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Soil C and Al availability in tropical single and mixed-species of *Eucalyptus* sp. and *Acacia mangium* plantations



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ABSTRACT

The Brazilian forestry sector has expanded to the Cerrado biome regions, where the soils are highly acid, with a high exchangeable Al content and a general nutritional deficiency. Nevertheless, the levels of soil organic matter (SOM) under the forest plantings are generally high and may play an important role in the complexation of Al in the soil solution. The objective of this study was to evaluate the effect of the introduction of *Acacia mangium* in monospecific *Eucalyptus* spp. plantations on the soil C content and its effect on the soil Al availability. The study was conducted at four experimental sites in São Paulo and Minas Gerais States in Brazil. We evaluated three treatments: monospecific stands (pure) of *Acacia mangium* and *Eucalyptus* and a mixed stand (constituted by *Acacia mangium* and *Eucalyptus* in 1:1 proportion), with four replicates at each site. The soil total C and the Al content in the soil depths of 0–5 and 5–10 cm. The introduction of *Acacia* in *Eucalyptus* plantations (mixed stand) significantly increased the soil C content by approximately 10% only at one of four sites studied, which was probably because the land was used as pasture before the introduction of the forest plantation and the because of high N input in a *Eucalyptus* plantation. Despite little improvement in the soil C content and consequently in the Al complexed by the soil organic matter, the introduction of *Acacia* in the *Eucalyptus* stands increased the Al availability by approximately 13% due to the reduction in the soil pH.

1. Introduction

Approximately 30% of the soils worldwide are classified as acidic, and this encompasses 60% of the soils in the humid tropics. Among the soil factors that affect agroforestry production, Al is the most limiting due to its toxicity. This is particularly important in Brazil, where > 50% of the soils are classified as acidic, with potentially toxic Al in the surface and subsurface soil layers (von-Uexküll and Mutert, 1995; Eswaran et al., 1997).

High Al concentrations usually inhibit root growth (Marschner, 1991), resulting in the inefficient absorption of water and nutrients (Silva et al., 2004), causing lower productivity, especially in regions where periods of drought (dry spells) occur such as in the Cerrado (Savannah) biome in Brazil. The forest plantations in Brazil have expanded to the Cerrado biome where the soils, despite having physical and topographical features favorable to mechanization, have detrimental chemical properties, such as high acidity, a high exchangeable Al

content and a general nutrient deficiency, especially in phosphorus. When chemically corrected, these soils have great potential for the implementation of technological management that results in high yields (Sousa et al., 2007).

Eucalyptus species are tolerant to Al in the soil solution (Neves et al., 1982a; Silva et al., 2004; Gonçalves et al., 2013), and they may also derive some benefits from its presence (Mullette, 1975; Keltjens and van Loenen, 1989; Vale et al., 1984; Huang and Bachelard, 1993; Watanabe et al., 1998; Silva et al., 2004), possibly due to root exudation of low molecular weight organic acids (Smith et al., 2003; Silva et al., 2004). Al complexation by these organic acids may protect against the damage caused by Al, allowing that plants to grow well in an acidic soil in the presence of high concentrations of exchangeable Al (Neves et al., 1982b; Vale et al., 1996). Another factor contributing to the reduced effect of Al in *Eucalyptus* plantations is the high content of soil organic matter under these plantations (Gonçalves et al., 2013; Gonçalves and Mello, 2000; Neves, 2000). Organic compounds (OA) formed by

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decomposition can lead to the formation of an Al-OA complex (Shotyk and Sposito, 1990; Hiradate et al., 1998), reducing the Al activity in the soil solution (Meurer et al., 2010).

The introduction of *Acacia mangium* Willd, a N-fixing tree, in singlespecies plantations of *Eucalyptus* can raise the soil organic C stocks (Forrester et al., 2013; Voigtlaender et al., 2012; Hoogmoed et al., 2014; Koutika et al., 2014). A change in the quality of the soil organic matter in N-fixing trees mixed with *Eucalyptus* plantations also has been reported. We propose the hypothesis that the improvement in the quantity and quality of the soil organic matter in multi-species plantations increases the amount of the Al-OA complex and consequently reduces the availability of toxic Al in the soil solution. Thus, our objective was to evaluate the effect of the introduction of *Acacia mangium* into monospecific plantations of *Eucalyptus* spp. on the soil C content and in its effect on four different forms of soil-available Al.

