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REGULAR ARTICLE

Understanding the enhanced litter decomposition of mixed-species plantations of *Eucalyptus* and *Acacia mangium*

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Abstract
Background and aims Soil microbial-derived litter decomposition represents an important step in the global carbon and nutrient cycling and, at the local level, is primarily driven by litter chemistry. Here, we assessed how mixed-species plantations with *Eucalyptus urograndis* and *Acacia mangium* could be a key to enhancing litter production, decomposition, and soil microbial activity.
Methods The relationships between litter decomposition and litter quality and quantity were compared among 6-year-old monocultures of *E. urograndis* and *A. mangium* (E100+N and A100, respectively) and a mixed plantation of both species (E50A50). Additionally, we evaluated soil microbial biomass carbon (MBC) and nitrogen (MBN), soil basal respiration (SBR), soil enzymes and the N mineralization potential.
Results The return to soil of N via litterfall in E50A50 was greater than E100+N, while the return of P in E100+N and E50A50 were higher than A100. The decomposition rate in A100 was slower than in the E50A50 and E100+N. The microbial activity, represented by soil enzyme activities (proteases and N-acetyl-β-glucosaminidases), was consistently higher in E50A50 than in A100.
Conclusion The E50A50 presented a more balanced supply of N and P associated to a better structural quality of the litter for microbial metabolism, with synergic reflections on decomposition rates and release of nitrogen.

Keywords Intercropping · N_2-fixing trees · Nutrient cycling · N:P stoichiometry · Enzyme activity · Decomposer starvation

Abbreviations

FTIR	Fourier-transform infrared spectroscopy
MBC	Soil microbial biomass carbon
MBN	Soil microbial biomass nitrogen
NAG	N-acetyl-β-glucosaminidase
qCO_2	Metabolic quotient
SBR	Soil basal respiration

Introduction

The replacement of *Eucalyptus* spp. monocultures with mixed plantations containing nitrogen-fixing leguminous trees has been proposed as a silvicultural system that can compensate for the high export of N caused by

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harvesting wood during successive rotations (Laclau et al. 2010; Voigtlaender et al. 2012). Studies have shown that the overall stemwood production of these mixed plantations can be similar to or greater than in monospecific *Eucalyptus* stands (Bouillet et al. 2013; Santos et al. 2016). Moreover, mixed plantations provide a series of benefits to the ecosystem, such as faster biochemical cycling of nutrients (Khanna 1997; Forrester et al. 2005b; Tang et al. 2013), increased diversity of microbial activity (Bini et al. 2013; Rachid et al. 2015) and higher stocks of C in the soil (Resh et al. 2002; Balieiro et al. 2008; Forrester et al. 2013).

However, the selection of species that promote interactions of facilitation and competitive reduction over intra- and interspecific competition for uptake and use of resources is a preponderant factor for the success of mixed plantations and the supply of expected environmental services (Forrester et al. 2005a, 2006, 2011). Briefly, facilitation occurs when at least one of the species acts positively on the development of the other species, as is the case of leguminous trees, which can increase the availability of the organic and inorganic forms of N in the system. On the other hand, competitive reduction is related to the contrasting morphological and physiological traits among the species, such as shade tolerance, growth rate, canopy structure (leaf area density) and effective root system depth. These differences can allow the more efficient use of field resources by the species, through complementary exploitation of niches (Vandermeer 1989; Forrester et al. 2006; Kelty 2006).

Acacia mangium Willd. has been recommended for mixed plantations with *Eucalyptus* spp. due to its ability to fix atmospheric N_2 through symbiotic association with diazotrophic bacteria (*Rhizobium* spp. and *Bradyrhizobium* spp.), the capacity to uptake nutrients of low mobility more efficiently (as P and Zn) intermediate by mycorrhizal fungi, and due to the diversity of wood and non-wood uses (Laclau et al. 2008; Krisnawati et al. 2011; Bouillet et al. 2013; Santos et al. 2016). Some studies have demonstrated that although *A. mangium* has high N_2 fixation potential (Galiana et al. 2002; Balieiro F de et al. 2010; Mercado et al. 2011) and N flow through the litter (Voigtlaender et al. 2012; Santos et al. 2017), the species has high demand for P (Kaye et al. 2000; Inagaki et al. 2011). However, *A. mangium* is very efficient in breaking down phosphate compounds before leaf abscission and reallocating them to other organs or to meet demand of microsymbionts (Inagaki et al. 2011; Santos et al. 2017). These characteristics can lead to a high N:P ratio litter, which can limit its decomposition, especially in tropical soils, which are naturally poor in P (Koutika et al. 2014, 2016). Conversely, *Eucalyptus* – derived leaf litter are P-richer than the *A. mangium,* and would improve the balance of N and P via litter production due to differences in the patterns of use and retranslocation of nutrients (Santos et al. 2017). Therefore, these differences in litter characteristics can be expected to have positive consequences on microbial activity and litter decomposition (Bonanomi et al. 2014).

Few studies have investigated the litter decomposition in mixed plantations of *Eucalyptus* and species of the genus *Acacia*. Xiang and Bauhus (2007) showed that in microcosm conditions the addition of litter from *Acacia mearnsii* Willd. accelerated the decomposition of the litter from *Eucalyptus globulus* (Labill.). Bachega et al. (2016) evaluated the mass loss of leaves and fine roots of *Eucalyptus grandis* (W. Hill ex Maiden) and *A. mangium* in litterbags placed in mixed and monoculture stands. The authors used the litter (fine roots and leaves) reciprocal transplant technique and observed that the plant community did not influence litter decomposition, disagreeing to the *Home Field Advantage hypothesis* (also see Austin et al. 2014). Santos et al. (2017) stated that mixed plantations can improve the balance of N and P via litter production in relation to monocultures, due to differences in the patterns of use and retranslocation of nutrients. In addition, the authors suggested that the decomposition and release of nutrients from the litter probably favors trees' nutrition earlier than in monocultures, especially by their complementary litter traits.

Soil microbes are the main drivers of litter decomposition and play an important role in the global and local carbon and nutrient cycling; however, litter decomposition is primarily influenced by litter chemistry in forest stands (Hättenschwiler et al. 2011). Thus, field studies involving litter decomposition associated to soil microbial activity are important to better understand the functioning of mixed plantations of *Eucalyptus* spp. with leguminous nitrogen-fixing trees. Here, we attempted to fill the gap of lack of litter decomposition studies in the context of mixed-species plantations, by comparing this system with their respective monoculture-based systems. Our hypothesis is that mixed-species plantation of *Eucalyptus urograndis* (*Eucalyptus urophylla* S. T.

Blake x *Eucalyptus grandis* W. Hill ex Maiden) and *Acacia mangium* outperforms their monospecific stands in terms of litter production and decomposition, by providing a more balanced supply of N and P to the soil microbial community.

Material and methods

Site description

The experiment was established in January 2009 in Seropédica, Brazil. The experimental area has gentle relief (<5% slope) and had been fallow for more than 15 years. The predominant vegetation before planting was *Andropogon bicornis* L. (Poaceae). The soil is a Haplic Planosol (Brazilian Soil Taxonomy) or Planosol (World Reference Base/FAO), which has a highly sandy surface horizon (0–20 cm) (>90% sand), with low levels of C (~0.32%), total N (~0.03%) and P (7.0 mg kg^{-1}), as well as low base saturation (<30%) and cation exchange capacity (<0.7 cmolc kg^{-1}). The climate is Aw according to the Köppen classification, with dry winters and rainy summers. The mean annual rainfall recorded during the study was 1370 mm, with average monthly temperatures ranging from 16 °C (June to August – dry season) to 36 °C (January to March – rainy season, with occurrence of dry spells) and a yearly average of 24 °C. The average relative humidity in the period was 81%. A complete characterization of the site can be found in Santos et al. (2016).

Experimental design and treatments

The study was performed based on an experiment with five combinations of plantations of the species *E. urograndis* and *A. mangium* in monocultures and mixed stands, established in randomized blocks in January 2009 (Santos et al. 2016). We studied three of the five combinations of three experimental blocks: (i) monoculture of *E. urograndis* fertilized with 120 kg N ha^{-1} (E100 + N); (ii) monoculture of *A. mangium* (A100); and (iii) mixed plantation with 50% of each species (E50A50). The tree spacing was 3 × 3 m (1111 plants ha^{-1}). The effective plot was considered as the inner area occupied by the 16 central plants.

