



# Litter removal impacts on soil biodiversity and eucalypt plantation development in the seasonal tropics

Jonas Inkotte<sup>1</sup> · Barbara Bomfim<sup>2</sup> · Sarah Camelo da Silva<sup>1</sup> ·  
Marco Bruno Xavier Valadão<sup>1</sup> · Márcio Gonçalves da Rosa<sup>3</sup> · Roberta Batista Viana<sup>1</sup> ·  
Alcides Gatto<sup>1</sup> · Reginaldo S. Pereira<sup>1</sup>

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**Abstract** The little layer of tree plantations provides primary nutrients for uptake, buffers changes in soil moisture, and provides habitat and substrate to soil epigeic fauna. However, this layer in eucalypt plantations is often removed to reduce fuel load during the fire season in the Brazilian savanna (Cerrado). Therefore, it is necessary to quantify the effects of changes in litter dynamics on the function of these plantations, on key nutrient cycling processes and on epigeic fauna diversity and abundance. In two adjacent stands (one juvenile and one mature), the consequences of two years of litter removal were quantified as monthly litterfall, leaf and fine wood litter decomposition, epigeic fauna abundance and diversity, soil biogeochemical variables, and tree diameter and basal area increments. Monthly litterfall rates in juvenile and mature stands did not change with litter removal over the study period. Annual litterfall ranged from

4.1 to 4.9 Mg ha<sup>-1</sup>a<sup>-1</sup> in litter removal plots and from 3.9 to 4.8 Mg ha<sup>-1</sup>a<sup>-1</sup> in control plots. Fine wood litter decomposition was slower in litter removal plots compared to controls, while leaf decomposition rates were similar in both. Two years of litter removal in the juvenile stand did not affect topsoil biogeochemical parameters but decreased available phosphorus at 20–40 cm depth relative to controls. In the mature stand, total cation exchange capacity (0–20 cm) was higher in controls (6.4 cmol<sub>c</sub> dm<sup>-3</sup>) relative to litter removal plots (6.3 cmol<sub>c</sub> dm<sup>-3</sup>), while soil moisture (0–40 cm depth) was lower in litter removal (25.45 m<sup>3</sup> m<sup>-3</sup>) compared to control plots (26 m<sup>3</sup> m<sup>-3</sup>) in the dry season. A non-metric multidimensional scaling ordination revealed an increased homogeneity in epigeic fauna where litter was removed. Litterfall, decomposition, diameter increment, four soil physical parameters and fourteen chemical parameters at 0–20 and 20–40 cm depth explained the differences in soil epigeic fauna composition between litter removal and control plots. Diameter increment decreased with litter removal only in the juvenile stand, which had reached its growth peak. The results indicate that removing excess litter to decrease fuel volume can alter soil biodiversity and edaphic conditions that negatively affect nutrient cycling and tree growth.

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✉ Barbara Bomfim  
bbomfim@lbi.gov

- <sup>1</sup> Department of Forestry Engineering, University of Brasilia, Brasília, Distrito Federal, Brazil
- <sup>2</sup> Climate and Ecosystem Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA
- <sup>3</sup> SESI/SENAI Social Service Industry, Santa Catarina State, Chapecó, Brazil

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## Introduction

Eucalyptus is one of the most widely planted tree genera in tropical and subtropical regions worldwide. Success of the numerous species often relates to their adaptation to various environmental conditions in addition to short, highly productive cycles (Turnbull 1999; Castro et al. 2016; Boeno et al.

2020). In Brazil, eucalyptus stands cover almost 7.0 million ha, approximately 77% of all planted areas, providing timber for domestic and international markets, and especially paper and cellulose (IBÁ 2020). However, the increasing demand for forest products from eucalyptus plantations has forced the conversion of native vegetation to monocrops, and combined with land-use changes for agriculture, has led to the degradation of the Brazilian savanna (Cerrado) (Ribas et al. 2016).

The Cerrado is the second largest biome of South America and one of the world's hotspots for biodiversity conservation due to high species endemism and heterogeneity of landscapes (Myers et al. 2000; Morandi et al. 2018). Because approximately 40% of the original Cerrado has been deforested and degraded (Zuin 2020), establishing eucalypt plantations has been a mechanism to meet forest product demands while alleviating the pressure on native forests and savannas (Boulmane et al. 2017). Restoring degraded areas with eucalypt plantations can improve soil productivity, promote nutrient mineralization, and increase water holding capacity (Paulucio et al. 2017; Sena et al. 2017). However, introducing exotic species like eucalyptus as monodominant stands is controversial because of possible negative impacts on soil biodiversity (Souza et al. 2016; Balieiro et al. 2020; Correa et al. 2020).

Eucalyptus plantations established in areas once covered by savanna vegetation alter nutrient cycles due to substantial above- and below-ground changes. Because of changes in vegetation, nutrient inputs via litterfall are altered, leading to shifts in soil fauna composition and diversity (Penã-Penã and Irmeler 2016). This impact on soil biodiversity results from the alteration of a high substrate diversity (i.e., litter from several species) to a single type of substrate favoring certain soil fauna groups and causing a biological imbalance (Baretta et al. 2003). However, studies suggest that eucalypt plantations, compared to other monocropping systems, have less impact on soil fauna communities (Rosa et al. 2015; Souza et al. 2016). For example, soil biological quality was restored eight years after the conversion of bare soil to a eucalyptus plantation (Boeno et al. 2020).

Litterfall is a critical ecosystem process in eucalyptus plantations, acting as a natural nutrient conduit and the main pathway to replenish plant-available nutrients (Giweta 2020). This process has a unique role in the maintenance of eucalypt plantations on low-nutrient soils like latosols, tropical red earth, that cover nearly 40% of the Cerrado (Haridasan 1994). Generally, commercial eucalyptus stands are fertilized during the first two years after planting because the trees acquire nutrients in lower concentrations and in chemically protected forms through their extensive root systems and mycorrhizal symbionts (McMahon et al. 2019). Thus, after initial fertilization, nutrient cycling occurs naturally through litterfall and decomposition throughout stand

development (Schumacher and Vieira 2015). Plant material deposited on the soil surface are decomposed by organisms, which convert their nutrient contents to mineral forms incorporated into the soil for eucalyptus uptake (Pritchett 1979). The litter layer, therefore, provides nutrients for plant uptake, buffers changes in soil water content and temperature, prevents erosion and nutrient leaching, and provides habitat and substrate to soil fauna (Giweta 2020).

Because litter affects many soil processes, it is necessary to assess the consequences of changes in litter dynamics in eucalyptus plantations. Manipulating the litter layer is a direct way to study the effects of litterfall mass and nutrient fluxes on ecosystem processes (Chen et al. 2014). Harvesting litter from managed forests for fuel and for farming has been a common practice in many countries (Zhao et al. 2016). In the Cerrado, removing excess litter to decrease fuel volume is a means of reducing the impact of wildfires (Alvarado et al. 2017; Gomes et al. 2018). The removed litter can be used in nucleation restoration practices for bare soil cover (Reis et al. 2014), improving several soil properties and providing habitat for terrestrial fauna that contribute to restoration. However, understanding how litter production, soil epigeic fauna, and soil physicochemistry interact on eucalyptus plantations are largely unknown (Sayer et al. 2020).