2. Materials and methods

2.1. Description of the study areas

This study was conducted at four experimental sites located in Itatinga (IT), Bofete (BO) and Luís Antônio (LA) in the São Paulo State, and in Santana do Paraíso (SdP) in the Minas Gerais state (Fig. 1), Brazil. These areas experience edaphoclimatic conditions representative of the main forest plantation regions with *Eucalyptus* spp. in Southeast Brazil (Tables 1 and 2).

Table 1 Location, altitude, relief, soil and climate characteristics of the experimental sites.

The experimental sites experienced historical reforestation with *Eucalyptus* spp., between 22 and 62 years ago (Table 2). At all sites, we used a randomized complete block design with three treatments and four replications (blocks). The treatments were as follows:

monospecific Acacia mangium stand;

monospecific Eucalyptus sp. stand; and

mixed stand with *Eucalyptus* sp. and *Acacia mangium* with a 50% planting density for each species.

All forest residues remained on the soil after the previous rotation was harvested to the installation of the experiment. Seedlings were planted after the planting lines were subsoiled to a depth of 40 cm. The *Acacia mangium* seedlings were inoculated with the N-fixing bacteria BR 3609 T and BR 6009, specifically selected for this species by EMBRAPA Agrobiologia. Further details of the treatments as well as the installation and conduct of the experiment can be found in Laclau et al. (2008). Table 2 Characteristics of the experimental sites.

2.2. Soil sampling

Soil sampling was carried out with the aid of an auger-type stainless steel probe. Five sampling points were selected on the planting lines and five points between the planting lines, a total of 10 sampling points evaluated per plot. Eight samples were collected from each point at soil depths of 0–5 and 5–10 cm for a total of 80 simple samples per soil layer (depth), from which we made a pooled soil sample for each depth per plot.

The BO site was sampled in September 2012 when the forest stands was almost seven and a half years old. Sampling of the LA and IT sites was done in November 2012, when the forest stands were almost eight and three years old (for the second rotation), respectively. Soil from the SdP site was collected in January 2013, six months after the forest stands were harvested at seven years and eight months old. The soil samples were dried at 45 °C to constant weight and sieved through a 2 mm sieve.

2.3. Soil analysis

The total soil C content was determined by dry combustion in an LECO TruSpec[®] CHNS Micro elemental analyzer (Saint Joseph, MI, USA). The soil Al was extracted with 0.01 mol L^{-1} CaCl₂, 1 mol L^{-1} KCl and 0.5 mol L^{-1} CuCl₂. It was assumed that the CaCl₂ extracted the available Al from the soil solution (Al_s) (Dougan and Wilson, 1974; Prosser et al., 1993), the KCl extracted the exchangeable aluminum (Al_e) (Jardine and Zelazny, 1996) and the CuCl₂ extracted the non-exchangeable Al (Juo and Kamprath, 1979; Urrutia et al., 1995).

The non-exchangeable Al is predominantly bound to the soil organic matter in the surface layer of the soil (0–10 cm) (Kaiser and Zech, 1996; Zambrosi et al., 2007). Thus, the Al complexed by the soil organic matter (Al_{OM}) was obtained by the difference between the Al extracted by the 0.5 mol L⁻¹ CuCl₂ and the Al extracted by the 1 mol L⁻¹ KCl.

For the Al extraction, 2 g of dried soil was placed in an 80 ml plastic jar, and 20 ml of the extraction solvent was added. After addition of the extraction solvent, the samples were shaken for 5 min on a horizontal table shaker at 220 rpm and left to precipitate for 16 h. The Al extracted by CaCl₂ and KCl was determined by titration with 0.025 mol L⁻¹ NaOH, using phenolphthalein as an indicator. For the determination of



Fig. 1. Location of the four experimental sites.

Table 1

Location, altitude, relief, soil and climate characteristics of the experimental sites.

