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REGULAR ARTICLE

Contrasting phenology of *Eucalyptus grandis* fine roots in upper and very deep soil layers in Brazil

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Abstract

Background and aims While the role of deep roots in major ecosystem services has been shown for tropical forests, there have been few direct measurements of fine root dynamics at depths of more than 2 m. The factors influencing root phenology remain poorly understood, creating a gap in the knowledge required for predicting the effects of climate change. We set out to gain an insight into the fine root phenology of fast-growing trees in deep tropical soils.

Methods Fine root growth and mortality of *Eucalyptus grandis* trees were observed fortnightly using minirhizotrons down to a soil depth of 6 m, from 2 to 4 years after planting.

Results In the topsoil, the highest live root length production was during the rainy summer (20 cm m^{-2} d^{-1}) whereas, below 2 m deep, it was at the end of the dry winter (51 cm m^{-2} d^{-1}). The maximum root elongation rates increased with soil depth to 3.6 cm d^{-1} in the 5–6 m soil layer.

Conclusions Our study shows that the effect of the soil depth on the seasonal variations in fine root growth should be taken into account when modelling the carbon, water and nutrient cycles in forests growing on deep tropical soils.

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Keywords Plantation · Fine root dynamics · Turnover · Lifespan · Minirhizotron · Oxisol

Introduction

Although fine roots play a major role in global biogeochemical cycles, the factors controlling their growth and mortality remain poorly understood (Pierret et al. 2016; Radville et al. 2016). Many studies conducted over the last decades show fine root turnover rates from 0.1 to 0.5 $yr.^{-1}$ in boreal forests to 0.6–1.0 $yr.^{-1}$ in tropical forests (Gill and Jackson 2000). However there are both conceptual and methodological problems with the methods used to estimate fine root dynamics and fine root mortality (Hendricks et al. 2006; Joslin et al. 2006; Jourdan et al. 2008). A few studies based on ^{14}C measurements suggest that fine root longevity in forests could be greater than the values estimated using sequential coring or ingrowth core methods (Gaudinski et al. 2001; Trumbore et al. 2006). However, an overestimation of fine-root life span using radio carbon methods has been shown in forest ecosystems, partly due to utilization of stored or recycled C to construct new fine roots (Strand et al. 2008; Vargas et al. 2009; Vargas and Allen 2008). While the effects of environmental changes on the phenology of aboveground plant components are well documented, our poor understanding of the factors driving root phenology leads to large uncertainties in the predictions of the response of terrestrial ecosystems to climate change (Radville et al. 2016). Studies in forest ecosystems showed that fine root lifespan depends strongly on root branch order (Wells and Eissenstat 2001; Tierney and Fahey 2002; Guo et al. 2008a, 2008b) as well as soil temperature, soil moisture, and soil nutrient availability (Eissenstat and Yanai 1997; Godbold et al. 2003; Joslin et al. 2006; Jourdan et al. 2008; Rytter 2013). Mycorrhizal colonization has been shown to increase fine root lifespan (King et al. 2002; Guo et al. 2008b). In temperate forests, higher seasonal variations of fine root growth and mortality in the topsoil than at depths >0.5 m have commonly been attributed to rapid changes in soil water content and temperature close to the soil surface (Hendrick and Pregitzer 1996; Schenk and Jackson 2002a). However, the processes controlling fine root phenology remain largely unknown and a recent review pointed out the need for more research based on direct observations of root growth using minirhizotrons and rhizotrons (Radville et al. 2016).

Although the major role of deep roots in carbon and water cycles has been shown for several decades in Amazonia (Nepstad et al. 1994; Saleska et al. 2007; Brando et al. 2008; Davidson et al. 2011), the phenology of deep roots in tropical forests remains poorly documented (Schenk and Jackson 2002b; Graefe et al. 2008; Radville et al. 2016). As far as we are aware, direct observations of the same roots throughout their lifespan using rhizotrons at depths >2 m are scarce and have only been performed in a rubber tree plantation in Thailand down to 4.5 m deep and never deeper using minirhizotrons (Maeght et al. 2013, 2015). Deep roots are likely to take up transient water and nutrient resources in tropical forests (Schenk 2006; Lambers et al. 2008; Christina et al. 2017), and during the dry season can provide access to large amounts of water stored in deep soil layers during the wet season (Alton 2014; Oliveira et al. 2005; Nardini et al. 2016). Fine root longevity estimated from ^{14}C measurements ranged from 0.4 to 1.3 yr. down to 6 m in pasture and from 1.0 to 3.4 yr. for mature and secondary forests in Eastern Amazonia without any clear dependence on the soil depth (Trumbore et al. 2006).

Tropical eucalypt plantations are simple forest ecosystems with only one plant species (sometimes one clone) growing in highly weathered soils which are often very deep. Such simple, single age, tropical forests can be useful to investigate the effects of the root characteristics (e.g. diameter, mycorrhizal colonization) and the depth on the phenology, without confounding effects resulting from a mixture of species and tree ages in individual root samples. Eucalypt plantations cover about 20 million hectares throughout the world and are expanding rapidly in tropical and subtropical regions (Booth 2013). The gross primary production (GPP) in Brazilian *Eucalyptus* plantations is commonly >4 kg C m^{-2} $year^{-1}$ (Stape et al. 2008; Ryan et al. 2010; Campoe et al. 2012; Nouvellon et al. 2012), among the highest in the world for forests (Luyssaert et al. 2007). GPP peaks 2–3 years after planting in tropical eucalypt plantations and the trees are harvested at 5 to 7 years of age (Ryan et al. 2004; Christina et al. 2015). *Eucalyptus* trees rapidly explore very deep soil layers (Laclau et al. 2013; Pinheiro et al. 2016), which is an important factor explaining low rates of nutrient leaching in fertilized plantations (Laclau et al. 2010; Mareschal et al. 2013). A recent study based on ^{15}N, Sr^{2+} and Rb^{+} tracers

suggested a functional specialization of fine roots in *Eucalyptus* plantations, increasing the efficiency of cation uptake in deep soil layers relative to the topsoil (da Silva et al. 2011).

We set out to gain an insight into fine root phenology in highly productive forests growing on very deep tropical soils. Fine roots were scanned fortnightly in minirhizotrons down to a depth of 6 m, from 2 to 4 years after planting *Eucalyptus grandis* trees. The study tested the hypothesis that (i) root elongation and mortality is more seasonal in the topsoil than in the deep soil layers, (ii) the longevity increases with the root diameter and mycorrhizal colonization, and (iii) fine root longevity is not influenced by soil depth.

Materials and methods

Study site

The study was carried out in the EUCFLUX experimental site (http://www.ipef.br/eucflux/en/), in a plantation of *Eucalyptus* hybrid trees belonging to the Duratex Company. The site is in the São Paulo state in Brazil (22°58′04″S, 48°43′40″W), 20 km from the Itatinga experimental station (ESALQ, University of Sao Paulo). The region has a Cwa climate, with rainy summers and dry winters. Over the last 20 years, the mean annual rainfall was 1360 mm, with 75% concentrated from October to March (spring and summer). Mean annual temperature was 20 °C, ranging from 13 °C in the coldest months (June to August) to 27 °C in the warmest months (December to February). Temperatures occasionally fall below 5 °C during the cold season. The mean annual air relative humidity was 75%, with minimum values during winter (close to 45%).

The soils were very deep Ferralsols (FAO classification), developed on Cretaceous sandstone, Marilia formation, Bauru group, with a slope < 5%, the maximum elevation was 760 m above sea level. Main physical and chemical soil properties are given in Table 1. Clay content down to a depth of 6 m at the study site ranged from 15 to 27% and the soil was acidic (pH ranging from 3.8 to 4.6). The mineral content was dominated by quartz, kaolinite and oxhydroxides (Maquère 2008). This soil is representative of the most common soil type where eucalypt plantations are established in Brazil.

The experimental site (90 ha) was planted in December 2009 with a single clone of the *Eucalyptus grandis* (W. Hill ex Maiden) x *E. urophylla* (S. T. Blake) hybrid at a spacing of 3 × 2 m. The area has been intensively managed for more than 20 years as commercial eucalypt plantations. The understory was eliminated by repeated herbicide application. Fertilizers were applied at planting, then at 6, 12 and 18 months of age. The total amount of nutrients applied was 80 kg N ha^{-1}, 90 kg P ha^{-1}, 284 kg K ha^{-1}, 76 kg S ha^{-1}, 5.4 kg B ha^{-1}, 3.4 kg Zn ha^{-1}, and 1.5 kg Cu ha^{-1}.

The study was carried out from 2 to 4 years after planting a monoclonal *E. grandis* x *E. urophylla* stand. Intensive measurements of carbon and water fluxes over the entire rotation at this site (including eddy-covariance) showed that 2–4 years after planting is the period with the highest leaf area index (about 5 m^2 m^{-2}), evapotranspiration (3–6 mm d^{-1}) and gross primary productivity (Christina et al. 2017).

