Article

Nutrient supply of nitrogen-fixing tree species in agroforestry systems with coffee

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Abstract

An experiment was carried out in the Huatusco region of Veracruz to test the effect of the proper use of fertilizer on the production of coffee under the shade of nitrogen-fixing tree species. For this, four treatments were proposed in four agroforestry systems (coffee-*Inga vera*, coffee-*Juglans pyriformis*, coffee-*Erythrina poeppigiana* and coffee-*E. Poeppigiana-Grevillea robusta*), with three blocks of 10 plants each, which consisted of : a) without fertilization; b) soil fertilization; c) foliar fertilization; d) soil and foliar fertilization. The nutritional balance was evaluated by means of two foliar analyzes carried out before and after fertilization. The yield was evaluated during the months of November and December. With the proposed nutritional management, the concentrations of N, P, K, Ca, Mg and Mn increased, while those of Fe, Cu and Zn decreased, approaching the optimum levels for cultivation. No significant differences ($p \leq 0.05$) in nutrient concentrations were found between the four treatments in systems IV and EG. In EP, there was a difference for Ca and Mn, in treatments 2 and 3. In JP, there was a difference for Mg in the three treatments.

Keywords: foliar analysis, nitrogen fixation, nutritional balance, yield.

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Introduction

Coffee cultivation in Mexico is carried out predominantly under shade with some leguminous species that form associations with nitrogen-fixing bacteria. These bacteria are found in almost all ecosystems freely or in association with legumes and other species, the latter having a competitive advantage by obtaining carbohydrates from their host (Saha *et al*., 2017).

The nodulation process begins when the plant sends chemical signals through the root system, which attract the bacteria, which enters through the root hair, causing cell division and root curling, which will become a node to fix N_2 (Murray, 2011). For this, the protein reductase provides electrons to nitrogenase, which reduces N_2 to NH_3, whose synthesis depends on both the host and the bacteria.

They develop mechanisms such as compartmentalization or the synthesis of leghemoglobin to prevent oxygen from coming into contact with nitrogenase, since it causes its inhibition (Juliana, 2004). This process involves a considerable energy expenditure for the plant, since in ideal conditions, 16 ATP molecules are hydrolyzed, while under natural conditions it is less efficient.

Bacterial nodules show a positive response to chemical fertilization as long as there is a nutritional balance, since the excessive application of nitrogen reduces their effectiveness until they are inactivated (Zheng *et al*., 2016). In some cases, nitrifying bacteria can supply the N that the crop needs; however, inoculation of these organisms is essential for the fixation process, as are the Mo, Fe, Ca and Co elements (Saha *et al*., 2017; Pedrozo *et al*., 2018).

It has been shown that coffee plantations under shade with leguminous species, the yield can reach 3.43 t ha^{-1} while in coffee systems open pit, yield is reduced to 1.48 t ha^{-1} with a maximum of 2 t ha^{-1} (Peeters *et al*., 2003; Farfán-Valencia and Mestre-Mestre, 2004; Benítez *et al*., 2015).

The objective of the present study was to evaluate the response to foliar and soil fertilization of four coffee plantations with and without leguminous species, using foliar analysis to propose a management plan that promotes biological nitrogen fixation, recycling of nutrients, improve the nutritional state of the crop and obtain maximum yields.

Materials and methods

The present study was carried out in the municipality of Huatusco, Veracruz, where a randomized complete block (BCA) experiment was carried out, with four fertilization treatments: 1) control; 2) soil fertilization; 3) foliar fertilization; 4) soil and foliar fertilization. Four agroforestry systems were chosen which were: 1) coffee-*Inga vera*; 2) coffee-*Juglans pyriformis*; 3) coffee-*Erythrina poeppigiana*; and 4) coffee-*E. poeppigiana-Grevillea robusta*. Three blocks of ten plants were chosen for each treatment, which made a total of 480 plants.

After applying the fertilizer to the coffee crop, the harvest yield in the month of December was evaluated, the production of cherry coffee per block was weighed and dried to determine the moisture content. Two foliar analyzes were performed, one before applying the fertilizers and the second four months after. The leaf sample was integrated by collecting the pair of leaves located in the fourth position of the apex towards the base of the branch, at the four cardinal points, for each plant in each block (Lara, 2005).

