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Nutritional efficiency in *Acacia Mangium* Willd seedling under nitrogen application

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Abstract

Even though *Acacia mangium* is able to perform Nitrogen Biological Fixation (NBF), seedlings are grown under N-fertilizer application during the initial stages of seedlings. The hypothesis tested herein is that the N supplying for Acacia seedling until the establishment of the symbiosis relationship is a required practice to enhance seedlings growth, N accumulation and Nitrogen Use Efficiency (NUE). In this sense, the goal of this study was to evaluate the implications of increasing N rates on Acacia seedlings' growth and nutritional status in earlier stages of growth. The study was performed along four months in greenhouse with increasing N rates (0, 50, 100 and 200 mg dm^{-3}) applied in pots. Plant parameters evaluated included: collar diameter, height, number of leaves, shoot and root dry matter. Plant nutritional status was evaluated through N content in leaves and NUE at the end of the trial. Findings herein showed that increased N rates applied enhanced all plant parameters, as follow: height (+53%), coller diameter (+43%), number of leaves (+70%) and shoot (+52%) and roots (+68%) dry matter production. Overall, increasing N rates promoted linear increasing on plant growth. Moreover, the N rates applied also improved the N content into the leaves, stem and roots in 55, 41 and 82%, respectively. The NUE was enhanced from 1.7 to 3.9 g mg^{-1} and plant dry matter was correlated with N content (r = 0.88). The findings herein indicate that N uptaken by plants was efficiently converted into dry matter. The high-N rates promoted higher N accumulation in roots (leaves>roots>stem) with a reduction in N fluxes, while N-low rates increased N fluxes and a reduction of N accumulation in roots (leaves>stem>roots) was observed. Based on our results, the N supplying during Acacia seedlings' earlier stages, when seedlings are not able to perform NBF, was a promising strategy to increase plant growth as well as enhancing NUE by plants.

Keywords: Seedling production, Forest Nutrition, Forestry, N_2-fixing.
Abbreviations: N_nitrogen; DQI_the dickson quality index; TMD_total dry matter weight; H_plant height; DMR_dry root matter weight; DMS_shoot part; NAE_absorption efficiency; NTE_N translocation efficiency; PTN_plant total N (shoot + roots); TNS_is the total N in shoot; NAsE_N assimilation efficiency.

Introduction

Brazil is a prominent player in roundwood production of planted forest, with approximately 7.8 million hectares under forest production, the country is also able to supply 91% of wood used at the local-industries. Planted forest with Acacia trees (*Acacia mangium* Willd) represents a small area with around 590.000 hectares, this area encompasses the planted forests with Rubber (*Hevea brasiliensis*), Teak (*Tectona grandis*), and Parica (*Schizolobium amazonicum*). Planted forest with Eucalyptus (*Eucalyptus* sp.) and Pine (*Pinus* sp.) represent the larger Brazilian area with a total of 7.3 million hectares (IBA, 2019).

The Acacia is a forest species native from Papua New Guinea, Indonesia and Australia, widely planted around the world. The Acacia trees are rusticity, present fast growth and they can reach 25 to 30 meters high (Krisnawati et al., 2011). Planted forest with Acacia has been recommended to roundwood production to supply the furniture industry and as an energy source for the cellulose industry. The Acacia also has been recommended as a promise alternative to recovery degraded areas due to its potential to recovery nutrient cycling in degraded systems, especially with N and carbon increase into the soil (Tonini et al., 2018). The Acacia is a leguminous tree able to fix N_2 from the atmosphere (Diouf et al., 2005; Dôbereiner, 1984; Galiana et al., 1990; Ribet and Drevon, 1996). Two Australian Acacia species (*A. mangium* Willd *and A. auriculiformis*) were compared regarding their potential to fix N_2 and the *A. mangium* Willd had higher N_2-fixation capacity than *A. auriculiformis*, , which explains higher productivity of *A. mangium* Willd under low fertility soil (Galiana et al. 1990). The high N-fixation efficiency of Acacia has been associated with the inoculation of microorganisms [*i.e*. mycorrhizal fungi, *Bradyrhizobium* spp. (Diouf et al., 2005; Galiana et al., 1990; Paula et al., 2015; Santos et al., 2017; Koutika and Richardson, 2019)], and phosphorus (P) supplying (Ribet and Drevon, 1996). Galiana et al. (2002) using ^{15}N showed that N_2-fixed by *A. mangium* varied from 19 to 90% with a high dependence upon soil fertility and inoculation of microorganisms.

