



Nitrogen accumulated and biologically fixed by uninoculated *Anadenanthera peregrina* (L.) Speg trees under monospecific stands in the Atlantic Forest biome

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Abstract

Nitrogen-fixing legumes are widely used to improve ecosystems. However, N accumulation in biomass, N₂ amounts biologically fixed, and the effects on soil N status are poorly understood for plantations with slow-growing N₂-fixing legume trees in seasonally dry environments. In this study, we assessed these questions in monospecific stands of uninoculated *Anadenanthera peregrina* (L.) var. *peregrina* (angico) trees established in the Atlantic Forest biome. Nine experimental plots with a stand density of 1111 tree ha⁻¹ were examined about five to six years after tree planting. N content in the leaves, branches, bark, and wood was estimated using allometric equations and N concentration in the tissues. To assess the N content, the topsoil layer was sampled in each plot and in one pasture area. The annual N₂ fixation rate at the stand scale was estimated by the natural abundance of ¹⁵N, using leaves of angico and a mix of leaves of herbs and shrubs growing in the understory in each plot. N accumulation in aboveground biomass was estimated at 260 kg ha⁻¹ and N allocated in the leaves and branches was four-fold that in the tree stem. N derived from biological N₂ fixation was estimated at 50% on average, with an annual rate of N fixed in the aboveground biomass of 22 kg ha⁻¹ year⁻¹. Soil N storage was similar between *A. peregrina* stands and the pasture area at six years after planting. Long-term gains of N₂ fixation may be greatly improved by the adoption of breeding programs for this slow-growing species and the use of an adequate rhizobium strain.

Keywords Forest plantation · Forest restoration · Legume trees · Natural abundance of ¹⁵N · Seasonally dry climate · Symbiotic N₂ fixation

1 Introduction

Seasonally dry tropical forests in the Americas cover more than 519,000 km² and are constantly undergoing anthropogenic degradation (Portillo-Quintero and Sánchez-Azofeifa 2010). Forest recovery in these regions is compromised, as

trees are subjected to increasingly severe droughts and low fertility of degraded soils. Legume trees are abundant in Central and South America and are largely used in plantations (Castro et al. 2017). Many legume trees adapted to dry climates have functional traits of survival strategies, such as high wood density and deciduous leaves (Souza et al.

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2012b; Reich 2014). Moreover, leguminous trees with the ability to fix N_2 from the atmosphere via symbiosis with diazotrophic bacteria have been widely used in land reclamation to increase OM, N, and other nutrients in tropical soils (Binkley and Giardina 1997; Franco and Faria 1997; Sprent et al. 2010; Chaer et al. 2011). Furthermore, fixed N can be transferred to neighboring species by different routes, such as litter, pruning of branches, mortality of roots and nodules, release of root exudates, and by mycorrhizae (Munroe and Isaac 2014; Paula et al. 2015).

N_2 -fixing legume trees planted or naturally regenerated in degraded lands may increase ecosystem services. Biomass accumulation and N_2 -fixing capacity constitute a competitive advantage that varies widely among species with either fast- or slow-growing strategies. However, the performance regarding N accumulation in biomass using biological N_2 fixation, as well as soil N effects, is better known for fast-growing than for slow-growing species (Cramer et al. 2007; Chaer et al. 2011; Shi et al. 2016; Voigtlaender et al. 2019). N accumulation and biological N_2 fixation have been investigated in tropical forest plantations, mainly for the fast-growing genera *Acacia*, *Leucaena*, and *Albizia*, where studies show high N_2 fixation rates in biomass when trees grow in satisfactory edaphoclimatic (e.g., high mean annual temperature and non-limiting water availability over the year) and silvicultural conditions (Binkley and Giardina 1997).

Anadenanthera peregrina (angico) is a slow-growing N_2 -fixing species with high wood density and deciduous leaves that grow naturally in seasonally dry regions of the Amazonia, Caatinga, Cerrado, and Atlantic Forest biomes, with two variations of the species reported: *A. peregrina* var. *peregrina* and *A. peregrina* var. *falcata* (Benth) Altschul (Lorenzi 2014; Morin 2015). *A. peregrina* has been used in agroforestry systems and in monospecific and mixed plantations for land reclamation in Brazil due to its potential use as wood products (e.g., charcoal, firewood, timber) and non-timber forestry products, mainly tannins (Souza et al. 2012b; Andrade et al. 2013; Lorenzi 2014). In spite of the potential of *A. peregrina* negatively affect the natural regeneration due to denser crown and allelopathy, this does not necessarily inhibit the natural regeneration over long-term since allelopathic effects might be species-specific as suggested for monoculture plantation of 30 years old (Souza et al. 2012b) and 70 years old (Nascimento et al. 2009). These studies found at least 29 and 66 tree species occurring in association with dominant *A. peregrina*, respectively. Nodulation and N_2 fixation capacity was observed in a natural population of *A. peregrina* in Pará State (Faria et al. 2011). However, rhizobia isolates from adult trees and inoculated in seedlings of *A. peregrina* var. *falcata* have shown low capacity of N_2 fixation (Gross et al. 2002).

