

Fine root biomass and seed bank in secondary and primary forests in Eastern Amazon

Massa de raízes finas e banco de sementes em florestas secundária e primária na Amazônia oriental

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Abstract: Amazon is made up of a mosaic of ecosystems that have important structure, flora, and ecological functions for Brazil and the world. Knowing aspects of this biome related to the production and biomass of fine roots is of major importance given the role that the plant root system plays in nutrient cycling and carbon storage. Therefore, this study aimed to quantify the fine root mass in two regeneration areas and in primary forest, with a forest edge, in order to verify whether in different environments there are significant differences in root mass, as well as to compare the seed bank of the three areas to try to identify similarities among banks. The native forest presented a higher root mass, which differs from the two regeneration areas. The seed bank of succession forests is more similar to each other. The native forest has a greater number of seeds per volume of soil and litter collected. Concerning morphospecies, a higher number was observed in succession forests. The three areas differ in terms of fine root mass; however, regarding the seed banks, a possible edge effect may be affecting the native forest.

Keywords: Biomass; Forest succession; Forest edge; Nutrient cycling.

Resumo: *A Amazônia é formada por um mosaico de ecossistemas que possuem estrutura, florística e funções ecológicas das mais importantes para o Brasil e o mundo. Conhecer aspectos desse bioma relacionado a produção e biomassa de raízes finas é de grande importância diante do papel que o sistema radicular vegetal possui na ciclagem de nutrientes e na armazenagem de carbono. O objetivo deste foi quantificar a massa de raízes finas em duas áreas de regeneração e em floresta primária, com presença de borda florestal, no intuito de verificar se em ambientes distintos existem diferenças significativas na massa de raízes, bem como comparar o banco de sementes das três áreas para tentar identificar similaridades entre os bancos. A floresta nativa apresentou maior massa de raízes, se diferenciando das duas áreas de regeneração. O banco de sementes das florestas de sucessão é mais similar entre si. A floresta nativa apresentou maior número de sementes por volume de solo e serapilheira coletado, enquanto as florestas de sucessão apresentaram maior número de morfoespécies. As três áreas diferem quanto a massa de raízes finas, no entanto quanto aos bancos de sementes um possível efeito de borda pode estar afetando a floresta nativa.*

Palavras-chave: *Biomassa; Sucessão florestal; Borda florestal; Ciclagem de nutrientes.*

INTRODUCTION

The high biological diversity of the Amazon Forest ensures its survival and productivity. This tropical rainforest is located predominantly on soils of low natural chemical fertility and comprises native species adapted to the region's climatic and nutritional conditions (SILVA *et al.*, 2021; GARLET and SCHUMACHER, 2020; LUIZÃO, 2007). Besides, the efficient recycling of organic matter is also a determining factor for the exuberance of life forms and biomass in the Amazon (LIMA *et al.*, 2003).

The species' root system is one of the ecological aspects that contribute to this efficiency, which is an important component in the acquisition of soil resources, adaptation, survival, and growth of plants in the initial stages of succession; thus, it provides the successional forest with the important ability to restore intensely degraded tropical environments (CATTANIO, 2017). The roots comprise some 33% of the global net primary productivity, and concerning the carbon and nutrients input into the soil, it is equal to or higher than that provided by the leaves (JACKSON *et al.*, 1997; NADELHOFFER; RAICH, 1992).

Studies by Valcarcel *et al.* (2007) emphasize the important role of microclimatic conditions (burlap x root interaction), diversity, and ecological function of species in the development of the fine root system; while Garlet and Schumacher (2020) state that the

highest fine root biomass is related to the availability of water, nutrients, and soil physical characteristics.

Fast-growing species, for instance, have a high metabolism and nutrient demand, producing fine roots with elevated absorption capacity and high specific length compared to slow-growing species (FINÉR *et al.*, 2011; BRASSARD *et al.*, 2013). Despite the importance of roots in understanding terrestrial ecology, nutrient, and carbon cycling, little information exists about their basic characteristics (biomass, length, surface area) and how they might respond to future global changes.

The study on root growth must be conducted using the evaluation of root characteristics such as mass, length, diameter, and surface; furthermore, it is important to consider the environmental factors that influence the distribution of roots, such as density and porosity of the soil, water and air available in the soil, the presence of litter, nutrients, soil pH and temperature, among others (BRAGA *et al.*, 2017; MARTINS *et al.*, 2004).

In this context, it is worth stressing the need for a better and deep knowledge in this area of ecology in tropical regions, especially for a better understanding of the carbon dynamics in the forest (MONROE *et al.*, 2020; LIMA *et al.*, 2012).

