Cultivos Tropicales, 2021, vol. 42, no. 2, e05
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

http://ediciones.inca.edu.cu

abril-junio
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

Original article

Quantification of biological nitrogen fixation in shadow trees from two coffee plantation in Cuba

Gloria M. Martín-Alonso[1*]

Carlos Bustamante-González[2]

Mario Varela-Nualles[1]

Alberto Pérez-Díaz[3]

Rolando Viñals-Núñez[4]

Anicel Delgado-Álvarez[1]

Luis R. Fundora-Sánchez[1]

[1]Instituto Nacional de Ciencias Agrícolas (INCA), carretera San José-Tapaste, km 3½, San José de las Lajas, Mayabeque, Cuba. CP 32 700

[2]Instituto de Investigaciones Agroforestales. UCTB Tercer Frente. Santiago de Cuba, Cuba. CP 92700

[3]Universidad de Guantánamo, Carretera a Santiago de Cuba, Km 2 ½ Guantánamo, Cuba

[4]Instituto de Investigaciones Agroforestales. UCTB Velasco. Holguín, Cuba

[*]Author for correspondence: gloriam@inca.edu.cu

ABSTRACT

In coffee agroecosystems, it is of much importance to maintain appropriate levels of nitrogen to guarantee stable and sustainable productions. In this agriculture type, it is fundamental to consider the insert of shade species that carry out the Biological Fixation of the Nitrogen like natural form of incorporating this element to the soil. In that sense, it was carried out an experiment in two coffee areas (*Coffea canephora* var. Robusta) of the East of Cuba, in Sagua-Nipe-Baracoa and Sierra Maestra mountains, with the objective of quantifying the production of dry biomass and content of two leguminous used as shade trees (*Samanea saman* and *Gliricidia sepium*) and starting, from those determinations, to estimate the nitrogen biological fixation by the total N difference method. The dry mass and N content quantified in

the shade trees were superior to the valued in the coffee, due to the different behavior among the species in study, coffee dry mass oscillated between 1.23 and 1.75 t ha^{-1} and the shade trees between 2 and 3 t ha^{-1}. The contents of foliate N of the shade species oscillated among 25-40 kg ha^{-1} and the coffee presented foliar concentrations of the element in appropriate ranges. The arboreal species present BFN between 20 and 55 %, that which transforms them into an important source for the contribution and recycling of the nitrogen inside the coffee ecosystems.
Key words: *Coffea canephora*, *Gliricidia sepium*, dry mass, *Samanea saman*

INTRODUCTION

Nitrogen (N) is an important element in the development of all organisms since it is part of the structure of amino acids, nucleic acids, proteins and cellular components [(1)]. However, in the soil it is one of the most limiting nutrients. The atmosphere contains 78 % N but this is found in forms not available to most living beings and can only be used by a group of highly specialized microorganisms, N-fixing bacteria, which are associated with different plant species mainly legumes, to carry out this important natural process [(2)].

In coffee agroecosystems, it is of utmost importance to maintain adequate levels of N to guarantee stable and sustainable productions. In this sense, results have been obtained that optimize the use of nitrogenous mineral fertilizers to achieve stable maximum yields. However, it is necessary to take into account the importance of other agroecosystem components to maintain and increase the inputs and element recycling, of form usable by the crop [(3)]. The high prices of mineral fertilizers in the world market and the negative effects on the environment that their inappropriate use can cause, make it necessary to investigate more exhaustively nitrogen contribution that other organic sources can make within coffee plantations, not just sources but, fundamentally, of coffee agroecosystem components.

In this sense, coffee plantations in agroforestry systems with shade trees exert control over water resources, since the water deficit is mitigated in periods of drought, in addition to helping soil fertility, minimizing erosion, recycling nutrients and providing large amount of organic matter [(4)]. In this type of agroecosystems, the inclusion of multiple-use trees is prioritized but it is also essential to consider the insertion of species that perform Biological Nitrogen Fixation (BNF) as a natural way of incorporating this element into the soil [(5)].

