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Distance from the trunk and depth of uptake of labelled nitrate for dominant and suppressed trees in Brazilian *Eucalyptus* plantations: Consequences for fertilization practices

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ABSTRACT

Climate changes will increase the probability of drought, which is likely to dramatically increase tree mortality. The capacity of trees to withdraw water in deep soil layers is an important trait likely to account for tree survival over prolonged droughts. Our study aimed to gain insight into the maximum distance from the trunk where *Eucalyptus* fine roots take up water and mobile nutrients in deep sandy soils during dry periods. NO_3^--^{15}N was injected in the soil at the end of the rainy season in commercial *Eucalyptus* stands planted with the same *E. urophylla* × *E. grandis* clone. The ^{15}N tracer was applied in the middle of the inter-row (replicated in 3 plots): at 5 depths (from 0.1 to 6 m) at age 0.6 year, at 4 depths (from 0.1 to 9 m) at age 1.2 years, at 5 depths (from 0.1 to 12 m) at age 2.2 years, and at 6 depths (from 0.1 to 15 m) at age 6.4 years. $\delta^{15}N$ was determined in leaves sampled in dominant and suppressed trees at different distances from each injection area, 4–5 months after NO_3^--^{15}N injection (after the dry season). While dominant trees took up NO_3^--^{15}N down to a depth of 6 m between 7 and 12 months after planting, the maximum depth of NO_3^--^{15}N uptake for suppressed trees was between 3 and 4.5 m. From 1.5 to 6 years after planting, a foliar enrichment in ^{15}N was mainly detected when the NO_3^--^{15}N tracer was injected in the upper soil layers and only for a few trees at a depth of 6 m. Most of the uptake of ^{15}N occurred within 2 m of horizontal distance from the injection site, whatever tree age and tree social status. Low amounts of NO_3^--^{15}N were taken up for injection sites located between 2 m and 5 m from the trunk, and ^{15}N uptake was never detected at horizontal distances greater than 6 m from the trunk. *Eucalyptus* fine roots can take up nitrates at depths between 6 and 8 m the first year after planting. However, the NO_3^--^{15}N tracer injected at a depth of 6 m was only taken up by dominant trees and a ^{15}N foliar enrichment of suppressed trees was only detected when the tracer was injected in the upper 3 m. Fertilizers must be applied within 2 m of the trunks in *Eucalyptus* plantations to be taken up by all trees, regardless of their social status. When fertilizations are concentrated the first months after planting in sandy soils, nutrient leaching in deep layers might increase the heterogeneity of the stands since mobile nutrients could only be taken up by dominant trees.

1. Introduction

Climate changes will increase the probability of exceptional droughts, which may dramatically increase tree mortality worldwide (Wu et al., 2011). Improving our understanding of the structural and physiological mechanisms by which trees maintain tissue hydration and photosynthesis in response to water deficit is therefore essential to predict the effects of climate change on tree survival, carbon sequestration, and use of water in forest ecosystems (McDowell et al., 2013; Klein et al., 2014; Venegas-González et al., 2018). A better

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understanding of the effects of tree nutrition on the mechanisms involved in tree response to drought is essential to adapt the silviculture to a probable future drier climate (Battie-Laclau et al., 2014). Both an increase of atmospheric carbon dioxide (Iversen, 2010; Nie et al., 2013) and prolonged droughts (Germon et al., 2019) should increase the exploration of deep soil layers by plant roots in the future. Deep rooting can enhance the tolerance of some plant species to drought making it possible to reach water stored in the subsoil (Chaves et al., 2003; Hoekstra et al., 2015). In a context of climate change, the maximum depth reached by plant roots is, therefore, an important trait to select genotypes tolerant to prolonged droughts (Comas et al., 2013; Pinheiro et al., 2016).

Roots have been little studied at depths of more than 5 m (Jackson et al., 1997; Pierret et al., 2016), and the maximum rooting depth has probably been underestimated in many tropical forests (Freycon et al., 2015). Most of the studies dealt with the description of fine root distributions and fine root dynamics throughout very deep soil profiles (Maeght et al., 2013; Pinheiro et al., 2016; Lambais et al., 2017). Even though labelling techniques have been used to locate the areas of nutrient uptake in the soil (Lehmann and Muraoka, 2001; Lehmann, 2003; Göttlicher et al., 2008), the contribution of deep roots to supply tree nutrient requirements is still poorly documented in highly weathered tropical soils (Poszwa et al., 2002; Pradier et al., 2017). A functional specialization of fine roots to take up cations depending on the depth has been shown for tree species (Göransson et al., 2008; da Silva et al., 2011) but the uptake of nitrate was little influenced by soil depth for *Eucalyptus grandis* trees (Bordron et al., 2019).

The depth of water uptake by tree roots is commonly estimated monitoring soil water contents throughout soil profiles (Guderle and Hildebrandt, 2015), through modelling approaches (e.g. Kumar et al., 2014; Christina et al., 2017), or using stable isotopes of hydrogen (deuterium, ^{2}H) and oxygen (^{18}O) (Beyer et al., 2016, 2018; Koeniger et al., 2016; Trogisch et al., 2016). Deuterium and ^{18}O techniques can be used to estimate the depth of water uptake when their natural abundances in soil solutions follow a clear gradient from the soil surface to deep soil layers (Roupsard et al., 1999; Brum et al., 2018). Injecting ^{2}H and ^{18}O at different depths makes it possible to detect the areas of water uptake in the soil at the date of sampling. For example, a dual-isotope labelling technique in a tropical rainforest (^{2}H applied at soil surface and ^{18}O injected at 120-cm depth) showed that some tree species had a high plasticity in their depth of water uptake, exhibiting an efficient strategy for water resource acquisition below 100-cm depth during dry periods (Stahl et al., 2013). However, the depth of water uptake in tropical forests can be highly variable from one week to another depending on the occurrence of rainfall events (Christina et al., 2017). It would be therefore necessary to repeat deuterium and ^{18}O sampling along the year to estimate the maximum distance of water uptake from trees. Moreover, isotopic techniques based on deuterium and ^{18}O measurements are expensive and time-consuming. Repeated measurements of ^{2}H and ^{18}O uptake at different depths in the soil throughout the year are therefore rare in tropical forests.

Da Silva et al. (2011) showed that ^{15}N from labelled nitrates injected at a depth of 3 m was only detected in young leaves of 6-year-old *Eucalyptus* trees when the gravitational soil solutions reached 3 m depth, which suggests that NO_3^--^{15}N uptake could be a tracer of water uptake in these plantations. Nitrate is highly mobile in soil solutions and the uptake of large amounts of nitrates is dependent on mass flow transport up to fine roots (Hinsinger et al., 2011; White et al., 2013; McMurtrie and Näsholm, 2018). Nitrate labelling could therefore be a proxy of the cumulative uptake of water between the date of NO_3^--^{15}N injection in the soil and leaf sampling a few months later, since a clear enrichment in foliar ^{15}N content can only occur if large amounts of NO_3^--^{15}N are transported through mass flow up to fine roots at the vicinity of the injection area.

