

## Fine root isotropy in *Eucalyptus grandis* plantations. Towards the prediction of root length densities from root counts on trench walls

Jérôme Maurice · Jean-Paul Laclau · Danilo Scorzoni Re ·  
José Leonardo de Moraes Gonçalves · Yann Nouvellon · Jean-Pierre Bouillet ·  
José Luiz Stape · Jacques Ranger · Maurel Behling · Jean-Louis Chopart

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**Abstract** The objectives of the study were to assess changes in fine root anisotropy and specific root lengths throughout the development of *Eucalyptus grandis* (W. Hill ex Maiden) plantations and to establish a predictive model of root length density (RLD) from root intercept counts on trench walls. Fine root densities (<1 mm in diameter) were studied in 6-, 12-, 22-, 28-, 54-, 68- and 72-month-old *E. grandis* plantations established on deep Ferralsols in southern Brazil. Fine root intercepts were counted on 3 faces of 90–198 soil cubes (1 dm<sup>3</sup> in volume) in each stand and fine root

lengths ( $L$ ) were measured inside 576 soil cubes, sampled between the depths of 10 cm and 290 cm. The number of fine root intercepts was counted on one vertical face perpendicular to the planting row ( $N_v$ ), one vertical face parallel to the planting row ( $N_i$ ) and one horizontal face ( $N_h$ ), for each soil cube sampled. An overall isotropy of fine roots was shown by paired Student's  $t$ -tests between the numbers of fine roots intersecting each face of soil cubes at most stand ages and soil depths. Specific root lengths decreased with stand age in the upper soil layers and tended to increase

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J. Maurice  
Engref, Fif, AGROPARISTECH,  
Nancy, France

J.-P. Laclau · Y. Nouvellon · J.-P. Bouillet  
UPR Ecosystèmes de Plantation, CIRAD,  
34398 Montpellier, France

D. Scorzoni Re  
Forest Science Department, UNESP,  
Botucatu, Brazil

J. L. M. Gonçalves · Y. Nouvellon · J.-P. Bouillet ·  
M. Behling  
Forest Department, USP, Esalq,  
Piracicaba, Brazil

J.-P. Laclau  
Ecology Department, Bioscience Institute, USP,  
São Paulo, Brazil

J. L. Stape  
Department of Forestry and Environmental Resources,  
NCSU,  
Raleigh, NC, USA

J. Ranger  
Biogéochimie des Ecosystèmes Forestiers, INRA,  
54280 Seichamps, France

J.-L. Chopart  
Upr Sca, CIRAD,  
97410 St. Pierre, Réunion, France

J.-P. Laclau (✉)  
Departamento de Ecologia / IB, CIRAD-USP,  
Rua do Matão, Trav. 14, n° 321, Cidade Universitária,  
São Paulo CEP 05508-900, Brazil  
e-mail: laclau@cirad.fr

in deep soil layers at the end of the rotation. A linear regression established between  $N_t$  and  $L$  for all the soil cubes sampled accounted for 36% of the variability of  $L$ . Such a regression computed for mean  $N_t$  and  $L$  values at each sampling depth and stand age explained only 55% of the variability, as a result of large differences in the relationship between  $L$  and  $N_t$  depending on stand productivity. The equation  $RLD = 1.89 \cdot LAI \cdot N_t$ , where LAI was the stand leaf area index ( $m^2 m^{-2}$ ) and  $N_t$  was expressed as the number of root intercepts per  $cm^2$ , made it possible to predict accurately ( $R^2 = 0.84$ ) and without bias the mean RLDs ( $cm cm^{-3}$ ) per depth in each stand, for the whole data set of 576 soil cubes sampled between 2 years of age and the end of the rotation.

**Keywords** Isotropy · Architecture · Fine root · Intercepts · Eucalypt · Brazil · Forest

## Introduction

Counting root intercepts on trench walls is a method commonly used to assess fine root distribution in forest soils (Bouillet et al. 2002; Adegbi et al. 2004; Schmid and Kazda 2005). This approach provides a map of root density on vertical soil profiles and the proportions of root lengths between soil layers can be deduced if roots have an isotropic development. A major advantage of this method over the conventional auger sampling method is that measuring root intercepts on trench walls requires only one fifth of the time necessary to measure root length (Vepraskas and Hoyt 1988). Developing a robust and low-cost methodology to assess root length densities (RLDs) across time and space from root counts on trench walls has been investigated for several decades for agricultural crops (e.g. Drew and Saker 1980; Vepraskas and Hoyt 1988; Chopart and Siband 1999). Melhuish and Lang (1968) showed from a geometrical probability theory the relationship  $L = 2N$ , between root length density ( $L$ , expressed as  $cm$  root length  $cm^{-3}$  soil volume) and the number of root intercepts ( $N$ , expressed in # roots  $cm^{-2}$ ) on three orthogonal faces of soil cubes, provided that the cubes are sufficiently small to make the assumption that a random root measured on one face goes through the soil volume and appears on another face (all the underlying hypoth-

eses are given by Lang and Melhuish 1970). The ratio ( $\alpha$ ) between  $L$  and  $N$  may be different from 2 when roots are counted on a single face if root distribution is anisotropic. Ranges of  $\alpha$  from 1 to 16 have been found for crops and discrepancies with the theoretical value of 2 were usually interpreted by a preferential orientation of fine roots (Vepraskas and Hoyt 1988; Escamilla et al. 1991). However, a few studies counted fine root intercepts on three orthogonal faces of soil cubes and  $\alpha$  ranging from 2 to 5 have been observed for maize and sugarcane crops (Chopart and Siband 1999; Chopart et al. 2008), and from 3 to 11 for *Melaleuca quinquinervia* and *Bactris gasipaes* Kunth (Lopez-Zamora et al. 2002). This feature shows that a deviation of  $\alpha$  from the theoretical value of two cannot be totally explained by root anisotropy. Including additional parameters taking into account plant development and root localization in the soil was likely to largely improve the predictive equations of RLDs (Chopart and Siband 1999).

Studies attempting to establish predictive equations of RLDs from root counts on trench walls are scarce in forest environments (Escamilla et al. 1991; Lopez-Zamora et al. 2002; Adegbi et al. 2004) and have yet to be carried out in *Eucalyptus* plantations. These plantations cover about 18 million hectares throughout the world and their environmental impact has been widely discussed (Cossalter and Pye-Smith 2003). Many studies have been carried out to assess root biomass in *Eucalyptus* plantations (e.g. O'Grady et al. 2005; Silva et al. 2009) and the role of fine roots in total belowground carbon allocation (Giardina et al. 2004; Marsden et al. 2010). Several process-based models have been developed recently to predict yields, water use, and resource use efficiencies in *Eucalyptus* plantations (e.g. Battaglia et al. 2004; Medlyn et al. 2007). Ecophysiological mechanisms are simulated with a wide range of complexity aboveground, but belowground processes are still modelled with great uncertainty. Predictive equations making it possible to estimate RLDs from root counts on trench walls would facilitate studies of fine root distributions in these tropical plantations and would contribute to improving current process-based models.