Among the trees used in the shade of coffee plantations are gliricidia (*Gliricidia sepium* (Jacq) Walp.), carob (*Samanea saman* (Jacq) Merril), among other leguminous tree species [(6)]. The coffee plantations established in systems associated with trees can generate additional

Cultivos Tropicales, 2021, vol. 42, no. 2, e05
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

income to producers, both from the point of view of fruits, wood, among other forest products sale, and for environmental services [7].

For these reasons it is necessary, in order to contribute to an estimate of the N balance in coffee agroecosystems, to quantify the contribution of shade trees that compose it, through the estimation of the biological N fixation process (BNF).

In this sense, this experiment was carried out in two coffee-growing areas in eastern Cuba, with the objective of quantifying the dry mass production and N content of two legumes used as shade trees in the coffee-growing ecosystem and, based on these determinations, estimate BNF by the total N difference method.

MATERIALS AND METHODS

The research was carried out over a period of four years (2004-2007) in two locations in the Sierra Maestra and Sagua–Nipe–Baracoa mountain ranges. One was Tercer Frente locality (Cruce de los Baños site), located at 20º09´ latitude N and 76º16´longitude W, 35 km NW from Santiago de Cuba city, 150 m above sea level, in Tercer Frente municipality. Sierra Maestra mountain, with the predominant shade of *Samanea saman* (Jacq) Merril.

The second locality was La Alcarraza, located in the Sagua de Tánamo municipality, Nipe - Sagua - Baracoa mountain ranges, at 20º35´ latitude N and 75º15´ longitude W, 118 km ESE of the city of Holguín, with a height of 300 m a.s.l, with a predominant shade of *Gliricidia sepium* (Jacq) Walp. Some properties of the soils are presented in Table 1.

Table 1. Main chemical characteristics of the cultivable horizon (0 - 30 cm) of the soils under study at the beginning of the experiments

Soils	pH	O.M (%)	P_2O_5 (mg 100 g^{-1})	K_2O	K^+	Ca^{2+}	Mg^{2+} (cmol$_c$ kg^{-1})	Na^+	CEC
Ochric brown without carbonates. Tercer Frente site	6.4	2.97	15.20	22.14	0.64	31.5	11.8	0.4	44.3
Gleyish brown without carbonates. La Alcarraza site	5.6	3.07	16.72	26.0	0.75	26.8	10.8	0.27	38.6

CEC: Cationic Exchange Capacity = ∑ Exchangeable cations

For soil chemical analysis, the following methods were used [8]: pH (H_2O) by the potentiometric method, with a soil: solution ratio of 1: 2.5; organic matter (% O.M) by the Walkley and Black method; P assimilable by extraction with H_2SO_4 0.1 N with soil: solution ratio 1: 2.5;

Exchangeable cations ($cmol_c$ kg^{-1}) by extraction with NH_4Ac 1 Mol L^{-1} at pH 7 and determination by complexometry (Ca and Mg) and flame photometry (K and Na).

The quantification of coffee tree phytomass (leaves) and of the shade trees (leaves, fruits, flowers and rachis) was carried out monthly from January to December in the two locations and during the four years evaluated.

The estimation of the amount of fallen litter from the shade trees in each locality was carried out using 1m x 1m polyethylene nylon blankets. Four shade trees were randomly selected in each experimental area and eight blankets were placed per tree, four in the street and four in the row of coffee tree (9). In the case of coffee tree, the contribution of the desiccation and pruning was also collected.

