TROPICAL LEGUMES AS IMPROVERS OF RANGELAND AND AGRICULTURAL SOILS†

[LEGUMBRES TROPICALES COMO MEJORADORES DE PASTIZALES Y SUELOS AGRÍCOLAS]

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SUMMARY

Legumes have been used for their nitrogen fixation properties; however, farmers in tropical countries they do not usually use them, because they do not appreciate visible improvement in the soil. The aim of this paper was to analyse the effect of three tropical legumes (*Phaseolus vulgaris*, *Canavalia ensiformis* and *Clitoria ternatea*) on growth promoting microorganisms [Free Life Nitrogen Fixers Bacteria (FLNFB), *Azospirillum* sp., *Azotobacter* sp. and Arbuscular Mycorrhizal Fungi (AMF) on the fertility of two rhizospheric soils; one for rangeland use (Ru) and the other for agricultural use (Au). The bioassays were established under a completely randomized design with three replicates per species. The evaluated soil properties were pH; Organic matter, OM; Total carbon, TC; Total nitrogen, N; Useful Phosphorus, P; Cation Exchange Capacity, CEC; and texture. In rhizospheric soil the populations of FLNFB, *Azospirillum* sp. and *Azotobacter* sp.; as well as AMF spores were estimated. The results indicate that *Ph. vulgaris* was the species that showed lower N fixation in Au soil, but higher P content was found in Ru. *Ca. ensiformis* and *Cl. ternatea* had higher N fixation, increased CEC, OM and TC. *Clitoria ternatea* favored the accumulation of OM and TC, promoting CEC, pH and the AMF population. *Canavalia ensiformis* was the only species to promote differentiated development of *Azospirillum* sp. and *Azotobacter* sp. in Ru, showing higher populations with this legume. Therefore, it is recommended that these data can be considered for the conservation of tropical species, both legumes and native microorganisms.
Keywords: Organic agriculture, soil conservation, biological nitrogen fixation, sustainability, *Fabaceae*.

RESUMEN

Las leguminosas han sido utilizadas por sus propiedades fijadoras de nitrógeno; sin embargo, agricultores de países tropicales no las han usado en gran medida, debido a que no aprecian una mejora visible en el suelo. El objetivo del trabajo fue analizar el efecto de tres leguminosas tropicales (*Phaseolus vulgaris*, *Canavalia ensiformis* y *Clitoria ternatea*) sobre los microorganismos promotores de crecimiento [Bacterias Fijadoras de Nitrógeno de Vida Libre (BFNVL), *Azospirillum* sp., *Azotobacter* sp. y hongos micorrizógenos arbusculares (HMA)] sobre la fertilidad de dos suelos rizosféricos, uno de uso pecuario (Up) y el otro de uso agrícola (Ua). Los bioensayos fueron establecidos bajo un diseño completamente al azar con tres repeticiones por cada especie. Las propiedades del suelo evaluadas fueron: pH; Materia Orgánica, MO; Carbono total, CT; Nitrógeno total, N; Fósforo aprovechable, P; Capacidad de Intercambio Catiónico, CIC, y textura. En suelo rizosférico se estimaron las poblaciones de BFNVL, *Azospirillum* sp. y *Azotobacter* sp.; así como las esporas de HMA. Los resultados indican que *Ph. vulgaris* fue la especie que mostró menor fijación de N en suelo de Ua; pero se encontró mayor contenido de P en suelo de Up. *Canavalia ensiformis* y *Cl. ternatea* tuvieron mayor fijación de N, incrementaron la CIC, la MO y el CT. *Clitoria ternatea* favoreció la acumulación de MO y CT, promoviendo la CIC, el pH y la población de HMA. *Canavalia ensiformis* fue la única especie en promover de forma diferenciada el desarrollo de *Azospirillum* sp. y *Azotobacter* sp. en suelo de Up, mostrando poblaciones más altas con esta leguminosa. Por tanto, se recomienda que estos datos se consideren en la conservación de especies tropicales tanto de leguminosas, como de microorganismos autóctonos.
Palabras clave: Agricultura orgánica; conservación de suelos; fijación biológica del nitrógeno; sustentabilidad; *Fabaceae*.

