RADICULAR BIOMASS AND ORGANIC CARBON OF THE SOIL IN FOREST FORMATIONS IN THE SOUTHERN AMAZONIAN MESOREGION

Ozias Cunha Bello[2], José Maurício da Cunha[3], Milton Cesár Costa Campos[4], Elilson Gomes de Brito Filho[5*], Marcos Gervasio Pereira[6], Guilherme Abadia da Silva[7], Wener Silva Simões[8] and Luís Antônio Coutrim dos Santos[9]

[1] Received on 31.10.2020 accepted for publication on 18.08.2021.
[2] Universidade Federal do Amazonas, Mestre em Ciências Ambientais, Humaitá, AM - Brasil. E-mail: <belloufam@hotmail.com>.
[3] Universidade Federal do Amazonas, Departamento de Física, Humaitá, AM - Brasil. E-mail: <maujmc@gmail.com>.
[4] Universidade Federal da Paraíba, Departamento de Solos e Engenharia Rural, Areia, PB - Brasil. E-mail: <mcesarsolos@gmail.com>.
[5] Universidade Federal da Paraíba, Graduando em Agronomia, Areia, PB - Brasil. E-mail: <bfsambiente@gmail.com>.
[6] Universidade Federal Rural do Rio de Janeiro , Departamento de Solos, Seropédica, RJ - Brasil. E-mail: <mgervasiopereira01@gmail.com>.
[7] Universidade Federal do Amazonas, Graduando em Biologia e Química, Humaitá, AM - Brasil. E-mail: <gui.abadia.s@gmail.com>.
[8] Universidade Federal do Amazonas, Graduando em Agronomia, Humaitá, AM - Brasil. E-mail: <wsimoes634@gmail.com>.
[9] Universidade do Estado do Amazonas, Departamento de Ciências Florestais, Itacoatiara, AM - Brasil. E-mail: <santoslac@gmail.com>.
*Corresponding author.

ABSTRACT – The soils of the Amazon region, despite being under one of the densest forests in the world, are mostly characterized by the low availability of nutrients, with litter being the main route of nutrient entry. The objective of this study was to quantify the biomass of fine roots in the dry and rainy seasons of the year, including the organic carbon of the soil, and to compare the results in different study environments. The study was carried out in environments of native forest and reforestation aged over 20 years, located in the municipality of Humaitá – AM state. To assess the root biomass, collections were carried out in two periods of the year: dry and rainy seasons. In each of the study areas, five trenches, 0.40 m deep by 0.40 m wide, were dug manually at depths of 0-5, 5-15, and 15-30 cm. For the organic carbon analysis, soil samples were collected in the form of clods at the same depths. The production of root biomass in the native forest environment occurred more intensely in the rainy season, reaching values of 8.19 t. ha^{-1}, greater than 3.57 t. ha^{-1} found in reforestation. The density as a function of the soil volume showed that the highest concentration is found in the first 5 centimeters of depth, differing significantly in the 5-15 and 15-30 cm layers for native forest area. The organic carbon of the soil showed significance between the dry and rainy seasons for the natural forest environments and reforestation with genipap.

Keywords: Amazon forest, Organic matter, Roots.

BIOMASSA RADICULAR E CARBONO ORGÂNICO DO SOLO EM FORMAÇÕES FLORESTAIS NA MESORREGIÃO SUL DA AMAZÔNIA

RESUMO – Os solos da região amazônica, apesar de estarem sob uma das florestas mais densas do mundo, são caracterizados, na sua maioria, pela baixa disponibilidade de nutrientes, sendo a serapilheira a principal via de entrada de nutrientes. O objetivo desse estudo foi quantificar a biomassa de raízes finas na época seca e chuvosa do ano, incluindo o carbono orgânico do solo, e comparar os resultados nos diferentes ambientes de estudo. O estudo foi desenvolvido em ambientes de floresta nativa e reflorestamento com idade acima de 20 anos, localizado no município de Humaitá- AM. Para avaliação da biomassa de raízes, foram realizadas coletas em dois períodos do ano: seco e chuvoso. Em cada uma das áreas de estudo foram escavadas manualmente cinco trincheiras, com dimensão de 0,40 m de profundidade por 0,40 m de largura, nas profundidades de 0-5, 5-15 e 15-30 cm. Para análise de carbono orgânico foram coletadas amostras de solo em forma de torrão nas mesmas profundidades. A produção de biomassa radicular no ambiente de floresta nativa ocorreu de forma mais intensa no período chuvoso, chegando a valores de 8,19 t. ha^{-1}, superior a 3,57 t. ha^{-1} encontrado em reflorestamento.