In all cases, the dry mass was determined (drying of each organ at 70 ºC until obtaining constant mass) and it was expressed in t ha^{-1}, as well as the corresponding chemical analysis of N, by means of wet digestion with H_2SO_4+Se and colorimetric determination with the Nessler reagent and expressed in % of dry mass per organs (8). With these values N extractions were calculated from each organ of the culture in all the evaluated years, according to the following expression:

$$\text{N extraction per organ (kg ha}^{-1}\text{)} = [\text{Dry mass (kg ha}^{-1}\text{)} * \text{\% N in each organ}]/100$$

From these data, the BNF was calculated by the total N difference method using the formula proposed (10):

$$\text{\% BNF} = [(\text{Content N fix} - \text{Content N ref})/(\text{Content N fix})] * 100$$

Where: fix = fixing plant, Ref = non-fixing plant

Shade trees of the *Gliricidia* or *Samanea* genera were considered as fixing plants, depending on the locality and as a non-fixing plant to the coffee tree, taking samples of this last species from plots that did not receive applications from nitrogen sources.

Statistical analysis: the arithmetic mean and the confidence interval of the means were determined for all the variables evaluated. The latter using the formula:

$$CI = \bar{x} \pm t_{\alpha(2)(n-1)}\, S/\sqrt{n}$$

Where: CI = confidence interval, $\bar{x}$ = arithmetic mean, S = standard deviation. n = number of observations, t=student's t-statistic of the hypothesis test, α = 0.05 for a confidence level of 95%. The statistical program STATGRAPHICS Centurion XV version 15.2.14 was used.

RESULTS AND DISCUSSION

The dry mass behavior of the coffee tree and the shade ones during the four years evaluated are shown in Figure 1. The values of coffee tree dry mass ranged between 1.23 and 1.75 t ha^{-1}, in correspondence with the reported for the *C. canephora* species in Cuba [(11)] and which in turn are higher than those obtained in different agroforestry systems with *C. arabica* under shade, where between 1.19 and 1.35 t ha^{-1} year $^{-1}$ were obtained dry mass [(12)].

In the two experimental sites, the amounts of coffee tree dry mass were similar, as was the dry mass of two shade tree species and these in turn were higher than values of coffee tree dry mass. In all the years that evaluations have been carried out, this is due to the different size between the species studied. The dry mass contributions of the shade trees had little variation from one year to another, oscillating between 2 to 3 t ha^{-1}, except in the third year evaluated, which reached values higher than 3 t ha^{-1}. This is due to the fact that the shade trees are in equilibrium and depending on the rainfall regime, a homogeneous behavior in the two zones can be expected annually, but varying between species [(13)].

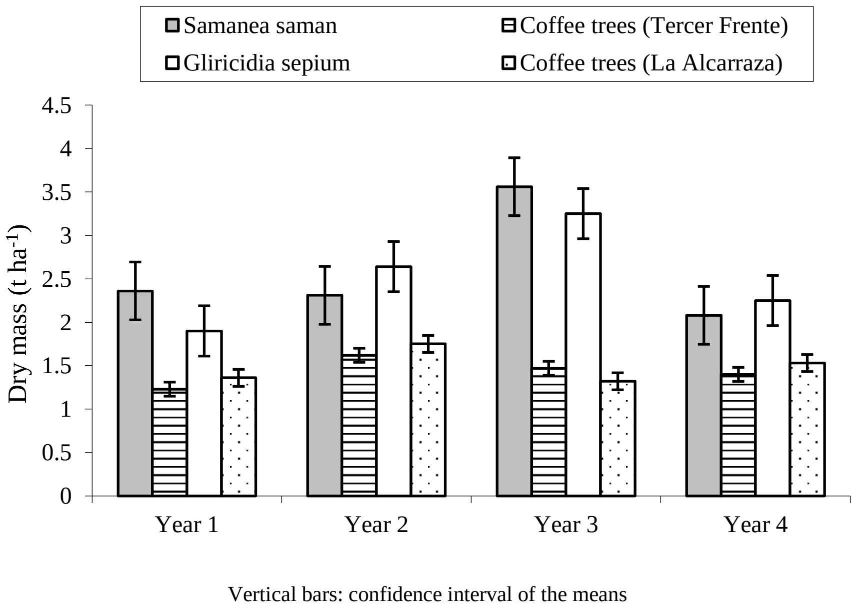


Figure 1. Behavior of coffee tree dry mass and the shade trees during the four years evaluated. *Samanea saman* at Tercer Frente site and *Gliricidia sepium* at La Alcarraza site

