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ARTICLE

Stratified sampling for roots biomass quantification in shifting cultivation in Amazon Brazil

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Abstract

Several countries have been paying attention to carbon stocks and balances in the soil, a characteristic related to land management and use. Among the biomes that have great participation in the maintenance of these stocks, the Amazon biome stands out, which has great diversity by area. With the advances in markets aimed at buying carbon credits, estimates of the values of these stocks are highly susceptible to the intrinsic characteristics of the location. In order to solve these problems, several soil sampling techniques have been used to estimate these values. However, soil sampling techniques vary greatly in the amount of soil sampled, directly impacting the values of these estimates. In this sense, the present work aims to evaluate the point and interval estimates of carbon stocks in the soil in a peripheral region of the Brazilian Amazon, in the state of Maranhão. For this, three soil sampling techniques were compared, the large monolith (LM), the small monolith (SM) and the auger (RA). Considering a stratified sampling plan (STR), in which the different sampled depths are considered as strata, its efficiency was compared to a simple random sampling (SRS) and its amplitudes with the simulation through the bootstrap technique. The samples were obtained by washing the samples and separating them into < 2 *mm* and > 2 *mm* for two biological groups (babassu roots and other roots). For interval estimates with the LM collection method, roots larger than 2 *mm* have a total of 2.56 to 4.62 $t\ ha^{-1}$, and for smaller roots, 1.67 to 4.33 $t\ ha^{-1}$. As for babassu roots, these values ranged from 0.38 to 1.44 $t\ ha^{-1}$ and those smaller than 2 *mm* from 0.86 to 2.43. In contrast, the LM collection method can be replaced by SM and RA only for thick roots (> 2 *mm*). Regarding the STR sampling plan, the variance of the total was reduced in relation to the SRS. The bootstrap technique managed to reduce the amplitude of the intervals to the total, showing an improvement in accuracy. Therefore, estimates of carbon stocks can be made for the RA method for stored carbon, but the method for carbon that will return to the atmosphere the LM method is the most suitable.

Keywords: Sampling plan; Efficiency; Design effect; Monolith; Root auger.

1. Introduction

The carbon balance in ecosystems is currently a global goal, since several public and private institutions invest too many resources to obtain such information. In particular, the quantification of carbon dioxide emissions and their redistribution in the atmosphere, ocean and terrestrial biosphere has motivated many scientists to provide quality information to support decision-making on global carbon stock budgets (Friedlingstein *et al.,* 2022).

Carbon balance studies in forest ecosystems are important to estimate the differences between emission sources and carbon sinks, which in itself is not an easy task, especially when the objective is to make predictions for possible scenarios. In a recent study, Zhao *et al.* (2021) using remote sensing tools warn for an unfavorable scenario until the year 2100 in which the forest ecosystems of northern South America will act as sources of carbon emissions into the atmosphere.

In this sense, Brazil is currently the largest contributor to carbon dioxide emissions from land use and land cover changes worldwide, representing a share between 17% and 29% of the global total (Friedlingstein *et al.,* 2019). However, the estimates and methods used to determine these values are still a matter of debate. Different remote sensing tools have improved the quality of published information, but the results can be even more accurate if observed data on land use *in situ* are added to the prediction models (Rosan *et al.,* 2021).

The carbon present in the ecosystem is compartmentalized in the two portions, above and belowground. In the soil fraction, it is present in living biomass (roots, microorganisms) and non-living biomass (plant and animal remains), as well as in soil organic matter in different compartments. Particularly, the quantification of carbon stored in the roots is still a very laborious task (Fidalgo *et al.,* 2007).

Agricultural systems play an important role in the production of goods and services, and food security. They are numerically the main source of food for the world, even with many flaws in the process, which lead to inefficiencies in several stages of the production chain, accounting for average losses and waste ranging from 20% to 50%, depending on the type of food (FAO, 2021). Even though it is a very risky activity, it has been performing well due to all the support for the production of knowledge and technological development, demonstrating its ability to overcome the challenges faced by population growth and the growing demand for food and production assets (Moraes & Souza, 2018).

