil layer. There were no significant differences ($P > 0.05$) in the fine root density between the monospecific and mixed eucalyptus stands at any depth for the different distances from the tree trunk.

After 8 months, the stand fine root biomass, in both the monospecific and the mixed stands, showed the same tendency for soil occupation, with a higher density in the 5 to 10 cm soil layers and close to the tree trunk (Figure 4). After 18 months, the fine root biomass density was higher in the monospecific and mixed black wattle stands, but with a tendency for large root biomass in the 5 to 10 cm depth layer and close to the trunk, for the planting row spacing, the planting line and for the diagonal between two planting lines.

The high density of fine roots in the deeper layers of the soil is due to the low soil fertility, the low organic matter content and soil moisture, as rainfall was low during most of the tree development period. These factors, in addition to oxygen availability and some other factors, have been reported to determine root development soil occupation at some depths (GONÇALVES; MELLO, 2004). Root exploitation has also been found to be most efficient in the layers in which water and nutrient availability are highest (SILVA et al., 2009; LACLAU et al., 2013).

Some studies have demonstrated that the fine root sample position in relation to the distance from the trunk does not interfere in evaluation of the distribution or quantification (GONÇALVES; MELLO, 2004). Nevertheless, a higher concentration of fine roots near the tree trunk may occur because of stem flow of the rain water down the trunk, resulting in a greater availability of water and nutrients (REYNOLDS, 1970). Moreover, there is also less intra and interspecific competition at this position, especially in water and nutrient-limited sites. This may be considered as one of the main factors controlling the fine roots in the stands under study, because rainfall was low, and included periods of drought, throughout the study. Although such effects may disappear throughout stand development (GONÇALVES; MELLO, 2004) there may be a tendency for more roots to grow near the trunk (LACLAU et al., 2004).

Variations in the morphological, physical and chemical characteristics of the soil profile may determine variations in the fine root distributions (GONÇALVES; MELLO, 2004). Côté et al. (2003) observed that root production increased as the concentrations of $NO_3$, $NH_4^+$ and $NO_3 + NH_4^+$ in the soil solution increased. Mello et al. (1998) reported that the root system growth and occupation of soil are intrinsic genotypic characteristics, which are closely related to the nutritional response, productive potential and adaptability to environmental stress conditions. Furthermore, the species may display mycorrhizal root associations that modify root system growth because of the increased availability of nutrients for the plants.

Eighteen months after stand establishment, the fine root biomass in all soil layers and occupation of the useful area around each tree were both higher than in the 8- month-old stands. In less productive sites than in the present study, root density has been found to increase due to a lower availability of water and nutrients in soil,

