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Abstract

Mixing N-fixing trees with eucalypts is an attractive option to improve the long-term soil N status in fast-growing plantations established in tropical soils. A randomized block design was replicated at four sites in Brazil to compare the biogeochemical cycles in mono-specific stands of *Eucalyptus* (100E) and *Acacia mangium* (100A) with mixed-species plantations in a proportion of 1:1 (50A50E). Our study aimed to assess the effects of introducing *A. mangium* trees in *Eucalyptus* plantations on atmospheric N_2 fixation, N cycling and soil organic matter stocks. Litterfall and soil N mineralization were measured over the last two years of the rotation (4-6 years after planting). Aboveground N accumulation in the trees and carbon (C) and N stocks in the forest floor and in the top soil were intensively sampled at harvesting age. N_2 fixation rates were estimated using the natural abundance of ^{15}N as well as by the difference between total N stocks in 100A and 50A50E relative to 100E (accretion method). While the ^{15}N natural abundance method was unsuitable, the accretion method showed consistently across the four sites that atmospheric N fixation reached about 250 and 400 kg N ha^{-1} $rotation^{-1}$ in 50A50E and 100A, respectively. Except at one site with high mortality, N contents within trees at harvesting were approximately 40% higher in 100A than in 100E. Mean N contents in litterfall and N mineralization rates were about 60% higher in 100A than in 100E, with intermediate values in 50A50E. The amounts of N in litterfall were much more dependent on soil N mineralization rates for acacia trees than for eucalypt trees. Soil C and N stocks were dependent on soil texture but not influenced by tree species. N budgets over a 6-year rotation were enhanced by about 65 kg N ha^{-1} yr^{-1} in 100A and 40 kg N ha^{-1} yr^{-1} in 50A50E relative to monospecific eucalypt plantations. Introducing N-fixing trees in eucalypt plantations might therefore contribute to reducing the need for mineral N fertilization in the long-term.

Key words: Organic matter, N_2 fixation, Eucalypt, litterfall, nutrition, Tree mixtures, tropical plantations.

1. Introduction

Mixed-species plantations associating nitrogen-fixing trees (NFT) with other highly productive tree species could be an attractive option to improve both the long-term soil nitrogen (N) status and the overall biomass production (Binkley et al., 2003; Forrester et al., 2004; Bouillet et al., 2013). Although total stem biomass production at the harvest was significantly enhanced in mixed-species stands relative to *Eucalyptus* monocultures in Australia (Forrester et al., 2004), Hawaii (Binkley et al., 2003), Congo (Bouillet et al., 2013) and in an experiment in Brazil (Santos et al., 2016), the mixture was less productive than monospecific *Eucalyptus* stands in other experiments established in Brazil (Bouillet et al., 2013). A growing body of evidence suggests an overall trend towards higher biomass production in a mixture than in monocultures of *Eucalyptus* and NFT in the areas where *Eucalyptus* growth is limited by N (Forrester, 2014). A positive effect of diversity on forest production in North America also suggested that facilitation between species could be enhanced relative to competition in stressful environments (Paquette & Messier 2011). However, light and water use efficiency to produce wood is commonly higher in *Eucalyptus* plantations growing under favourable conditions than in areas of low productivity (Binkley et al., 2004; Campoe et al., 2012), which supports an intensive management of highly productive plantations concentrated in small areas to satisfy the growing demand in wood (Stape et al., 2004; Battie-Laclau et al., 2016). While soil N availability is an important factor influencing the interactions between *Eucalyptus* and NFT, studies dealing with nutrient cycling over entire rotations are scarce in tropical regions (Forrester et al., 2006).

The gross primary productivity of Brazilian *Eucalyptus* plantations is higher than 3500 g C m^{-2} yr^{-1} (Ryan et al. 2010; Cabral et al. 2011), which ranks these forests among the most productive in the world (Luyssaert et al., 2007). Input-output budgets show consistently: i) that *Eucalyptus* plantations can benefit for several decades from an inherited soil fertility from the previous land use, and ii) that N fertilizer additions should increase over successive rotations (Laclau et al., 2005; Silva et al., 2013). NFT can be planted in N-deficient soils to enhance soil N status through large inputs of N derived from the atmosphere (Binkley et al., 2004; Forrester, 2014).

The ^{15}N natural abundance method is commonly used to estimate N_2 fixation in forest ecosystems (e.g. Galiana et al., 2002; Forrester et al., 2007; Bouillet et al. 2008) despite strong limitations (Shearer and Kohl, 1986; Chalk et al., 2016). N_2 fixation can also be estimated by the accretion method comparing total N stocks in the soil-plant system for NFT and non NFT, and considering that the difference is a result of N_2 fixation (similar N inputs and outputs except N_2 fixation). A comparison of N_2 fixation rates estimated with the accretion method, the ^{15}N natural abundance method and the ^{15}N labeling method is recommended to improve the reliability of the estimates (Shearer and Kohl, 1986; Parrota et al., 1996; Forrester et al., 2007; Bouillet et al., 2008). While some studies showed that carbon (C) and N stocks can rapidly increase in mixed-species plantations with NFT relative to eucalypt monocultures in Hawaii (Binkley et al. 2004), Puerto Rico (Resh et al. 2002), Brazil (Garay et al. 2004; Santos et al., 2016; 2017), Australia (Forrester and Smith, 2012), and Congo (Koutika et al., 2014), no significant changes in C and N stocks were detected after 1 rotation of NFT in Brazil (Voigtlaender et al., 2012; Rachid et al., 2013). The consequences of introducing NFT in eucalypt plantations might be less site-specific for N cycling than for C and N storage in the soil, with a rapid rise in N contents in litterfall and in soil N mineralization rates commonly observed after planting NFT (Binkley et al., 2004; Forrester et al., 2012; Voigtlaender et al., 2012; Sang et al., 2013; Tchichelle et al., 2017a).

A similar experimental design comparing monospecific and mixed-species plantations of *Eucalyptus* and *Acacia mangium* Wild has been replicated at 4 sites in Brazil. Bouillet et al. (2013) showed that the stemwood biomass at the time of harvest was 14%-57% higher in monospecific *Eucalyptus* stands (100E) than in *A. mangium* stands (100A) and an intermediate in mixture at a 1:1 ratio between *Eucalyptus* and *A. mangium* (50A50E) at those 4 sites. The biomass of aboveground tree components in 50A50E was strongly influenced by intra- and inter-specific interactions. Mixed-species plantations of *Eucalyptus* and *A. mangium* trees might be an interesting option to produce *Eucalyptus* saw logs with a much higher wood volume from individual *Eucalyptus* trees than in the commercial monospecific stands managed for pulpwood production (Bouillet et al., 2013).

The objective of our study was to gain insight into the potential of mixed-species plantations of *Eucalyptus* and NFT to enhance the long-term N status of tropical soils. We hypothesized that introducing *A. mangium* trees into highly productive eucalypt plantations leads to: 1) large N inputs through biological fixation, estimated consistently by the ^{15}N natural abundance method and the accretion method, 2) low changes in C and N stocks in the upper soil layers after 1 rotation despite a rise in N cycling through litterfall and soil N mineralization, and 3) an improvement of soil N budgets relative to monospecific eucalypt stands of the same order of magnitude as the amount of N fertilizer commonly applied in commercial plantations. Voigtlaender et al. (2012) showed results for soil C and N stocks, N contents in litterfall as well as soil N mineralization rates at Itatinga (IT). It is one of the 4 sites in the present study. Some results are shown for 3 sites here because the values already published at IT were excluded. Comparisons are made for 4 sites using the results of Voigtlaender et al.'s previously unpublished data for the IT site.

2. Materials and methods

2.1 Study sites

The same experimental design was replicated at Itatinga (IT), Bofete (BO), and Luiz Antônio (LA) in the São Paulo state and at Santana do Paraíso (SdP) in the Minas Gerais state (Figure 1). Large areas of commercial plantations of *Eucalyptus grandis* Hill ex Maiden and *Eucalyptus urophylla* S.T. Blake x *E. grandis* have been established in these regions. A detailed description of the 4 sites can be found in Bouillet et al. (2013). In brief, mean annual rainfall was similar at the 4 sites (1240 mm at SdP, 1390 mm at IT and 1420 mm at BO and LA), as well as mean atmospheric humidity (about 70%). The duration of the dry season (monthly rainfall < 30 mm) was 1-2 months at IT and BO, and 3-4 months at LA and SdP (Figure 2). Mean annual temperatures were 20°C at ITA, 21°C at BO, 23°C at LA and 24°C at SdP. The soils were Ferralsols at IT, BO and SdP and Ferralic arenosols at LA (FAO classification). The soil pH was acidic (between 4.5 and 5.8) and the amounts of available phosphorus and base cations were low at all the sites, decreasing sharply with soil depth (Table 1). The soils differed in texture, with clay contents in the 0-5 cm soil layer of 10% at LA, 11% at IT, 12% at BO and 51% at SdP. Our 4 experiments were representative of the range of soil properties and climatic conditions in the regions where the most productive *Eucalyptus* plantations are managed in Brazil.