Site ⁽¹⁾	Latitude	Longitude	Altitude	Relief	Soil class ⁽²⁾ , texture	Climate ⁽³⁾	AAR ⁽⁴⁾	AAT ⁽⁵⁾
IT BO LA SdP	(S) 23° 02′ 23° 11′ 21° 35′ 19° 16′	(W) 48° 37' 48° 25' 47° 31' 41° 47'	(m) 830 570 620 290	Gently undulating Gently undulating Gently undulating Wavy/undulating	Ferrasol, medium texture Ferrasol, medium texture Arenosols, sandy texture Ferrasol, clayey texture	Cfa Cfa Cwa Aw	(mm) 1319 1293 1463 1394	(°C) 19.4 19.9 20.7 22.0

¹ Location of the experimental sites: IT - Experimental Station of Forests Sciences of Itatinga/University of São Paulo; BO - Company Suzano Papel e Celulose S/A; LA - Company International Paper of Brasil Ltda; SdP - Company Celulose Nipo-Brasileira S/A.

² Soil classification according to FAO (2015).

³ Köppen's classification according to Álvares et al., 2013: Aw - altitude tropical, with winter dry season; Cfa; Cwa - temperate humid with dry winter and hot summer.

⁴ AAR: average anual rainfall.

⁵ AAT: average annual temperature of the air.

the Al extracted by CuCl₂, a flame atomic absorption spectrophotometer with nitrous oxide as an oxidizer was used.

3. Results

3.1. Soil C content

2.4. Statistical analysis

The data were analyzed according to a randomized complete block design with three treatments and four replications, for a total of 12 plots at each depth for each site (Fig. 1). The treatments evaluated were pure stands of Acacia mangium and Eucalyptus and a mixed stand at soil depths of 0-5 and 5-10 cm. The data were tested for normality (Shapiro-Wilk test) and homoscedasticity (homogeneity of variance -Box-Cox test). A general analysis of variance (ANOVA) was performed using the treatments, the soils (Arenosol, Ferralsol with medium texture and Ferralsol with clayey texture), the blocks and the treatment vs. soil interaction as the source of variation. Because interactions were found for most variables, we also performed a F test for each site with treatments and blocks as the source of variation. When the F test was significant (p < 0.10) we performed the LSD test at 5% probability to assess the difference between the treatments. To isolate the effect of soil organic matter on the availability of Al, the pH was treated as covariate in the analysis model. The relationship between the Al content extracted by the various solutions with the soil pH and the soil C content were assessed using a Spearman correlation analysis and linear and nonlinear regression analyses. The SAS University Edition software was used to perform the analyses.

Table 2 Characteristics of the experimental sites.

The ANOVA included all of the soil types, treatments and corresponding interactions and showed differences between soil class (p < 0.001) for soil C stabilization, specifically in terms of the soil texture (Table 3). The clayey soil (SdP) had 1.7-fold more C than the sandy soil (LA). We found a trend of highest C concentration in the soil under Acacia (8% higher than under *Eucalyptus* plantation) and the lowest under the *Eucalyptus* plantations, but it was significant

(p < 0.09) only at the BO site (Tables 3 and 4). Table 3. Effects of soil (S), treatment (T) and treatment and soil (T × S) interaction on soil C content (C), pH, the Al bound to the soil organic matter (Al_{OM}, mmolc kg⁻¹), the exchangeable Al (Al_e, mmolc kg⁻¹) and the Al in soil solution (Al_s, mmolc kg⁻¹) in pure stands of *Acacia mangium*, *Eucalyptus* or mixed forest stands.

Table 4 Soil C content in pure stands of *Acacia mangium* and *Eucalyptus* and in mixed forest stands.

3.2. Al availability in the soil

The Al_{OM}, Al_s and Al_e had different behavior according to soil type. The Al_{OM} was clearly higher in the Ferralsol with a clayey texture (33.58 mmolc kg⁻¹; 3.4-fold greater than the Arenosol), however the Al_s and Al_e were higher in the Ferralsol with a medium texture (5.54 mmolc kg⁻¹ and 3.33 mmolc kg⁻¹, respectively). For the treatments, pure stands of Acacia had higher values for all forms of extractable Al, with significant differences (p < 0.001) for Al_{OM} and