Fine root biomass

Soil samples were collected in November 2011 and November 2013 at 2 and 4 years after planting, respectively, in a stand of another *E. grandis* x *E. urophylla* clone at about 400 m from the minirhizotrons in the same commercial plot of 50 ha. A previous study showed that soil type, chemical properties and texture, historical land use and silvicultural practices and stand productivities were similar in both sites (Campoe et al. 2012). At age 2 years, a total of four soil cores were sampled from the 0–0.25 m, 0.25–0.50 m, 0.50–1.0 m, 1.0–1.5 m, 1.5–2.0 m layers then every 1 m down to a depth of 10 m, using a power auger with a diameter of 9 cm (see Pinheiro et al. 2016 for details). At age 4 years, 6 soil cores were sampled down to a depth of 2 m and 3 soil cores between 2 and 10 m. Soil samples were collected close to trees with a basal area similar to the mean of the stand, along a diagonal between trees in adjacent rows using the methodology described by Christina et al. (2011) to avoid contamination of deep soil samples by roots from the upper layers. Only intact soil blocks from the inner part of the auger were collected and all fragmented lumps of soil, which might have come from upper soil layers, were systematically discarded. Soil samples were weighed and gravimetric soil water content was measured. Fine roots (diameter ≤ 2 mm) were washed free of soil with tap water using two sieves (with mesh size of 150 and 500 μm)

Table 1 Main physical and chemical soil properties at the experimental site

Depth	Clay	Silt	Sand	pH	OM	P	H + Al	K	Ca	Mg	SB	CEC
(cm)	(%)			$CaCl_2$	g kg^{-1}	mg kg^{-1}	$mmol_c$ kg^{-1}					
0–25	14.8 ± 0.9	2.9 ± 0.6	82.3 ± 0.6	3.8 ± 0.2	17.1 ± 4.6	5.5 ± 1.0	61.8 ± 21.7	0.6 ± 0.1	5.2 ± 0.6	2.1 ± 0.5	7.9 ± 1.0	69.7 ± 22.6
25–50	17.4 ± 0.9	2.4 ± 0.2	80.3 ± 0.7	4.1 ± 0.1	10.6 ± 0.5	5.7 ± 0.1	36.1 ± 5.3	0.7 ± 0.1	4.8 ± 0.6	1.8 ± 0.0	7.3 ± 0.7	43.4 ± 4.9
50–100	19.3 ± 1.3	2.7 ± 0.5	78.0 ± 1.3	4.1 ± 0.1	9.3 ± 0.7	5.8 ± 0.5	31.3 ± 3.9	0.7 ± 0.2	4.5 ± 1.1	1.8 ± 0.0	6.9 ± 1.1	38.2 ± 2.8
100–200	22.6 ± 2.1	3.0 ± 0.5	74.4 ± 2.5	4.4 ± 0.2	12.3 ± 4.9	5.3 ± 0.6	21.9 ± 2.8	0.6 ± 0.1	5.6 ± 1.1	1.8 ± 0.0	7.9 ± 1.1	29.8 ± 2.6
200–400	24.9 ± 1.2	3.7 ± 0.5	71.4 ± 0.7	4.6 ± 0.1	6.6 ± 1.5	6.0 ± 0.4	14.9 ± 0.4	0.6 ± 0.1	4.8 ± 0.6	1.8 ± 0.0	7.2 ± 0.6	22.0 ± 0.3
400–600	27.3 ± 0.6	4.3 ± 0.5	68.4 ± 0.3	4.6 ± 0.1	5.3 ± 0.7	6.0 ± 0.5	14.3 ± 0.2	0.5 ± 0.1	4.5 ± 1.1	1.8 ± 0.0	6.8 ± 1.1	21.1 ± 1.2

Mean and standard deviations ($n = 3$). P was determined by Mehlich-1 and colorimetry; K was determined by Mehlich-1 and photometry; Ca and Mg were determined by KCl extraction and atomic absorption; OM, organic matter, was determined using sodium dichromate; SB, sum of base cations; CEC, cation exchange capacity

and separated carefully using tweezers. Live roots were sorted according to criteria such as a live stele, bright color and flexibility. Living fine roots separated from each soil sample were scanned (400 dpi resolution) and root lengths were estimated using WinRHIZO Version Pro V.2009c software (Regent Instruments, QC, Canada). Fine root length density (RLD, expressed in m kg^{-1}) was calculated for each soil layer by dividing the length of fine roots by the dry mass of the soil sample collected in each layer. Soil bulk densities for each layer, measured in a pit down to a depth of 10 m, were used to convert RLDs per kg of soil into RLDs per dm^3 of soil for each layer.

Soil water monitoring

Sub samples of 10 g of soil, from each layer sampled by auger for fine root biomass measurements, were dried at 105 °C to constant weight to determine the gravimetric water contents at 2 and 4 years after planting ($n = 4$ at age 2 years; $n = 6$ in the 0–2 m soil layer and $n = 3$ between the depths of 2 m and 10 m at age 4 years). Volumetric soil water content (θ) was also monitored over the whole study period at half-hourly intervals, using CS616 probes (Campbell Scientific Inc., Logan, UT, USA) installed at 12 depths down to 10 m (0.15, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 m) with five CS616 probes for each of the first three soil depths and three probes for each soil depth between 2 and 10 m (see Christina et al. 2017 for more details on the probe installation). Daily relative extractable water (REW) was calculated for each soil layer at which minirhizotrons were installed (see next section) as:

$$REW_i = \frac{\overline{\theta_i} - \overline{\theta min_i}}{\overline{\theta max_i} - \overline{\theta min_i}} \quad (1)$$

where $\overline{\theta_i}$ is the mean soil water content in the i^{th} soil layer calculated by interpolation of θ between measurement depths, and θmin_i and θmax_i are the minimum and maximum soil water contents observed over the study period in soil layer i.

Minirhizotron assessments

In November 2009, a permanent pit with concrete walls down to a depth of 7 m was built before clear-cutting the previous stand (Fig. S1). The pit was located diagonally between two trees of neighbor rows. Ten acrylic

minirhizotron tubes (1.8 m length, 6.35 cm internal diameter) were installed at approximately 45° to the vertical into the pit (at both ends) using a drill, at depths of 0–1, 1–2, 2–3, 3–4 and 5–6 m, with two tubes (replicates) at each depth (Fig. 1a). In four different plots at a distance from the pit, 12 minirhizotron tubes (60 cm length) were installed (July 2011) at 45° to the vertical to a depth of 30 cm leaving a few centimeters of the tube above the forest floor. In each plot, three minirhizotron tubes were located at a distance of 0.3, 0.9 and 1.5 m from the trees along a diagonal between two neighboring trees in adjacent rows (Fig. 1b). They were capped with a white PVC tube and aluminized tape, to prevent light infiltration and heating, and the top end was covered with a removable PVC cap. The bottom ends of the minirhizotron tubes were sealed to prevent water ingress into the tubes. The area around each tube was protected to avoid trampling.

Minirhizotron images were taken fortnightly using a scanner system (CI-600 Root Scanner, CID Inc., Camas, WA, USA) pushed by hand into each tube. Each scan (300 dpi) covered a 345° segment of the tube-soil interface and a length of about 21.6 cm, giving eight images per tube for the 1.8 m long minirhizotrons inside the pit and two images per tube for the 0.6 m long minirhizotrons (outside the pit). The effective soil area covered by each image was 422 cm^2. The images from the minirhizotron tubes were grouped for each of the six soil layers investigated: 0.0–0.3, 0.3–1.0, 1.0–2.0, 2.0–3.0, 3.0–4.0 and 5.0–6.0 m. At each observation date, there were 24 scans for the 0.0–0.3 m soil layer (2

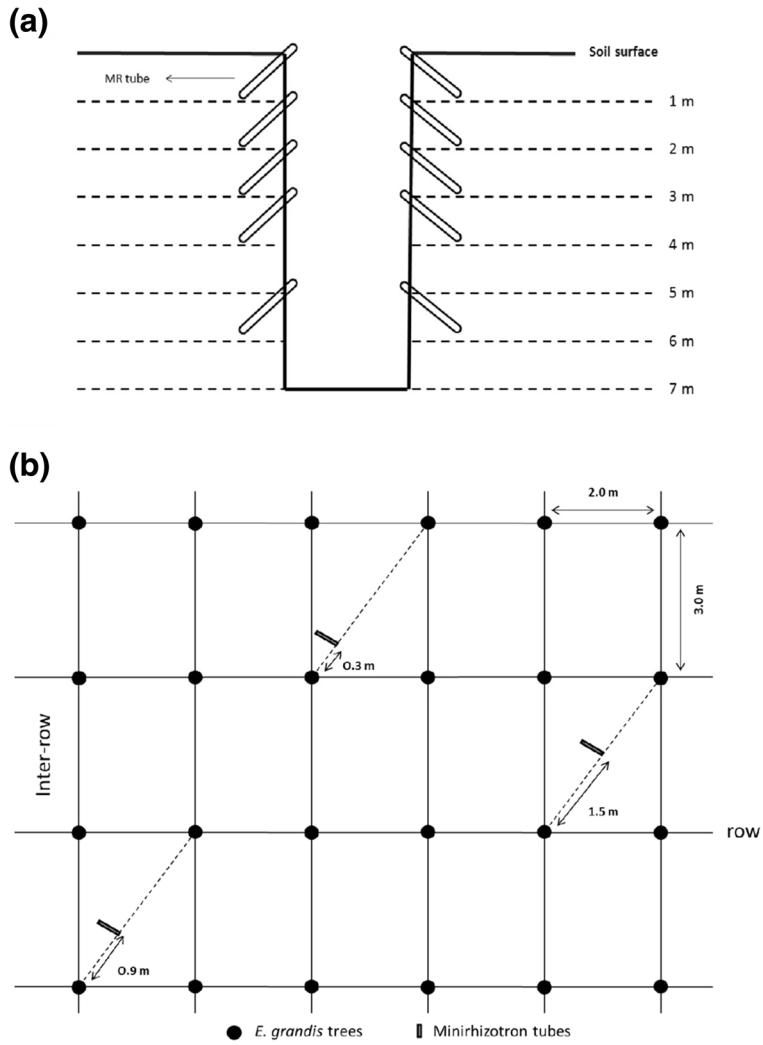

Fig. 1 Distribution of minirhizotron tubes in the permanent pit down to a depth of 6 m (**a**) and layout of the installation of minirhizotron tubes at 0.0–0.3 m depth (**b**)

scans from each of 12 minirhizotrons outside the pit), 9 scans in the 0.3–1.0 m layer (from 2 minirhizotrons, 3 images being unusable), 16 scans in soil layers 1.0–2.0, 2.0–3.0, and 3.0–4.0 m (from 2 minirhizotrons), and 12 scans in the 5.0–6.0 m soil layer (from 2 minirhizotrons, 4 images being unusable). Roots were scanned fortnightly from November 2011 to October 2013 in the pit and from February 2012 to January 2014 outside the pit. Roots were also scanned every 2 days during February 2013 (mid summer) and October 2013 (end of winter) to check that the turnover rates in our study were not underestimated as a result of a short lifespan of very fine roots being produced and then disappearing within the 14 days period between two successive scanning dates.