Mean annual increment of stemwood at the harvest (6 years after planting) ranged from 18.2 Mg ha^{-1} yr^{-1} at IT to 21.8 Mg ha^{-1} yr^{-1} at LA in 100E, from 14.7 Mg ha^{-1} yr^{-1} at IT to 18.9 Mg ha^{-1} yr^{-1} at BO in 50A50E, and from 4.5 Mg ha^{-1} yr^{-1} at LA to 11.5 Mg ha^{-1} yr^{-1} at BO in 100A (Bouillet et al. 2013). The biomass production of aboveground tree components at the 4 sites can be found in Bouillet et al. (2013).

2.2 Experimental design

The experiments were set up in former *Eucalyptus* stands that had been managed for 20–60 years at all the sites. Highly productive *Eucalyptus* seedlings and cuttings were planted: *E. grandis* mono-progeny at IT, one *E. grandis* clone used by the Suzano company at BO, and *E. urophylla* x *E. grandis* clones selected by the International Paper company at LA and the Cenibra company at SdP. All the *Acacia mangium* seedlings were produced from seeds collected in a single highly productive stand in Amazonia (Amapá state). Spacing was 3 m x 3m at all the sites, except at BO where it was 3 m x 2 m.

A complete randomized block design was established at each site with seven treatments and four blocks. Each plot had 10 x 10 trees with two buffer rows (except 8 x 10 trees at BO). Our study was carried out in three of the original seven treatments planted without N fertilization:

– 100A, monospecific *A. mangium* stand;
– 100E, monospecific *Eucalyptus* stand;
– 50A50E, mixture in a proportion of 1:1 between *Eucalyptus* and *A. mangium* with the same total stocking density as the monospecific stands.

Seedlings were planted between the rows of the previous plantation after soil cultivation with a ripping tine to 40 cm depth. *Acacia mangium* seedlings were inoculated with *Rhizobium* strains selected by EMBRAPA for their N_2 fixation capacities. In the 50A50E treatment, the two species were planted alternately in the row, and between adjacent rows. The amounts of fertilizer applied (P, K, Ca, Mg and micro-nutrients) were non-limiting to tree growth (Laclau et al. 2009) and the lack of N fertilization did not reduce significantly the stemwood biomass of eucalypt trees harvested at 6 years of age (Bouillet et al. 2013). Herbicide applications the first two years after planting eliminated grasses and shrubs in these experiments, as in most commercial plantations in Brazil.

2.3 Nitrogen contents within tree components

Circumference at breast height (CBH) and tree height (H) were measured in the inner plots at 66, 63, 72 and 73 months after planting at SdP, BO, IT, and LA, respectively. Nitrogen contents in above-ground tree components were estimated by sampling destructively 10 trees of each species in 100A, 100E and 50A50E at each site, distributed over the range of basal area (40 trees per site). The trees were separated into leaves, living branches, dead branches, stemwood and stembark. The stem of each tree was sawn into 3 m sections. The fresh mass of each tree component was measured in the field (± 20 g). The foliage was collected from three different sections of the trees' crown. The biomass of coarse roots, medium-size roots and fine roots were measured for the 2 species in 100A, 100E and 50A50E at the end of the rotation at IT (see Nouvellon et al. 2012 for the detailed methodology). Sub-samples of each component were dried at 65°C to constant weight, and the dry biomass of the components in each tree was calculated proportionally. The samples were then ground for chemical analysis. Quality control procedures were used in the laboratory. Allometric equations were established for each species in each treatment at each site and applied to the corresponding inventory to estimate N contents on a hectare basis from CBH and H measurements.

2.4 Litterfall

Litterfall was collected over the last two years of the rotation at BO, LA and SdP. The amounts of C and N returning to soil with litterfall at IT can be found in Voigtlaender et al. (2012). Leaf and fruit litterfall were collected in 4-5 traps (50 cm x 50 cm) per plot installed at various distances from the trees in 100A and 100E, and in 8-10 traps per plot in 50A50E.

Dead branches and bark were collected in an area of 9 m^2 (6 m^2 at BO) delimited between four trees in each plot (replicated in three blocks for the three treatments). The litter traps were set up in three blocks for the three treatments and the litter of the two species was distinguished in 50A50E.

The three sites were far from each other and we could go to each experiment only twice per trimester. Litterfall was collected every 4 weeks (periods of measurement of soil N mineralization) or every 8 weeks (between soil incubation periods). Litter samples were dried at 65°C for 72 h before weighing. Litter dry matter was measured in each plot. The replicates in the three blocks were mixed and composite samples were prepared for each litter component of each species in each treatment at each site every 3 months, then ground for chemical analysis.

2.5 Nitrogen contents in the forest floor and the upper soil layers

The methodology described by Voigtlaender et al. (2012) at IT was used at the end of the rotation at BO, LA and SdP. In brief, forest floor and the upper soil layers (0-5 cm, 5-15 cm and 15-30 cm) were sampled in three blocks. Nine positions in each plot were sampled for 100A and 100E and 18 positions were sampled in each plot for 50A50E (9 positions at different distances from each tree species). All the sampling positions were distributed throughout the plots, excluding 2 buffer rows, and replicated in 3 blocks.

The forest floor was sampled with a 15 cm-radius circular frame at each position and divided into three components: Oi (non-fragmented material), Oe (coarse fragments), and Oa (finely fragmented material). The nine samples per component collected in each plot were manually homogenized and one composite sample per plot in mono-specific stands (one sample for each species per plot in 50A50E) was ground for chemical analysis. The ash content was determined by heating sub-samples at 450 °C in an oven for 4 h and used as a correction to determine the ash-free dry mass.

Soils were sampled using 5 cm, 10 cm and 15 cm-long metal cylinders (5 cm in diameter) inserted into the upper 0–5 cm, 5–15 cm and 15-30 cm soil layers after collection of the forest floor. All soil samples were air-dried, weighed and the water content was determined from a subsample (dried at 105°C). The roots were removed and the samples were passed through a 2-mm sieve (no gravel in these soils). Bulk density was calculated for all the samples collected as the ratio between oven-dried soil mass and volume of the soil core.

2.6 Production of mineral nitrogen

The *in-situ* coring technique (Khanna and Raison, 2013) was used to estimate N mineralization in the 0–20 cm soil layer in 100A, 100E and 50A50E over the last two years of the rotation at BO, LA and SdP. Soil incubations were conducted for 4-week periods in 12 plots (3 treatments in four blocks) at each site in the middle of each trimester and repeated 8 times over 2 years.

At the onset of each sampling period, three pairs of cores (70 mm in diameter) were driven 20 cm into the soil with a hammer in each plot for 100A and 100E and 6 pairs of cores in each plot for 50A50E (3 pairs close to each tree species). The pairs of soil cores were located 35 cm, 105 cm, and 175 cm from the nearest tree in each plot for a representative sampling of the inter-row. One soil core from each pair was withdrawn immediately and the other core was covered with a plastic cap to prevent mineral N leaching and incubated for 4 weeks. Soil samples were transported in cooled insulated containers and then homogenized manually. Roots were removed and a subsample was collected for determining the water content at

105°C. Extractions were initiated on the same day for one composite sample in each plot. Mineral N was extracted by shaking 10 g of soil with 50 ml of 2 M KCl and the concentrations of NH_4^+-N and NO_3^--N in the extracts were determined by an automated flow injection system (Ruzicka and Hansen 1975). Net ammonification and nitrification were estimated by the difference between post- and pre-incubation concentrations of NH_4-N and NO_3-N, respectively. Net N mineralization was obtained for each sampling period by summing net ammonification and net nitrification.

Mean annual rates of net ammonification and net nitrification over the study period were estimated multiplying by 13 the mean values across the 8 incubation periods (there are thirteen 4-week periods in 1 year). Voigtlaender et al. (2012) estimated that annual net N mineralization at IT amounted to 123 kg ha^{-1} yr^{-1} in 100A and 63 kg ha^{-1} yr^{-1} in 100E, from 26 successive incubation periods of 4 weeks (from 4 to 6 yrs after planting). Applying the method used here to their data set (multiplying by 13 the mean mineralization rates estimated over 8 periods of 4 weeks in the middle of each trimester) would estimate mean annual net N mineralization rates at 148 and 65 kg ha^{-1} yr^{-1} in 100A and 100E, respectively, *i.e.* with an overestimation by 20% in 100A and by 3% in 100E. The method used in the present study should therefore be sufficiently accurate to detect the contrasting effects of treatments on the net production of mineral N across the 4 sites.

2.7 Assessment of nitrogen fixation

Natural abundance of ^{15}N

The samples collected in 10 trees per species and per site to measure biomass and N contents within aerial tree components (leaf, branch, stemwood and stembark) were used for δ^{15}N determinations in 100A, 100E and 50A50E. However, to reduce the number of isotopic analyses, we pooled the samples collected in the 3 dominant, 4 medium-size and 3 smallest trees of each species for each component in each treatment at each site (for a total of 192 samples analyzed). The method described by Bouillet et al. (2008) was used to estimate the percentage of N derived from atmospheric N_2 (%Ndfa) in *A. mangium* trees.