The *E. urograndis* seedlings were cloned and donated by Suzano Papel e Celulose S.A. The *A. mangium* seedlings, inoculated with strains of *Rhizobium* spp. (BR3609 and BR6009) (Faria et al. 2010), were produced from seeds collected from parent trees in the experimental field of Embrapa Agrobiologia, located in Seropédica, Brazil.

The base fertilization for all treatments consisted of a mixture of 100 g of P_2O_5 ($Ca(H_2PO_4).2H_2O$), 40 g of K_2O (KCl) and 25 g of a micronutrients cocktail (fritted trace elements - FTE BR12), applied in two lateral pits per plant. After the first month, 20 g of N ($(NH_4)_2SO_4$) was applied to the crown projection of the plants only in treatment E100 + N. The supplementary fertilization consisted of four applications of 20 g per plant of K_2O (KCl) (for all treatments) and 25 g of N ($(NH_2)_2CO$), timed at 3, 6, 9 and 12 months after planting, under the crown projection, with nitrogen only being supplied to the plants of treatment E100 + N. Finally, 2.0 Mg ha^{-1} of dolomitic limestone was applied to the entire area 12 months after planting, to raise the levels of Ca and Mg. The complete characterization of the treatments can be found in Santos et al. (2016).

Litter analysis

Litterfall production

The litterfall was quantified monthly between October 2013 (58 months after planting) and September 2014 (69 months after planting). Three conical litter traps with catchment area of 0.2375 m^2 were suspended 1 m from the ground and arranged randomly inside each effective plot, in the following positions: (i) in the planted row; (ii) in the center of the area between rows (between four trees) and (iii) at a diagonal distance of 1 m from the tree with diameter at breast height (DBH) nearest to the average of the plot, regardless of the species in the case of the mixed plantation.

The material collected was immediately oven-dried (65 °C for 48 h or to constant weight) and sorted into two fractions: leaves and miscellany (reproductive material, twigs with diameter smaller than 5 mm, bark and others). In the case of the E50A50, the sorting was done separately for each species. Finally, the dry weight of each fraction was determined.

In this study we only considered the leaf fraction (which accounted for 95% of the total dry weight of the material intercepted by the collectors – data not shown) to estimate the weight of the litter intercepted by the collectors, expressed as Mg of dry matter $hectare^{-1}$. We recognize this procedure slightly

underestimates the total content of nutrients contributed to the soil by disregarding the contribution of other fractions.

Litter stocks

The standing litter on the soil surface were quantified twice during the evaluation period (at 58 and 69 months after planting) with a steel quadrant frame (625 cm^2). The frame was placed on the residues stocked on the ground and the material inside was collected and placed in paper bags.

During the sampling, the litter was classified vertically regarding the presence of the layers L (recently produced material with little fragmentation) and F + H (highly fragmented material and humus). Four samples were collected randomly from each plot and were gathered to form a compound sample, representing the layers L and F + H in each plot. In the laboratory, the samples were oven-dried (65 °C for 48 h or to constant weight) and the dry weights were determined. The contamination of the mineral fraction (from the soil) in the litter samples was corrected by calculating the ash content in each sample, by heating sub-samples (previously ground and homogenized) in a muffle furnace at 450 °C for 5 h.

Litter decomposition

The method proposed by Olson (1963) for tropical ecosystems was used to calculate the decomposition coefficient (k), which considers the ratio between the litter produced yearly (L) and the average quantity of litter stocked on the ground in the same period (Xss), as described in Eq. 1. The average renewal of the litter stock was obtained from the inverse of the decomposition coefficient ($1/k$). According to this method, it is assumed that the litter mass on the ground is in steady state:

$$k = \frac{L}{\mathrm{Xss}} \tag{1}$$

The model proposed by Olson (1963) assumes that the litter decomposition ratio diminishes exponentially as a function of time. For this, the half-life ($t_{0.5}$), which is the time (in years) for the initial dry weight of the litter on the ground to decrease by half, was calculated by Eq. 2. The same concept can be applied to ascertain the time necessary for a 95% reduction ($t_{0.95}$) of the initial weight, according to Eq. 3:

$$t_{0.5} = \frac{\ln(2)}{k} \tag{2}$$

$$t_{0.95} = \frac{3}{k} \tag{3}$$

where k represents the decomposition coefficient obtained from Eq. (1).

Chemical characterization of leaf litter

The leaf litter samples were digested in a nitroperchloric acid solution in a microwave oven and then analyzed in a colorimeter (P), as described in Embrapa (2011). The N concentration was measured by distillation according to the Kjeldhal method after digestion in sulfuric acid (Embrapa 2011). The amount of N and P returned via litter in each plot were estimated by multiplying the concentration of each nutrient in the senescent leaves (g kg^{-1}) and the biomass of the leaf fraction produced (kg ha^{-1}). The results were expressed in Mg ha^{-1}.

The concentrations of cellulose and lignin were determined according to the method proposed by Van Soest (1994). Briefly, this method is based on the use of a specific detergent acid (20 g of cetyltrimethylammonium bromide per liter of sulfuric acid at 1 mol L^{-1}), to dissolve all the cell content and hemicellulose present in the samples, besides most of the insoluble protein, thus obtaining the detergent acid fiber, composed basically of lignin and cellulose. Then, a potassium permanganate solution was used to dissolve the lignin. Finally, the samples were heated in a muffle furnace (500 °C for 5 h) to obtain the ash content (remaining inorganic fraction) and the concentrations of cellulose and lignin present in the samples were determined by difference.

The content of total extractable polyphenols (soluble polyphenols, condensed and hydrosoluble tannins, and non-tannin polyphenols) as described by Anderson and Ingram (1993). Briefly, the method is based on the extraction of the polyphenols and tannins in 50% methanol (*v*/v), followed by filtration. We used Folin-Denis' reagent in a basic medium, with 17% sodium carbonate (m/v) to form the colour in the samples (blue). Then, we quantified the polyphenols using tannic acid as a standard solution. This is a colorimetric method based on the

absorbance values found in the individual samples and in the standard spectrophotometric curve at 760 nm.

Soil sampling

Fresh soil samples at 10 cm of depth were used to analyze the microbial activity, collected by a Dutch auger at six random points within the effective plots, always respecting the following positioning: three samples collected in the row and three others between the rows. After homogenization, 250 g of soil samples were placed in plastic bags and taken to the laboratory in an ice-filled chest. In the laboratory, the samples were immediately passed through a 2-mm sieve and small organic fragments were removed using metal tweezers. Finally, the samples were stored in a refrigerator until analysis (up to 15 days).

Soil analysis

Microbial analysis

The fumigation-extraction method described by Brookes et al. (1985) and Vance et al. (1987) was used to obtain the extracts for quantification of C and N in the microbial biomass, with some adaptations. Briefly, for the non-fumigated samples (NF), 20 g of soil (in duplicate) was submitted to extraction with 50 mL of K_2SO_4 0.5 M for 30 min in an orbital agitator at 150 rpm. The extracts were filtered and frozen until analysis. The fumigation of the samples (F) was done by directly adding ethanol-free chloroform (1 mL) inside glass flasks containing 20 g of soil (in duplicate). The flasks were placed in a glass desiccator containing a beaker with 25 mL of chloroform in the lower compartment. Then vacuum was applied to the desiccator and the chloroform was kept boiling for 1 min. The samples were kept under vacuum for 48 h. Then the extraction was performed as described previously for the NF samples, and the extracts were also preserved. The microbial biomass C (MBC) was ascertained by colorimetry (Bartlett and Ross 1988). The values of carbon associated with the microbial biomass were measured by applying a constant (Kc) equal to 0.35 (Anderson et al. 2008). The data were expressed as mg of microbial C kg^{-1} of dry soil. To quantify the microbial biomass N (MBN), 20 ml of the NF or F extract was used. First the samples were digested with sulfuric acid (with gradual increase of temperature to 350 °C), followed by distillation by steam dragging (Kjeldahl). Then the samples were neutralized by acid-base volumetry with sulfuric acid 0.0015 M. The N concentration in the extracts was calculated according to Silva et al. (2007a), applying a correction factor of 0.54 (Brookes et al. 1985). The results were expressed as mg of microbial N kg^{-1} of dry soil.

