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A fast exploration of very deep soil layers by *Eucalyptus* seedlings and clones in Brazil

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ABSTRACT

Although pioneer studies showed several decades ago that deep rooting is common in tropical forests, direct measurements of fine root distributions over the entire soil profile explored by the roots are still scarce. Our study aimed to compare, 2 years after planting, fine root traits of *Eucalyptus* trees planted from cuttings and from seedlings in order to assess whether the propagation mode has an influence on the capacity of the trees to explore very deep soils. Soils cores were sampled down to a depth of 13.5 m at the peak of leaf area index (LAI), 2 years after planting, under three *Eucalyptus* clones (belonging to species *E. saligna*, *E. grandis* × *E. urophylla*, *E. grandis* × *E. camaldulensis*) and under *E. grandis* seedlings in the same Ferralsol soil. LAI was estimated using allometric equations based on destructive sampling of eight trees per genotype.

All the genotypes exhibited fine root densities roughly constant between the depths of 0.25 and 6.00 m. Changes in fine root traits (diameter, specific root length and specific root area) were low between the topsoil and the root front. The ratios between mean tree height and root front depth ranged from 0.8 to 1.2 for the four genotypes. Although tree vertical extension was roughly symmetric above and below-ground for all the genotypes, the depth of the root front ranged from 8.0 m for the seedlings and the *E. grandis* × *E. urophylla* clone to 11.5 m for the *E. saligna* clone. Soil water content profiles suggested that the four genotypes had the capacity to withdraw water down to a depth of 8–10 m over the first 2 years after planting. Total fine root length ranged from 3.3 to 6.0 km per m^2 of soil depending on the genotype. The root area/leaf area ratio ranged from 1.3 to 3.2 and was negatively correlated with LAI across the four genotypes. This pattern suggests that the genotypes more conservative for water use (with a low LAI) invest more in fine root area relative to leaf area than genotypes adapted to wet regions (with a high LAI). The velocity of downward movement of the root front might be a relevant criterion in the last stage of the breeding programs to select clones with a fast exploration of deep soil layers in drought prone regions.

1. Introduction

The major role of deep roots to supply water and nutrients in tropical forests has been pointed out for several decades (*e.g.* Nepstad et al., 1994; Schenk and Jackson, 2002a), however, studies quantifying fine root distributions below a depth of 3 m remain scarce (Maeght et al., 2013; Freycon et al., 2015). The processes controlling water and nutrient uptake in very deep soil layers are still poorly understood in forest ecosystems and require further attention (Iversen, 2010; Binkley, 2015). The comparisons of measured (with the eddy-covariance technique) and modeled evapotranspiration across forests and savannas in Africa and Amazonia showed that the rooting depth is a major variable controlling the predictions of evapotranspiration during dry seasons in soil-vegetation-atmosphere-transfer (SVAT) models (Akkermans et al., 2012; Christoffersen et al., 2014). These studies suggest that the biological

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processes driving root dynamics in deep soil layers and leaf phenology should be urgently investigated, since these mechanisms mediate vegetation-climate feedbacks in the tropics through their control on evapotranspiration.

Simple forest ecosystems like *Eucalyptus* plantations can contribute to improving our understanding of the belowground strategy of fast-growing trees. *Eucalyptus* plantations are rapidly expanding in tropical regions and cover nowadays approximately 20 million ha worldwide (Grant et al., 2012; Booth, 2013). The area of these fast-growing plantations in Brazil shifted from 3.4 million ha in 2005 to 5.6 million ha in 2014 (ABRAF, 2006; IBÁ, 2015), which represents about 25% of the total area of eucalypt plantations in the world and almost 70% of the planted forests in Brazil. A recent study in a deep tropical soil showed that the maximum rooting depth of *Eucalyptus grandis* seedlings managed in short-rotation plantations was close to the mean stand height from planting to harvesting (Christina et al., 2011). However, the architecture of root systems is highly dependent on propagation techniques (seedlings *vs* cuttings) and genotypes (Fensham and Fairfax, 2007; Nibau et al., 2008; Bonneu et al., 2012), and most of the commercial plantations are now established with clones of several *Eucalyptus* species. Therefore, the relationship between stand height and maximum rooting depth observed for *E. grandis* seedlings might be different for the highly productive clones planted nowadays at large scale in tropical regions. Fertilization regimes are designed to supply tree nutrient requirements in commercial eucalypt plantations and the productivity is generally limited by water availability (du Toit et al., 2010; Stape et al., 2010). The root front velocity might be a relevant trait to consider in breeding programs of eucalypt species since an eventual access to the water table may explain different growth and survival rates between clones in exceptionally dry periods (Harper et al., 2009; Decker et al., 2013; Poot and Veneklaas, 2013; Matusick et al., 2013; Zolfaghar et al., 2014). Clones with deep roots may have access to a larger amount of water stored in deep soil layers compared to genotypes with a more superficial root distribution. Recent studies in Australian (Robinson et al., 2006; Mendham et al., 2011; Harper et al., 2014) and Brazilian eucalypt plantations (Battie-Laclau et al., 2014) showed that fine roots at depths >5 m can have an important functional role to supply water for tree growth during drought periods. Fine root traits are very challenging to measure (Maeght et al., 2013) and therefore cannot be used to screen a large number of genotypes. However, forest companies use a small number of new clones annually (commonly half a dozen) and the root front velocity of each clone planted at large scale might be an interesting criterion to measure in clonal tests at the last stage of the breeding programs. This basic information might be relevant to improve the matching between the root traits of each clone and the characteristics of the planting areas (in particular drought risk and soil depth).