Fine root intercepts were counted on three orthogonal faces of soil cubes ( $1 dm^3$  in volume) in a chronosequence of *Eucalyptus grandis* (W. Hill ex

Maiden) plantations established on deep Ferralsols in southern Brazil. Roots <1 mm in diameter were studied because the smallest distal roots play a disproportionately important role in ecosystem C and nutrient fluxes (Guo et al. 2008). The objectives of this study were to explore the changes in fine root anisotropy and specific root lengths throughout the development of *E. grandis* plantations, as well as the possibility of predicting RLDs from root intercept counts on trench walls. The relationships between the stand leaf area index (LAI) and the predictive equations of RLDs were also investigated, since similar root and leaf traits have been shown for *Eucalyptus* plantations (O'Grady et al. 2006; Laclau et al. 2008) and are suggested by theories linking organ structure and function (Magnani et al. 2002).

## Material and methods

### Study site

The study was conducted at the ESALQ/USP experimental station in Itatinga, State of São Paulo (23°02'S, 48°38'W). The mean annual rainfall over the 15 years prior to this study was 1,360 mm and the mean annual temperature was 19°C, with a seasonal cold period from June to September. The relief was typical of the São Paulo Western Plateau, with a gently undulating topography.

Fine root anisotropy was studied in a chronosequence of *E. grandis* plantations that covered the whole rotation (6 years) and was representative of commercial plantations in southern Brazil. After harvesting a 10-ha *Eucalyptus* plot located on a hill top (slope <3%) at an altitude of 850 m, two experiments with contrasting fertilizer applications were installed in 2004 and plots (about 0.25 ha each) were planted every year with the same silviculture, representative of commercial plantations. Fine roots were studied in 6-, 12-, 22-, 28-, and 54-month-old stands established in a 300-m radius area, with seedlings from the same half-sib family selected by the genetic improvement programme of the Suzano Bahia Sul Company. The soils were deep Ferralsols according to the FAO classification (>10 m), developed on Cretaceous sandstone, Marília formation, Bauru group, with a clay content ranging from 14–16% in the A<sub>1</sub> horizon to 20–25% in deep soil layers.

The mineralogy was dominated by quartz, kaolinite and oxyhydroxides, with acidic soil layers (pH between 3.9 and 5) containing very small amounts of available nutrients before the experiment was set up (sum of base cations <0.3 cmol<sub>c</sub> kg<sup>-1</sup>, whatever the soil layer) (Maquère 2008). All seedlings received standard commercial plantation fertilization, which was non-limiting for this soil type (120 kg N ha<sup>-1</sup>, 33 kg P ha<sup>-1</sup>, 100 kg K ha<sup>-1</sup>, 2 t ha<sup>-1</sup> of dolomitic lime, and micronutrients). Fertilizers were only applied at planting, except KCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> which were split: a quarter of the total amount was applied at planting, then at 6, 12 and 18 months of age. The oldest stand of the chronosequence was sampled 68 months after planting. This stand was located 13 km away in the same topographic position. Seedlings had the same origin and a similar silviculture was applied, except that the spacing was 1.6 m × 3.8 m whereas it was 2 m × 3 m in the other stands of this chronosequence. Growth curves were similar for all the stands and no biotic factors severely affected their growth.

Two other *E. grandis* stands were sampled in order to study the effect of contrasting growing conditions on fine root anisotropy. A low-productivity stand was sampled in the same experiment as the 54-month-old stand of the chronosequence, in a treatment with no potassium fertilizer application. At age 36 months, the aboveground biomass in this stand was 56% lower than in the stand of the chronosequence sampled in the same experiment (see -K vs +K treatments in Laclau et al. 2009). The stand with the highest productivity was sampled at age 72 months (harvest age) in a clayey soil (37–45% clay down to a depth of 10 m). The aboveground biomass was 44% higher than in the oldest stand of the chronosequence at age 6 years (unpublished data).

### Sampling methodology

One (in the 22-, 28-, 54, and 72-month-old stands) or two (in the 6-, 12-, and 68-month-old stands) set(s) of three soil profiles were sampled in each stand, in an area close to a tree of mean basal area (no weeds and missing trees in a radius of 10 m). Root growth is very fast in *Eucalyptus* plantations (e.g. O'Grady et al. 2005; Jourdan et al. 2008) and roots sampled from age 12 months onwards belonged to various trees around the soil profiles studied. The root character-

**Table 1** Number of soil profiles and soil cubes sampled in each stand for root anisotropy and root length density (RLD) measurements (roots <1 mm in diameter)

Stand age (months)	Site	Number of soil profiles	Sampling date	Anisotropy measurements	RLD measurements
6	Chronosequence	6	January 2007	180 cubes	–
12	Chronosequence	6	July 2007	180 cubes	–
22	Chronosequence	3	March 2007	90 cubes	–
28	Chronosequence	3	November 2008	90 cubes	90 cubes
54	Chronosequence	3	October 2008	90 cubes	90 cubes
54	Low productivity	3	October 2008	90 cubes	90 cubes
68	Chronosequence	6	July 2008	198 cubes	198 cubes
72	High productivity	3	November 2008	108 cubes	108 cubes
Total		33		1,026 cubes	576 cubes

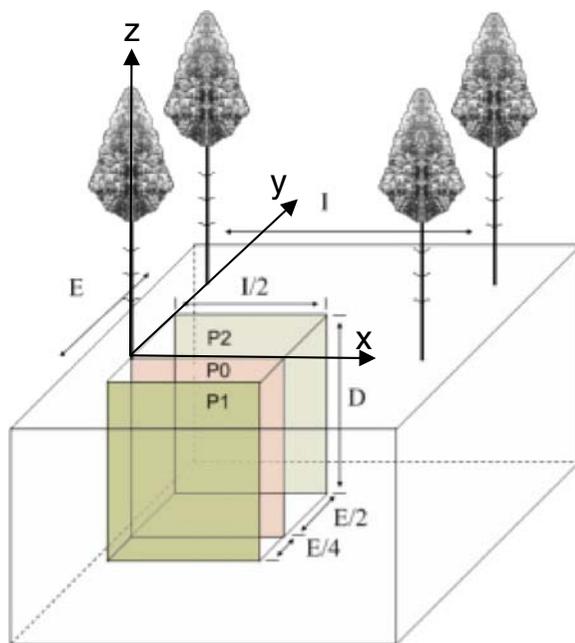
Two soil cubes (A and B, see Fig. 2) were sampled at each distance from the planting row, depth and soil profile. The distances from the planting row sampled were 20–30 cm, 80–90 cm and 140–150. The soil depths of 5–15 cm, 25–35 cm, 55–65 cm, 95–105 cm, 135–145 cm and 285–295 cm were sampled in all the stands (except at age 6 months where the depths of 5–15 cm and 135–145 cm were not sampled). Nine soil profiles were sampled at the depth of 285–295 cm at the end of the rotation: six in the chronosequence (at age 68 months) and three in a highly productive site (at age 72 months)

istics observed were therefore representative of the stand and not only influenced by the nearest tree. Roots were sampled on different dates in 2007 and 2008 (Table 1). In-growth core measurements in the same area showed that fine root production was higher at the onset of the wet season (October–January) than at the beginning of the winter (April–August) (Jourdan et al. 2008).