Eucalyptus planted forests cover more than 20 million hectares worldwide and are expanding rapidly in tropical and subtropical regions (Booth, 2013; Gonçalves et al. 2013). In Brazil, these fast-growing plantations are intensively managed over 7.4 million hectares and account for 75% of the total area of planted forests (IBGE, 2017). However, highly productive *Eucalyptus* plantations are sensitive to prolonged drought periods. The cumulative area affected by tree mortality as a result of drought over the last decade in the state of Minas Gerais (the largest producer of *Eucalyptus* in Brazil) was about 200,000 ha (Gonçalves et al., 2017). The variability among trees within the same stand to reach water and nutrients far from the trunk (horizontally and in depth) might be an important factor likely to help explain why only some trees survive during drought periods. While the influence of deep roots on drought-adaptative mechanisms of tree species is well documented (McDowell et al., 2008; Fang and Xiong, 2015), the relationship between the social status of trees and their ability to withdraw water in very deep soil layers has been little investigated in monospecific forests.

Our study aimed to assess the maximum distance from the trunk where *Eucalyptus* fine roots take up water and mobile nutrients in soil solutions in very deep sandy soils. We hypothesized that mobile nutrients are taken up farther from the trunk and more deeply for dominant trees than for suppressed trees.

2. Material and methods

2.1. Study site

Our study was carried out in one *Eucalyptus* stand at Avaré (23°00′41″S, 48°55′10″W) and in two neighboring *Eucalyptus* stands at Itatinga (23°14′54″S, 48°35′28″W), in southeast Brazil (São Paulo State). The distance between the stands sampled at Itatinga and Avaré was 40 km. The same clone of the *Eucalyptus urophylla* (S.T. Blake) × *Eucalyptus grandis* (Hill ex Maid) hybrid was managed by the Suzano SA Company at both sites with the same silvicultural practices. The annual rainfall and the mean temperature over the study period were 1578 mm and 20.2 °C at Itatinga, and 1980 mm and 20.6 °C at Avaré, respectively (Fig. 1). The soils at both sites were very deep Ferralic Arenosols (FAO classification) developed on Cretaceous sandstone of the Marilia formation, Bauru group. This soil type is common for commercial *Eucalyptus* plantations in Brazil (Table 1). The topography was a plateau at an elevation of 700–900 m at both sites and the water table was very deep (more than 15 m, which was the deepest hole made to inject the tracer in our study).

2.2. Experimental design

Experiments comparing plots with all trees planted in a single day (uniform treatment) with plots where planting was spread over 80 days (heterogeneous treatment) showed that the social status of the trees is established very early in monoclonal *Eucalyptus* plantations (Binkley et al., 2010). Suppressed trees the first months after planting in these experiments remain suppressed throughout the entire rotation.

In the youngest stand at Avaré, 15 circular plots (20 m in radius) were randomly located with a minimum distance of 50 m between the centre of each plot: 5 depths of tracer injection (0.1, 1.5, 3.0, 4.5 or 6.0 m) and 3 replications per depth. At 3 months after planting, 50% of the trees were totally defoliated manually in each plot to induce a variability of growth between trees making it possible to study dominant and suppressed trees. This manual defoliation mimicked the activity of ants, which can remove all the leaves from young *Eucalyptus* trees. The NO_3^--^{15}N tracer was applied in the middle of the inter-row at the centre of each plot, at age 0.6 years (7 months after planting) (Fig. 2).

At Itatinga, 27 circular plots (20 m in radius) were randomly located in a 9-month-old stand, with a minimum distance of 50 m between the centre of each plot: (i) 12 plots to apply the NO_3^--^{15}N tracer at 4 depths (0.1, 3.0, 6.0 or 9.0 m) and 3 replications per depth at 1.2 years after

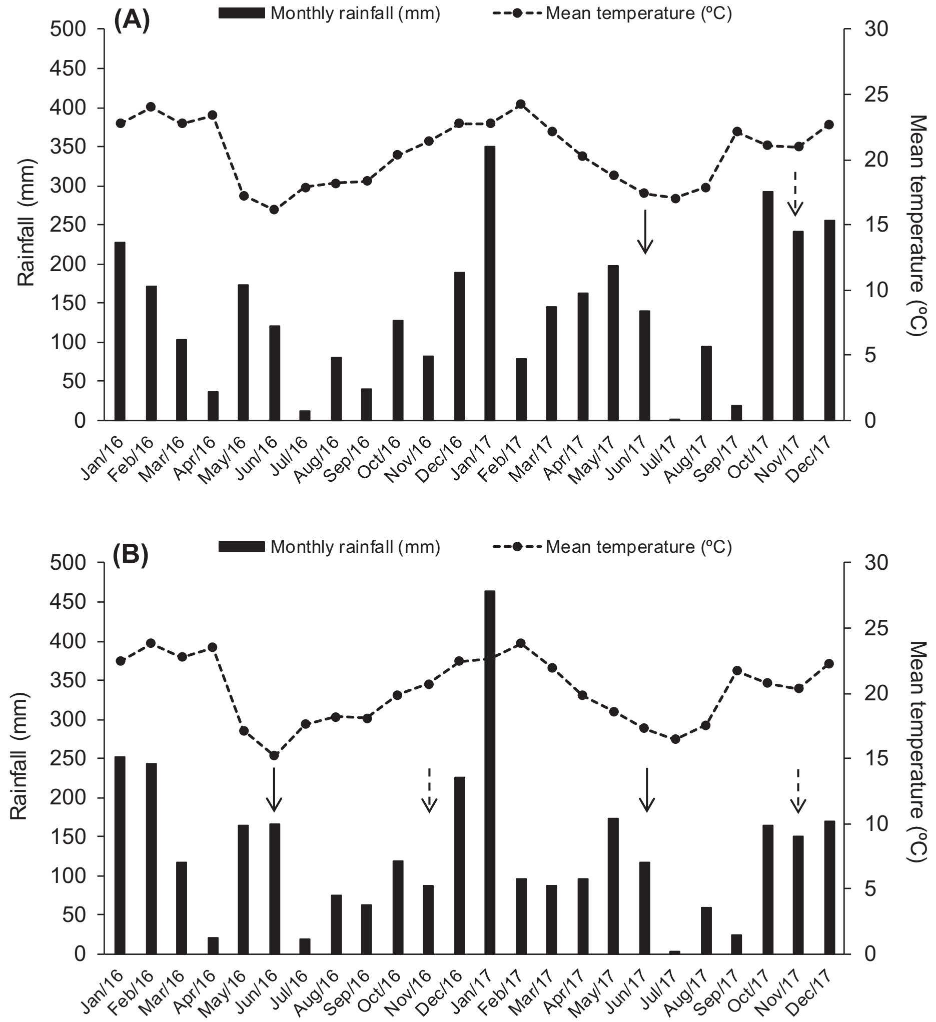

Fig. 1. Time course of monthly rainfall and mean temperature over the study period at Avaré (A) and at Itatinga (B). Continuous arrows show the ^{15}N tracer injection periods in 1.2- and 6.4-year-old stands in June 2016, and in 0.6- and 2.2-year-old stands in June 2017. Dashed arrows indicate the leaf sampling periods, five months after tracer injection.

planting, and (ii) 15 plots to apply the NO_3^--^{15}N tracer at 5 depths (0.1, 3.0, 6.0, 9.0 or 12.0 m) and 3 replications per depth at 2.2 years after planting. In order to induce a growth heterogeneity among trees in this stand, 50% of the trees were defoliated manually (about 80% of leaf area removed) at age 9 months. The NO_3^--^{15}N tracer was applied in the middle of the inter-row at the centre of each plot.

