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RESEARCH PAPER

Carbon stocks, partitioning, and wood composition in short-rotation forestry system under reduced planting spacing

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Abstract

• ***Key message*** **The use of reduced planting spacings is an important strategy to increase the carbon storage in the above-belowground biomass and should be recommended for future exploitation of forest energy plantations when the purpose intended is the production of biomass for energy.**

• ***Context*** Recent concerns about global warming have resulted in more concerted studies on quantifying carbon storage in forest systems. Forest energy plantations play an essential role in the carbon storage.

• ***Aims*** We proposed to evaluate the carbon storage and partitioning in short-rotation forest plantations and to characterize the elemental composition and energetic properties of the forest species *Eucalyptus grandis* W. Hill ex Maiden, *Mimosa scabrella* Benth, and *Ateleia glazioviana* Baill, grown under four planting spacings in Southern Brazil.

• ***Methods*** A field study was conducted in order to evaluate forest carbon stock and wood composition using samples collected by direct method. The four spacings evaluated were 2.0 × 1.0, 2.0 × 1.5, 3.0 × 1.0, and 3.0 × 1.5 m.

• ***Results*** The *Eucalyptus grandis* stored 327.1 Mg C ha^{-1} at 2.0 × 1.5-m spacing. When compared with the 3.0 × 1.5-m spacing, we observed a reduction of 29% in carbon stored. All forest species showed higher carbon storage in the following partitioning pattern: trunk>roots>branches>leaves>litter. Forest species energetic properties and elemental composition were not affected by planting spacing. On the other hand, variations according the tree portions were observed. For the carbon stocks in the soil, we observed an average accumulated carbon stock for the forest species studied of 77.4 Mg C ha^{-1} (0–40 cm).

• ***Conclusion*** Forest managers can accelerate growth and increase the forest carbon storage and biomass yield by using reduced planting spacing that are smaller than the current pattern used by the majority of the forest producers, which is 3.0 × 1.5 m. For *Eucalyptus grandis* and *Mimosa scabrella*, the planting spacings recommended to produce biomass and improve carbon stocks were 2.0 × 1.5 and 2.0 × 1.0 m, respectively.

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Contribution of the co-authors: FS: initiated the study. FS, BOC, DDN, and EFE: designed the experiment. FS, JS, LBT, and EE: performed and collecting the data of the experiment. FS and DDN: performed data processing and statistics. All the authors discussed and interpreted the results. FS: wrote the first version of the paper. DDN, BOC, EFE, EE, and LGC: discussed and modified the text. All co-authors gave their final approval for publication.

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Keywords Carbon sequestration · Forest biomass · Forest management · Short-rotation coppice · Soil carbon stocks

1 Introduction

Forest energy plantations represent an important carbon sink. Moreover, forest plantations are regarded as important contributors to offset the greenhouse gas emissions. Studies aiming to increase the area of forest plantations have been suggested for inclusion under the clean development mechanism as defined in Kyoto Protocol (Van Vliet et al. 2003). Forest energy plantations play an essential role in the carbon storage. Fast-growing forest plantations are considered highly efficient carbon sinks capable of contributing to the mitigation of the increase of CO_2 levels in the atmosphere (Laclau 2003; Coleman 2018; Trotsiuk et al. 2015; Bhattacharya 2019).

Forest management practices, including managing forest biomass and soil, that can be employed to curb the rate increase in CO_2 in the atmosphere can be grouped into two categories: (i) management for C storage and (ii) management for C substitution (Brown et al. 1996). The perspective of C storage management is to expand the storage in forest ecosystems by increasing the above-belowground biomass and carbon stored in the soil of forest plantations. Substitution management aims at increasing the transfer of forest biomass C into products (e.g., biofuels) rather than using fossil-fuel-based energy. In this context, this paper provides information about carbon sequestration in forest systems, taking into account the importance of carbon stocks in the whole forest system and the characterization of forest biomass in order to use as a biofuel.

The use of woody biomass as energy-fuel source provides substantial benefits as far as the environment is concerned (Demirbas 2004; Vassilev et al. 2010; Jha and Puppala 2017; Shuba and Kifle 2018). In this context, the characterization of composition and energetic properties of a given biomass source is the initial and most important step during the study and application of this feedstock for energy generation. For example, data from structural, gross calorific value, elemental composition, and energetic properties analyses have been used to characterize biomass sources (Van Loo and Koppejan 2008; Saidur et al. 2011).

Forest energy plantations are capable of storing large amounts of CO_2 in a relatively short period of time. This is related especially to the forest species capacity to store carbon in their structure (wood, branches, leaves, roots). Carbon storage in forest plantations involves different components including biomass C and soil C. Management systems that maintain a continuous canopy cover are likely to achieve the best combination of high wood yield and C storage (Thornley and Cannell 2000; Lal 2005). In this context, new studies involving forest energy plantations considering different forest species and planting spacings are needed to understand and quantify the carbon stored and partitioned in the whole system, i. e., soil stocks and forest biomass, including roots biomass.

Considering the approach of C in forest energy plantations, one important question arise: Store the C in the forest plantations in order to mitigate the CO_2 emissions or use the forest biomass to produce energy and meet global energy demand? One thing is certain, the two possibilities are valid and relevant considering all the factors involved. Many countries consider biofuel and bioenergy sources as an important alternative when compared with fossil energy due to an increasing concern for climate change. In this context, an intensification of forest management (planting, harvesting, and processing) is an important strategy in the energy chain, mainly in order to produce more bioenergy and can be associated also with the direct benefit of the forest plantations as a carbon sink (Kirschbaum 2003; Hoel and Sletten 2016). Therefore, the use of short-rotation cycle of forest energy plantations is important in both cases, produces biomass for energy, and also stores carbon in forest biomass.

Carbon storage in planted forests may be affected by the tree species and the planting spacing of the forest stand. Studies with different forest species have shown that the use of a greater number of trees per unit area has resulted in greater carbon accumulation such as in plantations with Poplar (Fang et al. 2007) and *Eucalyptus* (Brianezi et al. 2019). Using appropriate planting spacing can accelerate plant development (Bouillet et al. 2013) and then the carbon storage. The tendency of reducing planting spacings for biomass production is justified by the need to reduce the crop cycle, resulting in gains in productivity and time (Gonçalves et al. 2004; Stape et al. 2010; Ribeiro et al. 2015). However, there is a lack of studies that evaluate carbon stocks of different tree species when grown under reduced planting spacings.

The forest management applied to the production of biomass for energy generation and carbon storage basically consists in three main factors: (i) forest species used; (ii) tree density and planting spacing; and (iii) rotation time of the forest plantations (Couto and Müller 2008; Welfle et al. 2017). In Brazil, forest plantations are carried out mainly by forest companies and rural producers. The most used spacings are those that provide a useful area varying from 3 to 9 m^2 (Couto et al. 2002; Gonçalves et al. 2013). The authors proposed in this study to evaluate the feasibility of the use of reduced planting spacings in order to enhance carbon storage and wood composition.

In this study, we hypothesized that planting spacing in forest plantations affects the carbon storage above-belowground, soil carbon, and elemental composition and energetic properties of forest trees, which the forest managers can accelerate growth and increase the forest carbon storage and biomass yield by using adequate planting spacing. Therefore, the aims of this study were: (i) to evaluate the carbon storage and partitioning in short-rotation forest plantations (above-belowground biomass + soil) and (ii) to characterize the elemental composition and energetic properties of the forest species *Eucalyptus grandis* W. Hill ex Maiden, *Mimosa scabrella* Benth, and *Ateleia glazioviana* Baill, grown under four planting spacings in Southern Brazil.