Root image processing

Originally, all images were captured in horizontal TIFF format. In total, more than 5000 images were processed using the WinRHIZO TRON MF 2009 software (Regent Instruments Inc., Quebec, Canada). Individual root length and diameter were traced manually on the screen for each image. To identify the changes of root length and characteristics, new images were superimposed over images from the previous session. This allowed root sections originating from recent root growth or sections that had disappeared due to root death and decomposition to be identified. Roots were considered dead when they became black or disappeared, visibly deteriorated between two sampling dates, sloughed off or shriveled to approximately half the original diameter (Wells and Eissenstat 2001; Satomura et al. 2007). Mycorrhizal colonization was considered to be positive when dichotomous structures and/or a fungal mantle was present. The mortality of mycorrhizal roots was evaluated based on a modification of color of the fungal mantle or a deterioration or absence of growth patterns (King et al. 2002; Guo et al. 2008b). For all roots observed, we recorded the diameter, length, mycorrhizal colonization, date of appearance and disappearance. Only fine roots (diameter ≤ 2 mm) were taken into account. They were grouped into three diameter classes (<0.3 mm, 0.3 to 0.5 mm and 0.5 to 2 mm).

Root length calculations

Live root length production between two successive scans at t_{-1} and t ($LLP_{t\text{-}1,t}$, cm m^{-2}) was calculated by adding the length increment of each observed live root between t_{-1} and t, divided by the observed soil area of each image. Similarly, *dead* root length production ($DLP_{t\text{-}1,t}$, cm m^{-2}) was calculated by adding the increase in dead root length between two successive scans, divided by the observed soil area. Daily *live* root length production ($DLLP_{t\text{-}1,t}$) and daily *dead* root length production ($DDLP_{t\text{-}1,t}$, cm m^{-2} d^{-1}) were obtained by dividing $LLP_{t\text{-}1,t}$ and $DLP_{t\text{-}1,t}$ by the number of days between successive scans (t_i-$t_{i\text{-}1}$, which was approximately 14 days). Cumulative *live* root length production (*CLLP*) and cumulative *dead* root length production (*CDLP*) were calculated by summing *LLP* and *DLP*, respectively, over the whole study period.

The elongation rate of a root n during the period between t and t_{-1} ($RER_{n,p_{t_{-1},t}}$, cm day^{-1}) was the increase in length of root n divided by the number of days between t_{-1} and t (approximately 14 days). Mean RER in soil layer i between 2 successive scans at t_{-1} and t ($RER_{i,p_{t_{-1},t}}$, cm day^{-1}) was calculated as the mean of $RER_{n,p_{t_{-1},t}}$ for all the roots growing in soil layer i during this period (roots with RER > 0 between t_{-1} and t). The mean RER in soil layer i over the whole study period was the mean of the RER values for each period of 14 days. The maximum root elongation rate in soil layer i (max RER) was the value of RER for the root with the highest elongation rate over the period of 14 days (one root among hundreds of roots observed in soil layer i over the period).

Fine root turnover and lifespan calculations

Root individual lifespan was calculated as the difference in days between the day of the first appearance of the root and the date of death. The non-parametric Kaplan-Meier method was used to estimate the median lifespan (MLS, in days) and the root survival probability over the time period (Majdi et al. 2001; Tierney and Fahey 2001; Goel et al. 2010; Germon et al. 2016). All roots were considered individually and had the same weight in the calculation. At the end of the study, roots being declared as dead were uncensored and roots alive at the end of the study were censored. With this

analytic approach, median root lifespan was estimated and predicted with the Cox's proportional hazards regression for each depth, diameter and mycorrhizal colonization. Fine root turnover (year^{-1}) was calculated as the inverse of the MLS.

Statistics

For each soil layer (n = 12 in 0–0.3 m and n = 2 from 0.3 to 6 m deep), Pearson correlation coefficients were calculated between LLP (and DLP) and REW for the same 14 day period and the 4 preceding 14 day periods, to allow for a possible delay in the effect of changes in soil water content. Kaplan-Meier survival analyses were used to calculate survivorship statistics and to test fine root survival as a function of diameter, depth and mycorrhizal colonization. The semi-parametric Cox Proportional Hazard Model (Cox 1972) was used to identify whether depth, diameter and mycorrhizal colonization had a significant effect on fine root lifespan. The "Survival" package in R (Therneau 2014) was used and all calculations and analyses were performed using the R software version 3.2.5 (Team R 2013) with a level of significance of 0.05.

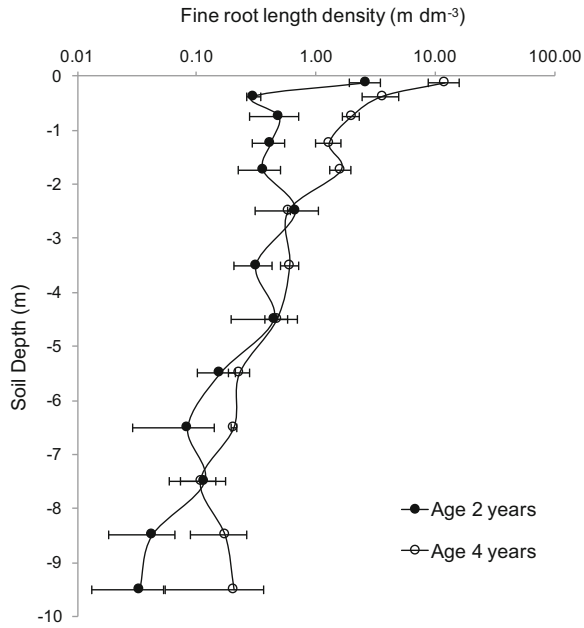

Fig. 2 Fine root length densities (m dm^{-3}) down to a depth of 10 m in a stand of another *E. grandis* x *E. urophylla* clone situated at about 400 m from the minirhizotrons, on the same soil type, at ages 2 (black circle) and 4 years (white circle). The error bars represent the standard errors (n = 4 at age 2 years; n = 6 in the 0–2 m soil layer and n = 3 between the depths of 2 m and 10 m at age 4 years)

Results

Fine root densities

At the start of the study period (in November 2011) fine root length densities (RLDs) sharply decreased with increasing depth from the topsoil and remained roughly constant between the depths of 0.5 and 5 m (Fig. 2). RLDs increased between 2 and 4 years after planting in most of the soil layers and the increase was particularly high in the 0–2 m layer and in the deepest soil layers (soil layers 6–7 m and 8–10 m).

Soil water contents

Volumetric soil water content at 50-cm depth ranged from about 8% over dry periods to 16% after rainfall events (Fig. 3). In 2012, annual rainfall was 1558 mm and gravitational soil solutions reached 2 m depth on 2 occasions, 4 m depth only once at the end of the rainy season, and never reached 6 m depth. In 2013, annual rainfall was 1511 mm, and gravitational solutions reached 2 m depth on 5 occasions, 4 m depth 3 times, and 6 m depth at the end of the rainy season. Volumetric soil water content ranged from about 9 to 18% at 2 m depth, and from 12 to 16% at 4 m and 6 m. The level of the water table decreased from a depth of 13.5 m at age 2 years to a depth of 15.5 m at age 4 years (data not shown).

Live root length production

Daily live root length productions (DLLPs) were highly dependent on the soil layer and the season (Fig. 4). While the highest DLLPs generally occurred during the summer in the superficial soil layers, at depths >2 m highest DLLPs were mainly at the end of the winter. The DLLP was much more seasonal in deep soil layers than in the topsoil. DLLPs ranged from 2 to 20 cm m^{-2} d^{-1} in the 0–0.3 m soil layer and reached a peak of 51 cm m^{-2} d^{-1} in the 5–6 m soil layer at the end of the first winter (September–October 2012). DLLP peaked at the end of the winter in all the soil layers at depths >2 m, when there was a sharp decrease in soil water availability for the trees (Fig. 3). The highest DLLPs in deep soil layers occurred when the mean soil water content in the

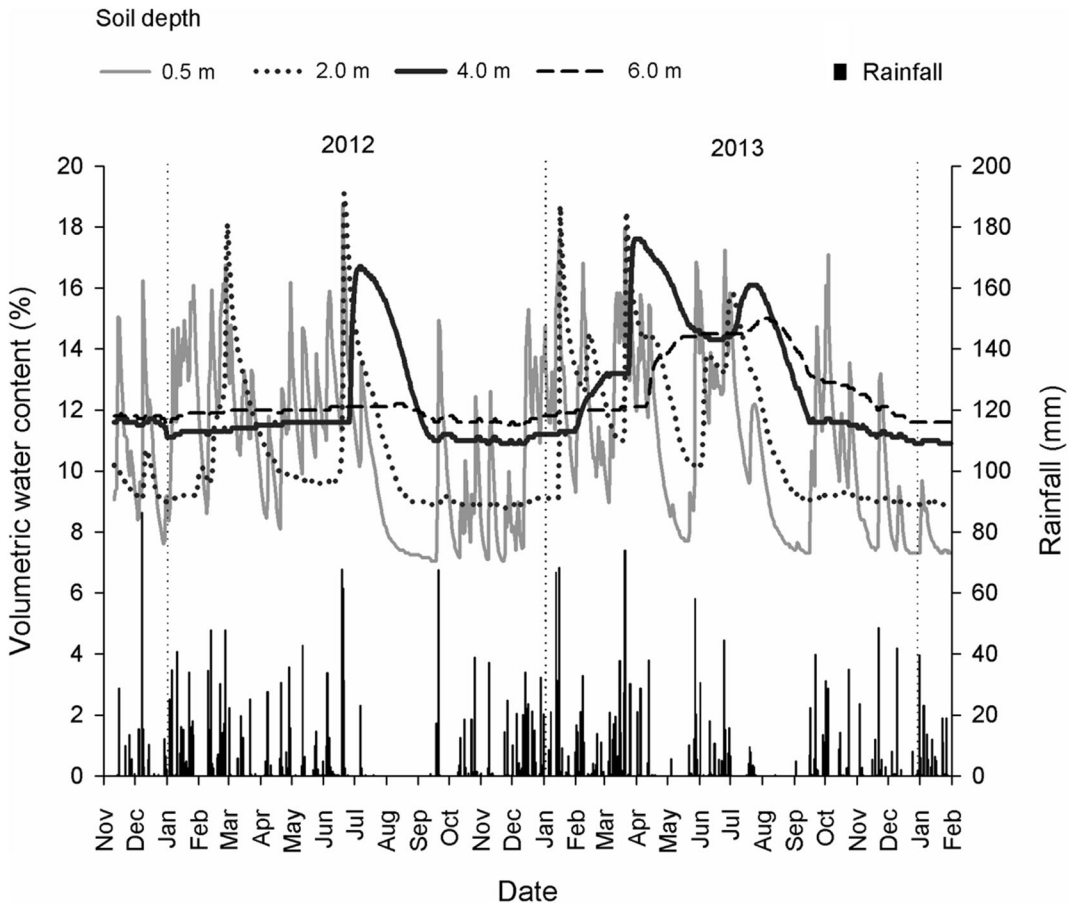

Fig. 3 Daily rainfall (bars) and time-course of volumetric soil water contents at depths of 0.5 m, 2 m, 4 m and 6 m (lines) over the study period

0–2 m soil layer was below about 8% (Fig. 5). The Pearson correlation coefficients between REW and DLLP during the same 14-days periods were not significant ($P < 0.05$), for all soil layers, which suggests that that DLLPs were not directly influenced by the changes in soil water content in each individual soil layer. The Pearson correlation coefficients between DLLP and REW measured during the 14-days periods preceding the DLLP determinations were also non-significant suggesting that fine root growth in each soil layer cannot be explained by a delayed effect of changes in soil water content in the same soil layer.