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INTRODUCTION

Legumes can be trees, shrubs and perennial or annual herbs, of cosmopolitan distribution, with approximately 730 genera and 19,400 species, characterized by allowing the fixation of atmospheric nitrogen to the soil, through their association with diverse strains of *Rhizobium* spp., which inhabit the nodules of the roots in the form of bacteroids and allow the Biological Fixation of Nitrogen (BFN), transformed the nitrogen into a compound assimilable by the plants (Rascio and La Rocca, 2013). Therefore, the use of legumes represents an alternative that decreases the use of nitrogen fertilizers and increases the yields of crops of agricultural interest (St. Luce *et al*., 2015). In Brazil, US $ 3.3 trillion in fertilizers have been saved (Moreira *et al*., 2012).

The *Fabaceae* family is also known for its multiple uses and functions (Thomas and Sumberg, 1995), such is the case of *Phaseolus vulgaris* L. used as a source of vegetable protein for human consumption (Iqbal *et al*., 2006), *Canavalia ensiformis* L. used as green manure (Ramos *et al*., 2001) y *Clitoria ternatea* L. appreciated as a source of fodder protein for feeding ruminants (Juma *et al*., 2006).

Some important agronomic practices are especially reserved for legumes, due to their soil improvement characteristics, such as their incorporation as green manure, in addition to their use in rotation, succession and alternation of crops (Whitmore, 2000). In principle, green manures can improve the organic carbon content because of their high percentage of water, sugars, starches and nutrients (Dabin *et al*., 2016); however, the roots also allow for the increase of organic matter in the soil and the increase in the populations of beneficial microorganisms in the rhizosphere, allowing the stabilization of the pH and the release of nutrients during its mineralization (Zotarelli *et al*., 2012), which in turn allow the development of bacteria growth promoters in plants and arbuscular mycorrhizal fungi (AMF), which are a set of different species of microorganisms that can increase plant growth and productivity; the most important genres are *Rhizobium* sp., *Pseudomonas* sp., *Azospirillum* sp. and *Azotobacter* sp., and AMF (De-Bashan *et al*., 2007). On the other hand, these bacteria promote plant growth through mechanisms such as nitrogen fixation, phosphate solubilization and the secretion of hormones; however, root colonization depends on the strain, the species, environmental conditions such as humidity in the soil, temperature and pH; as well as chemical, physiological and nutritional factors (Correa *et al*., 2017).

However, many farmers in tropical countries do not use, to a large extent, the planting of tropical legumes, because they do not appreciate a visible improvement in the soil, in proportion to the work involved (Sumberg, 2002). Therefore, the aim of the study was to analyze the effect of three tropical legumes (*Phaseolus vulgaris, Canavalia ensiformis* and *Clitoria ternatea*) on the microorganisms that promote growth [Free Life Nitrogen Fixing Bacteria (FLNFB), *Azospirillum* sp., *Azotobacter* sp. and AMF] on the fertility of two rhizospheric soils, one for rangeland use (Ru) and the other for agricultural use (Au).

MATERIALS AND METHODS

The present research work consisted of five stages: 1) Collection and characterization of the soil, 2) Establishment of bioassays; 3) Determination of the chemical and physical properties of the soils, during the flowering of the three species of legumes, 4) Viable account of Free Life Nitrogen Fixing Bacteria (FLNFB), *Azospirillum* sp. and *Azotobacter* sp., and AMF, and 5) Analysis of the results.