The samples were oven dried at 75 °C for 72 hours and milled in a stainless steel mill. They were then digested with 4 ml of diacid mixture and 2 ml of hydrogen peroxide. The elements N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B were then analyzed (Álvarez-Sánchez and Marín-Campos, 2015).

The nodules were analyzed with a scanning electron microscope in search of the bacteroide and the percentage of nutrients within the image frame was quantified using the EDAX analysis (Energy Dispersive X-ray Analysis). Subsequently, nodules from the three systems were collected with legumes to determine their nutrient concentrations.

Results and discussion

Foliar analysis prior to fertilization

The first foliar analysis (Table 1) was carried out during the month of July and showed deficiencies in N, K, Ca, Mg and Zn, according to the intervals proposed by Osorio (2012). On the other hand, P, B, Mo and Mn are located in the sufficiency range, but Fe and Cu exceeded the recommended concentrations, which is normal in acid pH soils, where Al, Fe, S and Mn in leaves are usually high (Bernal-Forero and Almanza-Merchan, 2016).

Table 1. Initial foliar nutrient concentration values of agroforestry systems for each treatment.

Treatment	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B	Mo
	(%)					(mg kg^{-1})					
EP	1.5	0.27	1.17	0.14	0.24	154.15	11.92	9.12	74.28	31	0.31
IV	1.63	0.29	1.34	0.21	0.35	157.25	17.06	7.64	67.54	29	0.28
JP	1.51	0.28	1.31	0.15	0.38	160.08	16.73	7.38	81.81	31.75	0.3
EG	1.6	0.26	1.39	0.16	0.27	147.43	10.18	9.22	47.98	39.25	0.29

EP= coffee system-*Erythrina poeppigiana*; IV= coffee system-*Inga vera*; JP= coffee system-*Juglans pyriformis*; EG= coffee system-*Erythrina poeppigiana* and *Grevillea robusta*; 0= control; 1= soil fertilization; 2= foliar fertilization; 3= soil and foliar fertilization.

Percent optimal deviation (DOP)

The DOP of the first foliar analysis is shown below (Table 2), where it can be seen that, in most systems, the deficient nutrients are Ca, N, B, K, P, Mg and Mn, the first being the of greater scarcity, while Fe was the only element that was not deficient in all systems.

Table 2. Deviation from the optimal percentage for foliar analysis prior to fertilization.

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
	(%)					(mg kg^{-1})				
Initial nutritional diagnosis (EP)										
CN	1.5	0.27	1.17	0.14	0.24	31	11.92	154.15	74.28	9.12
DOP	-48	-10	-43	-89	-29	-39	6	26	-5	-30
ORN	Ca> N> K> B> Zn> Mg> P> Mn> Cu> Fe									
Initial nutritional diagnosis (IV)										
CN	1.63	0.29	1.34	0.21	0.35	29	17.06	157.25	67.54	7.64
DOP	-44	-4	-35	-84	2	-43	52	29	-13	-42
ORN	Ca> N> B> Zn> K> Mn> P> Mg> Fe> Cu									
Initial nutritional diagnosis (JP)										
CN	1.51	0.29	1.31	0.15	0.38	28	15.68	160.08	81.81	15.68
DOP	-48	-8	-37	-88	11	-45	40	31	5	-44
ORN	Ca> N> B> Zn> K> P> Mn> Mg> Fe> Cu									
Initial nutritional diagnosis (EG)										
CN	1.6	0.26	1.39	0.16	0.27	39.25	10.18	147.43	47.98	9.22
DOP	-45	-13	-33	-88	-22	-23	-9	21	-38	-30
ORN	Ca> N> Mn> K> Zn>B> Mg> P> Cu> Fe									

EP= coffee system-*Erythrina poeppigiana*; IV= coffee system-*Inga vera*; JP= coffee system-*Juglans pyriformis*; EG= coffee system-*Erythrina poeppigiana* and *Grevillea robusta*; CN= nutritional composition; DOP= percentage optimum deviation; ORN= nutritional requirement order.

Kenworthy balance index (IBK)

To perform the calculation of the Kenworthy Balance Index, the values reported by Fonseca *et al*. (2018), from which the results presented in Table 3 were obtained, where the data from the initial analysis are compared.