The highest cumulative live root length productions (CLLP) over 2 years were found in the upper and in the lower soil layers (28 and 20 m m^{-2} in the 0.0–0.3 m and 5.0–6.0 m soil layers, respectively). CLLPs over 2 years ranged between 10 and 14 m m^{-2} in all the intermediate soil layers (Table 2). While CLLPs increased steadily over the study period in the topsoil, the time series of cumulative root lengths reflected a strong increase in seasonal variations of root length production in deep soil layers (Fig. 6).

Dead root length production

Cumulative dead root length production (CDLP) was considerably lower than cumulative live length production (CLLP) in all the soil layers. While CLLPs over 2 years ranged from 10.1 to 27.8 m m^{-2} depending on the soil layer, CDLPs ranged from 0.4 m m^{-2} in the 3.0–4.0 m layer to 4.3 m m^{-2} in the 0.0–0.3 m layer (Table 2). CDLP over 2 years was 4–8 times higher in the upper 0.3 m than in the subsoil layers. Surprisingly, daily dead root length production (DDLP) was not higher during the winter, when the REW was low, than during the other seasons, for all soil layers (Fig. 7). Seasonal variations of DDLPs were lower in the 0.0–0.3 m layer than in soil layers at depths >2 m.

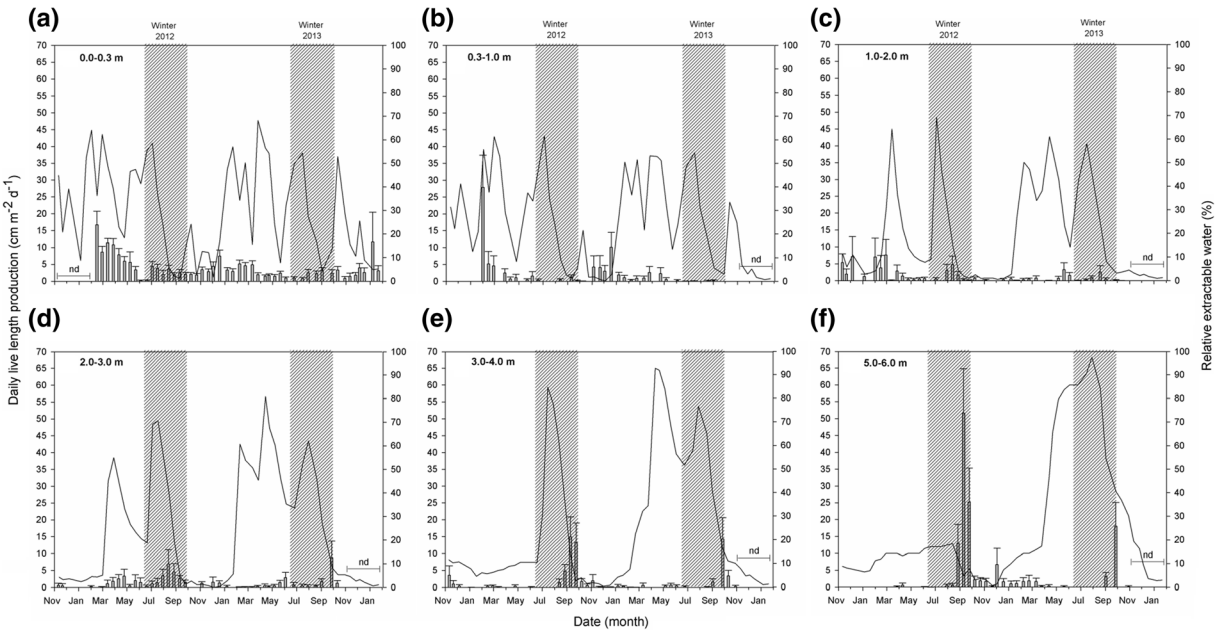

Fig. 4 Daily live root length production (cm m^{-2} day^{-1}) estimated every 14 days from 11 November 2011 to 21 January 2014 in soil layers **a** 0.0–0.3 m, **b** 0.3–1.0 m, **c** 1.0–2.0 m, **d** 2.0–3.0 m, **e** 3.0–4.0 m, and **f** 5.0–6.0 m. The error bars represent the standard errors (n = 24, 9, 12 and 16 in soil layers 0.0–0.3 m, 0.3–1.0 m, 5.0–6.0 m, and the others layers, respectively). Mean relative extractable water (%) during each period is shown as a solid line. *ND = no data* for daily fine root length production. Vertical shaded areas represent the winter (cold and dry period)

Root elongation rates

Mean RERs increased with soil depth, from 0.08 cm day^{-1} on average over the study period in the 0–30 cm layer to 0.17–0.29 cm day^{-1} between the depths of 2 and 6 m (Table 2). Maximum RERs were also higher in deep soil layers than in the topsoil, up to 3.6 cm d^{-1} in the 5–6 m soil layer. While the maximum RERs were at the end of the summer (from February to May) in the upper soil layers, in the 3.0–4.0 m and 5.0–6.0 m soil layers,

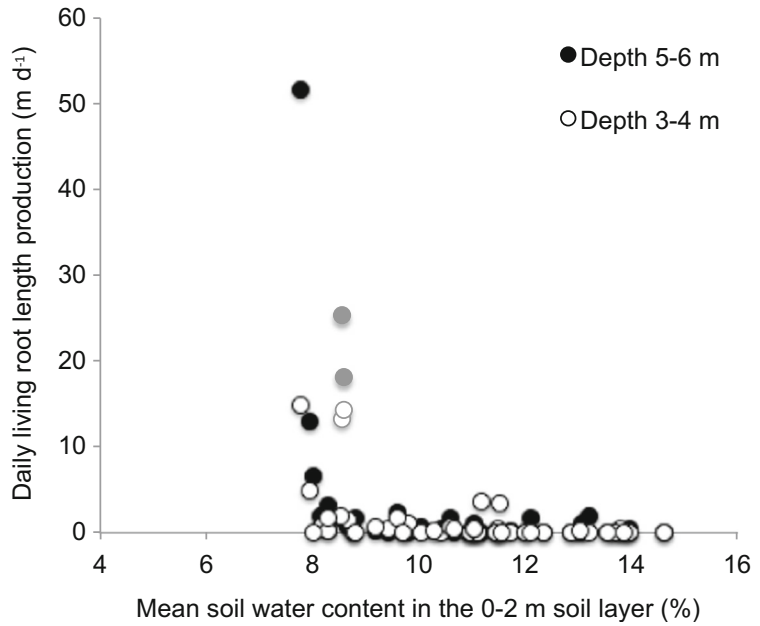

Fig. 5 Relationship between daily live root length production (cm m^{-2} day^{-1}) estimated every 14 days from 11 November 2011 to 21 January 2014 and mean soil water content (%) in the 0–2 m soil layer. Grey circles correspond to the 14-day period following the lowest water content of the year in the 0–2 m soil layer in 2012 and 2013, which might reflect a delay-effect of soil dryness in the 0–2 m layer on daily live root length productions at the depths of 3–4 m and 5–6 m

Table 2 Cumulative live root length production (CLLP, m m^{-2}), cumulative dead root length production (CDLP, m m^{-2}), and mean and maximum root elongation rates (RER, cm day^{-1}) over 2 years in each soil layer

	Soil layer depth (m)					
	0.0–0.3	0.3–1.0	1.0–2.0	2.0–3.0	3.0–4.0	5.0–6.0
CLLP (m m^{-2})	27.8 ± 13.9	12.1 ± 9.0	12.4 ± 10.0	10.1 ± 8.5	13.7 ± 11.9	20.2 ± 16.3
CDLP (m m^{-2})	4.3 ± 2.8	0.5 ± 0.6	1.0 ± 1.5	0.9 ± 1.4	0.4 ± 0.5	1.0 ± 1.2
Mean RER (cm d^{-1})	0.08 ± 0.14	0.12 ± 0.15	0.10 ± 0.15	0.20 ± 0.29	0.29 ± 0.44	0.17 ± 0.38
Max. RER* (cm d^{-1})	1.7	0.7	1.1	1.5	3.1	3.6

CLLP and CDLP means and standard errors were calculated for all the scans at each depth (n = 24 in soil layer 0.0–0.3 m, n = 9 in soil layer 0.3–1.0 m, n = 12 in soil layer 5.0–6.0 m, and n = 16 in the others soil layers). RER mean and standard deviations were calculated for all the elongated roots observed at each depth. * Maximum value measured for the root with the highest elongation rate in each soil layer (one root per layer during the period of 14 days with the fastest growth among hundreds of observed roots)

the maximum RERs were in late winter (September) (data not shown).