http://dx.doi.org/10.1590/1806-908820210000037

A densidade em função do volume de solo mostrou que a maior concentração se encontra nos primeiros 5 centímetros de profundidade, diferenciando significativamente nas camadas de 5-15 e 15-30 cm para área de floresta nativa. O carbono orgânico do solo apresentou significância entre o período seco e chuvoso para os ambientes de floresta natural e reflorestamento com jenipapo.

Palavras-Chave: Floresta Amazônica, Matéria orgânica, Raízes.

1.INTRODUCTION

The tropical forests play an important role in the storage and absorption of carbon from the atmosphere, as well as in climate change on a global scale. The survival of this ecosystem and its productivity is mainly attributed to its high plant diversity, composed of native species adapted to the climatic and nutritional conditions of the soil, which, in turn, developed efficient mechanisms in nutrient cycling over time (Jordan, 1985; Mendes, 2018).

The impacts resulting from forest clearing imply higher levels of erosion and soil compaction, promoting the exhaustion of nutrients from forest ecosystems (Fearnside, 2006). Roots are important in sustaining trees, and fine roots are one of the main means of capturing soil resources, and their length and number are indicators of nutrient absorption capacity (Freitas et al., 2008).

Carbon partitioning and its dynamics in plant components can also serve to increase the efficiency of the cycling process and improve carbon sequestration models (Block et al., 2006). Therefore, knowing the biomass production, as well as the density of the root system and the stock of organic carbon in the soil of forest ecosystems is fundamental for decision-making in order to mitigate possible impacts arising from human activities.

The production of fine roots can be affected by several factors, such as, by the type of vegetation formation and by species diversity (Finér et al., 2011; Brassard et al., 2013), by rainfall seasonality (Lima et al., 2012), and soil characteristics (Wright et al., 2011).

Root biomass plays an effective role in the processes that occur in the soil once its rapid renewal significantly contributes to the addition of organic compounds to the soil, having an important regulatory role in the carbon and nitrogen cycle in forest ecosystems (Menezes et al., 2010). According to Gill and Jackson (2000), the behavior of fine roots in relation to temperature and regional precipitation can help identify species that are more sensitive to climate change.

Given the importance of the root system for the maintenance and for the perpetuation of forest species and, above all, given the scarcity of studies focused on the Amazon region, the objective of this study was to quantify and compare, in two periods of the year, the production of root biomass and the soil organic carbon in reforestation areas and native forest in the southern Amazonian mesoregion.

2.MATERIAL AND METHODS

The study was carried out in areas of native forest and reforestation, located in the municipality of Humaitá-AM state, southern Amazonian mesoregion. The municipality of Humaitá is located about 200 km north of the capital of Rondônia state, Porto Velho, and 675 km south of Manaus, capital of Amazonas state. The study areas were divided into four distinct parcels, one of native forest, one of reforestation with Teak (*Tectona grandis* L.), one of reforestation with Jenipapo (*Genipa americana* L.), and another of reforestation considered mixed, composed by the species Mahogany (*Swietenia macrophylla* King.), Andiroba (*Carapa guianensis* Aubl.), Jenipapo (*Genipa americana* L.), Teak (*Tectona grandis* L.) and Sumauma (*Ceiba* pentandra).

Based on the coordinates, the area of native forest (NF) is located at coordinates 07°34’27’’S and 63°06’53’’W, altitude 57 m; the area of reforestation with Teak (TRE), located at coordinates 07°34’33’’S and 63°06’51’’W, altitude of 58 m; the reforestation area with Jenipap (JRE) is located at coordinates 07°34’41’’S and 63°06’49’’W, altitude 57 m; finally, the reforestation area considered mixed (MRE) is located at the geographic coordinates 07°34’54’’S and 63°06’55’’W, altitude of 58m.

