Functional specialization of *Eucalyptus* fine roots: contrasting potential uptake rates for nitrogen, potassium and calcium tracers at varying soil depths

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Summary

1. Little is known about the role of deep roots in the nutrition of forest trees and their ability to provide a safety-net service taking up nutrients leached from the topsoil.

2. To address this issue, we studied the potential uptake of N, K and Ca by *Eucalyptus grandis* trees (6 years of age – 25 m mean height), in Brazil, as a function of soil depth, texture and water content. We injected $NO_3^- $-$^{15}N$, Rb^+ (analogue of K^+) and Sr^{2+} (analogue of Ca^{2+}) tracers simultaneously in a solution through plastic tubes at 10, 50, 150 and 300 cm in depth in a sandy and a clayey Ferralsol soil. A complete randomized design was set up with three replicates of paired trees per injection depth and soil type. Recently expanded leaves were sampled at various times after tracer injection in the summer, and the experiment was repeated in the winter. Soil water contents were continuously monitored at the different depths in the two soils.

3. Determination of foliar Rb and Sr concentrations and ^{15}N atom % made it possible to estimate the relative uptake potential (RUP) of tracer injections from the four soil depths and the specific RUP (SRUP), defined as RUP, per unit of fine root length density in the corresponding soil layer.

4. The highest tracer uptake rates were found in the topsoil, but contrasting RUP distributions were observed for the three tracers. Whilst the RUP was higher for $NO_3^- $-$^{15}N$ than for Rb^+ and Sr^{2+} in the upper 50 cm of soil, the highest SRUP values for Sr^{2+} and Rb^+ were found at a depth of 300 cm in the sandy soil, as well as in the clayey soil when gravitational solutions reached that depth.

5. Our results suggest that the fine roots of *E. grandis* trees exhibit contrasting potential uptake rates with depth depending on the nutrient. This functional specialization of roots might contribute to the high growth rates of *E. grandis* trees, efficiently providing the large amounts of nutrients required throughout the development of these fast-growing plantations.

Key-words: deep root system, *Eucalyptus grandis*, ^{15}N, nutrient uptake potential, rubidium, soil depth, strontium, tropical soil

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Introduction

Even though tree roots are usually concentrated in the upper soil layers (Jackson *et al.* 1996; Eamus *et al.* 2002; Brassard, Chen & Bergeron 2009), some tree species can develop root systems down to several dozen metres (Canadell *et al.* 1996; Casper & Jackson 1997; Feikema, Morris & Connell 2010). Water uptake in deep soil layers has been shown in forests established in arid environments throughout the world (Roupsard *et al.* 1999; Nadezhdina *et al.* 2008; Bleby, McElrone & Jackson 2010), but also in humid equatorial and tropical climates (Oliveira *et al.* 2005; Schenk & Jackson 2005; Bruno *et al.* 2006; Markewitz *et al.* 2010). In the case of *Eucalyptus* plantations, water uptake has been reported down to > 5 m in the Congo (Laclau *et al.* 2001) and 8 m in Brazil (Christina *et al.* 2011), South Africa (Dye 1996) and Australia (Robinson, Harper & Smettem 2006).

In contrast, little is known about the role of deep roots in nutrient uptake in forests (Buxbaum, Nowak & White 2005; Brassard, Chen & Bergeron 2009). Concentrations of exchangeable cations usually decrease markedly with depth (Jackson *et al.* 2000; Krishnaswamy & Richter 2002) and a decrease in nutrient uptake with soil depth has been shown in temperate forests (Burton, Pregitzer & Hendrick 2000; Bennett, Andrew & Prescott 2002; Göransson, Ingerslev & Wallander 2008) and tropical agroforestry systems (Lehmann & Muraoka 2001; Lehmann 2003). However, the highest concentrations of nutrients can be found in depth in some specific areas (Stone & Kalisz 1991; Moroni, Smethurst & Holz 2004), and the subsoil may temporarily contain a significant proportion of available nutrients when low soil moistures limit nutrient uptake in surface layers (Lehmann 2003) or when hydraulic redistribution and soil water recharge occur at depth (McCulley *et al.* 2004). Deep roots are also likely to provide a safety-net service, taking up nutrients leached from the topsoil and transferring nutrients available in deep layers to the soil surface (Allen *et al.* 2004; Jobbágy & Jackson 2004; Mulia & Dupraz 2006).

Fine root distribution partly drives tree nutrient uptake throughout soil profiles (Rowe *et al.* 2001; Lehmann 2003). Recent studies based on excised roots under standardized conditions showed physiological and functional differences between superficial and deep tree roots (Göransson *et al.* 2006; Göransson, Fransson & Jönsson-Belyazid 2007). The hypothesis was put forward that roots adapt to soil depths where specific nutrients are available during plant development. Complementary *in situ* studies based on non-destructive measurements are needed to confirm this important finding. Stable isotopes and rare elements have been used to study nutrient uptake in tropical agroforestry systems (Lehmann & Muraoka 2001; Lehmann 2003; Rowe *et al.* 2001, 2006) and rain forests (Poszwa *et al.* 2009), in pine plantations in Florida (Van Rees & Comerford 1986), in European pine and eucalypt plantations (Dambrine *et al.* 1997) and boreal forests (Göransson, Ingerslev & Wallander 2008). Such studies have not been conducted, to our knowledge, in

tropical fast-growing plantation forests. Few investigations have been made of the ability of trees to take up several tracers differing in their mobility in the soil, and applied at the same depths (Brandtberg, Bengtsson & Lundkvist 2004; Göransson, Ingerslev & Wallander 2008), or of the effects of soil characteristics and water dynamics on tracer uptake (Havlin *et al.* 2005).

The aim of our study was to assess the potential uptake of N, K and Ca by *Eucalyptus grandis* Hill ex Maid. trees as a function of soil depth, texture and water content. We injected NO_3^--^{15}N, Rb^+ and Sr^{2+} tracers simultaneously close to different trees, at depths of 10, 50, 150 or 300 cm, both in a sandy and a clayey soil where soil water content was monitored. NO_3^- is more mobile in soil than K^+ (and its analogue Rb^+) and Ca^{2+} (and its analogue Sr^{2+}) (Barber 1995). The experiment was repeated in the warm and the cold seasons in southern Brazil, the last year before harvesting commercial plantations. These *E. grandis* plantations were chosen because they are among the most productive forest ecosystems in the world and they rapidly develop their root system in very deep soil layers (Christina *et al.* 2011). Deep fine roots were thus likely to play an important role in the nutrition of the trees, to maintain high growth rates, when adverse conditions lowered nutrient uptake in the upper soil layers.