Therefore "going back to the roots" of natural plant communities holds great promise for improving the sustainability of agricultural production of food, feed, fiber and fuel and ensuring the continuity of the various environmental services in scenarios of risk and uncertainty (Philippot *et al.,* 2013).

The root system is a still incipiently known component of the terrestrial ecosystem. This is attributed to difficulties in accessing roots both observationally and experimentally, the soil as an opaque body, its three-dimensional complexity and spatio-temporal variability (Böhm, 2012; Heuvelink & Webster, 2001; Mommer *et al.,* 2008). The primary functions of sustaining and absorbing water and nutrients are attributed to the roots, as well as some emerging functions arising from the need to adapt to the environmental conditions to which they are subjected (Bardgett *et al.,* 2014; Raven *et al.,* 2007).

Studies involving the determination of carbon from roots or root biomass still use excavation as the main method to access them as a way to obtain acceptable accuracy and precision in the estimates, which in turn, makes it necessary to process large volumes soil (Ratke *et al.,* 2019). The study should start with a sampling plan and then the choice of the sampling method. From previous information, it is known that roots have a dynamic behavior in the soil and that it depends on the species, soil use, growing season, and soil depth (Ratuchne *et al.,* 2016).

Low-input or low-input land use systems, such as shifting cultivation, also called slash-and-burn agriculture, are systems that rely on the burning of above-ground plant biomass as an entry route for nutrients and alkalizing agents present in soil ashes, to reduce the effects of soil acidity and provide a fertilizing effect aimed at managing crop productivity (Gomes *et al.,* 2020; Pivello, 2017). It is a ubiquitous form of agriculture in tropical and subtropical regions, in soils, generally, of low fertility, and practiced by traditional populations as a form of subsistence.

Burning is often carried out as a less costly way of preparing the soil before sowing and planting annual crops. Fire volatilizes most part of the nutrients present in the biomass, drastically reducing soil fauna, impoverishing the soil, and releasing carbon into the atmosphere, but on the other hand, it alkalinizes the soil, reducing active acidity and leaving some nutrients available for annual crops (Comte *et al.,* 2012).

Fire also causes harm from an ecological point of view, as it alters the composition and abundance of plant groups, selecting more adapted organisms such as babassu ruderal palms (*Attalea speciosa* Mart.). The fire burns the leaves of young plants but does not affect their underground stem or their root system, which, in turn, remobilizes their nutrient reserves as a survival strategy and the emission of new leaves. In adult plants, fire has little effect by only superficially damaging their stems and with very little effect on their leaves when they are at a great distance from the ground. In its fruits, it acts as a determinant in breaking dormancy when they are at ground level, weakening their rigid surface, which allows the entry of water and facilitates germination, thus increasing the abundance of individuals in the agroecosystem (Muniz, 2004).

Such adaptive characteristics of the babassu ruderal palm put it in a prominent adaptive position that guarantees it a competitive advantage in relation to agricultural crops, which have little response in production in the face of unfair competition in nutrient use efficiency with a ruderal species that develops better with little resource, which little perishes in relation to fire and with a root system that favors the remobilization of nutrients for regrowth and emission of new leaves (Gehring *et al.,* 2011). Strategies established in the study of root systems suggest their division into functional classes, based on root diameter and how they contribute to the entire system. This proposed division, for example into just two classes, allows a clearer understanding of their dynamics and specific importance in most ecosystem processes (Bardgett *et al.,* 2014; Böhm, 2012; Freschet *et al.,* 2021; Smith, 2007).

Fractions with larger diameters contribute in part to these processes but are more related to plant support and carbon accumulation processes (Mooney, 1972). On the other hand, fine roots and root hairs, on the other hand, contribute greatly to the surface area and root length, acting together in the absorption of water and nutrients, as well as in the exudation of organic solutes. Functionally, this fraction plays an important role in the formation and stability of soil aggregates (Materechera *et al.,* 1992).