Collection and characterization of the soil

The study was carried out with soil samples taken from a production unit located in Colonia Hidalgo, Municipality of Acayucan, Veracruz state, Mexico, with coordinates 18°04'15.6" N and -94°59'03.9 " W. The sites were located at 950 m distance between them, where 20 subsamples were collected, from which two composite samples were obtained, one for rangeland use (Ru) and another for agricultural use (Au), from which soil was collected at 30 cm depth, following the procedure in accordance with SEMARNAT (2000). The first zone has remained as a livestock system through cattle grazing and meadows [*Brachiaria brizantha* (A.Rich.) Stapf (1919) *Poaceae*], its properties were: pH 5.4; Organic matter, OM 3.9%; Total carbon, TC 2.3%; Total nitrogen, N 0.06%; available phosphorus, P 2.6 mg kg^{-1}; Cationic Exchange Capacity, CEC 7.8 cmol kg^{-1} and textural class Loam-clayey-sandy with 23% sand, 10% silt and 68% clay. The second site has been maintained under a traditional agricultural system with corn (*Zea mays* L. *Poaceae*) and soil characteristics were: pH 4.5; OM 5.0%; TC 2.9%; N 0.09%; P 20.6 mg kg^{-1}; CEC 8.6 cmol kg^{-1} and textural class Loam-clayey-sandy with 28% sand, 9% silt and 68% clay.

Bioassays

The soil was dried under shade, sifted and placed in 25 cm by 30 cm containers, placing 5 kg of soil (OECD, 1984). Subsequently, three seeds in three pots per each specie, were planted to ensure germination, one week after emergence, thinning was performed to leave only one plant with similar characteristics for all treatments (Table 1), humidity was maintained at 30%. The bioassays were established under a completely randomized design with three replications for each species. The species were selected due to their agronomic assessment in the tropics (Cáceres *et al*., 1995; Villanueva *et al*., 2004; Hernández-López *et al*., 2013). During the development of the trials, nutrients are not provided to avoid interferences between treatments. On the control only the chemical properties of the soil were evaluated, because the microbiological parameters came from the rhizosphere [the area of soil surrounding the plant roots (Gouda *et al.*, 2018)].

Table 1. Design of the experiment in soils with rangeland use (Ru) and agricultural use (Au).

Treatments	Characteristics	Species
T1	Ru soil	*Clitoria ternatea*
T2		*Phaseolus vulgaris*
T3		*Canavalia ensiformis*
T4	Au soil	*Clitoria ternatea*
T5		*Phaseolus vulgaris*
T6		*Canavalia ensiformis*
C1	Control Ru soil	Without plant (uncultivated)
C2	Control Au soil	Without plant (uncultivated)

Evaluation period

The evaluation was carried out until the flowering stage, since during this period the highest nitrate reductase activity was found in the root (Pliego *et al*., 2003), this period varied from 66 days for *Cl. ternatea*, 109 to *Ph. vulgaris* and 54 to *Ca. ensiformis*.

Soil fertility indicators

Laboratory work was governed by NOM-021-RECNAT-2000 from the preparation of three rhizospheric soil samples for each treatment, which included transfer, reception, registration, drying, milling, sieving, homogenization and storage for conservation, and soil analysis. The soil characteristics were analyzed: pH measured relative 1:2 soil-water, OM and TC by the method of Walkley and Black, total N (micro Kjeldahl, Sulfuric acid 0.01N), P usable by the procedure of Olsen, CEC with ammonium acetate 1N, pH 7.0 and texture by the Boyoucos method (SEMARNAT, 2000).

Promoting bacteria from rhizosphere of legumes

Three samples were taken from rhizospheric soil of each experimental unit (replicate). The count of colony forming units (CFU g^{-1} dry soil) was performed by means of the viable count method by serial dilution (Madigan *et al*., 2000), in media specific crops: combined coal for FLNFB, where two solutions were mixed: A (0.9 g K_2HPO_4, 0.2 g KH_2PO_4, 0.1 g KCl, 0.025 $Na_2MoO_4.2H_2O$, 4.9 g $Na_2FeEDTA$, 15 g agar and 1.0 mL distilled water), and B (0.2 g $MgSO_4.7H_2O$, 0.06 g $CaCl_2.H_2O$, 100 mL distilled water) (Rennie, 1981). The culture medium for bacteria of the genus *Azospirillum* was red Congo Döbereiner (D), composed of 5 g malic acid, 0.5 g K_2HPO, 0.2g $SO_4.7H_2O$, 0.1 g NaCl, 0.5 g yeast extract, 0.015 g $FeCl_3.6H_2O$, 4.8 g KOH, 15 ml red Congo, 15 g agar y 1.0 ml distilled water, pH 7.0 (Holguín *et al*., 1996). In the case of *Azotobacter* sp. we used the Ashby culture medium (5 g mannitol 5 g K_2HPO_4, 0.2 g $MgSO_47H_2O$, 0.5 g NaCl, 0.1 g K_2SO_4, 5 g $CaCO_3$, 15 g agar y 1.0 L distilled water, pH 7.0) (Podile and Kishore, 2007). The AMF spores were separated and counted by the wet sieving method, followed by centrifugation in a gradient 50% sucrose (w/v), according to the methodology suggested by Gerdemann and Nicolson (1963).

