Nutrient deficiency enhances the rate of short-term belowground transfer of nitrogen from Acacia mangium to Eucalyptus trees in mixed-species plantations

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Abstract

While a recent study showed that significant amounts of the nitrogen (N) requirements of young *Eucalyptus* trees can be provided by nitrogen-fixing trees (NFTs) in mixed-species plantations through short-term belowground N transfer, the consequences of soil fertility on this facilitation process remain unknown. We assessed the effect of fertilization on the percentage of N derived from transfer (%NDFT) from *Acacia mangium* trees to *Eucalyptus* trees in mixed-species plantations. A complete randomized block design with two treatments (fertilized vs unfertilized) and three blocks was set up in mixed-species plantations of *A. mangium* and *Eucalyptus* in Brazil, with 50% of each species at 2.5 m x 2.5 m spacing. Collection of litterfall and forest floor made it possible to estimate the annual N release from forest floor decomposition between 46 and 58 months after planting, close to harvest age. $^{15}N\text{-}NO_3^-$ was injected into the stem of one dominant *Acacia* tree in each plot, 58 months after planting. The $x(^{15}N)$ values of *Acacia* and *Eucalyptus* fine roots sampled within 1.8 m of the labelled *A. mangium* tree were determined at 7, 14, 30 and 60 days after labelling. The $x(^{15}N)$ values in wood, bark, branch and leaf samples were also determined for the 6 labelled *Acacia* trees and their two closest *Eucalyptus* neighbours, just before and 60 days after labelling. The amount of N released from forest floor decomposition was 31% higher in fertilized (F+) than in unfertilized (F-) plots. Sixty days after labelling, the aboveground compartments of *Eucalyptus* trees were significantly ^{15}N enriched in both treatments. The $x(^{15}N)$ values of *Acacia* fine roots were higher than background values from 7 days after labelling onwards in F+ and 30 days after labelling in F-. The $x(^{15}N)$ values of *Eucalyptus* fine roots were higher than background values in both treatments, from 30 days after labelling onwards. Mean %NDFT values were 18.0% in F+ and 33.9% in F- over the first 60 days after labelling, and 22.8% in F+ and 67.7% in F- from 30 to 60 days after labelling. Fertilization decreased short-term transfer belowground of N from *Acacia* trees to *Eucalyptus* trees. Our study suggests that belowground facilitation processes providing N from NFTs to *Eucalyptus* trees in mixed-species plantations are more pronounced in low-fertility soils than in nutrient-supplied stands.

Keywords: ^{15}N, fertilization, facilitation, mature trees, nitrogen-fixing trees, Brazil

1. Introduction

Most tropical forest plantations are established in nutrient-poor soils (Stape et al., 2010; Mareschal et al., 2011; Keenan et al., 2015). Large amounts of biomass harvested every 6-7 years in commercial *Eucalyptus* plantations can lead to unbalanced input-output nutrient budgets (Laclau et al., 2010; Voigtlaender et al., 2019). Nitrogen (N) fertilizers are commonly applied in commercial *Eucalyptus* plantations to enhance tree early growth and to balance the N budget in the soil for sustainable plantation management (Gonçalves et al., 2013; Koutika et al., 2014). However, the use of N fertilizers may be limited in the future because of their rising cost (Brunelle et al., 2015) and their environmental impact through the use of fossil energy for their production (Elser, 2011), nitrate leaching, NH_3 volatilization or nitrogen oxide emissions (Binkley and Fisher, 2019).

Acacia mangium (Willd.) is a fast-growing tree species largely planted in South-East Asia for the pulp industry that stands out among the nitrogen-fixing tree (NFT) species of high silvicultural interest (Yamashita et al., 2008). The association of *A. mangium* with *Eucalyptus* can be an alternative to the use of N fertilizers. *A. mangium* can fix large amounts of atmospheric N_2, as shown on 4 sites in Brazil with about 250 kg N ha^{-1} fixed in mixed-species plantations of *A. mangium* and *Eucalyptus grandis* (Hill ex Maiden) over a 6-year rotation (Voigtlaender et al., 2019). N_2 fixation by NFTs can improve the nitrogen status of companion species in mixed plantations, through the decomposition of N-rich above-ground litter (Munroe and Isaac, 2014; Santos et al., 2017; Tchichelle et al., 2017a) and fine roots (Bachega et al., 2016). Moreover, non-NFTs may benefit from short-term belowground transfer of N from NFTs, as observed in pot experiments (He et al., 2004, 2005; Yao et al., 2019), and in the field between *A. mangium* and *Eucalyptus* trees within a radius of 6.2 m around *Acacias* trees (Paula et al., 2015). However, as far as we are aware, the effects of soil fertility on this facilitation process have never been investigated.

Facilitation occurs when at least one species benefits from another. The balance between facilitation and competition between plant species depends on resource availability. According to the stress-gradient hypothesis, competitive interactions decrease and facilitation increases under stressful environmental conditions (Callaway and Walker, 1997; Maestre et al., 2009; Holmgren and Scheffer, 2010; Kikvidze et al., 2011). Consistently, some studies in forest ecosystems have shown that competition between species is lower in nutrient-poor soils than in nutrient-rich soils (Baribault and Kobe, 2011; Coates et al., 2013). However, the opposite results were also found with high interspecific competition in low-fertility sites (Newmann, 1973; Trinder et al., 2012). In addition, asymmetric competition between tree species can be highly dependent on soil fertility. In a mixed-species plantation of *Falcataria mollucana* and *Eucalyptus saligna* in Hawaii, *Falcataria* tree growth was reduced by *Eucalyptus* neighbours on phosphorus-rich soils and facilitated on phosphorus-poor soils (Boyden et al., 2005). Conversely, *Eucalyptus* tree growth was reduced by *Falcataria* neighbours on low-phosphorus soils, but increased on high-phosphorus soils.

The N_2 fixation rate of *Acacia mangium* trees decreased when soil fertility increased in *Acacia* monocultures (Galiana et al., 2002), and was higher in association with *Eucalyptus* than in *Acacia* monocultures, likely due to *Eucalyptus* competition for soil N (Paula et al., 2018). In a pot experiment, N addition reduced the N_2 fixation rate of *Dalbergia odorifera* and led to lower N transfer from *Dalbergia* to *Eucalyptus* seedlings (Yao et al., 2019). Comparing fertilized and non-fertilized mixed-species plantations, fine root monitoring in young mixed-species plantations suggested that soil nutrient deficiency could promote belowground facilitation in mixed-species plantations of *Eucalyptus* and *A. mangium*, through an increase in the density of *Eucalyptus* fine roots close to *Acacia* trees where *Eucalyptus* trees could take advantage of high soil N availability (Bordron et al., 2021). Short-term belowground transfer of N from *Acacia* trees to *Eucalyptus* trees can be another facilitation process, as shown 2 years after planting in a nearby experiment (Paula et al., 2015). However, no experimental evidence supported the hypothesis of higher belowground transfer of N in non-fertilized than in fertilized plots.