The soil basal respiration (SBR) rate was measured by the soil incubation method using NaOH as CO_2 trap, according to Silva et al. (2007b). Initially, 50 g of soil was weighed (in duplicate) in glass jars (2 L), which were hermetically sealed and pre-incubated in the dark at 25 °C for seven days. Then the jars were opened for ventilation and to add a plastic trap flask containing 10 mL of NaOH 0.5 M. The jars were immediately closed and incubated in the dark at 25 °C for 10 days. To quantify the CO_2, 2 mL of $BaCl_2$ was added to the trap flasks to precipitate the CO_2, followed by titration with HCl 0.5 M from the residual NaOH (Silva et al. 2007b).The SBR was expressed as μg CO_2-C kg^{-1} dry soil h^{-1}. Also, the metabolic quotient (qCO_2) was calculated by the ratio between SBR and MBC.

The activities of phosphatases and β-glucosidases were evaluated spectrophotometrically according to Tabatabai (1994) and Eivazi and Tabatabai (1988), respectively, with some modifications. The analysis of phosphatases was carried out in a non-buffered medium using two replicates in test tubes containing 0.5 g of soil, 1 mL of H_2O followed by 1 mL of the substrate *p*-nitrophenyl-phosphate (CAS Number: 333,338–18-4) at a concentration of 50 mM. The samples were incubated at 37 °C for 1 h and the reaction was stopped with 0.5 ml of $CaCl_2$ 0.5 M and 2 ml of NaOH 0.5 M. The analysis of β-glucosidases was performed under the same conditions except for the use of sodium acetate buffer at 100 mM pH 5.5 in the reaction medium and incubation with a solution of 50 mM of 4-nitrophenyl β-D-glucopyranoside (CAS Number 2492–87-7). The controls consisted of soil samples with the addition of 2 mL of H_2O or 2 mL of acetate buffer, for phosphatase and β-glucosidase, respectively. A test tube without soil containing 1 mL of H_2O (or acetate buffer for β-glucosidase) and 1 mL of substrate was used as a blank. All the samples were centrifuged and the supernatant was collected for reading at 410 nm in a spectrophotometer. The quantity of *p*-nitrophenol (PNP) formed in each sample was determined based on a standard curve with known concentrations of PNP, and the results were expressed as μmol PNP g^{-1} dry soil h^{-1}.

The activity of N-acetyl-β-glucosaminidases (NAG) was also evaluated spectrophotometrically, according to Parham and Deng (2000). In summary, the analysis was performed in a medium buffered with sodium acetate 100 mM pH 5,5 using two replicates, in test tubes containing 0.5 g of soil, 1 mL of acetate buffer followed by 1 mL of the substrate 4-nitrophenyl N-acetyl-β-D-glucosaminide (CAS Number: 3459–18-5) at a concentration of 10 mM. The samples were incubated at 37 °C for 1 h and the reaction was stopped by adding 0.5 ml of $CaCl_2$ 0.5 M and 2 ml of NaOH 0.5 M. The controls consisted of tubes containing 0.5 g of soil with 2 mL of acetate buffer. A tube without soil containing 1 mL of acetate buffer and 1 mL of substrate was used as blank. All the samples were centrifuged and the supernatant was read in a spectrophotometer with wavelength adjusted to 410 nm. The quantity of PNP formed in each sample was determined based on a standard PNP curve and the results were expressed as μmols PNP g^{-1} dry soil h^{-1}.

The activity of proteases was determined by the method proposed by Alef and Nannipieri (1995), which consists of incubating the soil sample together with a casein solution (substrate) for 2 h at pH 8.1 at 50 °C. Then the quantity of tyrosine released by the reaction medium was determined with the Folin-Ciocalteu reagent. The color was read with a spectrophotometer at 700 nm. The quantity of tyrosine formed in each sample was determined based on a standard curve and the results were expressed as mg tyrosine g^{-1} dry soil h^{-1}.

Nitrogen mineralization potential (ex situ incubation)

Subsamples of soil collected for the microbial analyses were used to determine the N mineralization potential in anaerobic incubations, according to the method proposed by Keeney and Bremner (1966), with some adaptations. For this, 5 g of soil from each sample (in triplicate) was placed in test tubes with length of 15 cm and internal diameter of 15 mm. After adding 12.5 mL of distilled H_2O, the tubes were vortexed and placed in a BOD incubator at 40 °C, where they remained for seven days. At the end of this period, 0.93 g of KCl was added to each tube to make a final concentration of 1 M. The tubes were again vortexed for 15 s and the soil + extract mixture was passed through filter paper. The concentration of NH_4^+ in the extracts was quantified before and after incubation, according to the method of Kempers and Zweers (1986).

Statistical analysis

After confirmation of normality and homogeneity of variance, the data were submitted to analysis of variance (ANOVA) considering a completely randomized block design, with n equal to 3 (blocks). The means of the variables were discriminated by the F-test ($p < 0.05$) and compared by the Tukey test ($p < 0.05$). The R software (R Development Core Team 2017) was used for the parametric analyses, with the support of the *ExpDes* package (Ferreira et al. 2013).

Results

Litter production and decomposition dynamic

Greater quantities of litter were produced by the E50A50 and E100 + N plantations during the period of analysis (Fig. 1), with accumulated values of 7207 kg ha^{-1} $year^{-1}$ and 5882 kg ha^{-1} $year^{-1}$, respectively (Table 1). In turn, the corresponding accumulation in A100 was 4264 kg ha^{-1} $year^{-1}$, significantly lower than in E50A50 ($p < 0.05$). The contribution of *E. urograndis* to the overall litter production in the mixed plantation (E50A50) was 68% of total dry matter (Table 1).

The monthly monitoring of litterfall revealed some patterns of the litter production dynamic in the mixed plantation (E50A50). The months of highest litter production of *E. urograndis* (May to July) coincided with the period of largest production of *A. mangium* (Fig. 1). Besides this, during the period evaluated, the litter production of the two species in E50A50 was almost always greater than in the single-species plantations (except for November, when the litter production of E100 + N was greater than in the other two plantations). The monthly input of residues in E50A50 varied between 343 and 1067 kg ha^{-1}, while in the monoculture stands of *E. urograndis* and *A. mangium* these figures were 241–806 kg ha^{-1} and 88–563 kg ha^{-1}, respectively (Fig. 1).

The largest accumulated litter stocks were found on the soil of A100 (7548 kg ha^{-1}) and E50A50 (5586 kg ha^{-1}) (Table 1). The stock in E100 + N (3680 kg ha^{-1}) was significantly lower than in A100, but did not differ statistically from E50A50 ($p > 0.05$). The litter stock in the plantations was inversely proportional to the decomposition constant of organic residues (k), which was more than two times higher in the plantations with

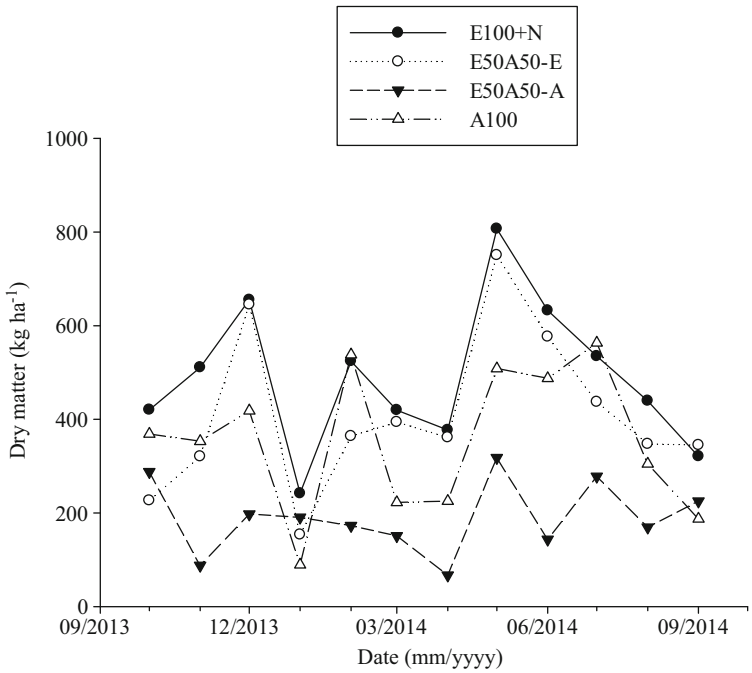

Fig. 1 Leaf litter production in monocultures (E100 and A100) and mixed plantation of *E. urograndis* with *A. mangium* (E50A50) between the fifth and sixth year of age. (E50A50-E and E50A50-A represent the litter contribution of *E. urograndis* and *A. mangium*, respectively, in the mixed plantation). *Software: SigmaPlot 11

E. urograndis (E100 + N and E50A50) (Table 1). The same pattern occurred with the parameters used to infer the litter decomposition, which indicated a faster litter stock renewal rate (1/k) in E100 + N and E50A50. The estimate of the average time for reduction, in terms of dry weight, of 50% and 95% of the accumulated *A. mangium* material was nearly three times greater than that produced by *E. urograndis* in E100 + N and two times the quantity of mixed litter in E50A50. Finally, the mixture of litter produced in E50A50 caused a positive effect on the decomposition of this material, since the parameters used did not differ in relation to the monoculture of *E. urograndis*, where the decomposition rate was faster (Table 1).

