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Edaphic nitrogen and nodulation of *Leucaena leucocephala* (Lam.) de Wit in silvopastoral systems

Nitrógeno edáfico y nodulación de *Leucaena leucocephala* (Lam.) de Wit en sistemas silvopastoriles

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Abstract

In order to reduce the impacts caused by cattle farming systems on the environment, strategies have been focused to include legume species within these systems to promote their potential for improving soil conditions. The symbiotic association between leguminous and bacteria from genus Rhizobium improves the nitrogen uptake by the legume plants and therefore their growth and productivity may be improved. Even though bacteria from this genus naturally occur, they also may be inoculated to plant seeds. Silvopastoral systems (SPS) are an alternative for cattle farming where trees are incorporated to spatial arranges. In this sense, woody legumes have been used as a possibility of being integrated to SPS. In order to evaluate changes associated with the availability of nitrogen and other nutriments within soils as well as the presence of rhizome nodules, in this work, the woody species *Leucaena leucocephala* was established within silvopastoral systems with high density (over 10,000 trees per ha) in a cattle farm, located in Balboa, Colombia. For assessing changes in soils after the establishment an experiment with a randomize complete block design and two treatments (inoculation and not inoculated) was used. Measurements were carried out during 7 months after the establishing the experiment, being the response variables the total and ammonia nitrogen as well as the amount of active nodules of *Rhizobium* on roots. Under the ecological conditions occurred during the experiment the presence of nodules did not show significant differences between treatments (P>0.05), however a total amount of 249, 31 kg/ha of nitrogen was provided to soils while the experimental period.

Key words: Nitrogen, nodulation, *Rhizobium*, senescence, soil nitrogen.

Resumen

La inclusión de leguminosas en sistemas silvopastoriles genera beneficios en la fertilidad de los suelos y en la calidad del forraje en oferta para los animales. La asociación simbiótica de leguminosas forrajeras ricas en bacterias del género *Rhizobium* mejora el contenido de nitrógeno y el desarrollo de la gramínea asociada. Las bacterias de este género se presentan en condiciones naturales cuando las cepas están presentes en el suelo, no obstante pueden ser inoculadas en las semillas antes de la siembra. El trabajo se realizó con la leguminosa arbustiva *Leucaena leucocephala* que fue establecida en sistemas silvopastoriles de alta densidad (10,000 plantas/ha), en una finca ubicada en el municipio de Balboa, departamento de Risaralda, Colombia. En un diseño de bloques completos al azar con dos tratamientos (con y sin inoculación con *Rhizobium* de semillas de *Leucaena*) 7 meses después del establecimiento de la *Leucaena*, se midieron los contenidos de N amoniacal y total en el suelo y las raíces y se evaluó la presencia de nódulos en estas últimas. No obstante que la inoculación no afectó la producción de biomasa, el aporte de N en el suelo fue equivalente a 250 kg/ha ($P < 0.05$).

Palabras clave: Nitrógeno, nodulación, *Rhizobium*, senescencia, nitrógeno del suelo.

Introduction

In Colombia cattle farming is an important economic sector at the national level, however when good management practices are not applied, serious environmental problems arises especially in the Andean region (Cuenca *et al.,* 2008). As part of the restructuring process of livestock, new management strategies have been raised in order to achieve a better balance between cattle farming systems and their environment. One of the strategies is the establishment of silvopastoral systems (SSP) by incorporating shrub legumes that bring different benefits, both in animal production and soil conservation (Chara *et al,* 2015); These include *Leucaena leucocephala* (Leucaena) a shrub of the subfamily Mimosoideae widely adapted to tropical conditions. According to the Plan for reducing GHG emissions submitted by the IDEAM in Colombia, silvopastoral systems are part of the mitigation options at sectoral level proposed by the Intergovernmental Panel on Climate Change -IPCC (Intergovernmental Panel on Climate Change) - (IDEAM, 2015). Moreover, there is a key challenge to improve the availability of nutrients in the soil, which can only be achieved with better farming practices such as intercropping, nitrogen-fixing plants, agroforestry systems and nutrient recycling (Powlson *et al.,* 2011). Thus, the implementation of silvopastoral models are considered as an opportunity to lead the livestock towards sustainable production, as it is mentioned in the strategic plan of the Colombian livestock 2019 (Fedegan, 2006).

