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Importance of Soil Health for *Coffea* spp Cultivation, from a Cooperative Society in Puebla, Mexico

Carol Meritxell Molina-Monteleón [1], Amparo Mauricio Gutiérrez [2,*], Rosalía Castelán-Vega [3,*] and José Victor Tamariz-Flores [3]

[1] Posgrado en Ciencias Ambientales. Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla 72570, Mexico; carol_sp@outlook.es
[2] CONAHCYT-Centro de Agroecología. Instituto de Ciencias, Benemérita Universidad Autónoma de Puebla. Edificio VAL 1, Km 1.7 Carretera a San Baltazar Tetela, San Pedro Zacachimalpa, Puebla 72960, Mexico; amg2510@hotmail.com
[3] Centro de Investigación en Ciencias Agrícolas. Instituto de Ciencias. Benemérita Universidad Autónoma de Puebla. Puebla, Puebla, 72570, Mexico; jrostamariz@hotmail.com
* Correspondence: amg2510@hotmail.com; Tel.: +52-222-229-55-00 (ext. 1315) (A.M.-G.); rosalia.castelan@correo.buap.mx; Tel.: +52-222-229-55-00 (ext. 7353) (R.C.-V.)

Abstract: The cultivation systems of *Coffea* spp in a cooperative society in Puebla, Mexico, include rustic, traditional polyculture, commercial polyculture, unshaded monoculture and shaded monoculture. In this work, the properties of the soil were analyzed through physical, chemical, and biological analysis to determine its nutritional status. Composite sample analyses were conducted to determine physical, chemical and microbiological parameters (fungi, actinomycetes, mesophilic bacteria, nitrifying and denitrifying bacteria). Leaf nutrients were determined. Rustic was the cropping system with the highest amount of K (0.76 cmol kg^{-1}) in soil and nutrient assimilation in leaf (N= 2.79%, P= 660.01, K= 17297.22 and Fe= 271.24 mg kg^{-1}) (p= 0.001); in addition to presenting high populations of mesophilic bacteria, fungi and actinomycetes (30.16, 0.59 and 0.83 respectively, x10^6 CFU g^{-1} soil) and very low nitrification and denitrification rates. The principal component analysis (PCA) (>3.25%) indicated that actinomycetes and K in soil favor the assimilation of Fe, K and P. This *Coffea* spp cultivation system had a lower impact on soil health than the rest of the systems and favored forest ecosystem conservation.

Keywords: soil quality; soil fertility; crop management; agroecosystem

1. Introduction

Primary activities in Mexico contribute 2.7% of the gross domestic product (GDP) [1], and the cultivation of *Coffea* spp occupies twelfth place [2] with a cultivated area of 702,686 ha [3]. Worldwide, Mexico is ranked tenth as a producer of *Coffea* spp, with Chiapas, Veracruz, and Puebla being the main producers [4].

Since its introduction, the cultivation of *Coffea* spp has been influenced by sociocultural and environmental factors [5], developing five cultivation systems: rustic or mountain, traditional polyculture, commercial polyculture, unshaded monoculture, and shaded monoculture [6].

Agricultural systems in the cultivation of *Coffea* spp depend on chemical fertilization [5], which intensifies chemical degradation, especially soil acidification, in addition to modifying pH values, loss of exchangeable bases, reducing microbial activity and causing toxicity from excess aluminum and manganese, coupled with overexploitation, leaching, erosion and soil runoff [7–9]. Over time, soil health has been affected by not fulfilling its function in the ecosystem, satisfying the needs of the organisms present [10,11]. Its evaluation allows us to obtain a complete position of the state of the soil through the analysis of its physical, chemical, and biological properties [11].

Physicochemical indicators such as gravimetric humidity, pH, CEC (cation exchange capacity), P, OC (organic C), N_t (total N), K, and micronutrients (Zn, Mn, Cu, Fe) can be considered, as can the biodiversity of micro and macroorganisms involved in the soil's biogeochemical cycles [12].

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In the municipality of Cuetzalan del Progreso, Puebla, Mexico, agricultural soils dedicated to the production of *Coffea* spp are susceptible to changes in humidity and temperature, which hinders high yields in cherry coffee, coupled with the change in land use, expensive inputs, and migration [13] (pp. 84,85). Due to the poverty and marginalization in which the majority of producers find themselves, it is necessary to generate information on the health of the soil in the different cultivation systems of a cooperative society in Puebla, Mexico.