The climate in the region is of the Am type, tropical rainy (monsoon-type rain), according to the Köppen classification, with average air temperature ranging between 25 and 27°C, relative humidity between 85% and 90% and average annual precipitation of 2,500 mm (Alvares et al., 2014). The region is characterized by two defined seasons, the rainy season, which occurs from October to April, concentrating the greatest volume of rain in the year, and the dry season, which occurs from May to September, with months presenting rainfall below 60 mm (Almeida et al., 2015).

Regarding the description of the study areas, the native forest environment is characterized by containing an enormous diversity of flora and fauna species, typical of Amazonian environments, maintained by the Brazilian Army (EB), with no presence of anthropic activities. The reforestation environment was implemented in early 2000, replacing a pasture area. In this area, an average of 1.5 tons of limestone per hectare were incorporated, before planting forest species. After approximately three years of reforestation implementation, cattle were introduced, again and sporadically, to control grass regrowth.

For the evaluation of fine root biomass (≤ 2 mm) samples were taken in two periods of the year: dry (June 2018) and rainy (January 2019). The sampling was an adaptation of the monolith method described in Bohm (1979). For each of the study areas, five trenches were manually excavated, with dimensions of 0.40 m in depth and 0.40 m in width.

The trenches were opened by adopting a distance of approximately 0.80 m from the base of the plant, collecting samples with the aid of a steel cylinder 10 cm high and 7 cm in diameter, at depths of 0-5 cm, 5-15cm and 15-30cm, with three repetitions per depth, per area. The samples were stored in plastic bags, identified, and taken to the soil laboratory of the Institute of Education, Agriculture and Environment of Humaitá (IEAA).

In separating the roots from the soil, the samples were subjected to manual washing in running water until total removal of the soil, using a set of overlapping sieves with meshes of 2mm, 1mm and 0.500mm, respectively. After washing the effective roots (≤ 2 mm), they were taken to a forced air circulation oven for 72 hours. After drying, with the aid of tweezers, they were weighed on an analytical balance of 0.0001 g precision.

This expression. (1) was used to calculate dry root density (DRS):

$$DRS=\frac{MS}{VM} \qquad \text{Eq.1}$$

Where:

DRS = dry root density, in g dm^{-3};

MS = root dry mass, in grams;

VM = volume of collected monolith, in dm^{-3}.

To estimate the Fine Root Biomass ≤ 2 mm (BRF) of each study environment, in a given spatial area and over a given period of time, the production of fine roots was calculated, disregarding the soil density by expression (2), according to Vogt et al. (1998) and used by Mendes (2018).

$$BRF=\frac{MS}{AC} \qquad \text{Eq.2}$$

Where:

DRS = dry root density, in g dm^{-3};

MS = root dry mass, in grams;

VM = volume of collected monolith, in dm^{-3}.

Soil sample collections for further analysis of organic carbon were carried out at the same time and periods of the year as the root biomass. In each study area, in the four plots, soil samples with preserved structure in clumps were collected at three different depths, 0-5, 5-15, 15-30 cm. After the soil was shade-dried and sieved in a 2 mm sieve, Air Dry Earth (TFSA), chemical were performed.

To determine the pH in water, the soil: the water ratio of 1:2.5 was used, shaking for 1 minute and leaving it to rest for 60 minutes, then reading the pH in a pH meter.

The soil organic carbon (COS) was determined by the Walkley-Black method, modified by Yeomans and Bremner (1988), according to the expression (3), Teixeira et al. (2017):

$$Corg = \frac{0{,}003 \times VD \times (40-VA) \times 40 \div VB \times 10}{M} \qquad \text{Eq.3}$$

Where: Corg = Soil organic carbon concentration, in g kg^{-1}; DV = Total volume of potassium dichromate solution added in the sample digestion, in ml; VA = Volume of the ammoniacal ferrous sulfate solution used in the sample titration, in ml; VB = Volume of the ammoniacal ferrous sulphate solution used in the titration of the heated blank, in ml; Value 0.003 = Milliequivalents of carbon mass (atomic weight/valence – 12/4, divided by 1000); Value 10 = Transformation from % to g kg^{-1}; M = Mass of the soil sample, in g.