There are several difficulties in studying root systems since the soil is a non-transparent body that offers a barrier to its observation and evaluation (Bengough, 2003; Böhm, 2012). Soil excavation is still used as a procedure for extracting and quantifying roots, although there are other methods used in the studies of root systems available, such as methods based on x-ray and NIRS spectrometry, biochemical, molecular and radioactive or isotope markers stable (Caldwell *et al.,* 1996; Caldwell & Eissenstat, 1987; Mamolos *et al.,* 1995; Roumet *et al.,* 2006).

For the studies to evaluate root biomass under field conditions, some destructive techniques are available, such as opening trenches, extraction of soil volumes using monoliths, augers, and root excavation associated with the separation and washing of the roots present in the extracted volume (Böhm, 2012; Majdi *et al.,* 1992). Another problem inherent to these studies involving destructive volumetric sampling of soil is the presence of extraneous organic matter (EOM), in addition to the impossibility of separating what is fresh or dead root (Ottman & Timm, 1984). In many biomes, the plant biomass below ground is superior to that above ground, although many efforts have been made in studies to quantify surface biomass, this is not what is observed for the subsoil portion (Maarel & Titlyanova, 1989), showing how much root quantification still needs to advance. All these problems configure sources of error in the estimation of root biomass in the ecosystem, together with the limitations imposed by the soil and the variability of the distribution of the root system, do not allow the accurate quantification of the real root biomass in different environments (Addo-Danso *et al.,* 2016; Keller *et al.,* 2001).

Soil carbon quantification studies are important to understand the effects of climate change on the composition of plant communities and how these changes impact the functions that organisms perform in these ecosystems (Falloon *et al.,* 2007; Silva *et al.,* 2018) since there is a lack of information on root biomass compared to aboveground biomass, which limits the establishment of an accurate carbon balance model of the ecosystem. The objective of this work is to establish a comparison between sampling methods for the estimation of root biomass in the eastern portion of the Amazon. The specific objectives are (i) to estimate the total root biomass of babassu under agricultural conditions using point and interval estimates, (ii) to compare the efficiency of sampling methods and (iii) to determine the efficiency of soil sampling methods to estimate the root biomass.

2. Matherials and Methods

2.1 Location and soil sampling methods

The analyzed data come from a survey conducted between 2007 and 2008 in the southeast periphery of the Amazon, on São Luís Island, Maranhão State (2°41' S, 44 °16' W). The climate is classified as Aw (Köppen, 2020) and the soil as Neossolo Quartzarenico Órtico (Embrapa, 2006). In this region, rainfall is concentrated between the months of January and June, with an annual average of $2,000mm$.

Collections took place at depths of $0-10$, $10-20$ and $20-30\,cm$, considered as stratum. To carry out the soil collection, three methods were used, the large monolith (LM) being a piece of cutting metal that has a dimension of 25 dm^3 ($5x5x1dm$) used as a reference for capturing all classes of roots, the small monolith (SM) with a dimension of 1 dm^3 ($2x1x0.5dm$) and the root auger (RA) with a volume of 196.3 cm^3 (2.5 cm radius and 10cm height).

2.2 Sampling technique

The best soil collection method was defined for the conditions proposed by the study, using the stratified sampling technique, since it is possible to separate the population into strata, thus improving the efficiency of the sampling process for determining the characteristic of interest (Bolfarine & de Oliveira Bussab, 2005; Cochran, 1977).

The sample sizing was based on the assumption that the distribution was uniform, that is, the same number of samples was taken for each stratum (Oliveira & Aquino, 2007), in which the strata were related to the depths sampled ($0-10$, $10-20$ and $20-30\,cm$). Five points of sampling units were used for each method (LM, SM and RA). The LM for having a larger volume of soil collected, 5 (n_h) total points were sampled, for the SM $15n_h$ total samples were collected, for the RA $45n_h$ samples were obtained.