In a 6-year-old stand at Itatinga (near the other sampled stand), 18 circular plots (20 m in radius) were randomly located with a minimum distance of 50 m between the centre of each plot. The NO_3^--^{15}N tracer was applied at age 6.4 years at the centre of each plot. The 18 plots in this stand corresponded to 6 depths of tracer injection (0.1, 3.0, 6.0, 9.0, 12.0 or 15.0 m) and 3 replications per depth. The dominant and suppressed trees sampled in this stand were selected based on the natural variability of tree growth during development (50% of the young trees were not defoliated as in the other sampled stands).

An inventory in each stand at the date of NO_3^--^{15}N tracer injection showed large differences in the basal area between dominant and suppressed trees (Appendix 1). In each plot, 4 dominant and 4 suppressed trees were selected at different distances from the injection site to sample recently expanded leaves in the upper part of the crown. The distances between the trunk of the sampled trees and the injection site were approx. 2 m, 4 m, 6 m and 10 m in the youngest stand, and approx. 2 m, 6 m, 10 m and 14 m in the other stands (Fig. 2). For each depth of tracer injection at each stand age, a total of 24 trees were sampled (4 dominant and 4 suppressed trees in 3 plots).

2.3. Tracer applications

Our study (aiming to assess the maximum depth where labelled nitrates can be taken up) was carried out during the dry season because a recent study showed that *Eucalyptus* trees withdraw preferentially water in the topsoil after rainfall events (Christina et al., 2017). One day before application in the field, a labelled solution was prepared in the laboratory and maintained at 4 °C. 16.6 g of $NH_4{}^{15}NO_3$ commercial compound (10 atom% or 29,221.98‰ NO_3^--^{15}N) dissolved in 20 ml of distilled water were injected at a single depth at the centre of each plot. The holes were drilled down to the target application depth using a cylindrical auger with an inner diameter of 9 cm and the soil water content was measured every meter in all samples collected during drilling (Appendix 2). Soil blocks from the inner part of the auger (to avoid any contamination with fine roots from upper soil layers) were collected for the deepest meter in each hole to assess fine root densities close to the area of NO_3^--^{15}N injection.

A PVC tube (2.5 cm in diameter) was inserted into each hole to avoid any contamination of the soil with NO_3^--^{15}N during tracer application. A 0.4-cm polyethylene tube, attached to an iron rod, was inserted into the PVC tube, with a length depending on the application depth. A plastic sheet was placed around each hole to avoid any contamination with NO_3^--^{15}N at the soil surface. 20 ml of the labelled solution was applied at the selected depth using a syringe. Thereafter, 280 ml of distilled water were injected to rinse the polyethylene tube

Table 1
Main physical and chemical soil properties at Itatinga and Avaré. Mean values and standard errors are indicated (n = 3).

Soil layer	Sand	Silt	Clay	pH	O. M.	P_{resin}	H + Al	K	Ca	Mg	SB	CEC
(m)	Particle size distribution (%)			$CaCl_2$	g·kg^{-1}	mg·kg^{-1}	mmolc·kg^{-1}					
Itatinga												
0–0.25	92.8 ± 0.3	1.4 ± 0.5	5.8 ± 0.4	4.20 ± 0.18	14.13 ± 3.50	12.59 ± 3.47	27.94 ± 4.96	0.36 ± 0.04	22.16 ± 4.95	4.54 ± 0.33	27.05 ± 4.79	55.00 ± 6.20
0.25–0.5	91.0 ± 0.4	2.1 ± 0.4	6.9 ± 0.5	4.04 ± 0.06	6.48 ± 0.35	3.87 ± 0.91	24.26 ± 2.59	0.26 ± 0.11	15.48 ± 0.55	2.68 ± 0.58	18.42 ± 0.68	42.68 ± 3.22
0.5–1	89.3 ± 0.4	1.9 ± 0.4	8.8 ± 0.1	4.03 ± 0.06	5.52 ± 0.36	2.48 ± 0.41	18.87 ± 2.41	0.22 ± 0.01	14.36 ± 1.01	2.05 ± 0.35	16.63 ± 0.76	35.49 ± 2.94
1–2	88.7 ± 0.4	2.0 ± 0.9	9.3 ± 0.6	4.07 ± 0.11	5.63 ± 0.25	2.28 ± 0.27	17.92 ± 2.71	0.35 ± 0.22	12.58 ± 3.20	2.29 ± 0.33	15.21 ± 3.35	33.14 ± 4.01
2–4	88.8 ± 0.7	1.8 ± 0.8	9.4 ± 0.2	4.23 ± 0.20	3.28 ± 0.88	1.87 ± 0.37	11.59 ± 1.74	0.35 ± 0.24	12.54 ± 1.52	2.57 ± 0.34	15.46 ± 1.86	27.04 ± 1.87
4–6	86.9 ± 1.3	2.6 ± 0.5	10.5 ± 0.8	4.17 ± 0.10	2.66 ± 0.67	1.59 ± 0.21	10.38 ± 0.80	0.16 ± 0.04	12.27 ± 2.05	2.47 ± 0.20	14.89 ± 2.15	25.27 ± 2.53
6–8	87.1 ± 0.5	2.3 ± 1.0	10.7 ± 0.6	4.20 ± 0.03	1.99 ± 0.23	2.08 ± 0.61	10.29 ± 0.52	0.14 ± 0.00	12.44 ± 0.49	2.12 ± 0.13	14.70 ± 0.44	24.99 ± 0.14
8–10	86.3 ± 0.2	3.8 ± 1.5	9.9 ± 1.5	4.19 ± 0.07	1.93 ± 0.14	1.73 ± 0.09	9.81 ± 0.42	0.14 ± 0.00	15.32 ± 3.39	1.87 ± 0.24	17.34 ± 3.16	27.15 ± 3.42
10–12	87.0 ± 0.4	2.4 ± 0.3	10.6 ± 0.3	4.12 ± 0.08	2.27 ± 0.21	1.77 ± 0.18	10.17 ± 0.44	0.14 ± 0.00	13.63 ± 3.48	2.11 ± 0.27	15.88 ± 3.66	26.05 ± 3.96
Avaré												
0–0.25	84.7 ± 2.4	3.4 ± 1.6	11.9 ± 1.6	4.13 ± 0.39	22.5 ± 4.84	8.24 ± 2.51	43.36 ± 10.73	0.81 ± 0.47	8.82 ± 6.27	3.16 ± 2.13	12.79 ± 8.38	56.15 ± 5.92
0.25–0.5	81.8 ± 2.3	4.8 ± 1.9	13.4 ± 1.1	3.94 ± 0.10	9.82 ± 0.46	4.27 ± 1.13	37.78 ± 4.66	0.38 ± 0.17	6.59 ± 8.96	1.41 ± 1.52	8.38 ± 10.64	46.16 ± 8.05
0.5–1	81.5 ± 1.6	4.7 ± 0.9	13.8 ± 1.2	3.87 ± 0.06	8.81 ± 1.54	2.49 ± 0.61	30.34 ± 2.07	0.26 ± 0.18	4.88 ± 6.85	1.11 ± 1.03	6.25 ± 8.07	36.59 ± 7.65
1–2	77.3 ± 3.7	6.3 ± 3.8	16.4 ± 0.6	3.97 ± 0.05	6.43 ± ± 1.24	1.89 ± 0.13	23.83 ± 3.64	0.24 ± 0.08	7.63 ± 11.58	1.51 ± 1.70	9.39 ± 13.35	33.23 ± 9.98
2–4	77.6 ± 2.8	5.6 ± 2.8	16.7 ± 0.3	4.21 ± 0.05	4.12 ± 0.79	1.93 ± 0.14	14.75 ± 0.72	0.13 ± 0.05	6.38 ± 8.63	1.34 ± 1.41	7.86 ± 10.07	22.61 ± 10.03
4–6	75.9 ± 1.5	5.6 ± 1.0	18.5 ± 0.9	4.33 ± 0.04	2.55 ± 0.58	1.96 ± 0.12	11.80 ± 0.40	0.16 ± 0.00	4.96 ± 6.17	1.22 ± 1.20	6.33 ± 7.37	18.13 ± 7.59