Fine root anisotropy was studied for all the soil profiles but RLDs were only studied for the soil profiles sampled in the 28-, 54-, 68- and 72-month-old stands. The soil profiles were perpendicular to the planting row (Fig. 1). They were sampled between the middle of the interrow ( $x=150$  or  $190$  cm in Fig. 1, depending on stand age) and: 1) the bottom of a tree of mean basal area ( $P_0$  for  $y=0$  cm), 2) 25% of the distance between two trees in the interrow ( $P_1$  for  $y=40$  or  $50$  cm, depending on stand age), and 3) 50% of the distance between two trees in the interrow ( $P_2$  for  $y=80$  or  $100$  cm, depending on stand age). Soil cubes were sampled at three distances from the planting row in each profile ( $x=20$ – $30$  cm,  $80$ – $90$  cm and  $140$ – $150$  cm) and five or six depths (5–15 cm, 25–35 cm, 55–65 cm, 95–105 cm, 135–145 cm and 285–295 cm) (Table 1 and Fig. 2).

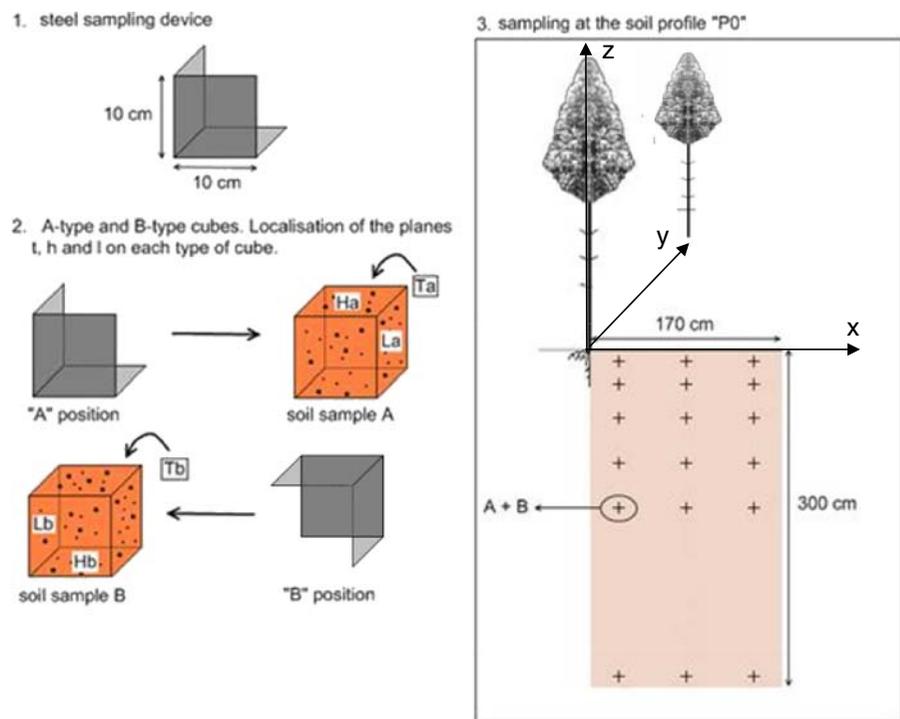
The sampling method was based on root intercept counts on undisturbed soil cubes (Chopart and Siband 1999). The soil cubes measured  $10\text{ cm} \times 10\text{ cm} \times 10\text{ cm}$ , and were extracted from the soil with square metal sampling devices, with three faces perpendicularly

oriented to each other. They were driven into the soil and drawn out carefully to preserve the integrity of the soil contained in the cubes. The three open faces were



**Fig. 1** Layout of the soil profiles sampled in each stand.  $E$  was the distance between 2 adjacent trees in the planting row,  $I$  was the inter-row length and  $D$  was the maximum depth sampled. In the 6-, 12-, 22-, 28- and 54-month-old stands:  $E=200$  cm,  $I=300$  cm and  $D=140$  cm. In the 68- and 72-month-old stands:  $E=160$  cm,  $I=380$  cm and  $D=290$  cm. A coordinate system ( $x$ ,  $y$ ,  $z$ ) is indicated in the figure

**Fig. 2** Layout of the positions sampled in each vertical trench wall and orientation of the sampling devices to collect the A and B soil cubes at each position. The faces where the number of root intercepts was counted are indicated on the A (subscript a) and B (subscript b) cubes: Ha and Hb (*horizontal*), Ta and Tb (*transversal, vertical and perpendicular* to the planting row), La and Lb (longitudinal, vertical and parallel to the planting row). A coordinate system (x, y, z) is indicated in the figure



flattened with a knife in order to obtain as perfect a cube as possible. Only living roots with a diameter  $< 1$  mm were studied. Living roots were identified according to the same criterion for root intercept counts on cube faces and root measurements inside the cubes: while living roots were pale and pliable, dead roots were brittle and dark. The number (#) of living root intercepts was counted on the three open faces which were given a specific name according to their orientation in the soil (h: horizontal; t: transversal, vertical and perpendicular to the planting row; l: longitudinal, vertical and parallel to the planting row). These numbers were converted into root intercept densities ( $\# \text{ root cm}^{-2}$ ) and identified as  $N_h$ ,  $N_t$  and  $N_l$  respectively, for the horizontal, vertical-transversal and vertical-longitudinal faces. Two types of cubes were sampled in each position: A-type cubes were 'open' toward the plantation row, with the h-face at the top of the sample and B-type cubes were 'open' towards the opposite planting row, the h-face at the bottom of the sample (Fig. 2). A-type cubes and B-type cubes were contiguous: if the A-type cube was sampled at the position (x, y, z), the B-type cube was sampled at the position (x, y+10 cm, z).

We assumed that the complementarities between A-type and B-type cubes (collected one after the other) avoided gradient effects inside the cubes

(Chopart and Siband 1999). Statistical analyses were therefore carried out for mean values between A-type and B-type cubes sampled at each position. RLDs were measured in the laboratory. The soil samples were washed carefully with a 1 mm-mesh sieve half immersed in water and dead roots were removed. Living roots were manually separated from the remaining soil particles and conserved at 2–4°C until processing. Roots were then scanned at a resolution of 200 dpi. The images were analysed with Analyra software (CIRAD, France) which provided root length measurements after calibration. The root samples were dried at 65°C to constant weight and the specific root length (SRL, root length divided by root mass) was calculated.