To calculate the sampling factor ($f_h = n_h/N_h$), an area of $10,000\ m^2$ was considered, that is, 1 hectare, and in this area the possible total number of samples was estimated for each method. For LM (25 dm^2), considering 5 sampled points (n) and a total number of samples for LM of 40 thousand (N) results in an f of 0.000125. The same happened for SM ($N_h = 500,000$) resulting in an $f_h = 0.0003$ and root auger ($N_h = 5,094,244$) with $f_h = 0.0000049$.

2.3 Response variable and estimates

After carrying out the collections in the different propositions listed above, the samples were washed, sieved and dried in individual paper bags through a forced hot air circulation oven, for approximately 48 h at 55 °C or until their weight was constant. The roots were separated into roots smaller than 2 *mm* (thin) and larger than 2 *mm* (thick) as proposed by Majdi *et al.* (2005), and their respective fresh masses were quantified using an analytical balance. Visual separation was also performed between two biological groups, babassu roots and roots of other species.

Estimates of total root mass were performed by point and intervals with 95% confidence. The following expressions were used for this:

$$N = \sum_{h=1}^{L} N_h$$

The estimate of the total

$$\hat{\tau} = (N\bar{y}_h)$$

where $\bar{y}_h$ is the estimated average in the h-th stratum.

Confidence intervals

$$C.I.(\tau, \gamma\%) = \left(N\bar{y}_h \pm t_{\alpha/2} \frac{N_h S_h}{\sqrt{n_h}} \sqrt{1 - f_h} \right)$$

To evaluate the sampling plan, the metrics of the estimates of confidence intervals (95% *C.I.*), standard error and the design effect of plan (DEFF) described by Kish (1985) were considered. The DEFF is considered a product of the ratio between the variance of an estimator obtained from any sampling plan in relation to the sampling plan determined as standard, used as a basis for simple random sampling (SRS), whose expression is as follows:

$$DEFF = \frac{VAR_{srt}\hat{\tau}}{VAR_{srs}\hat{\tau}}$$

Where STR is the total variance for the stratified sampling plan and SRS the total variance for the simple random sampling plan.

The total mass of roots in tons was also estimated using the bootstrap methodology, with the aim of improving the estimates (Tibshirani & Efron, 1993), by points and intervals with 95% confidence. 10,000 resamples were considered and their respective intervals were constructed.

Each bootstrap replica was defined as

$$\hat{\theta}^* = T(y^*)$$

The bootstrap estimate of the standard error of $\hat{\theta}$ was defined as

$$\hat{se}_{boot} = \sqrt{\frac{1}{B-1} \sum_{j=1}^{B} (\hat{\theta}_j^* - \bar{\hat{\theta}}^*)^2}$$

Where B is 10,000 and $\bar{\hat{\theta}}^*$ the average of replicates bootstrap. The bootstrap resampling technique was considered as a reference for comparing the confidence intervals of the proposed sampling plans (SRS and SRT).

3. Results and Discussion

The estimates of the total root mass (expressed in tons per hectare) with their respective confidence intervals are shown in table 1. In this work we consider that the total root mass obtained is the most reliable value since it was obtained with a larger volume of soil than the other sampling methods.

Table 1. Total root mass, expressed in tons per hectare, classified according to the sampling method

Root mass > 2 mm ($t\ ha^{-1}$)						
	SRS		STR		BOOSTRAP	
	$\hat{\tau}$	C.I. (95%)	$\hat{\tau}$	C.I. (95%)	$\hat{\tau}$	C.I. (95%)
LM	3.59	(2.54; 3.59)	3.59	(2.56; 4.62)	3.59	(3.23; 3.94)
SM	2.61	(1.65; 3.57)	2.61	(1.63; 3.59)	2.61	(2.19; 3.04)
RA	2.12	(0.98; 3.26)	2.13	(0.97; 3.28)	2.13	(1.24; 3.01)
Root mass < 2 mm ($t\ ha^{-1}$)						
LM	3.00	(1.19; 4.81)	3.00	(1.67; 4.33)	3.00	(2.59; 3.40)
SM	4.98	(3.97; 6.00)	4.98	(4.09; 5.87)	4.97	(4.73; 5.22)
RA	19.14	(15.51; 22.76)	19.02	(16.36; 21.68)	19.02	(16.16; 21.89)