The return of N via litter during the year was greater in the stands with the presence of *A. mangium*: 83 and 73 kg N ha^{-1} $year^{-1}$, respectively, in E50A50 and A100, while in E100 + N it was 56 kg N ha^{-1} $year^{-1}$ (Fig. 2a).

Table 1 Annual production (kg ha^{-1}), litter L and F + H layers (kg ha^{-1}) and litter decomposition parameters in *E. urograndis* (E100 + N) and *A. mangium* (A100) monocultures and in the mixed plantation (E50A50) between the fifth and sixth year of age

Parameter	E100 + N	E50A50			A100
		E. urograndis	*A. mangium*	total	
Annual production	5882.0 (±620) ab	4920.6 (±93)	2286.3 (±75)	7206.9 (±18) a	4264.2 (±91) b
Litter *L*	1476.2 (±61) a	–	–	1657.0 (±204) a	1262.3 (±167) a
Litter *F* + *H*	2203.8 (±37) b	–	–	3929.0 (±354) b	6285.7 (±653) a
Total	3680.0 (±27) b	–	–	5586.0 (±545) ab	7548.1 (±820) a
k	1.6 (±0.2) a	–	–	1.3 (±0.1) a	0.6 (±0.1) b
1/k	0.6 (±0.1) b	–	–	0.8 (±0.1) b	1.8 (±0.2) a
$t_{0.5}$	0.4 (±0.1) b	–	–	0.5 (±0.1) b	1.2 (±0.1) a
$t_{0.05}$	1.9 (±0.2) b	–	–	2.3 (±0.2) b	5.3 (±0.5) a

k: Decomposition constant; 1/k: mean time in years of litter renewal; $t_{0.5}$ and $t_{0.05}$: time in years required for loss of 50% and 95% of litter mass, respectively. Equal letters in the lines indicate absence of statistical difference by the Tukey test ($P < 0.05$). Values in parentheses represent the standard error of the samples between the experimental blocks

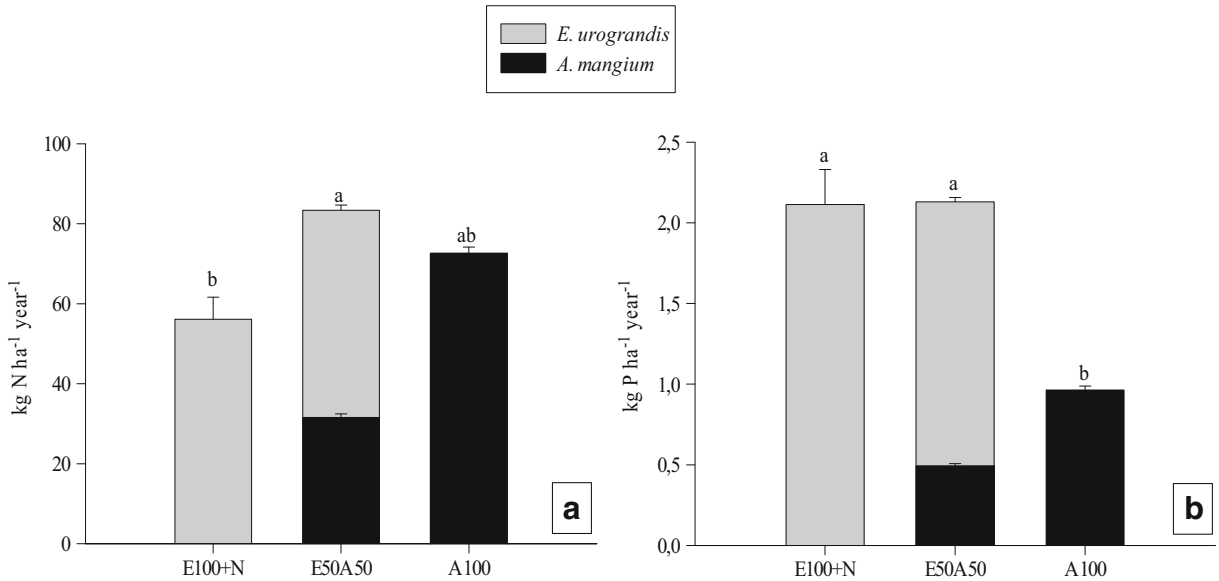

Fig. 2 Annual deposition of nitrogen (**a**) and phosphorus (**b**) via leaf litter in monocultures of *E. urograndis* (E100 + N) and *A. mangium* (A100) and mixed plantation (E50A50) between the fifth and sixth year of age. The vertical bars represent the standard error of the mean between the experimental blocks. Equal letters indicate absence of statistical difference according to the Tukey test ($p < 0.05$). *Software: SigmaPlot 11

In the mixed plantation, *A. mangium* contributed 38% of the total N supplied via litter.

In contrast to N, the supply of P via litter was greater when eucalyptus was present, both in the monoculture and mixed plantation (Fig. 2b). In this case, the return of P via litter was similar between E100 + N and E50A50, reaching values of about 2.1 kg P ha^{-1} $year^{-1}$ (Fig. 2b), while in A100, it was only 0.9 kg P ha^{-1} $year^{-1}$. The contribution of *E. urograndis* to returns of P in E50A50 was 79% of the total (Fig. 2b).

Chemical quality of the litter

The N:P ratio was three times higher in the *A. mangium* litterfall (85) than in the *E. urograndis* (27) (Table 2). In the mixed plantation, the N:P ratio was intermediate between the values of the two monocultures (Table 2). The levels of cellulose and lignin found in the *A. mangium* material were significantly higher ($p < 0.05$) in relation to *E. urograndis* (Table 2). The lignin:N and lignin:P ratios were both highest in E100 + N and lowest in A100. On the other hand, the *E. urograndis* litter contained higher concentrations of tannins (hydrosoluble + condensate) and polyphenols than produced by *A. mangium*. On average, the levels of these compounds were nearly double those in the *A. mangium* litter (Table 2).

Microbiological analyses and N mineralization rate (ex situ)

The microbial biomass C (MBC) in E100 + N was 94 mg C kg^{-1}, more than twice the value in A100 (46 mg C kg^{-1}) ($p < 0.05$) (Table 3). In turn, the microbial biomass N (MBN) did not differ between the stands, a fact that significantly altered the C:N ratio of the biomass. Therefore, the C:N ratio of the microbial biomass was significantly higher in E100 + N (14.2) than in A100 (9.8), suggesting a distinction in the composition of the microbial community between these environments. The values of MBC, MBN and C:N ratio were intermediate in E50A50 (Table 3).

The soil basal respiration (SBR) rate was higher in the monospecific *E. urograndis* stand (E100 + N) than in the mixed (E50:A50) and monospecific *A. mangium* (A100) areas (Table 3). Nevertheless, this fact was not reflected in significant differences in the metabolic quotient (qCO_2), an indicator of stress of the microbial community in the soil (Anderson and Domsch 1985).