Statistical analysis

The results were analysed using analysis of variance and comparison of means by the Tukey test ($P \leq 0.05$). The results were processed with statistical software SAS version 9.1 using PROC GLM (SAS, 2004).

RESULTS

The analysis of variance for FLNFB in the bioassays showed significant statistical differences ($P \leq 0.05$) between means in soil of Ru. On the other hand, *Azospirillum* sp. was greater in both soil types with *Ca. ensiformis* and *Cl. ternatea*; while *Azotobacter* sp. only showed statistical differences ($P \leq 0.05$) with *Ca. ensiformis* in soil with Ru (Figure 1 A, B).

The analysis of variance for the AMF populations in the bioassays showed significant statistical differences ($P \leq 0.05$) between the soil in Ru and Au, being the soil with *Clitoria ternatea* where the highest number of spores was found (Table 2).

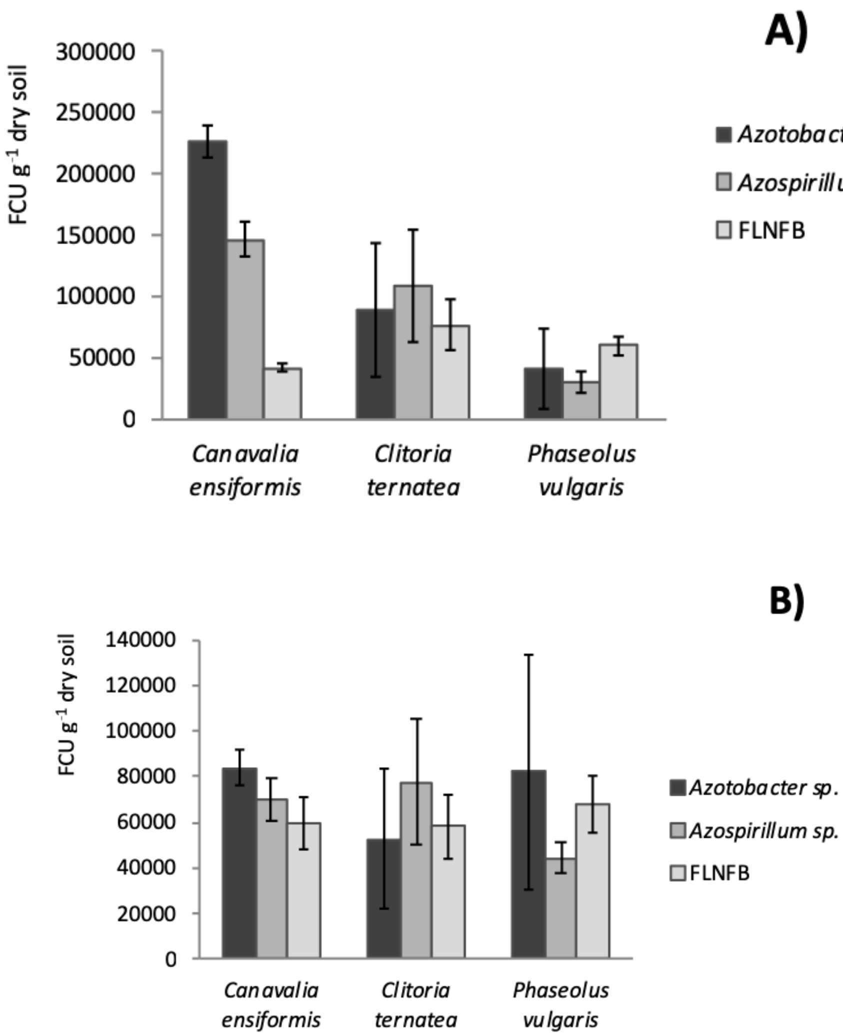

Figure 1. Populations of Free Life Nitrogen Fixing Bacteria (FLNFB), *Azotobacter* sp. and *Azospirillum* sp. in rhizospheric soil of *Phaseolus vulgaris* L., *Clitoria ternatea* L. and *Canavalia ensiformis* L. in soils with: a) rangeland use (Ru) and b) agricultural use (Au).