The data were tabulated and submitted to the normality test of error distributions by the Shapiro-Wilk test and Levene's homogeneity test. Given the requirements for analysis of variance (ANOVA), for significance ($p<0.05$), the Tukey 5% test was used as a *posteriori* test in comparisons of means between the respective environments. To compare the means between the collection periods, the T test of paired samples was applied, using the computer application SPSS statistics 23.3.

3.RESULTS

3.1. Climate conditions

In the rainy period (October to April), with April considered the transition month for the dry period, the volume of rainfall occurred in a regular and well distributed way, with an average of approximately 400 mm for the month of January, sustaining one of the outstanding characteristics, not only in the region, but in the Amazon forest as a whole, the regularity of rainfall and high temperatures for most of the year (Figure 1).

Source: Instituto Nacional de Meteorologia – INMET
Fonte. Instituto Nacional de Meteorologia – INMET

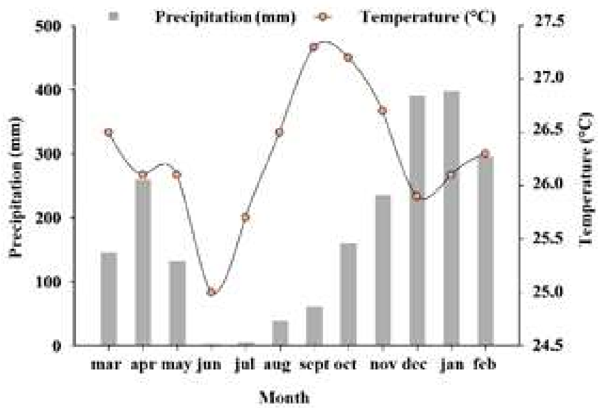

Figure 1 – Average temperatures and accumulated precipitation in the study period.
Figura 1 – *Temperaturas médias e precipitação acumulada no período de estudo.*

In the dry period (May to September), the greatest water deficit occurred in June, with an average of 3.6 mm, coinciding with the lowest monthly average temperature of 25°C, a fact that is correlated with cold fronts from other regions, producing cold, a very common phenomenon in the region. Another result observed was the precipitation in April with an average of 260 mm, considered atypical for the transition month.

3.2. Biomass dynamics

The results obtained corresponding to the dry period (June), at a depth of 0-5 cm, were not significant, it is possible to observe in Table 1 that the values of 3.13 t. ha^{-1} for NF; 2.78 t. ha^{-1} for JRE; 1.45 t. ha^{-1} for MRE and 0.94 t. ha^{-1} for TRE, they resemble a 5% significance level.

At the depth of 5-15 cm, still for the month of June, the NF environment showed significance for the other environments, with an average value of 1.44 t. ha^{-1} and coefficient of variation around 35.45%, supporting the accuracy of the data. At a depth of 15-30 cm, the production of fine root biomass was similar to the previous depth, with emphasis again on the NF environment, with significance for the other environments, with an average value of 1.24 t. ha^{-1}.

The results corresponding to the collection in January showed a behavior similar to the month of June, with higher biomass production in the 0-5 cm depth, with an emphasis on the NF environment, differing significantly from the other environments in the 5-layer layer. 15 with an average of 2.05 t. ha^{-1} and 2.34 t. ha^{-1} in the 15-30 cm layer, in the same month.

The production of fine root biomass occurred more intensely in the rainy season (Figure 2), with the exception of RJ which occurred in the dry season, with the NF environment being the one that most contributed to the production of biomass with 8.19 t. ha^{-1} in the rainy season. The biomass concentration at different depths was somewhat less discrepant in the native forest environment. However, in the reforestation areas the biomass production occurred more strongly in the 0-5 cm layer compared to the forest area native, reaching the highest percentage of 72.18% for JRE in the rainy season. In a work carried out by Witschoreck et al. (2003), under similar conditions found that 72% of root biomass is

Table 1 – Root biomass in different environments and depth of study, Humaitá-AM.
Tabela 1 *– Biomassa de raízes nos diferentes ambientes e profundidade de estudo, Humaitá-AM.*