Table 3. Nutritional diagnosis using the Kenworthy balance index for foliar analysis prior to fertilization.

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
	(%)					(mg kg^{-1})				
Initial nutritional diagnosis (EP)										
CN	1.5	0.27	1.17	0.14	0.24	31	11.92	154.15	74.28	9.12
IBK	54	89	60	19	74	75	108	123	97	75
C	B	N	B	MB	B	B	N	A	N	B
ORN	Ca> N> K> B> Mg> Zn> P> Mn> Cu> Fe									
Initial nutritional diagnosis (IV)										
CN	1.63	0.29	1.34	0.21	0.35	29	17.06	157.25	67.54	7.64
IBK	58	95	67	24	102	64	168	125	92	66
C	B	N	B	MB	N	B	MA	A	N	B
ORN	Ca> N> B> Zn> K> Mn> P> Mg> Fe> Cu									

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
	(%)					(mg kg^{-1})				
Initial nutritional diagnosis (JP)										
CN	1.51	0.29	1.31	0.15	0.38	28	15.68	160.08	81.81	15.68
IBK	54	90	66	20	110	62	152	127	107	64
C	B	N	B	MB	N	B	MA	A	N	B
ORN	Ca> N> B> Zn> K> P> Mn> Mg> Fe> Cu									
Initial nutritional diagnosis (EG)										
CN	1.6	0.26	1.39	0.16	0.27	39.25	10.18	147.43	47.98	9.22
IBK	57	85	70	20	81	81	93	118	78	76
C	B	N	B	MB	B	B	N	A	B	B
ORN	Ca> N> K> Zn> Mn> Mg> B> P> Cu> Fe									

EP= coffee system-*Erythrina poeppigiana*; IV= coffee system-*Inga vera*; JP= coffee system-*Juglans pyriformis*; EG= coffee system-*Erythrina poeppigiana* and *Grevillea robusta*; CN= nutritional composition; IBK= Kenworthy Balance Index; C= condition; MB= very low; B= low; N= normal; A= high; MA= very high; ORN= nutritional requirement order.

Both foliar analysis interpretation methodologies agree that the most deficient elements in the culture are Ca, N, B, K and Mg, while Fe and Cu reach excess levels, which could cause problems in the culture due to toxicity (López-García *et al*., 2016).

Foliar analysis after fertilization

The second foliar analysis was carried out during the month of November, which reflects a difference in the concentration of N in systems IV, EG and EP superior for treatments 2 and 3, demonstrating the effectiveness of foliar fertilization and soil. Table 4 shows the result of the foliar analysis.

Table 4. Nutritional status of the systems by treatment after applying the products.

Treatment	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu	B
	(%)					(mg kg^{-1})				
EP	1.89	0.21	2.89	0.54	0.27	61.9	5.17	84.74	7.33	129.53
IV	1.9	0.24	2.85	0.95	0.36	146.43	7.7	212.56	7.69	80.48
JP	2.05	0.18	3.06	0.82	0.33	69.47	3.73	201.39	8.41	117.14
EG	1.99	0.23	2.74	0.8	0.26	69.77	5.41	115.38	8.63	119.05

EP= coffee system-*Erythrina poeppigiana*; IV= coffee system-*Inga vera*; JP= coffee system-*Juglans pyriformis*; EG= coffee system-*Erythrina poeppigiana* and *Grevillea robusta*.

The P and K had a slight response to the treatments, however, in the EP and JP systems a small increase in the concentration of these nutrients can be noted. On the other hand, Ca and Mg had a positive response to treatments 2 and 3, with the exception of the EG system, which had a low value.

In the case of the microelements, Fe, Zn, Mn and Cu showed a positive response to foliar fertilization, but in treatment 3 the values decreased, however, according to what was reported by Fonseca *et al*. (2018), the values reach levels that can cause toxicity in the crop, therefore, by reducing its concentration in plants, a better nutritional balance is obtained.

Figure 1 and Figure 2 show that both K and B had a considerable increase, while the concentrations of P, Fe, Zn and Cu decreased slightly, which represents a benefit for the crop, since Fe exceeded the recommended concentrations in literature (Osorio, 2012). Similarly, an increase in Ca and Mg concentrations is observed, elements whose availability is limited in soils with acidic pH.

