School of Earth, Environmental, and Marine Sciences Faculty Publications and Presentations

College of Sciences

5-30-2023

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Article

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Abstract: Tree residue removal from Eucalyptus plantations after timber harvest can reduce soil functioning by reducing the organic matter input. To assess the effects of residue management systems (RMS) on soil aggregation, carbon (C) and nitrogen (N) content, and biological activities, a field trial was conducted in a commercial Eucalyptus plantation (loamy sand soil) in Mato Grosso do Sul, Brazil. The study assessed three RMS: cut-to-length (CTL), tree-length (TL), and bare litter (BL), respectively. After 21 months, undisturbed soil samples were collected and physically isolated into three aggregate-size fractions: large macroaggregates (LM), medium macroaggregates (SM), and microaggregates (MI). Results showed that these soils are mostly composed of LM (54%), and that removing harvest residues from the growing site included total organic carbon (TOC) by 28%, microbial biomass-C by 20%, fluorescein diacetate hydrolysis by 17%, and β-glucosidase activity by 26%, when compared to CTL. TL outperformed CTL for the proportion of LM and LM-associated TOC. Across fractions, a higher microbial quotient was observed in SM and MI fractions, suggesting that the TOC has higher stability inside the LM. This study suggests that leaving harvest residues on the soil should be recommended for Eucalyptus plantations, especially in low-fertility sandy soils, as it helps in maintaining the soil structure and biological activities critical for soil health and ecosystem function.

Keywords: sandy soils; soil health; aggregate stability; soil enzyme activity

1. Introduction

The global abundance of Eucalyptus plantations underscores their high species diversity and ecological adaptability, combined with their high production and rapid growth, even on sandy soils [1]. Such soils are typically infertile and cannot maintain adequate soil organic matter (SOM) levels [2], thereby requiring management practices that promote OM accumulation and biological activities as well as improve soil fertility [3–5].

For sustainable agriculture in sandy soils, physical and chemical properties can be preserved through reduced tillage or no-till systems, crop residue management, carbon inputs, and increased macro-aggregation and total porosity [2,6,7]. Understandably, harvesting methods can impose substantial differences in the amounts of OM deposited onto the soil surface.

The "cut-to-length" system dominates as the most common eucalyptus harvesting method in Brazil [8], returning the most tree residues to the soil as trees are delimbed in situ. By leaving all harvest residues on the ground, this boosts soil OM levels, biological activities, nutrient cycling, and soil structure. On the other hand, the industry has identified that removing some (tree-length) or all residues (bare litter) facilitates mechanical operations, reduces costs, and increases the operational efficiency of the business [8].

Tree-length harvest returns a moderate level of Eucalyptus residues to the soil, as they cut the trees and semi-process on-site to remove the pointer and bark, leaving on the field leaves, branches, and litter. The most intensive system, "bare litter", removes all trees from the site to be delimbed outside of the cultivation area at processing facilities. By removing all harvest residues from the site, only litter fall remains on the forest floor through the current cycle.

When the non-commercial components of the eucalyptus logs are maintained in the field, nutrient cycling through litter decomposition and forest harvest residues give the soils a great potential to meet the tree's nutritional demands, especially those of calcium and magnesium [4,9,10]. After harvesting, eucalyptus residues offer a source of OM and nutrients for the following eucalyptus growth cycles, apart from protecting the soil, increasing the water retention capacity, and preventing compaction, erosion, and direct sunlight exposure [11].

Organic matter improves soil aggregation, mainly in sandy soils, but soil aggregate stabilization is only possible through coupling organic material with mineral particles, which can be introduced by roots, fungal hyphae, or other microbial combinations [12,13]. A higher soil aggregate stability improves structural stability, and facilitates the movement of water, solutes, microorganisms, gases, and plant root growth, thereby benefiting the soil functions [14,15].

Biochemical attributes and microbial activities can serve as indicators of soil functioning. The microbial quotient (qMic) indicates the percentage of total organic carbon in the soil, while the metabolic quotient (qCO$_2$) shows the specific respiration rates of microbial biomass [16]. In this way, stressed soils typically have higher qCO$_2$ values [17], while areas with low organic compound stocks have corresponding low values of the microbial quotient [18].

Aside from the importance of maintaining harvest residues to ensure the entry of OM, it is also essential to maintain the soil structure, the carbon storage, and soil aggregates under different habitats (such as aerobic and anaerobic microsites) and support the activities of a diverse microbial community [19,20].

The aggregate size has a significant influence on microbiological activities, for example, in sandy soils under native pasture, basal respiration is higher in 250–4000 μm than in <250 μm aggregates [21]. Other studies have also stated increased basal respiration and microbial biomass carbon at greater soil aggregate sizes [22,23].