Evolution may have led to a nutrient-specific specialization in root functioning for *E. grandis* trees depending on soil depths, as suggested for oak trees in boreal forests (Göransson, Fransson & Jönsson-Belyazid 2007). Whilst nitrogen mineralization decreases rapidly with soil depth in natural areas of *E. grandis* trees in Australia (Connell, Raison & Khanna 1995), not insubstantial amounts of available K and Ca can be found in deep soil layers (Batjes 2008). We put forward the hypothesis of a strong relationship between soil nutrient availability under *E. grandis* forests and tree uptake, with (i) a higher proportional uptake in the topsoil for NO_3^- than for analogues of cations potentially released by weathering in deep soil layers (Rb^+ and Sr^{2+}) and (ii) greater influence of soil water contents on nutrient uptake for the most mobile ions.

Materials and methods

SITE CHARACTERISTICS

The study was conducted 15 km from Itatinga, State of São Paulo (22°58′04″S, 48°43′40″W). The mean annual rainfall over the 15 years prior to this study was 1360 mm and the mean annual temperature was 19·0 °C, with a cold and dry seasonal period from June to September with a mean temperature of 17·2 °C and cumulated rainfall of 225 mm. The relief was typical of the São Paulo Western Plateau, with a gently undulating topography.

The soils were deep Ferralsols according to the FAO classification, developed on Cretaceous sandstone, Marília formation, Bauru group. The slope was < 5%, and the maximum elevation was 760 m above sea level. Clay contents down to a depth of 300 cm at the study site ranged from 16% to 28% and from 39% to 41% at the top and the bottom of the hill, respectively (Table 1). The mineralogy was dominated by quartz, kaolinite and oxyhydroxides (Maquère 2008),

Table 1. Main physical and chemical soil attributes

Depth (cm)	Clay (%)	Silt (%)	Sand (%)	pH H$_2$O	P (mg kg^{-1})	Total C (%)	δ^{15}N (‰)	Total N (%)	K (mmol$_c$ kg^{-1})	Na (mmol$_c$ kg^{-1})	Ca (mmol$_c$ kg^{-1})	Mg (mmol$_c$ kg^{-1})	H (mmol$_c$ kg^{-1})	Al (mmol$_c$ kg^{-1})	SB (mmol$_c$ kg^{-1})	CEC (mmol$_c$ kg^{-1})	BS (%)
Sandy soil																
0–5	17.4	5.5	77.0	4.7	3.2	1.61	4.81	0.07	0.6	0.4	6.7	6.3	48.3	6.7	14.0	69.0	20.3
5–15	16.2	1.3	82.5	4.3	2.0	0.54	8.22	0.03	0.2	0.2	1.8	1.5	26.0	8.8	3.7	38.4	9.5
15–50	18.2	2.1	79.7	4.5	1.5	0.40	9.80	0.02	0.1	0.1	1.0	1.0	14.3	5.8	2.2	22.2	10.0
50–100	20.2	1.4	78.4	4.5	1.1	0.38	10.64	0.02	0.1	0.1	1.0	1.0	13.3	5.0	2.2	20.5	10.8
100–200	22.9	3.6	73.6	4.5	1.1	0.27	10.66	0.01	0.1	0.1	1.0	1.0	8.3	2.5	2.2	12.9	16.8
200–300	23.4	4.0	72.6	5.0	0.9	0.18	10.30	0.01	0.1	0.1	1.0	1.0	4.3	1.0	2.2	7.4	29.3
Clayey soil																
0–5	35.2	7.8	57.1	4.9	2.9	1.32	7.26	0.07	0.9	0.4	7.0	2.9	38.5	6.0	11.2	55.7	20.1
5–15	34.4	9.7	55.9	4.7	1.8	0.73	10.37	0.04	0.5	0.2	1.8	1.8	25.5	6.5	4.3	36.3	11.7
15–50	39.0	10.0	51.0	4.7	1.4	0.70	10.87	0.04	0.7	0.2	1.0	1.4	22.0	5.5	3.3	30.8	10.8
50–100	41.5	9.8	48.7	4.8	1.1	0.51	11.51	0.03	0.3	0.2	1.0	1.1	16.3	2.3	2.6	21.1	12.4
100–200	42.2	12.1	45.7	5.3	1.0	0.34	10.72	0.02	0.1	0.2	1.0	1.0	10.8	0.0	2.2	13.0	17.1
200–300	43.5	12.5	44.0	5.5	0.9	0.22	9.30	0.01	0.2	0.2	1.0	0.9	8.5	0.0	2.3	10.8	20.9

Total C, total N and ^{15}N were obtained with a Hydra 20–20 mass spectrometer coupled to an automatic N analyzer (ANCA-GSL; SERCON Co.). P was determined by Mehlich-1 and colorimetry; K and Na were determined by Mellich-1 and photometry; Ca and Mg were determined by KCl extraction and atomic absorption. SB, sum of bases; CEC, cation exchange capacity; BS, base saturation.

with acidic soil layers (pH$_{water}$ between 4·3 and 5·7). Both the sandy and the clayey soils were characterized by small amounts of nutrients (sum of base cations < 4·0 mmol$_c$ kg^{-1} below 15 cm). Exchangeable K concentrations were 0·1 mmol$_c$ kg^{-1} below 15 cm in the sandy soil and 0·2–0·3 mmol$_c$ kg^{-1} below 50 cm in the clayey soil. Exchangeable Ca concentration was 1·0 mmol$_c$ kg^{-1} below 15 cm in both soils. Total N concentrations decreased regularly with depth in both soils. δ^{15}N natural abundance ranged from 4·8‰ to 10·7‰ in the sandy and from 7·3‰ to 11·5‰ in the clayey soil.

The experimental area (200 ha) was replanted in December 2002 with *E. grandis* seedlings at a spacing of 3·75 × 1·60 m, after harvesting a previous *E. grandis* stand and herbicide application on the stumps. All seedlings received complete fertilization (62 kg ha^{-1} N, 52 kg ha^{-1} P, 131 kg ha^{-1} K, 1·5 Mg ha^{-1} of lime and micronutrients). Fertilizers were applied at planting, then at 6, 12 and 24 months of age. A complementary application of KCl (53 kg ha^{-1} K) was broadcast at 42 months of age. Mean tree heights were 22·7 and 27·4 m at 60 months of age on the sandy soil and the clayey soil, respectively. The corresponding mean annual increments in total bole volume were 35 and 55 m^3 ha^{-1} year^{-1}, respectively.