2 Materials and methods

2.1 Study site and experimental design

This study was performed to evaluate carbon storage and partitioning, as well as wood composition and energetic properties. In this context, a field study was conducted a field study in the city of Frederico Westphalen in the state of Rio Grande do Sul, Brazil, at the coordinates 27°22'S, 53°25'W and an altitude of 480 m. The study was conducted from September 2008 to September 2018.

The climate of the study area is Cfa (humid subtropical climate), which is characterized with mean annual temperatures of 19.1 °C, varying with maximum of 38 °C and minimum of 0 °C, according to Köppen's climates classification (Alvares et al. 2013). The soil was classified as Oxisol typical, clayey texture, deep, and well-drained. The establishment of the forest species was performed in September 2008 throughout seedlings transplantation. Fertilization was performed using 150 g of formulated fertilizer NPK (4-30-16) for each seedling at time of transplantation.

The experimental design of this study was a complete random block, with factorial arrangement of 3 × 4, with three forest species (*Eucalyptus grandis*, *Mimosa scabrella,* and *Ateleia glazioviana*), and four planting spacings (2.0 × 1.0; 2.0 × 1.5; 3.0 × 1.0 and 3.0 × 1.5 m), with three replications. Each block contemplated 12 experimental units, where the four planting spacings were allocated. Each plot showed 45 trees, and the size of each plot was relative to the spacing used. The sizes of the plots were 64, 96, 96, and 144 m^2, for the spacings (2.0 × 1.0; 2.0 × 1.5; 3.0 × 1.0; and 3.0 × 1.5 m), respectively. A sketch of an experimental unit can be seen in Schwerz et al. (2019).

2.2 Forest species studied

We proposed the study of three forest species: (i) eucalyptus (*Eucalyptus grandis* W. Hill ex Maiden), (ii) bracatinga (*Mimosa scabrella* Benth), and (iii) timbó (*Ateleia glazioviana* Baill). *Eucalyptus* (Myrtaceae) is native to Australia. This species presents relatively short rotation cycle in Brazil (6 to 8 years) and good adaptation to different Brazilian edaphoclimatic conditions (Flores et al. 2016). *Eucalyptus* is the most grown forestry species in Brazil, covering 72% (5.6 million ha) of the total forest planted area in the country (IBA 2016). It is one of the best options to produce charcoal, cellulose, paper, and energy. According Elli et al. (2020), the potential mean annual increment for *Eucalyptus* plantations ranged from 36 to 69 m^3 ha^{-1} $year^{-1}$. Bracatinga (Fabaceae) is originally from the Araucaria Forest (mixed ombrophylous forest) of Brazil. It is grown in Brazil mainly as energy forests (Mazuchowski et al. 2014) and has drawn the attention worldwide for its use in the production of pharmaceutical compounds (Seraglio et al. 2017). It is also a leguminous species that contributes to nitrogen fixation and has been used to compose agroforestry systems (Caron et al. 2018). The potential mean annual increment for this species is 36 m^3 ha^{-1} $year^{-1}$ (Carvalho 2003). Timbó is native to Brazil and belongs to the Fabaceae family, being a deciduous tree. It is grown mainly for recovering degraded areas, intending to produce sawn, and round wood for energy, cellulose, and paper (Carvalho 2003). The potential mean annual increment for this species is 9.8 m^3 ha^{-1} $year^{-1}$ (Carvalho 2003).

2.3 Destructive assessments and sampling

The destructive assessment for the aboveground carbon stocks of the forest species was performed in September 2015,7 years after planting the experiment, characterizing a short-rotation cycle. For each planting spacing, we evaluated nine trees, resulting in a total of 36 trees of each forest species. Sampled trees were previously demarcated at experiment implementation time. The data reported were extrapolated to Mg ha^{-1}, using the average carbon per tree and the final tree density as 1978, 2967, 2967, and 4450 tree ha^{-1}, for the spacings (2.0 × 1.0; 2.0 × 1.5; 3.0 × 1.0; and 3.0 × 1.5 m), respectively. We observed an average reduction of 11% on the final tree stand for the forest species studied. These reductions in forest stand are related with the mortality of the trees, caused mainly by diseases and pests.

The assessment of the trees was performed using direct method, which consist on cutting and weighing the different tree portions (Sanquetta 2002). Under field conditions, the total fresh biomass of sampled trees was assessed (trunk, branches, and leaves). The moisture content was determined by the samples from each portion in laboratory. The destructive assessments and sampling collecting can be seen in Fig. 1.

Destructive samples were collected by strict cubing (Fig. 1b). The samples were collected along the trunk, in the following sections: 0% (basis), 1.30 m (diameter at breast height—DBH), 25%, 50%, 75%, and 100% of the total height. For trunk, discs with 2-cm thick were collected, while for branches and leaves, a stratified sample was considered, including lower, middle, and upper tree canopy stratum

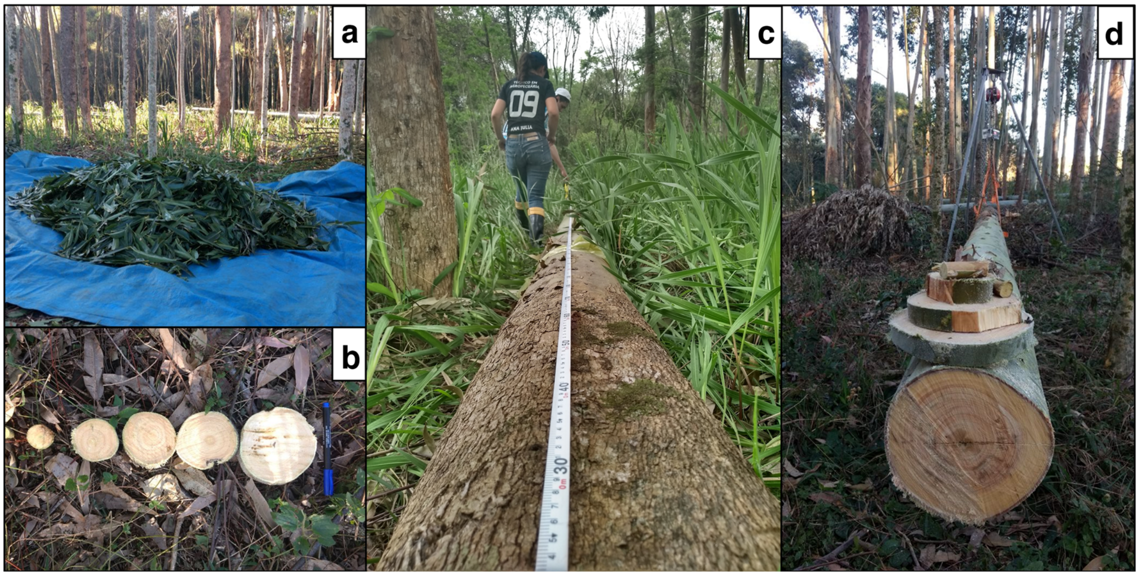


Fig. 1 Destructive assessments represented by strict cubing (**a**, **b**, and **c**) and trunk weighing using dynamometer balance (D) to determine aboveground biomass

(Fig. 1a). For the *Ateleia glazioviana* species was not possible to quantify the leaf area because this species presents deciduous characteristics, so, at time of tree assessments, the leaves were not computed.

The samples were allocated into a forced circulation oven at 103 ± 2 °C until they have reached a constant mass. Subsequently, the collected samples were macerated into a slicer, and the fraction retained on the 270-mesh sieve was used. The carbon content of the forest species (including wood, branches, and leaves) studied was assessed using a universal elemental analyzer (Model—Vario micro cube).