We investigated N accumulation in aboveground biomass and N rate derived from N_2 fixation, as well as the effects on

soil N status of adult trees of *A. peregrina* var. *peregrina* in monospecific stands established in an area formerly occupied by grass pasture in seasonally dry Atlantic Forest biome in Southeast Brazil.

2 Material and methods

2.1 Study site

The study was carried out in stands of *A. peregrina* at the Instituto Federal do Espírito Santo (IFES), in the municipality of Alegre, Espírito Santo State (20°46'15.46" S and 41°27'13.04" W), Southeast Brazil. The climate of the region is Aw (Köppen climate classification) with rainy summers and dry winters, annual rainfall 1200 mm, and mean annual temperature 23 °C (Alvares et al. 2013). The dry months (rainfall < 30 mm) in the region are June, July, and August, but in May and September, rainfall reaches roughly 50 mm. The region relief varies from wavy to mountainous with the presence of chains of mountains and culverts and average altitude 150 m (Instituto Brasileiro de Geografia e Estatística 1987).

This study was conducted in nine experimental plots, equally subdivided into three blocks, with spacing 3 × 3 m (1111 tree ha⁻¹). Each plot comprised an area of 1500 m². *A. peregrina* was planted in June 2011 in an area previously occupied by *Brachiaria* sp. pasture for at least 40 years without the application of lime and fertilizers (Fig. 1). The seedlings were grown in the Reserva Natural Vale in the municipality of Linhares, Espírito Santo State, Brazil (Instituto Ambiental Vale). The seeds were obtained from stock plants in the municipality of Dom Elizeu, Pará State, Brazil. The seedlings were not inoculated with rhizobia strains; however, the presence of nodules on fine roots of adult trees was observed during exploratory collection (Fig. 1). Cattle were removed before planting and the pasture was desiccated by glyphosate. The seedlings were planted in pits of 30 × 30 × 30 cm. Each seedling received 220 g of commercial fertilizer NPK 06-30-06 and micronutrients (0.2% B; 0.2% Cu; 0.2% Zn). The amounts of N, P, and K were 13.2, 66.0, and 13.2 g per seedling, respectively. Maintenance occurred during 12 months after planting with replanting, crowning seedlings, and the control of leafcutter ants.

The soils were classified by IFES technicians in the 1980s and adapted according to Embrapa Classification (2006) (Souza 2018). These soils correspond to the order of Acrisols, Ferralsols, and Cambisols (FAO classification), in blocks 1, 2 and 3, respectively. The altitude of plots ranged between 133 and 210 m, while the slope ranged between 2 and 39% (Souza 2018). The physical and chemical attributes in the surface soil layer (0–20 cm) were determined about six years after planting in experimental plots (Campanharo



Fig. 1 *A. peregrina* trees in the experimental site and nodules sampled from fine roots during exploratory collection (topsoil 0–20 cm) in the fall of 2016

2017, unpublished data). In brief, the average clay concentration (\pm standard deviation) was $35.1 \pm 6.3\%$, the pH (in H_2O) was 5.3 ± 0.2 , the P and K concentrations were 2.8 ± 2.4 and 53.3 ± 24.3 mg dm^{-3} , the Ca and Mg concentrations were 3.1 ± 2.0 and 2.2 ± 1.1 cmol dm^{-3} , and the average soil organic matter (SOM) was 21.4 ± 4.9 g kg^{-1} . Texture and soil fertility values were statistically equal between blocks, except for Ca and Mg concentrations, which

were slightly higher in block 2 than in block 3 (Table 1 Supplementary material).

2.2 Topsoil sampling and total N analysis

The soil was sampled at depths 0–5, 5–10, and 10–20 cm. N concentration was determined about six years after planting in the plots with angico trees and in an area occupied with *Brachiaria* sp. for at least 40 years, close to the experimental plots. In each plot, soil layers were sampled at six different distances from six angico trees, under $\frac{1}{4}$ of the hypothetical area of 9 m^2 occupied by each tree. The sampling points were spaced 0.5 m each. The six simple soil samples collected in each plot were mixed per soil layer, and one fraction was recovered for analyses. Eighteen samples were collected per soil layer in the pasture area and three composite samples were formed from a set of six repetitions. Total N concentration was determined using the Kjeldahl method, described by Mendonça and Matos (2005).