In this context, considering the economic importance of cultivating *Coffea* spp in Mexico and the impact it can have on soil health, this research proposes to study the properties of the soil through physical, chemical, and biological analysis to determine its nutritional status under different cultivation systems of *Coffea* spp in a cooperative society in Puebla, Mexico.

2. Materials and Methods

2.1. Sampling Site

Sampling was carried out in 15 plots of producers located in the "Taposontok" cooperative society of the municipality of Cuetzalan del Progreso, Puebla, with a latitude between 19º 57' 00" N and 20º 05' 18" N, and a length between 97º 24' 36" W and 97º 34' 54" W [14]. The altitude varies between 180 and 1,600 m above sea level. The predominant climate is semi-warm and humid, with year-round rain and an average temperature of 20.3 °C [15] (Figure 1).

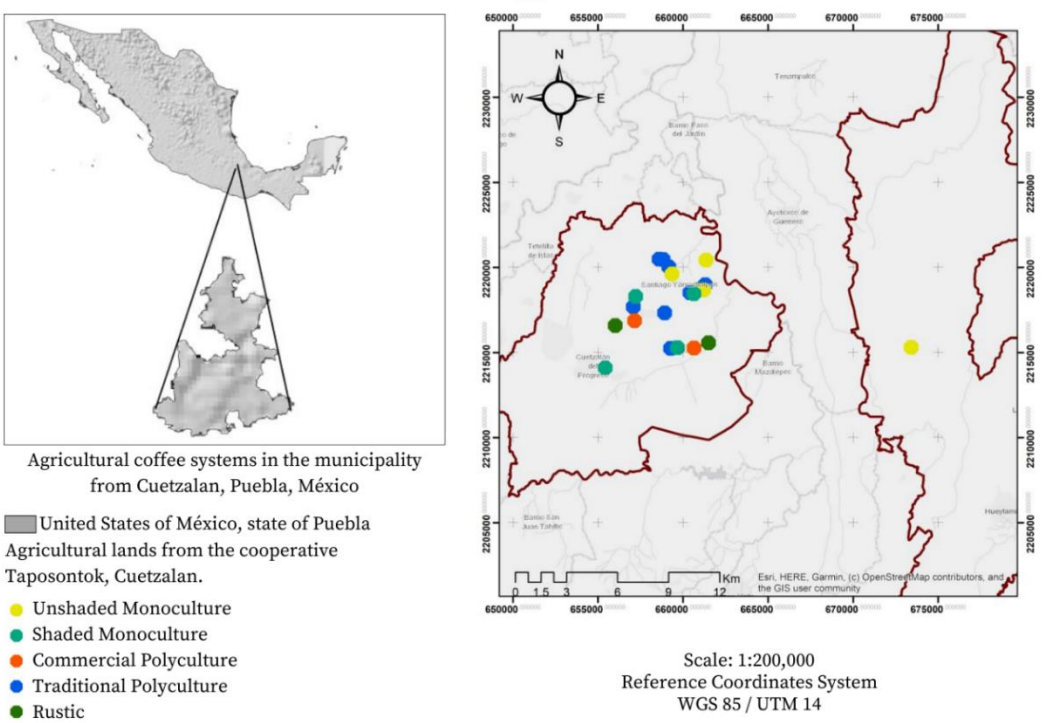

Figure 1. Sampling sites in the cooperative society, Puebla, Mexico. Cultivation methods: unshaded monoculture, shaded monoculture, commercial polyculture, traditional polyculture, and rustic.

Sampling was carried out in February 2023. In each cropping system, three plots were selected: unshaded monoculture (without the presence of shade), shaded monoculture (with species introduced for shade), commercial polyculture (with the introduction of plant species for shade and marketing), traditional polyculture (with shade from the forest ecosystem and introduction of valuable species), rustic (with shade from the forest ecosystem) [6]. For the physical and chemical analyses of the soil, a sample composed of a plot from 0 to 30 cm deep was taken, and for biological analysis, rhizospheric soil was sampled in sterile plastic containers. Thus, leaf samples from coffee plantations without pests and diseases. All samples were stored at 4°C until further analysis.