Our study, conducted in the same trial as Bordron et al. (2021), set out to gain insights into the effect of soil fertility on the short-term belowground N transfer from NFTs to non-NFTs in mixed-species plantations. We estimated the short-term belowground transfer of N from *Acacia mangium* to *Eucalyptus grandis* x *E. urophylla* S.T Blake neighbours under two contrasting levels of NPK fertilization. We hypothesized that: (1) N contents in litterfall, N stocks in the forest floor and the rates of N release during forest floor decomposition are higher in fertilized than in non-fertilized plots, and

(2) the rate of short-term belowground N transfer between *Acacia* and *Eucalyptus* is higher in non-fertilized plots than in fertilized plots.

2. Material and methods

2.1 Study site

The study was carried out at the Itatinga experimental station of São Paulo University, Brazil (23°02′S, 48°38′W), at 860 m above mean sea level. The total rainfall over the study period from April 2017 to May 2018 was 2300 mm and the mean temperature was 19.6 ºC, with an average of 13.6 ºC for the coldest month (July 2017) and 22.9 °C for the hottest month (March 2018). The soils were deep Ferralsols (FAO classification), acidic and of low fertility. In the 0-1.0 m layer, soil pH_{H20} was about 4.0, clay content ranged from 18 to 24% and CEC from 3.2 to 7.6 $cmol_c$ kg^{-1} (KCl extraction), the sum of base cations was around 0.4 $cmol_c$ kg^{-1}, organic matter ranged from 6 to 16 g kg^{-1} and total N from 0.6 to 0.9 g kg^{-1} (Bordron et al., 2021).

2.2 Experimental layout

In May 2013, a complete randomized block design was set up with two treatments (fertilization *vs* non-fertilization) and three blocks in mixed-species plantations of *Eucalyptus* hybrid (*E. grandis* x *E. urophylla*) and *A. mangium*. Each plot consisted of 10 x 10 plants at a spacing of 2.5 m x 2.5 m with two buffer rows. Mixed-species stands were established in a proportion of 1:1 between *Eucalyptus* and *A. mangium*, with the two species planted alternately in the row, and between adjacent rows. *A. mangium* seeds originated from Papua New Guinea, and were inoculated with *Rhizobium* strains (BR 3609T and BR6009 provided by EMBRAPA Agrobiologia, Seropédica - Rio de Janeiro state) selected for their high levels of nodulation in nursery and high N_2 fixation efficiency. *Eucalyptus* cuttings (H13 clone) were provided by the Instituto de Pesquisa e Estudos Florestais (IPEF - São Paulo state).

In fertilized plots (F+), 2000 kg ha^{-1} of dolomite limestone was applied at planting, as well as 32.0 kg ha^{-1} of FTE-BR (Fritted Trace Element, micronutrients and Borogran), 150 kg ha^{-1} of K, 35 kg ha^{-1} of P, and 24 kg ha^{-1} of N only close to the *Eucalyptus* plants, to not reduce N_2 fixation by *Acacia* trees (Paula et al., 2018). One year after planting, 166 kg ha^{-1} of K and 37 kg ha^{-1} of P were broadcast at the soil surface. In non-fertilized plots (F-), no mineral fertilizer was applied.

2.3 Litterfall and forest floor

Litterfall was collected every month from April 2017 (47 months after planting) to March 2018 (58 months after planting). Leaf litterfall was collected in 12 traps (50 cm x 50 cm) per plot installed at different distances from the trees (Fig. S1). Bark and dead branches were collected in an area of 6.25 m^2 delimited between four trees in each plot in the 3 blocks. All compartments were separated by species and dried at 65°C to constant weight. For a given species, the components were gathered for each season (autumn from April to June 2017, winter from July to September 2017, spring from October to December 2017, summer from January to March 2018) and ground for N analysis.

The forest floor was sampled in all plots at the beginning (April 2017) and at the end of the study period (March 2018). Forest floor material was collected in twelve quadrats (50 cm x 50 cm) representing the spatial variability within the plot (Fig. S1). For each position, the forest floor was divided into two components: Lf (intact material or coarse fragments) and Hf (highly fragmented material). For each component and each plot, the samples were manually homogenized and dried at 65ºC to constant weight. A composite sample was then ground for N analysis. For both litter floor and forest floor, the ash content was determined by heating sub-samples at a 500 °C in an oven for 4 h and the ash content was used as a correction to determine the ash-free dry mass and N concentration.

2.4 Rate of N release from forest floor decomposition

The amount of N released from the decomposition of the forest floor was estimated in each plot as:

$$N_{release} = N_{Forest\text{-}floor\_2017} + N_{Litterfall\_2017\text{-}2018} - N_{Forest\text{-}floor\_2018} \quad (1)$$

Where:

- $N_{Forest\text{-}floor\_2017}$ is the amount of N in the forest floor in April 2017 (in kg N ha^{-1}).

- $N_{Forest\text{-}floor\_2018}$ is the amount of N in the forest floor in March 2018 (in kg N ha^{-1}).

- $N_{Litterfall\_2017-2018}$ is the total amount of N in the litterfall between April 2017 and March 2018 (in kg N ha^{-1}).