The activities of enzymes associated with the nitrogen cycle, such as proteases and N-acetyl-β-

Table 2 Chemical quality of deposited litter (only for newly deposited leaves belonging to the L layer) in monocultures of *E. urograndis* (E100 + N) and *A. mangium* (A100) and mixed planting (E50A50)

Treatment	Tannins (%)	Polyphenols	Cellulose	Lignin	N:P (% $\%^{-1}$)	Lignin:N	Lignin:P
E100 + N	0.2 (±0.0) a	4.6 (±0.6) a	21.0 (±0.6) b	18.1 (±0.5) b	26.7 (± 1.6) c	18.1 (± 0.5) a	453.2 (± 13.4) c
%	0.5	10.6	47.8	41.2			
E50A50	0.2 (±0.0) a	4.5 (±0.7) a	26.7 (±1.2) b	20.6 (±0.9) ab	50.1 (± 2.7) b	15.2 (± 0.7) b	685.3 (± 31.1) b
%	0.4	8.7	51.3	39.1			
A100	0.1 (±0.0) b	1.9 (±0.1) b	34.9 (±1.0) a	22.7 (±1.3) a	85.3 (± 1.3) a	13.4 (± 0.8) c	1135.0 (± 65.0) a
%	0.2	3.2	58.6	38.1			

Means followed by equal letters in the same column indicate absence of statistical difference by the Tukey test ($p < 0.05$). The values in parentheses indicate the standard error of the mean between the experimental blocks

glucosaminidases (NAG), presented significant differences ($p < 0.05$) between the treatments. The highest activity of proteases occurred in E50A50, followed by E100 + N, and these two presented activity levels two and three times higher than in A100 respectively (Table 3). In turn, the activity of NAG in E100 + N and E50A50 was more than three times higher than in A100 (Table 3). There were no statistical differences among the treatments regarding activities of phosphatases and β-glucosidases (Table 3).

The N mineralization potential in the soil under A100, expressed in mg NH_4^+ during 7 days in laboratory conditions, was on average 30% lower in relation to E100 + N and E50A50 (Table 3).

Discussion

Higher litter production and nutrient return in mixed-species plantations

The stratification of the canopies in mixed *Eucalyptus* and *Acacia* stands has been reported to be a complementary niche strategy of these species to light capture (Bauhus et al. 2004; Forrester et al. 2006; le Maire et al. 2013). In our experiment, *E. urograndis* had competitive superiority in relation to *A. mangium* in terms of growth rate and net primary productivity (Santos et al. 2016). This complementarity of niches can be related to the greater litter production in the mixed plantation in our experiment. It

Table 3 Characterization of microbial biomass and soil biochemical activity in monoculture areas of *E. urograndis* (E100 + N) and *A. mangium* (A100), and mixed plantation of the two species (E50A50) at 5 years of age

Variable	Treatment			
	E100 + N	E50A50	A100	p-value
MBC (mg C kg^{-1})	94.4 (±9.7) a	60.6 (±4.6) ab	45.9 (±3.4) b	0.044
MBN (mg N kg^{-1})	6.7 (±0.7) a	5.4 (±0.2) a	4.6 (±0.1) a	0.262
Microbial C/N ratio (mg kg^{-1} mg kg^{-1})	14.2 (±0.3) a	11.1 (±0.4) ab	9.8 (±0.5) b	0.049
SBR (mg C-CO_2 kg^{-1} h^{-1})	0.6 (±0.1) a	0.4 (±0.1) b	0.3 (±0.0) b	0.084
qCO_2 (mg g^{-1} h^{-1} C-CO_2 do CBM)	6.5 (±0.8) a	6.0 (±0.3) a	8.3 (±0.6) a	0.363
Phosphatase (μg PNP g^{-1} h^{-1})	319.2 (±29.1) a	313.8 (±18.7)a	280.4 (±14.7) a	0.810
β-glucosidase (μg PNP g^{-1} h^{-1})	18.6 (±2.3) a	19.0 (±0.5) a	13.0 (±1.3) a	0.274
Protease (μg tyrosine g^{-1} h^{-1})	1522.8 (±231.7) b	2291.9 (±149.4) a	883.6 (±283.6) c	0.002
NAG (μg PNP g^{-1} h^{-1})	57.4 (±10.3) a	50.0 (±11.8) a	16.6 (±5.1) b	0.024
Potential N mineralization rate (mg NH_4^+ kg dry $soil^{-1}$ 7 d^{-1})	23.2 (±0.57) a	23.4 (±0.53) a	18.1 (±0.4) b	0.004

Means followed by equal letters do not differ by Tukey test ($p < 0.10$). The values in parentheses indicate the standard error between the experimental blocks

should be noted that the quantity of litter produced by *E. urograndis* was underestimated, since normally this species produces a high proportion of woody litter, which we did not quantify (Epron et al. 2013).

In E50A50, *E. urograndis* contributed nearly two-thirds of the overall litter dry mass produced by the trees, and although presenting lower levels of N (~7 g kg^{-1} versus ~13 g kg^{-1} in senescent leaves of *E. urograndis* and *A. mangium*, respectively), the *E. urograndis* trees returned a greater quantity of N than did the *A. mangium* trees (Fig. 2). The contribution of N of both species in E50A50 was higher than in the *E. urograndis* monoculture (E100 + N). The *A. mangium* trees (A100) produced N-richer litter due to their greater biological fixation of N_2 (Franco and Faria 1997; Galiana et al. 2002; Bouillet et al. 2008; Balieiro et al. 2010), but the quantity of dry matter was lower in the period analyzed. This pattern was also observed in monocultures and mixed plantations of *E. globulus* with *A. mearnsii* in Australia, where the content of N supplied by the leguminous monoculture did not differ from that of the mixed plantation (1:1 proportion between species), but both were higher than the *E. globulus* monoculture (Forrester et al. 2005b). A similar result was found by Voigtlaender et al. (2012), who argued that the flow of N returned to the soil by litter production functions as a good indicator of the availability of N in the soil.

The input of P via leaf litter was influenced by the patterns of utilization of P during leaf senescence, as previously reported by Santos et al. (2017). *A. mangium* is a species that retranslocate phosphate compounds and redirects them to other plant organs more efficiently than *Eucalyptus*, especially in environments that are poor in P (Inagaki et al. 2011). The high internal cycling leads to low P cycling by leaf litter production. However, in the mixed plantation (E50A50), the return of P was influenced by the large quantity of dry matter provided by *E. urograndis*. In this respect, diversifying the system with at least one arboreal species that has complementary strategies for use of nutrients (mainly N and P) can represent a gain in terms of decomposition and release of nutrients from the litter.

Factors controlling the litter decomposition in mixed plantations of *E. urograndis* and *A. mangium*

Our results show that although the litter from *Acacia* is N-richer, its decomposition was slower, principally in the monoculture stand, in relation to the mixed plantation with *E. urograndis* (E50A50). We believe this pattern is the result of a series of factors, which will be presented and explained below.

We think that a major factor limiting the litter decomposition process of *A. mangium* in the monoculture stands was the low entry of P due specifically the production of P-poor litter. On the other hand, the N-rich litter from *A. mangium* admixed with the litter P-rich from *E. urograndis* can act synergistically in favor of faster litter decomposition in the mixed stand. This hypothesis is corroborated by previous studies that have demonstrated high efficiency of internal recycling of P by *A. mangium* (Inagaki et al. 2011; Santos et al. 2017), as well as decomposition studies investigating the Home Field Advantage hypothesis carried by our group. Novotny et al. (2013) incubated litterbags with leaf litter of *E. urograndis* in their own stand (E100 + N) and on litter layer stocked by monocultures of *A. mangium* (A100). The same was done for *A. mangium* leaf litter. Their results showed (by means of FTIR spectroscopy combined with principal component analysis) that the microbiota associated with *E. urograndis* leaf litter drained part of the N when the litterbags were deposited on litter from *A. mangium*. On the other hand, the mass loss of the *A. mangium* deposited on litter from *E. urograndis* was almost irrelevant. The authors also found faster decomposition of litter from *E. urograndis* than *A. mangium*, with intermediate decomposition rates of the mixed litter from both species.