Concerning secondary forest, the seed bank is considered an important ecological aspect in these ecosystems since it helps to understand the natural recovery processes of an altered forest, besides allowing the identification of growth patterns in the forest and their different species.

The seed banks, which are composed of seeds buried in soil and those on the surface, vary considerably between primary and secondary forests and disturbed areas (CORDEIRO *et al.*, 2021). Freitas *et al.* (2021) highlighted that with the advance of forest succession, the seed bank decreases, showing higher density in younger forests and low representation in primary forest species.

Thereby, this study aimed to compare the amount of fine-root mass from three different forests and evaluate the soil seed bank from these forests.

MATERIAL AND METHODS

Study Area

Jari Ecological Station (Jari Esec), crossed by the Jari River, is an Integral Protection Conservation Unit in Brazil, located in the north region (Calha Norte) of the Amazon River, in the states of Amapa and Para. It is managed by the Chico Mendes Institute for Biodiversity Conservation. Geographically, it is located between parallels

00°08'33''S and 00°51'15''S and between meridians 052°31'02''W and 053°24'52''W of Greenwich. Of the total area (227,126 ha), 40% are located in the state of Amapá (in the municipality of Laranjal do Jari) and lies eastern part of another conservation unit, the Rio Iratapuru Sustainable Development Reserve. In the State of Para, the remaining 60% of Jari Esec area is located in the municipality of Almerim, where the reserve is bounded by the Paru State Fores to the western, ending the Ecological Station on the banks of the Paru River (IBAMA, nd).

The region's climate is classified as equatorial humid (average temperature of around 26.3 °C). Variations in the average temperature are registered in rainy or dry seasons (COUTINHO; PIRES, 1996).

As the most dominant species in the succession areas is the *Cecropia* genus, the collection area became known as the embaúba (*Cecropia pachystachya*) branch. This branch or secondary road is what separates the old plantation environments from the native forest.

The maintenance activities of the *Eucalyptus deglupta* (31ha), *Gmelina arborea* (12 ha), and *Pinus caribea* (10 ha) plantations at this site were ended 29 years ago, providing an ideal condition for natural succession processes within Esec.

This area is classified as Submontane Forest with a strong presence of the Burseraceae family and expressive participation of the *Protium* genus (SOUSA, GUEDES, APARÍCIO, 2011)

Data collection

For each treatment, three plots were established: abandoned *Pinus* plantation (1), abandoned *Eucalyptus* plantation (2), and one control for native forest (3).

Each plot measured 20 x 20 meters. In all treatments, the first plot started parallel to the road, in the first planting line towards the inner edge (0 m); the second plot followed the same alignment, at a distance of 50 meters from the end of the first plot (70 m from the edge); and the third, also in similar conditions, located at 140 meters away from the edge.

Samples from the soil/litter interface and the soil (0-5 cm) were taken in the central areas of each plot. The fine roots and seeds were collected using a wooden template measuring 25 x 25 cm. In total, 0.0625 m² of soil was collected per plot. The samples were stored in plastic bags. Before carrying out the analysis, the samples were washed under running water, and the roots with a diameter less than or equal to 2 mm were

separated (HERTEL *et al.*, 2003). During washing, the collection of seeds was also carried out.

Thereafter, the roots were stored in a tray and exposed to the open air for approximately 24 hours to uniform the humidity of all samples. To obtain the information on the total weight of roots, a Pesola Weighing Scale, 1000 g x 5 g, with Hook End was used.

The representation in the seed bank of individuals was performed deemed each different seed as a morphospecies.

Data analysis

The difference in fine root mass between forests and between distances was calculated using Analysis of Covariance (ANCOVA). The comparison of means for significant differences was made using the Tukey test.

To analyze the similarity between the seed banks of different forests, a binary matrix of the presence-absence of seeds was built. The data was submitted to multivariate analysis according to a clustering technique (Ward's method). The Euclidean distance was adopted as a measure of similarity. All analyzes were performed using the R software version 4.0.2.

RESULTS AND DISCUSSION

Fine root mass

Figure 1 presents the fine root mass values between the studied areas. The native forest stood out with the highest values, while the *Pinus caribaea* planting regeneration area had the lowest values. The plots sampled in the *Eucalyptus deglupta* plantation areas presented intermediate values to the other environments. It was observed that only in the first plot near the edge the value found was lower than the others for this distance, with 165 g.m⁻² of fine roots. Furthermore, the results indicated that the portion 140 meters from the edge of the native forest had the highest mass, with 335 g.m⁻² of fine roots, while the portion of the *Pinus* area 70 meters from the edge had the lowest value, with 50 g.m⁻².