and to promote nitrate uptake in the soil area where the tracer was applied. Lastly, the polyethylene and PVC tubes were carefully withdrawn from the holes, which were filled with the soil removed during drilling, respecting the original order of the soil layers. Similar methodologies were used by da Silva et al. (2011) and Bordron et al. (2019) to study fine root specialization to take up nutrients in *Eucalyptus* plantations.

2.4. Leaf sampling

Thirty young leaves (from 2 weeks to 3 months after bud emergence) were collected in the upper half of the crown of each sampled tree, about 5 months after tracer injection. da Silva et al. (2011) showed that the enrichment with ^{15}N of leaves sampled in the upper crown of *Eucalyptus grandis* trees was roughly constant between 2 and 7 months after NO_3^--^{15}N injection in the soil.

In each *Eucalyptus* stand, young leaves were also sampled in 12 control trees (6 dominant and 6 suppressed trees) located far from the injection site (> 50 m) in order to estimate the prediction interval of foliar $\delta^{15}N$ values in natural abundance. Control trees were sampled away from each other throughout the stand to cover the variability of the ^{15}N natural abundance. In the young stands (1 year and 1.6 years after planting), the leaves were collected from the ground using a long pruner. In the 2.7- and in the 6.8-year-old stands, a professional team of tree climbers used a 5-m pruner to cut the branches from the selected trees. In total, 30 young leaves (from 2 to 4 branches per tree) were collected on 132, 108, 132 and 156 trees at 1, 1.6, 2.7 and 6.8 years after planting, respectively.

2.5. Isotopic analyses of ^{15}N

In the laboratory, the leaves were washed in deionized water and oven-dried at 65 °C for 72 h. The dried samples were ground (< 60 µm) in a cryogenic mill (2010 Geno Grinder, SPEX Sample Prep, Metuchen, USA) at −196 °C for homogenization and stored in sealed acrylic pots until isotopic analysis. An aliquot of 4500–5000 µg of each dry and milled sample was weighed into a 6 mm height, 4 mm diameter cylindrical tin capsule (D1106 - Elemental Microanalysis, Okehampton, UK) with a 1 µg resolution scale (XP6 - Mettler Toledo, Greifensee, Switzerland). The ^{15}N analyses were performed at the Centre for Stable Isotopes at São Paulo State University using a continuous flow system in a CF-IRMS isotope ratio mass spectrometer (Delta V Advantage - Thermo Scientific, Bremen, Germany) coupled to an elemental analyzer (Flash 2000 OEA - Thermo Scientific, Bremen, Germany) with an interface (ConFlo IV Universal - Thermo Scientific, Bremen, Germany) that determines the isotope ratio of the sample ($R_{sample} = {}^{15}N/{}^{14}N$).

The ^{15}N values in isotopic delta ($\delta^{15}N$, expressed in ‰) were calculated according to the following equation (Coplen, 2011):

$$\delta^{15}N = \left(\frac{R_{sample}}{R_{air}} - 1\right)\bullet 1000‰$$

where R_{sample} is the $^{15}N/^{14}N$ ratio in the sample and R_{air} is the $^{15}N/^{14}N$ ratio of atmospheric air (R_{air} = 0.0036765) as an international standard. The standard uncertainty in $\delta^{15}N$ was ± 0.3‰ for samples slightly enriched with ^{15}N.

2.6. Data analysis

For each *Eucalyptus* stand, a prediction interval with a threshold of 99% for $\delta^{15}N$ natural abundance was calculated from the $\delta^{15}N$ values of the 12 control trees using the equation $\bar{X}_n \pm T_a s_n \sqrt{1 + 1/n}$, where $\bar{X}_n$ and s_n are the average and standard deviation of the control values, n the number of observations, and T_a the 99.5 percentile of a Student's t-distribution with (n-1) degrees of freedom (Geisser, 1993). The statistical software R was used (R Core Team, 2019). Foliar $\delta^{15}N$ values