Exploration of deep soil layers can have an important metabolic cost for plants (Iversen, 2010). Genotypes maximizing fine root length and fine root area for a given investment in belowground biomass are likely to improve water and nutrient acquisition. Fast growth requires fast and efficient acquisition both of above and belowground resources, and thus fast-growing and invasive tree species generally have higher specific root area (SRA), specific root length (SRL), smaller root diameter, and higher specific leaf area (SLA) than slow-growing species (Reich, 2014; Jo et al., 2015). Despite the growing body of evidence that deep roots can play a major role in functional ecology for a broad range of terrestrial ecosystems, studies investigating the changes in fine root traits between the topsoil and soil layers at depths >3 m remain scarce (Roupsard et al., 1999; Maeght et al., 2013). Insufficient sampling depths in many studies contributed to underestimates of actual rooting depths, especially in tropical forests (Schenk and Jackson, 2002b). Water and nutrient availabilities in tropical eucalypt plantations strongly change with depth in the soil (*e.g.* Mareschal et al., 2013; Versini et al., 2014), which might lead to contrasting suites of traits with depth since root traits are highly responsive to heterogeneous resource distributions (Ostonen et al., 2007; Prieto et al., 2015).

Fine root distributions cannot be measured for a large number of genotypes in deep soil layers and indicators of soil exploration by fine roots would be useful in breeding programs for a rapid screening of genotypes adapted to seasonally dry areas and to coarse-textured soils with low water retention capacity (Hamer et al., 2015). Leaf and fine root areas are hydraulically interdependent, thus constraining trees to adjust their area of water uptake to their area of water loss (Magnani et al., 2002; Zeppel, 2013; Mackay et al., 2015). Strong relationships between leaf biomass and fine root biomass have been shown throughout the early growth of *Eucalyptus globulus* (O'Grady et al., 2006) and *E. grandis* plantations (Laclau et al., 2008). A positive correlation between root area index (RAI) and leaf area index (LAI) was found across five poplar clones (Al Afas et al., 2008). However, the root area to leaf area ratio, or the root length to leaf area ratio were reported to vary depending on stand age (O'Grady et al., 2006), soil water availability (Rhizopoulou and Davies, 1993; Costa e Silva et al., 2004; Martin-StPaul et al., 2013), and genotype (Costa e Silva et al., 2004; Hamer et al., 2015). A modeling approach suggested that the root area to leaf area ratio (RAI/LAI) should be higher for anisohydric species than for isohydric species (Mackay et al., 2015). This ratio is therefore likely to greatly vary among eucalypt species growing in the same environment, with lower values for species from wet regions than for species from dry regions (Hamer et al., 2015).

Our study aimed to compare, two years after planting, fine root traits of *Eucalyptus* trees planted from cuttings and from seedlings in order to assess whether the propagation mode has an influence on the capacity of the trees to explore very deep soils. We hypothesized that: (i) the genotypes with the highest growth rates exhibit the highest SRL and SRA values, (ii) the pattern of exploration of deep soil layers is similar for seedlings and clones at the peak of LAI (2 years after planting) with an almost symmetrical vertical tree extension above and belowground, and (iii) the genotypes with the lowest LAI, presumably more conservative for water-use, have the highest root area to leaf area ratio. Although the range of productivity of the genotypes used in our study was narrow relative to the diversity of growth rates of eucalypt species and hybrids planted worldwide, we studied genotypes representative of the range of production observed in Brazilian eucalypt plantations. As far as we are aware, this study is the first investigating major fine root traits (root front velocity as well as fine root diameter, specific root length and specific root area) down to the root front at depths >10 m for genotypes propagated by cuttings in tropical planted forests.

2. Material and methods

2.1. Study site

Our study was carried out in commercial *Eucalyptus* plantations managed by the EucFlux project (http://www.ipef.br/eucflux/en/) at Itatinga (22°58′04″S, 48°43′40″W) in southeast Brazil (São Paulo State). Annual rainfall at the study site was 1278 mm in 2010 and 1758 mm in 2011 (~90% concentrated from October to May) and mean annual temperature was 19.5 °C, (16.3 °C on average from June to August and 22.2 °C on average from December to February). The mean annual air relative humidity was 76%, with minimum values of ~30% between July and September.

The soils were deep Ferralsols (FAO classification), developed on Cretaceous sandstone of the Marilia formation, Bauru group. The relief was typical of the São Paulo Western Plateau, with a gently undulating topography. The slope was <5%, and the maximum elevation was 760 m above sea level. Soil properties were representative of the most common soil type where eucalypt plantations are established in Brazil (Table 1). Clay contents ranged from about 16% in the topsoil to 22–25% in the 2–10 m soil layer. Whatever the soil layer down to a depth of 14 m, soil pH_{CaCl2} was approx. 4 and the concentrations of nutrients were approx. 5 mg kg^{-1} for P_{resin} and 6 $mmol_c$ kg^{-1} for the sum of base cations (exch. K^+, Ca^{2+} and Mg^{2+}). The monitoring of the water table at approx. 500 m from our experiment showed that the depth decreased by 6 m the first 1.5 years after clear-cutting the previous stand, then slowly increased over the rotation (unpublished data). Soil coring down to a depth of 13.5 m in the present study and down to 17 m in the same plots 4 years after planting (data not shown) suggested that the water table was at a depth of approximately 14–15 m when our study was carried out. The mineralogy was dominated by quartz, kaolinite and oxyhydroxides (Maquère, 2008).

2.2. Experimental design

Our study was carried out in two genotype tests (two blocks) replicated at about 300 m apart on the same soil type. Four genotypes selected by forest companies for their high growth rates were studied in each test:

Seedlings: seed lot of the *E. grandis* species used in the past by the Duratex Company in the São Paulo state (mean temperature of 19 °C and mean annual rainfall of 1340 mm in the region). These seedlings were less productive than the best clones planted nowadays.
Clone 8: clone of the *E. grandis* × *E. urophylla* hybrid selected by the Cenibra Company in the Minas Gerais state (mean temperature of 23 °C and mean annual rainfall of 1380 mm in the region).
Clone 14: clone of the *Eucalyptus saligna* species selected by the Klabin Company in the Rio Grande do Sul state (mean temperature of 19 °C and mean annual rainfall of 1680 mm in the region).
Clone 16: clone of the *E. grandis* × *E. camaldulensis* hybrid selected by the Copener Company in the Bahia state (mean temperature of 25 °C and mean annual rainfall of 700 mm in the region).

