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Fertilization increases the functional specialization of fine roots in deep soil layers for young *Eucalyptus grandis* trees

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ABSTRACT

Functional specialization of fine roots was found for *Eucalyptus grandis* trees at harvesting age (6 years) on tropical soils. Aiming to elucidate whether functional specialization is a ubiquitous feature of eucalypts, we focused on its changes with ontogeny, tree nutrient status and soil depth. We studied the potential uptake of N, K and Ca by 2-year-old *E. grandis* trees, as a function of soil depth and NPK fertilization. We injected NO^{-3}-^{15}N, Rb^+ (K^+ analogue) and Sr^{2+} (Ca^{2+} analogue) tracers simultaneously in a solution at depths of 10, 50, 150 and 300 cm in a sandy Ferralsol soil. A complete randomized block design was set up with three replicates of paired trees per injection depth, in fertilized and non-fertilized plots. Recently expanded leaves were sampled at 70 days after tracer injection. Determination of foliar Rb, Sr concentrations and x(^{15}N) allowed estimating the relative uptake potential (RUP) and the specific RUP (SRUP), defined as the ratio between RUP and fine root length density (RLD) in the corresponding soil layer. Various root traits were measured at each depth. Foliar N and K concentrations were higher in fertilized than in non-fertilized trees. The RUP of NO_3^--^{15}N decreased sharply with soil depth and the highest values of the SRUP of NO_3^--^{15}N were found at a depth of 50 cm. The RUP of Rb^+ and Sr^{2+} did not change with soil depth, whilst the SRUP of Rb^+ and Sr^{2+} were higher at the depth of 300 cm than in the topsoil, concomitant with an increase in root diameter and a decrease in root tissue density with depth. The SRUP of Rb^+ and Sr^{2+} at a depth of 300 cm were on average 136 and 61% higher for fertilized trees than for non-fertilized trees, respectively. Fine roots of young *E. grandis* trees showed contrasting potential uptake rates with soil depth depending on the nutrient. Fertilization increased the uptake rate of Rb^+ and Sr^{2+} by unit of root length in deep soil layers. Functional specialization of fine roots for cations of low mobility depending on depth previously shown at harvesting age also occurs in young *E. grandis* plantations and increases with fertilization application. This mechanism helps explaining very low amounts of cations lost by leaching in *Eucalyptus* plantations established in deep tropical soils, even in highly fertilized stands.

1. Introduction

Forest plantations accounted in 2015 for some 291 million hectares (7% of the world forest areas), (FAO, 2015) and play an increasing role to satisfy the increase in global wood demand (Keenan et al., 2015; Paquette and Messier, 2010). Eucalypt plantations cover about 20 million hectares around the world and are expanding rapidly in tropical and subtropical regions to provide raw material for wood, paper, and biofuel products as well as large amounts of firewood and charcoal for domestic uses (Booth, 2013). *Eucalyptus grandis* Hill ex Maiden, is one of the most planted *Eucalyptus* species owing to its high productivity (Stape et al., 2010), and adaptation to various environments (Binkley et al., 2017; Costa et al., 2017).

High productivity of commercial eucalypt plantations in Brazil largely depends on nitrogen (N), phosphorus (P) and potassium (K) applications (Gonçalves et al., 2013). However, the potential negative

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environmental impacts of fertilizers might limit their use in eucalypt plantations in the future (Brunelle et al., 2015). Even though fertilizers are applied in the topsoil, significant amounts of K and N are leached and taken up by *Eucalyptus* trees down to a depth of 3 m (Laclau et al., 2010). Fine roots, usually defined according to a diameter-based cut-off ≤2 mm (Freschet et al., 2017) play a major role in the capture and transport of water and nutrients needed for plant growth (Pregitzer et al., 2002; McCormack et al., 2015; Fort et al., 2017). Although tree fine roots are usually more abundant in the shallow soil layers than in depth (Brassard et al., 2009; Laclau et al., 2013; Pinheiro et al., 2016), their presence was observed in very deep soil layers in tropical or subtropical fast-growing plantations and natural forests (Nepstad et al., 1994; Christina et al., 2017; Germon et al., 2017; Fan et al., 2017), e.g. 17 m deep in 3.5 year-old *Eucalyptus* plantation in Brazil (Christina et al., 2011). The physiological function of deep fine roots is still questioned (Al Afas et al., 2008; Maeght et al., 2013). Deep-rooting is an important strategy to increase the amount of water available for the trees, and to cope with seasonal droughts (Maeght et al., 2013; Christina et al., 2017; Broedel et al., 2017; Fan et al., 2017). However, the processes that control the uptake of ions such as NO_3^-, K^+ or Ca^{2+} in very deep soil layers are still poorly understood in forest ecosystems (Iversen, 2010; Hinsinger et al., 2011; Binkley, 2015). Such information would be useful to assess the need to apply fertilization at various dates after planting (Laclau et al., 2010).