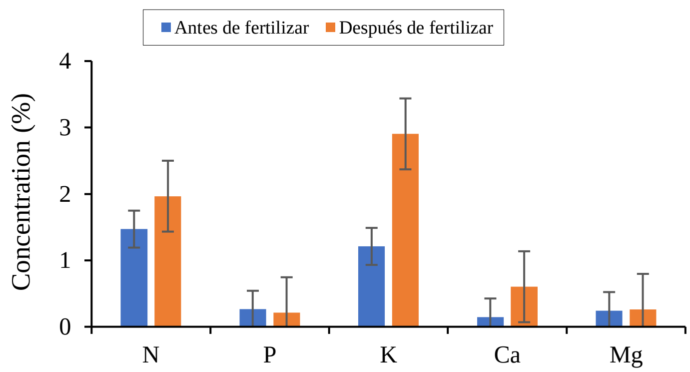

Figure 1. Average concentrations of nutrients in the EP system before and after fertilizing.

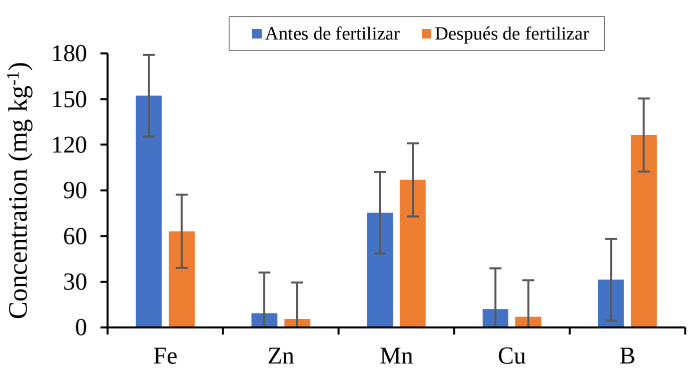

Figure 2. Average concentrations of micronutrients in the EP system before and after fertilizing.

In Figure 3 and Figure 4 it can be seen that the concentrations of K, Ca and Mn presented a positive response to fertilization, since they increased considerably, while N, Fe, P, Mg, Zn and Cu did not modify their concentrations in such a way considerable, as Rodríguez *et al*. (2014).

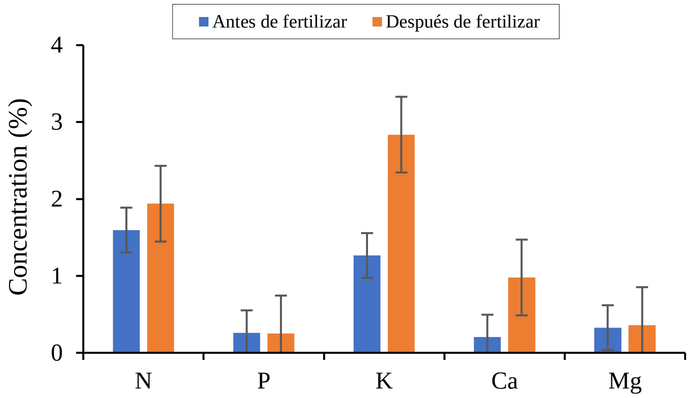

Figure 3. Macronutrient concentrations in the IV system before and after fertilizing.

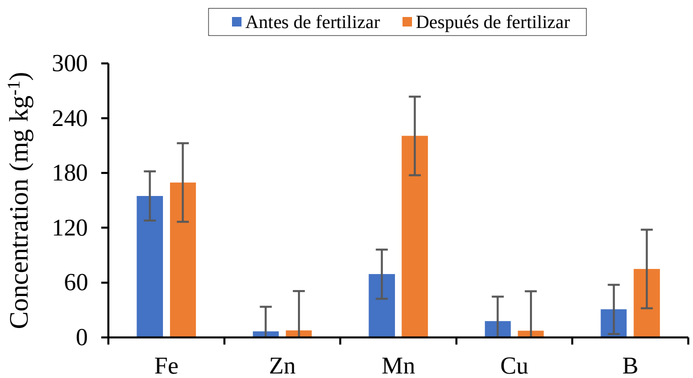

Figure 4. Micronutrients concentrations in the IV system before and after fertilizing.