The total of thick roots (greater than $2mm$ in diameter) obtained from the large monolith yielded a value of 3.59 $t\ ha^{-1}$. With 95% confidence, we believe that the total of thick roots is between 2.56 and 4.62 $t\ ha^{-1}$ and with a sampling error of 1.03 $t\ ha^{-1}$. The values of the estimates of the total of these roots obtained with the small monolith and with the root auger differ in their punctual estimate of the large monolith (2.61 and 2.13 $t\ ha^{-1}$ respectively) but not so, in the estimates by interval of confidence, since these overlap table 1. This result is very promising since, under the same sampling plane, a good estimate of the total masses of thick roots can be obtained with the manipulation of low volumes of soil.

Although the overlap between two confidence intervals is just an indication that the total roots quantified by one method or another may be equal, pointing out that behavior serves to have an idea of how the different collection methods could be similar in the estimation of roots. The mass of roots less than 2 mm in diameter with the large monolith was 3.0 $t\ ha^{-1}$, with 95% confidence we believe that the total number of roots less than 2 mm is between 1.67 and 4.33 $t\ ha^{-1}$ and with a sampling error of 1.33 $t\ ha^{-1}$. These results are not different from the values obtained with the small monolith since both intervals overlap. But, the values obtained with the root auger method are comparatively higher, both punctually and by confidence interval (table 1). This result suggests that with a small monolith values of total fine roots equal to the large monolith can be obtained, but not with the root auger. The latter overestimates the total number of fine roots.

For simulations through bootstrap, considering the $10,000$ resamples provide a better estimate, a fact by which resampling reduces the impact of variance and provides an interval with smaller amplitude (Dixon, 2006). In table 1 it is possible to verify this effect, since all the intervals for the collection methods were superimposed on the total ($\hat{\tau}$).

Although the intervals overlap, the amplitudes observed in SM together with stratified sampling are smaller when compared to the reference techniques (LM and BOOSTR). When observing the amplitude of the confidence intervals for roots > 2 mm, the CI via bootstrap were 0.71 (LM), 0.85 (SM) and 1.77 (RA). The amplitudes observed in the SRS plane were 1.05 (LM), 1.92 (SM) and 2.28 (RA) and in the STR plane they were 2.06 (LM), 1.96 (SM) and 2.31 (RA). This confirms that regardless of the method used (SM or RA) the quantification of roots < 2 mm is neither underestimated nor overestimated. As for roots <2 mm, a greater amplitude of the C.I. was observed and the SRT plane was what provided a better approximation of the simulations via boostrap, a technique that reduces the confidence intervals without losing information.

For babassu roots (Table 2) there is a similar trend, since the bootstrap method reduces the amplitude of the interval to the total. In contrast, the bootstrap method is not always the best tool for accurately estimating a parameter such as the total. In our research the RA collection method with a stratified sampling plan the amplitude was 0.9 $t\ ha^{-1}$ but with the bootstrap technique this amplitude was attenuated to no significant value ($p = 0.01$).

Table 2. Total root mass of babassu, expressed in tons per hectare, classified according to the sampling method

Root mass of babassu > 2 mm ($t\ ha^{-1}$)						
	SRS		STR		BOOSTRAP	
	$\hat{\tau}$	C.I. (95%)	$\hat{\tau}$	C.I. (95%)	$\hat{\tau}$	C.I. (95%)
LM	0.91	(0.42; 1.40)	0.91	(0.38; 1.44)	0.91	(0.81; 1.01)
SM	0.78	(0.24; 1.33)	0.78	(0.24; 1.33)	0.78	(0.61; 0.95)
RA	0.79	(0.33; 1.24)	0.79	(0.34; 1.24)	0.79	(0.79; 0.80)
Root mass of babassu < 2 mm ($t\ ha^{-1}$)						
LM	1.64	(0.81; 2.48)	1.64	(0.86; 2.43)	1.64	(1.48; 1.80)
SM	4.97	(3.78; 6.16)	4.97	(3.81; 6.13)	4.96	(4.63; 5.29)
RA	8.29	(7.00; 9.58)	8.23	(7.29; 9.18)	8.23	(8.05; 8.41)