2.5 ¹⁵N labelling of Acacia trees

In March 2018 (58 months after planting), a ^{15}N-labelled solution was injected into the stem of one *Acacia* tree in each plot, following the methodology described in Paula et al. (2015). The labelled *Acacia* trees were dominant trees, and did not show any disease or damage. The mean height of *Acacia* trees across the 3 blocks was 16.0 m in F+ and 15.6 m in F-. No tree mortality was observed within a radius of 2.5 m around the labelled *Acacia* trees. A hole was drilled into the stem (6 mm in diameter and 20 mm in depth). The trees with a single stem were drilled at 1.30 m in height and the trees with two stems were drilled below the fork. The drill was lubricated using distilled water to prevent damage to the xylem vessels. After removing the drill, a polyethylene tube (6 mm in diameter) attached to a bottle containing 500 mL of distilled water was pushed 20 mm into the drilled hole. As a preliminary experiment had shown possible reflux of the injected solution, 3 mL of acetic acid was added to the distilled water to prevent vascular clogging (Johansen, 1940). This bottle was then connected to a second one, containing 2.0 g of N (98 atom% ^{15}N-NO^{3}) as potassium nitrate, dissolved in 500 mL of distilled water. ^{15}N contamination was avoided by packing the bark around the tube with non-toxic mineral putty (Terostat®) and placing a plastic bag around the trees before labelling. The solution was absorbed by the stem between 28 and 60 days after labelling depending on the treatments and trees. Over the 60 days, the few *Acacia* leaves that fell were removed within 24 h after the fall.

2.6 Sampling

In each plot, samples of leaves, living branches, stem wood, stem bark and fine roots of one acacia and one eucalypt tree were collected before labelling to measure the corresponding ^{15}N background values. Seven, 14, 30 and 60 days after the first day of ^{15}N labelling, four soil samples were collected from each plot using a PVC tube (5 cm in diameter and 10 cm in length), at four positions randomly located within a radius of 1.8 m around the ^{15}N-labelled *Acacia* trees (Fig. 1). The mean height of the four *Eucalyptus* trees neighbouring the labelled *Acacia* tree across the 3 blocks was

20.6 m in F+ and 18.7 m in F-. For each plot, the soil samples were then bulked and rapidly brought to the laboratory. Living fine roots (diameter < 2 cm) of *Acacia* and *Eucalyptus* were then carefully separated. The colour, thickness and branching patterns were good indications of the species to which they belonged, with *Acacia* roots that are brighter, rougher, thicker and less branched than *Eucalyptus* roots (Germon et al., 2018). At 60 days after labelling, the labelled *Acacia* tree and two *Eucalyptus* trees neighbouring the labelled *Acacia* tree, in the row and in the adjacent row (Fig. 1), were destructively sampled in each plot (6 *Acacia* trees and 12 *Eucalyptus* trees in total). The N concentration and $x(^{15}N)$ values of leaves, living branches, stem wood, stem bark and fine roots were determined for all the sampled trees (see below, equation 2).

2.7 *Isotopic analyses*

The fine roots were gently washed in tap water. All samples were dried at 65°C to constant weight. The bark, wood, leaves and living branches were ground in Retsch mill Zm 200 (120 micra) and the fine roots were ground in a porcelain mortar. The $x(^{15}N)$ value and N concentration of the samples were determined using a Hydra 20-20 mass spectrometer coupled to an automatic N analyzer (ANCA-GSL, SERCON Co., Crewe, UK), using 10 mg of dry mass of dry plant material, with a precision of 0.0001 ^{15}N atom%. The $x(^{15}N)$ value of a given sample was expressed as:

$$x(^{15}N)_{sample}\ (‰) = ([(^{15}N/^{14}N)_{sample} - (^{15}N/^{14}N)_{air}] / (^{15}N/^{14}N)_{air}) * 1000 \quad (2)$$

2.8 *N derived from transfer*

The proportion of *Eucalyptus* N derived from transfer from *Acacia* was estimated at the collection date from fine root $x(^{15}N)$ values of the two species using the equation (Jalonen et al., 2009; Isaac et al., 2012; Paula et al., 2015):

$$\%NDFT = (x(^{15}N)_{Euca}(0) - x(^{15}N)_{Euca}(t)) / (x(^{15}N)_{Euca}(0) - x(^{15}N)_{Acacia}(t)) * 100 \quad (3)$$

Where:

- $x(^{15}N)_{Euca}$ is the $x(^{15}N)$ value of fine *Eucalyptus* roots collected at 0 - 1.8 m from the ^{15}N labelled *Acacia*.

- $x(^{15}N)_{Acacia}$ is the $x(^{15}N)$ value of fine *Acacia* roots collected at 0 - 1.8 m from the ^{15}N labelled *Acacia.*
- $x(^{15}N)_{Euca}(0)$ and $x(^{15}N)_{Euca}(t)$ are the $x(^{15}N)$ values of fine *Eucalyptus* roots before *Acacia* labelling and at the end of each collection date.
- $x(^{15}N)_{Acacia}(t)$ is the $x(^{15}N)$ of fine *Acacia* roots at the end of each collection date.

2.9 Statistical analyses

For each collection date and treatment, the ^{15}N enrichment of *Acacia* and *Eucalyptus* material was tested against ^{15}N background values using a one-tailed paired t test. Differences between treatments and blocks in dry matter, N concentration and N content in litterfall and forest floor, $x(^{15}N)$ of *Acacia* and *Eucalyptus* materials and %NDFT were tested using two-way ANOVA. The homogeneity of variances was tested using Levene's test. When the variances were unequal, the values were log-transformed. When ANOVA indicated significant effects, the means were compared with Bonferroni's multiple range test. Statistical analyses were carried out using R 3.5.2 (R Core Team 2018). The significance level was 0.05.

3 Results

3.1 Litterfall and forest floor

Fertilization greatly influenced litterfall in our mixed-species stands (Fig. 2). Litterfall dry matter was 38% higher in F+ than in F- with values of 7.8 and 5.6 Mg ha^{-1} yr^{-1}, respectively. The proportion of *Eucalyptus* dry matter in the total litterfall was 81.9% on average in F+ and 70.3% in F-. Leaves accounted for 92.4% of the dry matter of *Acacia* litterfall and 45.3% of that of *Eucalyptus* litterfall. Mean N concentrations in litterfall were 17.3 g kg^{-1} in F+ and 14.8 g kg^{-1} in F- for *Acacia* leaves, and 6.5 g kg^{-1} in F+ and 6.4 g kg^{-1} in F- for *Eucalyptus* leaves (data not shown). On average, branches accounted for 7.6% of the dry matter of *Acacia* litterfall and 50.0% of *Eucalyptus* litterfall. Mean N concentrations in branch litterfall were 6.6 g kg^{-1} in F+ and 8.1 g kg^{-1} in F- for *Acacia*, and 2.0 g kg^{-1} in F+ and 1.9 g kg^{-1} in F- for *Eucalyptus* (data not shown). The total amount of N in litterfall was 26% higher in F+ than in F- with values of 49 and 39 kg N ha^{-1} yr^{-1}, respectively (Fig. 2). *Acacia* material accounted for 46.3% of the amount of N in litterfall in F+ and 62.4% in F-. On average, leaves accounted for 72% of the total N content in *Eucalyptus* litterfall and 97% of the total N content in *Acacia* litterfall.