Soil density was measured from samples collected in the central position of each experimental plot and three points in the pasture area, using a 5-cm-diameter metal ring. The soil samples were dried at 105 °C for 72 h and weighed to obtain the soil dry mass. The mean values of soil density for the three blocks were 1160, 1273, and 1265 $kg\ m^{-3}$ at 0–5, 5–10, and 10–20 cm soil layers, respectively. In the pasture area, values were 1173, 1242, and 1236 $kg\ m^{-3}$ at 0–5, 5–10, and 10–20 cm, respectively.

Soil N storage ($kg\ ha^{-1}$) was estimated for each soil layer by multiplying the soil N concentration by the mean value of the soil density determined for each block with angico trees or by the mean value determined for the pasture area.

2.3 Estimation of N accumulated in aboveground biomass

The circumference at breast height (CBH) of all the trees in the plot was measured when the trees were about six years old using a millimetrically graduated tape. The CBH values were converted into diameter at breast height (DBH) using the formula $DBH = CBH/\pi$. Total height (H) was measured using a clinometer Haglöf model (ECII) for 30 trees distributed inside the plot, excluding two lines of buffer rows. A linear model was adjusted using data on H and DBH of 270 trees in total, measured in nine plots (Fig. 1, Supplementary Material). This equation was used to estimate the heights of the other trees in the plot.

To obtain the aboveground biomass, data on DBH and H measured at six years after planting were applied to the equations adjusted by Souza (2018) (unpublished data) to estimate biomass of leaves, branches, bark, and wood (Table 2, Supplementary Material). In brief, biomass equations were fitted with the measurement of aboveground

biomass of trees about five years after planting based on Souza (2018). The trees were selected according to the mean diameter \pm standard deviation, sampling one tree per plot. After cutting, all leaves, live and dead branches, and stems (wood + bark) were weighed in the field. Subsequently, subsamples of all components were taken to the laboratory to obtain DM at 65 °C until constant weight. Stembark biomass was estimated using the average percentage of bark on discs removed at five different heights along the stem. The above-ground biomass for each plot was converted into hectares using the plot area (1500 m²).

N concentration was determined using the Kjeldahl method (Malavolta et al. 1989) in the samples of leaves, live branches, stembark, and stem wood collected from nine trees (72 samples) five years after planting. The samples were grounded in a Wiley mill (2 mm mesh). The mean N concentration (g kg⁻¹) in each aboveground component was multiplied by its respective biomass to estimate the N accumulated in the stand six years after planting. We assume that the small interannual growth rate observed in *A. peregrina* trees possibly does not cause large changes in allometry and N status at five and six years of age.

2.4 Estimation of N derived from biological fixation

We used the technique of natural abundance of ¹⁵N to estimate N from the atmosphere (%Ndfa) (Boddey et al. 2000; Unkovich et al. 2008; Chalk et al. 2016). When the trees reached about six years old, three healthy trees of different sizes (small, medium, and big) were selected in the central area in each plot. For each tree, all leaves in four branches (one branch per cardinal point) in the lower, intermediate, and upper portions of the canopy were sampled. Leaves of all plots were then mixed for the isotopic analysis.

Leaves from the main species of herbs and shrubs growing in the understory were sampled randomly in each plot. The species identified were: *Lantana camara* L.; *Solanum asperum* L. C. Rich.; *Commelina erecta*. L.; *Baccharis* sp.; *Bidens* sp.; *Centrosema pubescens* Benth.; *Vernonanthura phosphorica* (Vell.) H. Rob.; and *Gomphrena* sp.; *Chromolaena* sp. The species unidentified belong to families Amaranthaceae, Asteraceae, and Malvaceae.

All leaves were dried at 65 °C to constant weight, grounded in a Wiley mill (2.0 mm mesh), and one subsample was stored. The equipment was cleaned between each milling using a vacuum cleaner, compressed air jet, and ethyl alcohol. The $x(^{15}\text{N})$ (Coplen 2011) values were determined in the mass spectrometer model Hydra 20–20 coupled to an automatic N analyzer model ANCA-GSL (Sercon Co., Krewe, UK). Approximately 10 mg of leaves were used in each analysis of ¹⁵N and the precision of isotopic measurements was 0.0001 $x(^{15}\text{N})$.