In the JP system (Figure 5 and Figure 6) elements K, Mn and B had a marked increase in response to fertilization. Fe reduced its toxicity concentration to normal levels, while the other elements did not have such a marked response.

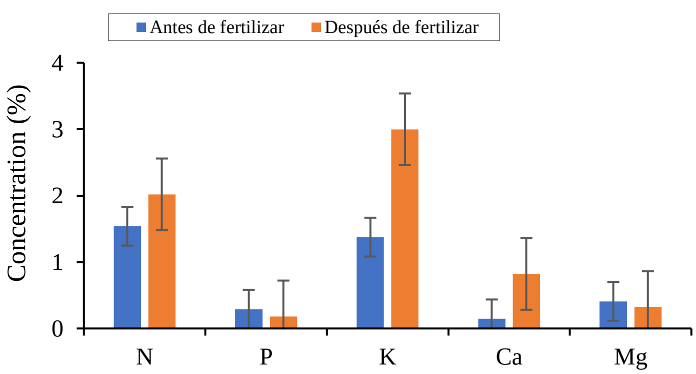

Figure 5. Macronutrient concentrations in the JP system before and after fertilizing.

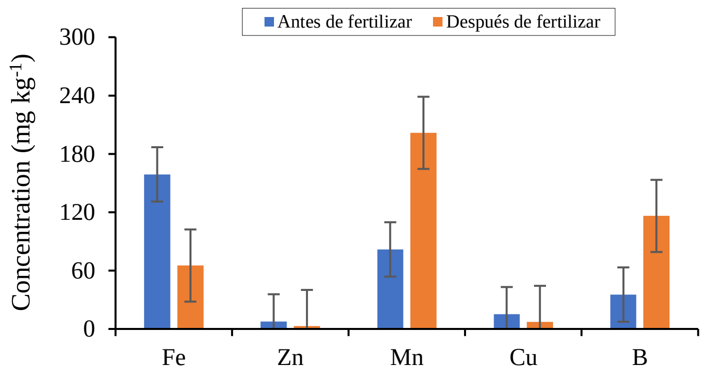

Figure 6. Micronutrients concentrations in the JP system before and after fertilizing.

In the EG system (Figure 7 and Figure 8), the concentrations of nutrients that were in deficiency conditions (K, Ca, Mn and B) were elevated, while Fe was considerably reduced. Similarly, P was reduced, since the crop had very high concentrations of it, as evidenced by research such as those carried out by Wintgens (2004a).

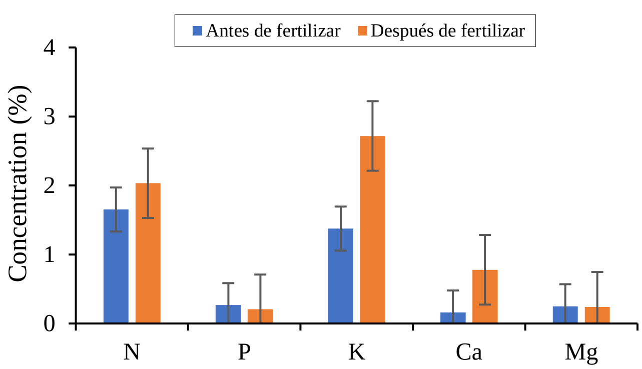

Figure 7. Macronutrient concentrations in the EG system before and after fertilizing.

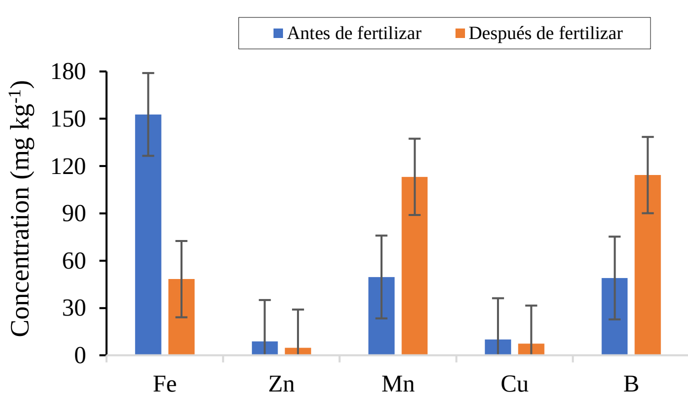

Figure 8. Micronutrients concentrations in the EG system before and after fertilizing.