In soil with rangeland use (Ru), the pH was classified as moderately acidic in most cases, except for soils with *Cl. ternatea*, which showed lower values than that registered in the control, which was identified as strongly acidic (SEMARNAT, 2000). OM, TC and N increased significantly with the seeding of *Cl. ternatea* and *Ca. ensiformis*.

On the other hand, *Ph. vulgaris* was the legume that least fixed N to the soil; but in which more P was found after flowering. In general, according to NOM-021-RECNAT-2000, all the values obtained for the CEC were interpreted as low, and texture were classified as franc-clay-sandy (Table 1).

In contrast, in the Au soil, the pH was favored with the presence of *Cl. ternatea* and *Ca. ensiformis*; while there was no variation between the control (C2) and sowing with *Ph. vulgaris*. OM and TC decreased significantly with the seeding of *Cl. ternatea* but there were no differences in the fixation of P and N, nor textural changes (franc-clay-sandy). On the other hand, *Ph. vulgaris* had lower CIC, classified as low according to NOM-021-RECNAT-2000 (Table 2). The highest degree of AMF infection was found in the root of *Cl. ternatea*, corresponding to an abundance of 1501 spores in rhizosphere dry soil g-1 (Figure 2).

DISCUSSION

The soil of productive livestock origin had a moderately acidic pH, and the contribution of nitrogen by the three legumes (*Ph. vulgaris*, *Ca. ensiformis* and *Cl. ternatea*) was significant.

Table 2. Fertility of the land for livestock or rangeland use (Ru): pH; Organic Matter (OM); Total carbon (C); Total nitrogen (N); Usable phosphorus (P); Cation exchange capacity (CEC); percentages of Clay, Silt and Sand evaluated after the flowering of tropical legumes and AMF spore's abundance in in rhizospheric soil of *Clitoria ternatea* L., *Phaseolus vulgaris* L. and *Canavalia ensiformis* L.

Identification	pH	MO	C	N	P	CEC	Clay	Silt	Sand	AMF
			%		mg kg^{-1}	cmol kg^{-1}		%		Spores abundance/ dry soil g^{-1}
Control soil (uncultivated)*	5.4 ^{a}	3.9^{b†}	2.3^{c}	0.06^{c}	2.6^{b}	7.8^{ab}	23^{ab}	10^{a}	68^{a}	---
Phaseolus vulgaris	5.2^{ab}	4.2^{b}	2.4^{bc}	0.07^{bc}	6.3^{a}	6.7^{b}	22^{ab}	6^{a}	71^{a}	392^{b}
Canavalia ensiformis	5.3^{a}	4.6^{a}	2.6^{ab}	0.09^{a}	3.8^{b}	7.3^{ab}	19^{b}	9^{a}	73^{a}	472^{b}
Clitoria ternatea	4.8^{b}	5.0^{a}	2.9^{a}	0.08^{ab}	2.4^{b}	8.2^{a}	24^{a}	7^{a}	70^{a}	1501^{a}

† Means with the same letter are not significantly different (Tukey, $p \leq 0.05$) (a>b).
* Textural class: Loam-clayey-sandy, (Cra).