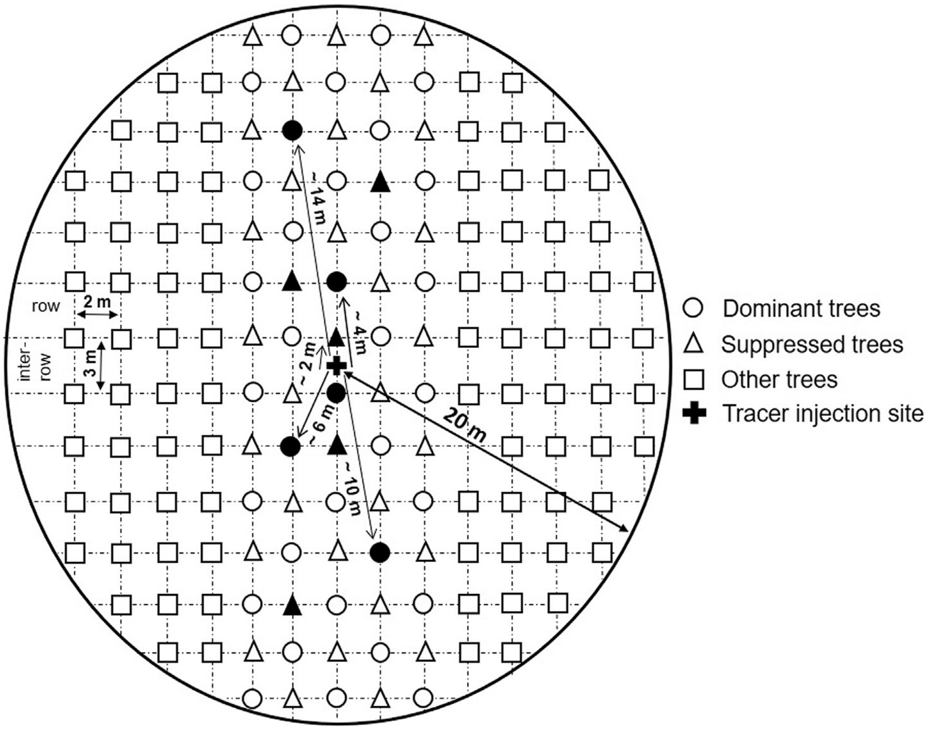

Fig. 2. Layout of a sampled plot. The ^{15}N tracer was injected at the centre of the plot. Solid circles and triangles indicate the location of dominant and suppressed sampled trees, respectively. Undisturbed trees (square symbol) in the plot are indicated. The horizontal distances between the sampled trees and the injection site ranged between 1 m and 14 m.

higher than the upper boundary of the prediction interval were considered significantly different from the control population ($p < 0.01$), which indicated that the sampled trees took up the NO_3^--^{15}N tracer injected in the same plot. The $\delta^{15}N$ values of all the sampled tree (dominant and suppressed) were plotted relative to the horizontal distance between the trunk and the tracer injection site.

3. Results

3.1. NO_3^--^{15}N uptake from 0.6 to 1 year after planting

Foliar $\delta^{15}N$ values showed that the trees within 2 m from the injection site (both dominant and suppressed) took up the largest amounts of the NO_3^--^{15}N tracer injected at a depth of 0.1 m (Fig. 3A). The highest $\delta^{15}N$ value was measured in a suppressed tree (273‰). The foliar $\delta^{15}N$ value was slightly above the upper boundary of the prediction interval of natural abundance for one dominant tree at a distance of 4 m from the injection site. The behavior was similar for the injection depth of 1.5 m, but with lower foliar $\delta^{15}N$ peaks in dominant and suppressed trees than for the injection depth of 0.1 m (data not shown).

When NO_3^--^{15}N was injected at a depth of 3 m, both dominant and suppressed trees took up the tracer within 2 m from the injection site (Fig. 3B). Only dominant trees took up the NO_3^--^{15}N tracer applied at a depth of 4.5 m and only within 2 m from the injection site. High foliar $\delta^{15}N$ values (greater than 100‰) showed that large amounts of tracer were taken up by those trees (Fig. 3C). At an injection depth of 6 m, only dominant trees took up the NO_3^--^{15}N tracer (foliar $\delta^{15}N$ values reached 94.4‰ in one tree) and one dominant tree took up the tracer at a distance of 3.7 m from the injection site (Fig. 3D). Soil coring to inject NO_3^--^{15}N showed that fine roots were present in all the areas of tracer injection down to a depth of 4.5 m in the 0.6-year-old stand (Appendix 3). However, it cannot be excluded that fine roots belonging to the previous *Eucalyptus* stand (before clearcutting and replanting) were not distinguishable from the fine roots of young trees. While soil texture was roughly constant throughout the soil profile (Table 1), soil water contents sharply increased below the depth of 4 m at age 0.6 years (Appendix 2), which suggests that water was mainly withdrawn by tree roots in the upper 4 m before the first dry season.

3.2. NO_3^--^{15}N uptake from 1.2 to 1.6 years after planting

Dominant and suppressed trees took up large amounts of the NO_3^--^{15}N tracer applied at a depth of 0.1 m, within 2 m from the injection site with a peak of foliar $\delta^{15}N$ at 420‰ for a suppressed tree. The foliar $\delta^{15}N$ value in one suppressed tree at a distance of 7.5 m from the injection site was slightly above the upper boundary of the prediction interval of natural abundance (Fig. 4A). When the NO_3^--^{15}N tracer was applied at a depth of 3 m, foliar $\delta^{15}N$ values were higher than the upper boundary of the prediction interval of natural abundance for all the dominant trees located within 2 m from the injection site and only for one suppressed tree. No tracer uptake was detected at greater distances from the injection site (Fig. 4B). Only dominant trees within a distance of 2 m from the injection site took up large amounts of NO_3^--^{15}N applied at a depth of 6 m. Foliar $\delta^{15}N$ values were slightly above the upper boundary of the prediction interval of natural abundance for two trees at a distance of 6 m from the injection site (Fig. 4C). Although some fine roots were sampled at a depth of 9 m when the NO_3^--^{15}N tracer was injected (Appendix 3), the foliar $\delta^{15}N$ values for all the sampled trees were within the interval of prediction of natural abundance, which showed that large amounts of the tracer were not taken up by the sampled trees at this depth.

3.3. NO_3^--^{15}N uptake from 2.2 to 2.7 years after planting

Large amounts of the NO_3^--^{15}N tracer injected at a depth of 0.1 m were taken up both by dominant and suppressed trees within 2 m from the injection site (Fig. 5A). The same pattern was observed when the NO_3^--^{15}N tracer was applied at a depth of 3 m (Fig. 5B). Only one dominant tree took up a detectable amount of NO_3^--^{15}N at a depth of 6 m and it was located within 2 m from the injection site (Fig. 5C). Foliar $\delta^{15}N$ values for all the sampled trees were within the prediction interval of natural abundance in the plots where the NO_3^--^{15}N tracer was injected at depths of 9 m and 12 m (data not shown). Soil coring to inject NO_3^--^{15}N showed that fine roots were present in all the areas of tracer injection down to a depth of 12 m in the 2.2-year-old stand