Even though Acacia is able to perform NBF, the establishment of Acacia seedling is done with mineral N fertilizers in tropical conditions. According to Faquin (2005), N supplying is needed during the early stages of seedling growth, when the symbiosis (i.e., the plant-microorganisms relationship) is not established

yet. In this sense, there is not a consensus regarding the optimal N rate in tropical condition due to the high dependence on soil texture, N_2-fixation, and the stages of plant development. According to Schumacher et al. (2013), 40 and 79 kg ha^{-1} N and P respectively, are required for Acacia transplanted in a clay loam soil with low P content. Santos et al. (2019) recommended 800 mg dm^{-3} of P in a sandy soil with low P content for better seedling development for transplant done 120 days after planting. In general, the suitable N management for Acacia is challenging due to the lack of information about their nutritional requirements (Gonçalves et al., 2013), such missing information may explain the low area planted with Acacia as compared to the area under Eucalyptus and Pine in Brazil (Reis et al., 2012; Stahl et al., 2013).
With sustainable development and the pursuit of 4R nutrient management principles (i.e., right source, right rate, right time and right place), studies presenting the N-fertilizer demand for the Acacia is needed for suitable N management, to achieve the circular economy with harmony among the economy, environment and society (Ghisellini et al., 2016). Forest seedling suitable management is mandatory to promote high vigor and good nutritional status of plants late on the fields. In contrast, malformed seedlings show a low tolerance to adverse conditions in the fields when transplanted, under such conditions plants are susceptible to weather stress, pests' attack and diseases (Turchetto et al., 2016). Moreover, the seedling with low quality may lead to re-planting operation, which increases the production costs and makes plants growth heterogeneous in the planted forest, affecting the management, planning and economic productivity (Reis et al., 2012).
The hypothesis tested herein is that N supplying is needed in the earlier stages of Acacia development until symbiotic relationship to be established and then BNF occurs. Thus, the goal of this study is to evaluate the N rates implications on Acacia seedlings' growth and NUE.

Results

Morphological parameters

The seedlings height, collar diameter and number of leaves as affected by increasing N rates were adjusted to linear model ($R^2 \geq 89\%$; $P \leq 0.05$), represented by positive balances of +32 and +53; +25 and +43; and +46 and +70%, respectively as compared to the same parameters observed for plants grown within the control under the low-N and high-N rates (Fig 1). These results indicated that N application increased leaves number $plant^{-1}$, plant height and collar diameter, which were 63 leaves, 73.3 cm and 8.4 mm, respectively.
The positive effects of N for plant height and collar diameter reflected in the HD with maximum ratio of 8.8 cm. This shows that there was an increase of 8.8 cm in height for each mm of collar diameter increased. There was low variation of HD and the values ranged from 6.9 to 8.8 cm mm^{-1}, and low balances of +9 and +17% under low and high-N rates. The low variation into HD was expected due to natural structure of plant, and high dependence on plant height and collar diameter, represented by r = 0.74.
The dry matter of shoot and roots under increased N rates was also adjusted to linear model [$R^2 \geq 97\%$; $P \leq 0.05$, (Fig 2)]. The dry matter of roots presented positive balances represented by the increase of +22 and +68%, following by dry matter of shoot with balances of +25 and +52%, respectively in N-lowest and N-highest rates. The general average of total dry matter production was 32.2 and 12.6 g $plant^{-1}$, respectively for the shoot and roots.
The positive implications of N application for the dry matter of shoot and roots impacted the total dry matter and DQI with linear responses and - r^2 higher than 97% (Fig 2). There was a high positive correlation between DQI and dry matter of roots ($r = 0.75$; $P \leq 0.05$), as well as the DQI and shoot ($r = 0.90$; $P \leq 0.05$), which indicated that both outcomes were more expressive for the DQI calculus as compared with the data of height ($r = 0.48$; $P \leq 0.05$) and plant collar diameter($r = 0.73$; $P \leq 0.05$). The highest total dry matter production was 63.7 g $plant^{-1}$ under 200 mg N dm^{-3}, which lead represented a positive balance of +57% using the N-high rate. The DQI also presented a similar balance with an increase of +55%, and the highest value of 6.1 under 200 mg dm^{-3} of N.