From the aboveground destructive assessments, the samples obtained were used to determine the gross calorific value, elemental composition, and energetic properties. The collected samples were evaluated in the Forest Biomass Energy Laboratory of the Department of Forestry Engineering and Technology of the Federal University of Paraná (UFPR). The gross calorific value was determined using a digital bomb calorimeter, C5000 Cooling System model, according to the technical standard NBR 8633 (ABNT 1984).

2.4 Energetic properties and elemental analysis

The energetic properties and elemental analysis were performed during the years (2009, 2011, and 2013). The energetic properties analysis was determined on weight percent in dry basis (wt% in dry basis), according to the technical standard NBR 8112 (ABNT 1986), from which the concentrations of volatile compounds, ash, and fixed carbon compounds were determined. The elemental analysis to evaluate carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S) contents were determined on weight percent in dry basis (wt% in dry basis), using a universal elemental analyzer (Model—Vario micro cube). The oxygen content was obtained by subtracting from 100% the sum of C, H, N, S, and ash contents in percentage. The average carbon content used for the root portion for the three forest species studied was obtained by Dallagnol et al. (2011).

2.5 Roots biomass evaluations

The belowground carbon storage determination was performed in September 2018, according to the methodology proposed by Sanquetta (2002). The root biomass was quantified using the direct method (destructive sampling). The method used is based on root excavation, cleaning, weighing, and sample collection in a stratified way, including fine, medium, and gross roots (Ratuchne et al. 2016). The sampling area changed according to the planting spacing. The useful area collected of each planting spacings were 1.0 × 0.5, 1.0 × 0.75, 1.5 × 0.5, and 1.5 × 0.75 m for the planting spacings 2.0 × 1.0, 2.0 × 1.5, 3.0 × 1.0, and 3.0 × 1.5 m, respectively, using a fixed depth of 1 m (Sanquetta et al. 2004). According to Morais et al. (2017), approximately 80% of the roots of the forest species are in the depth of 1 m. Twenty-four sample trees were randomly evaluated in order to collect representative and homogeneous samples, i.e., eight trees per forest species, two for each planting spacing. The destructive assessments and sampling collecting can be seen in Fig. 7 in Annex.

2.6 Soil carbon assessment

Soil carbon content and stock across two soil depths (0–20 and 20–40 cm) were evaluated in September 2017. For this, volumetric rings were used to collect samples to determine carbon content and soil density in order to quantify the carbon

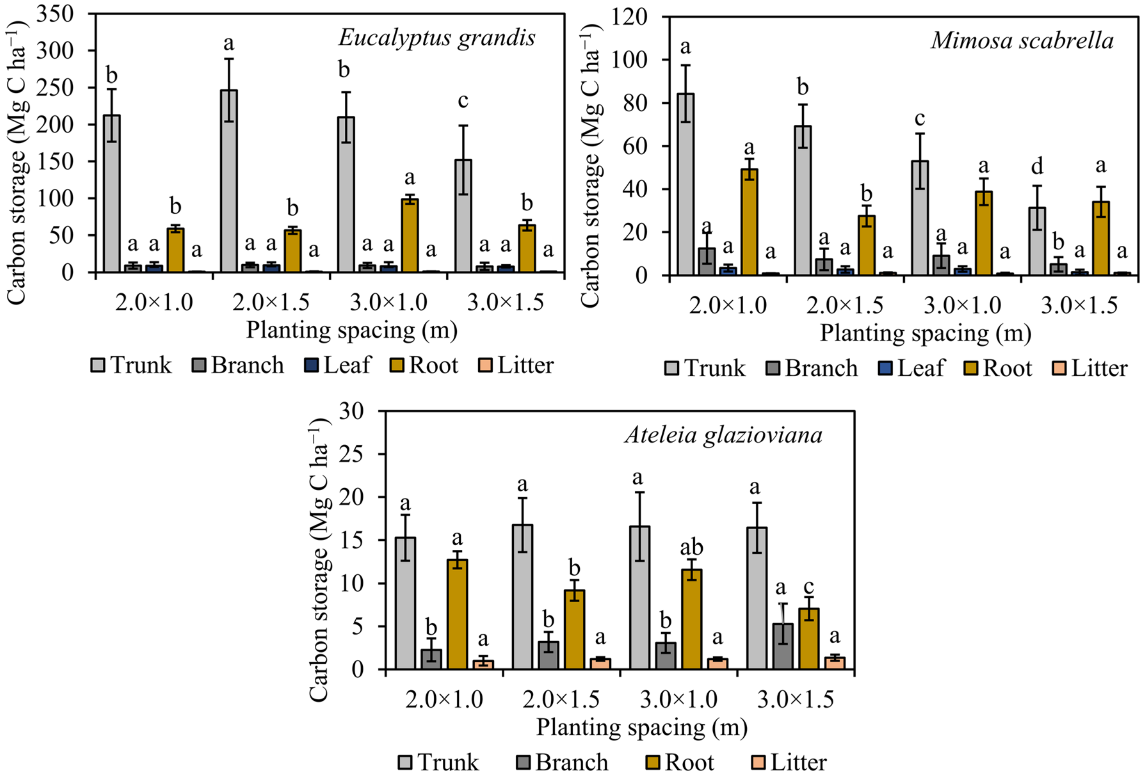
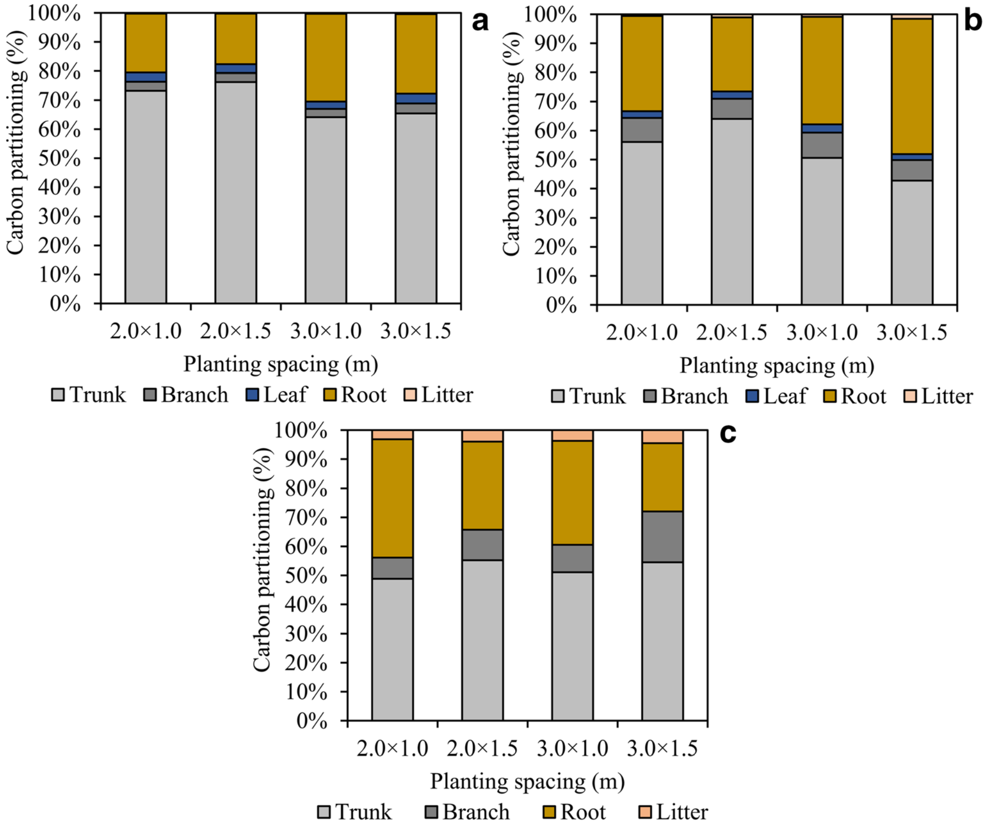