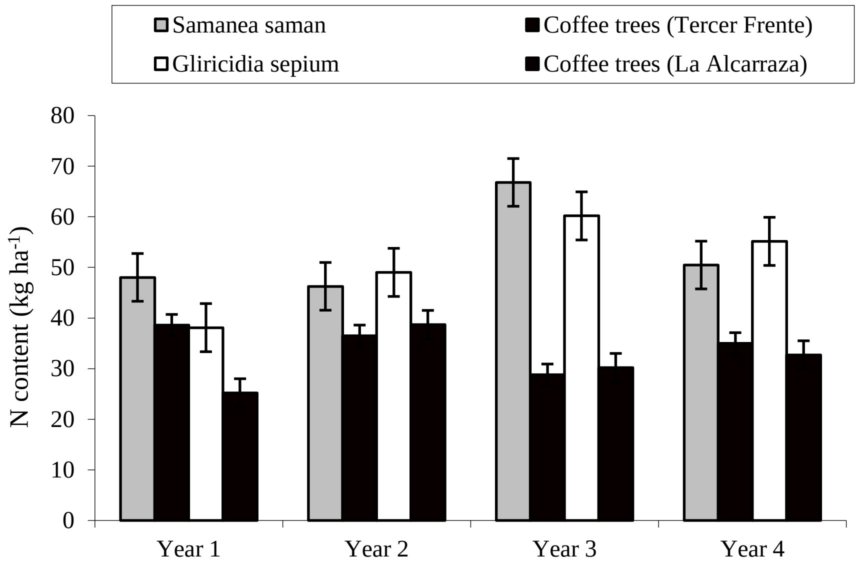
Thus, *Gliricidia sepium* litter can contribute 2.51 t ha^{-1} of dry mass as an average of the evaluated years, while *Samanea saman* does it annually (average of the evaluated years) for 2.58 t ha^{-1} of mass dry leaves.

The contribution to the coffee agroecosystem by *Samanea saman* can reach 2.3 t ha^{-1} of dry mass [(14)], values similar to those found in this study and for the case of *Gliricidia sepium*, values from 1.5 to 6 are reported t ha^{-1} $year^{-1}$ depending on the number of trees present in the system [(4,15)].

In Brazil, studies have been carried out in organic coffee plantations and it has been found that the contribution of *Gliricidia sepium* as shade of the coffee tree can reach 3.5 t ha^{-1} $year^{-1}$, providing a biomass composed of leaves, branches and flowers, the leaves being the main source of supply and recycling of nutrients. Under these conditions, *Gliricidia sepium* is a species that contributes more leaves to the ground than other shade species, such as *Erythrina* sp., that contribute more branches [(16)].

In Mexico, several forest species are used as shade in coffee plantations, whether or not they are legumes, with various functions within the ecosystem, such as producers of food, firewood, wood, among others, and they reach annual biomass contributions of up to 14 t ha^{-1} $year^{-1}$, thus complying with several of the requirements of a species to be used as a shade tree, among them, being of rapid growth and with a high production of biomass and litter [(17)].

Figure 2 shows the total N contents of the species under study: *Gliricidia sepium*, *Samanea saman* and *C. canephora*. In the evaluated sites and years (2004-2007), the total N content of the legumes was higher than that of the coffee tree, except in the first evaluated year and in the Tercer Frente site, in which the total N content values of coffee trees were similar to those found in shade trees. In the coffee tree, the total N content ranged between 25 and 40 kg ha^{-1}. It is important to note that N concentrations of the coffee tree were in the appropriate ranges for the *C. canephora* species, ranging from 2.91 to 3.09 % of total N [(18)].