Nitrogen accretion method

Nitrogen fixation at the 4 sites was also estimated using the N accretion method (Forrester et al., 2007) by comparing the N contents within soil, forest floor and biomass in 100A, 100E and 50A50E. All the N stocks were measured at each site, and for IT we used the values in the mineral soil and in the forest floor shown by Voigtlaender et al. (2012). The amount of N in the belowground biomass (N_{BG}) was only measured at IT (unpublished data), and we considered that the proportions of N in aboveground (N_{AG}) and belowground tree components were similar at the other sites for each treatment. N_{BG} was therefore estimated in each plot at BO, LA and SdP, multiplying the aboveground N content by the N_{BG}/N_{AG} ratio measured at IT.

Rates of N_2 fixation over six years after planting were estimated by calculating the difference in total N stock (summing N contents in tree biomass, forest floor and 0-15 cm soil layer) between 100E and the plots containing *A. mangium* (100A and 50A50E). We hypothesized that differences among treatments resulted only from N_2 fixation and that N losses via leaching or denitrification were negligible in our plots, which did not receive N fertilization. It was shown at IT that N leaching at a depth of 3 m was lower than atmospheric inputs over a *Eucalyptus* rotation (Binkley et al. 2018), and the same feature was observed at IT the first four years after planting in 100A and 50A50E (unpublished data). In addition, some measurements during the rainy season at IT also showed very low denitrification fluxes (unpublished data), as commonly reported in forest plantations and savanna woodland (Livesley et al. 2011).

2.8 Nitrogen budgets relative to monospecific Eucalyptus stands

A simple N budget was calculated in the soil to estimate the enrichment in soil N over the first rotation after introducing N fixing trees, relative to monospecific *Eucalyptus* plantations. In each experiment, we used the equation:

$$B_N = (F_N - E_A) - (0 - E_E) = F_N - E_A + E_E \quad (1)$$

where B_N is the soil N budget in 100A (resp. in 50A50E) relative to 100E, F_N is the biological fixation of N_2 in 100A estimated from the N accretion method (resp. in 50A50E), E_A is the exportation of N within stemwood at the harvest in 100A (resp. in 50A50E), and E_E is the amount of N exported with stemwood at the harvest in 100E.

2.9 Laboratory analyses

Total N in plant samples was determined by acid–base titration (TE036/01-Tecnal, Piracicaba, Brazil) after Kjeldahl mineralization. Carbon contents in the forest floor were estimated from dry matter values, assuming that the C concentration in each litter layer was similar to that measured at IT by Voigtlaender et al. (2012) for the same treatments. Carbon and N contents in the mineral soil at SdP, LA and BO were determined using NIR Spectrometry (Foss NIRSystems 5000, Silver Spring, MD, USA) for the 810 samples collected (Brunet et al., 2007). A CHN analyzer (Fisons/Carlo Erba NA 2000, Milan, Italy) was used to determine C and N concentrations for 150 samples selected to cover the range of spectra (110 samples for a specific calibration and 40 for cross-validation). The validation data set showed that NIRS predictions were accurate ($R^2 = 0.94$ for C and 0.95 for N).

2.10 Statistical analyses

We analyzed the main effects and interactions between treatments in each experimental area using the GLM procedure in SAS. When significant differences (P < 0.05) between treatment levels were detected, the Tukey test was used to compare treatment means. Then we used the average results for each treatment at each site to test the effects of the treatments and the sites as well as their interaction, as a global response study. Nitrogen content models were adjusted for each tree component by NPL procedure of SAS 9.2 (SAS Institute, Cary, NC, USA). Global and local models were established by treatment with up to three parameters ($y = a + bx^C$) and the Akaike's information criterion was used to select the best models. Homogeneity of variance was tested by Levene's test and original values were transformed when the variances were unequal. The data were analyzed with SAS statistical software (SAS Institute Inc., 2000), where linear regressions were established to assess the relationships between annual values of net N mineralization and N content in litterfall for each tree species across the plots studied.

3. Results

3.1 Nitrogen accumulation in the trees

Mean annual N accumulation in the aboveground (ABG) tree components over the rotation of 6 years ranged from 40 to 110 kg ha^{-1} yr^{-1} depending on the treatment and the site, and was approx. 40% higher in 100A than in 100E at IT, SdP, and BO (Figure 3). At LA by contrast, the ABG N content in 100A was only half of the amount in 100E. The ABG N content in the trees was intermediate in 50A50E relative to 100A and 100E, regardless of the site. About half of the N content in the ABG biomass in 100A was accumulated in the leaves and the stembark. In 100E and 50A50E, the stemwood contained from 40 to 60% of the ABG N content. Only 5-20% of the total amount of N in ABG tree components was accumulated in the branches whatever the site and the treatment.

3.2 Dry matter and N content in litterfall

Litterfall dry matter differed between treatments (Table 2). It was 50% lower in 100A than in 100E at LA, and 25% lower in 100A than in 100E at BO, and amounted to about 9 Mg ha^{-1} yr^{-1} in all the treatments at SdP (Figure 4a). In 50A50E, the proportion of *A. mangium* dry matter in the total litterfall was highly dependent on the site, ranging from 15% at LA to 40% at SdP. Nitrogen contents in litterfall were on average 1.7-fold as high in 100A as in 100E across the three sites (Figure 4b). The amounts of N in litterfall ranged from 75 to 103 kg ha^{-1} yr^{-1} in 100A depending on the site and from 49 to 62 kg N ha^{-1} yr^{-1} in 100E. N contents in litterfall were intermediate in 50A50E and *A. mangium* components represented between 30% (at LA) and 60% (at SdP) of the total amount of N in litterfall.

3.3 Carbon and nitrogen stocks in the forest floor and in the upper soil layers

The C stocks in the forest floor and in the 0-15 cm soil layer were not significantly influenced by the treatments but they differed between the sites (Table 2 and Appendix 1). While the total amounts of C in the forest floor ranged from 7.2 to 9.5 Mg ha^{-1} at BO and LA, they were close to 5 Mg ha^{-1} at SdP (Appendix 1). The pattern was different in the 0-15 cm soil layer, with higher C stocks at SdP (35 Mg ha^{-1}) than at BO (28 Mg ha^{-1}) and LA (23 Mg ha^{-1}).

The N content in the forest floor (F_N) was 30-90% higher in 100A than in 100E and intermediate in 50A50E at the three sites (Table 2 and Appendix 2). The effect of the site was much more marked in 100A (F_N ranging from 136 kg N ha^{-1} at SdP to 231 kg N ha^{-1} at LA) than in 100E (from 107 kg N ha^{-1} at SdP to 141 kg N ha^{-1} at BO) and in 50A50E (from 133 kg

N ha^{-1} at SdP to 151 kg N ha^{-1} at BO). In the 0-15 cm soil layer, the N stocks were not significantly different between treatments but were higher at SdP (about 2500 kg N ha^{-1}) than at BO (about 1500 kg N ha^{-1}) and LA (about 1200 kg N ha^{-1}) (Table 3). The total N stocks in the upper soil layers (summing the amounts in the forest floor and in the 0-15 cm layer) were not significantly influenced by the treatments 6 years after planting, whatever the site.

3.4 Nitrogen mineralization rates

The pools of mineral N in the 0–20 cm soil layer (non-incubated) and the N mineralization rates were significantly different between treatments and between sites (Table 2). On average over the study period, mineral N pools in the 0–20 cm layer were 16.6 kg ha^{-1} in 100A, 12.8 kg ha^{-1} in 50A50E and 12.4kg ha^{-1} in 100E with minimum values during the cold and dry season (data not shown). Mean N mineralization rates across the three sites amounted to 158.5 kg N ha^{-1} yr^{-1} in 100A, 131.2 kg N ha^{-1} yr^{-1} in 50A50E and 99.8 kg N ha^{-1} yr^{-1} in 100E (Figure 5 and Table 2). Net N mineralization rates were significantly higher at BO (199 kg N ha^{-1} yr^{-1} on average) than at LA (100 kg N ha^{-1} yr^{-1}) and at SdP (95 kg N ha^{-1} yr^{-1}). Net ammonification represented about 20% of the total net N mineralization in the three treatments at BO and was negligible at the two other sites (Figure 5).

3.5 Relationships between N content in litterfall and soil N mineralization

The amount of N returning to soil in litterfall was positively correlated with net soil N mineralization across the studied plots at the end of the rotation (Figure 6). However, the relationship was very different for eucalypt and acacia monocultures (not significant in *A. mangium* stands). Litterfall N return to soil could be used as a proxy for net soil N mineralization at the end of the rotation in eucalypt plantations. Tree plasticity to recycle N in litterfall when the soil N availability increased was much higher for *A. mangium* than for *E.*

grandis. For soil N mineralization rates ranging from 50 to 300 kg ha^{-1} yr^{-1} in *E. grandis* monocultures, N content in litterfall ranged from 50 to 70 kg ha^{-1} yr^{-1}. N content in litterfall ranged from 70 to 130 kg ha^{-1} yr^{-1} for soil N mineralization rates between 100 and 250 kg ha^{-1} yr^{-1}.