The term "trait" has been used to describe proxies of the performance of determined organisms in studies ranging from the organism to ecosystem level (Violle et al. 2007). The stoichiometric balance between N and P in litter content is one of the traits cited most often in studies that have investigated the decomposition dynamics (Smith 2002; Güsewell and Gessner 2009). Increased metabolic activity is also accompanied by higher demand for P, needed to synthesize proteins (a constituent of ribosomal RNA) and to constitute ATP molecules, necessary for the storage and transfer of energy from the reactions of intracellular metabolism (Elser et al. 2000). Therefore, the high capacity to fix atmospheric N_2 in symbioses by *A. mangium* and to recycle it via litter production (Galiana et al. 2002; Mercado et al. 2011) contrasts with its high ability to retranslocate P internally (Balieiro et al. 2004; Inagaki et al. 2011; Santos et al. 2017), leading the decomposition system to possible collapse due

to the high N:P ratio of the litter, influenced by the production of P-poor residues.

A secondary factor that can influence the decomposition process of *A. mangium* is the litter quality regarding the proportion of easily assimilated C compounds. Hättenschwiler et al. (2011) proposed the expression *decomposer starvation* to describe the slower decomposition process with low concentrations of labile C in the litter. Studies with global scale have shown that the availability of energy-rich C compounds, easily leachable and promptly accessible to the microbiota, can have a stronger influence on the decomposition rate than on the quantity of nutrients present in the litter (Hättenschwiler and Jørgensen 2010). Bachega et al. (2016) found higher concentrations of water-soluble compounds in litter from *Eucalyptus grandis* than from *A. mangium*, and attributed these findings to the lower activity of decomposer microorganisms associated with the decomposition residues from *A. mangium*. Our results suggest that the larger proportion of recalcitrant components (e.g., lignin) in the litter from *A. mangium* is related to the slower decomposition rates. This was corroborated by the positive correlation found between higher litter decomposition rates and greater lignin concentrations ($r = 0.75$; $p < 0.05$). Nevertheless, we believe that the effect of lignin by itself was not a determining factor, because the difference in the lignin content between the species was very small (18.1% vs. 22.7% for *E. urograndis* and *A. mangium*, respectively). Moreover, the supposition of the prevalent effect of lignin does not fit with the higher concentrations of tannins and polyphenols found in *E. urograndis* and the smaller concentrations of cellulose in *A. mangium*, which theoretically would lead to slower decomposition of the *E. urograndis* litter.

Nevertheless, it should be stressed that the litter N-richer produced by *A. mangium* also might have acted to delay the processes of degrading lignin and lignified carbohydrates that occur in the later decomposition stages, which implies lower litter mass loss rates, as reported by decomposition studies of other species (Berg and Ekbohm 1991; Carreiro et al. 2000). Nitrogen is an essential nutrient to regulate microbial growth and support the production of enzymes that act in the initial stages of litter decomposition, when water-soluble compounds and non-lignified carbohydrates are broken down by enzymes produced by bacteria and fungi (Berg 2000). However, some studies have shown that ammonium ions and amino acids can: (i) inhibit the production of ligninolytic enzymes in various species of fungi (e.g., white-rot fungi); and (ii) react with some products of lignin degradation, forming stable complexes with greater recalcitrance (Berg and Ekbohm 1991; Berg 2000). Therefore, we speculate that the mass loss of the N-rich litter (*A. mangium*) is slower in relation to the N-poor litter (*E. urograndis*) due to the difficulty of breaking down lignin compounds, imposed by the high availability of N in the litter and soil (Santos et al. 2017). However, further studies are necessary to determine the aliphatic and aromatic proportion of C and true role of N in the decomposition of litter from *A. mangium*.

The inhibition of decomposer communities by decomposer starvation or by means of secondary compounds inherited from green leaves (e.g., tannins and lignin) is consistent with the slower decomposition of litter (Hättenschwiler et al. 2011). Additionally, we believe that a strong influence exists of the trees on the fungal community in the soil of these plantations (E100 + N, A100 and E50:A50), as also reported by Rachid et al. (2015) in a study conducted in the same experimental area. Those authors observed that the plant community determines the strategy for using P by the saprophytic community in the litter. The integration of communities, greater abundance of genes and higher diversity indexes in the mixed plantation of *E. urograndis* and *A. mangium* suggest that the breakdown of organic compounds is more efficient by ectomycorrhizal fungi with a better state of P than N.

According to Rachid et al. (2015), 75% and 19% of the DNA sequences found in soil samples from the *E. urograndis* stands belonged to the phyla Basidiomycota and Ascomycota, respectively, while in the *A. mangium* monoculture these figures were 28% and 62%, respectively. Basidiomycete fungi generally act to decompose more recalcitrant compounds in the litter, such as lignin and holocellulose. Ascomycetes, in turn, specifically decompose cellulose and other less recalcitrant compounds in relation to lignin (Osono and Takeda 2006). Therefore, fungi with ligninolytic capacity are more efficient in reducing the litter mass stocked on the ground (Osono 2007). This fact might also be another factor responsible for the faster decomposition rates of the *E. urograndis* in relation to the *A. mangium* litter in the monocultures, including with positive reflections for the environment from the mixture of species (E50A50), where the decomposers are more diverse, as reported by Rachid (2013). The microbial integration was assessed

by pyrosequencing techniques in soil samples from the monocultures and mixed plantation, and the results indicated the existence of sharing of the community of bacteria and fungi that are predominant in the monocultures when the two species are combined. Besides this, the authors reported that the proportion of fungi in the *E. urograndis* monoculture was greater than in the mixed plantation, followed by the *A. mangium* monoculture, but that with the progress of the decomposition process, the fungal community became more similar to that presented by *E. urograndis*.

Litter quality as a mediator of microbial activity and N release

Only a few studies have reported the effect of mixing *Eucalyptus* spp. with leguminous trees on the microbial activity and potential release of N. Bini et al. (2013) showed that combined planting of *Eucalyptus grandis* and *Acacia mangium,* during the second rotation, supplied better quality litter (i.e., richer in N and P), although this did not cause greater microbial activity in the soil up to 30 months after the start of the second rotation.

We found the microbial biomass in the *A. mangium* monoculture to be inferior to that in the monospecific *E. urograndis* stand, probably because of the low quality of the *A. mangium* litter, especially the small concentrations of C and greater concentrations of P as well as the higher concentrations of more recalcitrant C compounds (mainly lignin). These factors limit the microbial metabolism (as discussed in section 4.2). Furthermore, the activities of the enzymes NAG and protease, both associated to the N cycle, were lower in the litter from the *A. mangium* monoculture. These enzymes are responsible for breaking down chitin and proteins, respectively, and are produced by microorganisms and plants (Parham and Deng 2000). Studies have shown an inverse relation between availability of a determined nutrient and the activity of enzymes specialized in breaking down organic compounds, because the increased production of enzymes by the microorganisms is only sustained when this leads to increased acquisition of resources (Sinsabaugh et al. 1993; Allison and Vitousek 2005). Olander and Vitousek (2000), for example, showed that the activity of NAG diminished significantly as the age and availability of N increased, when studying a chronosequence with three sites dominated by the arboreal species *Metrosideros polymorpha* Gaud. in Hawaii. Therefore, in the site with dominance of *A. mangium* (A100), we expected the activities of protease and NAG to be reduced because of the N-richer substrate formed by the leaf litter produced during the more than five years of the stand.

In contrast, in the mixed stand we observed higher microbial biomass, activities of proteases and NAG, and litter decomposition rate. This suggests that the mixture of residues from *A. mangium* and *E. urograndis* was more easily decomposed by the microbiota, influenced by the greater contribution of P supplied by *E. urograndis* and perhaps by the integration of the microorganisms colonizing the litter from the two species (Rachid 2013; Rachid et al. 2015). The greater N mineralization potential in soil samples from E100 + N and E50A50 corroborates the results obtained by the microbial activity indicators. These findings allow inferring that the improvements expected from introducing the leguminous nitrogen-fixing species on the litter decomposition, microbial activity and release of nutrients (especially N) are influenced by the structural quality of the residues produced by that species, the N:P ratio of the litter and the interaction with other microorganisms due to the increased diversity of the plant community. We observed all these benefits in the mixed plantation of *E. urograndis* and *A. mangium* (E50A50), indicating the advantage of this system regarding litter decomposition and microbial activity in relation to the two corresponding monocultures.