Áreas de estudo	Biomass (t ha^{-1})	CV (%)	Biomass (t ha^{-1})	CV (%)
	(June)		(January)	
		0-5 cm		
TRE	0,94 aA	30,65	1,74 aA	28,38
JRE	2,78 aA	96,99	1,91 aA	56,57
MRE	1,45 aA	50,99	2,58 aA	82,98
NF	3,13 aA	70,31	3,79 aA	38,78
		5-15 cm		
TRE	0,53 bB	42,08	0,52 bB	59,35
JRE	0,64 bB	6,90	0,64 bB	25,02
MRE	0,58 bB	75,86	0,54 bB	51,15
NF	1,44 aA	35,45	2,05 aA	24,78
		15-30 cm		
TRE	0,18 bB	56,30	0,26 bB	65,99
JRE	0,43 bB	18,22	0,31 bB	67,98
MRE	0,53 bB	72,61	0,45 bB	64,03
NF	1,24 aA	42,62	2,34 aA	53,29

Means of five repetitions Values followed by the same lowercase letter in the column do not differ by Tukey test at 5% and uppercase in the row, do not differ by T test of paired samples; TRE = reforestation with teak; JRE = reforestation with genipap; MRE = silvopastoral with mixed species; NF = native forest.
Médias de cinco repetições. Valores seguidos da mesma letra minúscula na coluna não diferem pelo teste de Tukey a 5 % e maiúscula na linha, não diferem pelo teste de T de amostras pareadas; RET = reflorestamento com teca; REJ = reflorestamento com jenipapo; REM = silvipastoril com espécies mistas; FN = floresta nativa.

concentrated in the first 30 cm of soil, inferring that roots with diameters up to 2.0 mm thick are the main responsible for the processes of absorption of water and nutrients.

An explanation for the lower percentages in the deeper layers in reforestation environments may be related to the history of these environments, attributing part of these results to disturbances suffered by the trampling of animals that constantly grazed in these areas, hindering the distribution of roots in the deeper layers. Another explanation may be correlated with age (≤ 20 years) and characteristics of planted species (homogeneous population).

Regarding the density of fine roots, it was found that the lowest values were found in reforestation environments, both for dry and rainy periods, with the exception of RJ as (Table 2), showing significant a ($p<0, 05$) at a depth of 5-15 and 15-30 cm for the NF environment in the dry period, with an average of 3.11 g-dm^{-3} and 2.68 g-dm^{-3}, respectively.

The density of fine roots (Figure 3) presents a behavior very similar to that of biomass, changing according to the depth of the soil. This behavior can be explained by the high concentration of litter on the surface, favoring greater water retention and availability of oxygen and nutrients resulting from the decomposition.

Another likely explanation would be due to soil compaction, as the first layers exert pressure on the lower layers, making the root distribution difficult. Thus, it was proved in the field that the dynamics in the nutritional and productive behavior of native species occurred more intensely at an equilibrium level, very likely due to the non-occurrence of anthropic actions.

3.3.Carbon in the soil

The results of the analyzes showed that the concentrations of organic carbon (Corg) in the dry period did not differ between the layers of 0-5 and 5-15 cm, in the different environments, (Figure 4). At a depth of 15-30 cm for the same period, the JRE environment showed a significant difference to the other environments with a value of 3.22 g kg^{-1}, this being the lowest concentration in the dry period.

This fact supports the observations of this study, where the JRE, TRE and MRE areas suffered disturbances due to another economic activity (livestock), which may have influenced the results at depths of 15-30 cm, proving to be significant ($p<0, 05$).

The results of the analyzes in the rainy season (January) showed significant differences in the comparison between the areas of native forest and reforestation for a depth of 15-30 cm, with values of 12.41 g kg^{-1} for NF, 8.97 g kg^{-1} for JRE and 8.88 g kg^{-1}