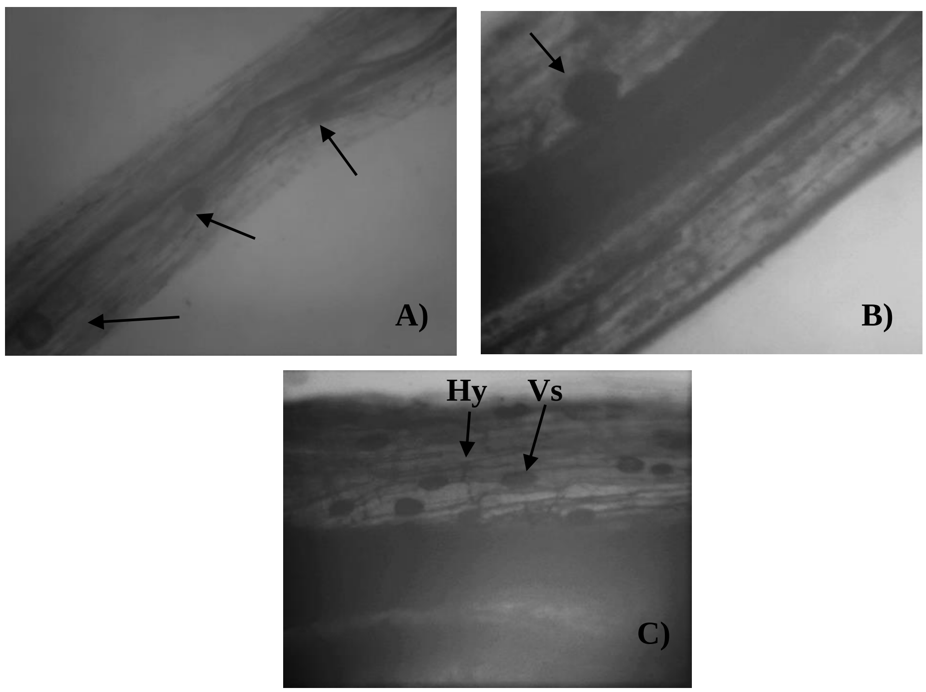

Figure 2. Shows the degree of AMF infection seen at 40x in roots of 0.05 cm in: A) Spores formed intraradically in *Phaseolus vulgaris*, B) *Canavalia ensiformis* root, and C) Vesicles (Vs) and hyphal (Hy) between root cells from *Clitoria ternatea* in rangeland use (Ru).

Table 3. Fertility of the for agricultural use (Au): pH; Organic Matter (OM); Total carbon (C); Total nitrogen (N); Usable phosphorus (P); Cation exchange capacity (CEC); percentages of Clay, Silt and Sand evaluated after the flowering of tropical legumes. and AMF spore's abundance in rhizospheric soil of *Clitoria ternatea* L., *Phaseolus vulgaris* L. and *Canavalia ensiformis* L.

Identification	pH	MO	C	N	P	CEC	Clay	Silt	Sand	AMF
			%		mg kg^{-1}	cmol kg^{-1}		%		Spores abundance/ dry soil g^{-1}
Control soil (uncultivated)*	4.6^{b†}	5.0^{a}	2.9^{a}	0.09^{a}	20.6^{a}	8.6^{a}	28^{a}	9^{a}	63^{a}	---
Phaseolus vulgaris	4.5^{b}	5.0^{a}	2.9^{a}	0.08^{a}	19.8^{a}	5.9^{b}	29^{a}	10^{a}	61^{a}	342^{b}
Canavalia ensiformis	5.0^{a}	5.0^{a}	2.9^{a}	0.08^{a}	18.4^{a}	7.3^{ab}	29^{a}	9^{a}	63^{a}	160^{c}
Clitoria ternatea	5.3^{a}	4.2^{b}	2.4^{b}	0.10^{a}	20.7^{a}	7.4^{ab}	26^{a}	8^{a}	67^{a}	2540^{a}

† Means with the same letter are not significantly different (Tukey, $p \leq 0.05$) (a>b).
* Textural class: Loam-clayey-sandy, (Cra).