2.2. Physical and Chemical Analysis

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The soil samples were dried and sieved through a 2 mm mesh, and the analyses were carried out in accordance with the Official Mexican Standard NOM-021-RECNAT-2000 [16]. The parameters analyzed in soil were humidity (gravimetric method), apparent density (test tube method), pH (electrometric method), EC (electrical conductivity by conductimetric method), OC (organic carbon) and OM (organic matter by method of Walkley and Black), soil texture (Bouyoucos method), exchangeable bases (Ca, Na, Mg, K) and CEC (Cationic Exchange Capacity) (ammonium acetate method). N_t (total nitrogen) (Kjeldahl method) and extractable P (Bray and Kurtz method) were determined in soil and leaves.

Zn, Mn, Cu and Fe were quantified in soil by extraction with diethylenetriamine penta-acetic acid (0.005 M); the solution was shaken for two h at 120 rpm and filtered through the Whatman No. 42 paper. For the determination of Ca, Mg, Na, K, Zn, Mn and Fe in leaf, an extract was prepared with H_2O_2 (30% w/w) and concentrated HNO_3 (analytical grade), a digestion was carried out at 200°C for 10 min in a CEM Mars Xpress microwave digestion; It was subsequently filtered with Whatman No. 42 paper and volumetric to 50 ml.

Finally, 0–8 ml was injected into a flame atomic absorption spectrometer (Agilent 55B AA). An N_2O/acetylene flame, a hollow cathode lamp with a current intensity of 10 mA and wavelength of 239.9 nm, was used for Ca quantification. An air/acetylene flame was used for the rest of the elements. For Mg and Cu, a hollow cathode lamp with a current intensity of 4 mA was used; Mg was read at a wavelength of 202.6 nm and Cu at 327.4 nm. A hollow cathode lamp with a current intensity of 5 mA was used to determine Na (330.2 nm), Zn (213.9 nm), Mn (279.5 nm), Fe (372 nm), K in sheet (404.4 nm) and K in soil (769.9 nm).

Calibration curves were made for K in leaf (0, 50, 100 and 250 mg L^{-1}), K in soil (0, 1 and 3 mg L^{-1}), Ca in leaf (0, 50, 100 and 250 mg L^{-1}), Ca in soil (0, 50 and 100 mg L^{-1}) and Cu in soil (0, 2.5 and 5 mg L^{-1}). And for the following elements in both soil and leaf: Mg (0, 5, 10 and 20 mg L^{-1}), Na (0, 50 and 100 mg L^{-1}), Zn (0, 0.5, 1 and 2 mg L^{-1}), Mn (0, 2, 3 and 5 mg L^{-1}) and Fe (0, 10, 15 and 20 mg L^{-1}).

2.3. Microbiological Analysis

The total mesophilic bacterial population was determined by the most probable number (MPN) technique in nutrient broth medium in triplicate of serial dilutions from 10^{-5} to 10^{-7} at 48 h, 120 rpm and 30°C [17].

Nitrifying bacteria were quantified in Nitrosomonas medium (g L^{-1}): $(NH_4)_2SO_4$, 1.7; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.02; K_2HPO_4, 0.015; Ferric EDTA, 0.001; pH 7.5; with 1 ml of trace element solution (g L^{-1}): $MnCl_2 \cdot 4H_2O$, 0.02; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, 0.01; $CuSO_4 \cdot 5H_2O$, 0.002; $CoCl_2 \cdot 6H_2O$, 0.0002. The nitrifying populations were determined using the MPN technique of 10^{-5} to 10^{-7} serial dilutions in triplicate for 8 d, 120 rpm and 30°C.

For the denitrifying bacteria, Nitrate reduction Broth Clark medium was used (g L^{-1}): Peptone, 20; KNO_3, 2; pH 7.0. Dilutions were made from 10^{-1} to 10^{-4} and monitored for 5 d at 30°C; Durham hoods were used for gas production as growth by MPN. The Sodium Salicylate and Sulfanilamide methods quantified the production of nitrates and nitrites to calculate nitrification and denitrification rates [18,19]. For the quantification of fungi and actinomycetes, the pour-plate method was used using malt mineral medium (g L^{-1}): NH_4NO_3, 7; K_2HPO_4, 1; KH_2PO_4, 1; Malta, 0.2; pH 5.6; enriched with 1 ml of micronutrient solution (g L^{-1}): $MgSO_4 \cdot 7H_2O$, 4; $FeSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.2. 100 µL was plated from 10^{-3} to 10^{-6} dilutions, incubated in triplicate for 6 d at 30°C, and quantified as Colony Forming Units (CFU) g^{-1} dry soil [20].