Vertical bars: confidence interval of the means

Figure 2. Behavior of the N content in the coffee tree and shade trees during the four years evaluated. *Samanea saman* at Tercer Frente site and *Gliricidia sepium* at La Alcarraza site

The total N content of shade trees was very similar between both species (Figure 2), *Samanea saman* contained between 46.26 to 66.80 kg ha^{-1} and as a mean of the evaluations 52.89 kg ha^{-1} $year^{-1}$. *Gliricidia sepium* leaves contained 38.1 to 60.18 kg ha^{-1}, with an average annual contribution of 50.61 kg ha^{-1} $year^{-1}$.

This contribution by the coffee tree and shade trees was similar to that reported in Valle del Cauca, Colombia, where *Gliricidia sepium* litter contributed 51.4 kg ha^{-1} $year^{-1}$, a contribution that is important within the contribution of this species by N biological fixation [(19)].

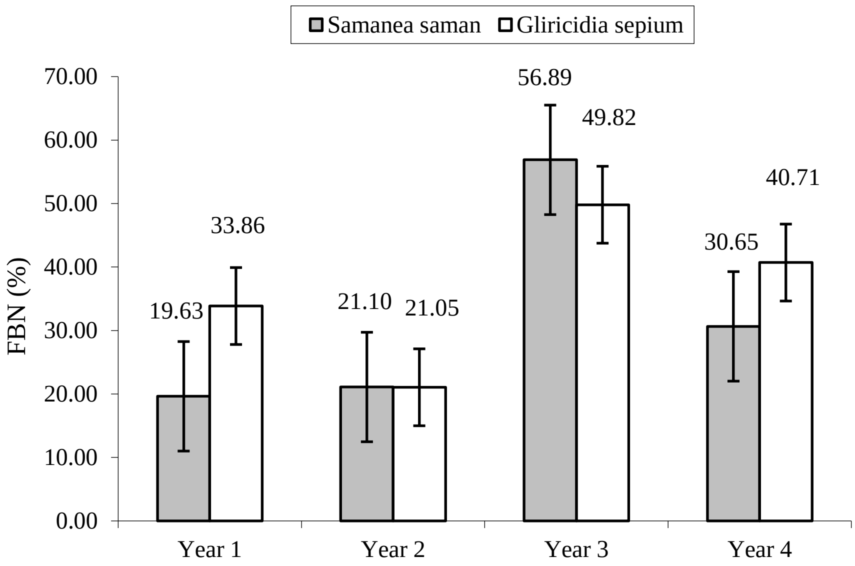
In Mexico it is reported that legume species such as *Inga* sp., make contributions close to 60 kg N ha^{-1} and these levels are due to the fact that nodulation in the roots occurs spontaneously and it is not customary to inoculate shrub legumes with efficient rhizobia species [(17)].

Figure 3 shows BNF percentages of the component tree legumes of coffee plantations under study, using the total N difference method and taking the coffee tree as the reference plant.

In three of the four years studied (years 1, 2 and 4), no differences were detected between the species and years evaluated in terms of this variable, with the two species found in ranges between 19 and 40 %, however, the third year did make a difference, with values much higher than those found in the rest of the years, between 49 and 56 % of the N derived from the BNF, however, *Gliricidia sepium* did not present differences in the BNF values between the third and the fourth year evaluated.

The total N content of shade trees was very similar between both species (Figure 2), *Samanea saman* contained between 46.26 to 66.80 kg ha^{-1} and as a mean of evaluations 52.89 kg ha^{-1} $year^{-1}$. *Gliricidia sepium* leaves contained 38.1 to 60.18 kg ha^{-1}, with an average annual contribution of 50.61 kg ha^{-1} $year^{-1}$.