The dry matter of the forest floor amounted to 6.7 Mg ha^{-1} in F+ and 6.0 Mg ha^{-1} in F- on average for the two sampling dates (Table 1). For a given layer, N contents in the forest floor were not significantly different between treatments, except for the Hf layer in 2017 with significantly higher N content in F- than in F+. The amount of N in the forest floor was not significantly influenced by the treatments, with average values of 65 kg N ha^{-1} in F+ and 62 kg N ha^{-1} in F-. Between 46 and 58 months after planting, the amount of N released from forest floor decomposition was 31% higher in F+ than in F-, with values of 43 and 33 kg N ha^{-1} yr^{-1}, respectively (Table 1).

3.2 x^{15}(N) in labelled Acacia trees

$x(^{15}N)$ values sharply increased in *Acacia* fine roots over the first 60 days after labelling in both fertilization treatments. In F+, mean $x(^{15}N)$ values of *Acacia* fine roots were multiplied by 3 relative to the mean background value of 2.73‰ from 7 days after labelling onwards, with average values of 10.78‰ over the 4 sampling dates after labelling (Fig. 3a). In F-, *Acacia* fine roots had higher values of $x(^{15}N)$ than the mean background value of 1.91‰ from 30 days after labelling onwards, with average values of 4.18‰ over the study period of 60 days after labelling. *Acacia* fine roots were significantly ^{15}N-enriched at 30 days in F-, and at 60 days in F+ and in F- (Fig. 3a).

Sixty days after labelling, the aboveground compartments of the labelled *Acacia* trees exhibited higher $x(^{15}N)$ values than background values, with significant differences for all compartments in both treatments (Table 2).

3.3 x^{15}(N) in Eucalyptus trees

Labelling *Acacia* trees increased the $x(^{15}N)$ values of the fine roots of neighbouring *Eucalyptus* trees. From 30 days after labelling onwards, mean $x(^{15}N)$ values of *Eucalyptus* fine roots were slightly higher than the mean background values of 1.27‰ in F+ and 0.36‰ in F-, with a significant enrichment in ^{15}N at 60 days after labelling in F+ and in F-. Over the 4 sampling dates after labelling, the average $x(^{15}N)$ values of *Eucalyptus* fine roots were 1.73‰ in F+ and 1.41‰ in F- (Fig. 3b). $x(^{15}N)$

values of *Eucalyptus* fine roots were highest at 60 days after labelling with values of 2.82‰ in F+ and 4.28‰ in F-. Fertilization did not change $x(^{15}N)$ values of *Eucalyptus* fine roots, except at 7 days after labelling, with significantly higher $x(^{15}N)$ values in F+ than in F-.

Sixty days after labelling, $x(^{15}N)$ values in the aboveground compartments of *Eucalyptus* neighbouring trees were significantly higher than background values for all compartments, in both treatments (Table 2).

3.4 Rates of N transfer from Acacia to Eucalyptus trees

The percentage of N of *Eucalyptus* trees derived from transfer (%NDFT) from *Acacia* trees estimated using Eq. (3) ranged from 9.5% to 29.5% in F+ and from 0 to 68.7% in F-, depending on the sampling dates (Fig. 4). Over the study period, the average values of %NDFT were 18.0% in F+ and 33.9% in F-.

4. Discussion

4.1 Fertilization increases N availability for Eucalyptus trees

In agreement with our first hypothesis, the amount of N released from forest floor decomposition was slightly higher in fertilized than in non-fertilized plots. The first 3 years after planting in the same experiment, fertilization increased the *Eucalyptus* fine root foraging strategy for nutrients in the topsoil (Bordron et al., 2021). The competition with *Eucalyptus* fine roots led to a partial exclusion of *Acacia* fine roots from the topsoil more marked in F+ than in F-, and a higher proportion of *Eucalyptus* fine roots at the vicinity of *Acacia* trees in F- (Bordron et al., 2021).

N concentrations were lower in *Eucalyptus* litterfall than in *Acacia* litterfall. Therefore, a higher percentage of *Eucalyptus* than *Acacia* material in litterfall in F+ than in F- led to only a 26% higher N content in F+, while the amount of dry matter was 38% higher in fertilized plots. Nitrogen contents in litterfall of 39 kg N ha^{-1} yr^{-1} in F- and 49 kg N ha^{-1} yr^{-1} in F+ were lower than in other mixed-species plantations of *Eucalyptus* and *A. mangium* in Brazil (from 55 to 85 kg N ha^{-1} yr^{-1}) (Voigtlaender et al., 2012, 2019; Santos et al., 2017), which could be consistent with the particularly low nutrient availability in the soil of this experiment, especially in non-fertilized stands.

The higher proportion of N-poor *Eucalyptus* litterfall in F+ than in F- did not prevent the decomposition of the forest floor. Nutrient concentrations, stoichiometry and C quality are major drivers of litter decomposition and nutrient release in forest ecosystems (Hobbie, 2000; Hättenschwiler et al., 2011). The decomposition rates are commonly positively correlated with the N concentrations in litter fractions (Cornwell et al., 2008). However, some negative correlations are also reported (Berg, 2000). High lignin contents can lower the decomposition of forest residues (Freschet et al., 2012). However, high N concentrations can delay the processes of degrading lignin and lower the rates of litter decomposition (Santos et al., 2017). In a nearby experiment, decomposition rates in litterbags were faster for *Eucalyptus* leaves than *Acacia* leaves, with initial higher water-soluble carbon and lower lignin concentrations in *Eucalyptus* leaves (Bachega et al., 2016). Fertilization can increase litter decomposition rates and nutrient availability in forest ecosystems (Aslam et al., 2015; Keuskamp et al., 2015). Litter decomposition can be stimulated at low levels of N addition (Zhang et al., 2018). The small amounts of N applied at planting might therefore have contributed to an increase in the rates of forest floor decomposition and N release in F+ relative to F-.