As silvopastoral systems, Leucaena has been evaluated in several tropical at high densities (10,000 trees/ ha) as an alternative to improve livestock production and N content in soils. Based on the above statement, the amount of N provided to the soil by the Leucaena crop was quantified during seven months after being established in a high density SPS.

Materials and methods

Study area

The study was conducted on a farm located in the municipality of Balboa, Risaralda, Colombia. This area is part of the sub-basin of the river Totuí, watershed of Risaralda River and the Great Basin of the Cauca River at 899 MASL and 27 °C average temperature. The average rainfall is 1500 mm/year with a bimodal distribution (Carder 2012). Hilly relief is the most predominant with soils of low content of organic matter (1.72%), high levels of calcium and magnesium (20.1 and 2.1 meq/100 g soil, respectively), medium potassium content (0.77 meq/100 g soil) and poor phosphorus content (5 ppm) (Carder, 2012).

Silvopastoral systems with *Leucaena* have been established four years ago in the study farm, as same as traditional livestock systems without trees. The soil in both systems was handled with some applications of ‘cenichaza’, ash residues of sugar cane resulted from its agroindustry.

Propagation of *Leucaena* plants

The initial phase started with the establishment of the nursery seedlings located at 1100 MASL and an average temperature of 24°C (Ruiz and Villa, 2007). The used seeds were *Leucaena leucocepahala* (Lam.) from Wit certification, which were placed in black plastic bags with 5 kg of soil that was previously analyzed for the presence of rhizobia in Mannitol Agar Yeast (MLA) medium (Perez *et al.*, 2011). The used soil had 5.7pH and 15.7% organic matter content. The seeds were irrigated every 2 days, germinated after 15 days and remained in the nursery until they reached a height of about 60 cm, before transplanting in the field (Solorio and Solorio, 2008).

Treatments and experimental design

The experimental design was a randomized complete block with two treatments, inoculated and non-inoculated plants. The mathematical model of the design was:

$$Y_{ijk} = \mu + I_i + T_j + (I * T)_{ij} + \beta_k + \epsilon_{ijk}$$

where, Y_{ij} is the response variable, μ is the average. I = factor (inoculation), T = factor (time), $I * T$ = interaction inoculation x time, β = block (slop of the land), ε = error, i = levels of I, j = levels of T, k = repetition.

The inoculation of the seeds was carried out during the nursery phase. Part of the seeds were inoculated with the product Ferbiol (ICA register 6214), which contains in the solution *Rhizobium loti* microorganisms. The experimental set up was on the field of 450 m^2, which was divided into three blocks according to the slope: Block 1 with 20%, block 2 with 10%, and block 3 which was plane (without slope). Each block was divided into two plots of 71.5 m^2 each, where 48 plants of *Leucaena* were planted at distances of 1 m x 1 m. The equivalent density was 10,000 plants/ha. Soil analysis were performed prior to the establishment of plants (pH, O.M, K, Ca, Mg, P and texture).

Tissue and soil analysis

Fifteen weeks after the experimental set up on the field, the number of nodules of *Rhizobium* in roots, total N and ammonia in leaves were determined. Soil composite samples were collected from each plot at 0-25 cm, and 25-50 cm deep. *Leucaena* whole plants were also collected, including roots for counting nodules (Figure 1). The first sample was taken 8 weeks after the establishment of the treatments, and then every 5 weeks until the 7 months, as recommended by Bueno (2009), corresponding to the time of low rainfall (Figure 2).

To determine the total and ammonium N, the Kjeldahl method was performed. Nodules were analyzed according to their internal and external color, shape, turgor, and location in the root. The count of the nodules was conducted at the Laboratory of Chemical Analysis of Soils, Technological University of Pereira.

Data analysis

All normal response variables were verified by the Kolmogorov-Smirnov and Shapiro-Wilk (SPSS 20) tests, and analysis of variance by ANOVA. Variables with a non-normal distribution were compared between treatments using the non-parametric Kruskal-Wallis test (SPSS 20). All analysis were performed using SPSS 20 software.