Additionally, the results suggest that root mass within the *Eucalyptus* environment tended to decrease as the distance from the edge increases. Whereas, in the other two environments, this tendency occurred only until the second plot, in the following plots, a considerable increase in root mass was observed; however, no significant differences

were found between distances in the *Eucalyptus* environment (ANCOVA, $F=0.340$, $p=0.585$, and $gl=1$) (Figure 1).

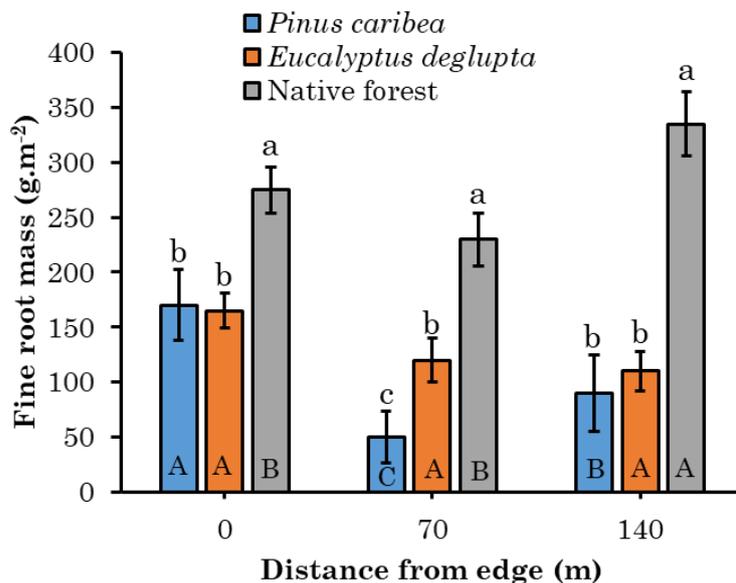


Figure 1. Fine root mass averages (g.m⁻²) at the three distances from edge and in the three different types of environments (*Pinus*, *Eucalyptus*, and Forest). Means followed by the same letter do not differ statistically from each other. Lowercase letters to compare environments, and uppercase letters to compare each environment at different distances by analysis of covariance (ANCOVA) using Tukey's Test for Post-Hoc Analysis at 5% probability. Error bars represent standard deviation.

The highest fine roots production occurred in the native forest with 840 g.m⁻², followed by the *Eucalyptus* with 395 g.m⁻² and *Pinus* 310 g.m⁻². The analysis of covariance showed a difference among the environments ($F=9.789$, $p=0.019$, $gl=2$). Also, when comparing the means of the three environments by the Tukey test, it was found that the native forest differed from *Pinus* ($p= 0.021$) and *Eucalyptus* ($p= 0.040$). Besides, the results revealed that the *Pinus* and *Eucalyptus* environments did not show significant differences between them ($p= 0.7950$) (Figure 2).

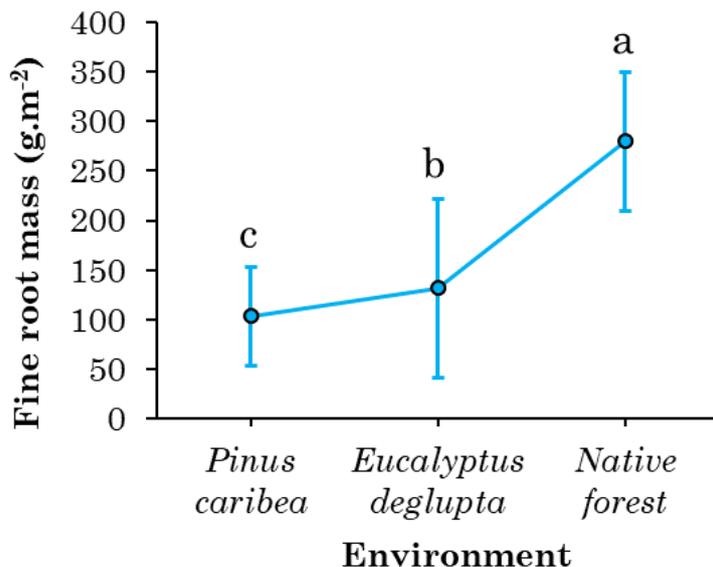


Figure 2. Root mass in the three environments studied at the Jari Ecological Station, Almeirim, Pará.