Four *Eucalyptus* genotypes, three clones of pure species and hybrids as well as one seedling lot (considered here as one "genotype"), were planted (spacing 3 m × 2 m) in each genotype test (12 × 14 trees per plot). The three clones were chosen to cover a large range of LAI at 2 years of age (from 2.8 to 4.0 m^2 m^{-2}), the range of productivities in commercial plantations and to represent the main species and hybrids currently planted in Brazil. Seedlings were also studied to compare fine root distributions for eucalypt trees propagated via seeds and cuttings. The seedlings were representative of the least productive genotypes still used in commercial plantations in Brazil. The main characteristics of the stands at the sampling age are shown in Table 2. Fertilization regimes used in commercial plantations were applied: 18 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, 30 kg K_2O ha^{-1} and 4 Mg ha^{-1} of dolomitic lime at planting, 31 kg N ha^{-1}, 36 kg K_2O ha^{-1} at age 4 months, 31 kg N ha^{-1}, 67 kg K_2O ha^{-1} at age 12 months and 151 kg K_2O ha^{-1} at age 24 months. Sulfuramide-based baits (1.3 kg ha^{-1}) were applied at planting to control ants and weeds were eliminated with glyphosate applications the first year after planting.

2.3. Fine root sampling

Fine roots (<2 mm in diameter) of the four genotypes were sampled 2 years after planting, at two distances from the trees in each inner plot (excluding three buffer rows). The two sampling positions in each plot were separated by at least 10 m of distance. The first sampling position was located in the middle of the inter-row (at 1.8 m from the nearest tree) and the second position was at 0.5 m from another tree. We sampled a total of four positions for each genotype (two distances from the trees × two blocks) down to a depth of 13.5 m. We preferred to collect a large number of samples down to the root front than to increase the sampling intensity in the upper soil layers, because previous studies in tropical eucalypt plantations showed that fine root densities do not sharply decrease down to a depth of 3–4 m at age 2 years. A concentration of fine roots in the upper soil layers occurs only from age 3 years onward in these fast-growing plantations (Bouillet et al., 2002; Laclau et al., 2013a).

Soil cores were sampled in the 0–0.25 m and 0.25–0.5 m layers, then every 0.5 m down to a depth of 13.5 m, using a cylindrical auger with an inner diameter of 9 cm and a length of 30 cm. We used the same methodology as Christina et al. (2011) to avoid contamination of the soil samples collected at depth by roots from the upper layers. After sampling down to a depth of 2 m, we enlarged the diameter of the hole and we installed a 2 meter-long plastic tube with a diameter of 20 cm to avoid surface soil falling further down. Only soil blocks from the inner part of the auger were collected and all fragmented soil pieces likely to come from upper soil layers were systematically discarded. Soil samples were put in plastic bags, identified and stored at 4 °C until processing. The root front was defined at each sampling position as the depth of the layer where the deepest root was observed. Soils were systematically collected a further 2 m down at each sampling position and the absence of roots below the layer identified as the root front

Table 1
Main physical and chemical soil properties across all the sampling positions in blocks 1 and 2.

Soil layer	Sand	Silt	Clay	pH	O.M.[a]	P_{resin}[b]	Base cations[b]	CEC
(cm)	Particle size distribution (%)			$CaCl_2$	g kg^{-1}	mg kg^{-1}	$mmol_c$ kg^{-1}	
0–25	80.8 ± 2.0	3.5 ± 0.8	15.8 ± 1.4	3.89 ± 0.12	13.2 ± 3.2	4.2 ± 0.8	6.5 ± 0.5	51.0 ± 15.4
25–50	79.2 ± 1.6	3.2 ± 0.9	17.7 ± 1.0	4.12 ± 0.06	8.8 ± 0.6	4.7 ± 0.5	5.8 ± 0.5	34.6 ± 1.8
50–100	77.0 ± 1.6	4.2 ± 1.9	18.9 ± 1.0	4.10 ± 0.02	7.4 ± 0.4	4.7 ± 0.6	5.8 ± 0.8	29.9 ± 2.2
100–400	71.9 ± 1.6	4.8 ± 2.1	23.3 ± 1.1	4.46 ± 0.08	7.4 ± 1.6	4.8 ± 0.3	6.4 ± 0.5	22.0 ± 1.0
400–800	67.3 ± 1.0	8.0 ± 4.1	24.7 ± 3.2	4.61 ± 0.12	4.7 ± 0.6	5.1 ± 0.6	6.4 ± 1.3	19.2 ± 1.6
800–1200	69.2 ± 1.9	9.1 ± 4.4	21.7 ± 3.1	4.37 ± 0.12	4.5 ± 0.2	4.5 ± 0.3	6.2 ± 0.3	21.0 ± 5.4
1200–1400	82.0 ± 8.6	4.1 ± 1.6	14.0 ± 7.1	4.39 ± 0.04	4.1 ± 0.3	4.8 ± 0.7	5.9 ± 0.8	15.4 ± 1.1

[a] Organic matter (O.M.) determined using sodium dichromate.
[b] The methodology described by van Raij et al. (2001) was used for resin extraction of phosphorus and exchangeable element determinations.

Table 2
Main characteristics of the stands at 2 years of age (the values in blocks 1 and 2 are given between parenthesis).

	Seedlings *E. grandis*	Clone 8 *E. grandis* × *E. urophylla*	Clone 14 *E. saligna*	Clone 16 *E. grandis* × *E. camaldulensis*
Stocking density (trees ha^{-1})	1406 (1476–1337)	1641 (1632–1649)	1606 (1615–1597)	1658 (1649–1666)
Stand basal area (m^2 ha^{-1})	8.5 (8.9–8.2)	10.6 (10.2–11.0)	9.0 (8.5–9.5)	11.0 (10.1–11.9)
Trunk biomass (Mg ha^{-1})	16.4 (17.1–15.6)	22.1 (21.1–23.0)	19.3 (17.8–20.8)	26.4 (24.0–28.7)
Woody production (Mg ha^{-1} y^{-1})	8.2 (8.6–7.8)	11.0 (10.5–11.5)	9.7 (8.9–10.4)	13.2 (12.0–14.4)
Dominant height (m)	11.7 (12.0–11.4)	12.0 (12.1–11.9)	10.6 (10.4–10.8)	11.9 (11.7–12.0)
Mean height (m)	8.5 (8.9–8.0)	10.8 (10.6–10.9)	9.5 (9.2–9.8)	11.1 (10.8–11.4)

was checked. The absence of any physical or chemical barrier to root growth down to a depth of 13.5 m was checked at all the sampling positions in our experiment.