This contribution by the coffee tree and shade trees was similar to that reported in Valle del Cauca, Colombia, where *Gliricidia sepium* litter contributed 51.4 kg ha^{-1} $year^{-1}$, a contribution that is important within the contribution of this species by biological fixation of N (19).



Reference plant: coffee tree. Vertical bars: confidence interval of the means

Figure 3. Quantification of the N biological fixation of shade trees during the four years evaluated. *Samanea saman* at the Tercer Frente site and *Gliricidia sepium* at La Alcarraza site

In some Latin American countries (Mexico, Brazil, Guadalupe, Venezuela, Cuba) it is reported that *Gliricidia* is capable of contributing more than 50 % of N via BNF (15). In Brazil, in organic coffee plantations under *Gliricidia sepium* shade, up to 66 % of N has been quantified in the system from the N biological fixation, although there are species such as *Erythrina poeppigiana* that contributes up to 78 % of N derived from BNF (16).

This behavior seems to be in correspondence with the values of dry mass and total N content, where the highest values were found in the third year of evaluation. In general, this result seems to be related to the incidence of rainfall that was higher in the third year (data not shown) and this higher humidity caused a growth stimulation of shade trees.

Regarding the importance of the inclusion of N-fixing shade trees within coffee plantations, it is suggested that *Gliricidia sepium* and *Samanea saman* are two N-fixing forest species of great importance and they are widely used in the world as living barriers to prevent the soil erosion and enriching it with N from the BNF process, which is very active in these species (6).

Samanea saman is a promising legume to promote shade in coffee plantations because it stands out for the high number and dry weight of *Rhizobium* nodules, indicative of the presence of an efficient BNF process. Regarding growth, it is fast growing, with more than 100 cm in height in 6 months, while *G. sepium* is of medium growth, with heights between 50 to 100 cm in 6 months and lower quantification of effective *Rhizobium* nodules (20) It is also stated that *Samanea saman* increases the height of associated plants in agroecosystems, both as a shade tree in coffee plantations, in agrosilvopastoral systems and as living fences (5,21).

Gliricidia sepium is a shrubby legume that benefits soil quality because it increases carbon capture (C) and has a high capacity for biological N fixation (15). Legumes are plants that have a C: N ratio of less than 30 in the foliage, so their decomposition in the soil is usually very fast, especially in the presence of high humidity and temperatures (22).

Shrub legumes raise C content in soils, which allows an increase in the cation exchange capacity, favoring the retention of cations and the consequent reduction in their washing or leaching, as well as an increase in N reserve and the improvement of the soil structure (23). In this way, arboreal legumes remain presence in coffee plantations correlates positively with the abundance of earthworm populations in the soil, and the large amount of litter that they release, favors the presence in quantity and variety of edaphic fauna, all of which make them widely preferred species in agroforestry systems (15).

In this regard, the field campaign of Mexican Carbon Program made it possible to quantify contributions to biodiversity caused by agroforestry systems of coffee under diversified shade that protects them from rainfall, preserves the soils while nourishing them with the fall of the leaves, promoting microorganisms and BNF in the case of legumes (24).

The presence of atmospheric N-fixing species in the agroforestry system makes it possible to improve the balance of this element in the soil. The methods used for quantification have been several, each with advantages and disadvantages and values that vary depending on the species evaluated, the methodology used and the edaphoclimatic conditions present at the plant growth site have been obtained (25). However, there are no doubts about the importance of this process in agroforestry systems with the presence of coffee trees and its relationship with the establishment of optimal supply systems for this nutrient. Associating the coffee tree with legume shade provides a foliar N content of this crop close to that indicated by the literature as optimal, thus evidencing the importance of this practice in agricultural systems where the nitrogen contribution is more limiting [16].