The results are in line with existing studies for tropical forests, indicating greater root production in primary forest areas when compared to areas of succession. These facts corroborate the fine root mass analysis since forest stands with greater species diversity are more balanced in fine root production, more efficient in self-sustainable soil construction processes, and consequently in the rehabilitation of degraded areas (VALCARCEL *et al.*, 2007). This higher root biomass in older forests also reflects on carbon stock; this is because, with the increasing age of the forest, greater will be the production of biomass and greater carbon storage (JONES *et al.*, 2019; MISHRA *et al.*, 2019).

The high value for submontane native forest (840 g.m⁻²) in the Jari region is close to that found for the montane forest by Rosado *et al.* (2011) in dry period in the Atlantic Forest in the state of São Paulo (875 g.m²).

Valcarcel *et al.* (2007) emphasize that the relationship of plants with litter and subsequent layers positively influences the development of fine roots, making the most of the nutrients released by cycling, besides having higher oxygen availability. This suggests that the litter decomposition process and nutrient release are more intense in native forests, which provides ideal conditions for the development of fine roots. In line with these authors, the present study obtained the largest root mass in the native forest.

It is worth mentioning that the large variation in fine root biomass between habitats may in part reflect the differences in species composition and environmental conditions

of forests, in the different methods used to extract fine roots, and also in the different depths investigated (Roderstein *et al.*, 2005).

The *Eucalyptus* area exhibited a more consistent relationship between the distance from edge and root mass; in this case, the distance from edge is inversely proportional to the root mass.

A possible edge effect can also be observed in the native forest, where the greatest fine roots mass was found in the portion farthest from the secondary road. This indicating that changes in the forest microclimate (higher light incidence, decreased humidity of air and soil, among others.) induced by the presence of edge (MURCIA, 1995; LAURENCE *et al.*, 1998) may affect the production of fine roots in areas more exposed to such effect.

The findings in this study showed that there were no significant differences between the distances, it is because the growth of secondary vegetation in abandoned areas, which have been previously deforested for anthropic use, decrease the intensity of edge effects since this new vegetation dampens the lateral entry of light and winds incidence (KAPOS, 1989).

When comparing the definitions of fine root turnover and calculation methods used in different studies with *minirhizotrons*, Satomura *et al.* (2007) highlighted that beyond these definitions and calculation, the measurement methods, soil depth, variations in physical qualities, forms, and functions of fine roots also interfered in the study of the production-death-decomposition cycle of fine roots. These authors also indicated that long-term research on these key factors associated with biotic and abiotic parameters might contribute to better knowledge in this area of ecology.

Additionally, the direct observations of root dynamics in the tropical forest in response to some environmental stimuli can facilitate the comprehension of processes related to current carbon fluxes and the future of tropical forests (CORDEIRO *et al.*, 2020).

About the fine root mass, the variation in treatments was corroborated by its great production, especially in the native forest, which presented the highest value, results that differ significantly from other environments.

Seed bank

Table 1 presents the morphospecies found in the seed banks of the three different forests. In total, 12 morphospecies were found, categorized as A-M, distributed in seven plots. No seeds were found in the soil in either native forest plots at the edge or in the

Eucalyptus plots, 140 meters away from the forest edge. Altogether, 38 seeds were collected from the soils of native forest (55.26%), *Pinus* (31.57%), and *Eucalyptus* (13.15%) areas. The morphotype D was the most evident species, with 17 seeds collected in the native forest plots, representing 44.73% of the total. Then morphotype C (18.42%), with seven seeds, six in the *Pinus* plots and one in the *Eucalyptus*. While seeds or strobili of *Pinus* (M) represented 10.52% of the collected seeds, presenting four individuals in the regeneration plots of this area.

Table 1. Seed morphospecies found in regenerating environments classified in abandoned *Pinus*, *Eucalyptus*, and Native Forest plantations at Jari Ecological Station (ESEC), Almeirim, Pará (Seeds. m⁻³).

Morphospecies	<i>Pinus</i>	<i>Eucalyptus</i>	Native Forest	Total
A	1			1
B	1			1
C	6	1		7
D			17	17
E			1	1
F			1	1
G			2	2
H		1		1
I		1		1
J		1		1
L		1		1
M	4			4
Total	12	5	21	38

Morphospecies D represented 80.95% of the seeds found in the native forest. Garwood (1989) states that although most pioneer species might produce high amounts of seeds in a short period, the persistent seed bank can be dominated by an individual species. Factors such as ease of dispersal over long distances, large seed production available to birds and bats, and post-dispersion dormancy mechanisms favor the permanence of these species in the seed banks in disturbed areas.