In this context, we hypothesize that residue management systems (RMSs) that maintain residual biomass including leaves, branches, and barks, on the soil surface after harvest, such as the cut-to-length (CTL) and tree-length (TL), contribute to improving the soil organic matter levels, biological activities, nutrient cycling, and soil structure, along with removing all harvest residues, such as in the bare litter method, reducing inputs of the organic material and consequently decreasing organic matter (OM) and nutrient contents. Thus, the RMSs CTL and TL, for increasing the OM and nutrient supply, are assumed to reduce the exposure and loss of C and N, which remain within the aggregates and stimulate microbial and enzymatic activities.

Given how fragile the structure of sandy soils is, and how relevant soil aggregates are for carbon storage and biological activities, the assessment of biological activities in soil aggregate samples is a relevant approach to demonstrate how small changes inside these aggregates due to management practices can significantly impact soil carbon and soil biology. The eucalyptus industry is increasingly opting for processing trees outside the growing site, resulting in no tree residues left on the soil. This practice reduces the amount of C input and nutrients that are returned to the soil. The objective of this research was to assess the impact of removing harvest residues from Eucalyptus harvest sites on soil health indicators assessed in soil aggregate samples, as compared to retaining tree residues on-site after harvest. Through this assessment of biological activities in soil aggregates, we aimed to provide new insights into the biological activity and soil C storage of sandy soils under tropical climate, and identify the best management practices to ensure the sustainability of

the eucalyptus industry and the health of the surrounding ecosystems. Our field trial in a commercial Eucalyptus farm compared a removal gradient of tree residues, and assessed indicators that play critical roles in soil health such as: (i) soil aggregation, (ii) carbon and nitrogen storage, and (iii) biological activities through microbial and enzymatic assays.

2. Materials and Methods

2.1. Location and Management History

In 2017, we established a field trial in the district of Água Clara—Mato Grosso do Sul State (latitude 20°38′57.22″ S, longitude 52°35′50.74″ W, 370 m asl), within a commercial *Eucalyptus urograndis* hybrid (*E. urophylla × E. grandis*) plantation (Figure 1). According to the Köppen classification, the regional climate was tropical with a dry winter and a rainy summer (Aw), with an annual mean rainfall of around 1370 mm at an average of 24.4 °C [24].
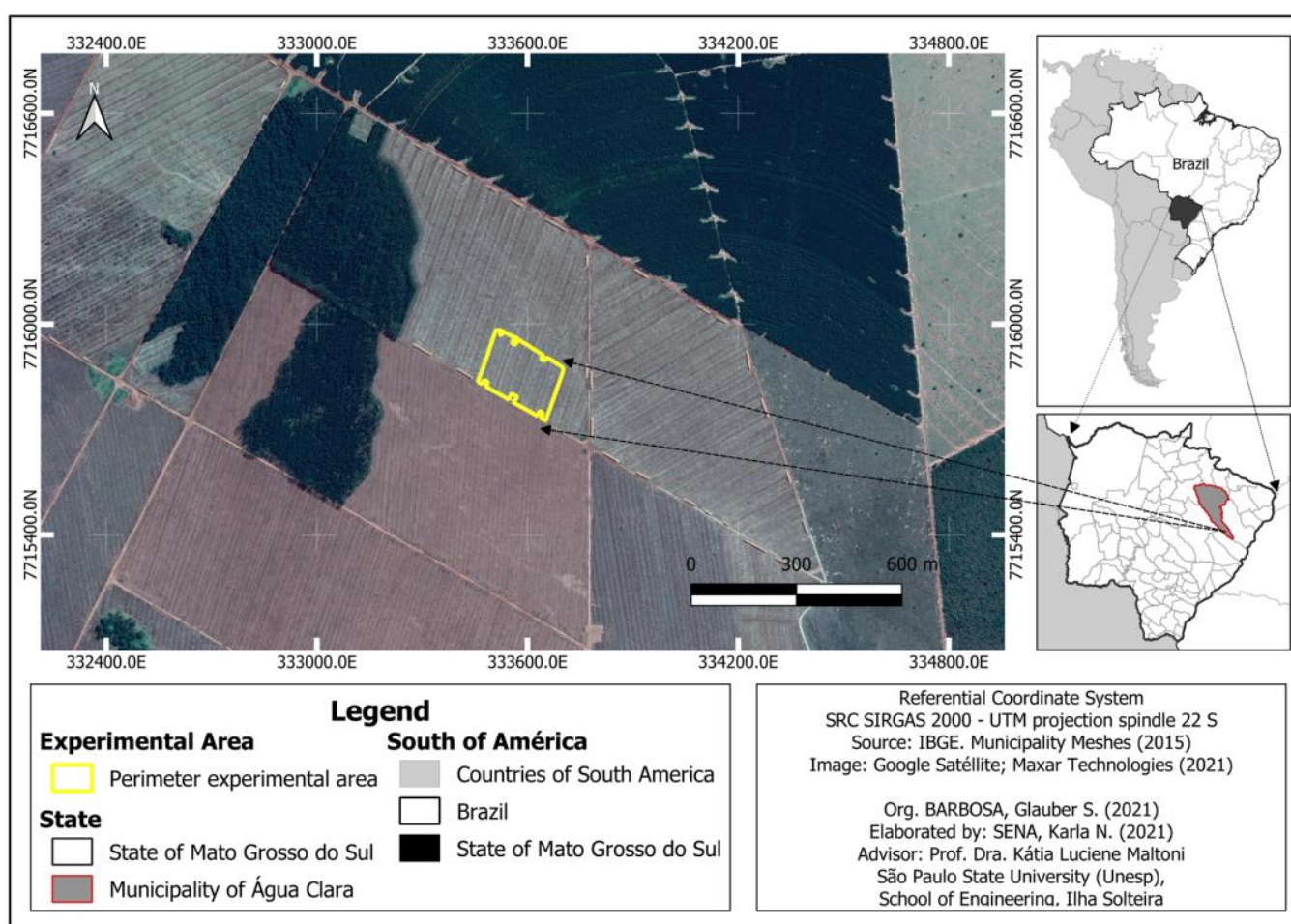