2.4. Statistical Analysis

The data generated were analyzed in the RStudio software version 4.2.2, and the normality of the data was evaluated using the Shapiro-Wilk test with the "stats" package. A Pearson correlation analysis (p= 0.001, 0.01 and 0.05) was performed with the "corrr" package for leaf properties. Subsequently, an analysis of variance (ANOVA) and a Tukey multiple comparison test of means were performed using the "agricolae" package to determine if there were statistically significant differences (p< 0.05) in the physical, chemical and biological properties of the soil and the leaf between the

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different cultivation methods. Finally, a principal component analysis (PCA) was performed to find the different relationships between the variables using the "prcomp" and "FactoMineR" packages. The optimal number of clusters for the data set was determined considering the methods "kl", "ch", "hartigan", "mcclain", "gamma", "gplus", "tau", "dunn", "sdindex", "sdbw", "cindex", "silhouette", "ball", "ptbiserial", and "frey"; distance measures "euclidean", "maximum", "manhattan", and "canberra" and graphs were generated using the "Elbow", "GAP" and "Silhouette" methods. Once the optimal number of clusters (two) was selected, the k-means algorithm was applied to both the variables and the individuals (cropping systems) [21].

3. Results

3.1. Comparison of Physical, Chemical, and Microbiological Characteristics in soil

The soils of the *Coffea* spp cultivation systems in the study area have a generally clayey texture, highlighting that rustic presented the Silt Sandy Loam and Silty Loam textures. The pH values range between 3.9 and 4.93, with an apparent density of 0.81 to 0.91 mg cm^{-3}, a humidity percentage range of 40.27 to 66.36%, and an EC range of 0.13 to 0.28 dS (Table 1). For macronutrients, shaded monoculture had the highest percentage of N_t (0.47%, p= 0.001) and OM (10.89%, p= 0.001). However, for K, the rustic system had the highest value (0.76 cmol kg^{-1}, p= 0.001). On the other hand, P was high in unshaded monoculture (7.96 mg kg^{-1}, p = 0.001).

Regarding micronutrients, the rustic system presented significantly lower concentrations (p= 0.001) of Cu (1.05 mg kg^{-1}), Zn (0.50 mg kg^{-1}), and Mn (0.86 mg kg^{-1}) and a higher concentration of Na (1.14 cmol kg^{-1}). The traditional polyculture had the significantly highest values (p= 0.001) of Mg (4.26 cmol kg^{-1}), Zn (2.88 mg kg^{-1}), Mn (8.96 mg kg^{-1}), and Cu (3.45 mg kg^{-1}).

Table 1. Physical, chemical, and microbiological analysis in coffee soils.