Results and discussion

Nodulation

Nodules were not seen during the nursery phase. After 8 weeks of the experimental set up, nodules were observed in roots of both plants inoculated as non-inoculated (Figure 3). This variable showed significant differences between treatments and between blocks ($P < 0.05$); the largest amount of nodules was present in the treatment without inoculum (18 ± 4). The effect of time did not show differences during the experiment and the highest average value (25 ± 8.7) was presented after 18 weeks.

Nodules on the roots of *Leucaena* had different shapes and sizes (Figure 4) with variable dimensions between 1 and 10 mm, with external light brown color and internal color ranging from pink to red (Figure 4). The biggest nodules were observed mainly in the main root.

Figure 1. Sampling of roots of *Leucaena leucocephala* tree.

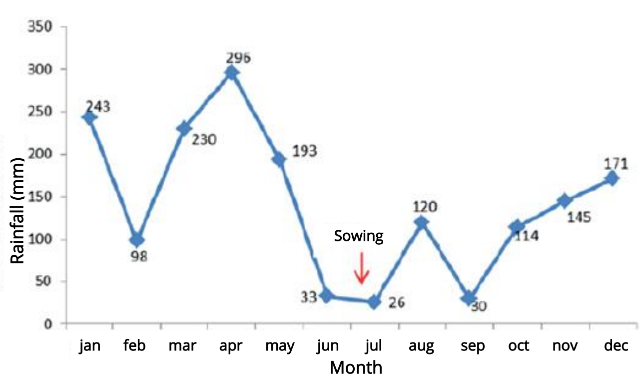

Figure 2. Annual precipitation 2012. The arrow indicates sowing time.

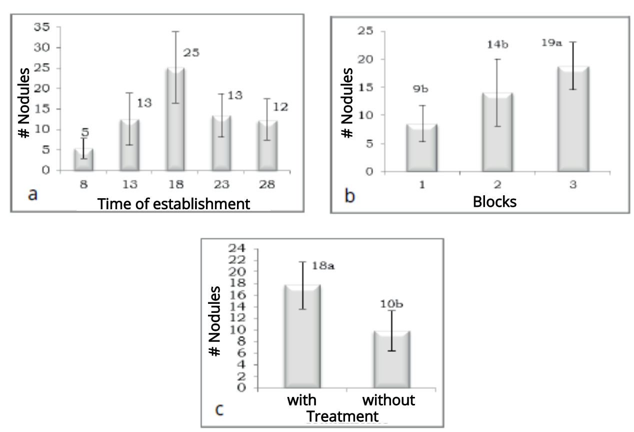

Figure 3. Average number of nodes: a. time of establishment, b. blocks, c. treatment.

Different letters indicate significant difference ($P < 0.05$). The lines indicate the standard error.

Figure 4. Nodules of rhizobia collected from roots of leucaena. Above, active nodules. Below, senescent nodules.

After the fifth month, nodules with noticeable changes in their structure were found (Figure 4), probably non active since they were dried or dehydrated with darker color than the root, and almost indistinguishable from the soil. Active nodules showed light external colorations and an inner tissue with defined turgor.

Total and ammonium nitrogen in the soil

Both total N and ammonium values showed differences (P < 0.05) in relation to the time of establishment, but no treatment effect (Figure 5). The total N showed no trend in relation with time, while the ammonium N showed a clear tendency to decrease. The highest percentage of the total N was found at week 28 in the sample taken at 25 cm depth. In this sample he found the lower value of ammonia nitrogen (0.17 ± 0.05 ppm).