4.2 Belowground transfer of N from Acacia to Eucalyptus trees

The ^{15}N enrichment of *Acacia* and *Eucalyptus* fine roots in our study was lower than in 26-month-old trees in a nearby experiment (Paula et al., 2015). The totality of ^{15}N solution was absorbed in the *Acacia* stem between 28 and 60 days after the start of labelling in our study, instead of 12-36 hours in Paula et al. (2015). This difference could be partly explained by a double volume of solution to be absorbed in the present study, as well as the difference in tree age between the two experiments. In the ecological conditions of São Paulo state, *Acacia* trees are much more suppressed by *Eucalyptus* trees at the end of stand rotation than in young stands (Bouillet et al., 2013; le Maire et al., 2013), which led to lower transpiration rates of mature *Acacia* trees than young *Acacia* trees in a nearby experiment (unpublished data). Other factors might have delayed the absorption of the ^{15}N solution by *Acacia* stems, in particular a partial clogging of xylem vessels after stem drilling, or narrow conductive sapwood width in mature trees (Pallardy, 2008; Debell and Lachenbruch, 2009) that would have needed holes drilled less than 20 mm into the stem. However, this slow ^{15}N absorption reduced the risk of artificial flush of ^{15}N in N root exudates after labelling, which is likely to overestimate the short-term belowground transfer (Paula et al., 2015). The limited number of replicates may have accounted for the high x(^{15}N) variability in *Acacia* and *Eucalyptus* fine roots between blocks and the little number of significant differences with background values over the study period.

^{15}N labelling of *Acacia* was heterogeneous between tree compartments and individuals (Table 2, Figure 3). Such heterogeneity was also found during the first weeks / months after ^{15}N stem injection for other tree species (Horwath et al. 1992; Swanston and Myrold 1998; Augusto et al. 2011). However, despite marked variability in the x(^{15}N) of fine roots between labelled acacias, the use of Equation 3 based on the relative differences in x(^{15}N) between fine roots of a given *Acacia* tree and *Eucalyptus* neighbours made it possible to reliably estimate the effect of nutrient deficiency on the belowground transfer of N.

Short-term belowground N transfer may occur directly via common mycorrhizal networks (CMNs) (Simard and Durall, 2004; Selosse et al., 2006; He et al., 2019) or root exudates of N compounds (Marschner and Dell, 1994; Fustec et al., 2010), or indirectly through rapid decomposition of very fine roots and microbial tissues (May and Attiwill, 2003; Staddon et al., 2003). Montesinos-Navarro et al. (2016) showed that short-term belowground N transfer between adult plants can be more effective by CMNs than via root exudates. CMNs can be formed by arbuscular mycorrhizal fungi (AMF) (Montesinos-Navarro et al., 2012) or ectomycorrhizal fungi (He et al., 2005). Mycorrhizal roots of both *Eucalyptus* and *A. mangium* were observed in our experiment (Bordron et al., 2021) and both species may potentially form CMNs. AMF are closely associated with *A. mangium* (Tawaraya et al., 2003; Dhar and Mridha, 2012) as well as with *Eucalyptus* species (Adjoud-Sadadou and Halli-Hargas, 2000). Intercropping *A. mangium* and *Eucalyptus* enhanced AMF colonization of *Eucalyptus* roots in the 0-10 cm layer (Bini et al., 2018) in a nearby stand, where 16 AMF species (across 6 AMF genera) were observed down to a depth of 8 m (Pereira et al., 2018). Ectomycorrhizas are associated with *Eucalyptus* (Horton et al., 2017; Robin et al., 2019) and *A. mangium* (Founoune et al., 2002; Diagne et al., 2013). *Pisolithus*, *Scleroderma*, *Thelephora* and *Boletellus* genera were found under *A. mangium* and *E. urophylla* trees (Aggangan et al., 2015), and *E. grandis* and *A. mangium* roots can both be colonized by *Pisolithus* sp. and *Scleroderma sp.* (Founoune et al., 2002; Ducousso et al., 2012).

4.3 Nutrient deficiency increases the rate of belowground transfer of N from Acacia to Eucalyptus

In agreement with our second hypothesis, the values of N transfer rates from *Acacia* to *Eucalyptus* trees were higher in non-fertilized than in fertilized mixed-species stands, with an average %NDFT of 33.9% in F- and 18.0% in F+-. The difference in %NDFT between the two treatments could be higher as a result of a faster enrichment in ^{15}N of *Acacia* roots in F+ than in F-. *Acacia* fine roots had higher $x(^{15}N)$ values than $x(^{15}N)$ background values from 7 days after labelling onwards in F+, and 30 days after labelling in F-. Eq. (3) was therefore applicable at 7 and 14 days after labelling in F+, but not in F-. Considering the last 30 days of the experiment, when the $x(^{15}N)$ values of the fine roots of both species were higher than the $x(^{15}N)$ background values in the two treatments, the average %NDFT would be 67.7% in F- and 22.8% in F+. %NDFTs were probably overestimated using Eq. 3 due to ^{15}N discrimination during this process (Paula et al., 2015). However, coherent results at successive sampling dates using the same equation for both treatments are consistent with higher belowground transfer of N in non-fertilized stands than in fertilized stands.

The average %NDFT was 43% in a nearby 26-month-old fertilized plantation of *Eucalyptus* and *A. mangium* (Paula et al., 2015). The higher rate of belowground transfer of N in Paula et al. (2015) relative to our study could be explained by the high N demand of young *Eucalyptus* trees to build the crown (Laclau et al., 2010). At the end of stand rotation, *Eucalyptus* trees are less dependent on soil N availability since a large share of the N requirements is provided by internal retranslocation (Laclau et al., 2010). The lower N release from forest floor decomposition in non-fertilized plots than in fertilized plots could be compensated for *Eucalyptus* trees through higher N belowground transfer rates from *Acacia* trees. In F-, *Eucalyptus* trees could proportionally benefit from higher belowground transfer of N from *Acacia* trees than in F+, through a higher exploration of *Eucalyptus* fine roots in the vicinity of *Acacia* trees. Bordron et al. (2021) showed at 34 months after planting in the same experiment that *Eucalyptus* fine root mass density in the topsoil (0-0.15 m) was higher in F- than in F+ near *Acacia* trees. The specific root length (ratio between length and dry mass of fine roots) of

Eucalyptus fine roots was also higher in F- than in F+ close to *Acacia* trees, which might enhance the belowground transfer of N between the two species by increasing the length of fine roots in this area of nitrogen-enriched soil.