Figure 1. Location of the perimeter experimental area.

The soil of the experimental area was a sandy Arenosol [25], with a loamy sand textural class (sand contents of 843–878 g kg^{-1}), originally covered by Cerrado *sensu stricto* (savanna-like woodland) [26]. At the time of evaluation, the trees were 1.7 years old.

2.2. Treatments and Experimental Design

The first cycle of 6-year-old Eucalyptus in the experimental area was cut in June 2017. The trees were harvested in the cut-to-length system, and at the end of September 2017 (120 days after harvest), the experimental plots were prepared, simulating the RMSs described below: cut-to-length (CTL)–maintenance of all residues (leaves, branches, bark, and litter) at the felling site, C:N ratio = 31; tree-length (TL)—maintenance of all residues except for the bark (leaves, branches, and litter), C:N ratio = 22; and bare litter (BL)—removal of all residues, i.e., the area was cleaned by sweeping, and only received plant biomass from the litterfall of the current cycle.

The experiment was conducted in a completely randomized design, with four replicates in the 0.00–0.10 m layer. Each management systems plot (40.8 × 27.6 m, i.e., 1126 m^2) had 12 tree rows, and were spaced 3.4 m between rows and 2.3 m between plants. Within each plot, we considered the eight central rows (500.8 m^2) for evaluations, thereby avoiding the border effects and interference from the neighboring areas.

Evaluations occurred 21 months after planting, in August 2019. The soil was sampled and analyzed to determine the effects of different eucalypt RMSs, their influence on the contents of nitrogen and organic carbon, and the microbiological and enzymatic activities of the soil.

2.3. Sampling and Aggregates from Dry Sieve Preparation

Undisturbed soil samples were collected in August 2019, in each plot. Four intact soil cores (0.10 m height × 0.10 m length × 0.10 m width) were taken at 3 m intervals from the center of each plot, and 0.00–0.10 m from the surface layer.

To minimize aggregate disruption, each core was manually removed and sieved through a 4.00 mm sieve crumbling along the natural fracture lines. According to [27,28], the fractionation procedure was performed with four composite samples in each treatment, consisting of three subsamples from the 0.00–0.10 m layer, and each composite sample was air-dried and then stored at room temperature.

All subsamples were shaken through sieves for 2 min, resulting in large macroaggregates that were retained from the 2.00 mm sieve, medium macroaggregates that were retained from the 0.250 mm sieve, and microaggregates that passed through the 0.250 mm sieve. The soil aggregates were then spread out in a thin layer and set to be air-dried. In summary, the subsamples were separated into large macroaggregates (LM: 4.00–2.00 mm), medium macroaggregates (SM: 2.00–0.25 mm), and microaggregates (MI: 0.250–0.00 mm), respectively.

2.4. Sand Correction of Aggregates

The sand correction procedure consisted of a combined 2 g of aggregate sample (LM, SM, and MI) with 2 mL of sodium hydroxide and 20 mL of deionized water, followed by shaking for 16 h on a horizontal shaker. After that, samples were passed through the 250 and 53 μm sieves for the macroaggregate sample, and only the 53 μm sieve for the micro-aggregate sample. All obtained fractions were oven dried at 60 °C and thus, the sand associated aggregate mass was subtracted from the initial aggregate amount, as indicated by Equation (1), resulting in sand free soil aggregate:

$$\text{Sand free soil aggregate (g) = aggregate fraction mass} - \text{sand associated aggregate mass} \tag{1}$$

where aggregate fraction mass represents the mass of the fraction obtained in physical fractionation, and sand associated aggregate mass represents the sand weight in the aggregate sample.