Diameter classes and mycorrhizal colonization effects on fine root lifespan

Median lifespan (MLS) of all the fine roots were not significantly different between soil layers, although the MLS estimates slightly decreased from 514 days in the upper soil layer to 501 days in the 5.0–6.0 m layer (Table 3). The mean turnover of the 3388 roots observed was 0.7 yr.$^{-1}$ in all soil layers. The fine root mortality in the 0.0–0.3 m layer was 19.9% on average (relative to the total amount of root produced), which was 2–5 times higher than in deeper soil layers. Median lifespans increased from 459 days for roots <0.3 mm in diameter to 511 days for diameters between 0.3 and 0.5 mm, and 525 days for roots between 0.5 and 2.0 mm in diameter (Fig. 8a and Table 4). Mycorrhizal roots represented about 40% of the fine roots in the 0.0–0.3 m layer, this proportion drastically dropped in deeper soil layers (data not shown). The median lifespan was about 1 month longer for mycorrhizal roots than for non-mycorrhizal roots (497 and 465 days, respectively) (Fig. 8b).

Discussion

Fine root phenology differs between soil horizons

Contrary to our first hypothesis, the seasonality of fine root growth and mortality was more marked in deep soil layers than in the topsoil. We then reject our first hypothesis stating that elongation and mortality was more seasonal in the topsoil than in the deep soil layers. While fine root growth occurred throughout the year in the upper soil layers, live fine root production was extremely low during the summer (hot-wet season) below a depth of 3 m and peaked at the end of the winter (dry season) in very deep soil layers. Surprisingly, mean RERs increased with soil depth and the maximum RERs (up to 3.6 cm day^{-1}) were measured below a depth of 3 m. The high values of RER of *E. grandis* trees in very deep soil layers are consistent with those derived from measurements of root front growth velocities (Christina et al. 2011), reaching 1.8 cm day^{-1} at about 10 months of age when the root front is 4 m deep, and then decreasing with stand age (e.g. root front growth velocity of about 1.2 cm day^{-1} at 2 years of age, when the root

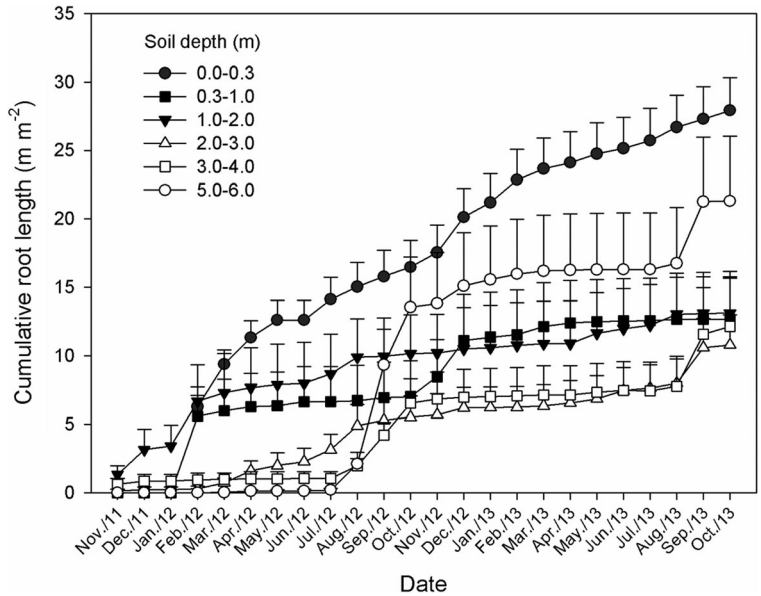

Fig. 6 Cumulative total root length (m m^{-2}) over 2 years. The error bars represent the standard errors (n = 24, 9, 12 and 16 in soil layers 0.0–0.3 m, 0.3–1.0 m, 5.0–6.0 m, and the other layers, respectively)

front is 11 m deep). High RER values were also reported for rhizotron-grown eucalypt seedlings (2.5 cm day^{-1}; Misra 1999). The maximum RER measured in the two upper layers (1.7 and 0.7 cm day^{-1} for the 0–0.3 m and 0.3–1.0 soil layers, respectively) are higher than those reported for similar depths in 1-year-old (0.6 cm day^{-1}) and 2-year-old (0.4 cm day^{-1}) eucalypt plantations in the Congo (Thongo M'bou et al. 2008), and for other tropical species such as rubber tree (0.3 cm day^{-1}; Chairungsee et al. 2013).

A study combining stable isotope and carbohydrate analyses in phloem sap and fine roots suggested that a

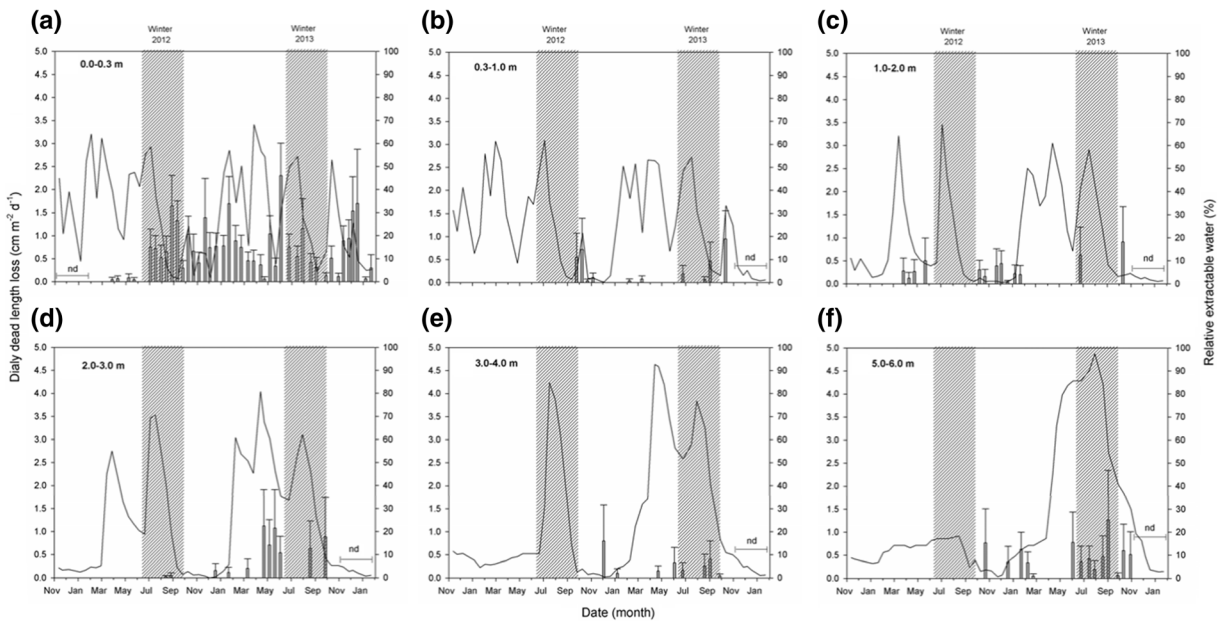

Fig. 7 Daily dead root length production (cm m^{-2} day^{-1}) estimated every 14 days from 11 Nov. 2011 to 21 Jan. 2014 in soil layers **a** 0.0–0.3 m, **b** 0.3–1.0 m, **c** 1.0–2.0 m, **d** 2.0–3.0 m, **e** 3.0–4.0 m, and **f** 5.0–6.0 m. The error bars represent the standard errors (n = 24, 9, 12 and 16 in soil layers 0.0–0.3 m, 0.3–1.0 m, 5.0–6.0 m, and the others layers, respectively). Mean relative extractable water (%) during each period is indicated (solid line). *ND = no data* for daily dead fine root length loss. Vertical shaded areas represent the winter (cold and dry period)

Table 3 Median lifespan (MLS, days), turnover rate (year^{-1}), and % of root mortality over 2 years in each soil layer

Cox proportional hazard model showed that MLS values were not significantly different between the soil layers ($p < 0.05$)

	Soil layer depth (m)					
	0.0–0.3	0.3–1.0	1.0–2.0	2.0–3.0	3.0–4.0	5.0–6.0
MLS (days)	514	509	507	505	506	501
Turnover (year^{-1})	0.71	0.71	0.71	0.72	0.72	0.72
Root mortality (%)	19.9	4.9	4.3	8.3	3.7	3.4
Roots observed (#)	2223	141	205	204	186	429

large share of the sucrose production was allocated towards fine roots during a drought period in a Mediterranean beech forest (Scartazza et al. 2015). This study suggests that the relative sink strength of deep fine roots might increase during dry periods, when the demand of aboveground sink tissues is reduced. A strong increase in sugar concentration within phloem sap has recently been shown during dry periods in Brazilian *E. grandis* plantations (Battie-Laclau et al. 2014), as also reported for *E. globulus* plantations in Australia (Pate and Arthur 1998). Even though a decrease in sink activity aboveground cannot be excluded to explain this pattern, the peaks of fine root growth observed in deep soil layers at the end of the dry periods in our study might be a consequence of an increase in sugar allocation belowground, as in the Mediterranean beech forest studied by Scartazza et al. (2015). Fine root growth was not correlated with soil water content, which suggests that soil water content in a particular soil layer was not the main driver of fine root growth. We speculate that the allocation of non-structural carbohydrates for deep root growth when rainfall becomes scarcer may be a physiological response to a rapid exhaustion of water in the topsoil, inducing tree roots to start growing in search of deep water resources during the dry periods. The environmental conditions controlling fine root dynamics in forest ecosystems are difficult to disentangle. Fine root growth can be influenced by both exogenous and endogenous factors in forest ecosystems (Moroni et al. 2003; Tierney et al. 2003). A recent meta-analysis of 40 studies in boreal, temperate and subtropical biomes concluded that although the root and shoot growth were positively correlated with monthly temperature and precipitation, the endogenous control of carbon allocation belowground was also a major driver of fine root phenology (Abramoff and Finzi 2015). The growth peaks of deep roots (below 2.0 m depth) of rubber trees in tropical climate (Maeght et al. 2015) and walnut trees in Mediterranean climate (Germon et al. 2016) occurred during the periods of aerial dormancy, whereas the growth of shallow fine roots was synchronised with aboveground tree components. We hypothesized that the asynchrony of fine root growth depending on the depth in our study might reflect a vertical shift of the major factors driving fine root growth, from soil environment for shallow roots to tree endogenous cues during periods of low rainfall for deep roots. However, further studies are needed to test this hypothesis.