Soil samples collected in the field were weighed and uniformly mixed. Gravimetric soil water contents were measured in each soil sample from 20 g of soil dried at 105 °C up to constant weight. All the fine roots in the samples were washed free of soil with tap water using sieves (with mesh sizes from 0.50 to 1.19 mm) and all the living roots with a length >1 cm were separated carefully by hand. Living roots were sorted using criteria such as a living stele, bright color and flexibility. A sub-sample (10% of the weight of each soil sample) was used to estimate the mass of extremely fine roots (less than 1 cm in length). All living fine roots with a length >1 cm separated from each soil sample were scanned (400 dpi resolution). Root lengths and areas were estimated in each sample using WinRHIZO Version Pro V.2009c software (Regent Instruments, QC, Canada). Fine roots were dried for 72 h at 65 °C and weighed (±0.1 mg) to estimate specific root length (SRL, length of scanned roots divided by their dry mass) and specific root area (SRA, area of scanned roots divided by their dry mass) in each soil sample. Total root length and total root area in each soil sample were calculated by multiplying the total root dry mass by SRL and SRA, respectively. Soil bulk densities measured in a pit down to a depth of 10 m in each layer were used to estimate fine root densities per dm^3 of soil in each layer. We considered that the soil bulk density was 1.4 kg dm^{-3} below a depth of 10 m (similar to the bulk density in the deepest soil layer sampled). Root Area Index (RAI, expressed in m^2 m^{-2}) in each soil layer was calculated multiplying the root area density by the thickness (m) of the soil layer.

2.4. Leaf sampling

Total leaf area was determined destructively on eight trees covering the range of basal areas for each genotype, following the same methodology as described in Nouvellon et al. (2010): foliage biomass was determined for each sampled tree by weighing all the leaves in the field and randomly subsampling 30 leaves in each third of the crown (upper, medium and lower). Leaf samples were immediately scanned at 300 dpi resolution and the fresh mass was measured. They were then weighed after oven drying at 65 °C for 48 h. The dry weights of these subsamples were used in conjunction with their measured area (using the ImageJ software) to calculate specific leaf area (SLA) for each crown section. The foliage dry weight of each crown section was calculated from the foliage fresh weight measured in the field and the water content of the subsamples. The foliage area of each crown section was calculated multiplying the foliage dry weight by the SLA of the subsample. Tree leaf area was obtained by summing the leaf areas of the three crown sections.

Genotype-specific allometric relationships were established ($R^2 > 0.90$) and applied to the inventory made at the same date (excluding 3 buffer rows in each plot) to estimate the total leaf area from tree attributes (diameter at breast height and height). LAI was calculated dividing the total leaf area estimated from the allometric relationships by the plot area.

2.5. Data analyses

RAI values in each soil layer and for the whole soil profile were compared among the four genotypes using SAS v.9.2 (SAS, Cary, NC, USA). Sampling positions were located close to different trees in each plot, therefore individual fine root measurements within a given soil layer were considered independent. A general linear model was used in a two-way analysis of variance (ANOVA) to test for differences in RAI due to genotypes and blocks. When significant differences were detected among genotypes, the Tukey test was used to compare treatment means. The Bartlett test was used to check homoscedasticity and the normal distribution of the residues was checked using the Shapiro–Wilk test. Mixed-effect models were used to test the effects of genotype, soil depth, sampling position, and interaction genotype × sampling position, genotype × soil depth, sampling position × soil depth as well as genotype × sampling position × soil depth (as fixed effects) on fine root diameter, SRL and SRA. Blocks were considered as random effects. Residues were modeled by a first-order autoregressive correlation model to account for the correlations between soil depths. A significance level of 5% was used in all the analyses. Pearson correlation coefficients were calculated for each genotype between soil depth and root diameter, SRL and SRA at each sampling position.

3. Results

3.1. Fine root distributions

Seedlings and clones exhibited a similar pattern of deep rooting at age 2 years (Fig. 1). Fine root densities dropped in the topsoil but remained roughly similar between the depths of 0.25 and 6 m whatever the genotype. Soil water contents at the end of the dry season were similar under the four genotypes down to a depth of 9 m and much more variable below a depth of 10 m. Distributions of fine root lengths across the soil profiles were roughly similar for the seedlings and the three clones with about 50% of the total fine root length in the upper 2 m and less than 5% of the total fine root length below 8 m (Fig. 2). Total fine root biomass was genotype-dependent, reaching 220 g m^{-2} of soil down to the root front for clone 8, 259 g m^{-2} for clone 16, 295 g m^{-2} for seedlings and 374 g m^{-2} for clone 14 (Table 3). Total fine root length ranged from 3.3 km per m^2 of soil (0.8 km per m^2 of leaf) for clone 8 to 6.0 km m^{-2} of soil (2.1 km per m^2 of leaf) for clone 14.

Vertical tree extensions were almost symmetrical above and belowground for the four studied genotypes (Fig. 3). The ratio between the maximum rooting depth and mean tree height was 1.04, 0.83, 1.23 and 0.92 for the seedlings, clone 8, clone 14 and clone 16, respectively. The ratios between maximum rooting depth and dominant tree height ranged from 0.74 to 1.10 for the four genotypes (Table 3). The root front reached a depth of 11.5 m on average for clone 14 (with a very low variability among the