2.5. Soil Aggregates Respiration Measurement and Microbial Biomass Carbon (MBC)

The air-dried aggregates (20 g dry weight) of each size class were placed in a thin and loose layer at the bottom of 100 mL jars, with three replicates. The moisture content was adjusted to 40% of the water holding capacity, and the samples were incubated in the dark at 25 °C for 24 h. Small vials with 5 mL of 1 M NaOH were placed in incubation jars to trap CO_2 after adding distilled water to the soil. In addition, three incubation jars containing only NaOH were used as blanks to correct for the CO_2 trapped inside the jars. The basal respiration of soil aggregates (BSR—μg CO_2-C g^{-1} dry soil day^{-1}) was estimated by titrating each jar with NaOH and 2 mL 1 M $BaCl_2$ (1:1) with 0.1 M HCl and phenolphthalein indicator (1% *w/v* in ethanol).

The soil microbial biomass carbon (MBC—μg C g^{-1} dry soil) of each aggregate size was determined using the chloroform-fumigation-extraction method described by [29]. After fumigation, the microbial carbon was extracted with 50 mL of 0.5 M K_2SO_4 from all the samples. The amount of MBC was determined by assessing the difference between the carbon extracted from the fumigation and non-fumigation controls using a coefficient (Kc) of 0.33 [29].

The metabolic quotient ($q CO_2$) was obtained by the ratio between the values of released CO_2-C and MBC (μg^1 CO_2-C soil day^{-1} of μg^{-1} MBC), according to [30]. The microbial quotient (qMic) was obtained by the ratio between the MBC and the soil total organic carbon (MBC/TOC) $\times$ 100 and was expressed as a percentage [31].

2.6. Enzyme Activity

Beta-Glucosidase (β-Glu) activity was determined according to [32], with the exception that toluene was omitted from our assays. Analyzes were conducted in triplicate with a non-substrate control. The method conducted was based on a colorimetric determination of the *p*-nitrophenol released by this soil enzyme when 1 g of soil sample is incubated with buffered substrate solutions. A total of 1 mL of 0.05 M *p*-nitrophenyl-a-D-Glucosidase (PNG) buffered solution was used as the substrate. The *p*-nitrophenol released was extracted by filtration and was measured using a microplate reader (BioTek Instruments, Winooski, VT, USA) set at a wavelength of 410 nm.

The fluorescein diacetate hydrolytic activity (FDA) was determined using the method described by [33]. The FDA hydrolysis assay can provide an estimate of the overall microbial activity in the soil sample by measuring it in a wide variety of enzymes, including esterases, proteases, and lipases [34]. We used 1.0 g of soil aggregates, air-dried, with 20 mL of 60 mM sodium phosphate buffer (pH 7.6), and mixed for 15 min at 100 rpm. Then, we added 100 μL of 4.9 mM FDA substrate solution (20 mg 3'6'-diacetyl-fluorescein in 10 mL acetone), and mixed for 1 h and 45 min at 100 rpm placed at 37 °C. After incubation, acetone (20 mL) was added and mixed into the suspension, which was centrifuged, and the supernatant was filtered through a Whatman No. 4 paper filter. Standard curves were generated using serial dilutions of a standard solution containing 0.5 g fluorescein sodium salt (Sigma F6377; $C_2H_{10}O_5Na_2$) in 500 mL distilled water. Samples and standards were determined with absorbance readings using a microplate reader (BioTek Instruments, Winooski, VT, USA) fixed at a wavelength of 490 nm.

2.7. Total Carbon and Nitrogen

For each soil aggregate fraction sample in each RMS, approximately 2 g of dry soil was finely ground and subsampled for total carbon (TC) and total nitrogen (TN) determined by combustion on an elemental analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA).

2.8. Active Carbon

Active C assessed through the amount of oxidized soil organic matter (SOM) has also been used to estimate the size of the labile C pool. Analysis was adapted from the method of [35], and cited by [36]. Briefly, 20 mL of 0.02 mol L^{-1} KMnO$_4$ was added to 50 mL screw-top centrifuge tubes containing 2.5 g air-dried soil. The tubes were shaken for 2 min at 240 oscillations min^{-1} and allowed to settle for 10 min. Following this, 0.5 mL of the supernatant was transferred into a second 50 mL centrifuge tube and mixed with 49.5 mL of deionized water. The sample absorbance was read with a spectrophotometer at 550 nm and active C (mg kg^{-1} soil) was calculated as Equation (2):

$$\text{Active C} \left(\text{mg kg}^{-1}\text{soil}\right) = \left[0.02 \text{ mol L}^{-1} \times (a + b \times \text{Abs})\right] \times \left(9000 \text{ mg C mol}^{-1}\right) \times \left(0.02 \text{ L solution kg}^{-1} \text{ soil}\right) \quad (2)$$

where 0.02 mol L^{-1} is the initial concentration of the KMnO$_4$ solution, a is the intercept of the standard curve, b is the slope of the standard curve, Abs is the absorbance of the sample, 9000 mg is the amount of C oxidized by 1 mol of MnO$_4$, with Mn$_7{}^+$ getting reduced to Mn$_4{}^+$, and 0.02 L is the volume of KMnO$_4$ solution that reacted with the soil. The samples consisted of soil aggregates and are expressed in kg.