Our second hypothesis was validated, the lifespan was longer for mycorrhizal roots than for non-mycorrhizal roots and increased with root diameter. A similar pattern has been shown in other forest ecosystems (King et al. 2002; Guo et al. 2008b). Many factors can be involved in the rise of lifespan of mycorrhizal fine roots, including an enhancement of the protection against pathogens (Smith and Read 1997) and the tolerance to drought (Wu and Xia 2006), as well as to temperature extremes (Bunn et al. 2009). Mycorrhizal fungi may also benefit individual roots through an improvement of the nutritional status and the water supply of the whole tree (McComarck and Guo 2014).

The soil depth did not significantly influence the median lifespan of fine roots, this result validates our third hypothesis. Median fine root lifespans around 1.4 yr., irrespective of the depth, were close to the values estimated from ^{14}C measurements in Eastern Amazonia. While fine root lifespan ranged from 2.0 to 2.7 yrs. in mature forests without a clear influence of the depth down to 6.7 m, the lifespan increased from 1.2 yr. in the topsoil to 3.3 yrs. in the 5.2–6.7 m layer in secondary forests (Trumbore et al. 2006). The fine root lifespan ranged between 1 and 2 yrs. in most of the soil layers under degraded and reformed pastures at the same site without a clear relationship between the depth and the fine root lifespan (Trumbore et al. 2006). Although direct observations of fine root lifespans using rhizotrons or minirhizotrons are scarce at great depths,

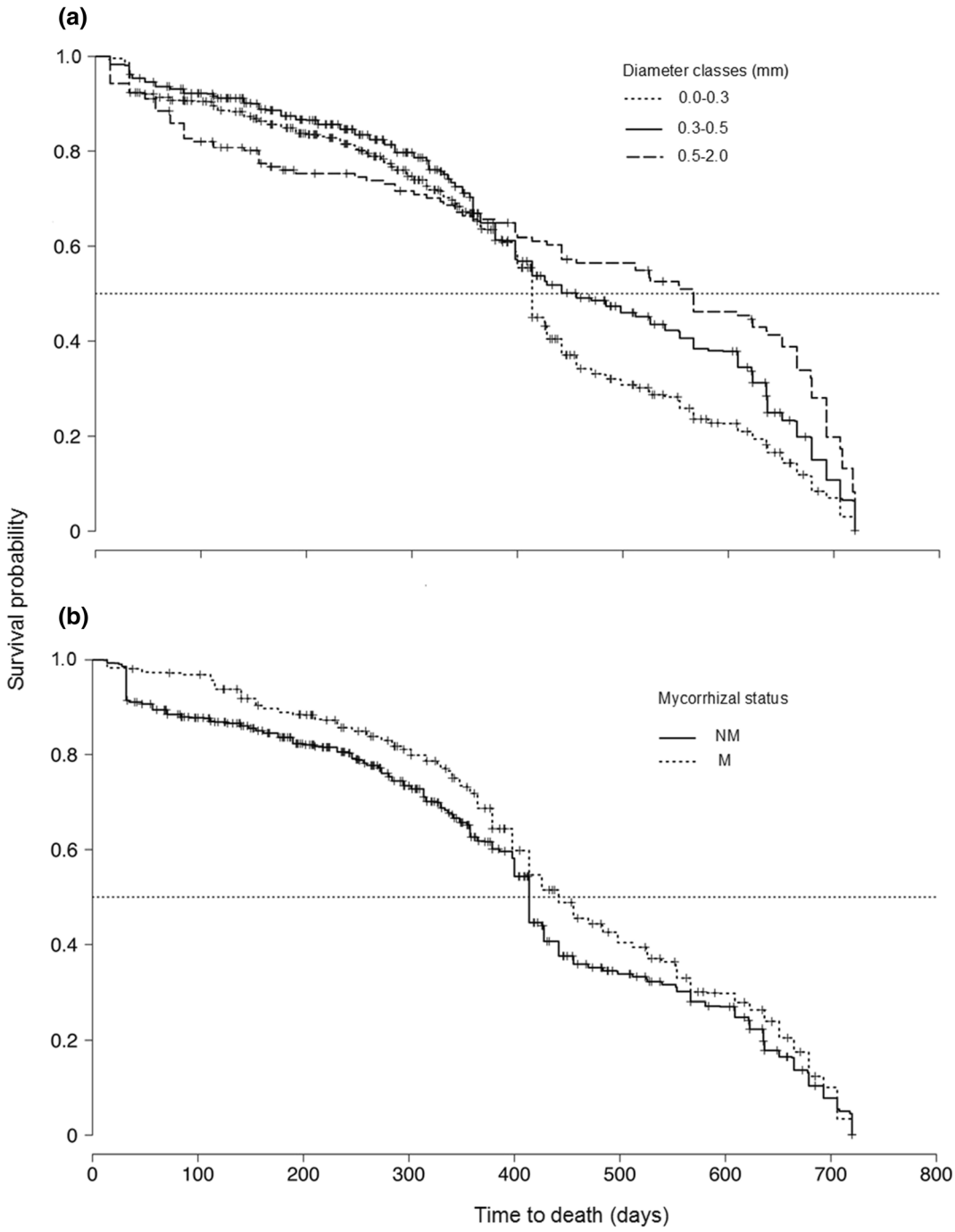

Fig. 8 Fine root survivorship across all soil layers between the depths of 0 and 6 m depth (**a**) for each root diameter class (0.0–0.3, 0.3–0.5 and 0.5–2.0 mm), and (**b**) for mycorrhizal (M) and non-mycorrhizal (NM) fine roots, from November 2011 to January 2014. Survivorships were estimated using the Cox's proportional hazards regression

available data suggest that the effect of soil depth is species and/or site dependent. Median fine root lifespan slightly increased from 0.5 yr. in the 0–1.7 m soil layer to 0.6 yr. in the 2.5–4.7 m soil layer for walnut trees in Mediterranean climate (Germon et al. 2016), and decreased from 1.4 yr. in the topsoil to 0.5 yr. in the 1.0–4.5 m soil layer in tropical rubber plantations (Maeght et al. 2015).

Table 4 Median lifespan (MLS, days), turnover rate (year^{-1}), mortality (%), and number of roots observed over 2 years depending on the diameter class (0.0–0.3, 0.3–0.5 and 0.5–2.0 mm) and mycorrhizal colonization (M, mycorrhizal roots and NM, non-mycorrhizal roots) across all soil layers

	Diameter classes (mm)			Mycorrhizal colonization	
	0.0–0.3	0.3–0.5	0.5–2.0	(M)	(NM)
MLS (days)	459 ± 21 a	511 ± 7 b	525 ± 2 c	497 ± 5 A	465 ± 7 B
Turnover (year^{-1})	0.79	0.71	0.69	0.73	0.78
Root mortality (%)	11.5	23.0	16.4	9.0	17.9
Roots observed (#)	2240	1000	158	1111	2287

MLS (±SD) was determined using the Kaplan Meier method. Different lower and upper case letters in the same row indicate significant differences between diameter classes and mycorrhizal colonization ($P < 0.05$), respectively

Key role of deep roots in tropical forests

Although our results showed a high concentration of fine roots in the topsoil layer, the fine root exploration in soil layers below 0.3 m did not conform to the pattern commonly described for forest ecosystems (Schenk and Jackson 2002a, 2002b). There was no exponential decrease in fine root density with increasing soil depth down to 6 m and fine roots reached depths of 10 m and 14 m in the second and fourth year after planting (Fig. 2a). The exploration of very deep soil layers has already been reported for other *Eucalyptus* species in the same soil type (Christina et al. 2011; Pinheiro et al. 2016). The strategy of fast root growth in the deep soil layers provides access to large amounts of water stored after clear-cutting the previous stand (Christina et al. 2017). This is consistent with the rapid increase in transpiration rates that reach maximum values when the leaf area index is at its maximum (from 2 to 4 years after planting) as observed for *Eucalyptus* plantations in Brazil and Australia (Cabral et al. 2010; Forrester et al. 2010; Christina et al. 2017). This is also consistent with an increase in exchangeable K content in the rhizosphere relative to the bulk soil recently observed down to large depths in the same area, which might reflect root-induced degradation of K-bearing minerals (Pradier et al. 2017). Thus, we speculate that the fast exploration of deep soil could be a plant strategy for maximizing the water and nutrient uptake needed to meet the high demand in water and nutrients during the early growth stage of fast growing trees. In their natural environment (e.g. Pfautsch et al. 2009) and in managed mixed-species plantations (le Maire et al. 2013), such a territorial strategy of fast-growing eucalypt species, aiming to explore rapidly a large volume of soil, might provide access to more water and nutrients than co-existing species, thus contributing to increased aboveground growth and competitiveness for light. With such ability to outcompete other species with a very fast initial growth, giant eucalypts such as *E. grandis* are considered to be long-lived rainforest pioneer tree species (Tng et al. 2012; Tng et al. 2013). A fast deep-rooting strategy also provides a strong competitive advantage to cope with the first severe drought events. Simulations using the MAESPA process-based model in the stand studied here (fitted using intensive monitoring data on soil water content and eddy covariance measurements) showed that deep rooting was an efficient strategy for increasing the amount of water available for the trees, allowing the uptake of transient gravitational water and possibly giving access to a deep water table (Christina et al. 2017). The decrease by 2 m of the water table level observed over the study period was a result of several phenomena: (i) water table recharge stopped after canopy closure at 2 years of age, (ii) water uptake by tree roots during the dry periods and (iii) lateral drainage of the water table (Christina et al. 2017). The major role of deep roots in supplying the water demand of natural forests and savannahs in Brazil during dry periods is well documented (e.g. Nepstad et al. 1994; Oliveira et al. 2005).

Minirhizotron technique considerations

The minirhizotron technique is commonly considered as a reliable approach for estimating fine root turnover and lifespan in the topsoil of terrestrial ecosystems (Majdi et al. 2005). However, few studies have used minirhizotrons to examine fine root phenology at depths >2 m (Maeght et al. 2013; Rewald and Ephrath 2013;

Germon et al. 2016). The soil environment can be modified around minirhizotrons installed in deep pits, which might bias the observations of fine root phenology. In particular, gas exchange between deep soil layers and the pit as well as a low resistance to root growth at the interface between the tube and the soil relative to undisturbed soil areas may modify fine root phenology. The unavoidable changes in soil environment close to the minirhizotrons, particularly at the soil-tube interface, may have led to overestimates of the fine root growth in deep soil layers in our study. However, soil cores collected in undisturbed areas in an adjacent stand, at the beginning and the end of our study period, confirmed the increase in fine root densities observed using minrhizotrons in deep soil layers. The consistency between the increase in fine root densities shown in soil cores and the fine root dynamics observed on the images from minirhizotrons suggests that they can be a useful tool for assessing the variation of fine root production and mortality with time in very deep soil layers, even though the absolute values of root length production should be considered with caution.