To understand the linkages between soil parameters and litter processes that influence eucalypt plantation development, the effects of litterfall and its decomposition, soil biogeochemistry and epigeic fauna communities were quantified in two stands in the Cerrado. Specifically, the following questions were proposed: (1) Does litter removal affect litter production and decomposition rates in eucalyptus stands? (2) Does litter removal affect soil physicochemical properties such as bulk density, water content, and fertility? (3) Does epigeic fauna respond to litter removal? And (4) Is tree diameter increment affected by litter manipulation? It was hypothesized that: (1) Litter removal would negatively influence litterfall and litter decomposition due to the reduction in nutrient inputs to the soil; (2) Soil bulk density, water content and fertility would be affected by litter removal; (3) Litter removal would negatively impact soil epigeic fauna communities due to habitat disturbance, and (4) Tree growth would be negatively influenced by litter removal.

## Materials and methods

### Site description

This study was carried out at the University of Brasília's experimental research station, Fazenda Água Limpa (15°56'–15°59' S and 47°55'–47°58' W). The property of over 4300 ha has approximately 153.5 ha of eucalyptus plantations. The climate is AW—tropical savanna, according

to Köppen's classification, with an annual average rainfall of 1552 mm and well-defined dry and rainy seasons. Monthly mean rainfall ranges from 9 mm in June to 249 mm in December (Nimer 1989). Oxisols (USDA classification system) or red latosols (EMBRAPA classification system) are the main soil order with a small area of yellow latosol (EMBRAPA classification system). Soils are highly acidic with low nutrient (e.g., phosphorus) availability (Haridasan 2000).

Two adjacent eucalyptus stands less than 1 km apart were selected. The mature plantation (3.3 ha) was established January 2010 and was 8 years old when this study began. *Eucalyptus urophylla* ST Blake  $\times$  *E. grandis* Hill ex-Maiden was the mature stand at 3  $\times$  2 m with tillage to 40 cm and 100 g of super simple phosphate and 100 g of NPK (4–30–16) applied (Fig. 1a). The juvenile plantation (23.0 ha) was established in 2013 with the same clonal hybrid at 3  $\times$  3 m spacing. Before planting, subsoiling was carried out to a 70 cm depth, and 600 kg ha<sup>-1</sup> of super simple phosphate applied in pits with 200 g per well 15 cm away from the

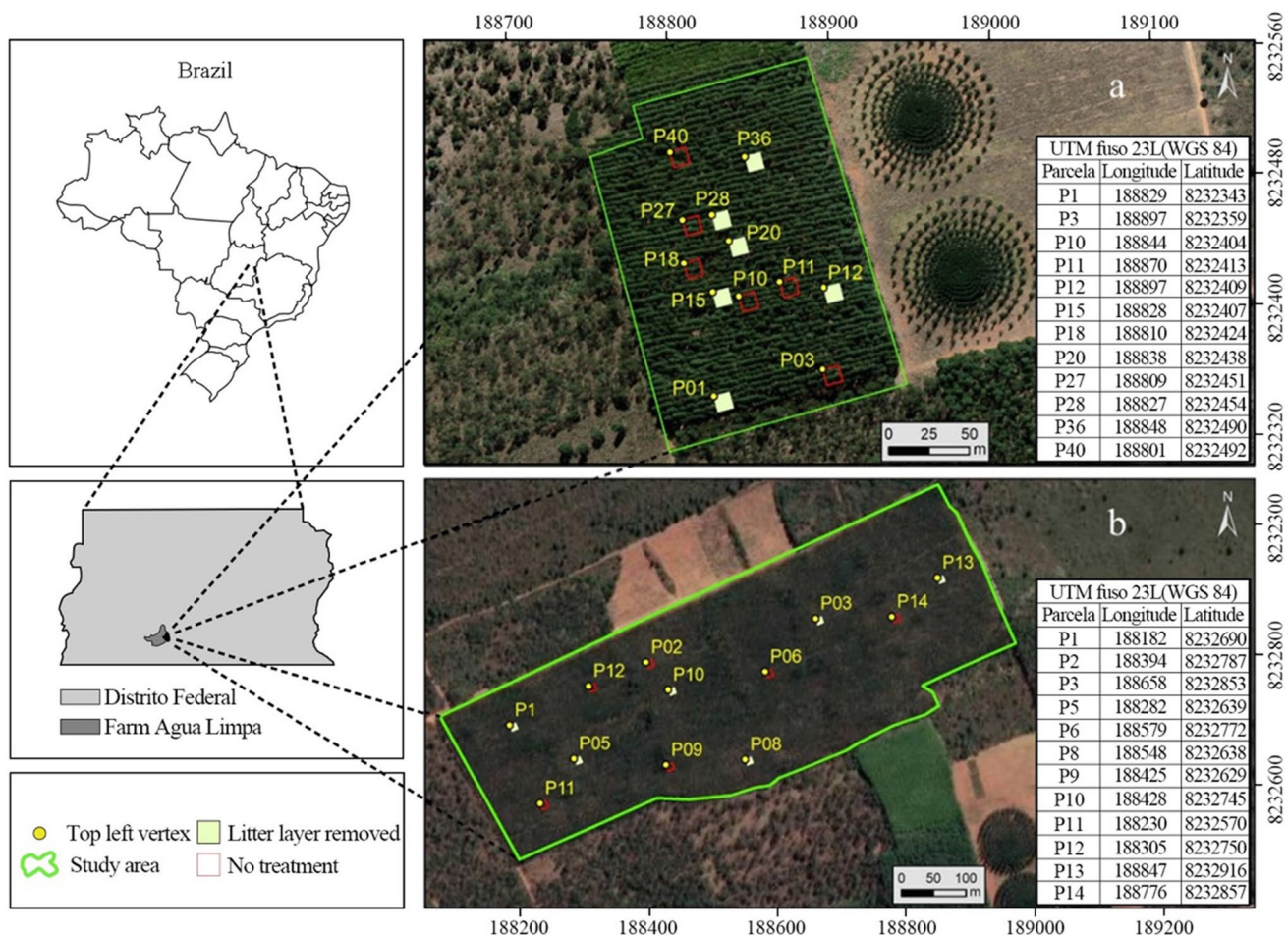
seedling, with applications at fifteen days, two months, one year, and two years after planting (Fig. 1b).

### Experimental design

Twelve 10  $\times$  10 m (100 m<sup>2</sup>) plots were established in each stand, half were plots with litter removal treatment. These plots remained uncovered and the litter removed every other month from March 2018 to April 2020. The other half was control plots where the litter on the surface was retained. In the mature stand, plots without litter were maintained for one year between November 2016 to October 2017 (i.e., the litter layer was removed). Afterwards, all plots remained without any intervention for five months before this study began (Fig. 1a).

### Litterfall sampling

Litterfall was collected monthly between May 2018 and April 2020. One 50  $\times$  50 cm (0.25 m<sup>2</sup>) collector with a



**Fig. 1** a Mature and b Juvenile eucalyptus stands in central Brazil. Twelve plots were randomly distributed within each stand, where in the red squares indicate litter removal plots and the white squares the control plots

nylon net 30 cm above the soil, as described by Scoriza et al. (2012), was installed in each of the 12 plots in both stands (Fig. 1a, b). All plant material deposited monthly was collected and placed in paper bags, taken to the University of Brasilia's Animal Nutrition Laboratory (LNA) and oven-dried at 65 °C until reaching constant weight. The oven-dried samples were sorted into the following fractions: leaves, reproductive parts (i.e., fruits, flowers, and seeds), branches (smaller than 1 cm diameter), and miscellaneous (i.e., unidentifiable material). Each fraction was weighed and their relative contribution to monthly total litterfall rates estimated by the averages per treatment by extrapolating the dry mass per area to  $\text{Mg ha}^{-1}$ .