N contents and efficiency

The content of N in leaves, stem and roots as affected by increasing N rates was adjusted to linear model ($R^2 \geq 78\%$; $P \leq 0.05$), represented by positive balances of +36 and +55%; +35 and +41%; and +44 and +82%, respectively in N-low and N-high (Fig 3).
The highest N content in plant was observed under 200 mg N dm^{-3} of N and the means recorded were 629.0, 165.4 and 318.3 g kg^{-1} in leaves, stem and roots, respectively. The general N average accumulation within the leaves, stem and roots were 484.6, 138.6, 194.8 g kg^{-1}, respectively. The NAE parameter as affected by the N rates was adjusted into a quadratic model ($R^2 \geq 50\%$; $P \leq 0.05$) and the optimal rate of absorption was 77.8 mg g^{-1} of N. The NAE presented a positive balance (+16%) with application of low-N represented by an increase of 17.2 mg g^{-1}, but under the increase of N rates there was a negative balance (-22%), which lead to a reduction from 71.8 to 59.5 mg g^{-1} (Fig 4).
The NTE presented negative linear response under increased N rates with a reduction from 163.7 to 144.9% ($R^2 \geq 82\%$; $P \leq 0.05$). If compared with control, there was negative balances under the application of N-low and high-N rates, which led to from 163.7 to 159.1% and from 163.7 to 131.1%, respectively (Fig 4). In contrast, there was a positive balance in NUE with increase of +7 and +50%, represented by an efficiency from 1.7 to 1.9 and 1.7 to 3.7 g mg^{-1}, respectively using N-low and high-N rates. The NUE as affected by increasing N rates was adjusted to linear model [$r = 0.95$, $P \leq 0.05$ (Fig 4)].
The N bilateral flow were intense between leaves and stem ($r = 0.80$; $P \leq 0.05$), and stem and roots ($r = 0.84$; $P \leq 0.05$) with the application of N-low rate, represented by the high content of N in leaves, followed by the high N content in steam and roots with a respective mean of 268.7; 90.4; 54.5 g kg^{-1}. Interestingly, under the increasing of N rates there was a reduction of N bilateral fluxes represented by lower values of correlations for the routes between leaves and stem ($r = 0.55$; $P \leq 0.05$), and stem and roots ($r = 0.14$; $P \leq 0.05$). This fact explains the high N content in roots 268.8 g kg^{-1} which was 42% higher than N content in stem, but lower than the N content in leaves (568.7 g kg^{-1}), Fig 5.

Discussion

The Positive effect of N rates on number of leaves, plant height and collar diameter of *Acacia mangium* were also reported in the literature (Santos et al., 2019; Sarcinelli et al., 2004; Schumacher et al., 2013), as well as in other forest species such as *Cassia grandis*, *Peltophorum dubium* (Soares et al., 2017), and *Citharexylum myrianthum*, a native species of the Atlantic Forest (Fernandes et al., 2019). In this study, under 200 mg dm^{-3} of N, the height and collar diameter of plants, and number of leaves, were increased 53, 43, and 70% respectively, as compared to the same parameters evaluated in plants growth within the control. Study of Schumacher et al. (2013) reported collar diameter equal to 12.5 cm under 40 kg N ha^{-1} applied in a clay loam soil under low P content. In our study, the collar diameter was lower (maximum of 7.0 cm) because the seedlings were evaluated within the initial plant development stage. The increasing in the number of leaves, followed by the height, and the collar diameter were expected because forest species devot energy to produce primary growth [i.e., height, stem diameter and number of leaves (Reis et al., 2016)] within the initial seedling growth stages.

The roots, shoot as well as the total dry matter were higher under N application, as also shown by Sarcinelli et al. (2004) and Inagaki et al. (2009). In this study, the increased N rates applied lead to linear dry matter production, thus, Acacia seedlings may respond to N rates even higher than 200 mg dm^{-3}. The findings herein are corroborated by Santos et al. (2019), who recommended 800 mg N dm^{-3} for Acacia seedling, under such N rate the seedling height was 172 cm at 120 days after planting. Sarcinelli et al. (2004) showed that the biomass production of *Acacia holosericea* decreased under the following order of nutrients deficiency: N = Mg > K > S > Ca > SC > P; with a high dependence on N within the initial of seedling development. Interestingly, the roots dry matter presented the highest positive balance, which was an increasing of 68% as compared to the same parameter observed under the control and N-high rate, following by dry matter of shoot with a balance of +52%. This result can be explained by the highest N accumulation under the low (+44%) and high N rates (+82). Inagaki et al. (2009) compared N and P implications on fine-roots production of *Acacia mangium* and both nutrients were limited, however, the N showed as the most effective in promoting the fine-roots production. Overall, the N deficiency weakens and delays seedling within the initial development stages (Gonçalves and Benedeti, 2000; Gonçalves and Benedetti, 2004). This fact is associated with plant metabolic disorder, leading to the production of small leaves, premature leaves death, roots without branches and collapse in the development of chloroplasts (Marschner, 2012). In addition, the high dry matter production of roots indicates the rusticity of the seedling (better vigor and photosynthetic capacity) (Caldeira et al., 2014).