3.6 N_2 fixation estimates

The accretion method estimated N_2 fixation in 100A over the rotation of 6 years at 413, 348 and 367 kg N ha^{-1} at IT, BO and SdP, respectively (Table 3). A high mortality of *A. mangium* trees at the end of the rotation prevented us from using this method at LA for the 100A treatment. In 50A50E, N_2 fixation over the rotation ranged from 84 kg N ha^{-1} at LA to 277 kg N ha^{-1} at SdP. Nitrogen concentrations in the aboveground tree components of the 50A50E treatment were significantly higher in *A. mangium* trees than in *Eucalyptus* trees (Table 4). N concentrations differed depending on the site and a species x site interaction was significant in living branches and stembark but not in leaves and stemwood.

Unexpectedly, $\delta^{15}N$ values in natural abundance within leaves and living branches were significantly lower for *Eucalyptus* than for *A. mangium* trees (Table 4). In stemwood and stembark, the differences between species were not significant. $\delta^{15}N$ values differed significantly depending on the site with higher values at SdP than at the other sites in all the aboveground tree components.

3.7 Nitrogen budgets

The amounts of N exported with stemwood at the harvest ranged from 148 to 238 kg ha^{-1} and were little influenced by the treatments at each site, except in 100A at LA where tree mortality was high (Table 3). Relative to a rotation of monospecific eucalypt trees, a monoculture of *A. mangium* increased the N budget by about 400 kg ha^{-1} over 6 years at IT, BO and SdP. The N budget over the rotation was about 200 kg ha^{-1} higher in 50A50E relative to 100E at the same sites, but lower at LA where the climate was not suitable for *A. mangium* trees.

4. Discussion

4.1 Biological N_2 fixation

While the accretion method showed large N inputs through biological N_2 fixation in 100A and 50A50E at the 4 sites, in agreement with our first hypothesis, the ^{15}N natural abundance method was unable to estimate biological N_2 fixation. Indeed, the $\delta^{15}N$ values in the leaves at the four sites were lower in non-NFT (both in 100E and 50A50E) than in NFT, which suggests that *A. mangium* trees did not fix N_2. However, the accretion method at the 4 sites in our study and ^{15}N labeling at IT (Bouillet et al., 2008; Paula et al., 2018) show that large amounts of N were actually fixed. Lower $\delta^{15}N$ values in *E. grandis* leaves than in *A. mangium* leaves consistently at the four sites confirm the risk of misuse of the ^{15}N natural abundance method pointed out in previous studies (Shearer and Kohl, 1986; Binkley and Fisher, 2013; Chalk et al., 2016). If the $\delta^{15}N$ values had been lower for *A. mangium* trees than for *E. grandis* trees, those values might have been used to estimate the N_2 fixation rates whereas the method would not be suitable. Lower $\delta^{15}N$ values in *E. grandis* trees than in *A. mangium* leaves might reflect different fractionation patterns of the ^{15}N taken up in the soil, which might be a consequence of the uptake of N by different mycorrhizal strains. Forrester et al. (2007) showed a strong relationship between the mycorrhizal status and $\delta^{15}N$ values within eucalypt and acacia trees in Australia and stressed the difficulties in using the ^{15}N natural abundance method for plants with different mycorrhizae strains. Further studies are needed to improve our understanding of the role of mycorrhiza in the nutrition of eucalypt and acacia trees in tropical plantations, and their effect on the isotopic fractionation of N.

Although the accretion method led to high standard errors of N_2 fixation in our study, the estimates were consistent across the four sites. This method requires an accurate quantification of N content in the trees, in the forest floor and in the soil. Despite an intensive sampling (160 trees harvested and N concentrations determined in 640, 144, and 972 samples of plant, forest floor and soil, respectively), the variability between blocks at each site was high. However, the global picture is consistent across the four sites and a large amounts of N input to the ecosystem through the N_2 fixation of acacia trees is consistent with more active N cycling in 100A and 50A50E than in 100E. The accretion method assumes that N inputs (except biological fixation) and N outputs in the soil are similar in the stands including NFT and in the stands with only non-NFT. The conditions for using the accretion method were propitious in our study. Nitrogen atmospheric deposition was probably of the same order of magnitude in eucalypt and acacia stands (low differences between eucalypt and acacia leaf areas probably led to similar filter effects on dry deposition) and very low losses of N by deep drainage have been shown in eucalypt plantations growing on deep tropical soils (Mareschal et al., 2013; Binkley et al., 2018). Monitoring soil solution chemistry in *A. mangium* stands and mixed-species stands with eucalypt and acacia over the first four years after planting at IT showed that N leaching at a depth of 3 m was of the same order of magnitude as atmospheric inputs (unpublished data). The depth of the soil was > 3 m at the four sites and whether the losses of N by deep drainage were higher under acacia than under eucalypt at some sites (as a result of higher soil N mineralization rates than in eucalypt plantations), this bias would lead to an underestimation of the N_2 fixation rates through the accretion method. The estimates of N_2 fixation at the end of one rotation of *A. mangium* (about 400 kg ha^{-1} from planting to age 6 years, except at LA) and in mixed-species plantations including 50% of *A. mangium* trees (about 250 kg ha^{-1} over one rotation, except at LA) are therefore conservative values that might be slightly underestimated at some sites. High N_2 fixation rates were also estimated, from ^{15}N labeling methods, in Congolese *Acacia mangium* plantations where the amount of N derived from the atmosphere in the mixture one year after labelling (220 kg ha^{-1}) was 60% higher than expected on the basis of the amount found in 100A (276 kg ha^{-1}), taking into account a 50% lower density of acacia trees in mixture (Tchichelle et al., 2017b). The low N_2 fixation rates at LA compared with the other sites can be explained by a high mortality of *A. mangium* trees at the end of the rotation resulting from unsuitable pedo-climatic conditions for this species.

4.2 Nitrogen cycling

Planting *A. mangium* trees in soils cultivated over several decades with eucalypt plantations dramatically changed nitrogen cycling in only 6 years. Even though a strong effect of NFTs on N cycling in planted forests is well documented (Binkley and Giardina, 1997; Forrester et al., 2006), the originality of our results relies on the speed of the changes in highly productive plantations (from 4 to 6 years after planting NFTs) and the consistency of the results at 4 sites. The main N fluxes of the biological cycle strongly increased in the stands including acacia trees in comparison with monospecific eucalypt plantations: N accumulation in the trees, N content in litterfall and soil N mineralization. A strong effect of NFTs on the production of mineral N in the soil has been shown in tropical plantations with eucalypts and acacias in the Congo (Tchichelle et al., 2017a), in Brazil (Rachid et al., 2013; Santos et al., 2016; 2017), in Australia (Adams and Attiwill, 1984; Forrester et al., 2005), and in Hawaii (Binkley et al. 2003).

N accumulation in the aboveground biomass was higher in 50A50E and 100A than in 100E at all the sites (except LA), which probably reflects the increase in N availability for the trees resulting from the high N_2 fixation rates of *A. mangium* trees (Wang et al., 2010; Inagaki and Tange, 2014). About half of the N content in the aboveground components of *A. mangium* trees was accumulated in leaves and stembark at 6 years of age whereas the stemwood contained half of the N stock in eucalypt trees. High accumulation rates of N in leaves and bark of acacia trees relative to eucalypt trees have already been reported (Bouillet et al., 2008; Koutika et al., 2014). The contrasting proportions of N accumulated in leaf, branch, stemwood and stembark for eucalypt and acacia trees at harvesting age suggest that the management of harvest residues could strongly influence the N bioavailability for the next rotation. ^{15}N-labeling of eucalypt harvest residues (leaf, branch, bark) showed that the amount of N released throughout the decomposition of the forest floor is highly dependent on the mixture between leaves and ligneous residues, which can strongly influence tree growth after replanting (Versini et al., 2014; 2016). Even though the amounts of N are higher in *A. mangium* residues than in eucalypt residues, the dynamics of N release is also influenced by the C quality of the litter (Freschet et al., 2012). The faster decomposition of *E. grandis* leaves than *A. mangium* leaves at IT might be a consequence of higher concentrations of lignin and condensed tannins as well as lower concentrations of water soluble compounds in acacia leaves (Bachega et al., 2016).

Soil N mineralization rates in the topsoil are generally higher under NFTs than under non-NFTs (e.g. Forrester et al., 2005; Wang et al., 2010**;** Koutika et al., 2014). This feature can be explained by larger amounts of N returning to soil with litterfall as well as rapid changes in soil biological activity and microbial community composition (Bini et al., 2013; Rachid et al., 2013; 2015). The most noticeable difference in net N mineralization among the sites in our study is the very high production of soil mineral N in eucalypt monocultures at BO, which could be a consequence of silvicultural practices over the rotations before trial establishment. About 200 kg N ha^{-1} $rotation^{-1}$ was applied at BO whereas the doses were commonly <100 kg N ha^{-1} $rotation^{-1}$ by other forest companies (unpublished data). A very fast root development in deep soil layers combined with high water and nutrient demand to produce leaves and fine roots after planting prevent the loss of large amounts of N in eucalypt plantations growing on deep tropical soils (Versini et al., 2014; Laclau et al., 2010). The large amounts of N applied at BO over the rotations before the establishment of our trial were therefore probably not lost, and might account for the very high N mineralization rates measured in the topsoil whatever the treatment (100A, 100E and 50A50E).