### Litter decomposition

Decomposition rates of leaf and fine wood fractions were measured following Bock and Gilbert (1957). The plant material was placed in 20 × 20 cm decomposition bags made of 2 mm nylon mesh. The bags were filled with 20 g of freshly deposited leaf and 20 g of fine wood material (i.e., branches < 1 cm in diameter) (IFN 2015). The collections included one litterbag per plot in each stand at 30-, 60-, 120-, 240-, and 365-d exposure between May 2018 and April 2019. The remaining leaf and wood material was taken to LNA to remove any impurities (roots, soil) and to determine the fresh weight. The materials were then oven-dried at 65 °C and re-weighed to determine the dry weight after reaching a constant (Scoriza et al. 2012). Decomposition rates were calculated as the ratio of the initial mass to the remaining mass after the exposure period, using the following equation:

$$L_{\text{MR}} = (L_{\text{MF}}/L_{\text{MI}}) \times 100 \quad (1)$$

where,  $L_{\text{MR}}$  is the remaining litter mass (%),  $L_{\text{MF}}$  is the final litter mass (g),  $L_{\text{MI}}$  is the initial litter mass (g).

The decomposition rate constant  $k$ , which indicates the annual mass loss, was calculated as:

$$X_t = X_0 \times e^{-kt} \quad (2)$$

where,  $X_t$  is the dry weight (g) of litter material remaining after  $t$  days and  $X_0$  is the dry weight (g) of litter material at  $t=0$ . The half-life (i.e., the time required for decomposition of 50% of the initial mass stored in the bags; Olson 1963) was calculated as:

$$T_{1/2} = \ln(2)/k \quad (3)$$

where,  $T_{1/2}$  is the estimated number of days needed to decompose 50% of the initial mass,  $\ln$  is the natural logarithm, and  $k$  is the decomposition constant.

### Soil chemical analysis

To quantify key biogeochemical variables in control and litter removal plots in both stands, soil samples were collected in May 2018 and March 2020. Four random samples were collected and combined into one composite sample per plot at two depths: 0–20 and 20–40 cm. The samples were air-dried in the shade and analyzed according to EMBRAPA (1998) by the following methods: exchangeable aluminum ( $\text{Al}^{3+}$ ) and potential acidity ( $\text{H} + \text{Al}$ ) by titration; available potassium (K) and phosphorus ( $\text{P}_{\text{av}}$ ) by Mehlich-1 extractor, whereas the remaining P ( $\text{P}_{\text{rem}}$ ) Determined using molecular absorption spectrophotometry as well as calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ); K by flame photometric analysis, and soil organic matter (SOM) by extraction and titration. For all samples, total cation exchange capacity (CECt), effective cation exchange capacity (CECe), the sum of bases (SB), base saturation (V), and aluminum saturation (m) were calculated.

Liming was not conducted in the mature stand, so the concentration of Ca and Mg were not increased before planting. Lime application increases the pH (Florentino et al. 2021) and enhances fertility. P and K concentrations and base saturation in the upper 20 cm layer were not within the range considered suitable to support eucalyptus productivity on soils originally covered by Brazilian savanna vegetation (Andrade 2004).

An initial soil chemical analysis was carried out on all plots in both stands to compare parameters before litter removal. There were no significant differences ( $p > 0.05$ ) between plots with litter removal and control plots at both depths, indicating similar fertility and chemical parameters across plots (Table S1).

### Soil physical analyses

Soil volumetric and gravimetric moisture, as bulk density and total porosity were measured following EMBRAPA (2011). Three randomly selected plots from the controls and litter removal plots of each stand were sampled every four months (three collections covering dry and rainy seasons) from randomly selected points where 40 × 40 × 100 cm (160 L) trenches were created. Using a steel ring (88.6  $\text{cm}^3$ ), undisturbed soil samples were collected at 0–20 and 20–40 cm depths, transported to the laboratory to determine wet weight, and oven-dried at 105 °C until a constant weight. Volumetric moisture, soil density, gravimetric moisture, and total porosity were calculated as:

$$VM = \left( \frac{a - b}{c} \right) / Dw \quad (4)$$

where  $VM$  is the volumetric moisture ( $\text{cm}^3 \text{cm}^{-3}$ ),  $a$  is the wet sample mass (g),  $b$  is the dry sample mass (g),  $c$  is the sample volume ( $\text{cm}^3$ ), and  $Dw$  is water density ( $1.0 \text{g cm}^{-3}$ ).

$$BD = a/b \quad (5)$$

where  $BD$  is the soil bulk density ( $\text{g cm}^{-3}$ ),  $a$  is the mass of the sample dried at  $105^\circ\text{C}$  (g), and  $b$  is the volume of the cylinder ( $\text{cm}^3$ ).

$$GM = \left( \frac{a-b}{b} \right) \quad (6)$$

where  $GM$  is the gravimetric moisture ( $\text{kg kg}^{-1}$ ),  $a$  is the wet sample mass (g), and  $b$  is the dry sample mass (g).

$$Tp = \left[ \frac{Pd - Ds}{Pd} \right] \quad (7)$$

where,  $Tp$  is the total porosity ( $\text{dm}^3 \text{dm}^{-3}$ ),  $Pd$  is the density of solid soil particles ( $\text{kg dm}^{-3}$ ), and  $Ds$  is the soil density ( $\text{kg dm}^{-3}$ ).

### Soil epigeic fauna

Soil epigeic fauna was sampled during the dry (September 2018) and rainy (January 2020) seasons. Five plastic fall traps (15 cm diameter) per plot containing ~200 mL of water and 10 ml of colorless neutral detergent were used as described by Baretta et al. (2007) and left in the field for 48 h. The traps were collected and taken to the laboratory where the contents were separated with 0.125 mesh sieves and transferred to flasks containing absolute ethyl alcohol for fixation. They were subsequently identified to the highest possible taxonomic level (genus, order, family) using a magnifying lens and taxonomic keys (Table 1). Shannon's diversity index to estimate species diversity was calculated according to Odum (1969):

$$H = - \sum [(pi) * \log(pi)] \quad (8)$$

where,  $H$  is Shannon's diversity index, and  $pi$  is proportion of individuals of  $i$ th species in a whole community:

$$pi = n/N \quad (9)$$

where  $n$  is the number of individuals of a given type/species,  $N$  is the total number of individuals in a community,  $\sum$  represents the sum, and  $\log$  is the natural logarithm but the base of the logarithm is arbitrary (10 and 2 based logarithms are also used).

### Tree diameter increment

Diameter increment was measured biannually at breast height (DBH ~ 1.3 m) in all individuals with diameters  $\geq 15$  cm in

all plots during April and October 2018, April and October 2019, and April 2020. Diameter ( $d$ ) in cm was calculated according to Yocom and Bower (1975) as:

$$d = c/\pi \quad (10)$$

where,  $d$  is the diameter (cm) and  $c$  the circumference (cm).