The DQI shows up as a good index to monitor seedlings development, the r^2 for this parameter was higher than 97%, and highly correlated with dry matter production. The use of DQI to evaluate seedling development was also suggested in previous studies (Fonseca et al., 2002; de Freitas et al., 2017; Soares et al., 2017). The greater DQI values are associated with better seedling quality and development (Caldeira et al., 2012). In this context, Soares et al. (2017) tested N rates ranging from 50 to 200 mg dm^{-3} and found DQI ranging from 1 to 7 and the highest DQI values were found under 200 mg N dm^{-3} for *Cassia grandis (*DQI = 3.9) and *Peltophorum dubium* (DQI = 3.9) seedlings. Moreover, Fernandes et al. (2019) showed DQI values ranging from 1 to 16 under N rates from 50 to 400 mg dm^{-3} and the highest DQI values were observed under 400 mg dm^{-3} applied for *Citharexylum myrianthum.* In this study, the DQI ranged from 2.5 to 6.1 and the highest values of DQI calculated under 200 mg N dm^{-3}. The optimal value of DQI depends upon the species, fertilization, substrate, volume of pot, irrigation and mainly the age of the seedling (Gomes et al., 2013).

Under N applications the content of the nutrient increased linearly within the leaves, stem and root (Fig. 3). The results are corroborated with the literature in planting regarding *Acacia mangium* (Sarcinelli et al., 2004) and other forest species such as *Khaya senegalensis* A. Juss (Araújo et al., 2019). In this study, the N contents in leaves ranged from 22 to 70 g kg^{-1}, and were within the plant sufficiency according to Marschner (2012), who recommends N content in leaves ranging from 20 to 50 g kg^{-1}. The N application promoted N accumulation in leaves, roots and stem such as 464, 194 and 138 g kg^{-1}, respectively. The lower N accumulation in stem was expected because this plant component is pointed out as an structure of water and nutrients translocation between the roots and the shoot (Marschner, 2012). The routes of N fluxes were intense under the low N rate (i.e., N-low) in leaves, roots, and stem, but it was reduced by increase of N rates with a higher accumulation of N in roots and leaves represented by an increase of 58 and 22%. When the N is taken up and converted in the plant metabolism, the N excess is accumulated in leaves, roots and later in fruit production (Gonçalves and Benedetti, 2004). Our data showed demonstrated that there was a higher accumulation in leaves>roots> stem (under high-N rates) and leaves>stem>roots (N-low rates). For *Khaya senegalensis* A. Juss, Araújo et al. (2019), showed the increasing in N content in leaves under N rates between 80 - 160 mg dm^{-3}. Moreover, for *Anadenanthera macrocarpa* seedling , Gonçalves et al. (2013) reported the highest N content in leaves with the highest N accumulation under 200 mg dm^{-3}. The high N contents were correlated with the dry matter production, which shows that the N was absorbed and converted into roots and shoot dry matter production.

Both, NAE and NTE were reduced under N application with optimal rates of efficiency fitted at 77.8 mg g^{-1} of N and 50 % respectively. This study indicated that increasing N rates, the absorption and translocation efficiency was reduced, this fact may be associated with N saturation in plant. Our data of NAE are corroborated by Carnevali et al. (2016), who noticed higher absorption efficiency (49,7 mg g^{-1}) under 62 mg N dm^{-3} for *Stryphnodendron polyphyllum* seedling. In plant, after the N to be absorpted in the plasma membrane, the nutrient is carried