CONCLUSIONS

- The dry mass and N content quantified in shade trees were higher than that evaluated in the coffee tree, due to the different size between the species under study, dry mass of the coffee tree ranged between 1.23 and 1.75 t ha^{-1} and the of shade trees between 2 and 3 t ha^{-1}.
- The foliar N contents of shade species ranged between 25-40 kg ha^{-1} and coffee tree presented foliar concentrations of the element in adequate ranges.
- The tree species *Gliricidia sepium* and *Samanea saman* present BNF between 20 and 40 %, which can rise up to 50-55 % in the years of greatest vegetative growth of trees, which makes them an important source for the contribution and recycling of nitrogen within coffee ecosystems.

BIBLIOGRAPHY

1. Sosa-Rodrígues BA, Sánchez-de Prager M, Sanclemente-Reyes OE. Influencia de abonos verdes sobre la dinámica de nitrógeno en un Typic Haplustert del Valle del Cauca, Colombia. Acta Agronómica. 2014;63(4):343-51.
2. Moreno-Conn LM, Pérez A, Ramírez M, Franco M. Efecto de la temperatura de almacenamiento sobre la viabilidad de bacterias simbióticas fijadoras de nitrógeno utilizadas en la elaboración de inoculantes biológicos para arveja *Pisum sativum* y soya *Glycine max*. Revista colombiana de biotecnología. 2014;16(2):45-56.
3. Perez-Diaz A, Bustamante-Gonzalez C, Martín-Alonso G, Rivera- Espinosa R. Efecto de la fertilización nitrogenada en el cafeto conilon sobre el rendimiento y algunos indicadores de calidad de suelos cambisoles de cuba. Holos Environment. 2014;14(1):49-61.
4. Gutiérrez YL, Lozano HL, Jimenez DAC. Ventajas y desventajas de cultivos de *Coffea arabica* L. y *Theobroma cacao* L. bajo sistemas agroforestales. Ingenierías & Amazonia. 2015;8(1):48-58.
5. Román Miranda ML, Mora Santacruz A, González Cueva GA. Sistemas agroforestales con especies de importancia maderable y no maderable, en el trópico seco de México. Avances en Investigación Agropecuaria. 2016;20(2):53-72.
6. Ferrari AE, Wall LG. Utilización de árboles fijadores de nitrógeno para la revegetación de suelos degradados. Revista de la Facultad de Agronomía, La Plata. 2004;105(2):63-87.

7. Vega GO, Ordoñez CME, Suarez JCS, Lopez CFP. Almacenamiento de carbono en arreglos agroforestales asociados con café *Coffea arabica* en el sur de Colombia. RIAA. 2014;5(1):213-21.
8. Paneque-Pérez VM. Manual de técnicas analíticas para análisis de suelo, foliar, abonos orgánicos y fertilizantes químicos. La Habana: Ediciones INCA; 2010. 157 p.
9. Anderson JM, Ingram JSI. Tropical soil biology and fertility: a handbook of methods. Vols. 36-40. Wallingford, Oxon, UK: CAB International; 1996.
10. Martín-Alonso GM, Tamayo-Aguilar Y, Hernández-Forte I, Varela-Nualles M, da Silva-Araujo E. Cuantificación de la fijación biológica de nitrógeno en *Canavalia ensiformis* crecida en un suelo pardo mullido carbonatado mediante los métodos de abundancia natural de ^{15}N y diferencia de N total. Cultivos Tropicales. 2017;38(1):122-30.
11. Bustamante González C, Pérez Díaz A, Viñals R, Martín Alonso GM, Rivera R, Rodríguez MI. Producción de fitomasa por *Coffea canephora* Pierre ex Froehner en dos ciclos productivos. Cultivos Tropicales. 2015;36(1):29-35.
12. Jiménez E, Martínez P. Estudios ecológicos del agroecosistema cafetalero: II. Producción de materia orgánica en diferentes tipos de estructura. 1979 p. 109-26.
13. Triana-González D, Loyola-Hernández O, Curbelo-Rodríguez LM, Guevara-Viera RV. Evaluación del aporte de hojarasca y la fauna edáfica asociada a *Gliricidia sepium* (Jacq) Kunth ex Walp como cercas vivas en sabanas ultramáficas. Revista de Producción Animal. 2013;25(2):14-9.
14. Bustamante González C, Rodríguez MI, Viñals R, Pérez A. Contribución al estudio del ciclo de nutrientes en el agroecosistema cafetalero: biomasa y aporte de nutrientes al suelo. Café Cacao,Cuba. 2007;8(2):29-35.
15. Canul-Solís J, Alvarado-Canché C, Castillo-Sánchez L, Sandoval-Gío J, Alayón-Gamboa J, Piñeiro-Vázquez A, et al. *Gliricidia sepium* (Jacq.) Kunth ex Walp. una especie arborea multiproposito para la sustentabilidad de los agroecosistemas tropicales. AGROProductividad. 2018;11(10):195-201.
16. Ricci M, da Silva-Araujo E, Alves B, de Almeida FFD, Rouws J, Junior D. Contribuição da adubação verde anual e do cultivo de espécies arbóreas para a ciclagem de nutrientes em cultivo orgânico do cafeeiro. Embrapa Agrobiologia-Boletim de Pesquisa e Desenvolvimento (INFOTECA-E). 2017;31.