Parameter		Unshaded Monoculture	Traditional Polyculture	Commercial Polyculture	Shaded Monoculture	Rustic	*p*-value
Textural Class		Clay, Silty Clay	Silty Loam, Clay	Silty Loam, Loam Clay	Loam, Clay	Silt Sandy Loam, Silty Loam	-
pH		3.90 ± 0.49	4.93 ± 0.55	4.58 ± 0.24	4.78 ± 0.52	4.63 ± 0.38	0.128
Bulk Density (mg cm^{-3})		0.85 ± 0.01	0.91 ± 0.05	0.88 ± 0.09	0.81 ± 0.07	0.87 ± 0.08	0.495
Moisture content (%)		58.06 ± 3.75 ab	40.27 ± 1.89 b	66.36 ± 1.48 a	55.72 ± 2.42 ab	48.31 ± 2.77 ab	0.010
EC (dS)		0.25 ± 0.03 a	0.28 ± 0.01 a	0.14 ± 0.02 b	0.24 ± 0.01 a	0.13 ± 0.01 b	0.001
P (mg kg^{-1})		7.96 ± 0.28 a	0.33 ± 0.05 b	0.68 ± 0.38 b	0.20 ± 0.01 b	0.26 ± 0.02 b	0.001
OM (%)		5.91 ± 0.39 bc	5.46 ± 0.90 c	4.79 ± 0.40 c	10.89 ± 0.40 a	7.14 ± 0.19 b	0.001
OC (%)		3.43 ± 0.23 bc	3.17 ± 0.52 c	2.78 ± 0.23 c	6.31 ± 0.23 a	4.14 ± 0.11 b	0.001
N_t (%)		0.25 ± 0.05 bc	0.15 ± 0.01 c	0.32 ± 0.03 b	0.47 ± 0.07 a	0.30 ± 0.02 b	0.001
Exchangeable Bases (cmol kg^{-1})	K	0.41 ± 0.02 b	0.36 ± 0.02 bc	0.33 ± 0.02 c	0.38 ± 0.02 bc	0.76 ± 0.05 a	0.001
	Ca	1.93 ± 0.38 c	6.79 ± 1.10 a	2.96 ± 0.47 bc	6.83 ± 0.35 a	4.12 ± 0.41 b	0.001
	Mg	1.13 ± 0.37 c	4.26 ± 0.64 a	1.43 ± 0.18 bc	2.29 ± 0.04 b	1.58 ± 0.08 bc	0.001
	Na	1.02 ± 0.02 ab	0.91 ± 0.01 bc	0.88 ± 0.02 c	0.90 ± 0.01 bc	1.14 ± 0.11 a	0.001
CEC (cmol kg^{-1})		7.88 ± 0.25 c	15.56 ± 1.48 b	15.02 ± 0.31 b	18.40 ± 0.32 a	14.29 ± 0.24 b	0.001
Micronutrients (mg kg^{-1})	Zn	0.66 ± 0.04 b	2.88 ± 0.18 a	0.53 ± 0.21 b	2.55 ± 0.47 a	0.50 ± 0.12 b	0.001
	Mn	1.40 ± 0.20 c	8.96 ± 0.20 a	2.05 ± 0.54 bc	3.67 ± 1.47 b	0.86 ± 0.10 c	0.001
	Cu	2.48 ± 0.46 b	3.45 ± 0.23 a	3.14 ± 0.08 a	2.80 ± 0.12 ab	1.05 ± 0.05 c	0.001
	Fe	140.45 ± 1.95 a	50.50 ± 0.90 b	11.46 ± 0.26 c	51.60 ± 0.20 b	20.79 ± 0.59 c	0.001
Mesophilic Bacteria		2.46 ± 0.07 e	22.56 ± 0.24 c	19.60 ± 0.31 d	27.59 ± 0.34 b	30.16 ± 0.08 a	0.001
Nitrifying Bacteria		336.54 ± 3.00 a	279.45 ± 1.88 b	226.73 ± 2.57 b	133.12 ± 5.72 c	1.51 ± 0.05 d	0.001
Denitrifying Bacteria		0.03 ± 0.01 a	0.02 ± 0.01 b	0.023 ± 0.01 b	0.02 ± 0.01 b	0.01 ± 0.01 c	0.001
Actinomycetes		0.35 ± 0.05 b	0.39 ± 0.01 b	0.23 ± 0.03 c	0.38 ± 0.02 b	0.83 ± 0.07 a	0.001
Fungi		0.28 ± 0.01 c	0.39 ± 0.01 b	0.05 ± 0.01 d	0.26 ± 0.03 c	0.59 ± 0.03 a	0.001

Data represent the mean of three repetitions ± standard deviation. Different letters in the column indicate significant statistical differences between cultivation systems according to the Tukey test (p< 0.05). EC: Electrical conductivity, OM: Organic matter, OC: Organic carbon, N_t: Total nitrogen, CEC: Cation exchange capacity. **1:** values x 10^6 cells g^{-1} soil dry; **2:** values x10^6 CFU g-1 soil dry.

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Shaded monoculture presented the highest CEC (18.40 cmol kg^{-1}, p= 0.001) and Ca (6.83 cmol kg^{-1}, p= 0.001). For Fe, the unshaded monoculture system had the highest concentration (140.45 mg kg^{-1}), and significantly lower values (p= 0.001) for Ca (1.93 cmol kg^{-1}), Mg (1.13 cmol kg^{-1}) and CEC (7.88 cmol kg^{-1}). In commercial polyculture, the significantly lowest values (p= 0.001) of Fe (11.46 mg kg^{-1}), OM (4.79 %), K (0.33 cmol kg^{-1}) and Na (0.88 cmol kg^{-1}) were found (Table 1).