Percent optimal deviation (DOP)

In the foliar analysis after the application of fertilizers, the DOP shows a significant increase in the levels of Ca, B and K, it is observed in Table 5, raising the first two to acceptable levels for the plant, while Zn, Fe, N and Cu remain in concentrations below the optimum proposed by Fonseca *et al*. (2018); INIFAP (2013).

Table 5. Deviation from the optimal percentage for foliar analysis after fertilization.

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
	(%)					(mg kg^{-1})				
	Final nutritional diagnosis (EP)									
CN	1.89	0.21	2.89	0.54	0.27	129.53	7.33	61.9	84.74	5.17
DOP	-35	-19	-1	54	-22	154	-35	-49	9	-60
ORN	Zn> Fe> N> Cu> Mg> P> K> Mn> Ca> B									
	Final nutritional diagnosis (IV)									
CN	1.9	0.24	2.85	0.95	0.36	80.48	7.69	146.43	212.56	7.7
DOP	-34	-19	38	-26	5	58	-31	20	173	-41
ORN	Zn> N> Cu> Ca> P> Mg> Fe> K> B> Mn									
	Final nutritional diagnosis (JP)									
CN	2.05	0.18	3.06	0.82	0.33	117.14	8.41	69.47	201.39	3.73
DOP	-29	-42	48	-37	-4	130	-25	31	159	-72
ORN	Zn> Fe> P> Ca> N> Cu> Mg> K> B> Mn									
	Final nutritional diagnosis (EG)									
CN	1.99	0.23	2.74	0.8	0.26	119.05	8.63	69.77	115.38	119.05
DOP	-31	-25	32	-38	-24	134	-23	-43	48	-59
ORN	Zn> Fe> Ca> N> P> Mg> Cu> K> Mn> B									

EP= coffee system-*Erythrina poeppigiana*; IV= coffee system-*Inga vera*; JP= coffee system-*Juglans pyriformis*; EG= coffee system-*Erythrina poeppigiana* and *Grevillea robusta*; CN= nutritional composition; DOP= percentage optimum deviation; ORN= nutritional requirement order.

Kenworthy balance index (IBK)

The IBK shows an increase in the concentrations of Ca in IV, EG and JP, while N, K, B and Mn increased in all systems, the last three reaching excess levels. On the other hand, Mg remained at normal concentrations and Fe, Cu, P and Zn decreased (Table 6).

Table 6. Nutritional diagnosis using the Kenworthy balance index for foliar analysis after fertilization.

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
	(%)					(mg kg^{-1})				
	Final nutritional diagnosis (EP)									
CN	1.89	0.21	2.88	0.54	0.27	129.5	7.33	61.9	84.74	5.17
IBK	67	66	137	47	81	229	55	58	105	50
C	B	B	A	MB	B	MA	B	B	N	B
ORN	Ca> Zn> Cu> Fe> P> N> Mg> Mn> K> B									

	N	P	K	Ca	Mg	B	Cu	Fe	Mn	Zn
	(%)					(mg kg^{-1})				
Final nutritional diagnosis (IV)										
CN	1.9	0.24	2.85	0.95	0.36	80.48	7.69	146.4	212.6	7.7
IBK	67	78	135	76	105	148	59	117	199	66
C	B	B	A	B	N	A	B	N	MA	B
ORN	Cu> Zn> N> Ca> P> Mg> Fe> K> B> Mn									
Final nutritional diagnosis (JP)										
CN	2.05	0.18	3.06	0.82	0.33	117.14	8.41	69.47	201.39	3.73
IBK	72	52	144	67	96	209	67	63	326	41
C	B	B	A	B	N	MA	B	B	MA	MB
ORN	Zn> P> Fe> Cu> Ca> N> Mg> K> B> Mn									
Final nutritional diagnosis (EG)										
CN	1.99	0.23	2.74	0.8	0.26	119.05	8.63	69.77	115.38	5.41
IBK	70	71	130	65	79	212	84	63	128	52
C	B	B	A	B	B	A	N	B	A	B
ORN	Zn> Fe> Ca> N> P> Mg> Cu> Mn> K> B									

EP= coffee system-*Erythrina poeppigiana*; IV= coffee system-*Inga vera*; JP= coffee system-*Juglans pyriformis*; EG= coffee system-*Erythrina poeppigiana* and *Grevillea robusta*; CN= nutritional composition; IBK= Kenworthy Balance Index; C= condition; MB= very low; B= low; N= normal; A= high; MA= very high; ORN= nutritional requirement order.