A stabilization period is required after the installation of the minirhizotrons to ensure that soil disturbance does not lead to a flush of fine roots that would overestimate root growth (Johnson et al. 2001; Germon et al. 2016). Minirhizotron studies in temperate and tropical forests are commonly started after a shakedown period of 6–12 months (Hendricks et al. 2006; Graefe et al. 2008). The shakedown time after minirhizotron installation for our fast-growing trees was 6 months in the topsoil (0.0–0.3 m layer) and >24 months below 0.3-m depth (the minirhizotrons were set up before planting the trees). The consistency between DLLP dynamics in layers 0.0–0.3 m and 0.3–1.0 m suggests that if there was an overestimate of root length production in the 0.0–0.3 m layer due to an inadequate period of soil stabilization, it was probably very low.

It is necessary to scan the minirhizotrons frequently to avoid an unknown proportion of fine roots appearing and disappearing between two successive scans. Fine root production, mortality and turnover can be underestimated if the lifespan of the finest roots is shorter than the time between two successive scans (Pregitzer and Hendrick 1996). A scanning interval of 2 weeks can reduce these underestimates to acceptable levels (Johnson et al. 2001). We scanned every 2–3 days over 1 month in the rainy summer and again over 1 month at the end of the dry winter to check that the 14 day interval between two successive scans over the study period of 2 years did not lead to an underestimate of the root turnover. While a few short-lived roots disappeared between 20 and 38 days after emergence, the vast majority of fine roots remained visible in the minirhizotron tubes for a much longer period. Collecting minirhizotron images fortnightly was therefore sufficient for a reliable estimate of fine root production in our fast-growing planted forest. Only a small proportion (<10%) of the fine roots which had appeared in the images below a depth of 30 cm from November 2011 onward had died by the end of our study period in October 2013, which leads to a non-negligible uncertainty in the lifespan values estimated from the Kaplan-Meier method. Our results suggest that minirhizotron studies should be carried out over several years to estimate accurately fine root turnover in tropical forests. As highlighted by Ahrens et al. (2014), future studies on fine root dynamics should integrate simultaneously ^{14}C measurements and minirhizotron observations in order to limit biases associated with each method and produce accurate fine root estimates in deep tropical soils.

Conclusion

Our study shows that the seasonality of fine root growth was much more marked at depth than in the topsoil. A lack of correlation between soil water contents and fine root growth in individual soil layers suggests that high growth rates starting at the end of the dry winter in deep soil layers might be controlled by physiological processes at the scale of the whole plant and in particular a response to a decrease in water availability in the topsoil. Both mean and maximum values of fine root elongation rates increased with the depth. Further studies examining fine root phenology down to the root front under temperature and rainfall gradients are needed to improve the predictions of response of tropical trees to climate change.

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References

Abramoff RZ, Finzi AC (2015) Are above and below-ground phenology in sync? New Phytol 205:1054–1061

Ahrens B, Hansson K, Solly EF, Schrumpf M (2014) Reconcilable differences: a joint calibration of fine-root turnover times with radiocarbon and minirhizotrons. New Phytol 204:932–942

Alton PB (2014) Reconciling simulations of seasonal carbon flux and soil water with observations using tap roots and hydraulic redistribution: a multi-biome FLUXNET study. Agric For Meteorol 198-199:309–319

Battie-Laclau P, Laclau JP, Domec JP, Christina M, Bouillet JP, de Cassia Piccolo M, de Moraes Gonçalves JL, Moreira RME, Krusche AV, Bouvet JM, Nouvellon Y (2014) Effects of potassium and sodium supply on drought-adaptive mechanisms in *Eucalyptus grandis* plantations. New Phytol 203: 401–413

Booth TH (2013) Eucalypt plantations and climate change. For Ecol Manag 301:28–34

Brando PM, Nepstad DC, Davidson EA, Trumbore SE, Ray D, Camargo P (2008) Drought effects on litterfall, wood production and belowground carbon cycling in an Amazonian forest: results of a throughfall reduction experiment. Philos. Trans. R. Soc., B Biol Sci 363:1839–1848

Bunn R, Lekberg Y, Zabinski C (2009) Arbuscular mycorrhizal fungi ameliorate temperature stress in thermophilic plants. Ecology 90:1378–1388

Cabral OMR, Rocha HR, Gash JHC, Ligo MAV, Freitas HC, Tatsch JD (2010) The energy and water balance of eucalyptus plantation in southeast Brazil. J Hydrol 388:208–2016

Campoe OC, Stape JL, Laclau JP, Marsden C, Nouvellon Y (2012) Stand-level patterns of carbon fluxes and partitioning in a *Eucalyptus grandis* plantation across a gradient of productivity, in Sao Paulo state, Brazil. Tree Physiol 32:696–706

Chairungsee N, Gay F, Thaler P, Kasemsap P, Thanisawanyangkura S, Chantuma A, Jourdan C (2013) Impact of tapping and soil water status on fine root dynamics in a rubber tree plantation in Thailand. Front Plant Sci 4:538

Christina M, Laclau JP, Gonçalves JLM, Jourdan C, Nouvellon Y, Bouillet JP (2011) Almost symmetrical vertical growth rates above and below ground in one of the world's most productive forests. Ecosphere 2:1–10

Christina M, Le Maire G, Battie-Laclau P, Nouvellon Y, Bouillet JP, Jourdan C, Gonçalves JLM, Laclau JP (2015) Measured and modeled interactive effects of potassium deficiency and water deficit on gross primary productivity and light-use efficiency in *Eucalyptus grandis* plantations. Glob Chang Biol 21:2022–2039

Christina M, Nouvellon Y, Laclau JP, Stape JL, Bouillet JP, Lambais GR, le Maire G (2017) Importance of deep water uptake in tropical eucalypt forest. Funct Ecol 31:509–519

Cox DR (1972) Regression models and life-tables. J R Stat Soc Ser B Methodol 34:187–220

da Silva EV, Bouillet JP, Gonçalves JLM, Abreu Junior CH, Trivelin PCO, Hinsinger P, Jourdan C, Nouvellon Y, Stape JL, Laclau JP (2011) Functional specialization of *Eucalyptus* fine roots: contrasting potential uptake rates for nitrogen, potassium and calcium tracers at varying soil depths. Funct Ecol 25:996–1006

Davidson E, Lefebvre PA, Brando PM, Ray DM, Trumbore SE, Solorzano LA, Ferreira JN, Bustamante MMC, Nepstad DC (2011) Carbon inputs and water uptake in deep soils of an eastern Amazon forest. For Sci 57:51–58

Eissenstat DM, Yanai RD (1997) The ecology of root lifespan. Adv Ecol Res 27:1–60

Forrester DJ, Collopy JJ, Morris JD (2010) Transpiration along an age series of *Eucalyptus globulus* plantations in southeastern Australia. For Ecol Manag 259:1754–1760

Gaudinski JB, Trumbore SE, Davidson EA, Cook AC, Markewitz D, Richter DD (2001) The age of fine-root carbon in three forests of the eastern United States measured by radiocarbon. Oecologia 129:420–429

Germon A, Cardinael R, Prieto I, Mao Z, Kim JH, Stokes A, Dupraz C, Laclau JP, Jourdan C (2016) Unexpected phenology and lifespan of shallow and deep fine roots of walnut trees grown in a Mediterranean agroforestry system. Plant Soil 401:409–426

Gill RA, Jackson RB (2000) Global patterns of root turnover for terrestrial ecosystems. New Phytol 147:13–31

Godbold DL, Fritz HW, Jentschke G, Meesenburg H, Rademacher P (2003) Root turnover and root necromass accumulation of Norway spruce (*Picea abies*) are affected by soil acidity. Tree Physiol 23:915–921

Goel MK, Khanna P, Kishore J (2010) Understanding survival analysis: Kaplan-Meier estimate. Int J Ayurveda Res 1:274–278

Graefe S, Hertel D, Leuschner C (2008) Fine root dynamics along a 2.000-m elevation transect in south Ecuadorian mountain rainforests. Plant Soil 313:155–166

Guo D, Li H, Mitchell RJ, Han W, Hendricks JJ, Fahey TJ, Hendrick RL (2008a) Fine root heterogeneity by branch order: exploring the discrepancy in root turnover estimates between minirhizotron and carbon isotopic methods. New Phytol 177:443–456

Guo D, Mitchell RJ, Withington JM, Fan PP, Hendricks JJ (2008b) Endogenous and exogenous controls of root life span, mortality and nitrogen flux in a longleaf pine forest: root branch order predominates. J Ecol 96:737–745

Hendrick RL, Pregitzer KS (1996) Temporal and depth-related patterns of fine root dynamics in northern hardwood forests. J Ecol 84:167–176

Hendricks JJ, Hendrick RL, Wilson CA, Mitchell RJ, Pecot SD, Guo DL (2006) Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. J Ecol 94:40–57

Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. Environ Exp Bot 45:263–289

Joslin JD, Gaudinski JB, Torn MS, Riley WJ, Hanson PJ (2006) Fine-root turnover and patterns and their relationship to root diameter and soil depth in a ^{14}C-labeled hardwood forest. New Phytol 172:523–535

Jourdan C, Silva EV, Gonçalves JLM, Ranger J, Moreira RM, Laclau JP (2008) Fine root production and turnover in Brazil eucalyptus plantations under contrasting nitrogen fertilization regimes. For Ecol Manag 258:396–404

King JS, Albaugh TJ, Allen HL, Buford M, Strain BR, Dougherty P (2002) Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytol 154:389–398

Laclau JP, Ranger J, Gonçalves JLM, Maquère V, Krushe AV, Thongo M'Bou A, Nouvellon Y, Saint-André L, Bouillet JP, Piccolo MC, Deleporte P (2010) Biogeochemical cycles of nutrients in tropical eucalyptus plantations: main features shown by intensive monitoring in Congo and Brazil. For Ecol Manag 259:1771–1785