Basal area ( $g$ ) in  $\text{m}^2$  was calculated as:

$$g = \pi d^2 / 40000 \quad (11)$$

where,  $g$  is the basal area,  $d$  the diameter (cm), and 40,000 was used to transform g into  $\text{m}^2$ .

Measurements were taken at 6, 12, 18, and 24 months and compared to the initial date (April 2018) to estimate diameter increments and control and litter removal plots compared to quantify the influence of litter removal. By the sum of all individual increments in each plot, the increase in basal area per plot ( $10 \text{m}^2$ ) was obtained and extrapolated to  $\text{m}^2 \text{ha}^{-1}$  to compare across control and litter removal plots. The average number of trees measured in each plot in the mature and juvenile stands was 14 and 15, respectively.

### Statistical analysis

T-tests were used to compare different variables between the two plots. When a normal distribution was not achieved, the non-parametric Mann Whitney (u) test was used. Monthly litterfall rates (total, leaf, branch, reproductive and miscellaneous fractions), leaf and fine wood litter decomposition, soil biogeochemical variables—volumetric moisture, soil density, gravimetric moisture, total porosity,  $\text{Al}^{3+}$ ,  $\text{H} + \text{Al}$ ,  $\text{P}$ ,  $\text{K}$ ,  $\text{Prem}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{OM}$ ,  $\text{CEC}_t$ ,  $\text{CEC}_e$ ,  $\text{SB}$ ,  $\text{V}$ , and  $\text{m}$ —and soil epigeic fauna abundance were compared between controls and litter removal plots. To test the effect of litter removal on basal area over two years, a three-way ANOVA accounted for any effects between collection times, and t-tests used to compare diameter increment between controls and litter removal plots. A 95% confidence level was considered for all statistical tests.

Software R (version 3.6.2; R Core Team 2019) was used to conduct non-metric multidimensional scaling (NMDS) ordinations (metaMDS function in the vegan package; Dixon 2003) based on Bray–Curtis dissimilarity (vegdist function) and standardized data (Bray and Curtis 1957). In this analysis, soil epigeic fauna abundance was compared in the control and litter removal plots. Vectors significant at the 95% confidence level (group scores obtained by the envfit function with 999 permutations) were plotted as arrows on the NMDS ordinations. These included soil epigeic fauna Shannon's diversity index as described by Odum (1969) and environmental vectors (litterfall, litter decomposition, four soil physical parameters and fourteen chemical parameters at 0–20 and 20–40 cm depths) in addition to tree growth.

**Table 1** Soil epigeic fauna in control and litter removal plots in juvenile and mature eucalyptus stands analyzed by t-test and Mann–Whitney test at the 95% confidence level

	Epigeic fauna group	Litter removal mean	Litter removal SD	Control mean	Control SD	<i>p</i> -value
Juvenile	Formicidae	1544.8	3143.3	198.8	81.8	ns
	Collembola	8.3	7.9	23.8	18.3	ns
	Hemiptera	1.3	0.8	5.7	2.3	<i>p</i> < 0.05
	Araneae	3.8	3.7	25.7	47.7	ns
	Diptera	11.0	7.4	23.5	10.2	<i>p</i> < 0.05
	Hymenoptera	2.8	1.9	2.2	2.0	ns
	Blapoda	0.2	0.4	0.5	0.5	ns
	Isoptera	0.7	0.8	4.5	4.2	ns
	Coleoptera	3.3	3.5	9.7	8.9	ns
	Isopoda	0.0	0.0	0.3	0.8	ns
	Opiliones	0.3	0.8	0.0	0.0	ns
	Orthoptera	1.0	0.6	1.5	1.0	ns
	Neuroptera	0.2	0.4	0.7	0.8	ns
	Protura	0.2	0.4	0.0	0.0	ns
	Ixodida	0.2	0.4	0.0	0.0	ns
	Siphonaptera	0.0	0.0	0.5	1.2	ns
	Acarina	0.0	0.0	0.3	0.8	ns
	Oligochaeta	0.2	0.4	0.0	0.0	ns
	Molusca	0.0	0.0	0.3	0.8	ns
	Mature	Diplopoda	1.5	2.1	0.8	0.7
Embiodera		0.2	0.4	0.0	0.0	ns
Symphyla		0	0.0	0.2	0.4	ns
Others		1.3	1.4	1.8	0.7	ns
Formicidae		169.5	126.9	135.8	119.7	ns
Collembola		32.8	10.8	9.5	5.0	<i>p</i> < 0.05
Hemiptera		5.2	1.9	5.8	3.3	ns
Araneae		9.5	3.7	12.8	6.1	ns
Diptera		15.3	4.5	20.8	4.4	<i>p</i> < 0.05
Hymenoptera		4.00	3.0	4.5	3.3	ns
Blapoda		0.7	0.5	0.5	0.8	ns
Isoptera		1.2	1.2	1.8	2.3	ns
Coleoptera		1.3	1.2	2.2	1.8	ns
Diplopoda		12.7	8.4	57.0	35.9	<i>p</i> < 0.05
Scorpiones		0.2	0.4	0.0	0.0	ns
Orthoptera		1.00	0.6	2.0	2.3	ns
Phasmatodea		0.2	0.4	0.2	0.4	ns
Neuroptera		1.8	2.2	3.3	2.0	ns
Acarina		0.0	0.0	0.2	0.4	ns
Ixodida		0.0	0.0	0.3	0.5	ns
Lepidoptera	0.2	0.4	0.2	0.4	ns	
Zoraptera	0.2	0.4	0.0	0.0	ns	
Others	0.3	0.5	0.3	0.8	ns	