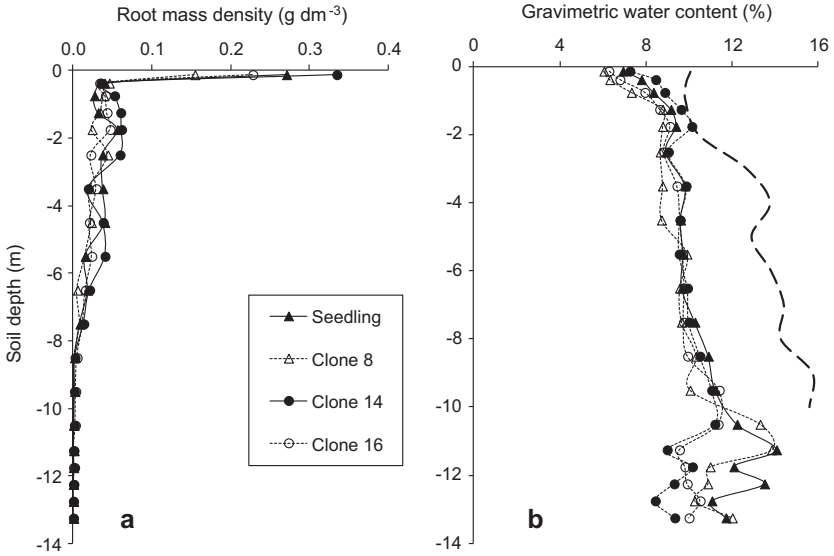

Fig. 1. Fine root distributions (a) and gravimetric water contents (b) down to the root front for 4 *Eucalyptus* genotypes at age 2 years. Mean values for 4 independent positions are indicated (sampling at 2 distances from different trees). The dashed line in figure (b) shows the gravimetric water contents during the first rainy season after replanting, on February 14th 2010.

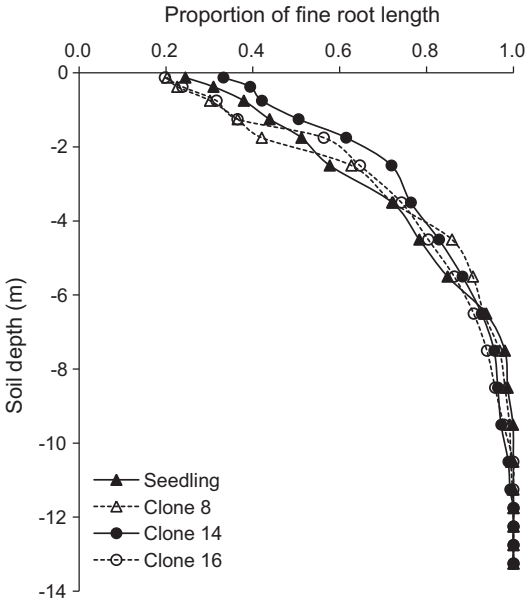

Fig. 2. Cumulated fraction of fine root lengths down to the root front (m) at age 2 years for four *Eucalyptus* genotypes.

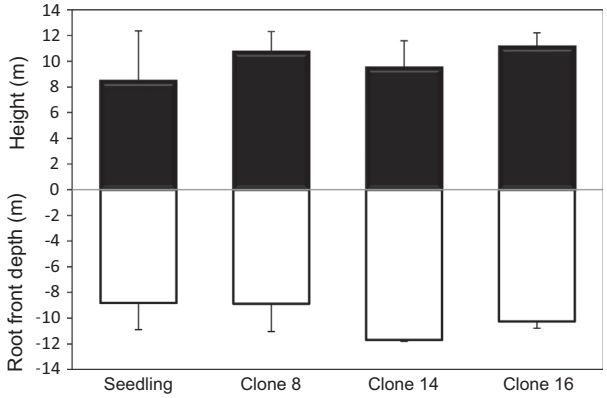

Fig. 3. Mean stand height and root front depth for each *Eucalyptus* genotype at age 2 years. Vertical bars indicate standard deviations between sampling positions for root front depth ($n = 4$) and trees measured in the inner plots for height ($n = 96$).

sampling positions), and less than 9 m for the seedlings and clone 8 (with a high variability among the sampling positions). The root front depth was intermediate for clone 16.

3.2. Fine root traits

While growth rates were higher for clone 16 than for seedlings (stand basal areas of 11.0 and 8.6 m^2 ha^{-1} at age 2 years, respectively), specific root length (SRL) and specific root area (SRA) were not significantly different between the four genotypes (Fig. 4 and Table 4). By contrast, fine root diameters differed between the genotypes across the sampling depths, with mean values ranging from 0.48 mm for the seedlings to 0.55 mm for clone 8. The

Table 3
Main characteristics of fine roots and leaves for the four genotypes at 2 years of age (the values in blocks 1 and 2 are given between parenthesis).

	Seedlings *E. grandis*	Clone 8 *E. grandis* × *E. urophylla*	Clone 14 *E. saligna*	Clone 16 *E. grandis* × *E. camaldulensis*
Root front depth (m)	8.7 (7.9–9.6)	8.9 (10.5–7.3)	11.7 (11.6–11.8)	10.2 (10.5–10.0)
Leaf area index (LAI, m^2 m^{-2})	3.4 (3.5–3.2)	4.0 (3.8–4.1)	2.9 (2.8–3.0)	2.8 (2.6–3.0)
Fine root biomass (g m^{-2})	295 (258–333)	220 (216–223)	374 (351–396)	259 (289–229)
Root area index (RAI, m^2 m^{-2})	5.7 (6.8–4.5)	4.9 (4.8–5.1)	9.3 (8.7–9.9)	6.7 (6.9–6.5)
Root length index (km m^{-2})	3.6 (3.8–3.3)	3.3 (2.7–3.9)	6.0 (5.6–6.4)	4.6 (4.5–4.6)
Root length/leaf area (km m^{-2})	1.1 (1.1–1.0)	0.8 (0.7–1.0)	2.1 (2.0–2.1)	1.6 (1.7–1.5)
RAI/LAI	1.7 (1.9–1.4)	1.3 (1.3–1.2)	3.2 (3.1–3.3)	2.4 (2.7–2.2)