EXPERIMENTAL DESIGN

A complete randomized block design was set up with four tracer application depths (i.e. 10, 50, 150 and 300 cm) and three blocks, on both the sandy and the clayey soil. The experiment was repeated with an application of tracers in the summer (March 2008) and in the winter (September 2008). Each block measured 200 × 100 m. For each depth and each application date, NO$_3^-$-^{15}N, Rb$^+$ and Sr^{2+} tracers were injected jointly in seven holes around two neighbouring trees with the same basal area as the mean of the stand in each soil type. The position of the holes was at ¼ (0·94 m) and ½ (1·88 m) of the inter-row on both sides of the planting row, at mid-distance (0·80 m) from the two sampled trees in the planting row and at mid-distance
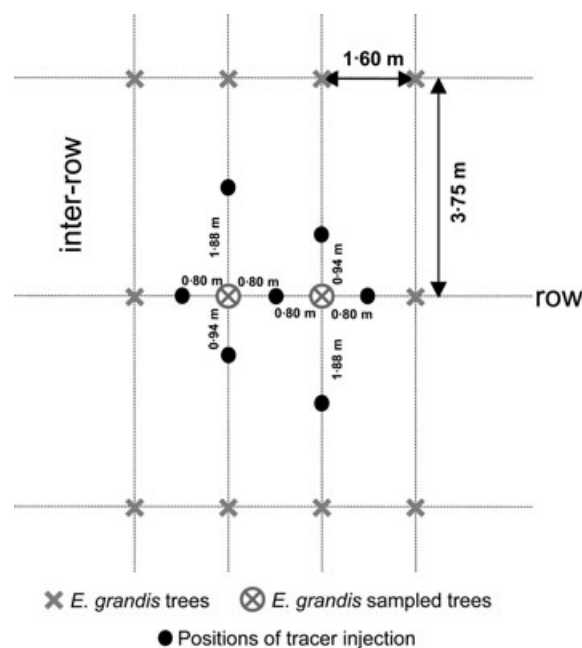

Fig. 1. Layout of the positions where tracers were injected for each depth and each pair of sampled trees. Leaf samples were collected from the two central trees at various times after tracer injection.

(0·80 m) from the two closest neighbours in the planting row (Fig. 1). The sets of two trees were located more than 30 m apart. Manual and chemical weeding were regularly carried out to keep the soil free of weeds within a radius of 20 m around the trees.

TRACER APPLICATION

A labelled solution was prepared in the laboratory dissolving $RbCl$ (119 g), $SrCl_2$ (256 g) and NH_4-$^{15}NO_3$ (10 atom % NO_3^--^{15}N) (956 g) in 3360 mL of distilled water. The solution was kept at 4 °C for 2 days; holes were drilled by hand using a 35-mm diameter stainless steel auger. A 25-mm diameter PVC tube was inserted into each hole to avoid contamination of the soil profile 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 labelled solution was injected at the selected depth. Thereafter, 10 mL of distilled water was injected to expel all tracers from the syringe and rinse the polyethylene tube. At each position, we applied 0·5 g of Rb, 0·5 g of Sr and 0·1 g of ^{15}N. The nutrient concentration in the 30 mL of solution injected at each position was 0·20, 0·19, 0·17 and 1·60 M for Rb^+, Sr^{2+}· NO_3^--^{15}N and N, respectively. The polyethylene and PVC tubes were carefully withdrawn after tracer application, and the hole filled with the soil removed during drilling, respecting the original order of the soil layers.

SAMPLING AND ANALYSIS

We climbed to a height of about 20 m on a neighbouring tree to collect young fully expanded leaves from the upper half of the crown of each pair of sampled trees. To obtain background values of Rb and Sr concentrations and ^{15}N atom %, we sampled four average sized trees in each soil before tracer application in the summer and four trees in each block (12 trees in total) in each soil before tracer application in the winter. Forty leaves (20 per labelled tree) were collected for each set of trees 15, 35, 70 and 115 days after tracer application. Additional leaves were collected 230 days after tracer application in the winter. The leaves were washed in deionized water, dried at 65 °C for 3 days, ground and stored in sealed acrylic bottles. The concentrations of Rb^+ and Sr^{2+} were determined by inductively coupled plasma mass spectrometry (ICP-MS Agilent 7500ce; Agilent Technologies, Tokyo, Japan) from 0·5 g of leaf powder digested in a mixture of 5 mL of nitric acid (16 M) and 1 ml of perchloric acids (12 M). ^{15}N contents (atom %) and total-N were determined with a Hydra 20–20 mass spectrometer coupled to an automatic N analyzer (ANCA-GSL; SERCON Co., Crewe, UK) from 10 mg of leaf powder dry matter (Barrie & Prosser 1996). The Method Detection Limit for Rb and Sr was 0·018 and 0·020 mg kg^{-1}, respectively. During the analysis, the relative standard deviation was lower than 0·5%. The precision of isotope measurements was 0·001 atom % ^{15}N.

ESTIMATION OF RUP AND SRUP

Foliar Rb and Sr concentrations and ^{15}N atom % were analysed for all sampling dates in one block for each soil type. The leaf samples collected in the remaining blocks were analysed 70 and 115 days after tracer injection. Additional analyses in all the blocks were performed 230 days after tracer application in the winter.

The RUP (Soethe, Lehmann & Engels 2006) was calculated in each soil type using

$$RUP_{ip}(\%) = (T_{ip} - T_{io})/[(T_{i10} - T_{io}) + (T_{i50} - T_{io}) \\ + (T_{i150} - T_{io}) + (T_{i300} - T_{io})] \times 100 \tag{1}$$

where RUP_{ip} was the RUP of tracer i at depth p, T_{ip} was the foliar concentration (Rb and Sr) or atom % (^{15}N) of tracer i at depth p, T_{io} was the background value of foliar concentration (Rb and Sr) or atom % (^{15}N) of tracer i and T_{i10}, T_{i50}, T_{i150}, T_{i300} were the foliar concentrations (Rb and Sr) or atom % (^{15}N) of tracer i at the depths of 10, 50, 150 and 300 cm.

The SRUP was calculated in each soil type using

$$SRUP_{ip}(\% \, cm^{-1}cm^3) = RUP_{ip}/RLD_p \tag{2}$$

where $SRUP_{ip}$ was the SRUP of tracer i at depth p and RLD_p was the fine root length density at depth p.

The RLDs were estimated from the number of intercepts of roots < 1 mm in diameter per cm^2 (Nt) using an empirical calibration equation established by Maurice *et al.* (2010) for the same *E. grandis* stand (for the two soil types and the same age as in our study). Nt was counted on a 5 × 5 cm grid down to a depth of 300 cm on six soil profiles perpendicular to the planting row in each soil type (see Bouillet *et al.* 2002 for method details). Mean RLD values for the six profiles in each soil type were estimated on the 5 cm layers above and below each depth (i.e. 5–15 cm for the depth of 10 cm, 45–55 cm for the depth of 50 cm, 145–155 cm for the depth of 150 cm and 295–305 cm for the depth of 300 cm).