2.9. Statistical Analyzes

The results were analyzed with the analysis of variance (ANOVA), followed by the Tukey test at $p < 0.05$, for means comparison. The relationship between the aggregate diameter classes (AgDi) and the harvest residue management systems (RMSs) was computed using the mean and standard error (SE) in R software [37].

3. Results

The statistical analysis revealed that most variables (MBC; qCO$_2$; qMic; TN; TOC; FDA; β-Glu; and active carbon) showed significant F-values for the impact of the residue management systems (RMSs), except for BSR. Moreover, significant F-values were observed for all variables concerning the effect of the aggregate diameter (AgDi) and the interaction between the RMS and AgDi (RMS × AgDi). The soil consisted of over 78% ± 8% in the largest macroaggregate class (LM), 3 ± 0.6% of SM, and 19 ± 8% of MI, respectively (Figure 2). The wide variation of each aggregate diameter significantly influenced the response of the evaluated properties ($p < 0.001$) (Table 1). Mean weight diameter estimated based on the proportion of each aggregate size particle [38] was 1795 μm in TL, 1600 μm in CTL, and 1531 μm in BL, respectively.

To better understand the use of energy resources by the microbial biomass, we compared the basal soil respiration (BSR), which uses CO$_2$ as a proxy for respiration, to the microbial biomass carbon (MBC), which denotes the size of the microbial community present, and defined the qCO$_2$ as BSR/MBC. A significant interaction was found between the RMS and AgDi for the aggregate classes MI and SM, respectively. Under TL, the qCO$_2$ in MI exceeded that than under BL and CTL three and four times, respectively, and was the highest qCO$_2$ observed, with no variations in LM between the RMSs. Higher qCO$_2$ values indicate a lower efficiency of the microorganisms in the use of organic material for energy [39], which was observed in the MI class under TL (Table 1), while the highest efficiency of microbial performance was observed in the SM class under BL and TL.
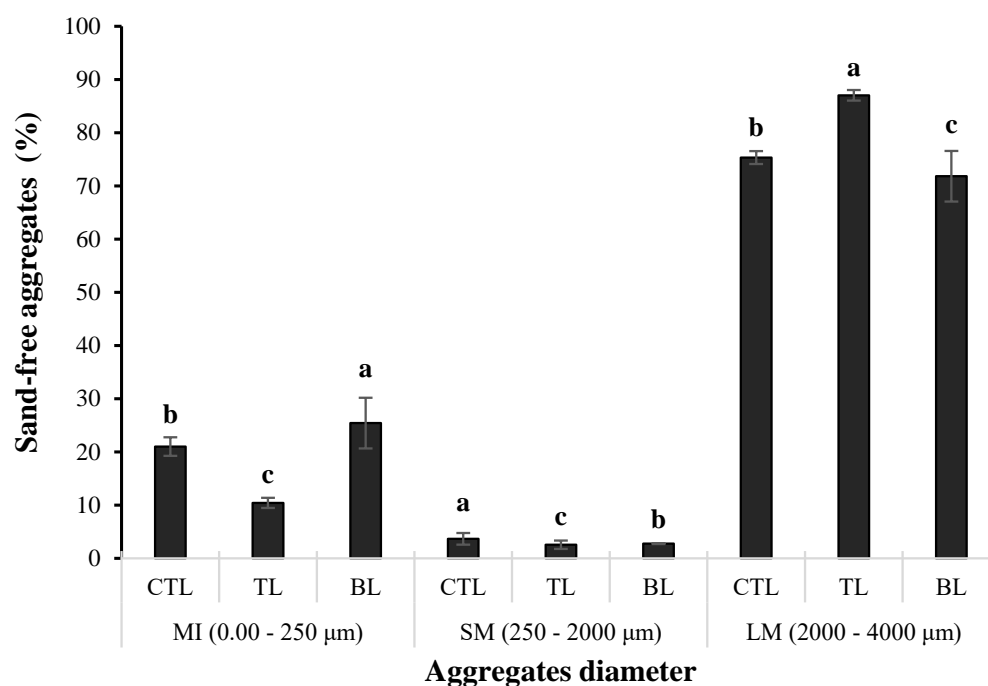
Figure 2. The proportion of each soil aggregate class within the whole soil, classified by the aggregate diameter for three *Eucalyptus urograndis* harvest residue management systems. Error bars represent the standard errors (*n* = 4). Bars followed by different lowercase letters within each aggregate diameter were found to be significantly different by the Tukey test, at $p \leq 0.05$. Abbreviations: LM, large macroaggregates (4.00–2.00 mm); SM, medium macroaggregates (2.00–0.25 mm); MI, microaggregates (0.250–0.00 mm), CTL, cut-to-length (maintenance of all harvest residues); TL, tree-length (removal of the bark and retention of the other residues); and BL, bare litter (removal of all harvest residues and receiving only the plant biomass from the current cycle's litterfall).