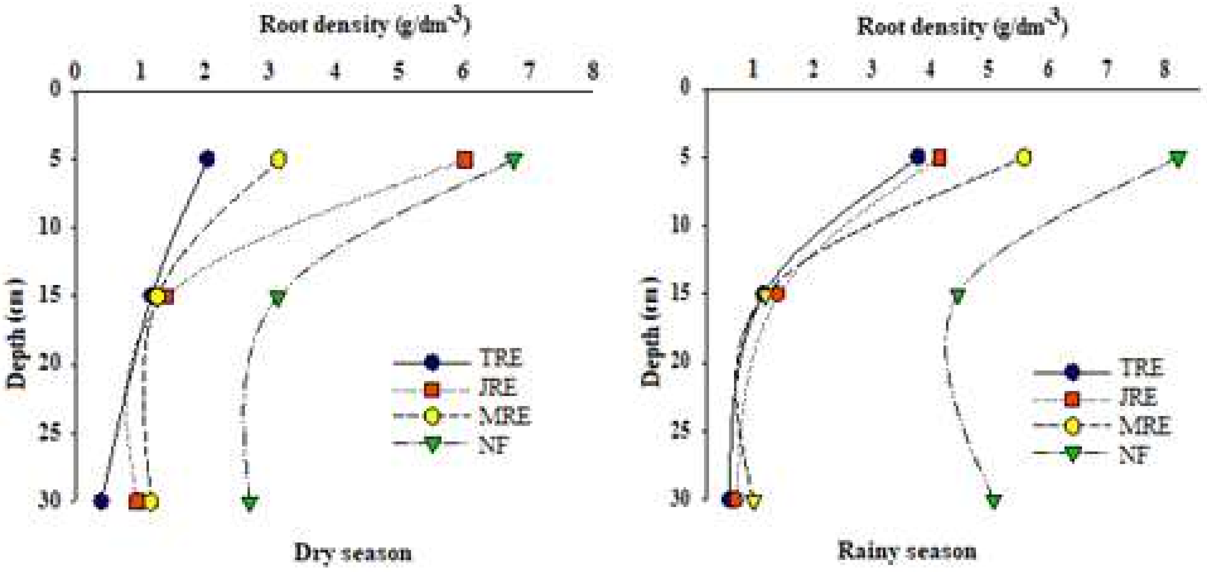

Figure 2 – Root biomass in the dry and rainy season of the year.
Figura 2 *– Biomassa de raízes no período seco e chuvoso do ano.*

for TRE, as shown in Figure 3. This may be correlated with the characteristic of less dense vegetation cover in natural forests, whereas in homogeneous plantations the soil is more exposed to climatic influences such as high temperature and high rainfall at this time of year in the region.

In the comparison between the dry and rainy periods, it was found that there was a significant difference only for the depth of 5-15 cm for the JRE environment and for the depth of 15-30 cm for NF, with higher concentrations in the rainy period. This fact may be associated with greater microbial activity,

Table 2 – Density of roots in differents in different study environments, Humaitá –AM.
Tabela 2 *– Densidade de raízes nos diferentes ambientes de estudo, Humaitá –AM.*

Áreas de estudo	Density (g/dm^{-3})	CV (%)	Density (g/dm^{-3})	CV (%)
	Junho(Dry)		Janeiro(Rainy)	
		0-5 cm		
TRE	2,03 aA	30,54	3,78 aA	28,30
JRE	6,01 aA	97,00	4,13 aA	56,65
MRE	3,13 aA	50,79	5,58 aA	82,97
NF	6,77 aA	70,31	8,19 aA	38,82
		5-15 cm		
TRE	1,16 bB	42,24	1,14 bB	59,64
JRE	1,39 bB	6,47	1,39 bB	24,46
MRE	1,25 bB	76,00	1,17 bB	51,28
NF	3,11 aA	35,36	4,45 aA	24,71
		15-30 cm		
TRE	0,39 bB	56,41	0,57 bB	64,91
JRE	0,94 bB	18,08	0,68 bB	67,64
MRE	1,15 bB	72,17	0,97 bB	63,91
NF	2,68 aB	42,53	5,06 aA	53,16

Five-repeat means Values followed by the same lowercase letter in the column do not differ by the Tukey test at 5% and uppercase in the row, do not differ by the T test of paired samples; TRE = reforestation with teak; JRE = reforestation with genipap; MRE = silvopastoral with mixed species; NF = native forest.
Médias de cinco repetições. Valores seguidos da mesma letra minúscula na coluna não diferem pelo teste de Tukey a 5 % e maiúscula na linha, não diferem pelo teste de T de amostras pareadas; RET = reflorestamento com teca; REJ = reflorestamento com jenipapo; REM = silvipastoril com espécies mistas; FN = floresta nativa.