SOIL WATER MONITORING

Soil water contents were monitored over the whole study period in the sandy soil and from May 2008 onwards in the clayey soil. Five and four replicates of CS616 probes (Campbell Scientific, Shepshed, UK) were buried horizontally at depths of 15, 50 and 100 cm from five and four trenches dug at different distances from the trees in the sandy and the clayey soils, respectively. Three replicates of CS616 probes were buried horizontally from three pits at depths of 200 and 300 cm in each soil type. The pits were backfilled after installation with the horizons in their natural arrangement. The measurement period was 30 s, averaged half-hourly using a CR1000 and two AM416 Multiplexers in each soil type (Campbell Scientific). Probes were calibrated by the gravimetric method.

STATISTICAL ANALYSES

The dynamics of Rb and Sr concentrations and ^{15}N atom % in leaves led to the estimation of the RUP and the SRUP 70 days after tracer injection to compare the uptake rates of the tracers when their accumulation in leaves was close to maximum values (Fig. 2). The hypothesis of a higher proportional uptake in the topsoil for NO_3^--^{15}N than for Rb^+ and Sr^{2+} was tested by analysing the variance of the RUP and the SRUP with three linear models including first-order interactions between factors. Model 1 compared the RUPs between depths for a given tracer based on the factors soil, depth, season and replicate nested within the soil. Model 2 compared the RUPs at a given depth for the different tracers based on the factors soil, tracer, season and replicate nested within the soil. Model 3 compared the SRUP between depths for a given tracer based on the factors soil, depth, season and replicate nested within the soil. The hypothesis of a greater influence of soil water contents on the uptake of NO_3^- than of Rb^+ and Sr^{2+} was tested by analysing the variance of the differences of the RUP (resp. SRUP) at a depth of 300 cm
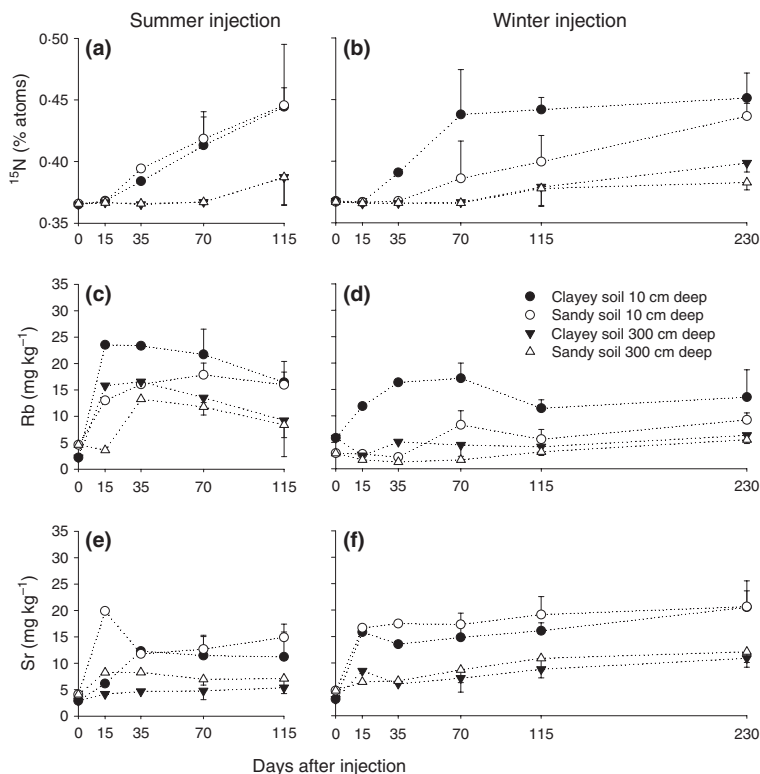
**Fig. 2.** Changes in foliar Rb and Sr concentrations and ^{15}N atom % after tracer injection in the summer and in the winter, in the clayey and the sandy soils. Standard deviations at each depth are indicated from 70 days after tracer injection onwards ($n = 3$).

between 70 and 230 days after tracer injection in the winter, when a marked increase in soil water content occurred (Fig. 3). A first-order interaction model (model 4) was used in which the factors were soil, tracer and replicate nested in the soil. The GLM procedure of SAS 6.11 was used (SAS Institute, Cary, NC, USA). Homogeneity of variances was tested by Levene's test, and the normal distribution of residues was tested with the Kolmogorov–Smirnov test. The RUP values were Arsin(sqrt)-transformed when variances were unequal. Bonferroni's test was used for multiple comparisons with a significance level of 0·05.
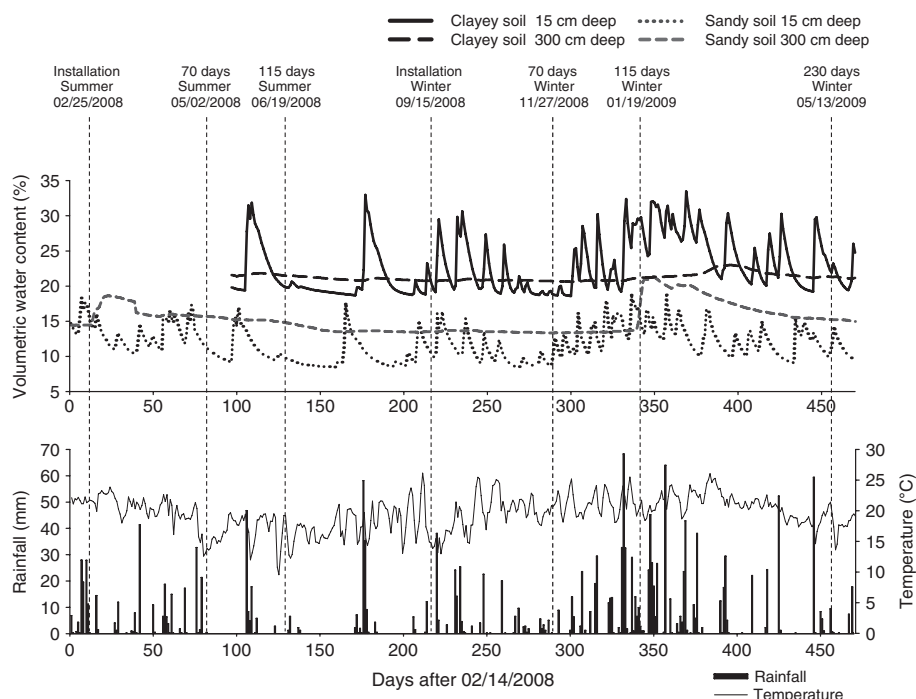
Fig. 3. Rainfall, air temperature and time-course of soil water contents at depths of 15 and 300 cm in the sandy soil and the clayey soil, over the whole study period. Soil water contents were measured with CS616 probes (4–5 probes in each soil at a depth of 15 cm and three probes at a depth of 3 m).
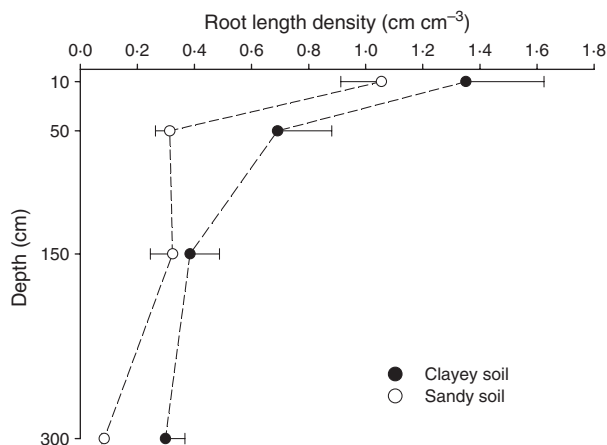
Fig. 4. Mean root length densities of fine roots (diameter < 1 mm) in the clayey soil and in the sandy soil at depths of 10, 50, 150 and 300 cm. Standard deviations at each depth are indicated ($n = 6$).