4.4 Perspectives

Higher rates of belowground transfer of N from *A. mangium* to *Eucalyptus* trees in non-fertilized than in fertilized mixed-species stands suggest that this facilitation process increases under harsh conditions. This finding reinforces the interest of associating *Acacia* with *Eucalyptus* in forest plantations to limit N deficiencies of *Eucalyptus* trees when plantations are established in low-fertility soils and/or when fertilizers are not applied, as commonly observed in smallholder plantations in tropical regions (Verhaegen et al., 2014; Nambiar, 2015). The evidence of significant short-term belowground transfer of N between NFT and companion trees could also promote association in temperate regions of NFT to non-NFT in short-rotation forests (Georgiadis et al., 2017) or agroforestry systems (López-Díaz et al., 2017). Higher values of belowground N transfer from NFT to companion trees in harsh conditions than in fertilized stands suggest that mixed-species plantations including NFTs could be particularly interesting to restore degraded soils (Du et al., 2019; Jourgholami et al., 2019).

Mixed-species plantations of *Eucalyptus* and *Acacia* can outperform *Eucalyptus* monocultures, as observed in Brazil (Santos et al., 2016), Congo (Bouillet et al., 2013; Tchichelle et al., 2017b) and Australia (Forrester et al., 2006). However, this pattern is not general, depending on the balance between competitive and facilitative processes (Forrester et al., 2006; Bouillet et al., 2013). Further studies dealing with belowground N transfer from NFTs to non-NFTs would be of interest for other tree species, silvicultural practices, soil types and ecological conditions. Such insights would be worthwhile for the management of both rural and commercial plantations, in particular in marginal zones where the extension of fast-growing forest species will mainly occur in the future (Booth, 2013).

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Figure captions

Fig. 1. Sampling scheme. Fine roots of *A. mangium* and *Eucalyptus* trees were sampled at 0, 7, 14, 30 and 60 days after ^{15}N-labelling of *Acacia* trees. At 60 days after labelling, the labelled *Acacia* tree and two neighbouring *Eucalyptus* trees (in the row and in the adjacent row) were harvested in each plot (6 *Acacia* trees and 12 *Eucalyptus* trees in total).

Fig. 2. Litterfall dry matter collected from April 2017 to March 2018 in mixed-species stands (50A:50E) with fertilization (F+) (a), and without fertilization (F-) (b). Corresponding N contents in litterfall in F+ (c) and in F- (d). For each treatment, total dry matter and total N content in litterfall, of bark, leaves and branches of *Acacia* trees (*Acacia*) and of *Eucalyptus* trees (*Eucalyptus)* are shown. Standard errors between blocks are indicated (n=3).

Fig. 3. Mean $x(^{15}N)$ values of *A. mangium* fine roots (a) and *Eucalyptus* fine roots (b) collected within 1.8 m of the ^{15}N-labelled *A. mangium*, at 0, 7, 14, 30 and 60 days after labelling in mixed-species stands with (F+) or without fertilization (F-). Vertical bars indicate standard errors between blocks (n=3). At a given collection date, * indicates $x(^{15}N)$ values significantly higher than background values ($P < 0.05$). Different letters indicate significant differences between treatments ($P < 0.05$).

Fig. 4. Estimates of the percentage of *Eucalyptus* nitrogen derived from *A. mangium* (%NDFT) at 7, 14, 30 and 60 days after *Acacia* labelling in mixed-species stands with (F+) or without fertilization (F-). Vertical bars indicate standard errors between blocks (n=3). The average %NDFT value over the study period of 60 days was 17.96% in F+ and 33.85% in F-.

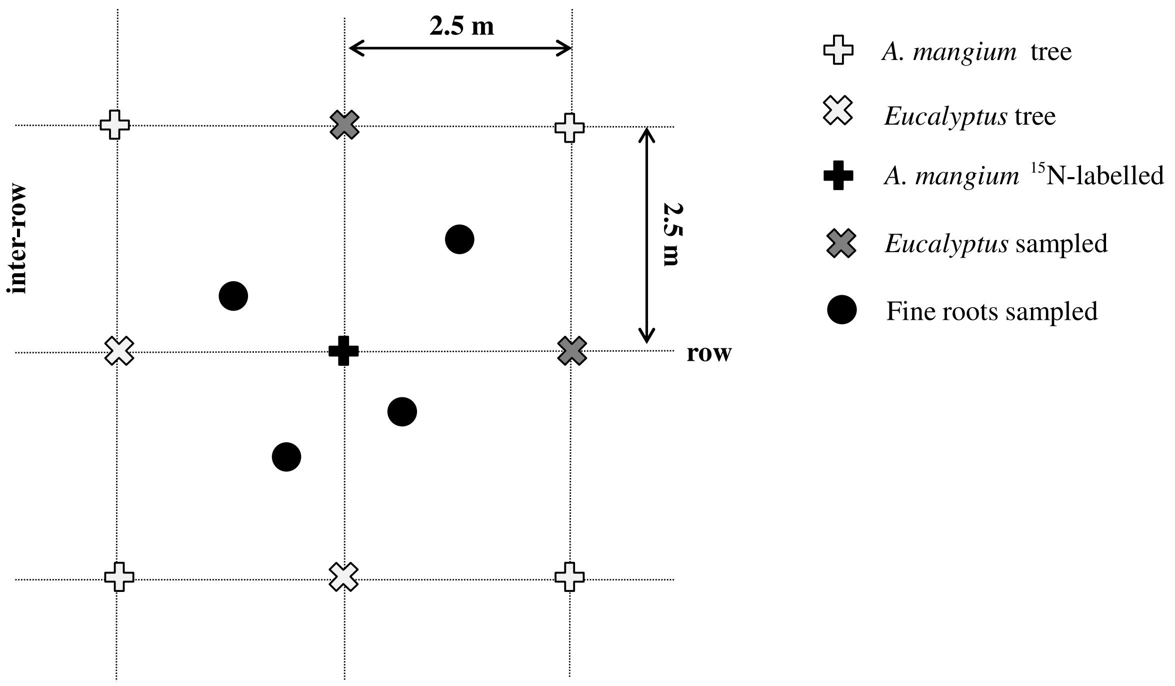

2.5 m
2.5 m
inter-row
row
A. mangium tree
Eucalyptus tree
A. mangium 15N-labelled
Eucalyptus sampled
Fine roots sampled

Fig. 1.