ns Not significant at the 95% confidence level

## Results

### Effects of litter removal on litterfall and litter decomposition between stands

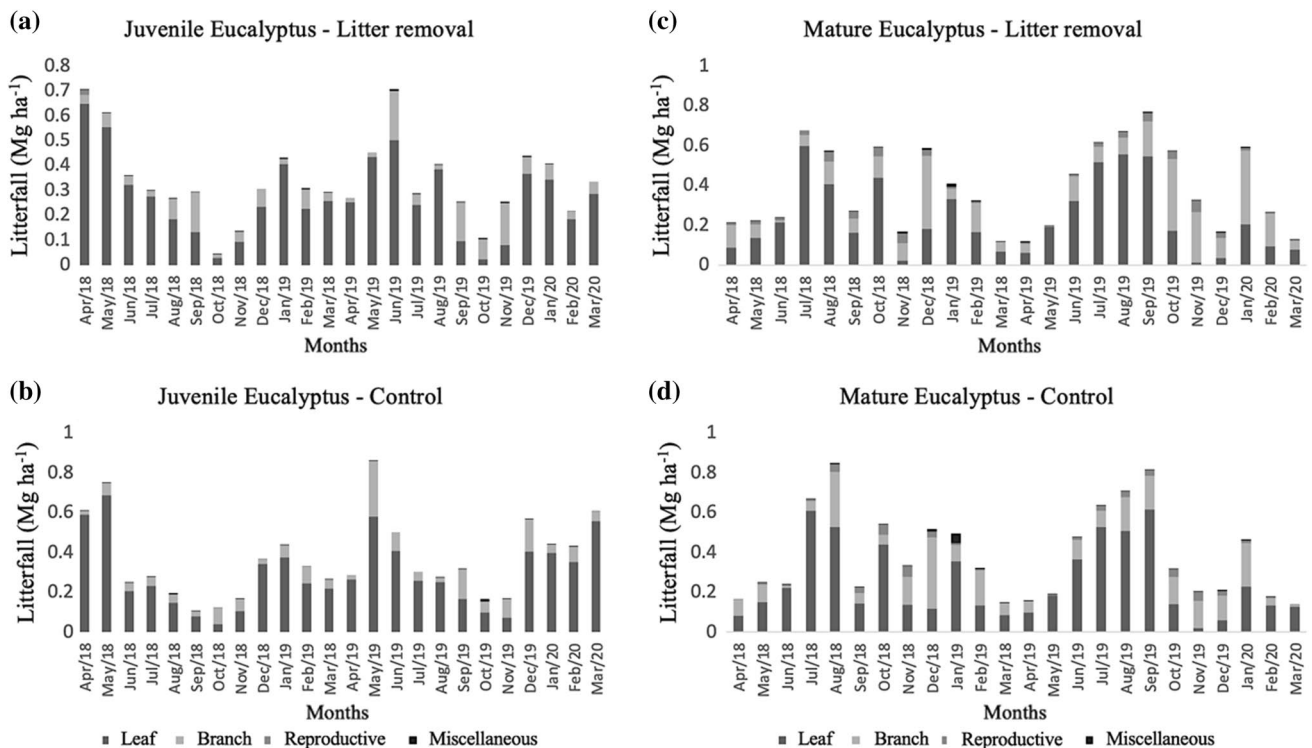
In the juvenile stand, litter removal had no effect on monthly litterfall rates for all fractions during both study years. The total litterfall in the first sampling year (2018–2019) was 4.1 Mg ha<sup>-1</sup> in litter removal plots (82.5% leaves, 16% branches, 0.8% reproductive material, and 0.5% miscellaneous), and 3.9 Mg ha<sup>-1</sup> in control plots (84.1% leaves, 15.4% branches, 0.1% reproductive material, and 0.7% miscellaneous) (Fig. 2a, b). In the following year (2019–2020), total litterfall in litter removal plots was 4.1 Mg ha<sup>-1</sup> (78.7% leaves, 22.4% branches, 0.2% reproductive material, and 0.8% miscellaneous), slightly lower than in control plots with 4.9 Mg ha<sup>-1</sup> (77.1% leaves, 21.9% branches, 0.2% reproductive material and 0.8% miscellaneous).

Litter removal in the mature stand did not affect monthly litterfall rates for all fractions in both study years. Annual litterfall between 2018 and 2019 was 4.4 Mg ha<sup>-1</sup>a<sup>-1</sup> in litter removal plots (63.7% leaves, 28.2% branches, 6.6% reproductive materials and 1.4% miscellaneous; Fig. 2). In control plots, annual litter deposition was 4.8 Mg ha<sup>-1</sup>a<sup>-1</sup> (63.0% leaves, 29.7% branches, 5.5% reproductive parts and 1.8% miscellaneous). In the second year (2019–2020), plots of

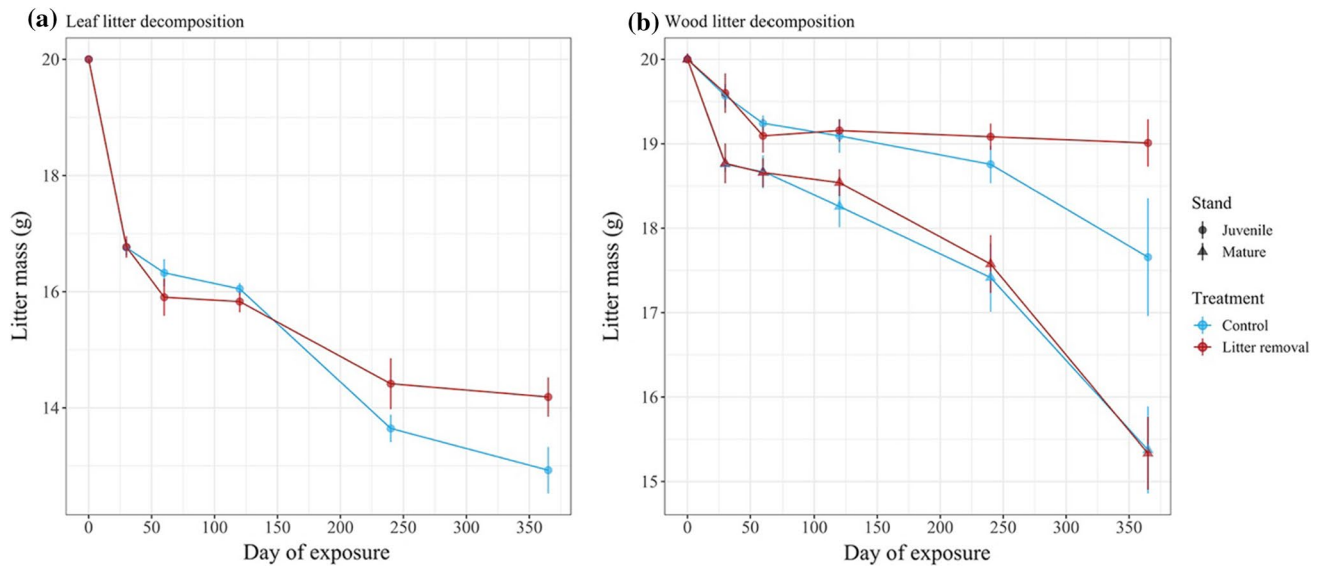
litter removal had an annual litterfall of 4.9 Mg ha<sup>-1</sup>a<sup>-1</sup> composed of 56.8% leaves and 36.8% branches (Fig. 2). Annual litterfall in control plots was 4.5 Mg ha<sup>-1</sup>a<sup>-1</sup>, where 66.4% was leaves and 27.4% branches.

In the juvenile stand, leaf, and fine wood litter decomposition rates (Fig. 3a, b; Table 2) were lower in litter removal plots ( $k_{\text{leaf}}=0.0009$ ;  $k_{\text{wood}}=0.0001$ ) compared to the control plots ( $k_{\text{leaf}}=0.001$ ;  $k_{\text{wood}}=0.0003$ ). These rates in control plots led to a half-life of 301 and 1003 d for leaves and wood, respectively. In the litter-removed plots, leaf litter half-life was 334 d and wood litter 3010 d. In terms of remaining litter mass through the collection times, even with different decomposition rates and half-life times, differences between both litter removal treatments were not significant ( $p>0.05$ ).

In the mature stand, the rate of leaf decomposition was lower in litter removal plots ( $k=0.0007$ ) compared to control plots ( $k=0.001$ ), indicating that removing litter reduced litter decomposition (Table 1). However, fine wood decomposition ( $k=0.0006$ ) did not differ between plots. Leaf half-life in control plots was 301 d, and 430 days for litter removal plots, more than four months difference. Branch half-life was 502 d, indicating a faster decomposition for leaves than for wood. Even with different  $k$  rates and half-life time, there was no significant effect of litter removal on the remaining litter mass.