Regarding microbial populations, the commercial polyculture had a significantly lower population (p= 0.001) of fungi (0.05 x 10^6 CFU g^{-1} soil) and actinomycetes (0.23 x 10^6 CFU g^{-1} soil). The rustic system presented the largest population significantly (p= 0.001) of mesophilic bacteria (30.16 x 10^6 cells g^{-1} soil), fungi (0.59 x 10^6 CFU g^{-1} soil) and actinomycetes (0.83 x 10^6 CFU g^{-1} soil); and a lower population of nitrifying bacteria (1.51 x 10^6 cells g^{-1} soil) and denitrifying bacteria (0.01 x 10^6 cells g^{-1} soil). On the other hand, in unshaded monoculture, the population of mesophilic bacteria (2.46 x 10^6 cells g^{-1} soil) was significantly lower (p= 0.001), with a greater population of nitrifying bacteria (336.54 x 10^6 cells g^{-1} soil) and denitrifying bacteria (0.03 x 10^6 cells g^{-1} soil) (Table 1).

In Figure 2, it was observed that there are significant differences in the nitrification and denitrification rates of the five cultivation systems, with unshaded monoculture being the one that had the highest rate of nitrification (0.506 mg g^{-1} soil day^{-1}) and denitrification (9.430 mg g^{-1} soil day^{-1}), while the rustic system presented the lowest nitrification rates (0.051 mg g^{-1} soil day^{-1}) and nitrite production was not detected (denitrification).

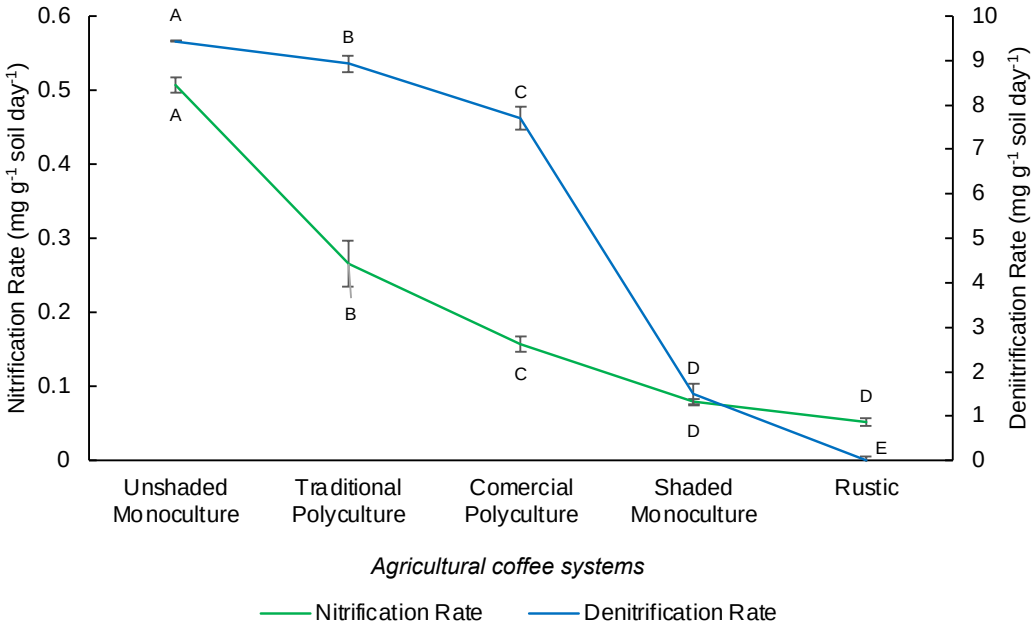

Figure 2. Nitrification and denitrification rates by cropping system in *Coffea* spp. Different letters indicate significant statistical differences between cultivation systems according to the Tukey test (p< 0.05).