Results

FINE ROOT DISTRIBUTIONS

The RLDs were 54% higher, on average, in the clayey soil than in the sandy soil (Fig. 4). They decreased sharply from 10 to 50 cm in depth then more steadily with soil depth down to 300 cm. The RLDs at depths of 50, 150 and 300 cm accounted for 18%, 19% and 6% of the RLDs at a depth of 10 cm in the clayey soil. In the sandy soil, the proportions were 35%, 19% and 11%, respectively.

Rb AND Sr CONCENTRATIONS AND ^{15}N ATOM % IN THE LEAVES

The highest tracer concentrations in recently fully expanded leaves were found on most of the sampling dates when they were applied in the upper soil layer (Fig. 2). Foliar Rb and Sr concentrations increased rapidly close to the maximum values observed, generally within 15 days after tracer application (except for Rb^+ applied in the winter at a depth of 300 cm in the sandy soil). When the $NO_3^- $-^{15}N tracer was injected in the topsoil, an increase in foliar ^{15}N atom % was observed from only 35–70 days after application. When this tracer was injected at depth, such an increase was delayed.

TRACER RELATIVE UPTAKE POTENTIAL

The RUPs of NO_3-^{15}N, Rb^+ and Sr^{2+} 70 days after application were significantly influenced by the depth of tracer injection ($F > 46\cdot9$, $P < 0\cdot01$), but not by the soil type and the season (model 1). The RUPs were significantly different between all the sampled depths, except between 150 and 300 cm for $NO_3^- $-^{15}N and between 50 and 150 cm for Rb^+ (Fig. 5). A significant depth × season interaction for Rb^+ ($F = 19\cdot0$, $P < 0\cdot01$) was the result of a decrease in RUP, in line with the depth of injection, which was much more pronounced in the winter than in the summer.

The RUPs were significantly influenced by the tracer type ($F > 12\cdot3$, $P < 0\cdot01$) at each injection depth, but not by the soil type and the season (model 2). The RUP was significantly higher for $NO_3^- $-^{15}N than for Rb^+ and Sr^{2+} at a depth of 10 cm, with mean values of 72·3%, 42·0% and 41·9%, respectively (Fig. 5). The RUP was significantly lower for $NO_3^- $-^{15}N than for the two other tracers at the other three depths. The RUPs of Rb^+ and Sr^{2+} were not significantly different, whatever the depth of tracer injection.
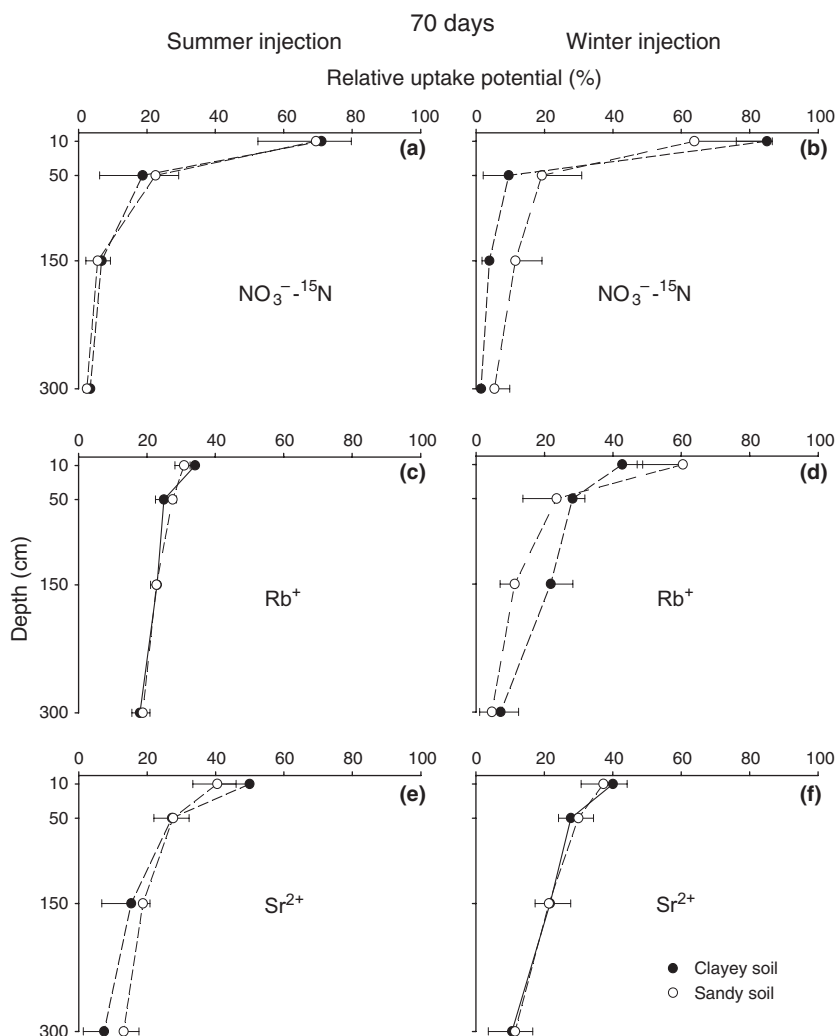
SPECIFIC RELATIVE UPTAKE POTENTIAL OF TRACERS