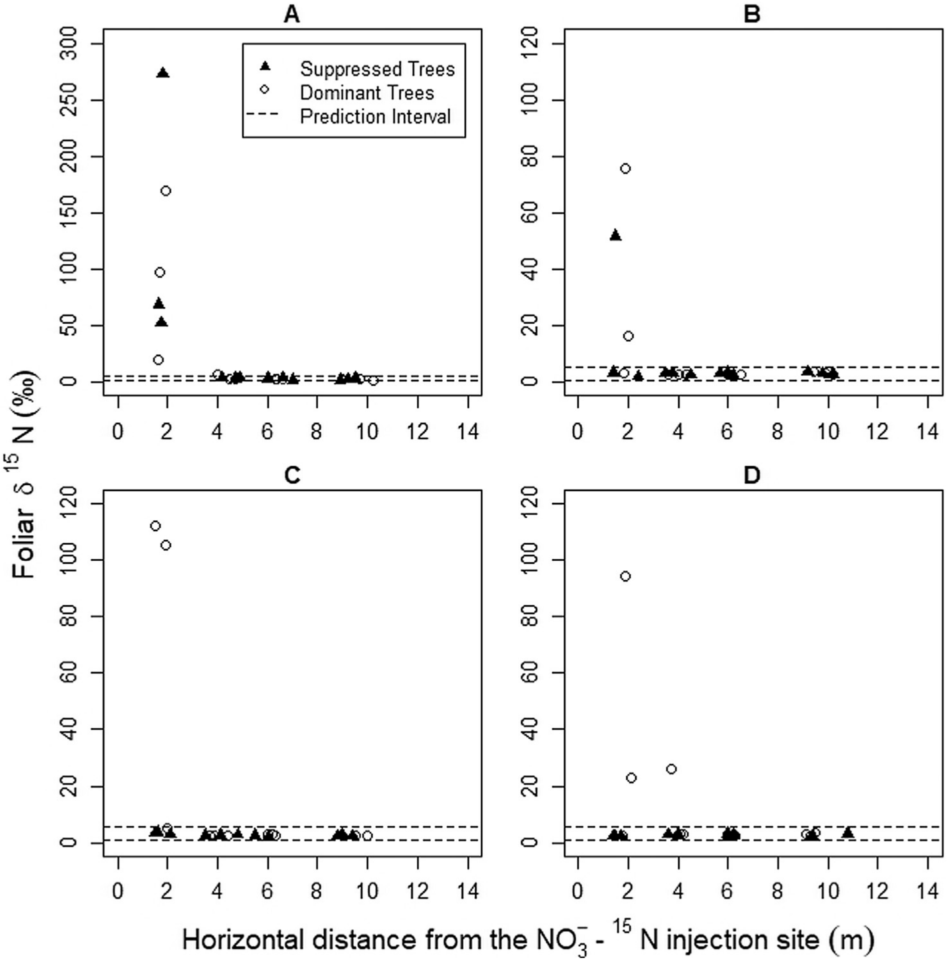

Fig. 3. Foliar δ^{15}N values in 1-year-old *E. urophylla* × *E. grandis* trees, 5 months after application of NO_3^--^{15}N tracer (A) at a depth of 0.1 m, (B) at a depth of 3 m, (C) at a depth of 4.5 m, and (D) at a depth of 6 m. Dominant trees (empty circle) and suppressed trees (solid triangle) were sampled. The prediction interval ($P < 0.01$) was calculated from δ^{15}N values in natural abundance measured for 12 trees (6 dominant and 6 suppressed trees). The lower and the upper boundaries of the prediction interval calculated at this age were 0.8‰ and 5.5‰, respectively.

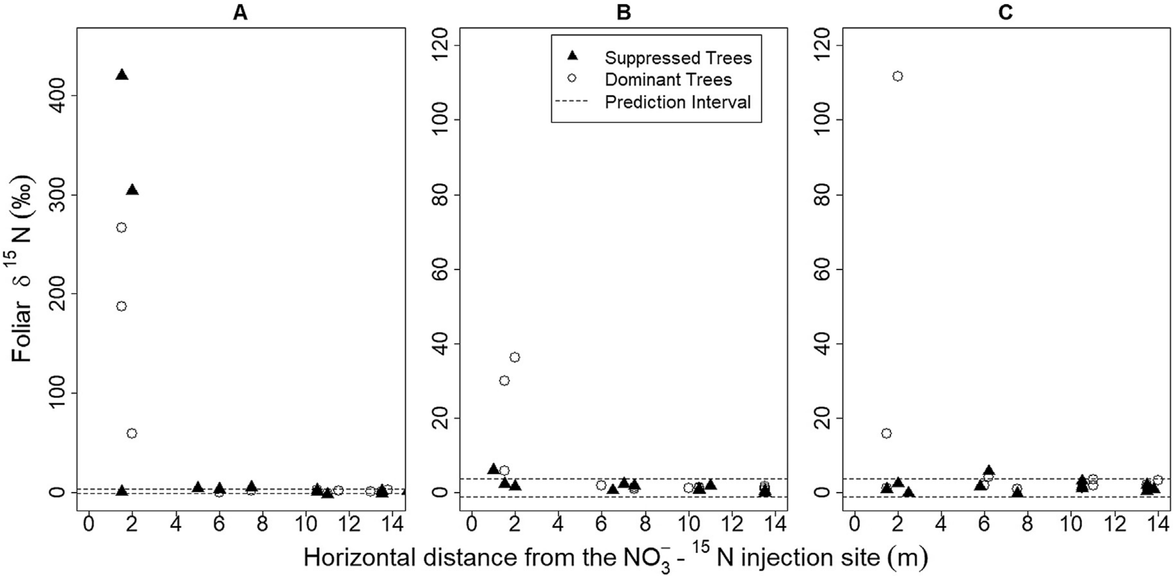

Fig. 4. Foliar δ^{15}N values in 1.6-year-old *E. urophylla* × *E. grandis* trees, 5 months after application of the NO_3^--^{15}N tracer (A) at a depth of 0.1 m, (B) at a depth of 3 m, and (C) at a depth of 6 m. Dominant trees (empty circle) and suppressed trees (solid triangle) were sampled. The prediction interval ($P < 0.01$) was calculated from δ^{15}N values in natural abundance measured for 12 trees (6 dominant and 6 suppressed trees). The lower and the upper boundaries of the prediction interval calculated at this age were −1.2‰ and 3.6‰, respectively.