Tree foliage was collected from three different sections of the crown for 10 trees sampled in each stand at the end of summer (March). Thirty leaves randomly selected in each section of the sampled tree foliage were scanned immediately after collection and dried at 65°C to estimate the specific leaf area (SLA, leaf surface divided by leaf mass) of the crown section. A Matlab program was used to measure the area of the sampled leaves and calibrated for scanner use. Models were established to predict tree leaf areas from the circumference at breast height and tree height at each age and applied to the inventories in

order to estimate leaf area on a hectare basis (see Nouvellon et al. (2009) for a detailed description of the method). Destructive sampling of LAI in March and October for the oldest stands in our chronosequence showed that LAI was about 20% lower at the end of the winter than at the end of the summer in the sandy soil and only 5% lower in the clayey soil studied (unpublished data). We estimated the stand LAI in sampling month  $i$  considering a sinusoidal variation of LAI throughout the year:

$$LAI_i = \frac{LAI_{\max} + LAI_{\min}}{2} - \frac{(LAI_{\max} - LAI_{\min})}{2} \times \sin \frac{\pi \times i}{6} \quad (1)$$

where  $LAI_i$  was the stand LAI in month  $i$  (comprised between 1 for January and 12 for December),  $LAI_{\max}$  was the LAI value measured in March of the same year in the same stand and  $LAI_{\min}$  was 80% of  $LAI_{\max}$  in the sandy soil. A remote sensing study on a network of 16 *Eucalyptus* stands in the same region showed seasonal changes in canopy reflectance consistent with our approach (Marsden et al. 2010).

#### Data analysis

Root anisotropy was studied at each soil depth comparing the  $N_t$ ,  $N_h$ , and  $N_l$  values. Student's  $t$ -test pair-wise comparisons were used considering each 1 dm<sup>3</sup> soil cube (mean of the A-type and B-type cubes for each position) as an individual estimate for the three  $N$  variables (Lopez-Zamora et al. 2002). A Bonferroni correction was used and the level of significance used for the three pairwise comparisons was 0.017. Linear regressions between fine root lengths inside the soil cubes ( $L$ ) and the  $N_t$ , and  $N_a$  (average of the six faces: three faces on two cubes) values were established in each stand. The  $t$  face of soil cubes corresponded to the plane of trench walls where root intercepts could be counted to study root development. Linear regressions were not computed for the  $h$  and  $l$  faces because horizontal planes and trench walls parallel to the planting row are of little interest for studying fine root distributions in the field. The models were restricted to force the intercept through zero. The slope of these regressions was defined as the  $\alpha$  coefficient thereafter. The confidence interval of  $\alpha$  was calculated to determine whether  $N_t$

and  $N_a$  provided comparable results and to test the  $L=2N$  relationship. Pearson correlation coefficients were calculated between selected variables dependent on the position of the cubes sampled in the soil profiles (depth and distance from the planting row) and selected variables dependent on fine root architecture ( $N_t$ ,  $N_h$ ,  $N_l$ ,  $L$ , SRL, and the  $N_t:N_a$  ratio).

For the whole dataset, linear regressions (forced through the origin) were computed between individual values of  $N_t$  and  $L$  (dependent variable), as well as between mean values per depth and stand age to reduce variability (Escamilla et al. 1991; Lopez-Zamora et al. 2002). Non-linear regressions were considered but they did not improve the predictions (data not shown). Preliminary observations showed that the  $\alpha$  coefficient was linearly dependent on stand LAI (data not shown). Linear regressions were thus established for the whole dataset between mean ( $LAI \times N_t$ ) and  $L$  values per depth and stand age. SAS software was used for all statistics (SAS Institute 1999), and the significance threshold was  $P < 0.05$ .

## Results

#### Main characteristics of fine roots

The number of fine roots intersecting the faces of soil cubes was negatively correlated with soil depth (Table 2). Correlation coefficients between soil depth and  $N_t$ ,  $N_l$ ,  $N_h$  were significant at mid rotation, but they were not significant for certain faces of the cubes at ages 6 and 68 months. The density of fine root intercepts on cube faces decreased significantly with the distance from the planting row at age 6 months, but was little influenced thereafter. Similar trends were observed for RLDs and fine root intercepts on cube faces. The  $L:N_t$  ratio was not significantly correlated with the distance from the planting row, or with soil depth, in the 28-, 54- and 68-month-old stands.

Paired Student's  $t$ -tests showed that the numbers of root intercepts were not significantly different between soil cube faces at most depths throughout stand development (Table 3). However,  $N_h$  was significantly higher than  $N_l$  (or  $N_t$ ) at ages 12 and 28 months in the chronosequence and at age 72 months in the highly productive site, when all the soil depths were considered. Fine roots were preferentially vertically

**Table 2** Pearson correlation coefficients between selected variables dependent on the position of the cubes sampled in the soil profiles (depth and distance from the planting row) andselected variables dependent on fine root (diameter <1 mm) architecture. Only the stands in the chronosequence are indicated. Significant correlations ( $P < 0.05$ ) are indicated in bold

Variables	$N_t$	$N_h$	$N_l$	SRL	$L$	$L:N_t$ ratio
<b>Age 6 months</b>						
Soil depth	-0.21	<b>-0.36**</b>	<b>-0.27*</b>			
Distance from the planting row	<b>-0.32***</b>	<b>-0.35**</b>	<b>-0.36**</b>			
<b>Age 12 months</b>						
Soil depth	<b>-0.63****</b>	<b>-0.65****</b>	<b>-0.46****</b>			
Distance from the planting row	-0.20	-0.20	-0.22			
<b>Age 22 months</b>						
Soil depth	<b>-0.55****</b>	<b>-0.51****</b>	<b>-0.53****</b>			
Distance from the planting row	-0.21	-0.16	-0.16			
<b>Age 28 months</b>						
Soil depth	<b>-0.41**</b>	<b>-0.55***</b>	<b>-0.50****</b>	0.07	<b>-0.40**</b>	-0.10
Distance from the planting row	-0.23	-0.11	-0.10	0.19	-0.19	0.10
<b>Age 54 months</b>						
Soil depth	<b>-0.42**</b>	<b>-0.45**</b>	<b>-0.40**</b>	<b>0.37*</b>	<b>-0.42**</b>	0.07
Distance from the planting row	-0.23	-0.29	<b>-0.30*</b>	-0.04	<b>-0.40**</b>	-0.23
<b>Age 68 months</b>						
Soil depth	-0.17	<b>-0.23*</b>	-0.06	<b>0.49****</b>	-0.11	-0.06
Distance from the planting row	<b>-0.27*</b>	-0.18	<b>-0.31*</b>	0.05	-0.16	0.10

$N_t$  number of fine root intercepts observed on the transversal faces of soil cubes,  $N_l$  number of fine root intercepts on the longitudinal face of soil cubes,  $N_h$  number of fine root intercepts on the horizontal face of soil cubes,  $SRL$  specific root length ( $m\ g^{-1}$ ),  $L$  fine root length measured inside the soil cubes

Significant correlations at  $P < 0.05$ , 0.01, 0.001 and 0.0001 are indicated by \*, \*\*, \*\*\*, and \*\*\*\*, respectively

oriented at these ages but this pattern was little pronounced since differences were not significant for most soil depths. Whenever significant differences in root intercept densities between soil faces were observed, the highest densities were found on the horizontal face but they were not significantly different from one of the lateral faces (either the t or the l face). The highest fine root anisotropy occurred at age 12 months. At that age, mean  $N_h$  and  $N_t$  values across all sampling depths were 20–25% higher than the mean  $N_l$  value. This pattern indicated that fine roots were preferentially oriented vertically and perpendicularly to the planting row at that age.