17. López M, Salazar G, García M. Crecimiento de árboles de sombreado y producción de café en un sistema agroforestal: resultados experimentales. In: Diagnóstico, productividad y ambiente en cafetales: estudios regionales y de caso. Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP); 2020. p. 200-30.
18. Pérez- Díaz A, Castañeda- Hidalgo E, Lozano- Trejo S, Bustamante-González CA, Rivera- Espinosa RA, Rodríguez GO, et al. Foliar Analysis as an Estimate on the Nutritional State of Conilon Coffee Plantations on Cambisoils. Journal of Life Sciences. 2014;8(2):181-7.
19. Gómez M, Preston TR. Ciclaje de nutrientes en un banco de proteína de matarratón *Gliricidia sepium*. Livestock Research for Rural Development. 1996;8(1):1-8.
20. Andújar F. Evaluación de leguminosas arbóreas en un Ultisol enmendado con cal y nutrientes. Revista Agropecuaria y Forestal APF. 2012;1(1):1-8.
21. Borges JA, Barrios M, Sánchez D, Quiróz Y, Dávila L. Evaluación del estrato herbáceo y aporte forrajero en una asociación natural entre *Cynodon plectostachyus* y *Erythrina poeppigiana* . Revista Agroforestería Neotropical. 2015;1(5):15-26.
22. Martín GM, Rivera R. Influencia de la inoculación micorrízica en los abonos verdes. Efecto sobre el cultivo principal. Estudio de caso: el maíz. Cultivos Tropicales. 2015;36(Especial):36-52.
23. Barreto AC, Fernandes MF. Cultivo de *Gliricidia sepium* e *Leucaena leucocephala* em alamedas visando a melhoria dos solos dos tabuleiros costeiros. Pesquisa Agropecuária Brasileira. 2001;36(10):1287-93. doi:10.1590/S0100-204X2001001000011
24. Libert-Amico A. Paisajes bioculturales ante el cambio climático: carbono y agrobiodiversidad en cafetales bajo sombra en Chiapas. In: Estado actual del conocimiento del ciclo del carbono y sus interacciones en México: síntesis a 2017. Mexico; 2017. p. 94-9.
25. Rapidel B, Allinne C, Cerdán C, Meylan L, Virginio-Filho E, Avelino J. Efectos ecológicos y productivos del asocio de árboles de sombra con café en sistemas agroforestales. Sistemas agroforestales: funciones productivas, socioeconómicas y ambientales. Serie técnica. Informe técnico. 2015;(42):5-20.