Digging deeper into the controlling factors of $q\text{CO}_2$, it was apparent that basal soil respiration (BSR) (Table 1) did not vary significantly across the evaluated RMSs (Table 1). However, AgDi and RMS × AgDi interactions revealed that while MI released on average 4.27 ± 0.92 μg CO_2 per gram of whole soil daily, LM respired four times as much, and SM only one-quarter of this amount (Table 1). For the MI class, BSR was the highest under BL, and LM respired most under TL, where 24% and 61% more CO_2-C was released than in CTL and BL, respectively. SM BSR did not differ across the RMSs.

The MBC varied for each RMS, AgDi, and RMS × AgDi interaction (Table 1). While LM retained the most carbon density and SM the least under each RMS, MI MBC decreased from a median range in CTL and BL to indistinguishable levels from SM under TL with a five times lower mean. Under the two RMSs where vegetation remains on the surface (CTL and TL) to LM, the MBC values were approximately 20% higher than with the complete residue removal under BL.

To see where excess organic matter remains available to the microbial community, the MBC/TOC ratio defines the $q\text{Mic}$. The MI $q\text{Mic}$ of the CTL as well as the BL, was found to significantly exceed that under TL by 27% on average. For the SM, BL had a significantly higher $q\text{Mic}$ than the RMS with residues, dwarfing CTL by 59% and 19% over TL, respectively. The lowest $q\text{Mic}$ was observed in LM (Table 1).

Table 1. Partitioning of the interaction between the Eucalyptus harvest residues management systems (RMSs) and the sandy free aggregate diameter (AgDi) for soil properties (whole soil).

Properties	RMS	AgDi		
		MI	**SM**	**LM**
		0–250	250–2000	2000–4000
TN (mg N g^{-1} soil)	CTL	0.08 bA	0.01 cA	0.55 aB
	TL	0.03 bA	0.01 bA	0.66 aA
	BL	0.08 bA	0.01 cA	0.47 aC
TOC (mg C g^{-1} soil) *	CTL	1.35 bA	0.27 bA	10.99 aB
	TL	0.55 bA	0.14 bA	13.67 aA
	BL	1.24 bA	0.17 bA	8.54 aC
TOC (mg C g^{-1} aggregate) *	CTL	7.42 bA	6.44 bA	14.59 aA
	TL	6.16 bA	5.29 bA	15.70 aA
	BL	7.04 bA	5.08 bA	13.98 aA
BSR (µg C-CO$_2$ g^{-1} soil day $^{-1}$)	CTL	4.51 bB	0.74 cA	15.33 aB
	TL	2.32 bC	0.48 cA	19.35 aA
	BL	5.99 bA	0.54 cA	15.50 aB
MBC (µg C g $^{-1}$ soil)	CTL	256.45 bA	32.81 cA	475.59 aA
	TL	53.80 bB	46.19 bA	500.60 aA
	BL	234.56 bA	55.82 cA	393.30 aB
qCO$_2$ (µg^1 CO$_2$-C soil day^{-1} of µg^{-1} MBC)	CTL	0.019 bB	0.023 abA	0.032 aA
	TL	0.094 aA	0.010 cB	0.040 bA
	BL	0.028 bB	0.010 cB	0.042 aA
qMic (%)	CTL	19.97 aA	13.05 bC	4.08 cA
	TL	14.05 bB	26.77 aB	3.56 cA
	BL	18.91 bA	32.99 aA	4.25 cA
FDA (mg fluorescein g^{-1} soil h^{-1})	CTL	6.41 bA	1.34 cA	31.43 aA
	TL	2.93 bA	0.70 bA	33.98 aA
	BL	6.86 bA	0.83 cA	26.77 aB
β-Glu (mg PNP g^{-1} soil h^{-1})	CTL	5.16 bA	1.75 cA	31.68 aA
	TL	2.12 bB	0.96 bA	28.79 aB
	BL	5.09 bA	1.09 cA	24.98 aC
Active Carbon (mg C g^{-1} soil)	CTL	0.299 bB	0.522 cA	1.072 aB
	TL	0.148 bC	0.364 cC	1.239 aA
	BL	0.361 bA	0.394 cB	1.022 aC

Averages followed by the same lowercase letters in the row for AgDi and uppercase letters in the column for between harvest RMS do not statistically differ, as determined by the Tukey test, $p > 0.05$. Abbreviations: BSR, basal soil respiration; MBC, microbial biomass carbon; qCO$_2$, metabolic quotient; qMic, microbial quotient; TN, total Nitrogen; TOC, total organic carbon; FDA, fluorescein diacetate hydrolytic activity, β-Glu, β-Glucosidase; CTL—cut-to-length, maintenance of all residues; TL, tree-length, removal of the bark and maintenance of the other residues; and BL, bare litter, removal of all residues and receiving only the plant biomass from the current cycle's litterfall. * TOC (mg C g^{-1} aggregate) represents the C concentration of an aggregate fraction, while TOC (mg C g^{-1} soil) represents the C concentration of an aggregate fraction corrected by the fraction's proportion within the whole soil.