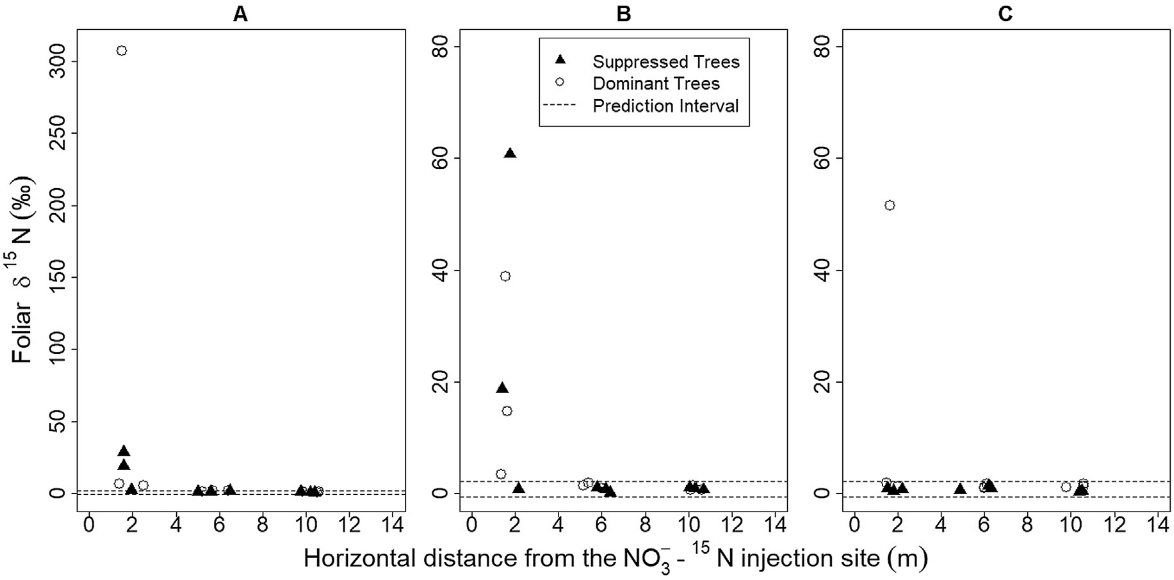

Fig. 5. Foliar $\delta^{15}N$ values in 2.7-year-old *E. urophylla* × *E. grandis* trees, 5 months after application of NO_3^--^{15}N tracer (A) at a depth of 0.1 m, (B) at a depth of 3 m, and (C) at a depth of 6 m. Dominant trees (empty circle) and suppressed trees (solid triangle) were sampled. The prediction interval ($P < 0.01$) was calculated from $\delta^{15}N$ values in natural abundance measured for 12 trees (6 dominant and 6 suppressed trees). The lower and the upper boundaries of the prediction interval calculated at this age were −0.6‰ and 2.2‰, respectively.

(Appendix 3). Changes in soil water contents between 1.2 and 2.2 years after planting, in the same stand (in June 2016 and June 2017), suggest that tree roots withdrew water between the depths of 4 m and 9 m (Appendix 2).

3.4. NO_3^--^{15}N uptake from 6.4 to 6.8 years after planting

A foliar enrichment with NO_3^--^{15}N was only detected when the NO_3^--^{15}N tracer was injected in the upper soil layer at a depth of 0.1 m. Both dominant and suppressed trees took up NO_3^--^{15}N within 3 m from the injection site. The suppressed trees had higher $\delta^{15}N$ values within leaves than the dominant trees, with a peak at 349‰ (Fig. 6A). At all the other depths of injection of the NO_3^--^{15}N tracer (i.e. 3, 6, 9, 12 and 15 m), foliar $\delta^{15}N$ values were within the prediction interval of natural abundance for all the sampled trees (Fig. 6B and 6C - data not shown below 6 m of depth). Soil coring to inject NO_3^--^{15}N in the 6.4-year-old stand showed that fine roots were present in all the areas of tracer injection down to a depth of 15 m (Appendix 3).

4. Discussion

4.1. Reliability of our method to detect the maximum distance of water uptake from trees

The depths of NO_3^--^{15}N uptake throughout the rotation in our study are consistent with other studies estimating the depth of water

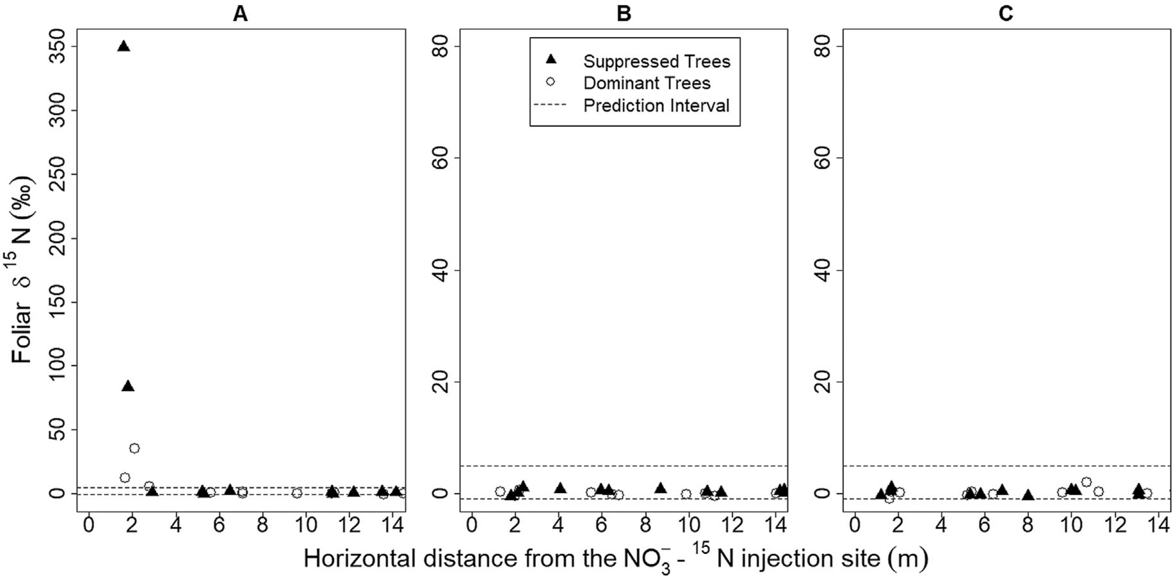

Fig. 6. Foliar $\delta^{15}N$ values in 6.8-year-old *E. urophylla* × *E. grandis* trees, 5 months after application of NO_3^--^{15}N tracer (A) at a depth of 0.1 m, (B) at a depth of 3 m, and (C) at a depth of 6 m. Dominant and suppressed trees were sampled. The prediction interval was calculated from $\delta^{15}N$ values in natural abundance measured for 12 trees sampled at more than 50 m from all the injection points. The lower and the upper boundaries of the prediction interval calculated at this age were −0.9‰ and 5.0‰, respectively.

withdrawal in tropical *Eucalyptus* plantations, which suggests that the uptake of labelled nitrate could be a simple proxy of cumulative water uptake in specific soil areas. In particular, a modelling approach based on soil moisture and eddy-covariance measurements over 4.5 years after planting in a commercial *Eucalyptus* stand at about 20 km from our study sites (in a similar soil) showed that water was withdrawn from the soil surface to a depth of 6–7 m the first year after planting, from the soil surface to a depth of 10 m the second dry season, then only in the upper soil layers and close to the water table at a depth of more than 12 m until harvesting (Christina et al., 2017). The depth of uptake of labelled nitrate by *Eucalyptus* trees in our study is consistent with the dynamics of water uptake throughout deep soil profiles over long periods in other *Eucalyptus* plantations in the same region (Laclau et al., 2013; Marsden et al., 2013; Christina et al., 2018). However, a strong demonstration of the validity of our method (enrichment in foliar ^{15}N after injection of NO_3^--^{15}N in the soil) as a proxy of water uptake by tree roots would require to monitor foliar ^{15}N as well as ^{2}H and ^{18}O in the trees after the injection in the same soil areas of NO_3^--^{15}N, ^{2}H and ^{18}O tracers.