The values of $x(^{15}\text{N})$ were converted to $10^3 \delta^{15}\text{N}$ according to Eq. (1):

$$10^3 \delta^{15}\text{N}_{\text{sample}} = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{air}}}{(^{15}\text{N}/^{14}\text{N})_{\text{air}}} \times 1000 \quad (1)$$

where ¹⁵N the value of $x(^{15}\text{N})$ determined in the sample and in the air (0.3663%); ¹⁴N = 100 — $x(^{15}\text{N})$.

The N percentage from biological N fixation (%Ndfa) was calculated using the equation proposed by Shearer and Kohl (1986) [Eq. (2)]:

$$\% \text{Ndfa} = 100 \left(\frac{\text{AE}_{\text{REFx}} - \text{AE}_{\text{Fx}}}{\text{AE}_{\text{REFx}} - \beta} \right) \quad (2)$$

where AE_{REFx} value of $10^3 \delta^{15}\text{N}$ determined for the set of leaves of understory plants in each plot; AE_{Fx} value of $10^3 \delta^{15}\text{N}$ determined for the set of leaves of *A. peregrina* trees in each plot; and β value of $10^3 \delta^{15}\text{N}$ is equal to zero referring to the $10^3 \delta^{15}\text{N}$ value from the atmosphere.

The technique of natural abundance of ¹⁵N is the most widely used to estimate %Ndfa in undisturbed plantations and natural forests. This technique demands knowledge of the β value, that is, the isotopic fractionation that occurs during N fixation when plants are supplied only with N from the atmosphere. To our knowledge, the β value has never been estimated for seedlings or adult *A. peregrina* trees. Furthermore, the β value may change according to tree species and age (Chalk et al. 2016) and can range from - 3 to + 1 in legume trees (Shearer and Kohl 1986; Boddey et al. 2000; Gehring and Vlek 2005).

2.5 Statistical analysis of the data

Differences in N concentration and N accumulation between aboveground components, and $10^3 \delta^{15}\text{N}$ values between leaves of *A. peregrina* trees and understory plants were tested using the t-test for normal data (and square root transformed) and, in some cases, using the Mann–Whitney rank sum test for non-normal data. Differences between understory plants and “zero” ($10^3 \delta^{15}\text{N}$ values from the atmosphere) were tested using the Mann–Whitney rank sum test. The relationship between values of $10^3 \delta^{15}\text{N}$ in leaves of trees and understory plants and the relationship between values of $10^3 \delta^{15}\text{N}$ in leaves and the %Ndfa of trees was studied using regressions. Moreover, the relationship between aboveground N and %Ndfa, as well as the amounts of aboveground N from N₂ fixation and soil N storage, was studied using regressions. Differences in N storage in topsoil between plots with trees and the pasture area were examined using mean values and standard deviation. Normality was verified using the Kolmogorov–Smirnov test with the Lilliefors correction. The statistical analysis was performed

with SigmaPlot 13 (Systat Software Inc. 2014), using a statistical threshold value of $P=0.05$.

3 Results

Angico trees were the only species occurring in the overstore six years after planting. The mean values of the survival rate of angico trees surpassed 83% in the experimental plots. The mean value of H and DBH of angico trees in experimental plots was 7.3 m and 9.0 cm, respectively, and the basal area was $7.0 \text{ m}^2 \text{ ha}^{-1}$.

N concentration was higher in leaf tissues, followed by bark, branches, and wood with mean values 22.16, 10.72, 6.63, and 2.08 g kg^{-1} , respectively (Fig. 2). The mean of N accumulated in aboveground biomass was 259.6 kg ha^{-1} with rates $44.8 \text{ kg ha}^{-1} \text{ year}^{-1}$. Leaves and branches accounted for 81.3% of total N accumulated in aboveground biomass on average, with 96.8 and $114.2 \text{ kg N ha}^{-1}$, respectively. By contrast, N accumulated in stem biomass was half of the values found for leaves, with a mean value $48.6 \text{ kg N ha}^{-1}$.

$\delta^{15}\text{N}$ ranged between -0.15 and 5.68‰ in angico leaves and between 1.17 and 7.0‰ in leaves of understory plants, with mean $\delta^{15}\text{N}$ values of 1.63 and 2.54‰ , respectively (Fig. 3a). The mean $\delta^{15}\text{N}$ in leaves of understory plants was significantly higher than in the atmosphere and angico leaves ($P < 0.05$). The values of $\delta^{15}\text{N}$ in angico leaves and understory plants were positively correlated.