Both analysis interpretation methodologies show that the levels of N, P, K, Ca, Mg and Mn rose, while the concentrations of Fe, Cu and Zn decreased to acceptable levels, demonstrating that the proposed management improves the nutritional balance of the coffee cultivation (Figure 9).

Figure 9. Comparison of coffee plants, on the left treatment 3 in the JP system, on the right a plant under the management of the producer.

Statistical analysis

The data obtained from the foliar analyzes were processed with the help of the R software (ver. 3.6.1) to obtain the Anova (summarized by the *p*-value) and comparison of means with Tukey and Duncan. Table 7 shows the ANOVA results by agroforestry system for each element, represented by the *p*-value. In cases where Ho is rejected the comparison of means with Tukey and Duncan tests is also shown, to indicate the treatment that had a significant difference. Systems IV and EG did not show a significant difference despite having changed the nutritional concentrations after fertilization (Pérez and Argueta, 2011).

Table 7. Statistical analysis of treatments for the EP system by element ($p\leq 0.05$).

System	Element	P-value	Decision
EP	N	0.1868	Ho
	P	0.7322	Ho
	K	0.2755	Ho
	Ca	0.0105	R Ho
	Mg	0.1954	Ho
	Fe	0.5635	Ho
	Cu	0.4011	Ho
	Zn	0.4175	Ho
	Mn	0.0021	R Ho
	B	0.3435	Ho

EP= system of *Erythrina poeppigiana*; Ho= the null hypothesis is accepted; R Ho= the null hypothesis is rejected, the alternative is accepted.

Nodulation

In the EP, IV systems, nodulation was observed, but when making cuts to the nodules, it could be seen that they lacked leghemoglobin (pink to red color) or were in extremely low concentrations. Extraordinarily little nodulation was observed in the EG system, possibly due to the age of the woodland, pH and lack of activators for nodulation (Co, Mo, Fe).

In EP nodules, the bacteria could be easily recognized in scanning electron microscope images (Figure 10), whereas in IV and EG it was more complicated (Figure 11), perhaps due to the low presence of leghemoglobin. However, the quantification of the percentage of each nutrient by means of the spectral response shows high Mo levels.

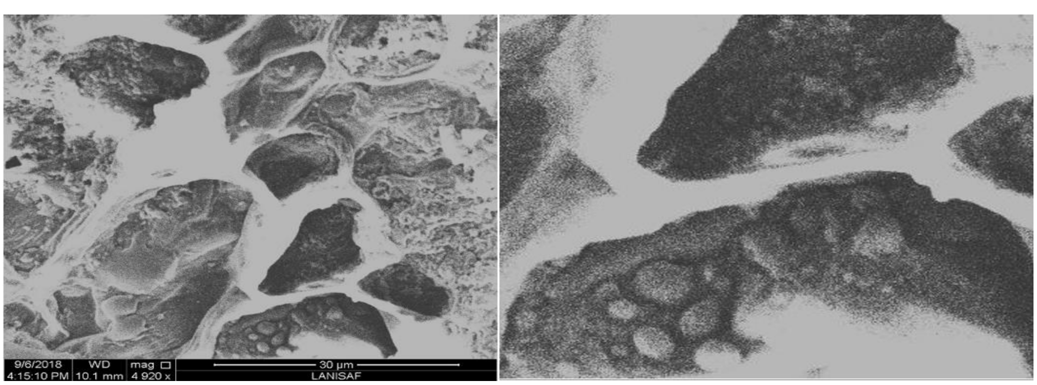

Figure 10. Scanning electron microscope image in an EP node. On the left, the complete image, on the right, a close-up of the area where the bacteroide was identified.