This directs insight towards nutrient levels, revealing that the contents of TOC and TN varied significantly different across the RMSs and AgDi, especially in the LM class. Twenty-one months after setting up the experiment, the harvest residues left on the surface significantly changed the TOC and TN contents only in the aggregates of the LM class, varying as TL > CTL > BL (Table 1). The TOC pattern differed only slightly from that of TN, with LM dominating MI by 90% and 88% respectively, and by 98% for both elements in SM, although TOC MI and SM were statistically indistinguishable (Table 1).

The total soil enzymatic activity (FDA), based on the measurement of fluorescein diacetate hydrolysis was not directly influenced by the different RMSs (Table 1); however, the RMS $\times$ AgDi interaction revealed a significant deviation of 15% less FDA under BL in the LM class, and generally CTL and TL presented the highest activities (31.43 and 33.98 mg fluorescein g^{-1} soil h^{-1}, respectively), again grouping where the harvest residues remained on the soil.

The activity of the β-Glu enzyme responded to the RMSs in that the highest activity occurred in LM under CTL, where all eucalyptus harvest residues, including the bark, persisted on the surface (Table 1). The total removal of the harvest residues in BL halted β-Glu activity by 13% and 21% compared to TL and CTL, respectively, in this AgDi. The limited processing in the TL RMS lowered β-Glu activity by 10% compared to CTL.

The FDA and β-Glu activities under the different RMSs were lowest in the SM, except under TL where MI and SM did not differ for either enzyme (Table 1). Nevertheless, comparing the proportions of MI and LM in the soil, accounting for approximately 20% and 75% of the sand-free aggregates, respectively, clearly shows the relevance of MI.

Soil active carbon differed significantly for RMSs, AgDi, and their interactions (RMS $\times$ AgDi) across all aggregate diameters. In each RMS, the active carbon contents were highest in the LM and lowest in the SM, with MI at an intermediate content. Importantly, active C in the LM was 20 times higher than in the SM, which reinforces the need to maintain the soil structure, ensuring that carbon remains readily available. In MI, BL had a higher active carbon content than CTL (17%) and TL (60%) (Table 1). In SM, the active carbon contents followed CTL > BL > TL, with decreases of 24 and 30%, respectively. LM was highest under the TL RMS and decreased by 13% under CTL and 18% under BL, respectively. These results emphasize the differences in soil carbon storage. Since active carbon is readily available carbon, a higher content in LM may be associated with a greater amount of labile C (Table 1).

4. Discussion

4.1. Availability and C:N Ratio of Residues Affect C Cycle-Related Indicators

The management of harvest residues greatly influences carbon cycling in the soil. Large macroaggregates (LM) constitute 70–85% of sand-free soil, followed by microaggregates (MI) and small macroaggregates (SM), which make up 10–25% and 2–5% of the soil, respectively. The bare litter (BL) system, which removes all the harvest residues from the soil surface, exhibited the lowest proportion of LM, resulting in a decrease in the carbon content compared to the CTL and TL systems, which maintained some harvest residue. Aggregate-associated carbon is protected from decomposition, and in loamy and clayey soils, aggregates represent an average of 60% of total soil, and can store up to 54 kg per hectare of carbon [40]. In contrast, aggregates only represent 13% of the total soil in sandy soils, storing substantially less carbon at an average of 18 kg per hectare [41]. In this study, the share of C inside the LM reached 6.8 tons C ha^{-1} under BL (top 5 cm), representing a reduction of 22.3% and 37.5% compared to CTL and TL, respectively.

For sandy soils, this indicates that even small decreases in the proportion of aggregates or the concentration of carbon inside the aggregates can result in proportionally large losses of soil carbon. After harvest, tree residual biomass serves as the primary carbon source for soil microbes. Additionally, higher microbial and enzymatic activities were expected in LM for CTL and TL than BL due to the known improvements in soil structure resulting from the increased organic matter input on the soil. Indeed, biological activities in LM, such as microbial biomass carbon (MCB), active carbon, and enzyme activities (FDA and β-Glu), were found to have also slowed down under BL, as demonstrated by β-Glu activity in the decreasing order of CTL > TL > BL (36.62, 28.79, and 24.98 mg pNP g^{-1} soil h^{-1}, respectively). Under similar climatic conditions, removing forest residues resulted in a 50% reduction in soil microbial respiration and a 40% reduction in MBC contents, respectively [3]. Our results also align with the findings of [21], who reported that aggregate size significantly affects microbiological activities in native pasture on sandy soil (loamy

sand texture), where BSR was higher in LM and SM than in MI. Other studies have also reported increases in BSR and MBC with increasing aggregate size [22,23]. Based on these responses, the CTL and TL systems maintain soil health and functioning better than BL, as the residue input stimulates microbial-related processes, thereby promoting nutrient cycling and primary productivity, in addition to soil conservation provided by the surface coverage [42].