Fig. 2 Carbon storage aboveground and belowground in a short-rotation cycle of *Eucalyptus grandis*, *Mimosa scabrella*, and *Ateleia glazioviana* grown under four planting spacings. Different small letters indicate significant differences ($p < 0.05$) by Tukey test among planting spacings

Fig. 3 Carbon partitioning in forest biomass in a short-rotation cycle of *Eucalyptus grandis* (**a**), *Mimosa scabrella* (**b**), and *Ateleia glazioviana* (**c**) grown under four planting spacings



storage. For each treatment, four repetitions were collected. Also, the soil samples were collected randomly within the plot in order to collect representative and homogeneous samples (Fig. 8 in Annex). Moreover, representative samples of the forest litter above the soil were collected. The litter samples were collected following the same experimental design of soil samples. To these ends, a useful area of 1 m^2 was considered. The litter samples were processed and evaluated according to the same method considered for forest biomass (wood, leaves, and branches), as described in the Section 2.3.

The soil carbon content was determined by the dry combustion method (CHNS/O), using an elemental analyzer, Perkin Elmer model, PE−2400 Series II, which is based on the quantification of CO_2 by infrared, where CO_2 is formed by the oxidation of the organic constituents at a temperature around 1500 °C. The soil samples collected were evaluated in the Forest Biomass Energy Laboratory of the Department of Forestry Engineering and Technology of the Federal University of Paraná (UFPR). The soil carbon stocks were calculated considering the soil density and the layer thickness according to the following equation: $SOC_{stock} = C \times SD \times \rho/10$, where SOC_{stock} is the soil carbon stock (Mg ha^{-1}); C is the soil carbon content (g kg^{-1} soil); SD is the soil density in the layer (g cm^{-3}); and ρ is the soil layer thickness (cm).

2.7 Statistical analysis

The results obtained in this study were statistically analyzed with the software "Statistical Analysis System" (SAS 2002). The analysis of variance (ANOVA) was performed to evaluate the effects of the planting spacing on carbon storage of the forest species and soil carbon storage. Also, differences were considered significant when $p < 0.05$ using Tukey test. The Bartlett test was used to verify the homogeneity of variances, and normality distribution of all data was checked using Shapiro–Wilk test. Additionally, the principal component analysis (PCA) (multivariate approach) was used to identify major patterns of variation and ordination of the elemental composition (C, H, O, N, and S) and properties (Ash, CV, FC, and VM) of the different tree portions. The principal components and biplot graphics were obtained using the PROC PRINCOMP procedure (SAS 2002).

The data used for the PCA were standardized by dividing the difference between each data point and the arithmetic

Table 1 Soil carbon storage for the different forest species grown under four planting spacings at 9 years old

Species	Spacing (m)	Depth (cm)	Soil carbon stock (Mg C ha^{-1})	Standard deviation
Eucalyptus grandis	3.0 × 1.5	0–20	45.860 aA*	0.431
		20–40	39.293 bA	0.748
	3.0 × 1.0	0–20	41.836 aA	0.463
		20–40	32.220 bB	0.844
	2.0 × 1.5	0–20	41.474 aA	0.384
		20–40	34.619 bB	0.961
	2.0 × 1.0	0–20	46.153 aA	0.967
		20–40	33.235 bB	0.756
Mimosa scabrella	3.0 × 1.5	0–20	37.623 aA	0.911
		20–40	31.280 bB	0.651
	3.0 × 1.0	0–20	45.642 aA	0.998
		20–40	34.279 bAB	0.658
	2.0 × 1.5	0–20	44.466 aA	0.455
		20–40	36.806 bA	0.623
	2.0 × 1.0	0–20	43.359 aA	0.539
		20–40	31.066 bB	0.649
Ateleia glazioviana	3.0 × 1.5	0–20	41.451 aA	0.220
		20–40	33.171 bA	0.183
	3.0 × 1.0	0–20	40.456 aA	0.317
		20–40	30.813 bA	0.643
	2.0 × 1.5	0–20	45.789 aA	0.377
		20–40	35.369 bA	0.769
	2.0 × 1.0	0–20	46.728 aA	0.397
		20–40	35.927 bA	0.852

*Different small letters indicate significant differences ($p < 0.05$) by Tukey test among two depths for each planting spacing, and capital letters indicate significant differences by planting spacings of each forest species mean of the variable of interest by the standard deviation of the variable. Two principal component vectors were used for the PCA analysis. Additionally, paired variables with apparent collinearity were excluded from the PCA analysis. The treatments evaluated in this study were coded as follows: W (wood), BA (bark), BR (branch), and L (leaf). For the first PCA, regarding the forest properties, we analyzed four planting spacings which were coded as follows: 1 (2.0 × 1.0 m), 2 (2.0 × 1.5 m), 3 (3.0 × 1.0 m), and 4 (3.0 × 1.5 m). For the second PCA, regarding the elemental composition, we analyzed three assessment years 1 (2009), 2 (2011), and 3 (2013).

3 Results

3.1 Carbon storage and partitioning in forest plantations

The carbon stocks of the forest species (Fig. 2) showed that the planting spacing had significantly effect on carbon storage for the different tree portions (Table 2 in Annex). Also, the soil carbon stock was influenced significantly by the two depths evaluated 0–20 cm and 20–40 cm (Table 3 in Annex). For the planting spacings studied, we observed a significant difference in the carbon stored in the soil for the forest species *Eucalyptus grandis* and *Mimosa scabrella*.

The carbon stock of the forest plantations was evaluated considering the carbon stored in the aboveground (trunk, branches, and leaves) and belowground (roots) parts of the trees. The average carbon content, considering an average of all planting spacings, for the three forest species and tree portions had the following pattern: *Eucalyptus grandis* leaves (48.2%), trunk (45.3%), branches (43.4%), and roots (42.6%); *Mimosa scabrella* leaves (46.7%), trunk (45.6%), branches (45.2%), and roots (44.7%); and *Ateleia glazioviana* trunk (45.0%), branches (46.5%), and roots (43.5%).

The values of carbon stock for the short-rotation cycle are presented in Fig. 2. We observed a significant difference on carbon storage of the forest species *Eucalyptus grandis* and *Mimosa scabrella* according the planting spacing studied. For *Ateleia glazioviana*, no significant difference was observed for carbon stored under different planting spacings.

The largest amount of carbon stored was obtained for *Eucalyptus grandis* at planting spacing 2.0 × 1.5 m, which was 327.1 Mg C ha^{-1}. In relative terms, 76.2% of the total carbon stored refers to the trunk, 3.1% branch, 2.9% leaf, 0.4% litter and 17.4% roots (Fig. 3). Furthermore, the widest planting spacing (3.0 × 1.5 m) stored 29% less carbon in forest biomass compared with the 2.0 × 1.5 m planting spacing. The average amount of carbon stored for *Eucalyptus grandis* was 293.2 Mg C ha^{-1}.