A decrease in specific root length (SRL) from 28 to 68 months after planting in the stands of the chronosequence showed that fine roots characteristics changed throughout stand development (Fig. 3a). This pattern was more pronounced at the depth of 10 cm than in deeper soil layers and an overall upward trend for SRL with soil depth was observed at the end of

the rotation for stands installed on the two soil types (Fig. 3b and Table 2). The highest SRLs at the end of the rotation were found in the stand with the highest productivity and LAI (clayey soil vs chronosequence), whatever the soil depths. This feature was not observed in response to K application 54 months after planting. The range of SRL was similar in the two stands studied at this age, despite aboveground biomass and LAI values in the stand of the chronosequence that were double those in the stand with no K application.

#### RLD predictive equation

The slopes of linear regressions between fine root lengths inside soil cubes and the number of fine roots intersecting the transversal faces ( $N_t$ ) decreased from 28 to 68 months after planting in the chronosequence, and were not significantly different when  $N_t$  and  $N_a$  were used as independent variables in the regressions

**Table 3** Mean number of fine roots (diameter <1 mm) intersecting each face of the soil cubes sampled in 3–6 soil profiles per stand. Significant differences using the Bonferroni correctionfor the paired Student's *t*-tests ( $P < 0.017$ ) are indicated in bold. Means followed by the same letters do not differ significantly

Stand age (months)	Site	Face	Mean number of roots intersecting each face (# roots cm <sup>-2</sup> )						
			Depth 10 cm	Depth 30 cm	Depth 60 cm	Depth 100 cm	Depth 140 cm	Depth 290 cm	All depths
6	Chronosequence	T	–	0.584	0.479	0.391	N.S.	–	0.484
		L	–	0.655	0.437	0.402	N.S.	–	0.499
		H	–	0.707	0.505	0.343	N.S.	–	0.516
12	Chronosequence	T	<b>1.681a</b>	<b>0.784ab</b>	0.720	0.762	0.376	–	<b>0.859a</b>
		L	<b>1.184b</b>	<b>0.638b</b>	0.670	0.735	0.388	–	<b>0.720b</b>
		H	<b>1.572ab</b>	<b>0.931a</b>	0.813	0.721	0.482	–	<b>0.898a</b>
22	Chronosequence	T	1.432	0.803	0.717	0.612	0.553	–	0.819
		L	1.356	0.806	0.694	0.571	0.503	–	0.782
		H	1.450	0.850	0.689	0.635	0.583	–	0.836
28	Chronosequence	T	<b>1.956b</b>	0.661	0.644	0.717	0.778	–	<b>0.951b</b>
		L	<b>2.433ab</b>	0.661	0.750	0.756	0.750	–	<b>1.070ab</b>
		H	<b>2.850a</b>	0.794	0.722	0.783	0.733	–	<b>1.177a</b>
54	Chronosequence	T	1.533	0.494	0.394	0.417	0.428	–	0.653
		L	1.528	0.433	0.417	0.617	0.450	–	0.689
		H	1.672	0.494	0.372	0.539	0.433	–	0.702
68	Chronosequence	T	0.786	0.344	0.483	0.689	0.547	0.294	0.545
		L	0.611	0.292	0.458	0.608	0.578	0.311	0.491
		H	0.806	0.367	0.572	0.619	0.614	0.217	0.561
54	Low productivity	T	2.017	0.872	0.672	0.739	0.839	–	1.028
		L	1.967	1.067	0.567	0.744	0.778	–	1.024
		H	2.356	1.061	0.567	0.639	0.633	–	1.051
72	High productivity	T	1.978	1.217	<b>1.422a</b>	0.811	1.039	0.372	<b>1.140ab</b>
		L	1.578	1.106	<b>1.094b</b>	0.989	0.928	0.406	<b>1.017b</b>
		H	1.794	1.356	<b>1.283ab</b>	0.939	1.067	0.483	<b>1.154a</b>

–: depth not sampled

(Table 4). A similar downward pattern was observed for stand LAI and mean RLDs in the chronosequence, from 28 to 68 months after planting. However, mean RLDs were similar in the 72-month-old stand installed on a clayey soil and in the low-productivity 54-month-old stand, despite a 58% higher LAI in the former stand.

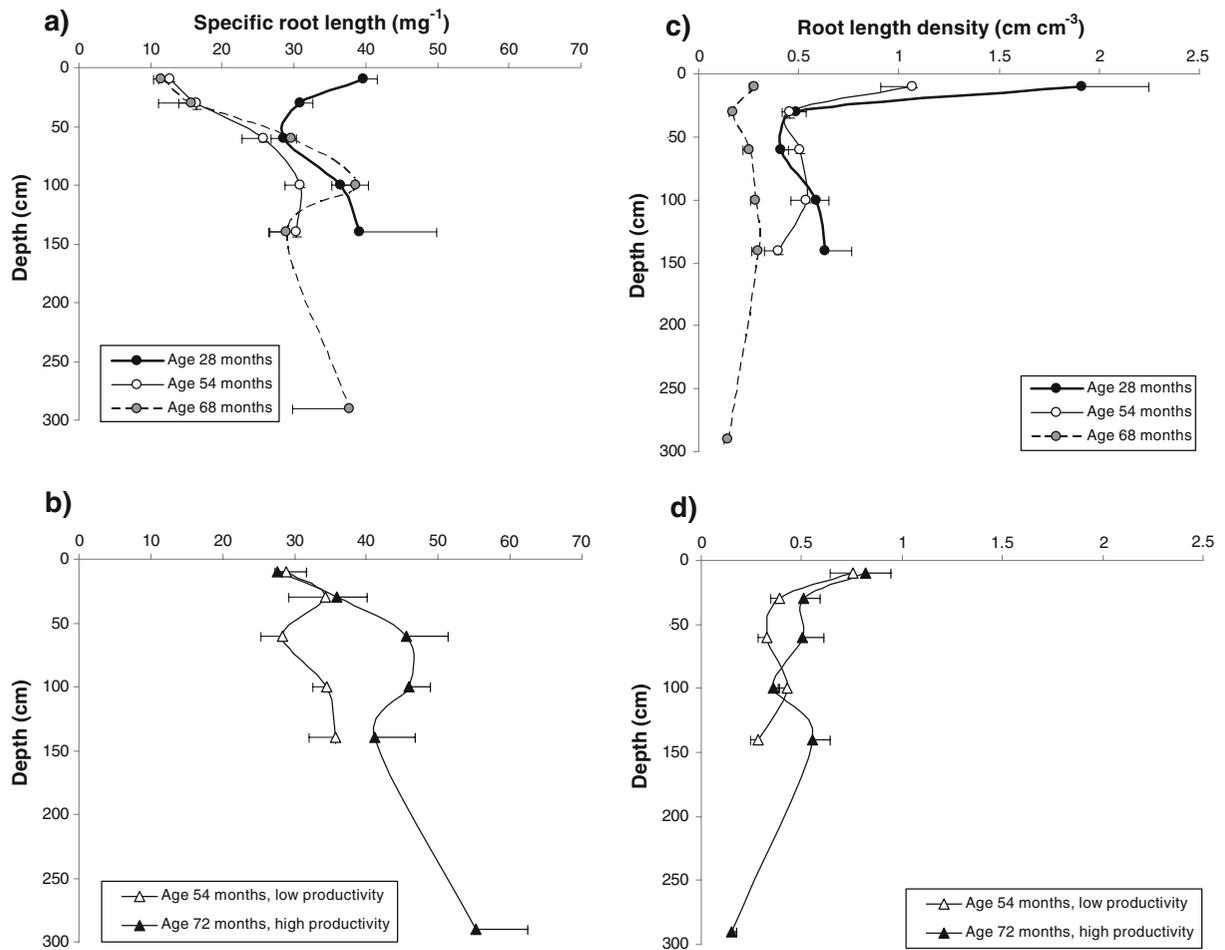
A linear regression between root lengths inside all the soil cubes sampled and the number of roots intersecting the transversal face of each cube accounted for 36% of the variability of RLDs. Fifty-one percent of this variability was explained when only the cubes sampled in the chronosequence were taken into account (data not shown). When the regression was established for mean