Conclusions

The microbial activity associated with the *A. mangium* monoculture was limited by the low structural quality of the litter from this species. Besides the higher content of lignin in the leaves, the smaller content of P in this litter caused a stoichiometric imbalance (N:P) of the organic substrate, and probably led to starvation/inhibition of the decomposers, attested by the low biomass and microbial activity of the soil under *A. mangium* monoculture. On the other hand, we raised evidence that mixed plantation of *E. urograndis* and *A. mangium*, with more diverse litter composition provides a better balanced supply of N and P able to sustain high microbial activity level with positive consequences on the litter decomposition, along with the probable integration of decomposing microorganisms. Therefore, we advocate that mixed plantation boost nutrient cycling efficiency and may

be a sustainable option to offset the high nitrogen export from successive monoculture-based silvicultural systems.

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References

Alef K, Nannipieri P (1995) Protease activities. In: Alef K, Nannipieri P (eds) Methods in applied soil microbiology and biochemistry. Academic Press, New York, pp 313–315

Allison SD, Vitousek PM (2005) Responses of extracellular enzymes to simple and complex nutrient inputs. Soil Biol Biochem 37:937–944. https://doi.org/10.1016/j.soilbio.2004.09.014

Anderson JPE, Domsch KH (1985) Determination of ecophysiological maintenance requirements of soil microorganisms in a dormant state. Biol Fertil Soils 1:81–89

Anderson JM, Ingram JSI (1993) Tropical soil biology and fertility: a handbook of methods. CAB International, Wallingford

Anderson JD, Ingram LJ, Stahl PD (2008) Influence of reclamation management practices on microbial biomass carbon and soil organic carbon accumulation in semiarid mined lands of Wyoming. Appl Soil Ecol 40:387–397. https://doi.org/10.1016/j.apsoil.2008.06.008

Austin AT, Vivanco L, González-Arzac A, Pérez LI (2014) There's no place like home? An exploration of the mechanisms behind plant litter-decomposer affinity in terrestrial ecosystems. New Phytol 204:307–314. https://doi.org/10.1111/nph.12959

Bachega LR, Bouillet J-P, de Cássia Piccolo M et al (2016) Decomposition of *Eucalyptus grandis* and *Acacia mangium* leaves and fine roots in tropical conditions did not meet the home field advantage hypothesis. For Ecol Manag 359:33–43. https://doi.org/10.1016/j.foreco.2015.09.026

Balieiro FC, Pereira MG, Alves BJR et al (2008) Soil carbon and nitrogen in pasture soil reforested with eucalyptus and guachapele. Rev Bras Ciênc Solo 32:1253–1260

Balieiro FC, Zilli JE, Prin Y, Faria SM (2010) Nodulação e fixação biológica de nitrogênio em *Acacia mangium*. In: Tonini H, Halfeld-Vieira BA (eds) *Acacia mangium*: características e seu cultivo em Roraima. Embrapa Informação Tecnológica, Brasília, pp 31–61

Balieiro FC, Franco AA, Pereira MG et al (2004) Contribution of litter and nitrogen to soil under *Pseudosamanea guachapele* and *Eucalyptus grandis* plantations. Pesq Agrop Brasileira 39:597–601

Bartlett RJ, Ross SD (1988) Colorimetric determination of oxidizable carbon in acid soil solutions. Soil Sci Soc Am J 52:1191–1192

Bauhus J, Winden AP, Nicotra AB (2004) Aboveground interactions and productivity in mixed species plantations of *Acacia mearnsii* and *Eucalyptus globulus*. Can J For Res 34:686–694

Berg B (2000) Litter decomposition and organic matter turnover in northern forest soils. For Ecol Manag 133:13–22

Berg B, Ekbohm G (1991) Litter mass-loss rates and decomposition patterns in some needle and leaf litter types. Long-term decomposition in a scots pine forest. VII. Can J Bot 69:1449–1456. https://doi.org/10.1139/b91-187

Bini D, Santos CA, Bouillet J-P et al (2013) *Eucalyptus grandis* and *Acacia mangium* in monoculture and intercropped plantations: evolution of soil and litter microbial and chemical attributes during early stages of plant development. Appl Soil Ecol 63:57–66. https://doi.org/10.1016/j.apsoil.2012.09.012

Bonanomi G, Capodilupo M, Incerti G, Mazzoleni S (2014) Nitrogen transfer in litter mixture enhances decomposition rate, temperature sensitivity, and C quality changes. Plant Soil 381:307–321. https://doi.org/10.1007/s11104-014-2119-4

Bouillet J-P, Laclau J-P, Gonçalves JL de M et al (2008) Mixed-species plantations of *Acacia mangium* and *Eucalyptus grandis* in Brazil 2. Nitrogen accumulation in the stands and biological N2 fixation. For Ecol Manag 255:3918–3930. https://doi.org/10.1016/j.foreco.2007.10.050

Bouillet J-P, Laclau J-P, Gonçalves JL de M et al (2013) *Eucalyptus* and *Acacia* tree growth over entire rotation in single-and mixed-species plantations across five sites in Brazil and Congo. For Ecol Manag 301:89–101. https://doi.org/10.1016/j.foreco.2012.09.019

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol Biochem 17:837–842. https://doi.org/10.1016/0038-0717(85)90144-0

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81:2359–2365. https://doi.org/10.1890/0012-9658(2000)081[2359:MESELD]2.0.CO;2

Eivazi F, Tabatabai MA (1988) Glucosidases and galactosidases in soils. Soil Biol Biochem 20:601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Elser JJ, Sterner RW, Gorokhova E et al (2000) Biological stoichiometry from genes to ecosystems. Ecol Lett 3:540–550. https://doi.org/10.1111/j.1461-0248.2000.00185.x

Embrapa (2011) Manual de métodos de análise de solo. Embrapa Solos, Rio de Janeiro

Epron D, Nouvellon Y, Mareschal L et al (2013) Partitioning of net primary production in *Eucalyptus* and *Acacia* stands and in mixed-species plantations: two case-studies in contrasting tropical environments. For Ecol Manag 301:102–111. https://doi.org/10.1016/j.foreco.2012.10.034

Faria SM, Diedhiou AG, Lima HC et al (2010) Evaluating the nodulation status of leguminous species from the Amazonian forest of Brazil. J Exp Bot 61:3119–3127

Ferreira EB, Cavalcanti P P, Nogueira DA (2013) ExpDes: Experimental Designs package

Forrester DI, Bauhus J, Cowie AL (2005a) On the success and failure of mixed-species tree plantations: lessons learned from a model system of *Eucalyptus globulus* and *Acacia mearnsii*. For Ecol Manag 209:147–155. https://doi.org/10.1016/j.foreco.2005.01.012

Forrester DI, Bauhus J, Cowie AL (2005b) Nutrient cycling in a mixed-species plantation of *Eucalyptus globulus* and *Acacia mearnsii*. Can J For Res 35:2942–2950. https://doi.org/10.1139/x05-214

Forrester DI, Bauhus J, Cowie AL (2006) Mixed-species plantations of *Eucalyptus* with nitrogen-fixing trees: a review. For Ecol Manag 233:211–230. https://doi.org/10.1016/j.foreco.2006.05.012

Forrester DI, Vanclay JK, Forrester RI (2011) The balance between facilitation and competition in mixtures of *Eucalyptus* and *Acacia* changes as stands develop. Oecologia 166:265–272. https://doi.org/10.1007/s00442-011-1937-9

Forrester DI, Pares A, Hara CO et al (2013) Soil organic carbon is increased in mixed-species plantations of *Eucalyptus* and nitrogen-fixing *Acacia*. Ecosystems 16:123–132

Franco AA, Faria SM (1997) The contribution of N2-fixing tree legumes to land reclamation and sustainability in the tropics. Soil Biol Biochem 29:897–903

Galiana A, Balle P, kang AN, Domenach AM (2002) Nitrogen fixation estimated by the 15N natural abundance method in *Acacia mangium* Willd. inoculated with Bradyrhizobium sp. and grown in silvicultural conditions. Soil Biol Biochem 34:251–262

Güsewell S, Gessner MO (2009) N : P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Funct Ecol 23:211–219. https://doi.org/10.1111/j.1365-2435.2008.01478.x

Hättenschwiler S, Jørgensen HB (2010) Carbon quality rather than stoichiometry controls litter decomposition in a tropical rain forest. J Ecol 98:754–763. https://doi.org/10.1111/j.1365-2745.2010.01671.x