Site	Eucalyptus genotipe	Spacing $(m \times m)$	Density trees (trees ha^{-1})	Planting date (month/year)	Total plot (trees)	Evaluated plot (trees)	Land use historical ⁽²⁾	Fertilizer applied ⁽³⁾ (4) (kg ha ^{-1})	
IT	E. grandis	3 × 3	1111	05/2003 11/2009 ⁽¹⁾	10 × 10	6 × 6	< 1940: Cerrado 1940–1988: <i>E saligna</i> (Coppice) 1988–2002: <i>E. grandis</i>	P: 44; K:85 Limestone: 2000 B, Fe, Zn, Mn	
во	E. grandis	3×2	1666	02/2005	8×10	4 × 6	< 1972: pasture 1972–2004: Eucaliptus sp.	P: 37; K: 186 Boillers ash: 3000 B. S	
LA	E. urophylla \times E. grandis	3 × 3	1111	02/2005	10 × 10	6 × 6	< 1982: Cerrado 1982–2004: E. grandis	N: 4 ⁽⁵⁾ ; P: 29; K: 147 Limestone: 1200 Cu, Zn,B	
SdP	E. urophylla \times E. grandis	3 × 3	1111	11/2004	10 × 10	6 × 6	< 1960: Cerrado 1960–2004: <i>Eucalyptus sp.</i>	N: 6; P: 45; K: 162 Dolomite: 1500 Cu, B, Zn	

¹ The first experimental rotation was from May 2003 to September 2009, and the experiment was reinstalled in November 2009, with the same characteristics and in the same area as the previous planting

 2 In all areas, the shoots of the previous rotations were suppressed by glyphosate application. At harvest, only the trunks (wood + bark) was removed, and the remaining residues were spread evenly in the field.

³ Conventional fertilization was routinely performed by Eucalyptus reforestation companies in their respective experimental areas.

⁴ N included in the NPK fertilizer was the same applied in commercial plantations. Fertilization was split between planting time and top dressing 1.5 years after planting.

Table 3

				-						
P values of fixed effects				Mean value per so	pil		Mean value per treatment			
	Soil	Treatment	$\mathbf{T}\times\mathbf{S}$	Arenosol sandy texture	Ferrasol medium texture	Ferrasol clay texture	Acacia mangium	Mixed	Eucalyptus	
				g	kg ⁻¹	g kg ⁻¹				
С	< 0.001	0.1773	0.5735	14.5b	13.3c	25.0a	17.10	16.70	15.90	
pН	< 0.001	0.0361	0.0100	4.10b	3.91b	4.95a	4.15b	4.36ab	4.44a	
Alom	< 0.0001	0.0019	< 0.0001	13.87c	16.23b	47.45a	28.03a	25.13b	24.39b	
Ale	< 0.001	0.0075	0.0138	5.89b	9.21a	3.77c	7.28a	6.14ab	5.45b	
Als	< 0.001	0.1610	0.3456	2.84b	4.83a	1.50c	3.32	3.13	2.72	

Effects of soil (S), treatment (T) and treatment and soil (T x S) interaction on soil C content (C), pH, the Al bound to the soil organic matter (Al_{OM} , mmolc kg^{-1}), the exchangeable Al (Al_e , mmolc kg^{-1}) and the Al in soil solution (Al_s , mmolc kg^{-1}) in pure stands of *Acacia mangium*, *Eucalyptus* or mixed forest stands.

Significant fixed effects (P < 0.05) are highlighted in bold. Different letters in the same line indicates significant differences between soil or/and treatment.

Al_e (Table 3).

The Al_s content ranged from 0.66 to 6.23 mmol_c kg⁻¹, with the highest values observed at the BO site with a medium soil texture. This site also had the highest Al_e content, which ranged from 1.77 to 11.40 mmol_c kg⁻¹. On the other hand, the SdP site with a clay texture had the lowest Al_s and Al_e content and the highest Al_{OM} content, and it was the only site that showed significant differences between treatments for all forms of extractable Al. Although in the 5–10-cm soil layer at IT, we also found significant differences for A and E in the pure stands, which were one unit lower for Al_s than in the mixed forest stand (Tables 3 and 5).

Significant differences in soil pH was found for soil type, treatment and interaction (Table 3). The lowest acidity was observed in the Ferralsol clayey soil and the highest in the Ferralsol medium texture soil and the Arenosol, which were significantly equivalent. An increase of *Eucalyptus* in the stands (from mixed to pure) was associated with higher pH.

At the SdP site, pure stands of Acacia had the lowest pH, the highest Al_s and Al_e content at the 0–5 and 5–10-cm soil depth. A difference in the Al_{OM} content was observed only in the 0–5-cm soil layer. In contrast, *Eucalyptus* in pure stands had the highest pH and the lowest soil Al content, and mixed forest stands had intermediate values (Table 5).