The mean %Ndfa was estimated at 50.3%, while N_2 fixation ranged between zero and 100% (Fig. 3b). N_2 fixation rates decreased significantly with increased $\delta^{15}\text{N}$ values

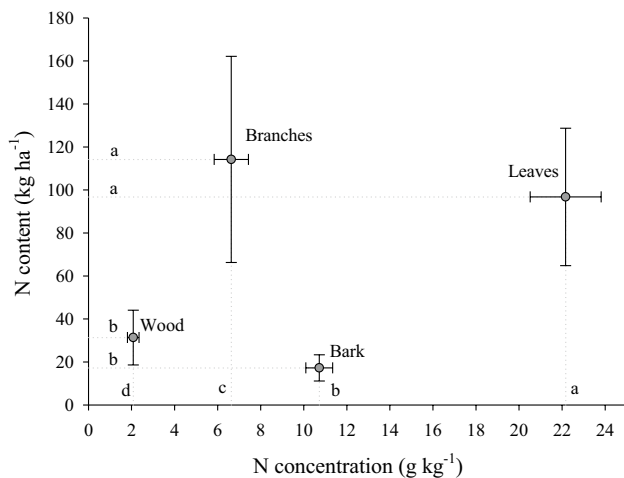


Fig. 2 N content and N concentration in aboveground biomass of *A. peregrina* trees. Vertical and horizontal error bars indicate standard deviations between the plots ($N=9$) and the trees ($N=9$), respectively. Different letters between biomass compartments indicate statistical differences ($P < 0.05$)

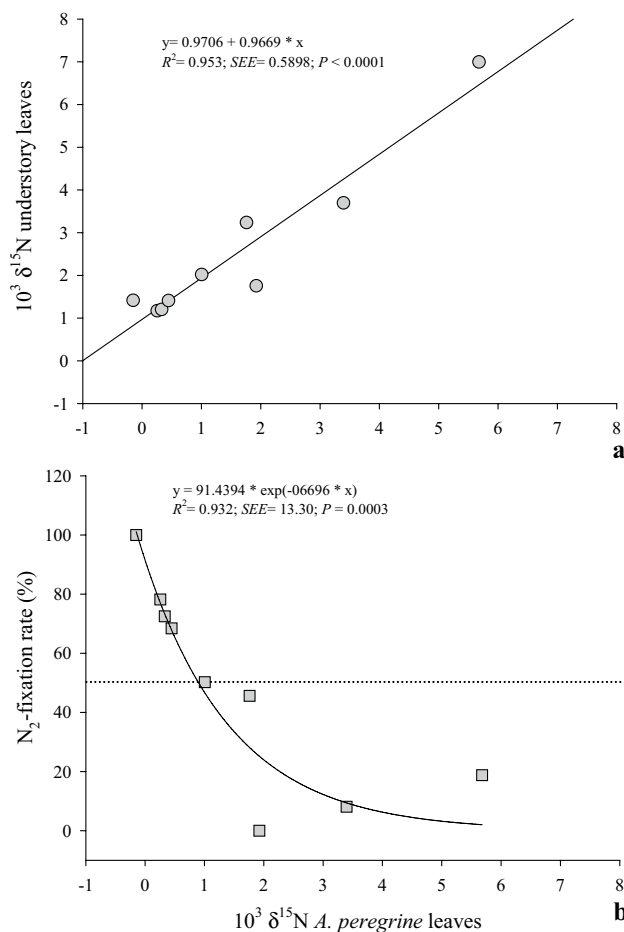


Fig. 3 Relationship between $10^3 \delta^{15}\text{N}$ in leaves of *A. peregrina* trees and leaves of understory plants (a), and between N_2 fixation rates and $10^3 \delta^{15}\text{N}$ values in leaves of *A. peregrina* trees (b). SEE standard error of the estimate. The dotted line indicates the mean N_2 fixation rate in the experimental site

in angico leaves. The N mean derived from biological fixation accumulated in the aboveground biomass was estimated at 130.6 kg ha^{-1} with a rate of N fixed equals to $22.5 \text{ kg ha}^{-1} \text{ year}^{-1}$ in the early stage of angico growth. Aboveground N accumulation in angico trees was inversely related to %Ndfa in experimental plots, with a significant P value for regression (Fig. 4a).

Amounts of aboveground N from biological N_2 fixation ranged from zero to 216.3 kg ha^{-1} (Fig. 4b). N storage at 0–20 cm soil depth ranged between 3584.2 and $5890.2 \text{ kg ha}^{-1}$. We observed a trend of decrease in the amounts of aboveground N from N_2 fixation with the increase of soil N storage in experimental plots; nevertheless, the reduction was not significant.