Although we injected NO_3^--^{15}N down to a depth of 15 m, we did not detect any significant uptake of labelled nitrate at depths of more than 9 m. This pattern does not demonstrate that water was not taken up at these depths but suggests that large amounts of labelled nitrate were not taken up by neighboring trees. Fine root densities are very low at depths of more than 6 m in *Eucalyptus* plantations (Christina et al., 2011; Laclau et al., 2013), and it is possible that the trees sampled to determine foliar ^{15}N contents in our study did not explore the small soil areas where the tracer was injected. The simple method used here to detect water and nitrate uptake is also probably less sensitive than isotopic methods based on ^{2}H and ^{18}O measurements to detect small amounts of water taken up throughout the study period.

4.2. Influence of the social status of the tree on the distance of uptake of the labelled nitrate

Our study clearly shows in monoclonal stands that dominant trees have a higher capacity to use soil resources in very deep soil layers than suppressed trees. From 0.6 to 1 year after planting, most of the trees close to the injection sites took up NO_3^--^{15}N in the upper 3 m of the soil profile and only dominant trees took up the tracer at depths of 4.5 m and 6 m. While some dominant trees took up large amounts of ^{15}N at a depth of 6 m the second and third years after planting, the lack of clear enrichment in foliar ^{15}N for all the suppressed trees suggests that their ability to access to soil resources in very deep soil layers was low relative to dominant trees.

This pattern is consistent with a recent study in a seasonal Amazon forest using the stable isotope ratios (δ^{18}O and δ^2H) of water collected from tree xylem and soils down to a depth of 10 m during an extreme dry event. Rooting depth differences were strongly related to tree size and water uptake by understory species was limited to shallow soil layers. Coexisting trees were mainly segregated along a single hydrological niche axis defined by root depth, access to light and tolerance of low water potential (Brum et al., 2018). Our results in monoclonal *Eucalyptus* stands suggest that the strong relationship between tree size and rooting depth showed for co-existing species in this tropical forest could be valid between big and small trees of the same species. However, another isotopic study in a tropical rainforest showed that tall trees preferentially extracted water from layers below a depth of 1 m, while smaller trees exhibited large variations in the mean depth of water uptake, which prevented the use of tree dimensions to parameterize functional models in this multi-specific forest (Stahl et al., 2013). Further studies on the relationship between tree size and water uptake down to a depth of 10 m (or more) are needed to get an insight into the sharing of limited water resources between plants during drought events in tropical forests.

Root exploration limited to shallow soil layers for small trees seems contradictory with the observation that suppressed trees can survive better to drought events than dominant trees (Bennett et al., 2015; McDowell and Allen, 2015). However, many factors other than the maximum depth reached by fine roots can account for tree survival during drought periods. Low water demand of suppressed trees relative to dominant trees might help avoid hydraulic failure during drought (Grote et al., 2016). While drought periods impacted less the growth of suppressed trees relative to dominant trees in some forests (Martín-Benito et al., 2008; Martínez-Vilalta et al., 2012; Zang et al., 2012), the pattern was less clear in other forests and a more pronounced impact of drought in the understory than for dominant trees has also been reported (Castagneri et al., 2012; Trouvé et al., 2014; Merlin et al., 2015).

The horizontal distance of NO_3^--^{15}N uptake from the trunk was not clearly influenced by the social status of the trees in our study. Only a small proportion of the sampled trees took up the NO_3^--^{15}N tracer, which is consistent with an uneven distribution of tree roots shown in other studies. In monospecific boreal forests, the average lateral root spread of trees was 4–5 m but not all trees took up the ^{15}N tracer close to the injection area, which suggested that the root system was highly asymmetric (Göttlicher et al., 2008). Throughout deuterium pulses, small trees in an Amazonian tropical forest showed different rooting patterns, while most species took up the deuterium tracer only close to the trunk, a few species had high concentrations of deuterium up to 10 m of horizontal distance from the injection area (Sternberg et al., 2002).

4.3. Consequences for the management of Eucalyptus stands in deep sandy soils

Our study was also designed to answer a question from forest managers in Brazil: which proportion of the inter-rows in *Eucalyptus* plantations must be fertilized (after weeding) to make it possible for all the trees to take up the applied nutrients? Fertilization and weeding are seasonal activities with a peak at the mid-rainy season, and an extension of these activities throughout the year could be useful to reduce the cost of the silviculture. The staff and the equipment needed to cope with the peak of activity over the rainy season could indeed be reduced if the intervention could be postponed of a few months in a significant proportion of the inter-rows within each stand.

Whatever stand age and tree social status, most of the labelled nitrate injected at different soil depths was taken up by trees located within 2–3 m from the injection site. Our results show that N fertilizers must be applied close to the trunks to be taken up by all the trees, whatever stand age. Spacing in commercial *Eucalyptus* plantations managed in short rotations is commonly 2 m × 3 m or 3 m × 3 m. Our results suggest that fertilization (and weeding) in half of the inter-rows during the rainy season could be sufficient to make it possible for all the trees to take up the applied nutrients. However, the proportion of fertilized inter-rows cannot be lower than 50%. Applying fertilizers in 1/3 of the inter-rows would prevent most trees that are not adjacent to the fertilized inter-row from taking up the nutrients and would probably lead to high heterogeneity of growth within the stand.

The risk of nutrient losses by deep drainage will be higher if only 50% of the inter-rows are fertilized because only about half of the root system would have access to the applied nutrients. Laclau et al. (2003) and Laclau et al. (2010) showed that nutrient requirements are high in the first two years after planting *Eucalyptus* trees to produce leaves and fine roots. A combination of high nutrient requirements and high transpiration rates of *Eucalyptus* trees in highly weathered tropical soils account for very low nutrient losses by deep drainage (Mareschal et al., 2013; Versini et al., 2014; Binkley et al., 2018). We show that nitrates leached in deep sandy soils can be taken up by tree roots at a depth of 6 m between 0.6 and 2.5 years after planting in commercial *Eucalyptus* plantations. However, the velocity of exploration of deep soil layers depends on the social status of the trees. When N fertilization is concentrated in the first months after planting (and even more if fertilizers are only applied in 50% of the inter-rows), nitrate leaching in very deep soil layers might increase the heterogeneity of stands since deep nitrates could only be available for dominant trees. Field trials are needed to assess if weeding and fertilizing only 50% of the inter-rows during the rainy season (and the other 50% a few months later) would affect stand productivity. In drought-prone areas, an increase in stand evapotranspiration due to weeds during the rainy season might reduce the availability of water for trees during the next dry season and therefore might increase the risk of tree mortality during prolonged drought periods. The suitability of an extension of weeding and fertilization throughout the year is therefore probably dependent on climate and soil properties. Further studies dealing with the relationship between the social status of the trees and the distance of water and nutrient uptake are also needed to improve tree-based modeling approaches that intend to predict the response of tropical forests to climate change.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2019.05.011.

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