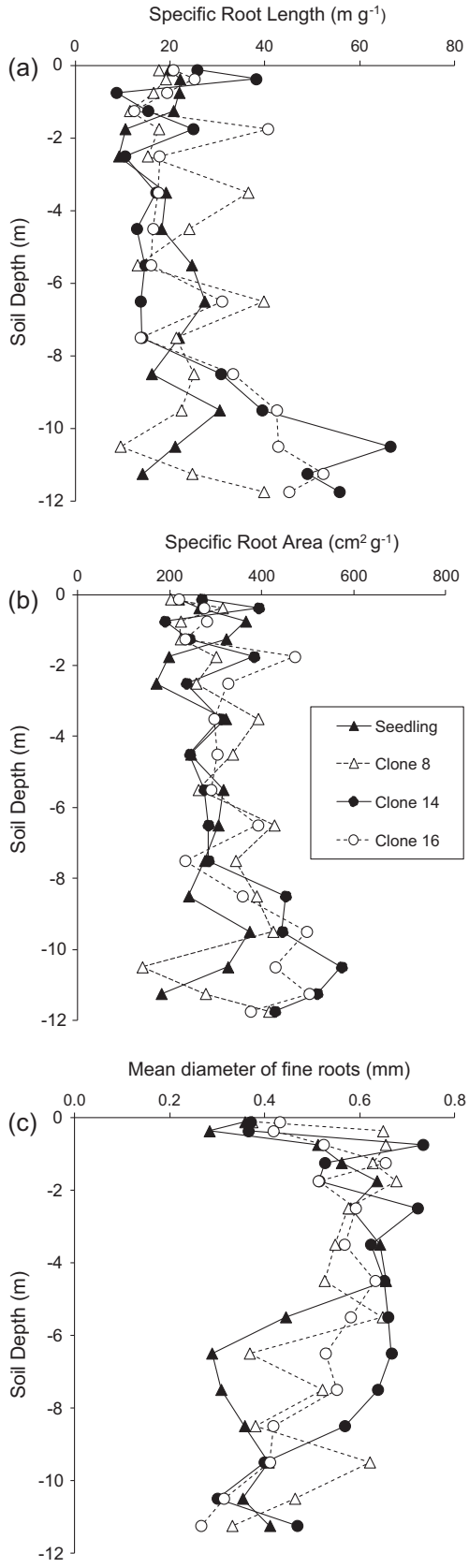

Fig. 4. Changes in specific root length (a), specific root area (b) and mean diameter of fine roots (c) with soil depth for four *Eucalyptus* genotypes.

sampling depth significantly influenced fine root diameter and SRL but not SRA across the four genotypes (Fig. 4 and Table 4). A positive correlation was found between soil depth and SRL as well as between soil depth and SRA for clones 14 and 16 only (R between 0.29 and 0.48, $P < 0.05$, $n = 57–63$). Regardless the genotype, changes in diameter, SRL and SRA were low between upper and lower soil layers relative to the differences observed between some adjacent soil layers.

Mean values of SRL, SRA and diameter of fine roots across all the soil depths, genotypes and sampling positions were 24 ± 12 m g^{-1}, 318 ± 90 cm^2 g^{-1} and 0.5 ± 0.1 mm, respectively. However, the variability was high. SRL ranged from 9 to 66 m g^{-1}, SRA from 140 to 573 cm^2 g^{-1} and mean root diameter from 0.3 to 0.7 mm depending on the soil layer and the genotype.

3.3. Relationship between leaf area index and root area index

While total RAI reached 9.3 m^2 m^{-2} for clone 14 (Table 3), it was significantly lower for clone 8 (4.9 m^2 m^{-2}) and intermediate for seedlings (5.7 m^2 m^{-2}) and clone 16 (6.7 m^2 m^{-2}). RAI was significantly higher in the 1–3 m soil layer for clone 14 (3.2 m^2 m^{-2}) than for seedlings (1.2 m^2 m^{-2}) and, although differences were not significant, the trend was similar in soil layers 0–1 m and >5 m (Fig. 5). The RAI/LAI ratio ranged from 3.2 for clone 14 to 1.3 for clone 8 with a strong negative relationship between the ratio of root area to leaf area and LAI across the four genotypes in the two blocks (Fig. 6). The ratio of root length to leaf area ranged from 0.8 for the genotype with the highest LAI to 1.6–2.1 for the genotypes with the lowest LAI (Table 3).

4. Discussion

4.1. Exploration of deep soil layers

The fine roots of the four genotypes explored a huge soil volume 2 years after planting. Similar soil water contents at the end of the dry season under the four genotypes suggested that the clones and the seedlings had the same capacity to withdraw soil water down to a depth of 9 m. Soil water contents were much more variable below a depth of 10 m, which might reflect a deeper uptake of soil water for certain genotypes and/or slight differences in soil texture for very deep soil layers. Piezometers in the same stand showed that the water table was at a depth of 14–15 m at the sampling age. Fine root densities sharply decreased below the upper layer (0–0.25 m), as commonly reported in forest ecosystems (Schenk and Jackson, 2002b). The pattern below the depth of 0.25 m was by contrast uncommon in our study, with fine root densities roughly constant down to a depth of 6 m for the four genotypes. While models commonly consider an exponential decrease in fine root densities with soil depth in forest ecosystems (Schenk and Jackson, 2002b), soils were also explored homogeneously by fine roots down to depths of more than 3 m at the peak of leaf area index (2 years after planting) in eucalypt plantations in Congo and Brazil (Bouillet et al., 2002; Laclau et al., 2013a). This pattern might be explained by a high water demand of eucalypt trees requiring the withdrawal of large amounts of water stored after clear-cutting in deep soil layers. A concentration of fine roots in the upper soil layers commonly observed close to the harvest age in these fast-growing plantations might reflect the nutrient inputs in the topsoil through the biological cycle (Laclau et al., 2010).

Although the sampling intensity was low in the topsoil in our experiment (only four sampling positions for each genotype) relative to most of root studies in forest ecosystems, the results confirm that an accurate sampling of deep soil layers is a priority in 2-year-old eucalypt plantations established in Ferralsol soils. Indeed, fine roots in the 0–0.5 m soil layer accounted for only 23–39% of the total fine root length down to the root front for the four genotypes. An exceptional sampling effort in deep soil

Table 4
F-values for the effects of genotype, depth and sampling position, interaction between genotype × depth, genotype × position, depth × position, and genotype × depth × position on fine root diameter (mm), specific root length (SRL, m g^{-1}), and specific root area (SRA, cm^2 g^{-1}).