4.3 Influence of A. mangium trees on soil C and N stocks

While soil C and N stocks commonly increase in NFTs relative to monospecific eucalypt stands (Resh et al., 2002; Wang et al., 2010; Forrester et al., 2013; Koutika et al., 2014), the changes were not significant after one rotation of *A. mangium* trees at the study sites, in agreement with our second hypothesis and the results already published at IT (Voigtlaender et al., 2012). Larger C and N stocks in the topsoil at SdP than at the other sites reflect the differences in clay contents and the well-documented relationship between soil texture and soil organic matter accumulation (Feller and Beare, 1997). While other studies showing that NFTs can greatly enhance soil N stocks were carried out in degraded tropical lands (Resh et al., 2002; Koutika et al., 2014), the field trials in our study were set up after decades of cultivation of highly productive eucalypt plantations. Soil C increased after 60 years of eucalypt monocultures relative to the native savanna at IT but, in agreement with the results in the present study, soil N stocks were not modified (Maquère et al., 2008).

4.4 Soil N budgets and consequences for fertilization regimes

In agreement with our third hypothesis, introducing *A. mangium* trees in commercial eucalypt plantations strongly enhanced soil N budgets in 100A and 50A50E. Atmospheric N_2 fixation led to an input of approx. 250 kg N ha^{-1} in mixed-species stands and 400 kg N ha^{-1} in monospecific *A. mangium* stands, which was much higher than the amounts of N fertilizer commonly applied (about 100 kg N ha^{-1}) in Brazilian commercial plantations (Gonçalves et al., 2008). However, the budgets have been computed here relative to eucalypt monocultures and comprehensive input-output budgets including all the major fluxes (in particular atmospheric deposition, run off and deep leaching) would be necessary to assess the long-term changes in soil N stocks. Moreover, fine roots of *E. grandis* trees were found 4 years after planting down to the water table at a depth of 17 m at Itatinga while the *A. mangium* roots reached a depth of 12 m (Germon et al., 2018), which might provide access to deep soil profile sources of soil N (Houlton et al., 2018). The larger amounts of N accumulated in *A. mangium* trees than in eucalypt trees at our study sites, consistent with the results of a meta-analysis (Inagaki and Tange, 2014), lead to much larger N inputs at the soil surface with harvest residues in *A. mangium* stands than in eucalypt plantations. A strong relationship between the N content in harvest residues, heterotrophic respiration, and early tree growth after replanting was shown in Congolese eucalypt plantations (Versini et al. 2013). Further studies are needed to improve our understanding of the biogeochemical processes driving the mineralization of harvest residues in short-rotation forests in order to optimize the fertilization regimes over successive rotations (Versini et al., 2014; Rocha et al., 2016).

In conclusion, the ^{15}N natural abundance method was unsuitable to estimate N_2 fixation despite high N fixation rates in *A. mangium* stands, consistently shown by the accretion method at 4 sites in Brazil. Planting *A. mangium* trees strongly increased the availability of mineral N in the topsoil as well as N cycling through litterfall. Nitrogen budgets suggest that introducing NFTs in eucalypt plantations can contribute to increasing their sustainability through a reduction of the need for N fertilizer addition. The new frontiers of afforestation in Brazil are close to the Amazonia region where high temperatures are more suitable for *A. mangium* trees than for eucalypt trees. Biomass production and N cycling in mixed-species plantations with eucalypt and *A. mangium* trees should be studied in this context where facilitation processes might lead to transgressive overyielding as shown recently under a similar climate in the Congo (Pretzsch and Schütze, 2009).

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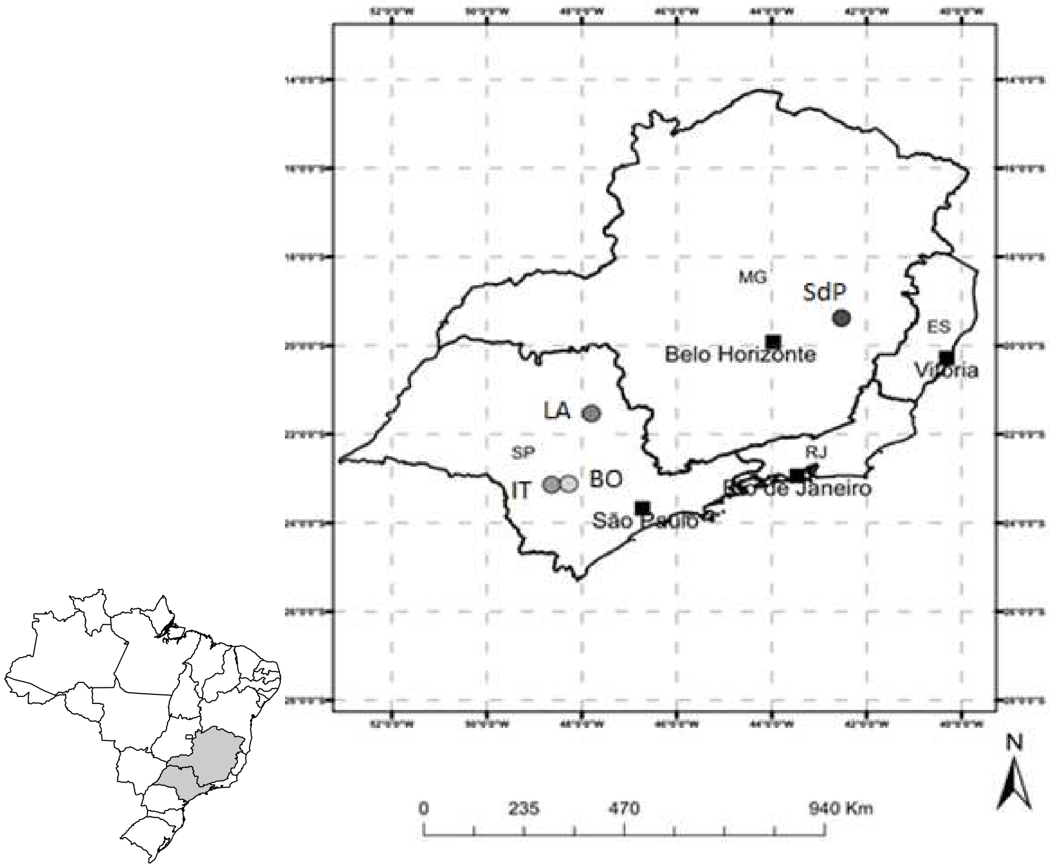

Figure 1. Localization of the experimental sites in Brazil.