The mean N content in topsoil was similar between angico plots and the reference pasture, with 1391 and 1396 kg ha^{-1} , 1288 and 1333 kg ha^{-1} , and 2201 and 2164 kg ha^{-1} for 0–5, 5–10, and 10–20 cm soil layers, respectively (Fig. 5). The

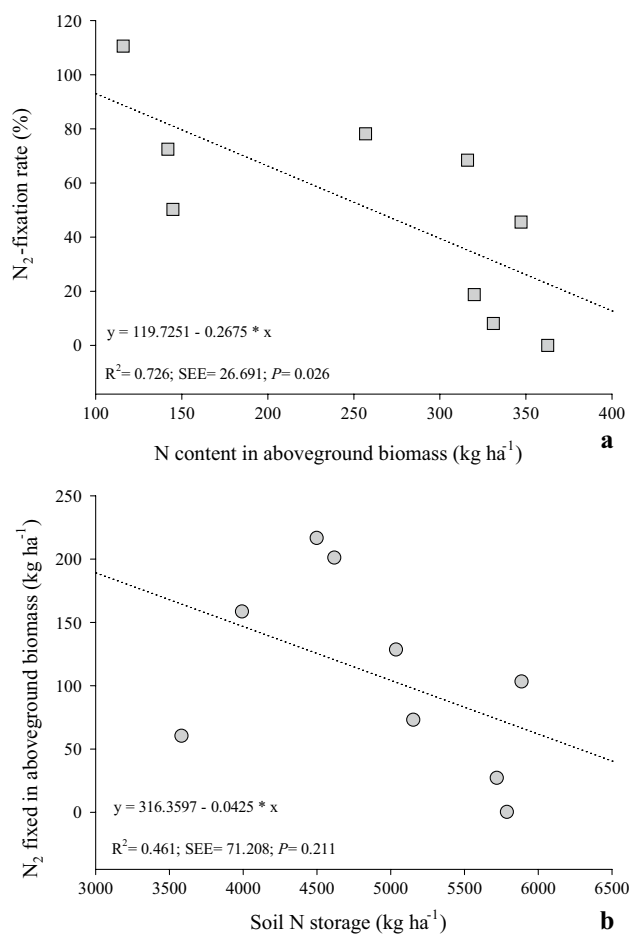


Fig. 4 Relationship between the N_2 fixation rate and the N content in aboveground biomass (a), and between N amounts fixed in aboveground biomass and N storage at 0–20 cm soil depth in the experimental plots. *SEE* standard error of the estimate

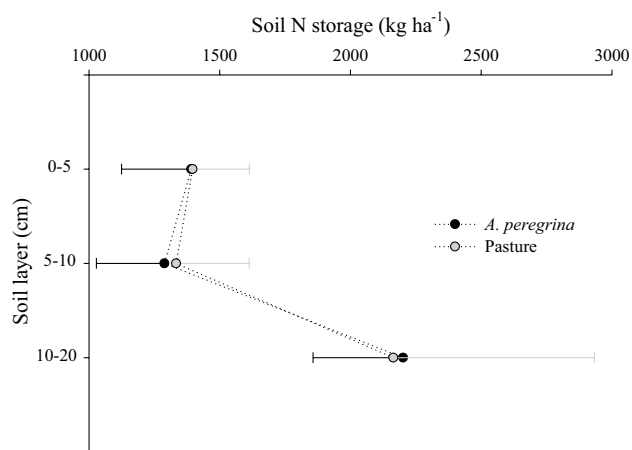


Fig. 5 N storage in topsoil layers in plots with *A. peregrina* trees and pasture areas with predominance of *Brachiaria* sp. Horizontal error bars indicate standard deviations between plots with *A. peregrina* ($N=9$) and *Brachiaria* sp. ($N=3$)

maximum and minimum values for 0–5, 5–10, and 10–20 cm soil layers of angico plots ranged between 950 and 1757, 795 and 1566, and 1442 and 2695 $kg\ ha^{-1}$, respectively.

4 Discussion

Growth and aboveground N accumulation in angico trees was lower than fast-growing legume species, such as *Acacia mangium* Willd. (Paula et al. 2018; Voigtlaender et al. 2019) and *Pseudosamanea guachapele* (Kunth) Harms (Balieiro et al. 2002); however, it was similar to several native Brazilian tree species (Coelho et al. 2007; Amazonas et al. 2018). For instance, N accumulated in aboveground biomass of angico trees was lower than 623 $kg\ N\ ha^{-1}$ accumulated in pure stands of 60-month-old *P. guachapele* (Balieiro et al. 2002) and 660 $kg\ N\ ha^{-1}$ for 72-month-old *A. mangium* growing in pure stands in four different sites in Brazil (Voigtlaender et al. 2019). The slow growth rate of *A. peregrina* trees implies wood density values higher than some tree species, such as *A. mangium* and *P. guachapele* (see Icrat database online). Trees with high wood density generally show lower growth rates, leaf photosynthetic rates, and stem hydraulic conductivity, but greater survival rates in stressful environments (Reich 2014).