	Diameter	SRL	SRA
Genotype	$\boldsymbol{F_{3,91} = 3.36}^{*}$	$F_{3,100} = 1.35$	$F_{3,100} = 1.54$
Depth	$\boldsymbol{F_{15,91} = 5.46}^{**}$	$\boldsymbol{F_{15,100} = 1.80}^{*}$	$F_{15,100} = 1.35$
Position	$F_{1,91} = 0.00$	$F_{1,100} = 0.25$	$F_{1,100} = 1.73$
Genotype × Depth	$F_{42,91} = 1.37$	$F_{44,100} = 0.79$	$F_{44,100} = 0.61$
Genotype × Position	$F_{3,91} = 0.60$	$F_{3,100} = 1.26$	$F_{3,100} = 2.18$
Position × Depth	$F_{14,91} = 1.05$	$F_{15,100} = 0.72$	$F_{15,100} = 0.72$
Genotype × Depth × Position	$\boldsymbol{F_{35,91} = 1.69}^{*}$	$F_{38,100} = 0.48$	$F_{38,100} = 0.49$

* Significant effects at $P < 0.05$.
** Significant effects at $P < 0.01$.

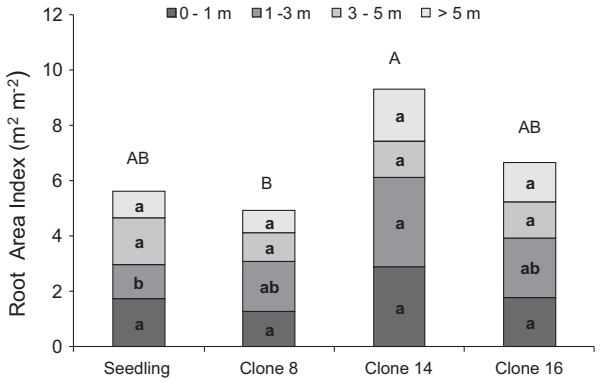

Fig. 5. Root area index in the 0–1 m, 1–3 m, 3–5 m and >5 m soil layers (m^2 m^{-2}) for each *Eucalyptus* genotype. Different lower-case letters in the same soil layer indicate significant differences between genotypes ($P < 0.05$). Different upper-case letters indicate significant differences between genotypes for the total root area index down to the root front.

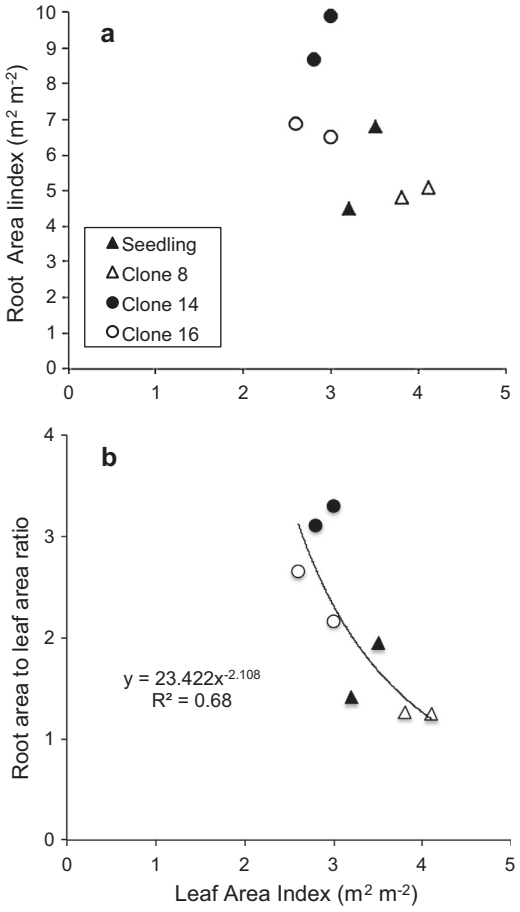

Fig. 6. Relationship between root area index (m^2 m^{-2}) and leaf area index (m^2 m^{-2}) (a) and between the root area: leaf area ratio and LAI (b), across the 4 *Eucalyptus* genotypes at age 2 years. Mean values per plot in each block are shown.

layers was therefore more relevant than a high sampling intensity in the topsoil to accurately estimate total fine root biomass, fine root length and fine root area. Although fine root densities were significantly higher close to trees than at mid inter-row in our study, the absolute differences between the two sampling positions were low (data not shown). Other studies suggest that the spatial variability in the upper soil layers is relatively low in tropical eucalypt plantations and that soil layers at depths >1 m contain a large share of the total fine root biomass (Bouillet et al., 2002; Laclau et al., 2013a,b).

Contrary to our first hypothesis, the genotypes with the highest growth rates did not exhibit higher SRL and SRA values than the genotypes with the slowest growth rates. This pattern might be a result of the low differences in growth rates between the four studied genotypes that were selected for their high biomass production. The effect of soil depth on fine root diameter, SRL and SRA was genotype-dependent with relatively low differences between the topsoil and the deepest soil layers. A small effect of soil depth on fine root diameter, SRL and SRA was also shown recently down to the root front (at a depth of 7–8 m) for *Citrus* trees in Brazil (Adriano, 2015). Minirhizotrons monitored over 2 years from the topsoil to a depth of 6 m in a nearby *E. grandis* plantation showed that peaks of fine root growth occurred at different dates depending on the soil depth (Lambais, 2015). Different stages of fine root development at the date of root sampling in our study might account for the significant differences in diameter and SRL observed between the soil layers. Morphological and chemical root traits compared in the topsoil and in the 1.0–1.5 m soil layer (or in the deepest soil layer when soils were shallower) in 20 plant communities located across three climatic zones (tropical, mediterranean and montane) suggested that soil depth influenced some root functions. Shallow, fine roots were thinner, richer in nitrogen and with lower lignin concentrations compared to deep fine roots. However, absolute differences were small for most of the traits measured and SRL values were not statistically different between shallow and deep soil layers (Prieto et al., 2015). SRL values in our study were within the range reported in *E. grandis* plantations sampled at different ages and in soil of contrasting fertility (Maurice et al., 2010). While fine root diameter, SRL and SRA were of the same order of magnitude for the four genotypes, fine root biomass was highly variable (from 220 to 374 g m^{-2} at age 2 years). This pattern suggests that tree breeding aiming to reduce the metabolic cost of soil exploration should focus on root biomass rather than on fine root traits like SRL or SRA in tropical eucalypt plantations.