3.2. Nutrient Analysis in Coffea spp Leaves

The quantification of macronutrients in *Coffea* spp leaves (Table 2), such as P (660.01 mg kg^{-1}) and K (17297.22 mg kg^{-1}) presented the highest values significantly (p= 0.001) in rustic. Thus, as in the content of micronutrients, Zn (12.50 mg kg^{-1}, p= 0.015) and Fe (271.24 mg kg^{-1}, p= 0.001). On the other hand, N (2.98%), Ca (15160.23 mg kg^{-1}) and Mg (5362.29 mg kg^{-1}) were significantly high (p= 0.001) in shaded monoculture and significantly low for rustic, with values of 10967.89 mg kg^{-1} and 2833.22 mg kg^{-1} respectively. Rustic also presented the significantly lowest values (p = 0.001) for Mn (33.33 mg kg^{-1}). The Na concentration for the five culture methods did not present statistically significant differences (p= 0.585).

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Table 2. Analysis of microelements in *Coffea* spp.

Parameter		Unshaded Monoculture			Traditional Polyculture			Commercial Polyculture			Shaded Monoculture			Rustic		
N (%)		1.20	± 0.16	c	2.60	± 0.11	ab	2.30	± 0.26	b	2.98	± 0.13	a	2.79	± 0.12	a
P (mg kg^{-1})		290.20	± 5.19	b	156.02	± 3.65	c	156.02	± 4.56	c	295.18	± 4.78	b	660.01	± 2.80	a
Micronutrients (mg kg^{-1})	K	9659.61	± 27.63	c	11558.43	± 27.80	b	8866.94	± 11.44	c	12029.73	± 25.15	b	17297.22	± 23.73	a
	Ca	10021.27	± 10.00	c	14233.60	± 14.97	b	16233.93	± 12.88	a	15160.23	± 17.44	b	10967.89	± 14.96	c
	Mg	2804.05	± 17.49	c	3745.70	± 19.14	b	3478.99	± 20.78	b	5362.29	± 15.82	a	2833.22	± 19.99	c
	Zn	10.42	± 0.42	c	12.92	± 0.42	a	12.92	± 0.42	a	11.45	± 0.46	b	12.50	± 0.43	a
	Mn	37.50	± 0.33	c	53.33	± 0.30	b	50.73	± 0.49	b	60.21	± 0.54	a	33.33	± 0.40	c
	Na	248.88	± 2.09		244.71	± 1.49		262.07	± 3.25		262.07	± 3.42		248.32	± 1.67	
	Fe	100.83	± 1.83	c	91.66	± 1.67	c	96.25	± 1.10	c	144.79	± 1.12	b	271.24	± 1.08	a

Data represent the mean of three repetitions ± standard deviation. Different letters in the column indicate significant statistical differences between cultivation systems according to the Tukey test (p= 0.001).

A total correlation analysis (Pearson) (Table 3) was performed for the relationship of nutrients in the leaves of *Coffea* spp. The significantly positively related macronutrients were P and K (0.7609674, p= 0.00098) and N and K (0.576633, p= 0.02443). The macronutrients that were positively related to the micronutrients were P and Fe (0.8290195, p= 0.00013), K and Fe (0.9139314, p= 0.00000), and P and Mn (-0.54382089, p= 0.03612) were negatively correlated. Micronutrients such as Ca and Mg (0.7383237, p= 0.00167), Na and Mg (0.655838, p= 0.00794) positively correlated.

Table 3. Pearson Correlation Matrix of microelements in *Coffea* spp leaf.