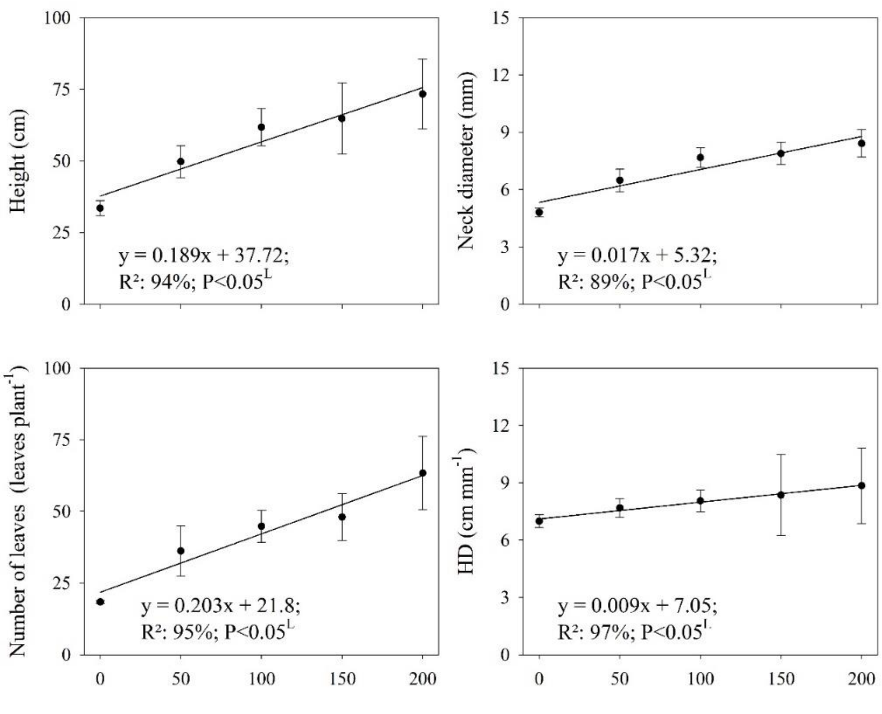

Fig 1. Height, collar diameter, number of leaves, and HD (height/diameter of plants) under increased N rates (0; 50; 100; 150; and 200 mg dm^{-3}) for Acacia (*Acacia mangium* Willd).The N rates were tested by the regression test (P≤0.05), and adjusted to a linear model (^{L}). Means are presented with their respective error deviation, a vertical bar in each mean.

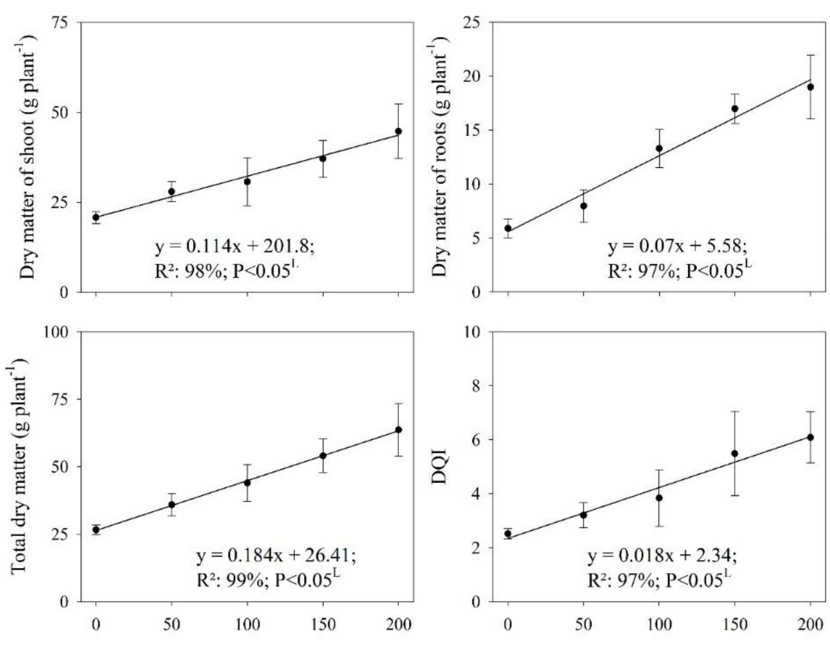

Fig 2. Dry matter (DM) of shoot, roots, and total, and Dickson quality index (DQI) under increased N rates (0; 50; 100; 150; and 200 mg dm^{-3}) applied for Acacia (*Acacia mangium* Willd). The N rates were tested by the regression test (P≤0.05), and fitted by a linear model (^{L}). Means are presented with their respective error deviation, a vertical bar in each mean.

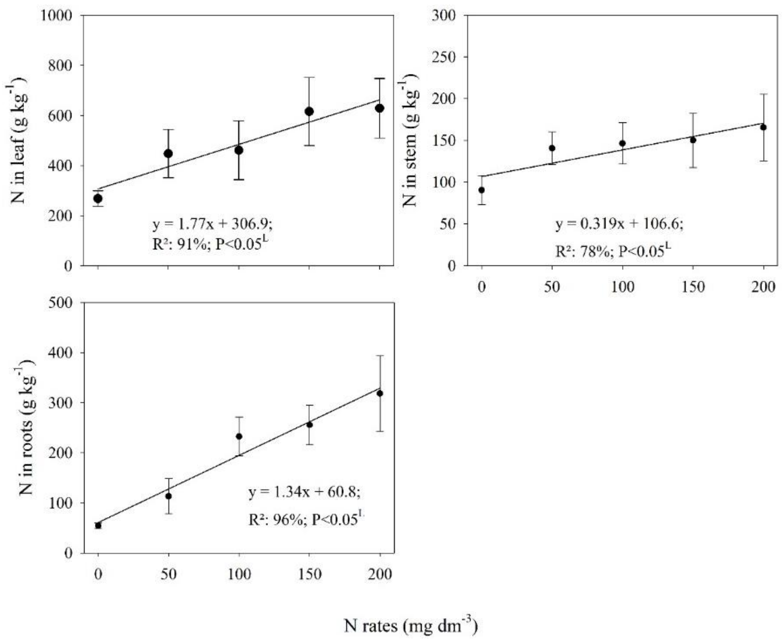

Fig 3. N content in leaves, stem and roots under increasing N rates (0; 50; 100; 150; and 200 mg dm^{-3}) in Acacia (*Acacia mangium* Willd). The N rates were tested by the regression test (P≤0.05), and adjusted to a linear model (^{L}). Means are presented with their respective error deviation, a vertical bar in each mean.