4.2. Relationship between mean stand height and root front depth

In agreement with our second hypothesis, the vertical tree extension above and belowground was almost symmetrical for the four genotypes, irrespective of the propagation technique (seeds or cuttings). The depth of the root front shown by soil coring for each genotype was consistent with soil water content profiles across the soil layers. Indeed, soil water contents between the depths of 10 m and 13.5 m were higher under seedlings and clone 8 than under clones 14 and 16, which suggests that clones 14 and 16 withdrew water more deeply than seedlings and clone 8. However, the variability in soil water contents below a depth of 10 m may also reflect small differences in soil texture between the plots. Fine roots below a depth of 8 m amounted to less than 5% of the total fine root biomass whatever the genotype in our study, which was consistent with previous results for *E. grandis* seedlings in Brazil (Christina et al., 2011; Laclau et al., 2013a). A recent study using time series of soil water contents and measurements of water table level over five years to parameterize a process-based model showed that very low densities of *E. grandis* fine roots at depths >10 m can provide most of tree water requirements during dry periods after canopy closure (Christina, 2015). The role of the deepest roots, even though their density is extremely low, can therefore mostly explain the behavior of eucalypt trees during drought events. The variability in root front depth observed in our study (between 8 and 11.5 m for 2-year-old trees) suggests that this trait might be interesting to measure before planting new clones at large scale in drought prone regions. Indeed, a fast access to large amounts of water stored in very deep soil layers after the harvest of the previous stand might contribute to improving the survival rates during exceptional droughts of eucalypt trees growing in very deep soils.

4.3. Root length and root area relative to leaf area in Eucalyptus plantations

A wide range of RAI has been reported in forest ecosystems and, as far as we are aware, our study is the first showing the proportions of RAI in each soil layer down to the root front in tropical planted forests. RAI values between 5 and 9 $m^2\ m^{-2}$ for our four genotypes were close to mean values of 6–7 $m^2\ m^{-2}$ reported by Jackson et al. (1997) for tropical forests. A large share of total RAI (34–47% depending on the genotype) was found below a depth of 3 m in our 2-year-old stands, which highlights the need to sample very deep soil layers to accurately estimate the potential of water uptake of *Eucalyptus* genotypes in deep soils. Despite the fast growth of the four studied genotypes and a high water demand with LAI values of 3–4 $m^2\ m^{-2}$ at age 2 years, higher RAI values have been reported in temperate forests (Jackson et al., 1997; Meinen et al., 2009) and in orange orchards (Adriano, 2015).

In agreement with our third hypothesis, the root area/leaf area ratio was negatively correlated with LAI. Although the predictive value of the relationship is probably weak as a result of the dependency of the variables (both contain a leaf area term in the figure), this trend is in agreement with previous studies. Comparing nine *Eucalyptus* species from a biodiversity hotspot, Hamer et al. (2015) showed that the species from the wetter regions had a lower root length to leaf area ratio than those from drier environments, when they were grown in the same experimental conditions. Similarly, Costa e Silva et al. (2004) showed that a drought resistant *E. globulus* clone had a higher root area to leaf area ratio and root length to leaf area ratio than a drought sensitive clone, especially under water-limited conditions. This pattern suggests that genotypes with the highest LAI (presumably genotypes from wet environments) could have the lowest root length/leaf area and root area/leaf area ratios when they are growing under the same environment, which is consistent with our results. However, we must be cautious because we are not sure that the genotypes with the lowest LAI in our study are the most adapted to drought, except clone 16 (*E. camaldulensis* hybrid selected in a dry region), which has indeed a low LAI and a high RAI/LAI ratio. A wide diversity of mechanisms have been shown within the *Eucalyptus* genus to cope with water restriction (White et al., 2000; Whitehead and Beadle, 2004). Depending on the species, structural adaptations maintaining homeostasis in water use can involve primarily a limitation of water loss through a reduction in leaf area (Pita and Pardos, 2001) or an increase in root area in deep soil layers to maximize water uptake (Dye, 1996). Further studies are needed to assess whether the decrease in RAI/LAI ratio when LAI increases is a general strategy of *Eucalyptus* trees to maintain homeostasis in water use or only valid for the four genotypes studied here.

A high diversity of form and function among fine-root orders suggests that the traditional definition of fine roots as all roots $\leqslant$2 mm in diameter may be unable to account for the root area of water capture (Pregitzer et al., 2002; McCormack et al., 2015). A study carried out in three Sitka spruce (*Picea sitchensis*) stands to compare RAI estimated for all the fine roots (diameter <2 mm as in our study) with the absorbing root area ($RAI_{absorbing}$) estimated using electrical impedance measurements showed that $RAI_{absorbing}$ was not proportional to the total fine root area and accounted for only 4–20% of the total RAI. Contrary to RAI for all the fine roots, $RAI_{absorbing}$ was proportional to the basal area across the three studied stands, which also suggested that only a fraction of total RAI was responsible for tree water uptake (Butler et al., 2010).

In conclusion, our study shows a fast exploration of deep soil layers by fine roots for the most productive clones and seedlings in Brazilian *Eucalyptus* plantations, with a depth of the root front corresponding roughly to mean stand height. This relationship between tree height and root front depth is probably only valid in absence of any physical or chemical barrier in the soil. A variability in root front depth between 8 m and 11.5 m depending on the genotype suggests that the velocity of root front movement downward might be an interesting criterion to consider before planting new clones at large scale, sampling soil cores down to depths of 10–15 m under the most promising genotypes between 1 and 2 years after the establishment of the clonal tests. The large variability in fine root biomass between the four genotypes in our study suggests that the carbon allocation patterns between above- and below-ground tree components may largely differ depending on the genotype. The belowground behavior of eucalypt trees deserves more attention to select the most adapted genotypes in a context of increasing abiotic stresses. Clones combining a high velocity of root front movement downward with high SRL values might be of interest to access to large amounts of water stored in deep soil layers before canopy closure.

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