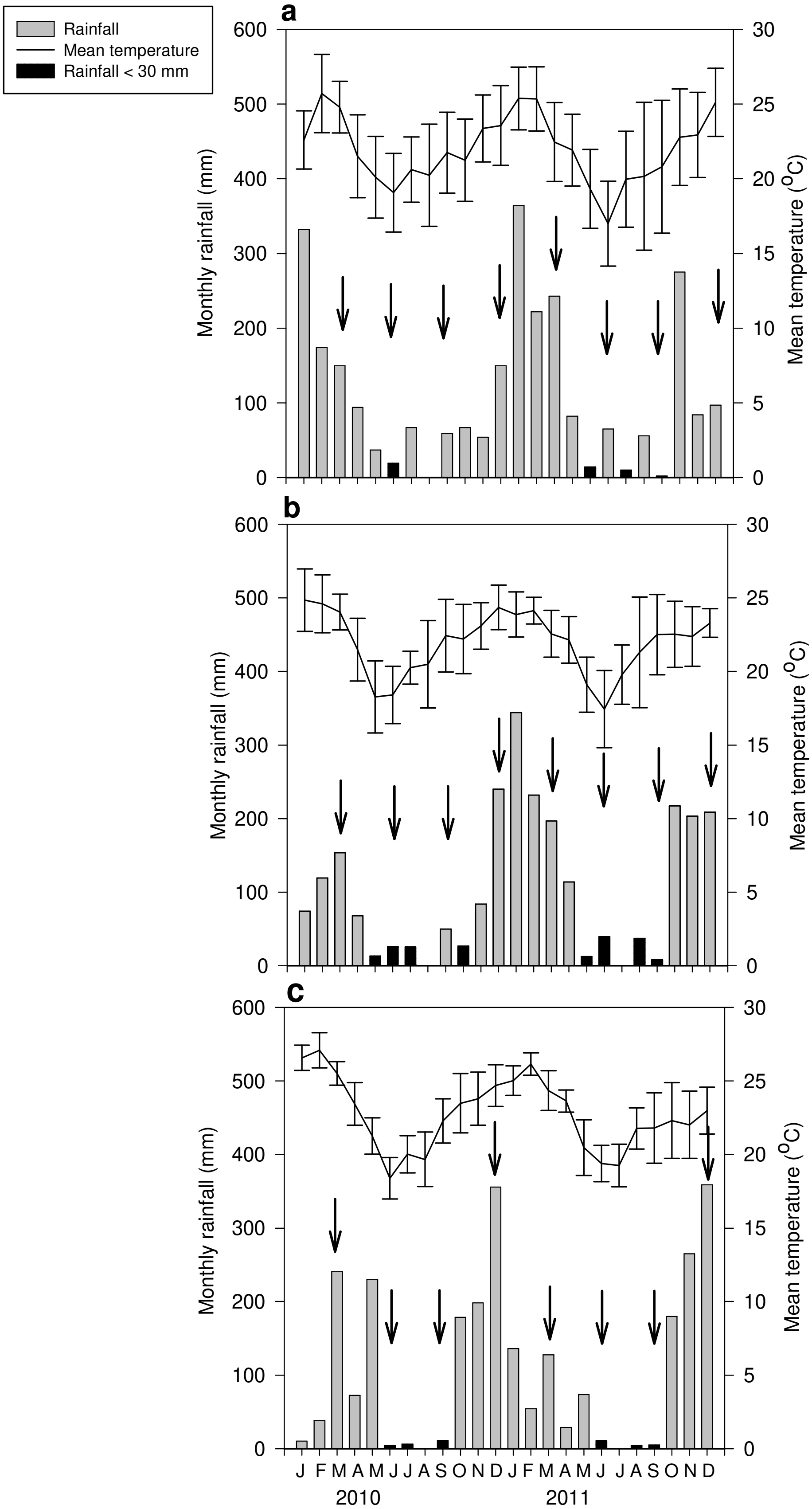

Figure 2. Monthly rainfall and temperature at BO (a), LA (b) and SdP (c). The arrows indicate the periods of measurement of soil N mineralization (one month every three months).

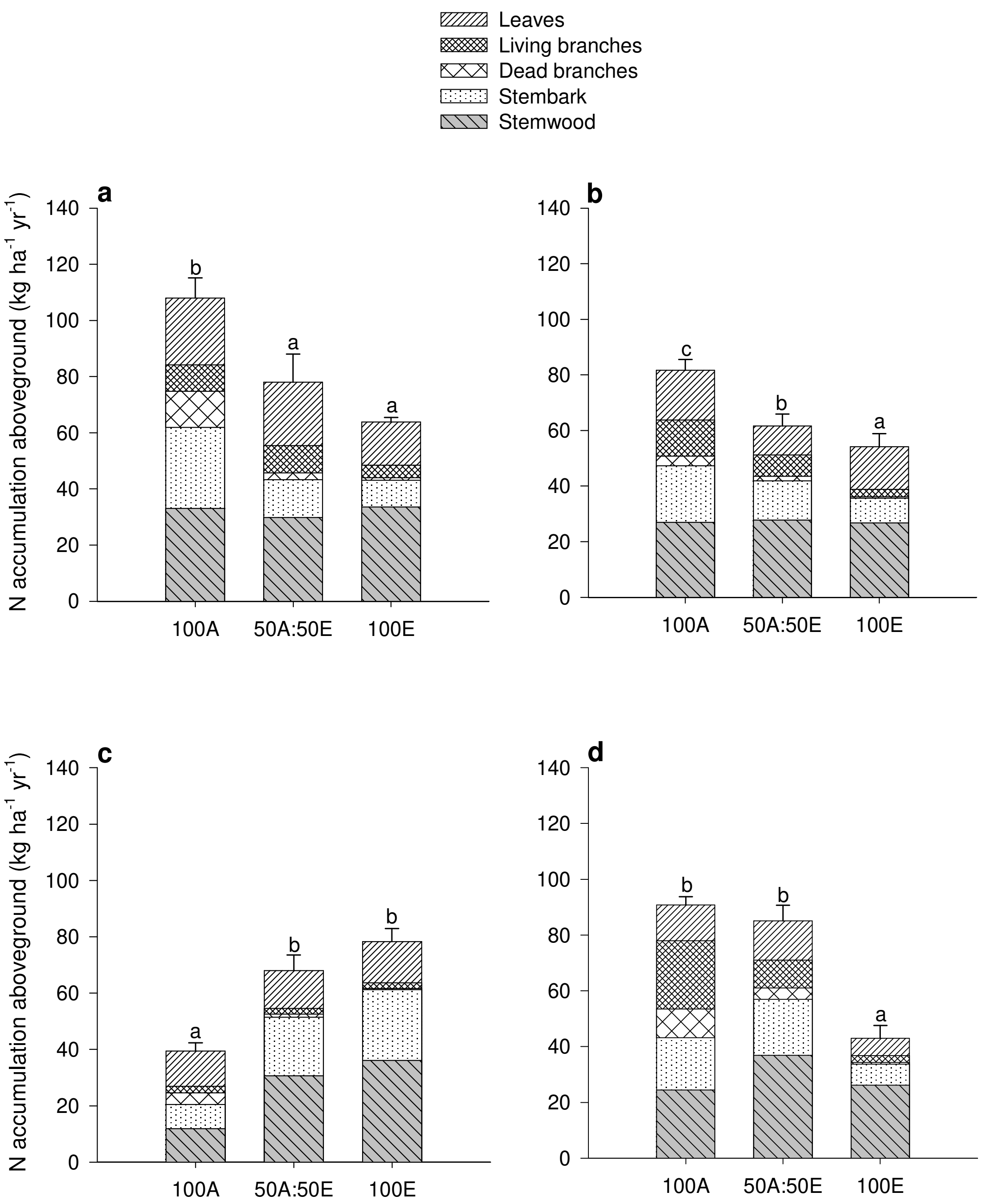

Figure 3. Mean annual N accumulation in the aboveground tree components over a rotation of 6 years (harvesting age) in 100A, 50A50E and 100E at IT (a), BO (b), LA (c) and SdP (d). Vertical bars show standard errors between blocks for each treatment (*n* = 3 at IT and *n* = 4 at BO, LA and SdP). Different letters indicate significant differences between treatments in total N content aboveground at the same site ($P < 0.05$).

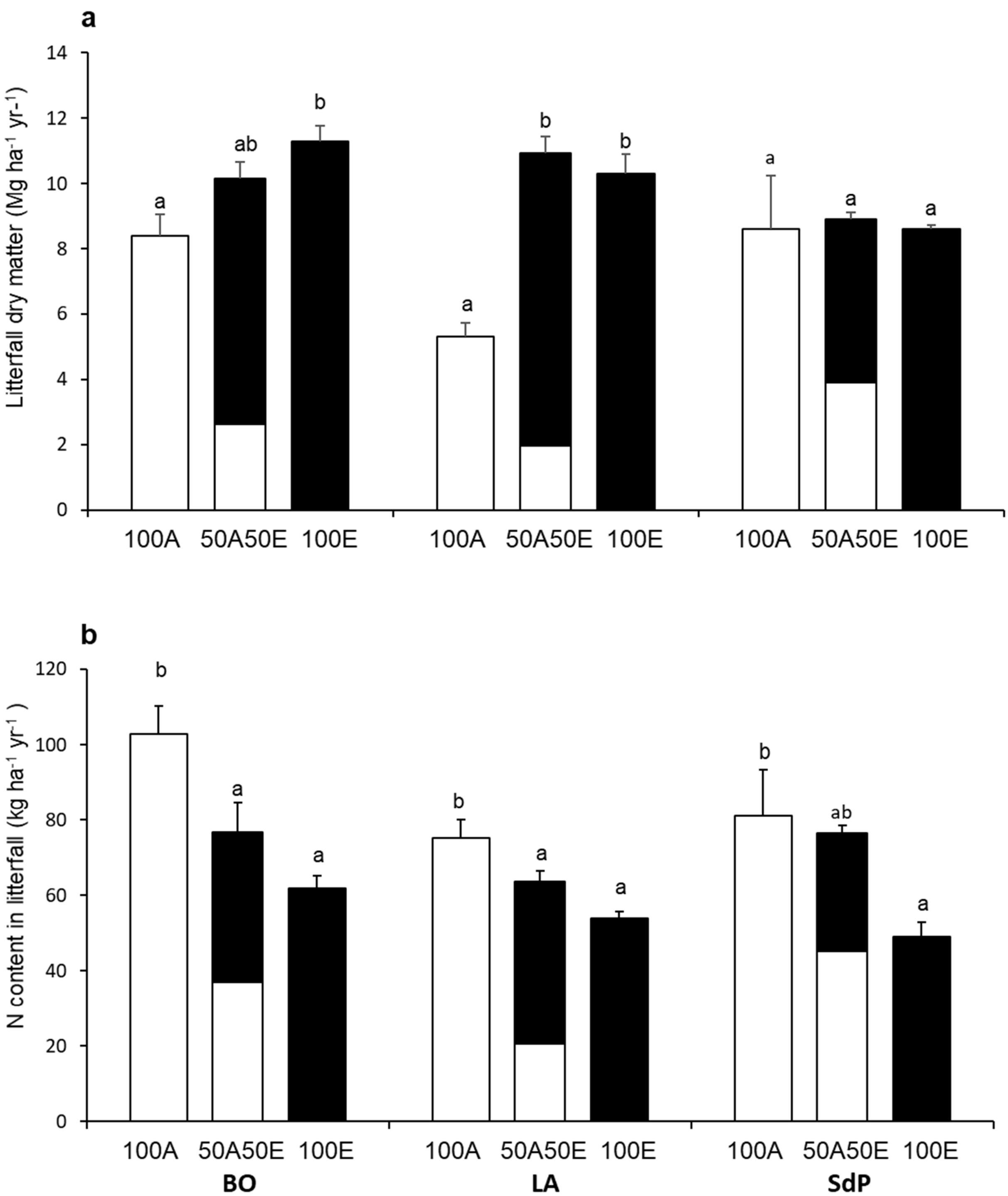

Figure 4. Annual litterfall dry matter (Mg ha^{-1} yr^{-1}) from 4 to 6 years after planting a) and annual N content (kg ha^{-1} yr^{-1}) in litterfall b) in 100A, 50A50E and 100E at BO, LA and SdP. Vertical bars show standard errors between blocks for each treatment (*n* = 3). Different letters indicate significant differences between treatments at the same site ($P < 0.05$).