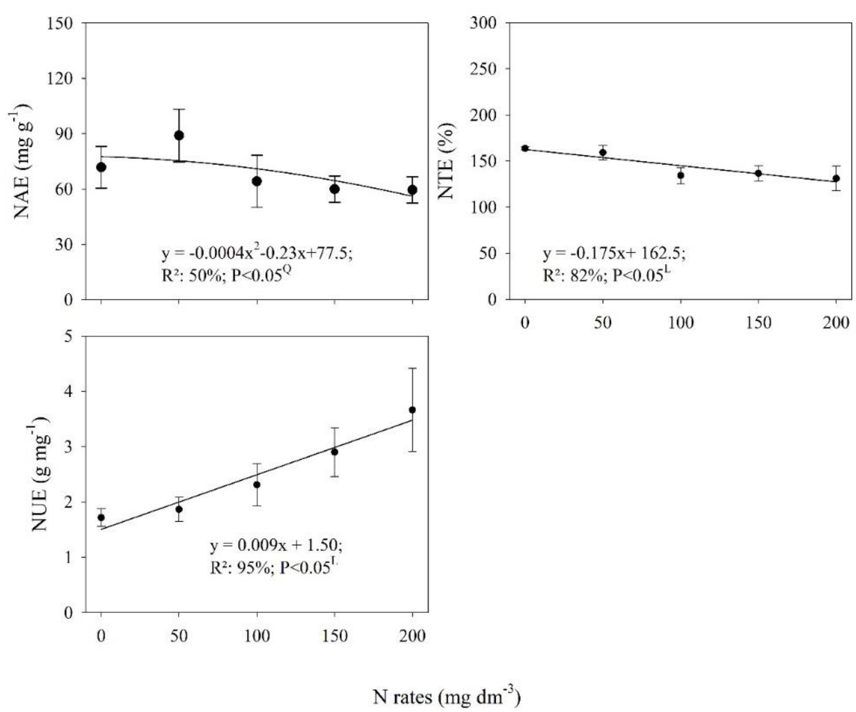

Fig 4. N absorption efficiency (NAE), the N translocation efficiency (NTE), and the N use efficiency (NUE) under increased nitrogen rates (0; 50; 100; 150; and 200 mg dm^{-3}) applied for Acacia (*Acacia mangium* Willd). The N rates were tested by the regression test (P≤0.05), and adjusted to a linear model (^{L}) and fitted by a linear (^{L}) and quadratic models (^{Q}). Means are presented with their respective error deviation, a vertical bar in each mean.

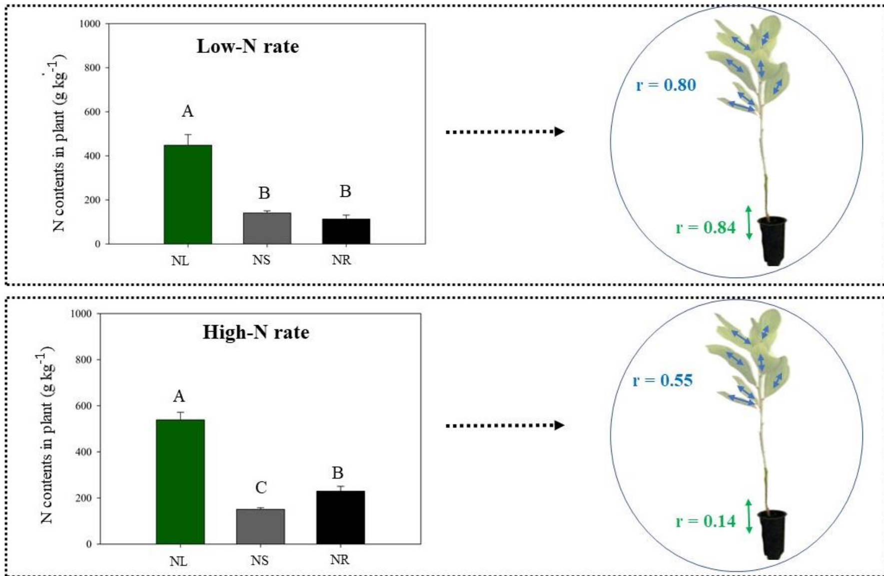

Fig 5. Fluxes in N content in leaves (NL), stem (NS) and roots (NR) under low-N (50 mg dm^{-3}) and high-N (100-200 mg dm^{-3}) rates applied for Acacia (*Acacia mangium* Willd). The N flux was tested trough the Pearson correlation (P≤0.05), and the average of N contents within the leaves (NL), stem (NS) or roots (NR) troughthe LSD test (P≤0.05). Green and blue correlations tested the flux between leaves to stem and from stem to roots, respectively