While removing all residues decreased total organic carbon (TOC) contents and slowed down biological activities, the presence of bark residue may have caused similar effects. TL (all residues minus bark) outperformed CTL (all residues including bark) for the proportion of LM (by 13.45%) and the LM's concentration of TOC and total nitrogen (TN) by 19.6% and 16.7%, respectively. Similarly, most biological activities in the LM were enhanced in TL compared to CTL, namely basal soil respiration (BSR) (by 21%), MBC (by 5%), FDA (by 7.5%), and active C (by 13.5%). The slightly faster decomposition of the soil organic material (C:N ratio = 22) contributed to the increase in the activity of the microorganisms, which participate more actively in the soil aggregate process through fungus hyphae and bacterial filaments, as well as by the presence of binding substances [43,44]. However, in the CTL, a slightly slower decomposition (C:N ratio = 33) was observed due to the presence of the bark, meaning this effect was not so intense. Aggregate formation is normally associated with the stabilization of organic carbon inside aggregates, where it is protected from decomposition, and in this situation it was possible to observe increments in organic carbon and aggregate size [45]. The expected faster decomposition of residues under TL may have resulted in an enhanced release of binding agents, contributing to the larger proportion of LM under TL compared to CTL.

The stability of aggregate-associated C in sandy soils of tropical climates is unknown. Estimating the long-term stability of aggregate-associate C would require considering turnover rates of aggregates [46], and the environmental drivers of soil organic matter dynamics. Identifying sustainable soil management practices is therefore critical to addressing long-term C storage [20].

4.2. Stability and C Accumulation in Soil Aggregates

Across all resource management systems (RMS), it was found that the proportion of SM and MI was on average 9.6 and 1.8% lower than in LM, respectively. Moreover, TOC concentration in SM and MI was, on average, 50% lower than in LM. Considering the proportion of each aggregate size class and their respective TOC concentration, the amount of TOC associated with LM was 57 and 10 times greater than that associated with MI and SM, respectively. The microbial quotients (qMic) can serve as indicators to describe the relationship between the amount of microbial biomass carbon (MBC) and the soil TOC [39,47]. Our results indicate that MI and SM had qMic's around 5.3 times larger than LM. In the LM fraction, there was no significant difference in the qMic observed between the treatments. High qMic values indicate that a large proportion of the TOC is actively cycled by the microbes, which can lead to increased CO_2 emissions and decreased soil carbon sequestration, showing the vulnerability of these fractions to C losses [48].

This highlights the vulnerability of these fractions to carbon losses [41] and supports the idea that large aggregate size fractions can help protect and stabilize soil carbon. The present findings support the conclusions of [49], who conducted a mechanistic study demonstrating a direct correlation between the C input and total organic carbon (TOC) levels. Furthermore, they showed that C inputs from high input systems are more likely to be sequestered in larger aggregates, including both large and small macroaggregates.

When comparing different RMSs, TL had the lowest proportion of MI and the largest proportion of LM. This indicates that the MI may have been used in the formation of LM in this system [6]. On the other hand, in BL, where no residues were left on the soil, there may have been a breakdown of LM, as this system had the highest proportion of MI. These results suggest that the presence of these residues can help stabilize LM by promoting the formation of new aggregates, while the absence of residues may lead to the breakdown of

existing LM [20]. Therefore, resource management practices that promote the formation and stabilization of LM, such as TL, should be encouraged to improve the soil structure and enhance soil health.

5. Conclusions

Identifying sustainable soil management practices is critical to addressing long-term issues of environmental degradation and global climate change. This study provides valuable insights into the stability of aggregates and carbon cycling in sandy soils under Eucalyptus plantations in tropical climates. Our research reveals that a significant proportion (about 54%) of these soils are made up of large macroaggregates (LM, 2000–4000 μm), which rely on harvest residues for their formation and stabilization. When trees were processed off-site and all harvest residues were removed, as in the bare litter treatment, we observed a significant decline in LM-associated total organic carbon (TOC), microbial biomass carbon, FDA, β-glucosidase activity, and active C after 21 months, compared to treatments where harvest residues were left on site. Removing these residues also led to an increase in the amount of microaggregates (MI, 0–25 μm) in the soil, indicating that some of the MI was not used to build LM, or that the absence of residue input contributed to the breakdown of LM. Our analysis of aggregate soil fractions revealed that LM was the most effective in protecting and stabilizing soil C, as evidenced by the lowest microbial quotient (*q*Mic), which was about one order of magnitude lower than MI and small macroaggregates (SM, 25–2000 μm). These findings demonstrate that the preserving of these residues on site (i.e., CTL) offers a greatly improved option for sandy soils in the West-Center of Brazil. Our results also indicate that the CTL has a great potential to improve soil biological conditions and the efficacy of nutrient cycling from residues, as verified by the microbial enzyme soil. Therefore, maintaining the harvest residues on site is essential to promote the formation of LM and the stabilization of soil C in sandy soils under Eucalyptus plantations, and the adoption of CTL can significantly contribute to sustainable soil management practices.

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