The *Mimosa scabrella* species showed a different response compared with that observed for *Eucalyptus grandis*. Higher

Fig. 4 Carbon partitioning in the system (tree biomass + soil) in a short-rotation cycle of *Eucalyptus grandis* (**a**), *Mimosa scabrella* (**b**), and *Ateleia glazioviana* (**c**) grown under four planting spacings

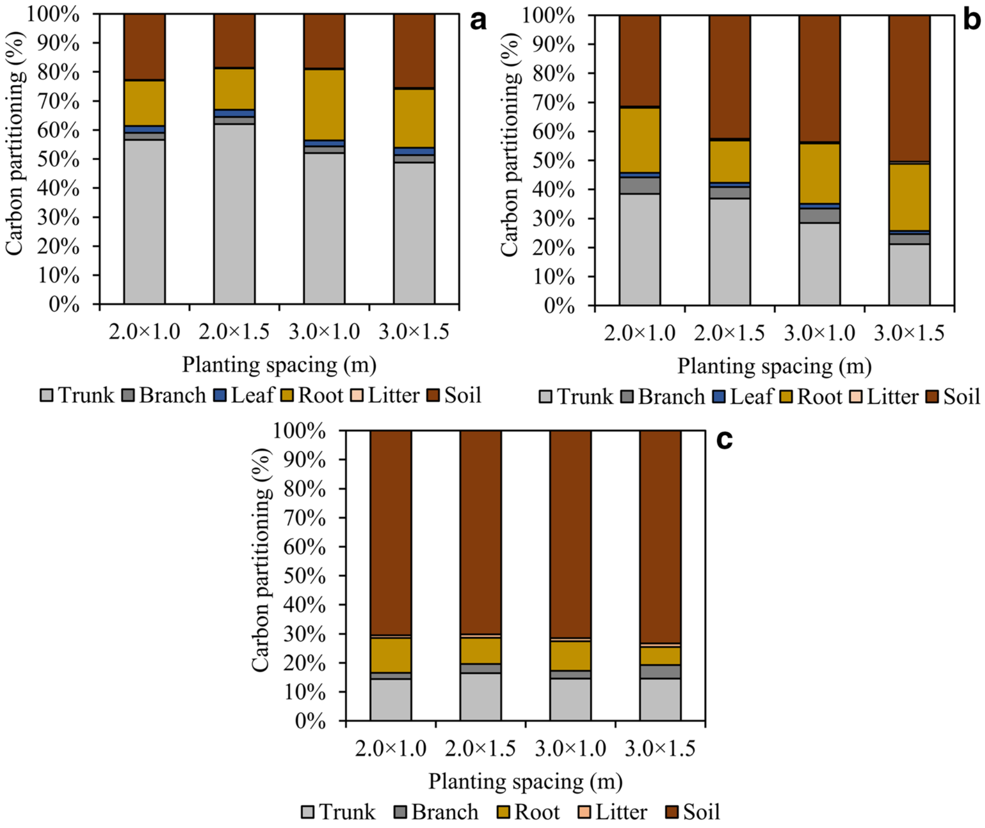


Fig. 5 PCA with biplot showing forest species properties grown under four planting spacings (previously identified). The variables fixed carbon (FC), volatile material (VM), ash content (Ash), and calorific value (CV) are indicated by arrows, while the four tree portions W (wood), BA (bark), BR (branch), and L (leaf) and four planting spacings are indicated as points 1 (2.0 × 1.0 m), 2 (2.0 × 1.5 m), 3 (3.0 × 1.0 m), and 4 (3.0 × 1.5 m)

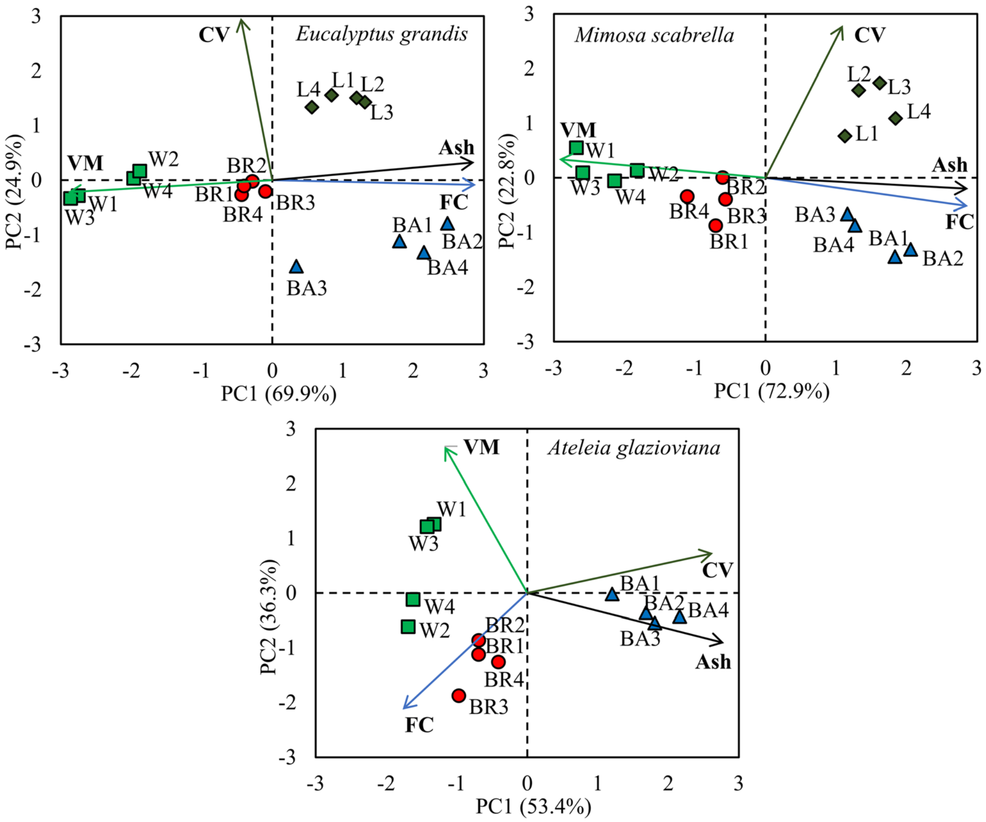
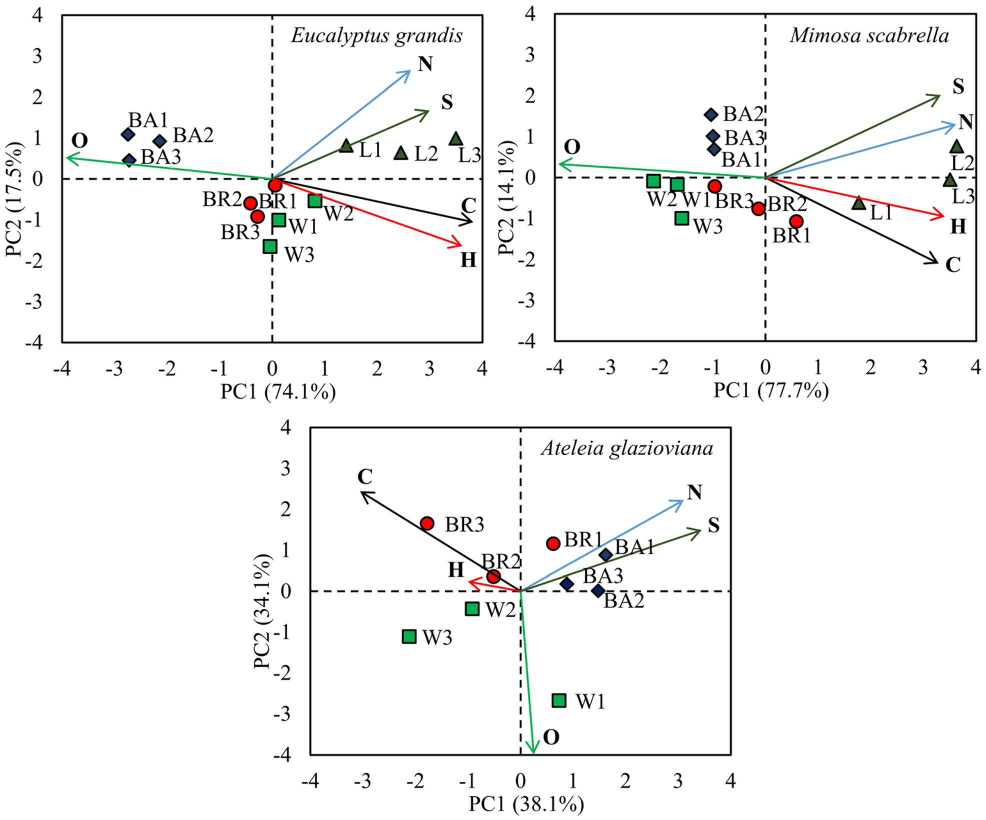