In this work, we decided to identify babassu roots due to the importance of this species in the Amazon region of Brazil. Under the collection with the large monolith with thick roots, the total mass was 0.91 $t\ ha^{-1}$. With 95% confidence, we believe that the total of coarse roots of babassu is between 0.38 and 1.44 $t\ ha^{-1}$ with a sampling error of 0.53 $t\ ha^{-1}$. Results very similar to these values can be obtained with the small monolith and root auger collection methods (table 1). This result is also very promising and suggests that it is possible to collect roots with the root auger, with low volumes of soil, and obtain good estimates of the total mass of babassu thick roots.

In the conditions presented in the study, the LM provided a better sampling of roots, whether they are smaller or larger than 2 *mm*, regardless of the sampled species, due to providing intervals with low amplitudes. However, it is the technique that requires more soil volume, that is, more costly.

An alternative to this type of collection is the use of the SM or RA method, since both types of collection are interesting because they allow samples with a smaller volume of soil, consequently it is possible to carry out a greater number of samples in the same place, increasing the accuracy of the sampling. For roots larger than 2 *mm*, regardless of the species, it is possible to replace LM with SM or RA.

Considering roots smaller than 2 *mm*, a promising result is the use of the SM collection method, as it offers a good estimate similar to the LM, but the use of the RA method is not recommended for this type of root, as it overestimates the root mass values. The stock of carbon in the soil, that is, that which is likely to return to the atmosphere quickly, should not be sampled with AR, due to its inaccuracy in point and interval estimates.

When babassu roots larger than 2 *mm* are considered, regardless of the collection method, both provide assertive and overlapping estimates, not differing in themselves. In roots smaller than 2 *mm*, the only technique that was able to capture the total number of roots with assertiveness was the LM. Because it is a species that represents more than 50% of the total fine roots in the soil, a fact that must be taken into account in soil sampling and cannot be estimated with low volumes of soil.

The variances of the totals for the SRT plan were equal to or less than the variances of the totals of the SRS plan among the three forms of collection. Thus confirming that a stratified sampling plan can be more efficient in terms of precision than a simple sampling plan (Cochran, 1977). According to table 3, observing the DEFF, it is possible to verify that the LM method has an efficiency greater than 18% (1.18) when compared to the other methods (SM and RA) in the quantification of roots larger than 2 *mm*. When roots smaller than 2 *mm* are considered, LM has an efficiency about 57% and 39% greater than SM and RA, respectively.

Table 3. Sampling plan efficiency (SPE) for three methods in different roots diameters

	DEFF		
	LM	SM	RA
> 2 mm	1.18	0.97	0.99
< 2 mm	3.08	1.32	1.88
> 2 mm babassu	0.96	1.03	1.04
< 2 mm babassu	1.63	1.07	1.90

When babassu roots larger than 2 *mm* are considered, the collection method did not interfere with the efficiency of the sampling plans, since the variances for both plans were not different. In roots smaller than 2 *mm*, considering the LM as a reference, the RA has 17 % lower efficiency, on the other hand, the SM did not demonstrate efficiency when using stratified sampling.

4. Conclusions

LM provides more accurate point and interval estimates when compared to other methods. This collection method can be replaced by SM and RA in the quantification of roots that form part of the carbon stock that remain in the soil. Carbon stocks, which comprise the fraction returnable to the atmosphere, should not be sampled by AR, due to its lack of accuracy in estimating carbon stocks.

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Conflicts of Interest

The authors declare no conflict of interest.

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