Various factors drive nutrient uptake by tree roots, including soil nutrient availability, ion mobility in soil solutions, root traits or root ion transporters (Chapman et al., 2012; Costa et al., 2017; Kulmatiski et al., 2017). Contrasting potential uptake rates with depth depending on the nutrient were found for *Quercus robur L., Fagus sylvatica L. and Picea abies* (L.) Karst. in Northern Europe (Göransson et al., 2007, 2008) and for *E. grandis* in Brazil (da Silva et al., 2011). Fine roots of mature *E. grandis* trees exhibited, by unit of root length, greater uptake capacity of Rb^+ (analogue of K^+) and Sr^{2+} (analogue of Ca^{2+}) at a depth of 3 m than in the topsoil (da Silva et al., 2011). By contrast, a specialization of upper fine roots was likely in NO_3^- uptake. These results found at harvesting age were consistent in dry and wet seasons, for clayey and sandy soils. However, no information is available for young trees and the consequences of tree nutrient status on the functional specialization of fine roots are still unknown. Root specialization might be explained by many factors, as changes in specific transporter activity and mass flow rates from the soil to the roots through changes in root hydraulic conductivity (Costa et al., 2017). High-affinity transporters in plant, such as K and N-transporters, can be activated at very low nutrient concentrations allowing efficient nutrient uptake (Schachtman and Schroeder, 1994; Kiba and Krapp, 2016). On the opposite, Bao et al. (2011) showed that AtNRT2.1, an inducible high-affinity NO_3^- transporter in *Arabidpsis thaliana* was upregulated by phosphate and sulphate supply. Higher nutrient availability can increase tree nutrient uptake as shown for *E. grandis* (Rowe et al., 2008; Costa et al., 2017).

Moreover, to the best of our knowledge, no specific root functional traits have been associated with the functional specialization of *Eucalyptus* fine roots along the soil profile. Water and nutrient availabilities are highly dependent on soil depth in tropical eucalypt plantations (Mareschal et al., 2013; Versini et al., 2014). This heterogeneity throughout the soil profile leads to contrasting root functional traits that are highly sensitive to heterogeneous resource distributions (Ostonen et al., 2007). Prieto et al. (2015) measured root functional traits in 20 plant communities located in 3 climatic zones (tropical, Mediterranean and montane) along a land-use gradient. Fine roots exhibited different suites of functional traits (e.g. root diameter or Specific Root Length, SRL) at different soil depths suggesting a difference in root function and foraging capacity. Roots with higher SRL were found in less fertile soils. Pate et al. (1995) showed for Australian species that deep roots had conductivities up to 15 times higher than roots of similar diameter in the topsoil layers, which could help to explain the ability of *Eucalyptus* trees to use efficiently the resources in deep soil layers despite very low fine root densities.

The study set out to assess the potential uptake of N, K and Ca by 20-month-old *E. grandis* trees as a function of soil depth and fertilization at planting. We used NO_3^--^{15}N, Rb^+ and Sr^{2+} tracers simultaneously injected close to trees, at several depths down to 3 m, in a completely randomized experiment including NPK-fertilized and control (non-fertilized) plots. We hypothesized: (i) a functional specialization of fine roots for young eucalypt trees with higher potential uptake rate (per unit of fine root length) for NO_3^--^{15}N in the surface soil and for Rb^+ and Sr^{2+} in the deep soil layers, as shown in mature eucalypt plantations, and (ii) an increase in functional specialization of fine roots when tree nutrient status is improved by fertilization.

2. Materials and methods

2.1. Study site

The study was carried out in the Itatinga experimental station of São Paulo University (23°02′S, 48°38′W), at 860 m above mean sea level. Over the 15 years prior to planting, the mean annual rainfall was 1360 mm, with a dry and cold season from June to September. The soils are very deep Ferralsols according to FAO classification (FAO, 2014) developed on Cretaceous sandstone with a water table at a depth of approximatively 17 m (Christina et al., 2011). Soil chemical analyses down to a depth of 3 m are given in Table 1.

2.2. Experimental design

The experiment was conducted in a complete randomized block design with three blocks and two treatments. Seedlings were planted at a density of 1111 trees ha^{-1} (3 m × 3 m spacing) on May 2014. Each plot had a total area of 48 m × 48 m and an inner plot of 36 m × 36 m with two buffer rows. Within each block, there were plots of *E. grandis* without (F−) or with fertilization (F+) applied at planting: 125 kg ha^{-1} P, 121 kg ha^{-1} N, 136 kg ha^{-1} K, 45 kg ha^{-1} B and 30 kg ha^{-1} FTE (*Fritted Trace Element*, micronutrients). Factorial fertilization trials at the study site showed that the amounts of nutrients applied were non-limiting for *Eucalyptus* tree growth (Laclau et al., 2009). Fertilizers were dug into the soil below each tree. Higher N and K concentrations were found in leaves of fertilized than non-fertilized trees (Table S1). The study was carried out on January 2016, at 20 months of age during the rainy period. The tracers were applied at four depths (i.e. 10, 50, 150 and 300 cm) in each block, in both F+ and F− treatments. For each depth, NO_3^--^{15}N, Rb^+ and Sr^{2+} tracers were injected together into seven holes around two neighbouring *Eucalyptus* trees with the same basal area as the average of the stand (da Silva et al., 2011). The position of the holes was ¼ (0.75 m) and ½ (1.5 m) of the inter row on both sides of the planting row, at mid-distance (1.5 m) from the two sampled trees in the planting row and at mid-distance (1.5 m) from the two nearest neighbours in the planting row (Fig. 1). The pairs of trees were located more than 16 m apart to prevent root competition for nutrient uptake. Gravimetric water contents (around 10%) were not significantly different between treatments regardless of the depths.

2.3. Tracer application

A labelled solution was prepared in the laboratory, one day before application. RbCl (100 g), $SrCl_2$ (215 g) and NH_4-$^{15}NO_3$ (10 atom% NO_3-^{15}N) (652 g) were dissolved in 3360 mL of distilled water. The solution was maintained at a temperature of 4 °C until application in the field. Holes were drilled down to the target application depth using a 35-mm diameter stainless steel auger. A 25-mm diameter PVC tube was inserted into each hole to avoid contamination of upper soil layers during tracer application. A 4-mm diameter polyethylene tube, attached to an iron rod, was inserted into the PVC tube and 20 mL of the