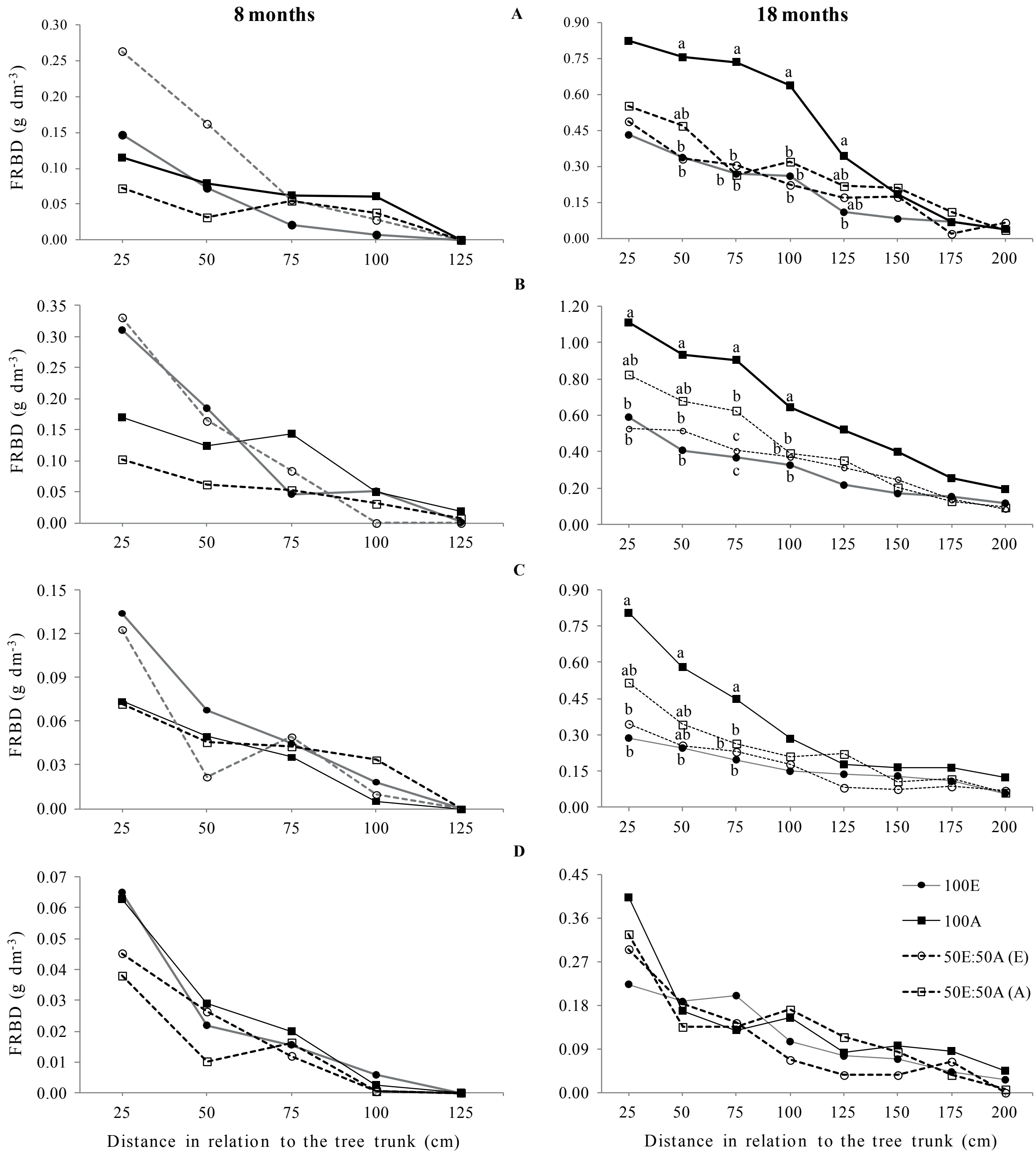


**FIGURE 3** Fine roots biomass density (FRBD - g.dm$^{-3}$) at 8 and 18 months in monospecific (100E and 100A) and mixed (50E:50A(E) and 50E:50A(A)) planting of eucalyptus and black-wattle, in the different depths (A. 0-5; B. 5-10; C. 10-20 e D. 20-30 cm), considering the distance in relation to the trees trunk. (The existence of letters indicate that there are significant differences between the treatments by the Tukey Test, at a significance level of 0.05).

**FIGURA 3** Densidade da biomassa de raízes finas (DBRF - g dm$^{-3}$) aos 8 e 18 meses de idade em plantios monoespecíficos (100E e 100A) e mistos (50E:50A(E) e 50E:50A(A)) de eucalipto e acácia-negra em diferentes profundidades (A. 0-5; B. 5-10; C. 10-20 e D. 20-30 cm) e distâncias do tronco da árvore. (A existência de letras indica que há diferenças significativas entre os tratamentos pelo teste de Tukey ao nível de 0,05 de significância).