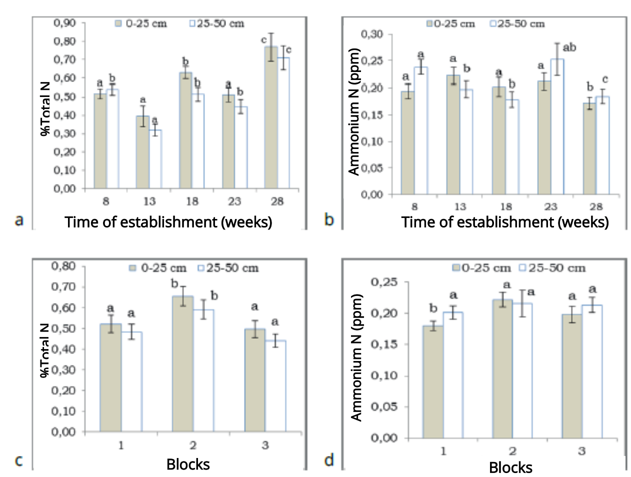

Figure 5. Variation of total and ammonia nitrogen in soils: a and b, time of establishment; c and d, blocks.

Different letters indicate statistically significant differences, the lines on the bars show standard deviation.

Seedlings of 13 weeks in the nursery stage showed no nodules on the roots. According to Rincón et *al.* (2004), cotyledon development 30 days after germination limits the action of rhizobia on nodule formation. Taking into account that soil contained high levels of OM (15.7%) and a carbon/nitrogen ratio of 15.97, it might have affected the formation of nodules on the root (Esquivel, 1963).

The formation of effective and active nodules in control plants (without inoculation) confirms the existence of strains of *Rhizobium* in the soils of the study area. This was confirmed with the specific microbiological analysis of soil, where colonies of bacteria were observed. The high specificity of *Leucaena* by rhizobia is important to highlight, since few strains produce nodulation in this species (Tang, 1994; Hernandez, 2012). Rincón *et al.* (2004) found *Leucaena nodula* with self-isolated strains, which explains the higher nodulation in plants without inoculation. Matus *et al.* (1990) suggest that the success of inoculation of legumes depends on the competitiveness of the new *Rhizobium* to invade the rhizosphere and compete with homologous strains in nodule formation.

The presence of reddish nodules indicates that nodulation is effective and efficient (Marquina, 2011). From week 23 the senescence of nodules was noticed because of the differences in morphology (Figure 4e, f, g). According to Fernandez-Luqueño and Espinosa-Victoria (2008), delay on senescence of nodules has beneficial effects on N fixation, whereas the early senescence leads to a poor N fixation. For these researchers senescence is related to the color and loss of turgor in the nodules, symptoms that could clearly be seen in the root nodules of *Leucaena* in this study. The above changes were accompanied by changes to the internal color of the nodules from light to dark pink, which sometimes coincides with the process of flowering and pod filling legume or moments of water deficit.

During this study a permanent attack of defoliating insects on the plants of *Leucaena* was observed, besides a dry period. Thus, the development of the plants might have been affected, together with the nodulation (Sanchez, 2007).

Total and ammonium nitrogen in the soil

The initial amount of total N in the soil before the planting of *Leucaena* was 0.39%, equivalent to 88.86 kg/ha between 0 and 50 cm deep. At the end of the experiment, 28 weeks after sowing, the averages of N in the soil were 0.77% at 25 cm, and 0.71% at 50 cm deep in the ground. Those values are equivalent to 175 kg/ha and 162 kg/ha respectively, with a total of 338.17 kg/ha.

The above results indicate a contribution of 249.31 kg/ha of N in this soil, taking into account the difference between the initial and final contents; indicating the benefits of *Leucaena* in silvo-pastoral systems. These values are consistent with those found by Bruning and Rozema (2013) who estimate that the use of legumes as green manure, contributing to an increase of 30 to 80 kg N /ha per year.

Although N has different forms in the soil, it is only absorbed by plants and microorganisms as nitrate (NO_3) or ammonium (NH_4+). The means of total N and ammonium N showed a great variation in this study. Since this element has large quantities in the soil, more than other essential elements for the plant (Navarro and Navarro, 2003), the variation in the means was expected. While the amount of ammonium N in the soil decreased to 9.37 g/ha, in plant tissue increased, possibly due to the phenological stage of the plant.

In this study *Leucaena* contributed to an increase of 250 kg N/ ha per year, indicating the advantages of this forage when included in silvo-pastoral systems.

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