Fig. 6 PCA with biplot showing the elemental composition of the different forest species. The elementary components carbon (C), hydrogen (H), oxygen (O), nitrogen (N), and sulfur (S) are indicated by arrows, while the four tree portions W (wood), BA (bark), BR (branch), and L (leaf) and three assessment years are indicated as points 1 (2009), 2 (2011), and 3 (2013)



amount of carbon stored were obtained in the reduced planting spacing 2.0 × 1.0 m, which represented an accumulated value of 150.4 Mg C ha^{-1}. A proportional reduction in carbon stored was observed with increasing planting spacing. For instance, the wide planting spacing 3.0 × 1.5 m stored 51.3% less carbon compared with the 2.0 × 1.0 m spacing. The average amount of carbon stored for *Mimosa scabrella* considering all planting spacings studied was 109.1 Mg C ha^{-1}. Regarding the carbon storage specifically for the trunk portion, trends were similar to the total carbon storage considering all tree portions, i.e., trees conducted under wider spacings had lesser carbon stored for *Eucalyptus grandis* and *Mimosa scabrella* species, while no significant differences were observed for *Ateleia glazioviana*.

All the forest species showed higher values of the carbon storage in the following pattern: trunk > roots > branches > leaves > litter (Fig. 3). For the *Eucalyptus grandis,* we observed that 69.7% of the carbon stored in the forest biomass was allocated in the trunk; while the other forest species presented average values of 52.9%. Also, we found an average partitioning of 23.8, 35.4, and 32.5% for *Eucalyptus grandis*, *Mimosa scabrella*, and *Ateleia glazioviana*, respectively, for the carbon allocated in the roots. The branches, leaves, and litter had little contribution to the carbon stored compared with the trunk and root.

The values of soil carbon stock for the forest species grown under different planting spacing are showed in Table 1. We observed a significant difference on soil carbon stock for the two depths evaluated. The soil carbon stock obtained in this study for the two depths evaluated ranged from 37.6 to 46.7 Mg ha^{-1} (0–20 cm) and from 30.8 to 39.3 Mg ha^{-1} (20–40 cm), considering all forest species and planting spacings. This result demonstrates high vertical variability of the soil carbon stock. Considering the average of the soil carbon stock for all forest species and planting spacing, we observed values of 43.4 Mg C ha^{-1} (0–20 cm) and 34.0 Mg C ha^{-1} (20–40 cm), i. e., a reduction of 21.7% of the C stored in the deepest layer.

For the trees grown under different planting spacings variations in soil carbon stock were observed for the forest species *Eucalyptus grandis* and *Mimosa scabrella*. Variations were observed only for the soil depth 20–40 cm, and no response pattern was observed between planting spacings. For *Eucalyptus grandis* trees, the average accumulated soil carbon stock was 78.7 Mg C ha^{-1} (0–40 cm), considering all planting spacings. For *Mimosa scabrella*, an average of 76.1 Mg C ha^{-1} (0–40 cm) was obtained, while *Ateleia glazioviana* showed values of 77.4 Mg C ha^{-1}. These results demonstrated that the growth of different forest species under different planting spacings in a rotation cycle of 9 years was not sufficient to cause variations in the topsoil carbon stocks.

The carbon partitioning in the forest system (soil + tree biomass) are showed in Fig. 4. We observed an average of 54.8%, 18.7%, and 21.4%, for the *Eucalyptus grandis* trunk, roots, and soil, respectively. The other tree portions accounted for the remainder. For the forest species *Mimosa scabrella* and *Ateleia glazioviana*, a greater contribution of the soil was observed in the total carbon stock in the forest plantation system compared with the *Eucalyptus grandis* species. This response was related to the lower potential of these species into produce biomass and consequently to store carbon both aboveground and belowground. For *Mimosa scabrella* species, 31.2% of the carbon was partitioned to trunk, 20.2% to roots, and 42.0 to soil, while for *Ateleia glazioviana*, the partitioning pattern was 15%, 9.4%, and 71.3%, in the same order (trunk, root, and soil).

3.2 Forest species composition and energetic properties

Main PCA results of forest species properties grown under four planting spacings are presented in Fig. 5. The *Eucalyptus grandis* PCA analysis indicated that primary and secondary components were responsible for, respectively, 69.9% and 24.9% of the cumulated variance for all investigated tree portions and planting spacing. For the other species, the primary and secondary components were responsible for, respectively, 72.9% and 22.8% for *Mimosa scabrella* and 53.4% and 36.6% for *Ateleia glazioviana*. In general, it was observed that for the three species studied, the principal component analysis allowed the explanation of more than 90% of the cumulated variance for all investigated tree portions and planting spacings.

For the *Eucalyptus grandis* energetic properties, PC1 was associated with fixed carbon and ash content in contrast with volatile material, while PC2 was associated especially with calorific value. This same response was observed for the forest species *Mimosa scabrella*. Regarding the tree portions, we observed that leaves were strongly associated with calorific value and ash content. On other hand, the wood and branches were associated with volatile material in contrast with fixed carbon. Also, the bark was characterized to be strongly associated with fixed carbon.

Regarding the *Ateleia glazioviana* forest species, PC1 was associated with calorific value and ash content in contrast with fixed carbon, while PC2 was associated especially with volatile material. For the tree portions, the wood was strongly associated with fixed carbon and volatile material in contrast with ash content. Also, the bark was characterized to be strongly associated with ash content and calorific value. Another important point that we need to emphasize is that the planting spacing had not influence on *Eucalyptus grandis* and *Mimosa scabrella* species properties since they presented a similar pattern of response (Fig. 5). For *Ateleia glazioviana* species, we observed variation according the planting spacing, which trees grown at the planting spacings (2.0 × 1.0 m and

3.0 × 1.0 m) showed more volatile material and trees grown in the other two planting spacings presented more fixed carbon.

For the elemental composition of the forest species studied, the *Eucalyptus grandis* PCA analysis indicated that primary and secondary components were responsible for, respectively, 74.1% and 17.5% of the cumulated variance for all studied tree portions and assessment years (Fig. 6). For *Mimosa scabrella* species, this pattern was 77.7% and 14.1%, and for *Ateleia glazioviana*, it was 38.1% and 34.1%.

We observed similar pattern for the *Eucalyptus grandis* and *Mimosa scabrella* species regarding the elemental composition, where PC1 was associated with C and H in contrast with O, while PC2 was associated especially with S and N. For the *Ateleia glazioviana*, we observed that PC1 was associated with N and S in contrast with O, while PC2 was associated especially with O in contrast with C. Similarly to the pattern observed for the forest properties, the assessment years had no significative influence on forest species composition. For the tree portions, the wood was associated with C and H in contrast with O.