A strong relationship between Al_s ($R^2 = 0.63$; p < 0.01) and Al_e ($R^2 = 0.67$; p < 0.01) with soil pH was observed. The Al_{OM} content and the CEC_e was strongly correlated to the soil C content (Fig. 2).

Table 5. Mean Al content of in the soil solution (Al_s) , exchangeable Al (Al_e) and Al bound to the soil organic matter (Al_{OM}) in pure stands of *Acacia mangium* or *Eucalyptus* and in mixed forest stands.

4. Discussion

4.1. Soil C content

Several studies have shown C sequestration in forest soils is greater under N_2 -fixing than under non- N_2 -fixing species in temperate and tropical regions, (Kaye et al., 2000; Johnson and Curtis, 2001; Sigrid et al., 2002; Resh et al., 2002; Binkley, 2005; Jeddi et al., 2009; Wang et al., 2010; Forrester et al., 2013; Koutika et al., 2014). However, in this study, we found significant differences in the total soil C content (Table 4) only at the BO site, a Ferralsol with a medium texture, which probably was an effect that resulted from the historic land use as pasture before the *Eucalyptus* plantation and was associated with the higher planting density and the high N fertilizer input in the experimental area compared to the other experimental sites (Bouillet et al., 2013) (Table 2). Variability among sites was also shown in Hoogmoed et al. (2014), in which increases, no change or decreases for young mixed-species restoration plantings in Australia were observed.

Previous land use has a strong impact on the soil C sequestration potential of forested sites. In addition, the C compounds present in residues drives the decomposition rate in a forest ecosystem (Hättenschwiler and Jørgensen, 2010), which is determined by the recalcitrant components (Bachega et al., 2016). On the other hand, pasture residues contain more labile compounds than forest residues, which are associated with higher decomposition rates (Eclesia et al., 2012). As result, pasture soils have high C stocks and high root densities in the upper part of the mineral soil that promote aggregate formation and soil C stabilization in the aggregates (Six et al., 2002); therefore, forestation may have little effect (Guo and Gifford, 2002; Murty et al., 2002).

A *Eucalyptus* forest plantation following the pasture at the BO site probably increased the litterfall dry matter that maintained the C cycle in the soil. In addition, the high N fertilizer input (Bouillet et al., 2013) contributed to the change in the decomposition rate for the medium texture. However, Berg and Matzner (1997) stated that high levels of soil N can slow down the decomposition of the recalcitrant fraction (lignin) of the organic matter. This finding could also explain the low increase in the soil C content in the Acacia pure stands, corroborating the lower decomposition rates for the Acacia residues observed by Bachega et al. (2016). Others researchers have found a general long-term stimulating effect on microbial activity due to N addition on the recalcitrant SOM (Fog, 1988; Berg and Matzner, 1997). Keyser et al. (1978) explained that white-rot fungus did not synthesize its lignin-degrading enzymes in the presence of low-molecular N-rich compounds

Table 4

Soil C content in pure stands of Acacia mangium and Eucalyptus or in mixed forest stands.

Soil class	Site	Depth	Acacia mangium		Eucalyptus	Eucalyptus			Mixed			
		(cm)	$CO g kg^{-1}$									
Arenosols	LA	0–5	10.42	(0.06)		9.82	(0.12)		11.23	(0.04)		0.4039
(sandy texture)		5-10	12.44	(0.15)		11.15	(0.10)		13.66	(0.08)		0.1403
Ferrasol	IT	0–5	16.25	(0.09)		15.27	(0.19)		15.82	(0.14)		0.9176
(medium texture)		5-10	14.25	(0.06)		12.46	(0.07)		13.34	(0.15)		0.4843
	BO	0-5	13.23	(0.13)	а	9.87	(0.10)	b	10.57	(0.08)	ab	0.0899
		5-10	14.78	(0.21)	а	11.35	(0.15)	b	13.16	(0.07)	ab	0.0874
Ferrasol	SdP	0–5	26.81	(0.20)		28.14	(0.22)		27.75	(0.32)		0.8471
(clayey texture)		5–10	23.68	(0.25)		22.09	(0.20)		21.54	(0.22)		0.4116

Values in parentheses refers to the standard error (±). Means followed by different letter on the row, at each depth and each site, differ by LSD test.