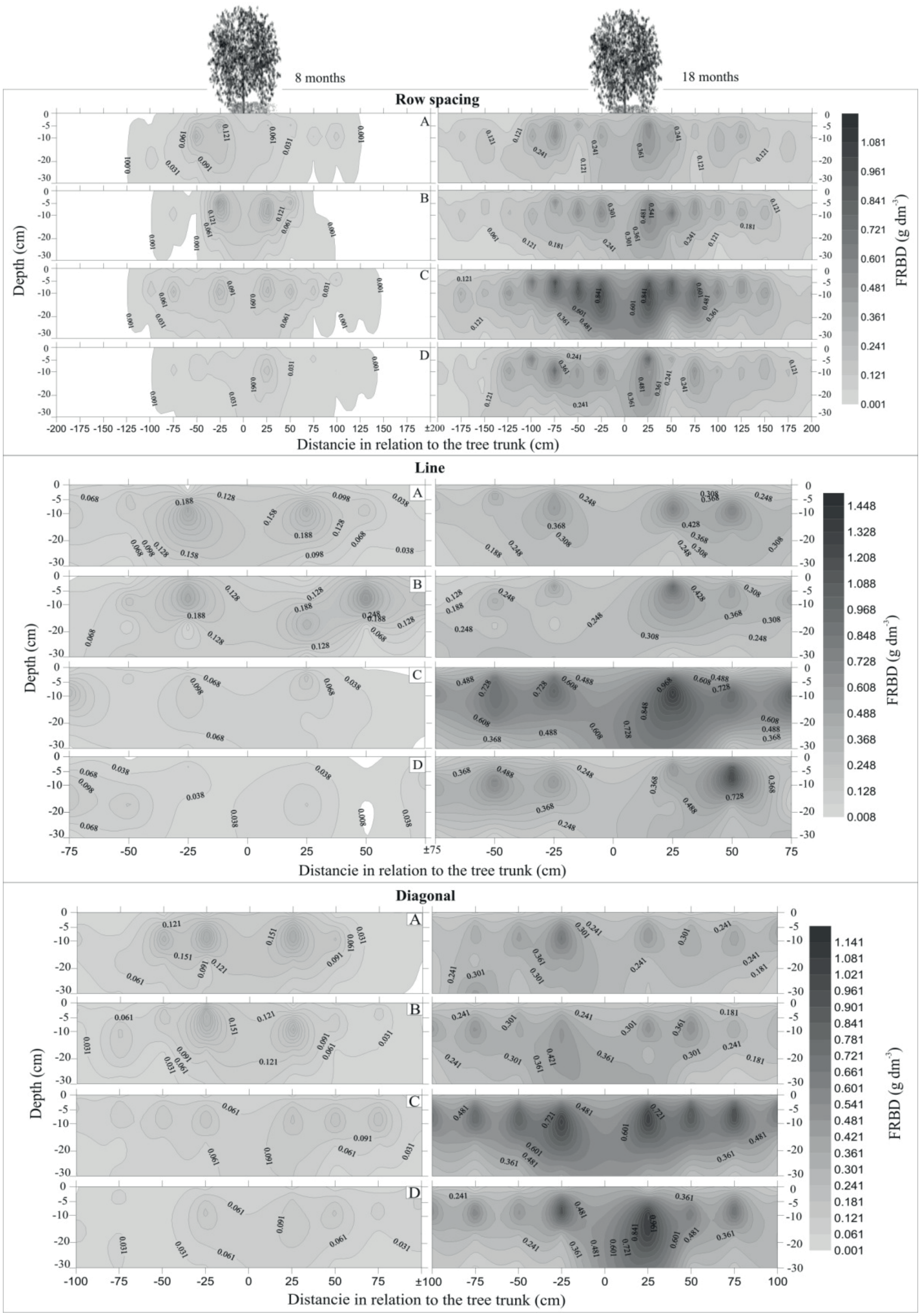


**FIGURE 4** Spatial distribution of fine roots biomass density (FRBD - g·dm$^{-3}$) in monospecific and mixed stands of eucalyptus and black-wattle (A = 100E; B = 50E:50A (E); C = 100A and D = 50E:50A (A)), at 8 and 18 months of age.

**FIGURA 4** Distribuição especial da densidade de biomassa de raízes finas (DBRF - g dm$^{-3}$) em plantios monoespecíficos e misto de eucalipto e acácia-negra (A = 100E; B = 50E:50A (E); C = 100A and D = 50E:50A (A)) aos 8 e 18 meses de idade.

which forces plants to extend their roots to increase their capacity to absorb water and nutrients (GONÇALVES; MELLO, 2004). The opposite is expected in more productive sites, with more fertile soils, in which the root system would not need to extend as far to absorb the necessary volume of water and nutrients that are vital for metabolic activity.

On evaluating the fine root system of *Eucalyptus globulus*, Fabião et al. (1995) found that interference in root growth would be more accentuated in a situation of water stress than for nutrient shortage. Nambiar (1983) reported that other factors may be important in the distribution of forest species fine roots (e.g. soil and air temperature) and that for *Pinus radiata* and a soil temperature higher than 15 °C, fine root development was higher. Tang et al. (2013) did not observe interactions between the roots of *Eucalyptus camaldulensis* and *Leocena leucocephala* 10 years after plantation establishment because rocky soil inhibited root growth.

We did not find any (positive or negative) interactions between the biomass of root systems of eucalyptus and black wattle during the first 18 months after planting. The same was found for fine root length, and the trees in mixed stands did not show significant differences relative to the monospecific stands (VIERA et al., 2012). Knowledge about fine root growth and distribution in soil during the initial stages of stand development may help in decision-making regarding intensive forestry practices to ensure more efficient use of soil resources.

## CONCLUSIONS

In 8-month-old trees, the distribution of fine root biomass density was the same in both species under study, reaching the maximum at a distance of 125 cm from the tree trunk. Eighteen months after planting, the root biomass density in the monospecific black wattle stand was higher than in the monospecific eucalyptus stand and the mixed stand. No negative interactions between the roots of the two species were observed, indicating than mixed stands may make more efficient use of the soil resources.

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