Laclau JP, da Silva EA, Lambais GR, Bernoux M, le Maire G, Stape JL, Bouillet JP, Gonçalves JLM, Jourdan C, Nouvellon Y (2013) Dynamics of soil exploration by fine roots down to a depth of 10 m throughout the entire rotation in *Eucalyptus grandis* plantations. Front Plant Sci 4:243

Lambers H, Haven JA, Shaver GR, Smith SE (2008) Plant nutrient-acquisition strategies change with soil age. Trends Ecol Evol 23:95–103

le Maire G, Nouvellon Y, Christina M, Ponzoni FJ, Gonçalves JLM, Bouillet JP, Laclau JP (2013) Tree and stand light use efficiencies over a full rotation of single- and mixed-species *Eucalyptus grandis* and *Acacia mangium* plantations. For Ecol Manag 288:31–42

Luyssaert S, Inglima I, Jung M et al (2007) CO_2 balance of boreal, temperate, and tropical forests derived from a global database. Glob Chang Biol 13:2509–2537

Maeght JL, Rewald B, Pierret A (2013) How to study deep roots – and why it matters. Front Plant Sci 4:299

Maeght JL, Gonkhamdee S, Clément C, Ayutthaya SIN, Stokes A, Pierret A (2015) Seasonal patterns of fine root production and turnover in a mature rubber tree (*Hevea brasiliensis* Müll. Arg.) stand-differentiation with soil depth and implications for soil carbon stocks. Front Plant Sci 6:1022

Majdi H, Damm E, Nylund JE (2001) Longevity of mycorrhizal roots depends on branching order and nutrient availability. New Phytol 150:195–202

Majdi H, Pregitzer K, Morén AS, Nylund JE, Agren GI (2005) Measuring fine root turnover in forest ecosystems. Plant Soil 276:1–8

Maquère V (2008) Dynamics of mineral elements under a fast-growing Eucalyptus plantation in Brazil: implication for soil sustainability. PhD thesis, Agroparitech, Paris, 369 pp.

Mareschal L, Laclau JP, Nizila JDD, Versini A, Koutika LS, Mazoumbou JC, Deleporte P, Bouillet JP, Ranger J (2013) Nutrient leaching and deep drainage under eucalyptus plantations managed in short rotations after afforestation of an African savanna: two 7-year time series. For Ecol Manag 307:242–254

McComarck ML, Guo D (2014) Impacts of environmental factors on fine root lifespan. Front Plant Sci 5:205. https://doi.org/10.3389/fpls.2014.00205

Misra RK (1999) Root and shoot elongation of rhizotron-grown seedlings of *Eucalyptus nitens* and *Eucalyptus globulus* in relation to temperature. Plant Soil 206:37–46

Moroni MT, Worledge D, Beadle CL (2003) Root distribution of *Eucalyptus nitens* and *E. globulus* in irrigated and droughted soil. For Ecol Manag 177:399–407

Nardini A, Casol V, Dal Borgo A, Savi T, Stenni B, Bertoncin P, Zini L, McDowell NG (2016) Rooting depth, water relations and non-structural carbohydrate dynamics in three woody angiosperms differentially affected by an extreme summer drought. Plant Cell Environ. 39:618–627

Nepstad DC, Carvalho CR, Davidson EA, Jipp PH, Lefebvre PA, Negreiros GH, Silva ED, Stone TA, Trumbore SE, Vieira S (1994) The role of deep roots in the hydrological and carbon cycles of Amazonian forest and pastures. Nature 372:666–669

Nouvellon Y, Laclau JP, Epron D, le Maire G, Bonnefond JM, Gonçalves JLM, Bouillet JP (2012) Production and carbon allocation in monocultures and mixed-species plantations of *Eucalyptus grandis* and *Acacia mangium* in Brazil. Tree Physiol 32:680–695

Oliveira RS, Bezerra L, Davidson EA, Pinto F, Klink CA, Nepstad DC, Moreira A (2005) Deep root function in soil water dynamics in cerrado savannas of central Brazil. Funct Ecol 19:574–581

Pate J, Arthur D (1998) $\delta^{13}C$ analysis of phloem sap carbon: novel means of evaluating seasonal water stress and interpreting carbon isotope signatures of foliage and trunk wood of *Eucalyptus globulus*. Oecologia 117:301–311

Pfautsch S, Rennenberg H, Bell TL, Adams MA (2009) Nitrogen uptake by *Eucalyptus regnans* and *Acacia* spp. - preferences, resource overlap and energetic costs. Tree Physiol 29:389–399

Pierret A, Maeght JL, Clément C, Montoroi JP, Hartmann C, Gonkhamdee S (2016) Understanding deep roots and their functions in ecosystems: an advocacy for more unconventional research. Ann Bot 118:621–635

Pinheiro RC, de Deus JC Jr, Nouvellon Y, Campoe OC, Stape JL, Aló LL, Guerrini IA, Jourdan C, Laclau JP (2016) A fast exploration of very deep soil layers by *Eucalyptus* seedlings and clones in Brazil. For Ecol Manag 366:143–152

Pradier C, Hinsinger P, Laclau JP, Bouillet JP, Guerrini IA, Gonçalves JLM, Asensio V, Abreu-Junior CH, Jourdan C (2017) Rainfall reduction impacts rhizosphere biogeochemistry in eucalypts grown in a deep Ferralsol in Brazil. Plant Soil:1–16. doi:https://doi.org/10.1007/s11104-016-3107-7

Pregitzer KS, Hendrick RL (1996) Applications of minirhizotrons to understand root function in forest and other natural ecosystem. Plant Soil 185:293–304

Radville L, McCormack ML, Post E, Eissenstat DM (2016) Root phenology in a changing climate. J Exp Bot 67:3617–3628

Rewald B, Ephrath JE (2013) Minirhizotron techniques. In: Eshel A, Beeckman T (eds) Plant Roots: The Hidden Half. CRC Press, New York, pp 42.1–42.15

Ryan MG, Binkley D, Fownes JH, Giardina CP, Senock RS (2004) An experimental test of the causes of forest growth decline with stand age. Ecol Monogr 74:393–414

Ryan MG, Stape JL, Binkley D, Fonseca S, Loos RA, Takahashi EN, Ferreira JM, Lima AMN, Gava JL, Leite FP, Andrade HB, Alves JM, Silva GGC (2010) Factors controlling *Eucalyptus* productivity: how resource availability and stand structure alter production and carbon allocation. For Ecol Manag 259:1695–1703

Rytter RM (2013) The effect of limited availability of N or water on C allocation to fine roots and annual fine root turnover in *Alnus incana* and *Salix viminalis*. Tree Physiol 33:924–939

Saleska SR, Didan K, Huete AR, da Rocha H (2007) Amazon forests green-up during 2005 drought. Science 318:612

Satomura T, Fukuzawa K, Horikoshi T (2007) Considerations in the study of tree fine-root turnover with minirhizotrons. Plant Root 1:34–45

Scartazza A, Moscatello S, Matteucci G, Battistelli A, Brugnoli E (2015) Combining stable isotope and carbohydrate analyses in phloem sap and fine roots to study seasonal changes of source–sink relationships in a Mediterranean beech forest. Tree Physiol 35:829–839

Schenk HJ (2006) Root competition: beyond resource depletion. J Ecol 94:725–739

Schenk HJ, Jackson RB (2002a) Rooting depths, lateral root spreads and below-ground/above-ground allometries of plants in water-limited ecosystems. J Ecol 90:480–494

Schenk HJ, Jackson RB (2002b) The global biogeography of roots. Ecol Monogr 3:311–328

Smith SE, Read DJ (1997) Mycorrhizal simbioses, 2nd edn. Academic Press, London

Stape JL, Binkley D, Ryan MG (2008) Production and carbon allocation in a clonal eucalyptus plantation with water and nutrient manipulations. For Ecol Manag 255:920–930

Strand AE, Pritchard SG, McCormack ML, Davis MA, Oren R (2008) Irreconcilable differences: fine-root life spans and soil carbon persistence. Science 319:456–458

Team R (2013) R development core team. RA Lang Environ Stat Comput 55:275–286

Therneau T (2014) A package for survival analysis in S. R package version 2.37–7

Thongo M'bou A, Jourdan C, Deleporte P, Nouvellon Y, Saint-André L, Bouillet JP, Mialoundama F, Mabiala A, Epron D (2008) Root elongation in tropical eucalyptus plantations: effects of soil water content. Ann For Sci 65:209

Tierney GL, Fahey TJ (2001) Evaluating minirhizotron estimates of fine root longevity and production in the forest floor of a temperate broadleaf forest. Plant Soil 229:167–176

Tierney GL, Fahey TJ (2002) Fine root turnover in a northern hardwood forest: a direct comparison of the radiocarbon and minirhizotron methods. Can J For Res 32:1692–1697

Tierney GL, Fahey TJ, Groffman PM, Hardy JP, Fitzhughs RD, Discroll CT, Yavitt JB (2003) Environmental control of fine root dynamics in a northern hardwood forest. Glob Chang Biol 9:670–679

Tng DYP, Williamson GJ, Jordan GJ, Bowman DMJS (2012) Giant eucalypts - globally unique fire-adapted rain-forest trees? New Phytol 196:1001–1014

Tng DYP, Jordan GJ, Bowman DMJS (2013) Plant traits demonstrate that temperate and tropical giant eucalypt forests are ecologically convergent with rainforest not savannah. PLoS ONE 8:e84378

Trumbore SE, da Costa ES, Nepstad DC, de Camargo PB, Martinelli LA, Ray D, Restom T, Silver W (2006) Dynamics of fine root carbon in Amazonian tropical ecosystems and the contribution of roots to soil respiration. Glob Chang Biol 12:217–229

Vargas R, Allen MF (2008) Dynamics of fine root, fungal rhizomorphs, and soil respiration in a mixed temperate Forest: integrating sensors and observations. Vadose Zone J 7:1055–1064

Vargas R, Trumbore SE, Allen MF (2009) Evidence of old carbon used to grow new fine roots in a tropical forest. New Phytol 182:710–718

Wells CE, Eissenstat DM (2001) Marked differences in survivorship among apple roots of different diameters. Ecology 82: 882–892

Wu QS, Xia RS (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425