The SRUPs of $NO_3^- $-^{15}N were significantly influenced by the soil type ($F = 17\cdot6$, $P < 0\cdot01$) and the injection depth ($F = 8\cdot6$, $P < 0\cdot01$), but not by the season (model 3). The SRUP of $NO_3^- $-^{15}N was significantly higher at a depth of 10 cm than at 150 and 300 cm, with mean values of 60·4, 20·2 and 27·6% cm^{-1} cm^3, respectively (Fig. 6a,b). The SRUP of $NO_3^- $-^{15}N was found to be twice as high in the sandy soil as in the clayey soil, with mean values of 50·8 and 25·1% cm^{-1} cm^3, respectively. The SRUPs of Rb^+ were significantly influenced by soil types ($F = 40\cdot6$, $P < 0\cdot01$), depths ($F = 15\cdot0$, $P < 0\cdot01$) and seasons ($F = 21\cdot2$, $P < 0\cdot01$), with significant interactions between these factors ($F > 11\cdot6$, $P < 0\cdot01$) (model 3). A tendency towards an increase in the SRUP of Rb^+ in line with the application depth was observed when that tracer was injected in the summer (Fig. 6c).

The SRUPs of Sr^{2+} were significantly influenced by soil types ($F = 48\cdot5$, $P < 0\cdot01$) and injection depths ($F = 11\cdot1$, $P < 0\cdot01$), with significant interactions between these two factors ($F = 14\cdot6$, $P < 0\cdot01$) (model 3). This interaction was mainly the result of an increase in the SRUP of Sr^{2+} in line with the application depth in the sandy soil (from 36·8 to 146·6% cm^{-1} cm^3, on average, at depths of 10 and 300 cm, respectively), whereas the SRUP values were little affected by depth in the clayey soil (ranging from 30·1 to 48·1% cm^{-1} cm^3, irrespective of the application depth and the season) (Fig. 6e,f). The SRUPs of Sr^{2+} were higher in the sandy soil than in the clayey soil, with mean values of 84·3 and 37·8% cm^{-1} cm^3, respectively.

INFLUENCE OF SOIL WATER CONTENT ON RUP AND SRUP

Rises in soil water contents occurred in the upper layers of the sandy and the clayey soils after each rainfall event (Fig. 3). In contrast, soil moisture remained close to minimum values at a depth of 300 cm over most of the study period, except during periods of high rainfall. An increase in soil water content at a depth of 300 cm occurred in the sandy soil just before tracer injection in the summer and in the two soils in the winter between 115 and 200 days after tracer application.

The RUPs and SRUPs increased from 70 to 230 days after tracer application in the winter at a depth of 300 cm (Fig. 7) in the two soils, whatever the tracer, except for Sr^{2+} in one replicate on the sandy soil. The increase in RUP from 70 to 230 days was not significantly influenced by soils and tracers,

Fig. 5. Relative uptake potential of $NO_3^- $-^{15}N, Rb^+ and Sr^{2+} at depths of 10, 50, 150 and 300 cm, in the clayey soil and in sandy soil. The RUPs of $NO_3^- $-^{15}N, Rb^+ and Sr^{2+} were calculated 70 days after tracer injection in the summer (a, c, e, respectively) and in the winter (b, d, f, respectively). Standard deviations at each depth are indicated ($n = 3$).

with mean values of $+9\cdot5$, $+9\cdot9$ and $+5\cdot7\%$ for $NO_3^- $-^{15}N, Rb^+ and Sr^{2+}, respectively (model 4). Significant differences in the increase in the SRUP from 70 to 230 days after injection were found between tracers ($F = 8\cdot5$, $P < 0\cdot02$), but not between soils. The increase in the SRUP was $+73\cdot2$, $+149\cdot0$ and $+64\cdot4\%$ cm^{-1} cm^3 for $NO_3^- $-^{15}N, Rb^+ and Sr^{2+}, respectively, in the sandy soil. The increase in the SRUP in the clayey soil was $+43\cdot1$, $+24\cdot3$ and $+20\cdot5\%$ cm^{-1} cm^3, respectively.

Discussion

DYNAMICS OF FOLIAR TRACER CONCENTRATIONS AND TRACER UPTAKE

Monitoring tracer accumulation in recently expanded leaves showed a delay in the increase in ^{15}N atom % compared with Rb and Sr concentrations, in both seasons. This pattern might partly be explained by the use of ammonium nitrate fertilizer, labelled with ^{15}N for the nitrate component only. Garnett *et al.* (2001, 2003) using ion-selective microelectrodes found a higher uptake for ammonium than for nitrate by 1-month-old *Eucalyptus nitens* and *Eucalyptus globulus*

seedlings. A key process for the uptake and assimilation of nitrate is nitrate reductase activity (NRA) in the plant (Eddy *et al.* 2008). High concentrations of NH_4^+ in soil solutions may lower both NO_3^- uptake (Boukcim & Plassard 2003) and NRA in the roots (Nicodemus, Salifu & Jacobs 2008). Whilst most herbaceous species exhibit higher NRA in the shoots than in the roots, the contrary is generally found for woody species (Miller & Cramer 2005). High concentrations of NH_4^+ in soil solutions close to injection zones, as well as the time needed for nitrate reduction in the roots and for amino acid transport in the xylem sap of *E. grandis* trees, might have contributed to delaying the increase in ^{15}N atom % in leaves compared with the accumulation of Rb and Sr. The microbial immobilization of $NO_3^- $-^{15}N in the soil might also partly explain this pattern (Bengtsson & Bergwall 2000).

A delay of 70 days after injection was selected to compare root potential uptakes when the foliar ^{15}N atom % and concentrations of Rb and Sr were close to their maximum. Leaf tagging of *E. grandis* trees in a nearby area showed that about 1 month was needed from leaf initiation to full expansion (Laclau *et al.* 2009), and the young fully expanded leaves sampled in our study were probably 1- to 2-month-old. Root potential uptake has been commonly assessed about