4 Discussion

This study showed that the planting spacing has significant influence on forest carbon stocks. Reduced planting spacings promoted higher carbon stocks for the forest species *Eucalyptus grandis* and *Mimosa scabrella*. On the other hand, the *Ateleia glazioviana* species showed no response pattern. Besides that, significant differences in the potential of carbon storage among forest species were observed. Such differences may be related to the ability of each forest species in acquiring available resources, efficiency of resource conversion into biomass and forest stand uniformity (Stape et al. 2010; Gonçalves et al. 2013). Also, differences in morphology, anatomical features, and growth behavior could also contribute to the differences observed on carbon stocks between forest species (Binkley et al. 2013; Forrester 2019; Le Maire et al. 2019). However, it is important highlight that the aim of this study was not to compare forest species. The main aim was to assess the potential of each forest species in storing carbon under different planting spacing and to characterize the wood composition.

4.1 Planting spacing affects the carbon storage and partitioning of forest plantations

The findings of this study show that carbon stocks and forest biomass partitioning changed according the planting spacing and forest species studied. Differences in the potential of carbon stocks among forest species can be related with the capacity of each forest species to produce forest biomass. Moreover, *Eucalyptus grandis* showed the greater amount of carbon stored compared with the other forest species studied. This can be related with the greatest investment in genetic improvement and edaphoclimatic adaptation (soil and climatic conditions) of this forest species which is globally used (Stape et al. 2010; Gonçalves et al. 2013; Flores et al. 2016).

Analyzing the carbon stocks for each forest species grown at different planting spacing, we can highlight that the use of reduced planting spacing provides larger carbon stock when compared with those commonly used by the forest producers (3.0 × 1.5 m). This result demonstrates that reduced planting spacing can be an important alternative to improve carbon stocks in forest plantations. However, the authors highlight that new studies are needed to confirm the technical, operational, and economic feasibility of using reduced planting spacing in forest energy plantations.

The carbon stock above-belowground of the forest species are related with the potential of the forest produce biomass and the carbon content of each tree portion. Regarding the carbon stored aboveground and belowground, we observed an average partition of 76.2 and 23.8%, respectively, for the *Eucalyptus grandis*; 64.58 and 35.42% for the *Mimosa scabrella*; and 67.47 and 32.53 for the *Ateleia glazioviana* species. The results found in the present study agree with that obtained by Ribeiro et al. (2015), who reported that the tree carbon stock in the stand level for the above-belowground parts were 64.2% and 35.8%, respectively. These values are within the carbon stock range for *Eucalyptus* plantations. For instance, in a stand of *Eucalyptus* in Brazil, Paixão et al. (2006) found an aboveground carbon stock of 76.4% and a belowground carbon stock of 23.6%.

Regarding the carbon content of the three forest species studied for the different tree portions, we observed similar pattern with those observed for Ribeiro et al. (2015), who reported for the *Eucalyptus grandis* species average carbon contents of 46.1%, 44.6%, 42.9%, and 37.8% for leaves, trunk, branches, and roots, respectively. Our results and those found by Ribeiro et al. (2015) suggest there are differences in carbon content among different tree portions. Several studies use a biomass conversion factor ($C = 0.50$) as average carbon content of the forest species (Brown et al. 1986). Therefore, we recommend caution in the use of this conversion factor, since there may be overestimation or underestimation depending on the portion of the tree as well as the species studied. IPCC (2006) recommends that in the absence of specific carbon content values, a default carbon content of 47% should be used to estimate the carbon fraction in the aboveground forest biomass.

4.2 Soil carbon stocks and partitioning (biomass + soil) in forest plantations

The soil carbon content presented a decaying trend with increasing soil depth, such as observed in other studies (Jobbágy and Jackson 2000; Sheikh et al. 2009; Salton et al. 2011; Zinn et al. 2012; Morais et al. 2017).

The soil carbon stocks observed in this study are within the values of soil carbon stocks in forest plantations worldwide. Gasparini and Di Cosmo (2015), studying the carbon stock in the biomass and the soil of native forests in Italy, found values between 20 and 110 Mg ha^{-1} of carbon stored at the biomass and between 70 and 90 Mg ha^{-1} of carbon stored at the soil. Our findings are in agreement with the results from Laclau (2003), who found levels of soil carbon from 80 to 100 Mg ha^{-1}. The results reported here emphasize the importance of soil in storing carbon in the forest systems. According to Pulrolnik (2007), 92% of the soil carbon stock in the *Eucalyptus* forest is concentrated up to 1-m deep and only 8% in the litter.

Our study showed that planting spacing and the forest species studied did not influence the carbon stock in the soil. This result may be related to two main reasons. The first one is related to the age of the forest stand and the change in soil use. To perform the experiment, implantation was needed to prepare the soil (harrowing and plowing) for planting the forest seedlings. These soil preparation and change in the soil use may be modified and homogenized the soil carbon stocks. Therefore, the duration of the forest species growth (9 years—2008 to 2017) was not sufficient to modify the carbon stock of the soil, because this reason, both planting spacing and forest species, showed no pattern and difference in the carbon stock.

This result agrees with that observed by Paul et al. (2003), who reported that forest plantation with *Eucalyptus grandis* and *Eucalyptus globulus* generated a decrease of carbon up to 30 cm in the first 10 years, increasing only from 10 to 14 years of age. The reduction was associated with the impact of the soil management practices to perform the forest implantation and that can be reestablished when the forest begins to stabilize and allows the significant return of carbon from the forest biomass cycling.

The second reason is related to the depth of the soil layer evaluated. In this study, we evaluated two layers (0–20 cm and 20–40 cm), which were defined in order to contemplate the soil layer that contains most of the carbon stored. However, due to the great interaction between forest litter and carbon stock in the superficial layers (0–5 cm and 5–10 cm), we believe that the 0 − 20 cm used in our study was not adequate to capture the effect of these superficial layers. Therefore, no differences were found in soil stock carbon in the different spacings and species studied.

The higher soil carbon stock in the topsoil may be related with the accumulation of vegetal residue, the amount of organic matter, the root activity, and the microorganism's activity (Lal 2005; Babujia et al. 2010; Dawud et al. 2016; Ahmed et al. 2019). This result agrees with that observed by Lima (2004), who reported the highest carbon content in the 0–5-cm layer in *Eucalyptus* forest compared with pasture, mostly justified by the presence of forest litter and microorganism's activity. Moreover, short-rotation forest plantations without any nutritional enrichment can cause carbon loss (Turner and Lambert 2000). Therefore, to increase carbon stocks in the soil, some forest management strategies need to be adopted including site preparation, species management/selection, use of fertilizers, and soil amendments.

When considered the forest carbon stock of the whole system (forest biomass + soil), we can highlight that, as there was no difference in soil carbon storage for the different species (Table 1), the total carbon partition in the forest system was basically related to the amount of carbon stored in the above-belowground forest biomass. From this, it was possible to highlight that in plantations with *Eucalyptus* species, the accumulation of carbon in the aboveground was greater than that stored in the soil. On the other hand, the species *Mimosa scabrella* and *Ateleia glazioviana* presented a greater amount of carbon stored in the soil, which is justified by the low potential in storing carbon in both above-belowground biomass.

Evaluating the total carbon stock in the soil-plant system in *Eucalyptus* plantations in Minas Gerais, Gatto et al. (2010) observed a partition of 29.0% (64.1 Mg ha^{-1}) to the wood; 16.0% (34.9 Mg ha^{-1}) to the crop residues; and 55% (122.7 Mg ha^{-1}) to the soil. Also, these authors highlighted that soils under fast-growing forest plantations in tropical regions can be considered the largest drain for C stock in the soil-plant system. This result is different from that observed in the present study, where a higher contribution of forest biomass compared with the soil was observed in *Eucalyptus* plantations for sub-tropical region. This response may be related with the climate conditions (Stape et al. 2010). Brazil forestry chain assumes a privileged position as one of the few countries in the world with the appropriate climate and technological conditions for forest energy production (Gonçalves et al. 2008).