Table 5

Mean Al content of in the soil solution (Al_s), exchangeable Al (Al_e) and Al bound to the soil organic matter (Al_{OM}) in pure stands of Acacia mangium or Eucalyptus and in mixed forest stands.

Soil class	Site	Depth	Acacia mangium		Eucalyptus	Eucalyptus		Mixed	
		(cm)	Al, (mmolc kg ⁻	⁻¹)					
Arenosols	LA	0-5	2.90	(0.30)	2.31	(0.26)	2.72	(0.31)	0.3161
(sandy texture)		5-10	2.96	(0.35)	3.20	(0.48)	3.32	(0.31)	0.1573
Ferrasol	IT	0–5	3.79	(0.28)	3.75	(0.40)	4.68	(0.38)	0.1588
(medium texture)		5-10	3.50	(0.37)b	3.61	(0.38)b	4.62	(0.35)a	0.0169
	BO	0–5	6.23	(0.65)	5.40	(0.53)	5.18	(0.55)	0.5344
		5-10	5.81	(0.38)	5.63	(0.38)	5.46	(0.34)	0.5911
Ferrasol	SdP	0–5	1.89	(0.57)a	0.66	(0.16)b	1.06	(0.32)ab	0.0485
(clayey texture)		5-10	3.04	(0.84)a	1.12	(0.30)b	2.01	(1.19)ab	0.0202
			Al_ (mmolc kg ⁻	-1)					
Arenosols	LA	0–5	5.87	(0.54)	4.62	(0.49)	4.98	(0.66)	0.3572
(sandy texture)		5-10	6.88	(0.44)	6.21	(0.78)	7.18	(0.50)	0.6061
Ferrasol	IT	0–5	6.94	(0.26)	7.63	(0.78)	8.01	(0.73)	0.5093
(medium texture)		5-10	8.37	(0.31)	8.01	(0.38)	8.84	(0.59)	0.5875
	BO	0–5	11.40	(1.11)	9.02	(0.85)	9.77	(1.48)	0.3453
		5-10	10.69	(0.53)	10.39	(0.73)	11.16	(0.59)	0.4318
Ferrasol	SdP	0-5	4.74	(2.07)a	1.77	(0.24)b	1.71	(0.75)b	0.0303
(clayey texture)		5-10	9.77	(1.40)a	2.66	(1.14)c	4.86	(3.57)b	0.0111
Alom (mmolc kg $^{-1}$)									
Arenosols	LA	0–5	13.41	(1.11)	12.37	(1.09)	13.74	(0.70)	0.5388
(sandy texture)		5-10	14.61	(1.62)	14.19	(1.22)	15.40	(1.77)	0.5754
Ferrasol	IT	0-5	18.37	(1.29)	17.01	(1.23)	16.67	(1.11)	0.5923
(medium texture)		5-10	18.71	(0.80)	16.82	(1.83)	19.26	(1.37)	0.2626
(во	0-5	11.05	(2.13)	12.82	(0.78)	14.71	(0.94)	0.3365
		5-10	17.93	(0.82)	16.31	(0.89)	15.65	(1.41)	0.0986
Ferrasol	SdP	0-5	53.68	(4.37)a	36.12	(10.92)b	46.85	(4.84)ab	0.0386
(clayey texture)		5-10	55.31	(4.77)	44.12	(8.90)	42.31	(6.74)	0.4186
рН (H ₂ O)									
Arenosols	LA	0-5	4.83	(0.05)	4 93	(0.05)	4 95	(0.06)	0 2441
(sandy texture)	1.2 1	5-10	4.80	(0.03)	4.90	(0.03)	4.95	(0.05)	0.0963
Ferrasol	IT	0-5	4.00	(0.04)	4.90	(0.04)	4.83	(0.03)	0.0505
(medium texture)	11	5-10	4.93	(0.00)	4.85	(0.05)	4.83	(0.03)	0.2441
(incutain texture)	RO	0.5	4.53	(0.03)	4.53	(0.05)	4.60	(0.03)	0.1664
	DO	5-10	4 75	(0.02)	4.68	(0.03)	4.00	(0.04)	0.4655
Ferrasol	SAD	0.5	T./J	(0.00) (0.17)b	5.00	(0.03)	5.62	(0.03) (0.18)ab	0.4033
(clavev texture)	Jur	5-10	473	(0.17)b	5.50	(0.25)a	5.08	(0.10)ab	0.0309
(clayey texture)		0-10	1.7 5	(0.10)0	0.10	(0.1 <i>0)</i> a	0.00	(0.17 Ju	0.0009

Values in parentheses refers to the standard error (±). Means followed by different letter on the row, at each depth and each site, differ by LSD test.

such as ammonium and amino acids.