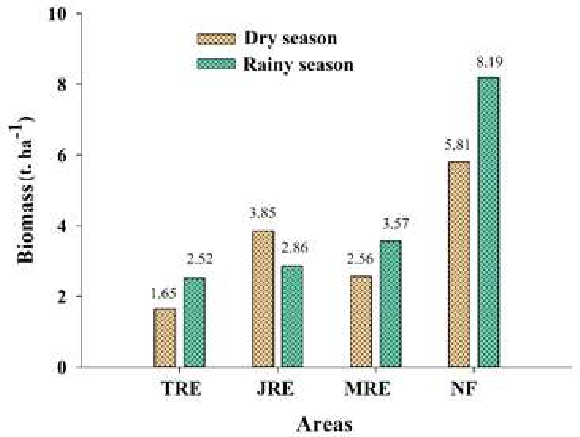

Figure 3 – Density of fine roots in different areas and depths.
***Figura 3** – Densidade de raízes finas nas diferentes áreas e profundidades.*

accelerating the decomposition process of organic compounds, making them assimilable to the soil-plant system.

4.DISCUSSION

In the comparison between the dry and rainy season, there were no significant differences in the biomass for the different collection depths and different study environments, showing a slight increase in the biomass production in the rainy season, except for JRE, as shown in Figure 2. Precipitation is one of the climatic variables that most influence the production of biomass in tropical forests (Green et al., 2005). Similar results were also observed by Metcalfe et al. (2008), in the Amazon rainforest.

A possible explanation for the higher values that occurred in the dry period in JRE, including all depths, is that the water deficit associated with lower concentrations of organic carbon in the soil, has stimulated the root production. Fine roots induce the production of more biomass when there are fewer resources available below the soil surface (Markesteijn and Poorter, 2009).

In the present study, it was observed that the more the depth of the soil increases, the number of fine roots decreases, as shown in Figure 3. According to Finér et al. (2011), the root biomass decreases exponentially from upper to lower soil layers in different forest biomes, alternating its behavior only in the total rooting depth. This behavior was also observed in a study of fine roots in young *Eucalyptus dunnii* Maiden trees by Dick and Schumacher (2019), corroborating the results presented in this study.

The density of fine roots (Figure 3) can be a factor related to the characteristic of the plant genotype, as well as to the nutritional behavior, productive