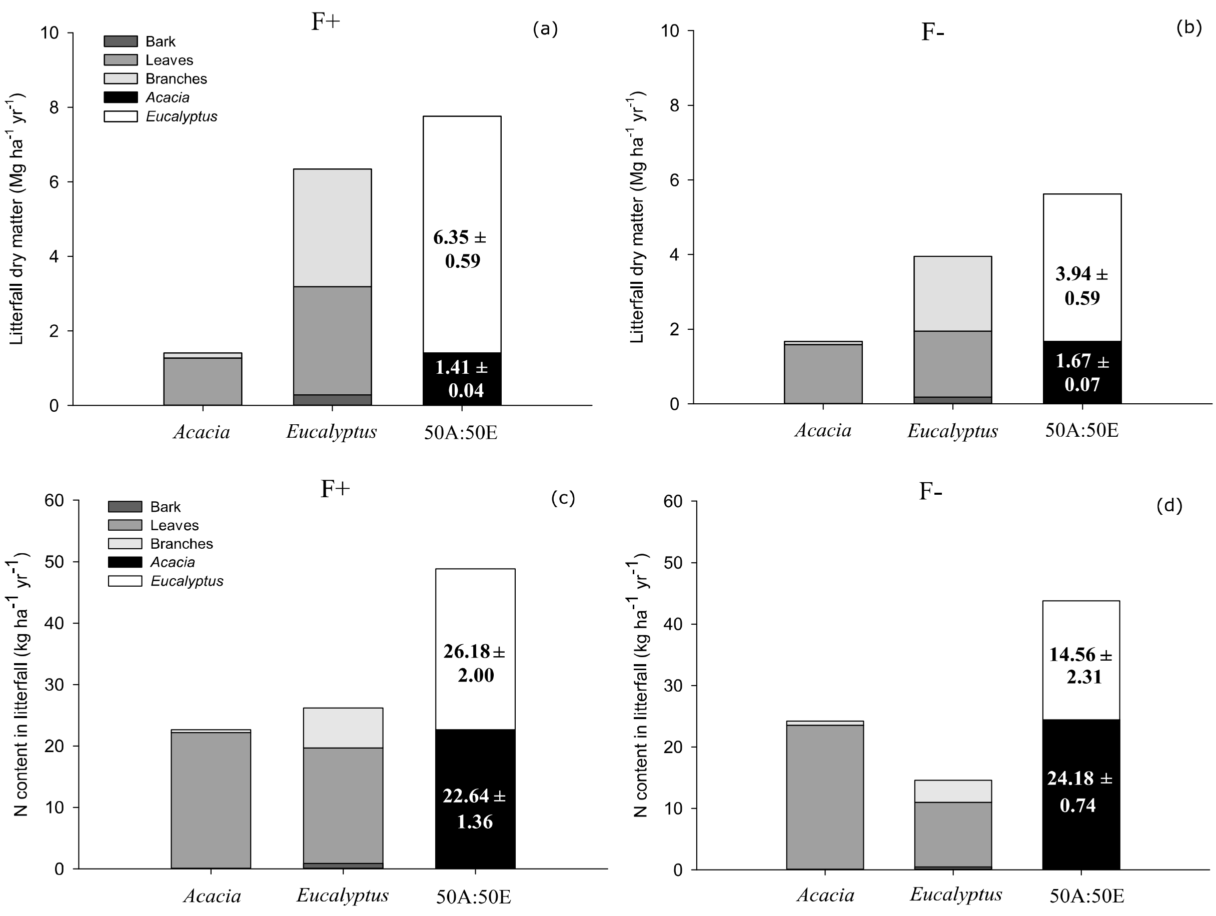
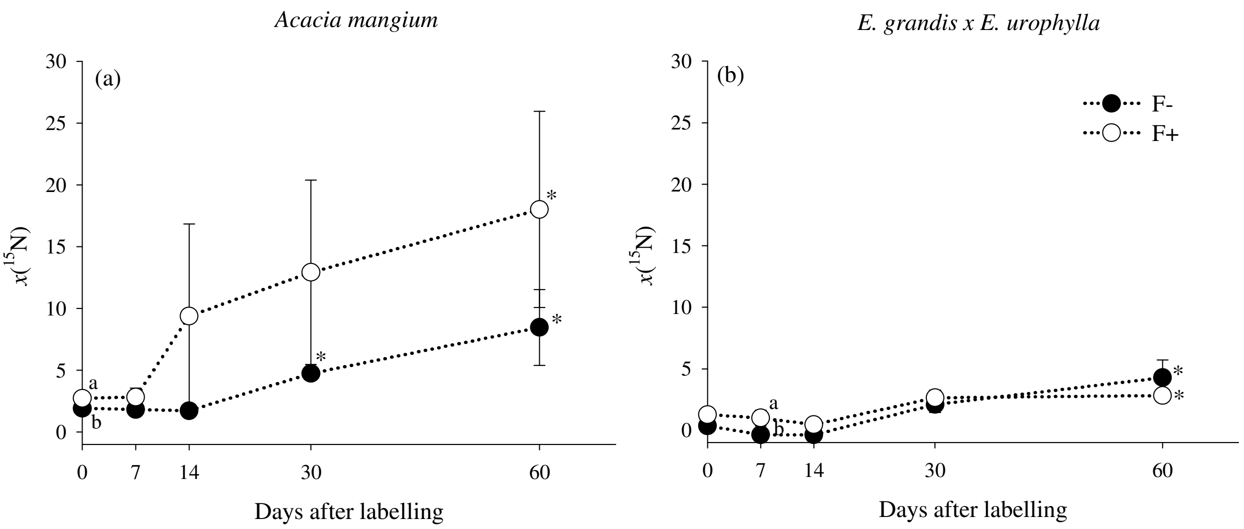

F+
(a)
Bark
Leaves
Branches
Acacia
Eucalyptus
Litterfall dry matter (Mg ha-1 yr-1)
0
2
4
6
8
10
6.35 ± 0.59
1.41 ± 0.04
Acacia
Eucalyptus
50A:50E
F-
(b)
3.94 ± 0.59
1.67 ± 0.07
F+
(c)
N content in litterfall (kg ha-1 yr-1)
0
10
20
30
40
50
60
26.18 ± 2.00
22.64 ± 1.36
F-
(d)
14.56 ± 2.31
24.18 ± 0.74

Fig. 2.



Acacia mangium
(a)
30
25
20
15
10
5
0
$x(^{15}N)$
a
b
*
*
*
0
7
14
30
60
Days after labelling
E. grandis x E. urophylla
(b)
F-
F+
a
b
*
*
Days after labelling

Fig. 3.