Soil texture evidenced a strong effect in this study. The highest clay content at the SdP site affected the soil C content, and showed the highest values, evidencing the noteworthy function of the clay content on soil C stabilization. Jandl et al. (2007) stated that the stabilization of soil C is not strongly related to site productivity, but that soil properties play a dominant role. Experiments using a ¹³C tracer showed that the net accumulation of new tree-derived C was greater in loamy soils with a low productivity than in fertile sandy soils with high productivity (Hagedorn et al., 2003). However, on a poor sandy mineral soil, a mixture of spruce was reported to store more C than on a fertile soil, which was attributed to the slow decomposition and complex formation between organic molecules and metal ions (Vesterdal et al., 2007).

An increase in the soil C content with an increase of Acacia in the stand was found in this study, which could be because the Acacia pure stand had the highest N content (Voigtlaender et al., 2012; Bachega et al., 2016), which was a potential driver of the change in the soil C content. However, this treatment did not show the highest aboveground biomass production (Bouillet et al., 2013). Forrester et al. (2013) stated that the soil C stock was not linearly related to the proportion of *A. mearnsii* in a stand, but showed a high positive correlation with the aboveground biomass, and because a mixed stand is significantly more productive than a monoculture, the soil C was also highest in a mixed stand. Hoogmoed et al. (2014)also provided the justification that N₂-fixing trees had the highest C content underneath them because their higher N fertility resulted in more rapid tree growth, which consequently increased the below-ground C input to the soil (e.g., Resh et al.,

2002). Nevertheless, the mechanisms behind the soil C differences under N_2 -fixing species are not well established and are likely to be a net effect of three processes (Binkley, 2005; Forrester et al., 2013). Soil C could be increase by increased by forest residue input, a decreased decomposition rate of the recent C pool or stabilization of the old C pool (from the last rotation), or by an increase of the rate at which the recent C from forest residue is incorporated into the pool of stabilized or slowly turning over soil C.

The use of N₂-fixing trees, alone or in mixed forest plantations may be a good strategy for future C sequestration through intense forest management and reforestation programs (Zhang et al., 2012). However, there is a lack of consensus about how N₂-fixing and non- N₂-fixing trees interact, what the drives differences among studies and how it is possible to maximize soil C sequestration by the introduction of N₂fixing trees into a *Eucalyptus* plantation. In addition, the effect of N₂fixing tree species on soil C sequestration is species-specific and should not be generalized, but requires more long-term planting experiments to determine if benefits to C sequestration will occur (Hoogmoed et al., 2014).

4.2. Availability of Al in the soil

The availability of Al in a highly weathered soil is strongly affected by pH (Alleoni et al., 2009; Zambrosi et al., 2007; Gruba et al., 2015). However, other factors such as the soil organic matter content may have a great effect on Al availability (Urrutia et al., 1995; Alleoni et al., 2009; Zambrosi et al., 2007; Gruba and Mulder, 2015), because the soil



Fig. 2. Relationships between soil pH in water with the Al content in the soil solution (Al_s) (a) exchangeable Al (Al_e) (c) Al complexed by soil organic matter (Al_{OM}) (e) and relationships between the total soil C content with the Al content complexed by the soil organic matter (b), the effective soil CEC (CEC_e) (d) and percentage of Al extracted by 0.5 mol L⁻¹ CuCl₂ that was in the exchangeable form (f).

organic matter can complex Al and decrease its availability (Walker et al., 1990; Berggren and Mulder, 1995; Walna et al., 2005; Brady and Weil, 2013). This fact has been confirmed and explains the low toxicity of Al on crops (Al sensitive species) in low pH soils cultivated with notillage (Alleoni et al., 2003, 2005; Caires et al., 1998, 2004, 2005).

