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

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ABSTRACT

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

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

1. Introduction

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

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

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

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

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

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

2. Material and methods

2.1. Study site

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