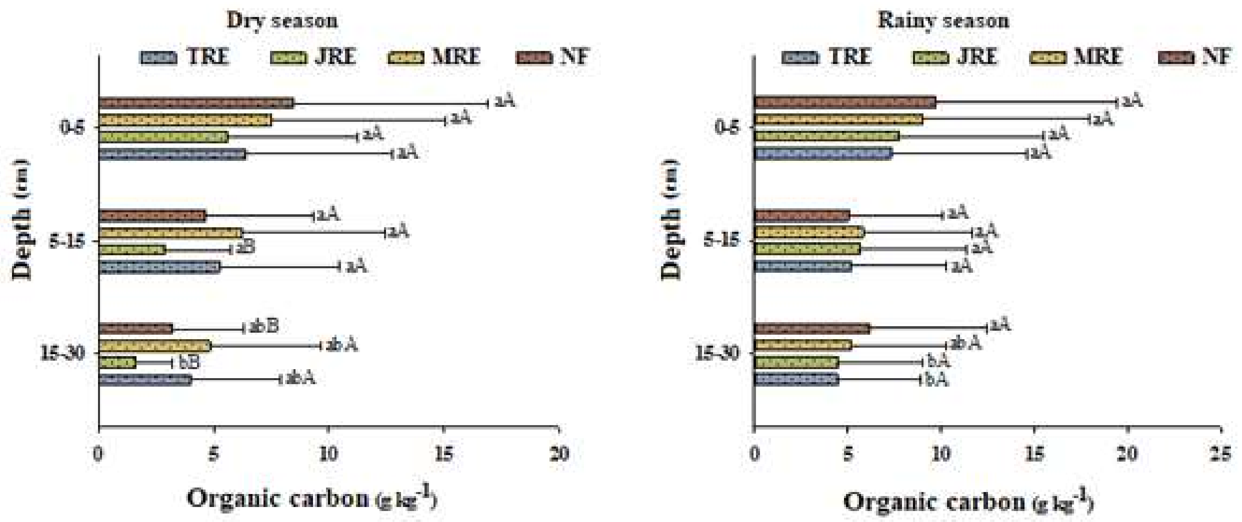

Figure 4 – Concentration of Organic Carbon in different areas, depths and period. Values followed by the same lowercase letter do not differ by Tukey test at 5% and upper case, do not differ by T test of paired samples.
***Figura 4** – Concentração de (Corg) nas diferentes áreas, profundidades e período. Valores seguidos da mesma letra minúscula não diferem pelo teste de Tukey a 5 % e maiúscula, não diferem pelo teste de T de amostras pareadas.*

potential and capacity to adapt to environmental stress conditions (Martins et al., 2004).

Similar results regarding the percentage of root biomass found in *Eucalyptus cloeziana* F. Muell plantations, with almost 50% of the biomass in the first 5 centimeters (Navroski et al., 2010), corroborate the results obtained in this study, where 66% of the biomass root of JRE, are concentrated in the first 5 centimeters (Table 2). The highest concentration of fine root biomass in a study with *Araucaria angustifólia* were found in the first 20 cm of soil depth, making up a total of 53.4% of the total biomass (Schumacher et al., 2004).

Organic carbon contents have been used as indicators of soil alterations, being lower in conditions of environmental disturbance (Mendes, 2018). Results found by Gatto et al. (2010), in the order of 22.44 g kg^{-1}, in Eucalyptus plantations are similar to the values obtained in MRE in the order of 18.92 g kg^{-1}, found in the rainy season. In the comparison between the two periods (dry) and (rainy), the behavior of organic carbon was similar to that of root biomass, with no significant differences, except for JRE, which presented significant concentrations at depths of 5-15 and 15-30 cm, in the comparison between dry and rainy season. This fact may have a strong correlation with the rugged relief associated with the high precipitation indices at this time of year. The higher concentrations of organic carbon found in the surface layers are explained by the fact that the soil surface suffers greater interference from organic matter from the fall of leaves and branches, promoting more intense nutrient cycling processes (Vital et al., 2004).

Native forest soils showed greater acidity, perhaps due to the degradation process of organic matter and its rapid mineralization, resulting in greater natural soil acidification in these environments. The efficiency of organic compounds increases the availability of nutrients in the soil, it is assumed that many of these acids are degraded within a few days after the release of plant residues (Costa et al., 2013).

Approximate pH values in native forest areas of 4.30 and pasture of 4.40 were found by Oliveira et al. (2015), corroborating the values found in this study. The higher pH values of the reforestation areas may have a correlation with management practices, notably the practice of liming, and soil preparation may have contributed to higher percentages of fine roots in the first layers of the soil.

5.CONCLUSIONS

The production of root biomass, adding the three study depths, was greater in the native forest environment, occurring more intensely in the rainy season of the year, reaching values of 8.19 t. ha^{-1}, decreasing as depth increases. However, the highest percentages, corresponding to the total produced, occurred in reforestation environments for a depth of 0-5 cm, reaching values of 72.18% for MRE in the rainy season.

The behavior of root density as a function of soil volume showed that the highest concentration of roots is found in the first 5 centimeters of depth, not differentiating between environments and periods of the year. For the other depths it was significant for the native forest environment, with higher values in the rainy season. Soil organic carbon (Corg) showed significance between the dry and rainy season for the NF and JRE environments.

It is assumed that, considering the results obtained, edaphoclimatic factors such as precipitation, soil and characteristics of forest species influenced both the production of fine root biomass and the estimation of soil organic carbon. We can also conclude from the results obtained that reforestation can be an alternative to improve soil quality in areas degraded by activities that constantly turn the soil over and make them susceptible to various factors of soil degradation.

6. AUTHOR CONTRIBUTIONS

Bello, O. C., Cunha, J. M, Brito Filho, E. G.: Investigation and Writing – Original Draft;

Pereira, M. G., Silva, G. A., Simões, W. S. Methodology.

Campos M. C. C., Brito Filho E. G., Santos, L. A. C.: Writing – Review and Editing.

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