Higher mean $\delta^{15}N$ values in leaves of understory plants compared to angico trees in eight out of nine experimental plots suggest the occurrence of N fixation. The maximum difference observed in $\delta^{15}N$ values between understory plants and angico trees was 1.75‰. A difference of $\delta^{15}N$ lower than 5‰ in leaves of N_2 -fixing and reference plants may have limited the estimation of N_2 fixation because of ^{15}N fractionation in plants and the precision of the isotopic analysis (Högberg 1997). However, previous studies reported estimations of N_2 fixation even when differences were smaller than 5‰ (Coelho et al. 2007; Reis et al. 2010; Souza et al. 2012a; Bigli et al. 2021). Boddey et al. (2000) reported that the ^{15}N natural abundance technique should be viable when $\delta^{15}N$ values in reference plants are significantly higher than 0‰ or when $\delta^{15}N$ of legumes obtain 100% of N from the atmosphere (β value). Sometimes, it is not possible to use the natural abundance method, as observed for trees in tropical rainforests (Gehring and Vlek 2005) and mixed-species plantations (Forrester et al. 2007; Bouillet et al. 2008; Voigtlaender et al. 2019). In these cases, $\delta^{15}N$ values are lower in leaves of several N_2 -fixing legume trees than in leaves of reference non-legume trees. In five plots in our study, the use of the β value equal to zero led to N fixation rates above 50%. However, the fractioning of ^{15}N during fixation may lead to β values generally smaller than 0‰ in leaves that could thus surpass the N_2 fixation rate if $\delta^{15}N$ is equal to 0‰ is used. β values are below zero in *Mimosa caesalpinifolia* Benth. (− 1.24‰) (Reis et al. 2010), *A.*

mangium (− 0.3‰) (Galiana et al. 2002), and in wild populations of *Cyclopia* sp. (− 1.29 to − 0.24‰) (Spriggs and Dakota 2009). A more accurate estimation of %Ndfa on stand development requires knowledge of the appropriated β value for the species. However, the exact β value is difficult to ascertain in the field, due to the fractionation of ^{15}N caused by a plant life stage, and rhizobia and mycorrhizae strains associated with trees (Högberg 1997; Forrester et al. 2007; Chalk et al. 2016).

The annual rate of N fixed in aboveground biomass of *A. peregrina* since the planting of $22.5 \text{ kg ha}^{-1} \text{ year}^{-1}$ was lower than the minimum $50 \text{ kg ha}^{-1} \text{ year}^{-1}$ reported for vigorous stands of legume trees planted in tropical climate conditions (Binkley and Giardina 1997). However, this amount of N fixed was similar to other leguminous and actinorhizal trees growing in seasonally dry environments, such as *Acacia senegal* L. Willd with $12 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Raddad et al. 2005), and *Casuarina equisetifolia* L. with $15 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Mariotti et al. 1992). Parrotta et al. (1996) reported declines in %Ndfa values with increasing tree age for fast-growth *Leucaena leucocephala* (Lam.) de Wit trees in Costa Rica because of greater N availability in the soil. Souza et al. (2012a) also showed higher N_2 fixation rates in slow-growth old legume trees in the final successional stage than in young legume trees in the first successional stage of the Caatinga dry forest biome. In the same stands of *A. peregrina*, the mean value of %Ndfa was similar (59%) to the present study, while *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby trees were the reference species for N_2 fixation. Moreover, the annual rate of N fixed in aboveground biomass to 44-months-old was also similar ($23.0 \text{ kg ha}^{-1} \text{ year}^{-1}$) (Bighi et al. 2021).

Variability of the N_2 fixation rate could be associated with different factors in our study site, namely lack of inoculation with specific strains of rhizobium during the seedling growth, the need for an adequate symbiont in the soil (Bala et al. 2003), such as mycorrhizal strains, N demand by trees and N availability in the soil, as well as competition with weeds and trees (Binkley and Giardina 1997; Cramer et al. 2007; Paula et al. 2018; Bighi et al. 2021). Competition between understory plants and adult angico trees is reduced mainly because solar radiation in the understory decreases significantly in the summer in our study site (unpublished data). Moreover, an allelopathic effect over the grass could have taken place for *Bachiara bryzantha* (Hochst.), as observed by Nogueira et al. (2018). The grass cover was sparse, especially in the plots in blocks 1 and 2, and absent in the plots in block 3 (unpublished data). The proportion of grass-covered soil ranged from 20 to 85% in four plots, while the N_2 fixation rate ranged from 50 to 100% in these plots. In the other five plots without grass incidence, the N_2 fixation rate ranged from 0 to 68%. Further studies could assess the effects more deeply of the interaction between *A. peregrina*

and *Brachiaria* grass on N_2 fixation rates in *A. peregrina* trees. The mean %Ndfa values equal to 50% found in the present study is similar to the average value of 42% estimated for leguminous plants growing in natural conditions (Andrews et al. 2011) and the minimum %Ndfa value of 47% reported for the species *A. columbrina* (Vell.) Brenan, growing in the Caatinga biome with more than 50 years of regeneration (Souza et al. 2012a).