4.3 Forest biomass composition and properties affect energy yield

The forest biomass that provides better results for energy generation has low moisture and ash content and high wood density, C content, and heating value (Labrecque et al. 1997; Klasnja et al. 2002; Eloy et al. 2014). According to Cardoso et al. (2015), several traits have an effect on biomass characterization for energy generation, such as the moisture content, wood density, heating values, ash, and C content. Moreover, several factors may modify the anatomical structure and properties of the wood, such as genetics and silvicultural practices (irrigation, fertilization) and the environment (soil, temperature, rainfall) (Raymond 2002; Tharakan et al. 2005). In forest energy plantations, the species, planting density, rotation length, and management practices may influence both wood yield and quality, affecting its ability to generate energy (Labrecque et al. 1997; Tharakan et al. 2003, 2005; Eloy et al. 2014).

The study and characterization of the properties and composition of a given forest-based biomass fuel is the initial and most important step during the investigation and application of such fuel. Our study highlights that as important as the amount of biomass produced, the forest biomass characterization is of essential importance since the forest species composition and energetic properties can influence the potential for energy generation.

Elemental composition is one of the most important characteristics for biomass utilization, when the purpose is energy generation. The results presented in this study indicate that forest species properties and composition did not vary substantially with planting spacing and age of the forest system (Figs. 5, 6). However, for the different tree portions, clear differences were observed among the elemental composition and properties. The elemental contents showed that the wood of the forest species contain higher proportion of carbon content compared with hydrogen and oxygen, which increase the energy value of the feedstock. This result was more evident for the *Eucalyptus grandis* species.

In this context, when it is intended to produce biomass for energy, it is desirable that the wood presents high levels of carbon and hydrogen and low levels of oxygen and ash content, which was observed in this study. The wood of the forest species presented high volatile matter and reduced values of ash content (Fig. 5). The higher amount of ash in biomass makes it less desirable as fuel (Demirbas and Demirbas 2009).

Information generated in this study highlight that all forest species studied presented suitable features to produce energy, such as energy properties and elemental composition (especially the forest species *Eucalyptus grandis*), and they could be recommended for energy plantations under reduced planting spacing. It is important to highlight that the recommendations suggested in this study are for short-rotation forestry plantations. The extrapolation of this information for long-term forest rotations needs to be considered with caution.

According to the results obtained in this study for short-rotation forestry plantation, it is possible to make the following final remarks: (i) findings obtained in this study indicate optimal planting spacings that are smaller than the current ongoing pattern, which is 3.0 × 1.5 m. Thus, forest managers can manipulate the planting spacing to provide greater amount of forest biomass per unit of area which is the feedstock to produce energy; (ii) our results can be used by industries and forest producers interested in producing forest biomass for energy; (iii) forest biomass use of the native species *Mimosa scabrella* and *Ateleia glazioviana* can play an important role in the regional energy supply; (iv) this study demonstrated that the different forest species and planting spacing did not affect the carbon stocks in the soil. We observed that the carbon stocks in the soil ranged only between soil layers; (v) the "carbon credits" program can provide a way for farmers to generate revenue, improving farmers profit by considering (forest biomass for energy + carbon pricing) and also reducing atmospheric carbon dioxide levels; and (vi) future studies should focus in evaluate the long-term impacts of repeated short-rotation plantations on soil C storage and soil traits.

5 Conclusion

The use of reduced planting spacing in forest systems was an important strategy to increase the C storage in the above-belowground biomass. The hypothesis of this study was confirmed since forest managers can accelerate growth and increase the forest carbon storage and biomass yield by using adequate planting spacing. All results indicate optimal planting spacings that are smaller than the current pattern used by the majority of the forest producers, which is 3.0 × 1.5 m. For *Eucalyptus grandis* and *Mimosa scabrella*, the planting spacings recommended to produce biomass and improve the carbon stocks were 2.0 × 1.5 and 2.0 × 1.0, respectively, while for *Ateleia glazioviana*, it was not possible to indicate the most appropriate spacing.

The pattern of carbon stored in the tree portions were trunk > roots > branches > leaves > litter. For the carbon stocks in the soil, we observed an average accumulated carbon stock for the forest species studied of 77.4 Mg C ha^{-1} (0–40 cm).

The characterization and use of forest biomass as energy source play an essential role in order to reduce the use of fossil-fuel-based energy. Forest species studied presented suitable features to produce energy related to energy properties and elemental composition. For instance, the *Eucalyptus grandis* wood was characterized to present high levels of carbon and hydrogen and low percentages of oxygen.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards Human images (Figs. 7d and 8a) are of authors.

Conflict of Interest The authors declare that they have no conflict of interest.

Annexes

Fig. 7 Roots assessments to determine belowground biomass based on root excavation (**a** and **b**), cleaning (**c**), and root weighing (**d**) using dynamometer balance

Fig. 8 Soil sampling at two depths 0–20 and 20–40 cm to quantify the soil carbon storage. Samples collection (**a** and **b**) and volumetric rings used (**c** and **d**)

Table 2 Analysis of variance for the carbon storage aboveground and belowground in a short-rotation cycle of *Eucalyptus grandis*, *Mimosa scabrella*, and *Ateleia glazioviana* grown under four planting spacings

Factor	DF	Mean square				
		Eucalyptus grandis				
		Trunk	Branches	Leaves	Roots	Litter
Planting spacing	3	10,457.99*	8.18^{ns}	6.56^{ns}	1271.05*	0.08^{ns}
Block	2	17,419.15	117.28	70.15	168.82	0.45
R^2	-	0.41	0.61	0.52	0.91	0.62
CV	-	27.91	26.69	25.90	10.06	24.63
		Mimosa scabrella				
Planting spacing	3	2312.46*	47.37*	3.57^{ns}	101.23*	0.07^{ns}
Block	2	1002.14	17.35	1.32	182.64	0.09
R^2	-	0.56	0.27	0.30	0.93	0.42
CV	-	27.89	35.51	42.50	13.34	18.20
		Ateleia glazioviana				
Planting spacing	3	6.95^{ns}	11.45*	-	1.91*	0.028^{ns}
Block	2	145.96	0.89	-	0.18	0.47
R^2	-	0.50	0.43	-	0.67	0.86
CV	-	16.83	38.90	-	13.94	8.06

*Significant at 5% of error probability; ns, non-significant at 5% of error probability; R^2 , coefficient of determination; DF, degrees of freedom; CV, coefficient of variation

Table 3 Analysis of variance for the soil carbon storage at two depths 0–20 and 20–40 cm for the different forest species grown under four planting spacings

	Mean square				
	Eucalyptus grandis				
Factor	Planting spacing (P)	Soil depth (S)	P*S	R^2	CV
Soil carbon stock	93.31*	1496.18*	33.61^{ns}	0.47	16.06
	Mean square				
	Mimosa scabrella				
Factor	Planting spacing (P)	Soil Depth (S)	P*S	R^2	CV
Soil carbon stock	143.94^{ns}	1595.36*	36.89^{ns}	0.41	19.25
	Mean square				
	Ateleia glazioviana				
Factor	Planting spacing (P)	Soil depth (S)	$P{*}S^{ns}$	R^2	CV
Soil carbon stock	$130.56^{\ ns}$	1723.73*	5.58	0.51	17.28

*Significant at 5% of error probability; ns, non-significant at 5% of error probability; R^2 , coefficient of determination; CV, coefficient of variation

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