out by different carriers, classified as low and high affinity carriers; the former acts when the external N concentrations are high and not subject to regulation, while carriers of high affinity operate under low external N concentrations (Von Wirén et al., 1997). While the N translocation is related with biochemical cycling in plant and the capacity of plant to translocate nutrient from a part to another (Gonçalves and Benedetti, 2004). The N is classified as a mobile nutrient (Marschner, 2012), which explained the positive results caused by high N translocation in plant. As compared with N translocation in other forest species, the translocation in *A. mangium* Willd was much higher (164%) than that reported for eucalyptus and *Cedrus* clones, which presented a NTE of 91% (Pinto et al., 2011) and 92% (Batista et al., 2015), respectively.

Interestingly, the NUE was increased under the N applications and the highest value was 3.7 g N mg^{-1}. The NUE is associated with plants ability to metabolize the N applied. Bredemeier and Mundstock (2000), in an extensive literature review showed that suitable N rate enhances the NUE due to better N use and translocation (Xing et al., 2019). The positive effect of NUE is closely associated with both increased dry matter production and N content into the plant. Overall, the N from N-fertilizer, such as urea, causes high N losses from the agroecosystems as ammonia volatilization (Gallucci et al., 2019) and nitrous oxide emissions (Almeida et al., 2015; Carmo et al., 2013), this is especially remarkable when urea is not properly managed. The NUE evaluated herein is associated with the assimilation of N uptaken by plants without relationship with N losses from the agroecosystems. If the NUE of *A. mangium* Willd was compared with other tropical forest species (i.e. *Piptadenia stipulacea*, NUE: 0.36 g mg^{-1}; *Mimosa ophthalmocentra*, NUE: 0.36 g mg^{-1}; and *Schinopsis brasiliensis*, NUE: 0.5 g mg^{-1}) studied by Barbosa et al. (2019), the assimilation of *A. mangium* Willd was much higher [*i.e.*, around 3 g mg^{-1}, Fig. 4)] . Thus, the highest NUE for *A. mangium* is reinforce the the ability of this specie to establish symbiotic association with microorganism and fixing N_2 at roots (Dôbereiner, 1984; Galiana et al., 1990).

Materials and Methods*Experiment characterization*

The study was carried out in a greenhouse in Ipameri, Goiás (17º 43’ 19” latitude S e 48º 09’ 35” longitude W). The experimental design adopted was randomized block with five N rates (0; 50; 100; 150 e 200 mg dm^{-3}) and five replications, totalizing 25 experimental units.

Soil was collected at areas cultivated with pasture (*Brachiaria* sp.) from the 0.2-0.4 m layer. Soil was classified as a Latossolo Vermelho-Amarelo distrófico (Embrapa, 2018). Soil was homogenized, sieved (4 mm mesh), and chemical parameters analyzed (Raij et al., 2001; Walkley and Black, 1934). Soil chemical and physical characterization was as follow: organic matter (7.0 g dm^{-3}), phosphorus (0.8 mg dm^{-3}; Mehlich I), potassium (0.04 cmol$_c$ dm^{-3}) calcium (0.2 cmol$_c$ dm^{-3}), magnesium (0.1 cmol$_c$ dm^{-3}), hydrogen plus aluminum (1.8 cmol$_c$ dm^{-3}), cationic exchange capacity (2.1 cmol$_c$ dm^{-3}), saturation of bases (16%), boron (0.3 mg dm^{-3}), copper (0.3 mg dm^{-3}), iron (34.0 mg dm^{-3}), manganese (10.0 mg dm^{-3}), and zinc (0.3 mg dm^{-3}). Soil bulk density was 1.21 g cm^{-3}, and a texture classified as sandy loam, with respective content of clay, silt and sand: 310; 80; and 610 g kg^{-1} (hydrometer method; Gee and Orr 2002).

Based on soil chemical characterization, soil acidity was increased with application of 0.51 g dm^{-3} of lime (Relative Total Neutralization Power of 92), to achieve 60% of base saturation. Soil and lime were placed in plastic bag at 60% of soil water holding capacity during 60 days to allow lime reacting.

Applications of macro and micronutrients were done with the following rates and respective nutrients sources: 150; 150; 0.1; 0.5; 0.5; 1.5; and 0.5 mg dm^{-3} of phosphorus (superphosphate triple), potassium (potassium chloride), molybdenum (ammonium molybdate), copper (copper sulfate), zinc (zinc chloride), manganese (manganese chloride), and boron (boric acid), respectively. The fertilizers were supplied through a nutritive solution, except for the phosphorus that was applied in soil.