70 days

Summer injection   Winter injection

Specific relative uptake potential (% cm^{-1} cm^3)
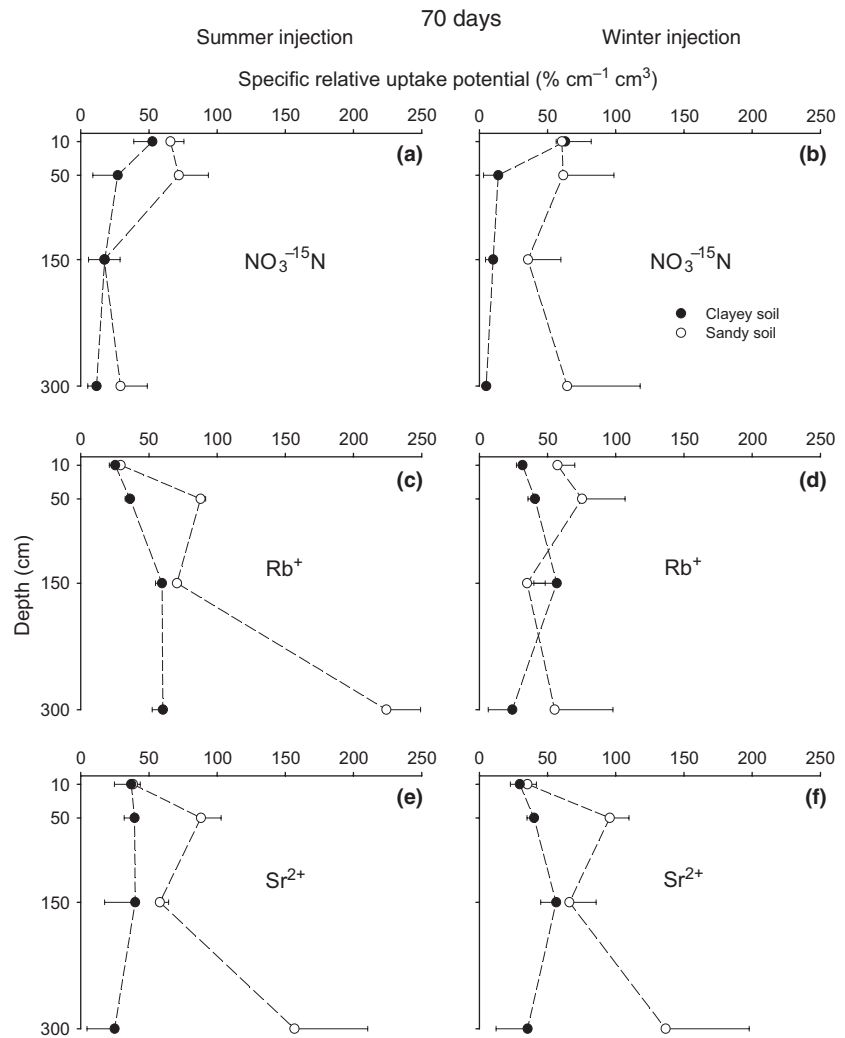
Fig. 6. Specific relative uptake potential (SRUP) of NO_3^--^{15}N, Rb^+ and Sr^{2+} at depths of 10, 50, 150 and 300 cm, in the clayey soil and in the sandy soil. The SRUPs of NO_3^--^{15}N, Rb^+ and Sr^{2+} were calculated 70 days after tracer injection in the summer (a, c, e, respectively) and in the winter (b, d, f, respectively). Standard deviations at each depth are indicated (n = 3).
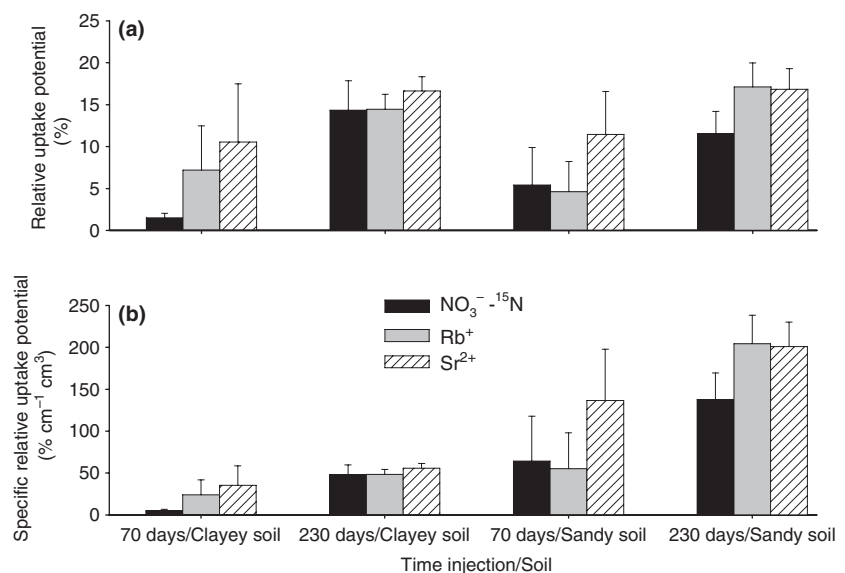
Fig. 7. The relative uptake potential (RUP) (a) and specific RUP (b) of NO_3^--^{15}N, Rb^+ and Sr^{2+} at a depth of 300 cm in the clayey soil and in the sandy soil, 70 and 230 days after tracer injection. Standard deviations at each depth are indicated (n = 3).

2 months after tracer injection in forests and agroforestry systems (Rowe *et al.* 2001; Soethe, Lehmann & Engels 2006; Göransson, Ingerslev & Wallander 2008). The vertical dis-placement of tracers within 70 days after their application was probably low and should not have biased the comparison of the RUP and the SRUP between depths. Soil trenches

down to a depth of 10 m at our study site (in the two soil types) did not reach the water table (unpublished data), and capillary rise in the upper 3 m of soil could therefore be ignored. Soil solution monitoring over the rotation in a nearby eucalypt plantation established on a sandy soil showed that the peaks of K^+ and NO_3^- observed in soil solutions at a depth of 15 cm after fertilizer application only reached a depth of 100 cm after 4–5 months of the rainy season (Laclau *et al.* 2010). Fine root densities did not change dramatically within the possible distance of tracer displacement 70 days after injection, and the overall pattern of root uptake was unlikely to be largely modified by the vertical displacement of tracers.

The dynamics of foliar tracer concentrations from 70 to 230 days after their application should be interpreted more cautiously. An accumulation of tracers within leaves during this period might result from uptake deeper than the depth of injection, and within-tree physiological processes may also influence the dynamics of foliar concentrations. In particular, large retranslocations of N and K (and their tracer ^{15}N and Rb) may occur in *Eucalyptus* leaves, whereas they are negligible for Ca (and its analogue Sr) (Fife, Nambiar & Saur 2008). Despite the difficulty in accurately interpreting the dynamics of foliar tracer concentrations over long periods, the increase in the RUPs and the SRUPs in the winter from 70 to 230 days after the injection of the three tracers at a depth of 300 cm suggested that root uptake in deep soil layers was significantly enhanced when gravitational waters reached that depth. A mass flow transport of tracers to roots might then have increased the uptake of the three tracers used in our study, despite their contrasting mobility in the soil.