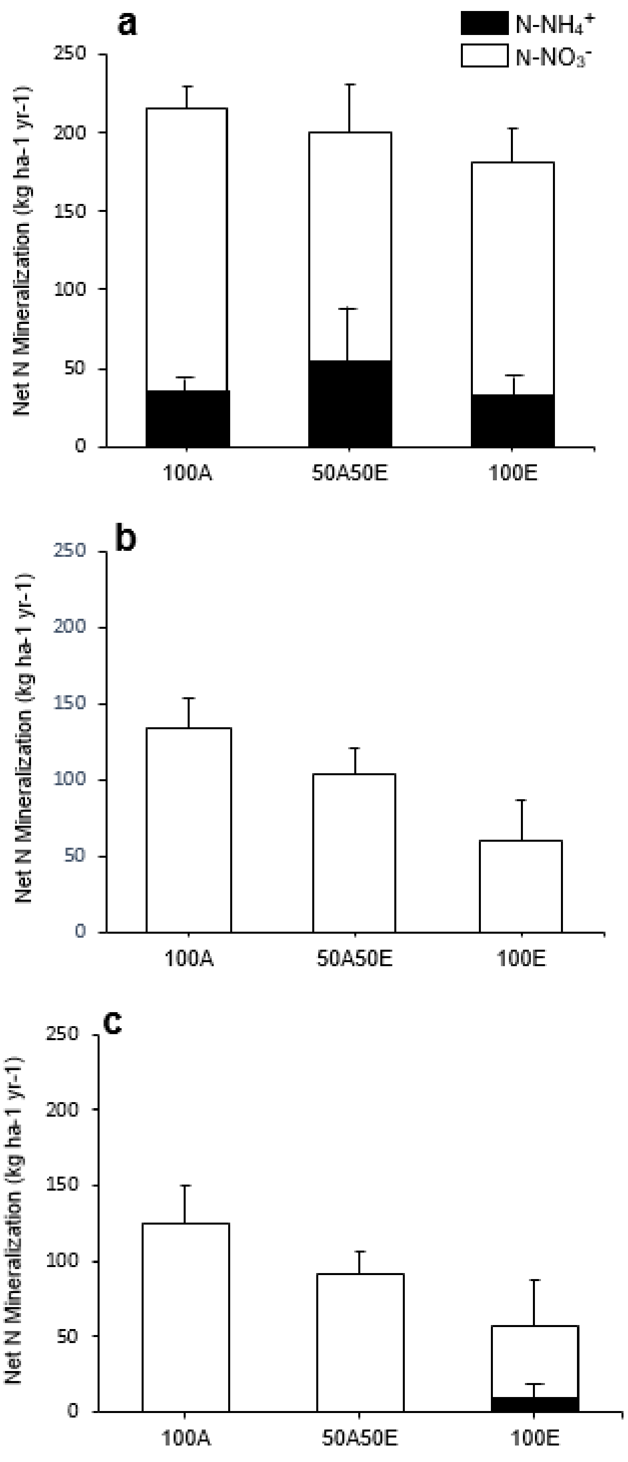

Figure 5. Net ammonification and nitrification rates in 100A, 50A50E and 100E at BO a), LA b), and SdP c). Vertical bars show standard errors between blocks (*n* = 3).

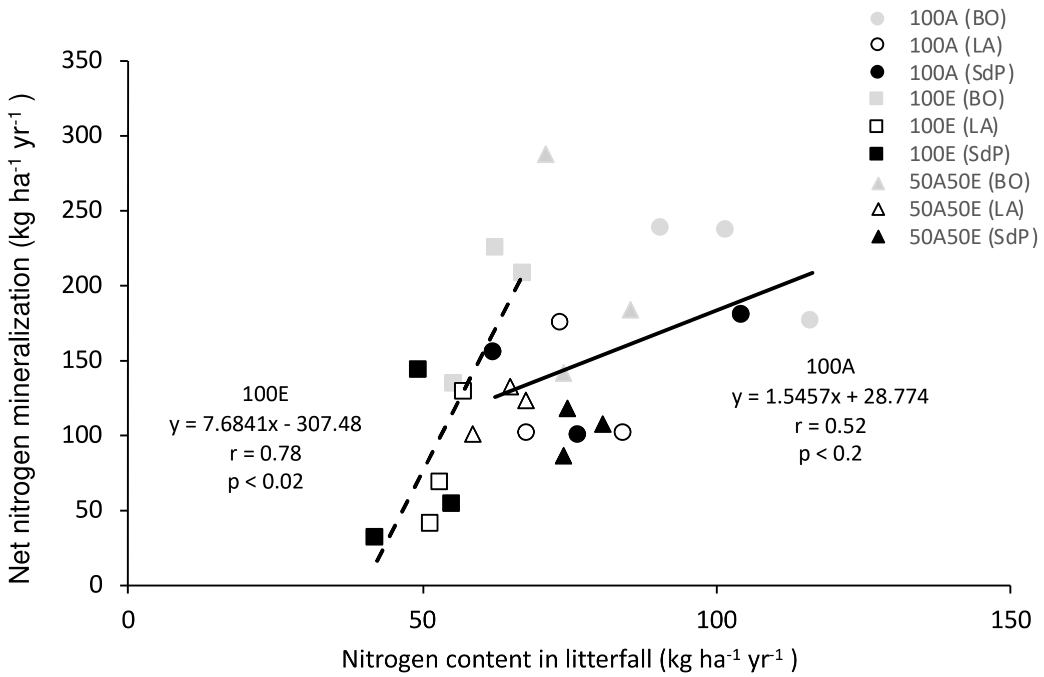

Figure 6. Relationship between net nitrogen mineralization in the top soil and nitrogen content in litterfall from 4 to 6 years after planting in 100A, 50A50E and 100E. The dotted and the full lines show linear regressions for 100A and 100E, respectively, across all the studied plots.

Table 1. Mean and standard deviation between blocks (n = 3) of selected soil properties in the 4 experiments. The fertilization regimes in each experiment are indicated.

Área	Soil layer (cm)	Bulk density ($g\ cm^{-3}$)	pH_{H2O}	Organic matter (%)	Resin P ($mg\ kg^{-1}$)	Sum of base cations ($cmol_c\ kg^{-1}$)	ECEC ($cmol_c\ kg^{-1}$)	Fertilization ($kg\ ha^{-1}$)
Itatinga	0-5	1.23 (0.20)	5.5 (0.2)	3.5 (0.8)	7.3 (1.5)	0.96 (0.23)	1.76 (0.27)	P: 44; K: 85
(IT)	50-100	1.42 (0.04)	5.8 (0.3)	0.7 (0.0)	2.0 (0.0)	0.02 (0.00)	0.58 (0.01)	dolomite: 2000
	200-300	1.49 (0.07)	5.7 (0.1)	0.4 (0.0)	1.0 (0.0)	0.02 (0.01)	0.21 (0.04)	B, Fe, Zn, Mn
Bofete	0-5	1.27 (0.16)	4.5 (0.2)	2.4 (0.5)	11.3 (7.8)	0.40 (0.21)	5.87 (0.19)	P:37; K: 186
(BO)	50-100	N.D.	4.6 (0.3)	0.5 (0.1)	1.0 (0.0)	0.11 (0.00)	1.90 (0.43)	Boiler ash: 3000*
	200-300	N.D.	5.1 (0.1)	0.1 (0.1)	1.0 (0.0)	0.11 (0.00)	0.85 (0.22)	B, S
Luiz Antônio	0-5	1.38 (0.11)	4.8 (0.1)	1.7 (0.3)	14.0 (4.6)	0.84 (0.12)	4.32 (0.08)	N:4; P:29; K:147
(LA)	50-100	N.D.	4.8 (0.2)	0.4 (0.1)	1.7 (0.6)	0.13 (0.04)	1.98 (0.27)	Lime: 1200
	200-300	N.D.	4.8 (0.2)	0.2 (0.0)	1.0 (0.0)	0.11 (0.00)	1.02 (0.05)	Cu, Zn, B
Santana do	0-5	1.04 (0.14)	5.5 (0.5)	3.8 (0.8)	6.4 (2.5)	5.78 (4.64)	10.75 (4.96)	N: 6; P: 45;
Paraíso	50-100	N.D.	4.7 (0.3)	1.2 (0.2)	1.3 (0.6)	0.14 (0.02)	3.79 (0.43)	K: 162; dolomite:
(SdP)	200-300	N.D.	5.0 (0.3)	0.1 (0.0)	1.0 (0.0)	0.16 (0.01)	2.15 (0.43)	1500; Cu, B, Zn

* N content in boiler ash was < 0.1%; N.D.: not determined.