Type D seed, despite having been found in primary forest, is possibly not representative of a canopy species, as its size characteristics are very similar to orthodox type seeds (relatively small); moreover, the proximity of the edge (70 and 140 meters) may also favor the dispersion of pioneer species seeds in the native forest area.

The largest number of species was observed in plot 2 (*Eucalyptus*) near the edge, with four species (H, I, C, and J), while the highest number of seeds were found in the native forest plot, at 70 meters from the edge, which had 14 in total (G=2 and D=12).

The native forest was the environment with the highest number of seeds (21), followed by *Pinus* (12) and *Eucalyptus* (5) areas. Despite having the smallest number of seeds, the plots of the environment with *Eucalyptus* presented the highest number of morphospecies (5), while native forest and *Pinus* had the same quantity (4). Several studies report that seed bank with high density is related to younger successional forests. Araújo *et al.* (2001), when analyzing three different age groups of succession (6,17, and 30 years old), found higher seed density in the soil of younger successional forests, even though the floristic composition of pioneer species was similar for the three areas.

This trend was not found at Jari Ecological Station and may be associated with the limited sampling carried out in the area, in a single day and at one point per plot in each environment. Caldato *et al.* (1996) recommend that studies of this nature, especially in the case of primary forests, have a minimum duration of one year or even two years, due to the great seasonality in the production and accumulation of seeds in the soil and due to species that flower and bear fruit in periods longer than one year.

The similarity between seed banks evidenced that the nearby environments were those of secondary forest (Figure 4). Garwood (1989) empathizes that there is a high similarity of species between samples, especially in the initial succession stage, and the proximity of the regeneration areas allows a more effective dispersion. The *Pinus* and *Eucalyptus* areas are near Jari Ecological Station, which allows greater interaction between them.

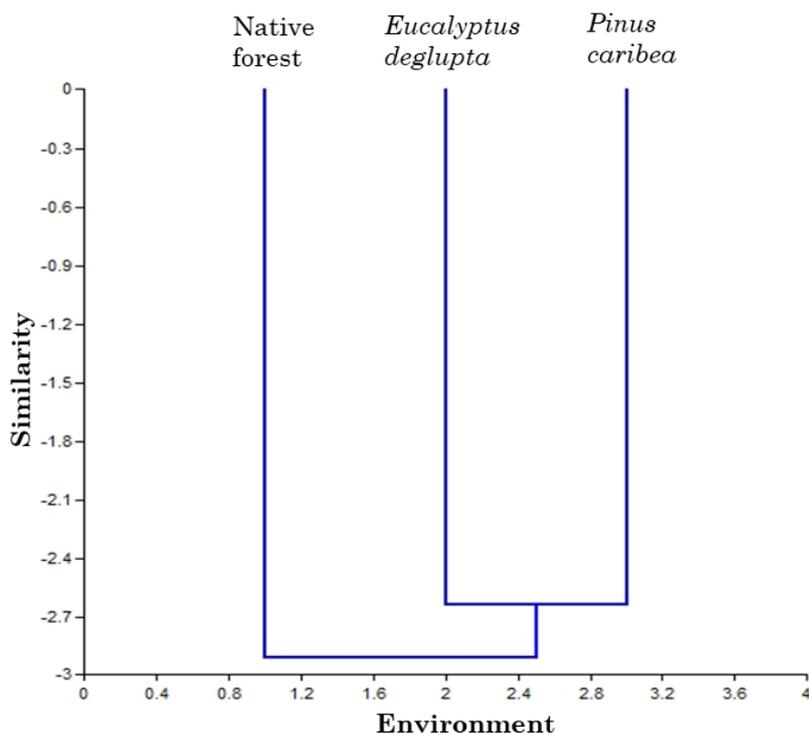


Figure 4. Dendrogram of a cluster analysis of the seed bank from two secondary forests and one primary in eastern Amazon, Almeirim, Pará

CONCLUSIONS

The composition of the seed bank in the investigated areas showed considerable diversity, especially in the regeneration areas that presented the greatest amount of morphospecies. The native forest presented a higher root mass, which differs from the two regeneration areas. The seed bank of succession forests is more similar to each other. The native forest has a greater number of seeds per volume of soil and litter collected. Concerning morphospecies, a higher number was observed in succession forests.

RECOMMENDATION

Further research is recommended to study the seed bank with the proper floristic and functional identification of the morphospecies in the area to the right identification of the lifestyle (bush, grass, vine, or tree) of the collected material. Also, it is recommended to extend the research period to verify whether the number of species in the seed bank reflects the floristic composition of vegetation or if new species are being spread in the areas.

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