Hättenschwiler S, Coq S, Barantal S, Handa IT (2011) Leaf traits and decomposition in tropical rainforests: revisiting some commonly held views and towards a new hypothesis. New Phytol 189:950–965. https://doi.org/10.1111/j.1469-8137.2010.03483.x

Inagaki M, Kamo K, Miyamoto K et al (2011) Nitrogen and phosphorus retranslocation and N:P ratios of litterfall in three tropical plantations: luxurious N and efficient P use by *Acacia mangium*. Plant Soil 341:295–307

Kaye JP, Resh SC, Kaye MW, Chimner RA (2000) Nutrient and carbon dynamics in a replacement series of *Eucalyptus* and *Albizia* trees. Ecology 81:3267–3273. https://doi.org/10.1890/0012-9658(2000)081[3267:NACDIA]2.0.CO;2

Keeney DR, Bremner JM (1966) Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability1. Agron J 58:498. https://doi.org/10.2134/agronj1966.00021962005800050013x

Kelty MJ (2006) The role of species mixtures in plantation forestry. For Ecol Manag 233:195–204. https://doi.org/10.1016/j.foreco.2006.05.011

Kempers AJ, Zweers A (1986) Ammonium determination in soil extracts by the salicylate method. Commun Soil Sci Plant Anal 17:715–723. https://doi.org/10.1080/00103628609367745

Khanna PK (1997) Comparison of growth and nutrition of young monocultures and mixed stands of *Eucalyptus globulus* and *Acacia mearnsii*. For Ecol Manag 94:105–113. https://doi.org/10.1016/S0378-1127(96)03971-0

Koutika LS, Epron D, Bouillet JP, Mareschal L (2014) Changes in N and C concentrations, soil acidity and P availability in tropical mixed acacia and eucalypt plantations on a nutrient-poor sandy soil. Plant Soil 379:1–12. https://doi.org/10.1007/s11104-014-2047-3

Koutika L-S, Mareschal L, Epron D (2016) Soil P availability under eucalypt and acacia on Ferralic Arenosols, republic of the Congo. Geoderma Reg 7:153–158. https://doi.org/10.1016/j.geodrs.2016.03.001

Krisnawati H, Kallio M, Kanninen M (2011) *Acacia mangium* Willd.: ecology, silviculture, and productivity. CIFOR, Bogor, Indonesia

Laclau J-P, Bouillet J-P, Gonçalves JL de M et al (2008) Mixed-species plantations of *Acacia mangium* and *Eucalyptus grandis* in Brazil: 1. Growth dynamics and aboveground net primary production. For Ecol Manag 255:3905–3917. https://doi.org/10.1016/j.foreco.2007.10.049

Laclau J-P, Ranger J, Gonçalves JL de M et al (2010) Biogeochemical cycles of nutrients in tropical *Eucalyptus* plantations: main features shown by intensive monitoring in Congo and Brazil. For Ecol Manag 259:1771–1785. https://doi.org/10.1016/j.foreco.2009.06.010

le Maire G, Nouvellon Y, Christina M, et al (2013) Tree and stand light use efficiencies over a full rotation of single- and mixed-species *Eucalyptus grandis* and *Acacia mangium* plantations. For Ecol Manag 288:31–42. https://doi.org/10.1016/j.foreco.2012.03.005

Mercado AN, van Noordwijk M, Cadisch G (2011) Positive nitrogen balance of *Acacia mangium* wood lots as fallows in the Philippines based on15N natural abundance data of N2 fixation. Agrofor Syst 81:221–233. https://doi.org/10.1007/s10457-010-9309-8

Novotny EH, Rodrigues AF, Balieiro FC, et al (2013) Avaliação de alterações estruturais durante a decomposição de serapilheira de eucalipto e *Acacia mangium* por meio de espectroscopia vibracional (FTIR) aliada à análise de componentes principais (PCA). In: Encontro Brasileiro de Substâncias Húmicas. Sociedade Internacional de Substâncias Húmicas, Santo Antônio de Goiás

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activityby N and P availability. Biogeochemistry 49:175–191. https://doi.org/10.1023/A:1006316117817

Olson JS (1963) Energy storage and the balance of producers and decomposers in ecological systems. Source Ecol 44:322–331

Osono T (2007) Ecology of ligninolytic fungi associated with leaf litter decomposition. Ecol Res 22:955–974. https://doi.org/10.1007/s11284-007-0390-z

Osono T, Takeda H (2006) Fungal decomposition of *Abies* needle and *Betula* leaf litter. Mycologia 98:172–179

Parham JA, Deng SP (2000) Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biol

Biochem 32:1183–1190. https://doi.org/10.1016/S0038-0717(00)00034-1

R Development Core Team (2017) R: a language and environment for statistical computing

Rachid CTCC (2013) Biodisponibilidade de nutrientes e estrutura microbiana do sistema solo-serapilheira em floresta plantada mista de *Eucalyptus urograndis* e *Acacia mangium*. Federal University of Rio de Janeiro

Rachid CTCC, Balieiro FC, Fonseca ES et al (2015) Intercropped silviculture systems, a key to achieving soil fungal community management in *Eucalyptus* plantations. PLoS One 10: e0118515. https://doi.org/10.1371/journal.pone.0118515

Resh SC, Binkley D, Parrotta JA (2002) Greater soil carbon sequestration under nitrogen - fixing trees compared with *Eucalyptus* species. Ecosystems 5:217–231. https://doi.org/10.1007/s10021-001-0067-3

Santos FM, Balieiro F, de C, Ataíde DH dos S et al (2016) Dynamics of aboveground biomass accumulation in monospecific and mixed-species plantations of *Eucalyptus* and *Acacia* on a Brazilian sandy soil. For Ecol Manag. https://doi.org/10.1016/j.foreco.2015.12.028

Santos FM, Chaer GM, Diniz AR, Balieiro F, de C (2017) Nutrient cycling over five years of mixed-species plantations of *Eucalyptus* and *Acacia* on a sandy tropical soil. For Ecol Manag 384:110–121. https://doi.org/10.1016/j.foreco.2016.10.041

Silva EE, Azevedo PHS, De-Polli H (2007a) Determinação do nitrogênio da biomassa microbiana (BMS-N). In: Comun. Técnico 96 Embrapa Agrobiol. https://www.infoteca.cnptia.embrapa.br/bitstream/doc/597310/1/cot096.pdf

Silva EE, Azevedo PHS, De-Polli H (2007b) Determinação da respiração basal (RBS) e quociente metabólico do solo (qCO2). In: Comun. técnico 99 Embrapa Agrobiol. https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/627577/1/cot099.pdf

Sinsabaugh RL, Antibus RK, Linkins AE et al (1993) Wood decomposition: nitrogen and phosphorus dynamics in relation to extracellular enzyme activity. Ecology 74:1586–1593. https://doi.org/10.2307/1940086

Smith VH (2002) Effects of resource supplies on the structure and function of microbial communities. Antonie Van Leeuwenhoek 81:99–106. https://doi.org/10.1023/A:1020533727307

Tabatabai MA (1994) Soil enzymes. In: Weaver RW (ed) Methods of soil analysis. Soil Science Society of America, Madison, pp 775–833

Tang G, Li K, Zhang C et al (2013) Accelerated nutrient cycling via leaf litter, and not root interaction, increases growth of *Eucalyptus* in mixed-species plantations with *Leucaena*. For Ecol Manag 310:45–53. https://doi.org/10.1016/j.foreco.2013.08.021

Van Soest PJ (1994) Nutritional ecology of the ruminant, 2nd edn. Cornell University Press, Ithaca

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vandermeer JH (1989) The ecology of intercropping. Cambridge University Press, New York

Violle C, Navas M-L, Vile D et al (2007) Let the concept of trait be functional! Oikos 116:882–892. https://doi.org/10.1111/j.0030-1299.2007.15559.x

Voigtlaender M, Laclau J-P, Gonçalves JL de M et al (2012) Introducing *Acacia mangium* trees in *Eucalyptus grandis* plantations: consequences for soil organic matter stocks and nitrogen mineralization. Plant Soil 352:99–111. https://doi.org/10.1007/s11104-011-0982-9

Xiang W, Bauhus J (2007) Does the addition of litter from N-fixing *Acacia mearnsii* accelerate leaf decomposition of *Eucalyptus globulus*? Aust J Bot 55:576–583. https://doi.org/10.1071/BT06083