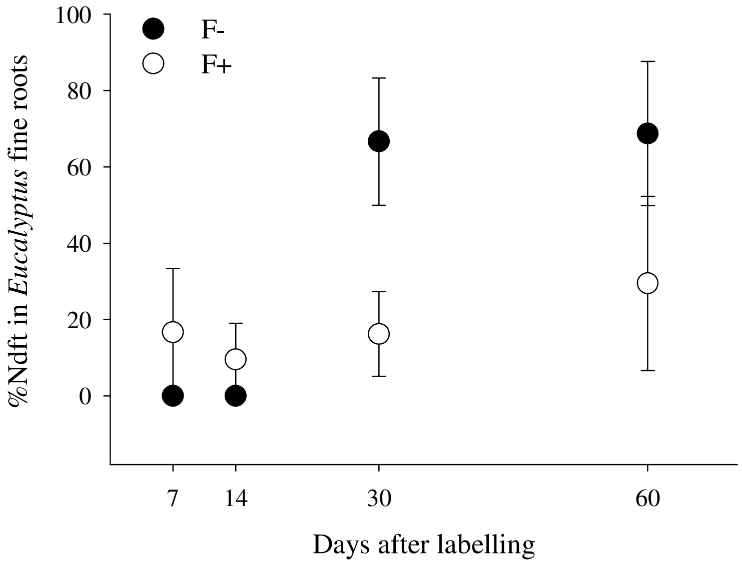

F-
F+
%Ndft in *Eucalyptus* fine roots
100
80
60
40
20
0
7
14
30
60
Days after labelling

Fig. 4.

Table 1

Dry matter and N content in the Lf and HF components of the forest floor collected in April 2017 and March 2018 in mixed-species stands (50A:50E) with (F+) or without (F-) fertilization. For a given plot, the values of dry matter and N content near *Acacia* and near *Eucalyptus* correspond to the mean of the 6 positions sampled close to 6 different *A. mangium* trees and 6 positions sampled close to 6 different *Eucalyptus* trees, respectively. Dry matter and N content in the litterfall collected between April 2017 and March 2018 in the same treatments are indicated, as well as the corresponding amounts of N released during forest floor decomposition during the same period. Standard errors between blocks are indicated (n=3). Different Latin uppercase letters indicate significant differences ($P < 0.05$) between treatments (F+ vs F-) for each species, and different Latin lowercase letters indicate significant differences between *Acacia* and *Eucalyptus* in each treatment. Different Greek letters indicate significant differences between treatments for the whole stand.

	F+			F-		
	Near *Acacia*	Near *Eucalyptus*	Whole stand	Near *Acacia*	Near *Eucalyptus*	Whole stand
Forest floor 2017						
Dry matter (Mg ha^{-1})						
Lf	4.44±0.34 Aa	4.08±0.54 Aa	4.26±0.35 α	3.46±0.32 Aa	3.61±0.19 Aa	3.53±0.25 α
Hf	1.75±0.05 Ba	1.80±0.09 Aa	1.77±0.05 □	2.37±0.21 Aa	1.94±0.16 Aa	2.15±0.03 α
Total	6.19±0.39 Aa	5.89±0.46 Aa	6.04±0.34 α	5.83±0.34 Aa	5.55±0.28 Aa	5.69±0.31 α
N content (kg N ha^{-1})						
Lf	46.81±1.08 Aa	37.20±6.79 Aa	42.01±3.80 α	36.52±1.45 Ba	30.43±5.23 Aa	33.47±3.14 α
Hf	19.93±0.27 Ba	20.02±3.00 Aa	19.97±1.41 □	27.26±1.17 Aa	24.35±2.76 Aa	25.81±1.56 α
Total	66.74±1.35 Aa	57.22±3.81 Aa	61.98±2.46 α	63.78±2.54 Aa	54.78±2.73 Aa	59.28±2.43 α
Forest floor 2018						
Dry matter (Mg ha^{-1})						
Lf	4.38±0.39 Aa	5.21±0.29 Aa	4.80±0.24 α	4.25±0.57 Aa	3.97±0.19 Ba	4.11±0.34 α
Hf	2.54±0.12 Aa	2.66±0.27 Aa	2.60±0.19 α	2.08±0.27 Aa	2.33±0.25 Aa	2.20±0.26 α
Total	6.92±0.36 Aa	7.88±0.56 Aa	7.40±0.37 α	6.32±0.37 Aa	6.30±0.29 Aa	6.31±0.33 α
N content (kg N ha^{-1})						
Lf	48.90±4.58 Aa	36.89±2.59 Aa	42.89±3.19 α	44.22±8.17 Aa	35.33±0.99 Aa	39.78±4.18 α
Hf	26.42±1.94	23.54±1.64	24.98±1.76	22.94±2.60	27.90±1.05	25.42±1.82

	Aa	Aa	α	Aa	Aa	α
Total	75.32±3.39	60.42±3.71	67.87±3.12	67.16±7.11	63.23±0.36	65.20±3.38
	Aa	Ab	α	Aa	Aa	α
Litterfall						
Dry matter (Mg $ha^{-1}yr^{-1}$)			7.76±0.62 α			5.62±0.65 α
N content (kg N $ha^{-1}yr^{-1}$)			48.81±2.96 α			38.73±3.00 α
N release (kg N ha^{-1} yr^{-1})			42.92±7.34 α			32.82±4.18 α

Table 2

Mean $x(^{15}N)$ values in aboveground compartments of *A. mangium* and *Eucalyptus* neighbours before (background values) and 60 days after *Acacia* ^{15}N-labelling. Standard errors between blocks are indicated (n=3). For a given tree compartment and a given treatment (F+ and F-), * indicates significantly higher $x(^{15}N)$ values than background values (P < 0.05).

Compartments	*A. mangium*				*Eucalyptus*			
	Background		60 days		Background		60 days	
	F+	F-	F+	F-	F+	F-	F+	F-
Bark	1.91±0.16	1.09±0.55	274.91*±85.65	219.77*±58.71	-0.73±0.40	-1.27±0.09	2.40*±0.79	2.28*±0.66
Wood	2.73±0.16	2.00±0.33	287.20*±37.01	347.07*±197.41	-0.27±0.42	-0.36±0.24	4.31*±0.78	2.87*±0.57
Branches	1.82±0.64	0.91±0.64	156.52*±29.07	168.90*±32.93	-1.37±0.42	-1.46±0.18	1.53*±0.41	0.77*±0.09
Leaves	3.00±0.55	2.09±0.74	165.89*±18.84	109.75*±38.46	-0.09±0.46	-0.18±0.36	0.96*±0.49	0.96*±0.08