**Fig. 2** Monthly litterfall (Mg ha<sup>-1</sup>) April 2018 to March 2020 in **a** litter removal plots and **b** control plots in the juvenile stand, **c** litter removal plots, and **d** control plots in the mature stand



**Fig. 3** Mean changes with 95% confidence interval in **a** leaf and **b** wood litter mass during one year of decay experiments in the litter removal (red) and control (blue) plots in the juvenile (circle) and mature (triangle) stands

**Table 2** Litter decay rates, coefficients of determination ( $R^2$ ) and  $p$ -values from exponential regressions for leaf and fine wood fractions in the litter removal and control plots in the juvenile and mature stands

Stand	Treatment	Litter fraction	Decay equation	$R^2$	$p$ -value
Juvenile	Litter removal	Leaf	$L_{MR} = 18.1924\exp(-0.0009x)$	0.87	0.02
Juvenile	Control	Leaf	$L_{MR} = 18.1985\exp(-0.001x)$	0.91	0.01
Juvenile	Litter removal	Wood	$L_{MR} = 19.5928\exp(-0.0001x)$	0.72	0.11
Juvenile	Control	Wood	$L_{MR} = 19.8188\exp(-0.003x)$	0.97	0.001
Mature	Litter removal	Leaf	$L_{MR} = 17.7729\exp(-0.0007x)$	0.01	0.05
Mature	Control	Leaf	$L_{MR} = 18.1656\exp(-0.001x)$	0.91	0.014
Mature	Litter removal	Wood	$L_{MR} = 19.6682\exp(-0.0006x)$	0.96	0.002
Mature	Control	Wood	$L_{MR} = 19.5990\exp(-0.0006x)$	0.97	0.001

LMR is the litter remaining mass (g)

### Soil chemical and physical parameters

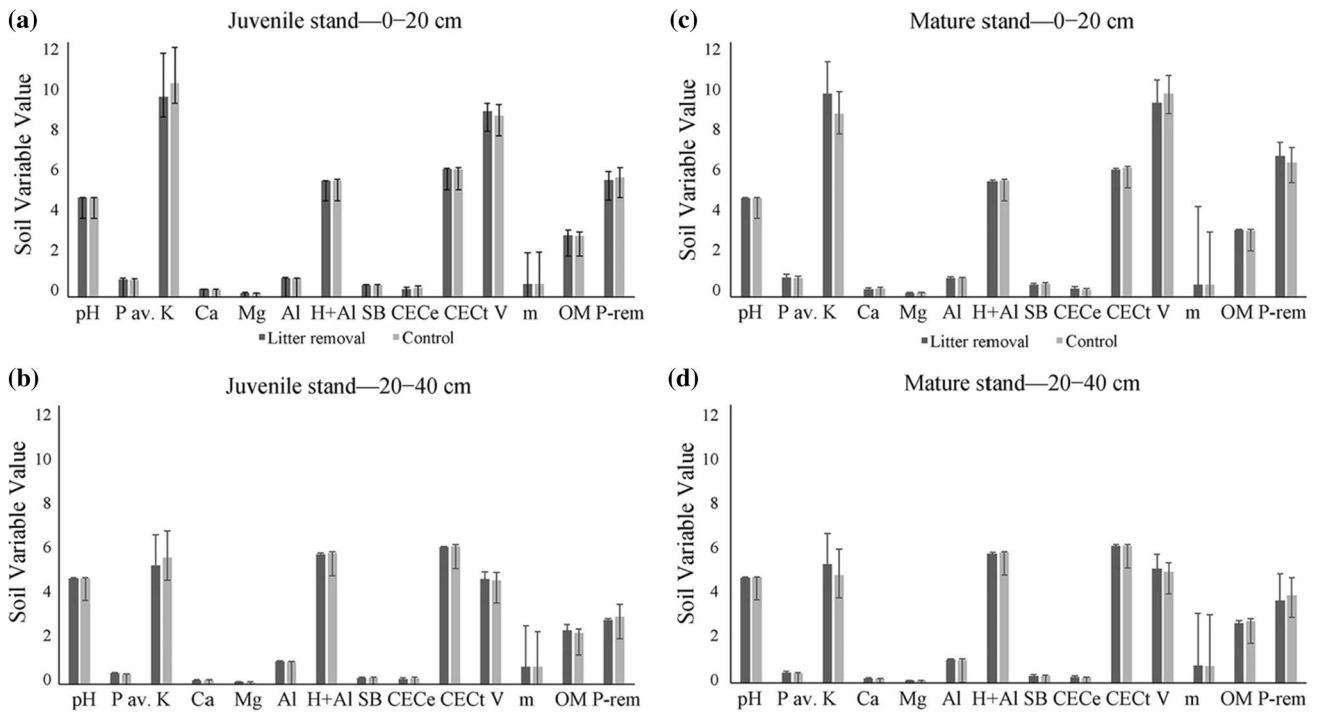
After two years of removing litter in the juvenile stand, there were no differences in topsoil (20 cm) biogeochemical parameters (Fig. 4a). However, available P at the 20–40 cm depth was higher in plots with litter removal. All other parameters did not differ between treatments in the juvenile stand (Table S2). In the mature stand, only total cation exchange capacity (CECt) in the 20 cm depth was higher in the control plots (Fig. 4c).

In terms of soil physical parameters, in the juvenile stand, there were no differences between control and litter removal plots in both periods (Table S3). However, during the dry period in the mature stand, soil gravimetric moisture at the 20–40 cm depth was lower in litter-removed plots compared to control plots. Soil moisture in the upper 20 cm was also lower in litter removal plots than in control plots (Fig. S1).

### Tree diameter increment

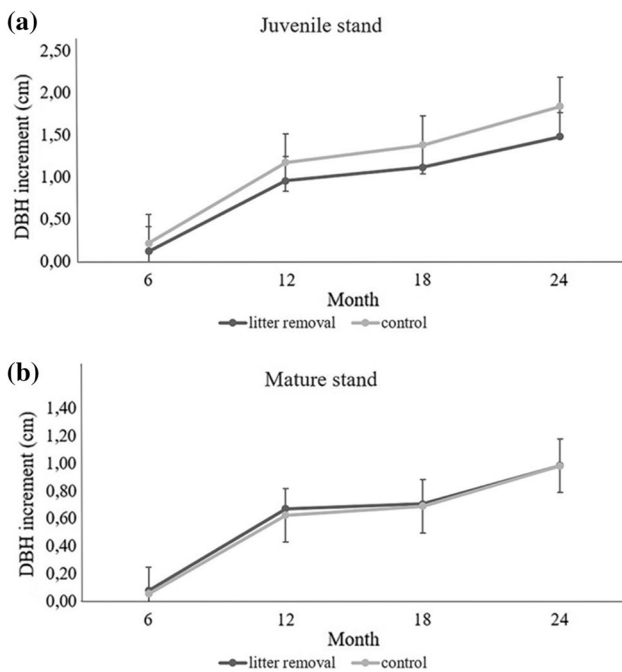
Basal area increments in both stands did not differ between plots. In the juvenile stand, basal area in the litter-removed plots showed a semiannual average increase of  $0.026 \text{ m}^2 \text{ ha}^{-1}$ , lower than the  $0.029 \text{ m}^2 \text{ ha}^{-1}$  in the control plots (Table S4). After two years of evaluation, this change represents a loss of  $0.42 \text{ m}^2$  per hectare in basal area increment due to litter removal. (Table S4). In addition, litter removal negatively affected individual tree diameter increments in the juvenile stand, a 19.5% reduction after two years (Fig. 5a). However, the effect of litter removal on diameter increment was not noted in the mature stand (Fig. 5b).