The introduction of leguminous trees in a *Eucalyptus* monospecific stand can increase the soil organic matter content and improve its quality (Forrester et al., 2013), which can also affect the soil Al availability. Nevertheless, such an effect was not observed in this study (Table 4). The SdP site was the only site that showed a difference in Al availability with treatment, however it did not show a difference in the C content. Using the pH as a co-variant in the ANCOVA model did not reveal differences at the SdP site, indicating that this difference was due to the pH difference with treatment and not to the soil C content.

It has been recognized that leguminous species cause soil acidification (Binkley, 1992; Yamashita et al., 2008; Kasongo et al., 2009; Koutika et al., 2014). A reduction in soil pH can occur due to three principal factors: i) The increased absorption of cations over anions by the legumes, which results in the increased exudation of H^+ and/or reduced exudation of OH^- (Yamashita et al., 2008). Koutika et al. (2014) argued that this does not apply to the conditions in this study, because of the higher growth and the consequent higher cation absorption in *Eucalyptus* pure stands (Bouillet et al., 2013). ii) An increase in the organic matter content that promotes an increase in the soil CEC, which results in soil acidification under conditions of low base availability (Binkley, 1992). However, an increase in the soil C content (Table 3) or in the CEC was not observed (data not shown). iii) The primary factor contributing to soil acidification was the high nitrification rate in the Acacia plantings that resulted from the higher deposition of N via the litterfall (Voigtlaender et al., 2012; Voigtlaender et al., in press).

The highest acidification was observed at the SdP site due to the higher initial pH (approximately 6), and that site had a high nitrification rate as did the BO site (data not shown; Voigtlaender et al., in press), especially for the Acacia pure stands. In general, nitrification is high in areas with high soil pH (Cantarella, 2007). However, the chemical processes of nitrification can reduce the soil pH by producing protons, resulting in a decrease in soil pH under N₂-fixing trees (Wang et al., 2010).

A high correlation was observed between Al_s (r = 0.79 p < 0.001) and Al_e (r = 0.78 p < 0.001) with soil pH, indicating that most of the variation (over than 70%) in the Al content could be explained by soil pH.

The amount of Al complexed by the organic matter (OM) was

observed to be a function of the soil C content and of the OM complexation capacity and not of the soil pH. The soil C content explained 74% (p < 0.001) of the variation in the Al_{MO} content (Fig. 2b).

The remaining variation can be explained by the difference in the OM complexation capacity. The highest Al complexation capacity by the OM was observed at the SdP site at which the soil under *Acacia mangium* pure stands had a higher Al complexation capacity (1.9 mmol_c g⁻¹ of C), while the mixed stands had an intermediate capacity (1.5 mmol_c g⁻¹ of C) and the *Eucalyptus* pure stands had a lower capacity (1.4 mmol_c g⁻¹ of C). No differences between sites or treatments were seen at the other sites, which had an average C complexation capacity of 1.2 mmol_c g⁻¹. This result may be associated with the higher clay content at the SdP site.

In general, the introduction of *Acacia mangium* into pure stands of *Eucalyptus* can increase soil organic matter (Forrester et al., 2013) and the Al complexation capacity by the OM, increasing the Al content complexed by the OM. Nevertheless, this did not result in a decrease in the soil Al availability (Al_s and Al_e), because the pH reduction promoted by *Acacia mangium* resulted in the dissolution of Al-OH polymers, as well as metastable forms of Al hydroxide (Berggren and Mulder, 1995; Zambrosi et al., 2007; Gruba et al., 2013).

5. Conclusion

- i) The introduction of *Acacia mangium* into pure *Eucalyptus* stands had few effects on the soil organic carbon (SOC) even without an increase in the aboveground mass production. However, the SOC increased by approximately 10% in one of the four sites studied, which was classified as Ferralsol, with medium texture;
- ii) Despite the strong relationship found between the SOC and the amount of Al complexed by the soil organic matter, no reduction in Al availability was noted with the introduction of *Acacia mangium*, not even at the site that had an improvement in the SOC. The opposite effect was observed with the introduction of the *Acacia mangium* into a *Eucalyptus* plantation, since it promoted soil acidification and consequently increased the soil Al availability.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi: http://dx.doi.org/10.1016/j.geodrs.2017.05. 001. These data include the Google maps of the most important areas described in this article.

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