RLDs per depth at each age, the predictive equation for the stands in the chronosequence was:

$$RLD = 7.89 \times N_t \quad (R^2 = 0.83, n = 16, P < 0.0001) \quad (2)$$

with RLD expressed in cm cm<sup>-3</sup> and  $N_t$  expressed in # roots cm<sup>-2</sup>. When the cubes sampled in the low and high productivity stands were included in the regression, the equation was:

$$RLD = 5.65 \times N_t \quad (R^2 = 0.55, n = 27, P < 0.0001) \quad (3)$$



**Fig. 3** Mean specific root lengths (SRL) of fine roots (diameter <1 mm) sampled in the chronosequence (a) and in the low and high productivity stands (b), as well as mean root length density of fine roots sampled in chronosequence (c) and in the low and

high productivity stands (d). Standard errors at each depth are indicated ( $n=9$ , except in the 68-month-old stand above the depth of 150 cm where  $n=18$ )

The comparison of predicted and measured mean RLDs per soil depth at each age showed poor prediction using Eq. 3 for most of the depths sampled (Fig. 4a).

Including stand LAI in the regression between mean RLDs and  $N_t$  per soil depth for all the stands sampled led to the predictive equation:

$$RLD = 1.89 \times LAI \times N_t \quad (R^2 = 0.84, n = 27, P < 0.0001) \quad (4)$$

The range of discrepancies between measured and predicted values was much lower using Eq. 4 than Eq. 3, whatever the age and the productivity of the

stands (Figs. 4a and b). Mean RLDs per soil depth for the whole data set were estimated with no bias with Eq. 4.

## Discussion

### Fine root characteristics

Paired Student's *t*-tests between the numbers of fine roots intersecting each face of soil cubes showed that fine roots exhibited an overall isotropy throughout tree development (Table 3). The mean number of roots intersecting the soil cubes across all depths was

**Table 4** Linear regressions (forced through the origin) between root length density (RLD) of fine roots (diam. <1 mm) measured inside soil cubes sampled in 3–6 soil profiles per stand (dependent variable expressed in  $\text{cm cm}^{-3}$ ), and the number of fine roots intersecting the transversal face ( $N_t$ ) or the average of the faces in three dimensions ( $N_a$ ) of soil cubes

Stand age (months)	Site	LAI	RLD Mean $\pm$ SD ( $\text{cm cm}^{-3}$ )	Independent variable	Slope ( $\alpha$ )	Confidence interval ( $P=0.95$ )	$R^2$
28	Chronosequence	4.2	0.81 $\pm$ 0.74	$N_t$	<b>8.66</b>	7.59–9.73	0.86
				$N_a$	<b>7.81</b>	6.96–8.67	0.88
54		3.8	0.61 $\pm$ 0.40	$N_t$	<b>6.55</b>	5.30–7.80	0.72
				$N_a$	<b>6.97</b>	5.89–8.04	0.79
68		2.2	0.25 $\pm$ 0.12	$N_t$	<b>4.45</b>	3.94–4.96	0.85
				$N_a$	<b>4.89</b>	4.45–5.33	0.90
54	Low productivity	1.9	0.44 $\pm$ 0.24	$N_t$	<b>3.38</b>	2.84–3.93	0.78
				$N_a$	<b>3.33</b>	2.91–3.97	0.77
72	High productivity	3.0	0.48 $\pm$ 0.32	$N_t$	<b>3.89</b>	3.45–4.34	0.85
				$N_a$	<b>4.34</b>	3.90–4.77	0.88

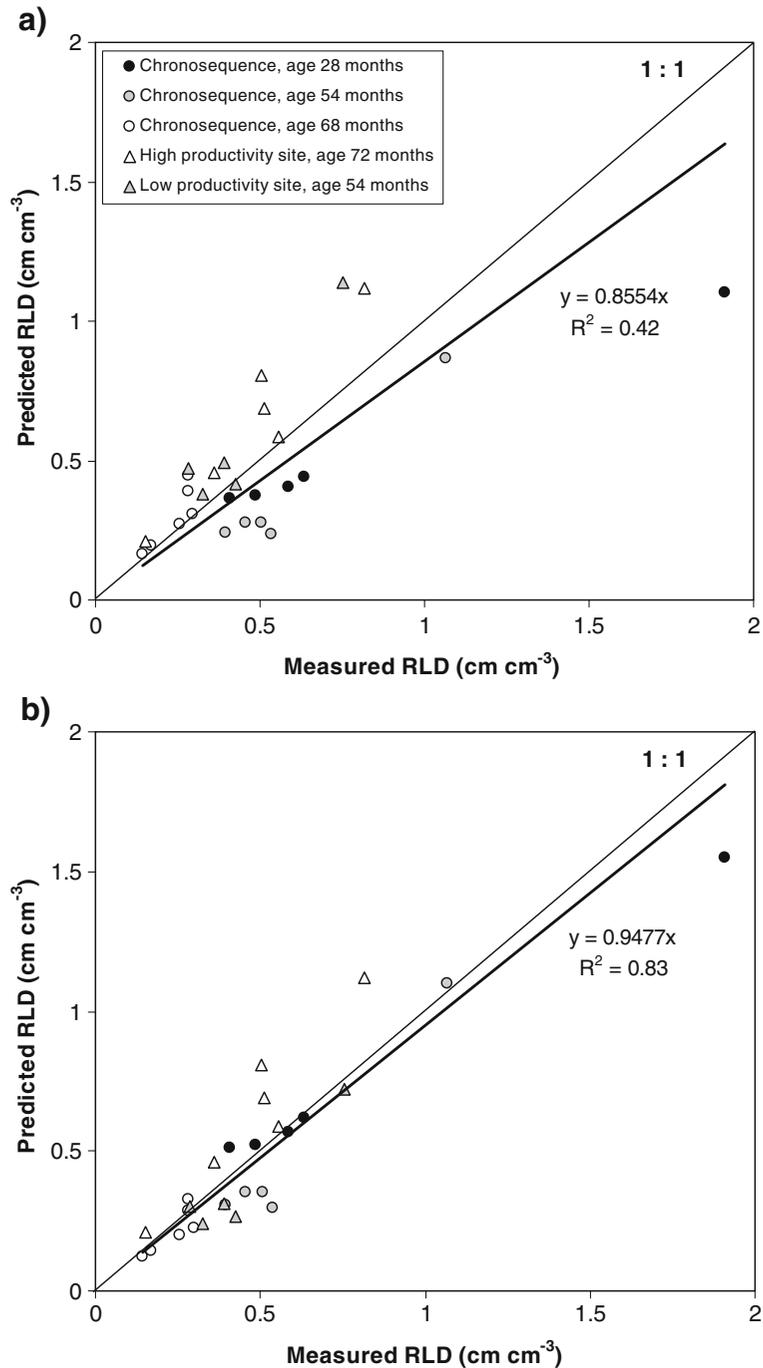
(independent variable, expressed in  $\#$  roots  $\text{cm}^{-2}$ ). The confidence intervals of the slopes ( $\alpha$ ) of the regressions are indicated as well as the leaf area index (LAI) and mean RLDs across sampling depths for the stands sampled. Slopes significantly different from 2 are indicated in bold ( $P<0.05$ ). All the regressions were highly significant ( $P<0.0001$ )