**Fig. 4** Soil chemical parameters in control and litter removal plots in juvenile and mature eucalypt stands over two years: **a** juvenile stand (0–20 cm depth); **b** juvenile stand (20–40 cm depth); **c** mature stand (0–20 cm depth); and **d** mature stand (20–40 cm depth). In all panels, SB=sum of bases ( $\text{cmol}_c \text{dm}^{-3}$ ), CECe=effective cation exchange capacity ( $\text{cmol}_c \text{dm}^{-3}$ ), CECt=total cation exchange capacity ( $\text{cmol}_c \text{dm}^{-3}$ ), V=base saturation (%), and m=aluminum

saturation (%), P av.=available phosphorus ( $\text{mg dm}^{-3}$ ), K=potassium ( $\text{mg dm}^{-3}$ ),  $\text{Ca}^{2+}$ =calcium ( $\text{cmol}_c \text{dm}^{-3}$ ),  $\text{Mg}^{2+}$ =magnesium ( $\text{cmol}_c \text{dm}^{-3}$ ),  $\text{Al}^{3+}$ =aluminum ( $\text{cmol}_c \text{dm}^{-3}$ ), H+Al=potential acidity ( $\text{cmol}_c \text{dm}^{-3}$ ), SB=sum of bases ( $\text{cmol}_c \text{dm}^{-3}$ ), OM=soil organic matter ( $10^{-2} \text{kg kg}^{-1}$ ), and P-rem=remaining phosphorus ( $\text{mg L}^{-1}$ ). Bars represent standard deviation

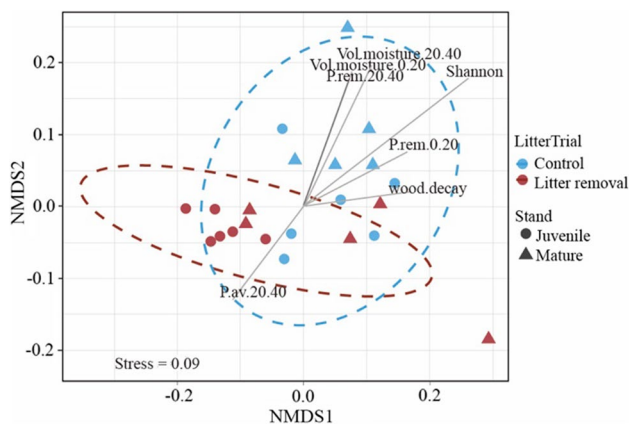


**Fig. 5** Two years of accumulative increment in DBH in litter removal and control plots established in **a** juvenile and **b** mature eucalypt stands

### Soil epigeic fauna abundance and diversity

Regardless of the season, litter removal had a negative effect on Hemiptera and Diptera abundance in the juvenile stand. Other epigeic fauna groups did not change (Table 1). In the mature stand, only Diplopoda and Collembola differed in abundance between treatments. Diplopoda was most abundant in control plots and Collembola most abundant in litter removal plots (Table 1).

Non-metric multidimensional scaling (NMDS) ordinations showed a low-stress level (0.09), indicating a good fit of observed and actual dissimilarities among epigeic fauna communities in control and litter removal plots in both stands (Fig. 6). There was a higher homogeneity of epigeic fauna within the plots of litter removal compared to control plots. Litter removal led to a reduction in beta diversity of epigeic fauna in the stands. Among the significant soil and litter vectors, Shannon's diversity covaried positively with control plots, indicating that litter removal also reduced soil epigeic fauna alpha diversity. The other significant vectors were soil moisture (at 0–20 and 20–40 cm depths), remaining P (both depths), available P (20–40 cm) and wood decay rate. Soil moisture (at



**Fig. 6** Non-metric multidimensional scaling (NMDS) plot of soil epigeic fauna including environmental vectors in control (blue) and litter removal (red) plots in juvenile (circles) and mature (triangles) stands. P. av.=available P ( $\text{mg dm}^{-3}$ ), P. rem.=remaining P ( $\text{mg L}^{-1}$ ), Vol. moisture=volumetric moisture ( $\text{g cm}^{-3}$ ), Shannon=Shannon's diversity index ( $h'$ ), and Wood decay=fine wood decomposition rate (k)

both depths) co-varied with control plots, suggesting a relationship between epigeic fauna and soil water content. The same trend was observed for the remaining phosphorous, which represents P buffer power in the soil. Wood decay also co-varied with control plots in terms of epigeic fauna diversity, while available P (20–40 cm) correlated negatively with Shannon's diversity and positively with the litter removal plots (Table S5).

## Discussion

### Litterfall and litter decomposition

Contrary to our predictions, litterfall was not affected by litter removal. It was expected that removing the litter would negatively impact litterfall as it is a major pathway for nutrient and organic matter transfer from the canopy to the soil, whose nutrient availability influences litter production (Sayer et al. 2020). However, litter production is also affected by climate and evapotranspiration (Giweta 2020). Therefore, it was speculated that if the litter removal experiment continued for over two years, litterfall would be affected by this management practice, as seen in other studies (e.g., Nzila et al. 2002).

In terms of litter decomposition, regardless of stands, litter removal practices, and the fact that samples were collected monthly, there was not an advanced stage of decomposition. Low decomposition rates in the Cerrado occur due to local precipitation patterns that have a long dry period that causes a reduction in biological activity (Inkotte et al. 2022).

Our results indicate that litter decomposition (leaf and wood) was negatively affected by litter removal regardless of stand age. The litter layer strongly influences soil moisture, temperature, and soil biogeochemical variables so that litter removal may have generally decreased decomposer activity. For instance, microbial communities that decompose plant litter are commonly negatively affected by changes in microclimate (Giweta 2020). In a study in tropical China, litter removal decreased litter decomposition in three successional forests by up to 27% (Chen et al. 2014). Litter removal in pine plantations in northern Argentina also showed a decrease in litter decomposition rates as early as after one month of exposure (Trentini et al. 2018). They also reported that litter removal substantially impacted soil fauna communities inhabiting the litter layer and not the soil. In the NMDS analyses, this was observed through the heterogeneous distribution of soil epigeic communities in control plots (Fig. 6).

However, short-term litter removal does not always affect microbial decomposer activity (Fahey et al. 1998). This may explain the lack of an effect of litter removal on wood decomposition rates in the mature stand. Similarly, Wang et al. (2019) found no differences in litter decomposition rates due to litter removal in a eucalyptus-dominated forest of southeast Queensland, Australia. Also, in older stands, litter decomposition tends to be slower (Valadão et al. 2019), and the effects of litter removal are less detectable in short-term experiments (<5 years). This could explain the null effect of litter removal on wood decomposition rates in the mature stand.

### Litter removal effects on water content, remaining P, and available P

Litter removal in the mature stand significantly reduced soil gravimetric moisture during the dry period, while all other physical parameters did not change. In contrast, litter removal in the juvenile stand did not affect soil physical parameters. In contrast to our findings, Versini et al. (2013) found that soil moisture decreased with litter removal in a young eucalyptus stand with a larger spacing ( $3.3 \text{ m} \times 3.7 \text{ m}$ ) compared to our study. This may be explained by the canopy structure of our juvenile and mature stands because there is less direct solar radiation on the soil surface when the canopy is closed (Lei et al. 2018). The litter layer also acts as a natural buffer for soil moisture (Giweta 2020) during the dry season, which corroborates the observed decrease in soil moisture by litter removal during that period. This might have influenced the lower litter decomposition rates found in the litter removal plots, as soil moisture directly influences soil fauna activity and, consequently, litter decomposition (Petraglia et al. 2019).