Acacia mangium seeds were harvested in the region of Ipameri, Goiás. Initially, the seeds were treated with hot-water washing, during 60 seconds, due to integumentary numbness in seeds (Mapa, 2009; Rodrigues et al., 2008). Treated seeds were planted in plastic tubes (volume of 53 cm^3) using a commercial substrate (Carolina Soil®; base of sphagnum peat, expanded vermiculite, and rice hulls). Seedling with size of 10 cm was transplanted 90 days after planting.

Limed and fertilized soil was added into plastic pots (black, volume of 5 dm^3), and seedling of Acacia transplanted. The N source used was urea (45% of N), which was equally- splitted into 4 applications, done at 1, 30, 60 and 90 days after seedlings transplanting. The urea was dissolved in water and applied in solution into the soil. During the experimental period, soil was kept at 60 % of soil water holding capacity, to keep such condition, the soil was constantly weighted and water added when needed. By sides, the greenhouse had a black sombrite of 50% and covered with a transparent plastic of 150 microns. The mean temperature inside the greenhouse was 23°C.

Morphological parameters

Height, collar diameter, and number of leaves were measured with graduated ruler and digital caliper at 120 days after transplanting. With the data of height and collar diameter of plants, the height:diameter ratio divided (HD) was calculated. Plants (roots + shoot part) were divided into leaves, stem and roots, and dried in a forced air circulation oven set up at 70 °C for three days. Thereafter, the plant components were weighted to obtain the dry matter of roots (DMR), shoot part (DMS: leaves + stem), then the total dry mass (TDM) was calculated through the sum of DMR and DMS.

The Dickson quality index (DQI), known as seedling quality index (Dickson *et al.*, 1960) was calculated through Eq. 1.

$$DQI = \frac{TMD_{(g)}}{\left(\frac{H_{(cm)}}{ND_{mm}} + \frac{DMS_{(g)}}{DMR_{(g)}}\right)} \qquad \text{Eq. 1.}$$

Where DQI, TMD, H, ND, DMR and DMS stand for the index of seedling quality, total dry matter, the height of planting, the collar diameter of plants, roots and shoot dry matter, respectively.

N contents and efficiencies

The N concentration in leaves, steam and roots were determined within the dried matter samples, which were homogenized and sieved (4 mm mesh), according to the Kjedahl method (Silva, 2009). With data of dry matter weight and N concentration in each plant part, the N content within those plants components was calculated. The N content and dry matter production were used to calculate the N absorption efficiency (NAE) (Swiader et al., 1994), the N translocation efficiency (NTE) (Li et al., 1991), and the N assimilation efficiency (NAsE) (Siddiqi and Glass, 1981), through the respective Equation 2, 3, and 4.

$$NAE\ (mg\ g^{-1}) = \frac{PTN}{DMR} \qquad \text{Eq. 2.}$$

$$NAE\ (mg\ g^{-1}) = \frac{TNS}{PTN} * 100 \qquad \text{Eq. 3.}$$

$$NAsE\ (mg\ g^{-1}) = \frac{TMD^2}{PTN} \qquad \text{Eq. 3.}$$

Where, PTN: is the plant total N (shoot + roots); TNS: is the total N in shoot; DMR: is the dry matter of roots; TDM: is the total dry matter. The N balance into the soil was calculated through the difference of the average calculated for the control (without N application) with the rates of 50 (lowest-N), and 200 mg dm^{-3} of N (highest -N). The N flux content between shoot, stem and roots was monitored in low (50 mg dm^{-3} of N) and high N rates (100-200 mg dm^{-3} of N). Correlations between leaves, stem and roots were done by the Pearson correlation ($P \leq 0.05$), and classified as negative (unilateral flux) and positive correlation (bilateral flux).

Statistical analysis

The assumptions of homogeneity of variance and normality of residues were evaluated by the Bartlett-Test and the Shapiro-Wilk-Test, respectively. Data were submitted to the F-test for the analysis of variance (ANOVA). When the F-Test was significant ($P \leq 0.05$), the N rates were tested by the Regression-Test using quadratic and linear models ($P \leq 0.05$). Statistical analysis was performed using R Statistical Software (version 4.0.0; R Foundation for Statistical Computing).

Conclusion

Acacia mangium Willd grown lineary as affected by increasing N rates ranging from 50 to 200 mg dm^{-3}. Similar pattern also occurred for the following parameters: plant high, collar diameter, number of leaves, dry matter production and N content plant.

The highest N rates also promoted the highest N accumulation in roots (leaves>roots>stem) with a reduction in N fluxes, while the lowest N rates increased N fluxes with a reduction of N accumulation into roots (leaves>stem>roots).

Based on our results, the N supplying during the earlier stages of Acacia enhanced seedlings development, as well as seedlings morphological parameters, N use efficiency, and N accumulation in roots.

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