higher for the h face than for the other two faces whatever the stand age, but the differences were not significant with either the l or the t face. This pattern showed that a weak trend of preferential vertical orientation was observed but an overall isotropy throughout stand development can be accepted. Most of the studies based on the root intersect method were carried out counting root intercepts in only one plane and comparisons of root intersect densities on 3 orthogonal faces of soil cubes are rare. Lopez-Zamora et al. (2002) showed that fine root isotropy is not a common feature for tree species. Whilst the growth of *Bractis gasipaes* roots on a clayey Oxisol was isotropic, the growth of *Melaleuca quinquenervia* roots on a sandy Spodosol was anisotropic with 50% fewer roots found on the basal face than on lateral faces of soil cubes. To our knowledge, the only other studies with root intercepts counted on 3 orthogonal faces of soil cubes were carried out by Baldwin et al. (1971) for onions, Chopart and Siband (1999) for maize, Chopart et al. (2008) for sugarcane crops, and Dusserre et al. (2009) for upland rice. Whilst strong anisotropy was observed for onion roots, overall isotropy of fine roots was observed throughout the development of maize and sugarcane crops.

Fine root isotropy was little influenced by spatial localization in the soil whatever the stand age in our study. Sampling down to a depth of 290 cm made it possible to check that the three-dimensional develop-

ment of fine roots in very deep soil layers was similar to that observed in the top soil. This feature is important for *Eucalyptus* plantations established on deep soils because fine roots are found down to depths of 8–10 m within 7 years of planting (Robinson et al. 2006). Even though fine root densities decrease largely in deep soil layers they are likely to supply a not insubstantial proportion of water and nutrient requirements for tree growth (e.g. Lehmann 2003; Bruno et al. 2006; Robinson et al. 2006). Most studies dealing with fine root anisotropy have been carried out in the upper 1 m of soil and an assessment in deep soil layers was lacking (e.g. Chopart and Siband 1999; Lopez-Zamora et al. 2002).

Specific root length (SRL) is an important characteristic of root systems influencing the volume of soil explored per unit of biomass invested in fine roots. High SRL are expected in fast-growing *Eucalyptus* plantations because fast growth requires fast and efficient acquisition of resources and a high SRL allows rapid nutrient uptake per unit root mass (Ryser 2006). A comparison of 11 mature temperate tree species showed that a faster growth rate was associated with a higher SRL and smaller root diameters, but differences in root tissue density were not significant between slow- and fast-growing species (Comas and Eissenstat 2004). SRL have been little studied in *Eucalyptus* plantations and comparisons with other studies are difficult since



**Fig. 4** Comparison of mean predicted and measured values of fine root length densities (RLDs) per soil depth in soil cubes sampled from 28 to 72 months after planting. The RLDs (expressed in  $\text{cm cm}^{-3}$ ) were predicted from: **a** the equation  $\text{RLD} = 5.65 \cdot N_t$  where  $N_t$  is the density of fine roots intersecting

the transversal faces of soil cubes (expressed in  $\# \text{ roots cm}^{-2}$ ), or **b** from the equation  $\text{RLD} = 1.89 \cdot \text{LAI} \cdot N_t$  where LAI is stand leaf area index (expressed in  $\text{m}^2 \text{ m}^{-2}$ ). Linear regressions between predicted and measured RLDs were established for all the soil depths and stand ages sampled

SRLs are largely dependent on the root diameter class studied and may vary significantly with seasons and soil depths (Wang et al. 2006). Whereas the SRL of fine roots (<1 mm in diameter) ranged from 10 to 60 m g<sup>-1</sup> in our study, the mean SRL of fine roots (<5 mm in diameter) in a 14-month-old *Eucalyptus globulus* plantation in Tasmania was 17 m g<sup>-1</sup> in the 0–10 cm soil layer and decreased with soil depth (O’Grady et al. 2005). A decrease in SRL with soil depth was also observed in maritime pine plantations (Bakker et al. 2009), contrary to the upward trend down to a depth of 3 m observed at the end of the rotation in our study. Increased nutrient supply led to a higher SRL in maritime pine plantations (Bakker et al. 2009). The higher nutrient availability during the early growth of *Eucalyptus* plantations than at the end of the rotation, resulting from large amounts of fertilizers applied at planting, might be involved in the decrease in SRL in the top soil from 28 to 54 months after planting in our study. However, there are contrasting results on the effects of nutrient supply on SRL in the literature, which might be due to variations in the components of SRL, root fineness and root mass density (Ryser 2006).

A decrease in LAI, RLD, and SRL was observed in our chronosequence from 2 to 6 years after planting. Positive correlations between tree foliage mass (or LAI), fine root lengths (or fine root area) and SRLs have been reported for temperate and boreal tree species (Adegbidi et al. 2004; Al Afas et al. 2008; Børja et al. 2008). However, the relationship between foliage biomass and RLD as well as SRL may be affected by nutrient availability in forest stands (Ostonen et al. 2007; Bakker et al. 2009). In our study, K deficiency at age 54 months led to a drop in stand LAI but had little effect on mean SRL, contrary to the positive correlation between LAI and SRL observed in the chronosequence. Fine root diameter distributions can be modified by nutrient availability (Zobel et al. 2007) as well as root tissue density (Ostonen et al. 2007), and K deficiencies are likely to modify root traits, as shown by a reduction in root longevity for K-deficient *Hibiscus rosa-sinensis* L. cv. Leprechaun (Egilla et al. 2001). Further studies would be necessary to assess whether modifications in fine root tissue densities are involved in the maintenance of SRLs by *Eucalyptus* trees under K deficiency.