The fact that $\delta^{15}\text{N}$ values seem positive between angico trees and understory plants suggests that both species access N pools with a similar abundance of ^{15}N (Craine et al. 2015). However, differences in preferred N sources (e.g., ammonia, nitrate, and amino acid) are known for various tree species and herbaceous species (McFarland et al. 2010). According to Unkovich et al. (2008), the application of the natural abundance method could be compromised when the main source of N uptake differs between the N_2 -fixing plant and the non-fixing reference plant. N transfer estimation was not assessed in this study because of uncertainties regarding the isotopic signature of the dominant N source that could be transferred from angico trees to understory plants (Peoples et al. 2015). However, N transfer may occur from angico trees to understory plants by litterfall, root, and nodule decomposition or direct transfer via mycorrhizal networks and roots exudates (Munroe and Isaac 2014; Paula et al. 2015). N transfer from N_2 -fixing legumes may modify $\delta^{15}\text{N}$ signature of soil N available to both N_2 -fixing and reference plants (Carlsson and Huss-Danell 2014), which could lead to small differences in $\delta^{15}\text{N}$ values in leaves of angico trees and understory plants in our study site.

Annual N mineralization in the soil may provide larger N amounts in comparison with aboveground accumulation of N in *A. peregrina* stands, for example, the rate of about 3% of the total N mineralized estimated from monospecific stands of *A. mangium* growing in Aw climate conditions in Santana do Paraíso, Southeast Brazil (Voigtlaender et al. 2019). Thus, soil N availability possibly did not limit the growth of *A. peregrina* in our study site. Moreover, N available in the soil could explain the decrease of %Ndfa in the plots with greater aboveground N accumulation. On the other hand, while N_2 fixation is crucial for tree growth and survival in degraded soil, the metabolic cost of symbiosis is higher than the uptake of soluble N compounds from the soil (Schubert 1986). Regulation of N_2 fixation according to N availability in the soil has been reported for leguminous species in natural tropical rainforest (Barron et al. 2011). The increase of soil N availability has been associated with the decrease of N derived from N_2 fixation in fast-growing *A. mangium* (Galiana et al. 2002) and *L. leucocephala* (Parrotta et al. 1996).

Furthermore, other factors may influence N pools in our study site, such as spatial differences in the landscape structure that influence resource availability for tree

growth, as well as SOC accumulation and loss, leading to changes in soil N pools. Paula et al. (unpublished data) found that soil N and C stocks were positively and significantly related, in our study site. Further studies could investigate deeper the environmental factors that affect the silvicultural performance of *A. peregrina* trees.

N accumulation in angico leaves was at least twofold higher than in wood, which is an important characteristic that could help accelerate N cycling in an ecosystem. However, in our study, there was no evidence of a significant increase in soil N content by angico trees in relation to the pasture area until six years after planting. Soil N status increases quickly after planting leguminous trees in barren land or in soil degraded by agriculture (Wang et al. 2010; Shi et al. 2016). These rapid increases are rare when trees are planted in pasture lands (Shi et al. 2016) or in eucalyptus plantations even after > 6 years of N-rich inputs from fast-growing, N₂-fixing *A. mangium* trees (Voigtlaender et al. 2019).

Plantations of *A. peregrina* trees may help reduce the initial costs associated with restoration, as these trees grow alongside grasslands in seasonally dry climates and have capacity to increase the N amount in the ecosystem via N₂ fixation. Inputs of N-rich tissues from *A. peregrina* trees are important for the ecosystem in the long term, as a large fraction of N accumulated in the aboveground biomass is allocated to leaves of deciduous species. In addition, belowground biomass of angico tree is possibly an important compartment for N storage (Bighi et al. 2021), as well as a source of N to the soil; however, this was not assessed in this study. Furthermore, breeding programs could use *A. peregrina* trees in forest plantations of economic interest in addition to adopting adequate rhizobium strains for this species.

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Isotopic analysis: PCO; Writing original draft: RRP, LJM. Review and Edition: RRP, LJM. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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