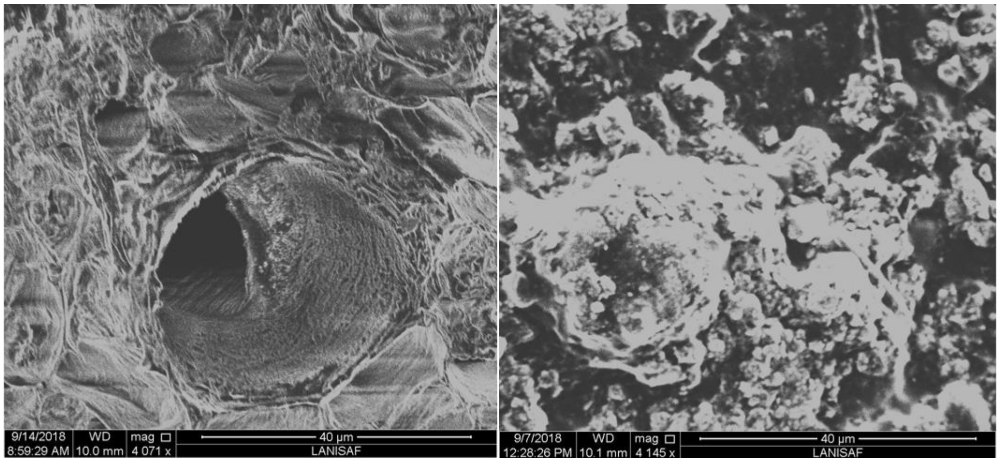

Figure 11. Scanning electron microscope images in IV (left) and EG (right) nodules.

In the three nodules analyzed (Table 8), since this element promotes the insertion of electrons in N_2 and the movement of this element within the plant, increasing the yield of crops such as beans (Olivé, 2009; Ocaña-Reyes, 2016; Pérez and Ruiz, 2017).

Table 8. Nitrogen, iron, cobalt and molybdenum concentrations in the images captured by the scanning electron microscope.

Element/system	EP	IV	EG
N (%) in weigh	3.52	22.52	6.86
Fe (%) in weigh	3.23	0.55	2.89
Co (%) in weigh	1.27	0.78	2.11
Mo (%) in weigh	13.9	17.77	9.64

In IV nodules, canal-like structures were observed that can be seen in EP, which indicates that the nodules formed correctly; however, Bacteroides were not observed within these structures. On the other hand, in EG, neither structural nor Bacteroide development is observed, due to the inactivity of the nodule.

In the quantitative analysis of the images, high concentrations of nitrogen were detected in the nodules of both species, demonstrating that the roots are absorbing this element, despite the low presence of leghemoglobin. Likewise, Fe and Co, which are the activating elements of FBN, are found in higher concentrations than those determined by soil analysis, despite the presence of antagonisms with Al^{3+}.

Conclusions

An abundant nodulation was not observed in the tree roots, but in those that are in contact with the runoff water on the edge of the plot, next to the road cut in the EP system. This may be because the availability of nutrients in this area is greater since the water carries cations such as Ca^{++} and Mg^{++}.

To solve this problem, it is necessary for the producer to continue fertilizing with the proposed scheme, to improve production and promote the recycling of essential nutrients for the nodules. When comparing both foliar analyzes, the concentrations of N, P, K, Ca, Mg and Mn rose to levels within the normal range, while the concentrations of Fe, Cu and Zn decreased, approaching the optimum range for the crop.

No statistically significant difference was found in systems IV and EG, while in EP there was a difference in the concentrations of Ca and Mn, elements whose assimilation is limited in acidic soils. In the case of Ca, treatments 2 and 3 had a positive and statistically different response to treatment 1, in Mn concentration, treatment 3 had a marked positive response with respect to the other treatments. In JP, a difference was found in Mg concentrations, with treatment 1 being the one with the lowest concentrations compared to the other treatments.

Fruit production was benefited by different treatments in each system, while in EP and EG high yields were obtained with treatment 3, in IV it was treatment 2 that produced the same effect and JP was benefited by treatment 1, this confirms the need to analyze each system independently to offer the producer specific fertilization schemes for each soil condition that allows maximum yields to be obtained.

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