## Predictive models of root length densities

The low anisotropy of fine roots in our study and the similar values of  $\alpha$  using  $N_t$  or  $N_a$  as independent variables showed that discrepancies from the theoretical value  $\alpha=2$  were not a result of a preferential orientation in a particular direction, as suggested by numerous studies (e.g. Vepraskas and Hoyt 1988; Escamilla et al. 1991). The slopes between  $N_t$  (or  $N_a$ ) and  $L$  ranged from 3 to 9 in the stands sampled in our study and a similar range of values was found by Lopez-Zamora et al. (2002) for two other tree species. Values approximately 50% higher than the theoretical expectation of 2 were found using  $N_a$  in maize and sugarcane crops (Chopart and Siband 1999; Chopart et al. 2008). The trend to underestimate RLDs multiplying  $N_t$  (or  $N_a$ ) by the theoretical value of 2 might be a result of the practical experimental difficulty in identifying very fine roots intersecting the cube faces under field conditions (Bengough et al. 1992; Lopez-Zamora et al. 2002; Chopart et al. 2008). An increase in the ratio between measured  $L$  and  $N_t$  ( $L:N_t$ ) with  $N_t$  observed in maize and sugarcane crops supported the following hypothesis: the more roots intersect cube faces, the more difficult it is to count all of them (Chopart and Siband 1999; Chopart et al. 2008). This trend was not observed in our study since  $L:N_t$  was negatively correlated with  $N_t$  for all the soil cubes sampled ( $r=-0.34$ ,  $P<0.0001$ ). The discrepancy of  $\alpha$  from the theoretical value of 2 might also result from using the geometrical theory outside its validity boundaries for roots <1 mm in diameter inside soil cubes of 1 dm<sup>3</sup> in volume (Lopez-Zamora et al. 2002). This theory is based on the assumption that a random straight line (a root) crosses 2 faces of the soil cubes (Melhuish and Lang 1968). A hypothesis to explain the influence of stand LAI on the relationship between  $L$  and  $N_t$  might be that fine roots are longer and less ramified in stands with the lowest LAI, and thus more effectively meet the assumptions required to apply Melhuish and Lang’s theory, than in stands with a higher LAI. Stand LAI increases with water and nutrient availabilities in *Eucalyptus* plantations (e.g. Battaglia et al. 1998). High phenotypic plasticity of fine roots was observed for temperate tree species with an upward trend for SRL with water and nutrient supply (e.g. Meier and Leuschner 2008; Bakker et al. 2009). Roots were broken during sample preparation in our study and we could not assess their

total length and branching pattern. However, Pregitzer et al. (2002) showed that mean root lengths increased while SRL decreased with increasing root order for nine tree species. This pattern suggests that the parallel decrease in SRL and stand LAI in our chronosequence from age 2 years onwards might be associated with an increase in mean root length within the 0–1 mm class. The assumption of the geometrical theory would then be more effectively met when stand LAI decreases, which would explain why the  $\alpha$  coefficient reached values closer to 2 in stands with low LAI values. Even though recent studies have shown that fine root architectural development (density of root tips, branching ratio, root diameter) is essential to gain insight into root turnover and nutrient fluxes belowground for tree species (Pregitzer et al. 2002; Guo et al. 2008), information in the literature on the relationship between stand LAI and fine root branching is scarce (Wang et al. 2006). For sugarcane crops, the  $\alpha$  coefficient was closer to the theoretical value of 2 for coarse roots than for fine roots (Chopart et al. 2008). This pattern might result from both a more accurate counting of root intercepts on the faces of the cube under field conditions and requirements to apply Melhuish and Lang's theory being better met for long roots with little branching than for very fine roots. Potassium starvation is likely to reduce the elongation of lateral fine roots (Nibau et al. 2008), and a reduction in fine root branching under K deficiency might explain why the lowest  $\alpha$  coefficient in our study was found in the K-deficient stand (Table 4).

Including stand LAI in the relationship between  $L$  and  $N_t$  greatly improved the predictions of RLDs. An increase in the slope of the relationship between  $L$  and  $N_t$  was observed throughout the development of maize and sugarcane crops and was accounted for using an empirical coefficient positively correlated with  $N_t$  for sugarcane and thermal time elapsed since sowing for maize. The relationships between crop LAI and these empirical coefficients were not investigated in those studies. However, since crop LAI is generally positively correlated with root densities and time elapsed since sowing for those crops, using LAI to account for the changes in the relationship between  $L$  and  $N_t$  might also be valid. Stand LAI is highly sensitive to growing conditions and this variable might make it possible to model the slope between  $L$  and  $N_t$  for a large range of environ-

ments, provided that the relationship between  $L$  and  $N_t$  has a biophysical support influenced by stand LAI (length of fine roots or branching pattern for example). The large improvement in RLD predictions when taking into account stand LAI in the K-deficient treatment despite growing conditions similar to those in the 54-month-old stand of the chronosequence supports this hypothesis (similar seedlings, age, soil, and climate for these two treatments in the same experiment). However, further studies are required to assess whether stand LAI is likely to improve the prediction of RLDs from root counts on trench walls for other species and growing conditions.

Our study shows that Eq. 4 can be used to estimate RLDs from root counts on trench walls in *E. grandis* plantations established on deep Ferralsols, from 2 to 6 years after planting (end of rotation for intensively-managed plantations). It would have been interesting to investigate the shape of the relationship between  $\alpha$  and stand LAI for the whole rotation, including young stands sampled before canopy closure. Measurements were considerably time consuming and we singled out the establishment of a predictive equation in stands of contrasting productivity in order to check that our results were not site- or silviculture-specific. We put forward the hypothesis that intra-annual variations in LAI occurred with a simultaneous variation in RLD for the very fine roots sampled. Many studies have shown that *Eucalyptus* fine roots are highly dynamic in response to water availability, and stand LAI should be estimated on the date of root intercept counting to use Eq. 4. Complementary studies are necessary to establish a predictive equation covering the whole rotation and to test the relationship between stand LAI,  $N_t$  and RLDs for other tree species and other environments. Moreover, further studies should test the influence of soil cube sizes on the relationship between RLDs and  $N_a$  to assess if the discrepancies in the  $\alpha$  coefficient from the theoretical value of 2 were due to an underestimation of the number of very fine roots intersecting the faces of soil cubes (little visible under field conditions) or resulted from non-applicability of the geometrical theory for ramified short roots that do not cross two faces of the cubes. Studies dealing with fine root architecture are necessary to improve our understanding of the relationship between fine root abundance and stand LAI in tropical plantations.

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