In terms of soil chemistry, total cation exchange capacity at the 0–20 cm depth was the only variable that decreased with litter removal in the mature stand. This may be explained by the reduction of organic residues that could affect SOM in litter-removed plots, given that soil organic matter is highly correlated to total cation exchange capacity in tropical regions (Novais et al. 2007). However, there was no significant difference in SOM levels in control and litter removal plots in both stands.

In the juvenile stand, subsurface soil P availability increased with litter removal. This may be related to the characteristics of this macronutrient and its buffering power that tends to equalize labile and non-labile soil pools (Novais et al. 2007). With litter removal and the consequent reduction of phosphorous from litter, soil P becomes more present in the soil solution; however, this may not represent greater plant availability due to low soil mobility. Because of the predominance of positive charges in the highly weathered soils in the Cerrado, soil P adsorption in Fe/Al oxyhydroxides is favored. Correlations between soil fauna diversity and the remaining P in control plots also support this assumption, as P inputs from litter are highly correlated to fauna activity. Soil P concentration after litter removal show significant interannual fluctuations (Sayer et al. 2020), which may also explain the increased P levels in our litter removal plots after two years.

The lack of an effect on most soil chemical parameters corroborates Wang et al. (2019) findings in eucalyptus-dominated forests in Australia, suggesting that the Australian stands were unaffected by 15 months of litter removal. On the other hand, several studies report significant impacts of litter removal on soil organic carbon (Cao et al. 2020) and total carbon since the amount of organic C stock in forest soils is the result of annual C inputs from litterfall and C outputs through litter decomposition (De Marco et al. 2016). Other studies have reported reductions in pH and P (Tanner et al. 2016), K, Ca, and Mg (Versini et al. 2014; Wang et al. 2019). Because litter decomposition rates were affected by litter removal, it is speculated that soil fertility would be impacted over the following years in the litter-removed plots. This is because litter decomposition is essential for plant nutrition in eucalyptus stands following two years post-planting, as after that period, natural nutrient cycling supports growth and stand development (Schumacher and Vieira 2015).

### Differential effects of litter removal on diameter increment

Litter removal negatively affected diameter increment in the juvenile stand but not in the mature stand. This corroborates other studies showing that the effect of organic residue removal on eucalyptus growth in the Democratic

Republic of the Congo and in Brazil also reduced average tree growth (Laclau et al. 2010a, b). Versini et al. (2013) reported that litter removal decreased above-ground biomass by 33 to 38%. Rocha et al. (2016) found that wood productivity, after removing or burning forest residues, was 6% lower than in control plots. However, Wood et al. (2009), studying the effects of litter removal on old-growth tropical wet forests, found that litter removal had no significant effect on tree growth, similar to our findings for the mature stand. The differences in growth response to litter removal between the juvenile and mature stands may be explained by the slower growth in older stands which have reached their growth stability. In mature stands, litter decomposition is slower (Valadão et al. 2019), such that the effects of litter removal on growth are less evident in a short-term experiment (<5 years).

### Litter removal impact on soil epigeic fauna abundance and diversity

Soil epigeic fauna organisms belonging to Hemiptera, Diptera, and Diplopoda orders were affected by removal of litter in both stands (Table 1). The reduction of Hemiptera in the juvenile stand, an order commonly found in soils under eucalyptus (Soliman et al. 2019), could have significant consequences for ecosystem functions. For example, this could affect the food chain due to a reduction in abundance of these groups (Goldman et al. 2020). The order Diptera—which decreased by over 50% due to litter removal relative to the controls in the juvenile stand—is one of the most crucial insect groups in savanna soils (Inkotte et al. 2022). This order is only temporarily present because of its remarkable litter-decaying activity (Assad 1977). Litter manipulation has severely impacted Diptera communities in *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson plantations in southeastern Brazil (Camara et al. 2019). Because Diptera is one of the most abundant soil fauna groups in eucalyptus plantations (Martins et al. 2017; Tacca et al. 2017), the impact of litter removal on their abundance is an ecological concern to the function of eucalyptus plantations.

Litter removal in the mature stand led to a reduction in Diplopoda organisms. According to Nsabimana (2013), Diplopoda is one of the most prevalent soil faunae across several eucalyptus stands and is dependent on litter for habitat and food (Assad 1977), contributing to transforming plant residues into organic matter. Thus, this order's disappearance can negatively affect nutrient cycling by reducing their contribution to litter decomposition and disturbing the food chain. Overall, our findings corroborate Trentini et al. (2018), who reported that litter removal in an exotic *Pinus* plantation in Argentina reduced the abundance of some epigeic fauna groups.

Regardless of the stand, litter removal homogenized the composition of soil epigeic fauna. Shannon's diversity index co-varied with control plots, indicating a reduction in soil epigeic fauna diversity. While there were no other studies of eucalyptus plantations in central Brazil, Sayer et al. (2020) found that litter removal reduced the abundance of soil arthropods in a 15 year experiment in an old-growth lowland tropical forest. They suggested that such a reduction partially explained the decrease in litter decomposition rates.

The correlation between soil moisture and epigeic fauna in control plots suggests that the removal of the litter layer in eucalyptus plantations is of concern due to the influence of soil moisture on epigeic fauna diversity. Each soil organism has an optimal moisture environment to support their survival (Wasis et al. 2018). Given that litter removal reduced soil moisture, especially during the dry seasons, soil epigeic fauna and nutrient cycling are directly affected. Soil organisms are partially dependent on litter deposition because most epigeic fauna species and populations are more dependent on litter as a habitat (Trentini et al. 2018). Therefore, the litter layer and soil epigeic fauna organisms are key components in nutrient cycling, not only in natural ecosystems but also in managed systems (Ashford et al. 2013) like the eucalyptus plantations in this study.

## Conclusions

The impacts of litter removal practices in two eucalyptus stands were quantified, with an emphasis on litter–soil–plant interactions and nutrient cycling processes. Our two-year study showed that litter removal reduced leaf litter decomposition rates which can, in turn, affect nutrient cycling. Individual tree diameter increment was only affected by litter removal in the juvenile stand, which may be a consequence of slower growth in the mature stand compared to the juvenile stand.

In addition to affecting remaining soil P and moisture, wood decay rates, and epigeic diversity, litter removal affected specific soil faunal groups. The abundance of Hemiptera, Diptera, and Diplopoda groups decreased with litter removal, suggesting impacts on edaphic food chains and nutrient cycling that can alter the functioning of eucalyptus plantations. Although litterfall was not affected by litter removal, in the long-term, this could be possible due to changes in nutrient cycling promoted by the reduction of nutrient inputs caused by litter management. Overall, our results suggest that litter removal practices in eucalyptus stands negatively affect their functioning and should be

avoided. Future long-term ecological studies covering larger spatial scales in eucalyptus stands in the seasonal tropics are needed to better understand the impacts of litter removal practices on litter–soil–plant interactions to support management decisions and prescriptions.

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