Cheng *et al*. (2013) affirm that in prairie soils the increase of the pH stimulates the nitrification rates; but the acidification of the soil decreasing the mineralization rates of nitrogen, this could have happened with *Ph. vulgaris* where the soil nitrogen decreased by 0.02%. There is evidence of some advantages and disadvantages that livestock systems have with respect to the biological fixation of nitrogen. Tippannavar and Ramachandra (1990) found cysts formed by *Azotobacter* spp. in samples of bovine excreta, aseptically extracted from the rectal passage, so that the population densities of these nitrogen-fixing bacteria could be inoculated to the soil through faeces. In contrast, the incorporation of urine can reduce nitrogen fixation by 70%, with chronic effects up to 286 days (Menneer *et al.*, 2004). On the other hand, de Klein *et al*. (2014) mention that cattle urine patches contain high concentrations of readily available nitrogen, which exceeds the immediate absorption potential of the plant, causing gaseous or leaching losses. With respect to the increase of rizospheric P, Jin *et al.* (2014) indicate that it is mainly attributed to the stimulation of microbial biomass because of the increase in the carbon flow of root systems in the form of CO_2.

The soil of agricultural origin had a strongly acid pH, possibly due to the application of large amounts of nitrogen fertilizers used in the cultivation of corn (*Zea mays*), which are anions that dissociate in water, leading to some acid reactions. In this studio, the determining factors on the growth of *Ca. ensiformis* was the excess of Al, the deficiency of P and the strongly acid pH; but in soils with a pH lower than 4.0, their growth and yield decrease (Torrealba *et al*., 1998). Tong and Xu (2012) found that adding urea to the soil stimulates the growth of ammonia-oxidizing bacteria populations, thereby accelerating the process of nitrification and soil acidification. Ammonium is related to soil acidification, this would explain the acidification of soil present with *Ph. vulgaris* in agricultural land.

The highest degree of AMF infection was found in the root of *Cl. ternatea*, this corresponded to a greater abundance of spores in rhizosphere and to higher concentrations of P, because the presence of AMF improve the nutrition of plants in low fertility soils, change pH, have a significant effect on the abundance of AMF spores in the soil (Peña-Venegas *et al*., 2007), and is positively related to contents of P and N (Pérez and Vertel, 2010), as it happened in this experiment due to the possible colonization by AMF in corn crop, which can oscillate up to 75% (Montaño *et al.*, 2001).

On the other hand, AMF can reduce the loss of nutrients by leaching after rain, however, this depends on the species of host plants and AM fungi species present in the soil; being nutrient retention one of the most important ecosystem services (Köhl and van der Heijden, 2016). Studies conducted in agricultural areas have found a positive correlation between arbuscular colonization with the intensity of land use and the application of mineral fertilizers (Jansa *et al.*, 2016); however in this study, it was better related to the use of pasture, coinciding with a research conducted in Brazil, where the culture of *Clitoria ternatea* and *Cenchrus ciliaris* resulted in the selection of AMF species that ensure the sustainability of the pasture (Menezes *et al.*, 2016).

Azospirillum and *Azotobacter* are efficient bacteria in the fixation of nitrogen, in the production of growth hormones and solubilization of phosphorus (Shukla, 2019). For example, *Azotobacter chroococcum* solubilize P (in the range of 1.5-1.7 $\mu g\ ml^{-1}$ of tricalcium phosphate and 0.19-0.22 $\mu g\ ml^{-1}$ of mussooric rock phosphate) in the rhizosphere of wheat (Kumar and Narula, 1999), this may have happened in *Phaseolus vulgaris* with prairie soil. In addition, Canavalia can fix up to 133 kg ha^{-1} N from the atmosphere (Wortmann *et al.*, 2000), this species being the one that most N fixed to the rangeland use.

CONCLUSION

The cultivation of three tropical legumes (*Ph. vulgaris*, *Ca. ensiformis* and *Cl. ternatea*), up to the flowering stage, had significant effects on soil fertility for both land uses, *Ca. ensiformis* and *Cl. ternatea* being the legumes that significantly favored N fixation, the CEC, the content of OM and C. In Au soil, *Ph. vulgaris* showed the lowest N content; but higher P content in the rhizosphere. On the other hand, *Cl. ternatea* increased the accumulation of MO and C, favouring the CEC and pH in this soil. The best microbiological quality was found in soil with Ru, having higher bacterial populations; however, in agricultural soil, a greater number of AMF spores was found. Therefore, it is recommended that this data may be the basis for the conservation of tropical species of both legumes and native microorganisms.

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