Table 2. Effects of treatments (T), sites (S) and interaction between treatment and site (T x S) on forest floor dry matter (M_F, kg ha^{-1} yr^{-1}), N stock in the forest floor (N_F, kg ha^{-1}), C stock in the 0-15 cm soil layer (S_C, kg ha^{-1}), N stock in the 0-15 cm soil layer (S_N, kg ha^{-1}), mineral N stock in the 0-20 cm soil layer (N_M, kg ha^{-1}), net N mineralization (M_N, kg ha^{-1} yr^{-1}), litterfall dry matter (L_{DM}, kg ha^{-1} yr^{-1}), N content in the litterfall (L_N, kg ha^{-1} yr^{-1}), and total N stock in trees + forest floor + 0-15 cm soil layer (N_T, kg ha^{-1}). Different letters in the same row are indicated when the differences between treatments or between sites are significant ($P < 0.05$).

	P values			Mean value per treatment			Mean value per site		
	Treatment	Site	T x S	100A	50A50E	100E	BO	LA	SdP
M_F	0.0776	**<0.0001**	0.6550	1391	1547	1744	**1712 b**	**1831 b**	**1139 a**
N_F	**<0.0001**	**0.0183**	0.1438	**86 c**	**36 a**	**55 b**	**68 b**	**54 a**	**56 a**
S_C	0.9525	**0.0115**	0.859	35712	36565	36384	**35927 ab**	**31400 b**	**41334 a**
S_N	0.7968	**<0.0001**	0.8311	1785	1775	1713	**1495 a**	**1215 a**	**2564 b**
N_M	**< 0.0001**	**< 0.0001**	**0.0239**	**16.6 a**	**12.8 b**	**12.4 b**	**12.0 b**	**11.6 b**	**18.2 a**
M_N	0.0678	**< 0.0001**	0.9475	158.5	137.9	99.8	**199.0 b**	**99.5 a**	**98.0 a**
L_{DM}	**<0.0001**	0.5840	0.3145	**7.5 b**	**5.0 a**	**10.0 b**	8.2	7.0	7.2
L_N	**<0.0001**	**0.0183**	0.1438	**173 a**	**72 c**	**110 b**	**135 a**	**107 b**	**112 b**
N_T	**0.0303**	**< 0.0001**	0.156	**2432 a**	**2360 ab**	**2064 b**	**2064 b**	**1754 b**	**3148 a**

Table 3: Nitrogen stocks in tree biomass, in the forest floor and in the 0-15 cm soil layer (kg ha^{-1}) approx. 6 years after planting in 100A, 100E, and 50A50E at 4 sites in Brazil. Nitrogen fixation rates in 100A and 50A50E were estimated by difference between total N stocks relative to 100E (accretion method). Standard errors between blocks are indicated (n = 3). Significant effects are indicated in bold ($P < 0.05$). Different letters in the same row are indicated when the differences between treatments are significant.

	IT			**BO**			**LA**			**SdP**		
	100A	**50A50E**	**100E**	**100A**	**50A50E**	**100E**	**100A**	**50A50E**	**100E**	**100A**	**50A50E**	**100E**
N in tree biomass	**648 ± 43 b**	**471 ± 14 a**	**383 ± 10 a**	**524 ± 10 c**	**416 ± 31 b**	**301 ± 17 a**	**216 ± 49 a**	**412 ± 15 a**	**465 ± 13 b**	**429 ± 68 ab**	**544 ± 19 b**	**278 ± 5 a**
N in forest floor	**192 ± 12 b**	**118 ± 5 a**	**104 ± 11 a**	230 ± 9	151 ± 26	141 ± 16	**231 ± 32 b**	**147 ± 7 a**	**119 ± 7 a**	136 ± 26	133 ± 1	107 ± 26
Soil N (0-15 cm)	1210 ± 95	1247 ± 70	1150 ± 70	1488 ± 76	1544 ± 47	1453 ± 39	1175 ± 47	1289 ± 69	1180 ± 59	2693 ± 100	2491 ± 253	2506 ± 266
Total N in trees and soil	2050 ± 141	1836 ± 82	1637 ± 52	**2242 ± 75 b**	**2111 ± 89 ab**	**1895 ± 47 a**	1622 ± 111	1848 ± 64	1764 ± 63	3258 ± 80	3168 ± 271	2891 ± 239
N_2 fixation	413 ± 170	199 ± 132	0	348 ± 111	217 ± 81	0	-	84 ± 103	0	367 ± 318	277 ± 476	0
N export within stemwood	198 ± 13	179 ± 6	201 ± 4	164 ± 8	169 ± 16	164 ± 9	64 ± 14	182 ± 2	217 ± 4	148 ± 26	238 ± 9	169 ± 5
N input relative to 100E	416 ±161	221 ± 130	0	348 ± 117	212 ± 90	0	-	119 ± 99	0	388 ± 332	209 ± 472	0

N input relative to 100E in 100A (resp. 50A50E) was computed as: N_2 fixation in 100A (resp. 50A50E) – N export within stemwood in 100A (resp. 50A50E) + N export within stemwood in 100E. High tree mortality at the end of the rotation in 100A at LA prevented from estimating the N_2 fixation rate and the N input relative to 100E.

Table 4. Mean N contents (%) and δ^{15}N (‰) in aboveground components of *Acacia* and *Eucalyptus* trees at Itatinga (IT), Bofete (BO), Luiz Antônio (LA) and Santana do Paraíso (SdP). *P* values show the effects of species, sites and the interaction species x site in 50A50E.

		Acacia				Eucalyptus				*P* value		
	Treatment	IT	BO	LA	SdP	IT	BO	LA	SdP	Species	Site	Species x Site
N content (%)												
Leaves	100E					1.89 ± 0.15	2.19 ± 0.10	2.12 ± 0.19	1.93 ± 0.16			
	50A50E	2.91 ± 0.13	2.49 ± 0.22	2.92 ± 0.35	2.50 ± 0.26	1.99 ± 0.07	2.05 ± 0.25	2.19 ± 0.32	1.78 ± 0.14	**<0.001**	**0.033**	0.165
Living branches	100E					0.23 ± 0.04	0.40 ± 0.10	0.30 ± 0.04	0.21 ± 0.04			
	50A50E	0.79 ± 0.10	0.64 ± 0.24	0.59 ± 0.06	0.59 ± 0.10	0.26 ± 0.03	0.39 ± 0.07	0.35 ± 0.03	0.29 ± 0.03	**<0.001**	**0.034**	**0.029**
Stembark	100E					0.26 ± 0.03	0.27 ± 0.01	0.23 ± 0.01	0.34 ± 0.01			
	50A50E	1.13 ± 0.01	1.26 ± 0.06	1.05 ± 0.03	1.30 ± 0.14	0.26 ± 0.01	0.28 ± 0.01	0.26 ± 0.03	0.36 ± 0.01	**<0.001**	**<0.001**	**0.021**
Stemwood	100E					0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01			
	50A50E	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.03	0.18 ± 0.02	0.06 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.00	**<0.001**	**0.011**	0.760
δ^{15}N (‰)												
Leaves	100E					-0.91 ± 0.56	-0.77 ± 1.94	-1.90 ± 1.54	2.49 ± 1.50			
	50A50E	-0.76 ± 0.93	3.73 ± 3.43	0.91 ± 0.28	8.81 ± 2.02	-1.14 ± 0.93	1.68 ± 1.44	-1.27 ± 1.96	2.93 ± 1.39	**<0.001**	**<0.001**	0.057
Living branches	100E					-5.98 ± 1.20	-1.78 ± 0.05	ND	0.95 ± 3.10			
	50A50E	-2.49 ± 1.16	-0.95 ± 2.26	ND	4.70 ± 2.34	-0.89 ± 0.93	-0.43 ± 0.93	ND	4.24 ± 2.16	**0.003**	**<0.001**	0.389
Stembark	100E					-2.88 ± 2.85	-2.78 ± 0.66	ND	0.73 ± 1.37			
	50A50E	-1.77 ± 4.21	-2.26 ± 0.68	ND	6.68 ± 1.40	-2.89 ± 3.77	-1.28 ± 2.01	ND	1.78 ± 1.90	0.106	**<0.001**	0.224
Stemwood	100E					3.48 ± 1.06	-0.61 ± 0.66	ND	-2.86 ±0.99			
	50A50E	-2.17 ± 0.58	-2.39 ± 2.80	ND	2.10 ± 1.43	0.53 ± 1.36	0.83 ± 2.00	ND	2.80 ± 8.84	0.881	**0.014**	0.010

ND: not determined.