Injecting large amounts of tracers close to the sampled trees made it possible to overlook possible drawbacks in the estimation of tracer uptake distributions. In our study, nutrient uptake was not compensated for differences in the immobilization of NO_3^--^{15}N, discrimination of ^{15}N against ^{14}N, dilution of the tracers in the soil or their sorption to soil particles between the studied depths. However, δ^{15}N natural abundance and soil properties were homogeneous within each soil type down to a depth of 300 cm at our study site (Table 1). A mineralogical study and adsorption isotherms of cations and anions on the same sandy soil in a nearby *E. grandis* plantation showed similar interactions between the solid phase and the soil solutions throughout the soil profile (Maquère 2008). Moreover, discrimination of K^+ against Rb^+ (Göransson, Ingerslev & Wallander 2008) and Ca^{2+} against Sr^{2+} (Blum *et al.* 2008) could be overlooked close to the injection zones where concentrations of tracers were much higher than the concentrations of NO_3^-, K^+ and Ca^{2+} monitored in soil solutions down to a depth of 300 cm in the two soils studied (unpublished data). The influence of antagonism or synergism processes (Barber 1995; Genenger *et al.* 2003; Rothstein & Cregg 2005) for the uptake of the three tracers applied simultaneously at the same location was ignored because we set out to assess relative tracer uptake potentials between depths and not absolute uptake values at a given depth. It cannot be excluded that leaves were the prime destination of the tracers taken up in the topsoil whereas a higher proportion of the tracers taken up in depth were transported to below-ground tree components. However, a similar pattern might occur for the three tracers and was unlikely to lead to a strong bias in the estimation of relative uptake rates with depth of *E. grandis* fine roots.

FUNCTIONAL SPECIALIZATION OF *EUCALYPTUS GRANDIS* FINE ROOTS

We found the RUP in the topsoil to be significantly higher for NO_3^--^{15}N than for Rb^+ and Sr^{2+}. A decrease in the RUP with soil depth occurred for the three tracers, but was much more marked for NO_3^- than for the analogues of K^+ and Ca^{2+}. In particular, the RUPs after tracer injection in the summer decreased sharply with depth for NO_3^--^{15}N whereas they were little affected by the injection depth for Rb^+ and were intermediate for Sr^{2+} (Fig. 5). This contrasting behaviour for tracers injected simultaneously at the same location suggests a functional specialization of *E. grandis* fine roots relative to soil depth. A strong relationship between the distributions of the RLDs and RUPs is commonly observed in forests and agroforestry systems established on deep tropical soils (Rowe *et al.* 2001; Soethe, Lehmann & Engels 2006), even though large differences in uptake rates across species have been found in deep soil layers (Lehmann 2003). The sharp decrease in the RLDs with soil depth found in our study is commonly observed at the end of the rotation for *Eucalyptus* plantations (Bouillet *et al.* 2002; Maurice *et al.* 2010). The RUP distributions in our study showed that fine root densities alone may not accurately reflect the nutrient uptake capacity of trees for certain nutrients and that the vertical distribution of nutrient uptake is largely dependent on soil water dynamics. As pointed out for other tree species (Göransson, Fransson & Jönsson-Belyazid 2007), our results showed that a better understanding of factors driving nutrient uptake in deep soil layers is required to improve current models of nutrient cycling in forest ecosystems that use the distribution of roots as the sole criterion for predicting uptake of nutrients from different soil depths.

The SRUP distributions suggested a specialization of upper fine roots in NO_3^- uptake and deep fine roots in potassium and calcium uptake. In agreement with our second hypothesis, the SRUPs were significantly higher at a depth of 300 cm than at a depth of 10 cm for Rb^+ and Sr^{2+}, whereas the contrary occurred for the NO_3^--^{15}N tracer applied simultaneously at the same positions. This pattern for the highly mobile NO_3^- showed that an overlap of the uptake zones around roots in the topsoil was unlikely to explain the large increase in the SRUP with soil depth observed for the analogues of K^+ and Ca^{2+} when soil water content was sufficient to transport ions by mass flow. Differences in the RLD between depths might have biased the estimation of the SRUP because tracers were added at a higher proportion for a given root length at depth than in the surface soil layers. Such bias should have been more pronounced for the highly mobile tracer

$NO_3^- $-$^{15}N$ than for Rb^+ and Sr^{2+} (Göransson, Ingerslev & Wallander 2008). However, differences in the SRUP between depths for a given tracer and between tracers for a given depth in our study were large, and consistent in the two soil types. That overall pattern strongly supported the hypothesis of root functional specialization.

Further studies will be needed to gain insight into the processes involved in fine root functioning of *Eucalyptus* trees. The SRUPs were about twice as high in the sandy soil as in the clayey soil for the three tracers. This pattern partly resulted from the 54% higher RLDs found in the clayey soil. However, it might also have resulted from differences in mycorrhizal abundance and diversity between the two soils (data not shown). Göransson, Fransson & Jönsson-Belyazid (2007) observed under standardized conditions that the uptake of *Quercus robur* excised roots decreased with increasing soil depth for NH_4^+ and Rb^+ but not for $H_2PO_4^-$. Another bioassay for excised roots showed that the decrease in Rb^+ uptake with soil depth observed for *Q. robur* roots was species dependent and did not occur for *Fagus sylvatica* L. and *Picea abies* (L.) Karst roots (Göransson *et al.* 2006). Göransson, Fransson & Jönsson-Belyazid (2007) suggested that (i) species evolution led to physiological and functional differences between roots depending on soil depth, as NH_4^+ and K^+ are mainly available in the topsoil in southern Sweden, whereas P availability is usually less depth dependent and (ii) depth-dependent uptake rates were because of morphological differences, such as the activity or number of transporters in the cell membranes. Physiological studies dealing with the influence of soil depth on the characteristics of transporters and channels on root cell membranes are required for a better understanding of the functional specialization of fine roots (Very & Sentenac 2003).

Conclusion

The highest uptake rates for the tracers were observed in the topsoil, but we found contrasting RUP distribution depending on nutrients and soil water contents. Whilst roots growing in the topsoil layer were likely to be specialized in nitrogen uptake, deep roots exhibited greater potentialities per unit of length to take up potassium and calcium analogues. The hypothesis of a relationship between soil nutrient availability in the natural area of *E. grandis* forests and tree uptake was supported by our study. Fine root functional specialization might be involved in the high growth rates of *E. grandis* plantations through efficient uptake of nutrients in considerable volumes of highly weathered soil. Further research will be needed to gain insights into the changes at depth in the physiology and mycorrhizal status of fine roots. In particular, the influence of mycorrhizal development in roots with soil depth on the differential patterns of uptake of nitrate vs. cations would deserve further attention. The occurrence of root functional specialization depending on soil depth for other tree species growing in their natural habitats on low-fertility tropical soils should be investigated.

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