Variables	N	P	Ca	Na	Mg	K	Zn	Fe	Mn
N	1	0.2467462 (0.37532)	0.15408777 (0.58349)	0.20881933 (0.45513)	0.4175778 (0.12145)	0.576633 (0.02443)*	0.46497838 (0.08074)	0.4765052 (0.07254)	0.15264905 (0.58705)
P	0.2467462 (0.37532)	1	-0.34567145 (0.20696)	-0.17495034 (0.53287)	-0.3109498 (0.25929)	0.7609674 (0.00098)***	0.31167246 (0.25812)	0.8290195 (0.00013)***	-0.54382089 (0.03612)*
Ca	0.1540878 (0.58349)	-0.3456715 (0.20696)	1	0.35947898 (0.18818)	0.7383237 (0.00167)**	-0.3460064 (0.20649)	-0.00839027 (0.97633)	-0.3072806 (0.26525)	0.3478592 (0.20390)
Na	0.2088193 (0.45513)	-0.1749503 (0.53287)	0.35947898 (0.18818)	1	0.655838 (0.00794)**	-0.1032731 (0.71417)	0.22520867 (0.41966)	-0.1655947 (0.55532)	0.08639506 (0.75949)
Mg	0.4175778 (0.12145)	-0.3109498 (0.25929)	0.73832374 (0.00167)**	0.65583796 (0.00794)**	1	-0.2822739 (0.30805)	0.35225978 (0.19785)	-0.2968784 (0.28259)	0.27643548 (0.31859)
K	0.576633 (0.02443)*	0.7609674 (0.00098)***	-0.34600645 (0.20649)	-0.10327305 (0.71417)	-0.2822739 (0.30805)	1	0.46540082 (0.08043)	0.9139314 (0.00000)***	-0.3976754 (0.14212)
Zn	0.4649784 (0.08074)	0.3116725 (0.25812)	-0.00839027 (0.97633)	0.22520867 (0.41966)	0.3522598 (0.19785)	0.4654008 (0.08043)	1	0.3405685 (0.21419)	-0.51123327 (0.05145)
Fe	0.4765052 (0.07254)	0.8290195 (0.00013)***	-0.30728059 (0.26525)	-0.16559474 (0.55532)	-0.2968784 (0.28259)	0.9139314 (0.00000)***	0.34056846 (0.21419)	1	-0.49853466 (0.05855)
Mn	0.1526491 (0.58705)	-0.5438209 (0.03612)*	0.3478592 (0.20390)	0.08639506 (0.75949)	0.2764355 (0.31859)	-0.3976754 (0.14213)	-0.51123327 (0.05145)	-0.4985347 (0.05855)	1

*The correlation is significant at a value of p< 0.05. **The correlation is significant for p< 0.01. ***The correlation is significant for p< 0.001. Two-sided significance values are in parentheses.

3.3. Principal Component Analysis (PCA)

The principal components analysis showed a grouping of treatments defining two groupings. It was found that 59.6% of the variability can be explained by two main dimensions, Dim 1 (33.6%) and Dim 2 (26%). Furthermore, it was highlighted that the plots belonging to the rustic system have similar properties to each other, which were grouped in Cluster 1 (20%), and are different from the plots of the unshaded monoculture systems, shaded monoculture, traditional polyculture and commercial polyculture, which were grouped in Cluster 2 (80%). The differences between the *Coffea* spp cultivation systems are visualized, separating the rustic system, with minimal intervention in its ecosystem, from those with some modification (Figure 3).

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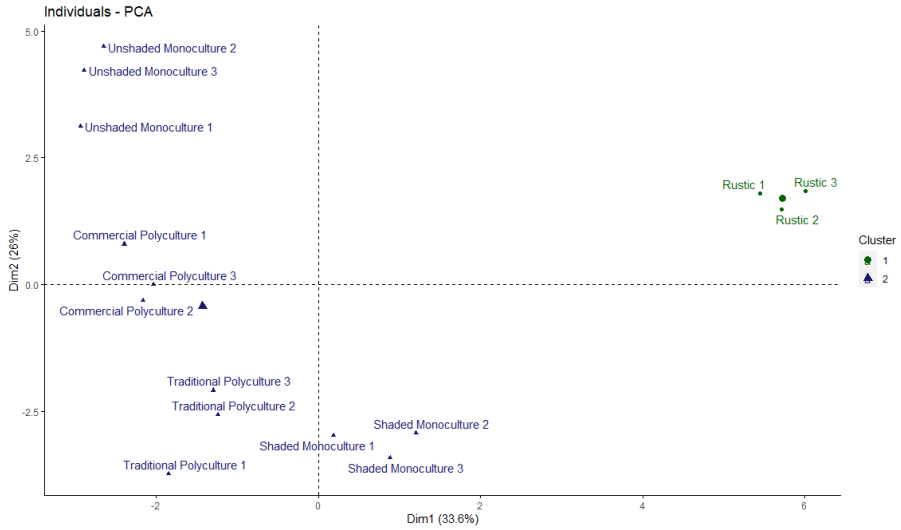

Figure 3. Principal component analysis (PCA) for the five *Coffea* spp cultivation systems.

The PCA was carried out with all the study variables. The results indicated that the most important variables to explain the variability of the main dimensions (Figure 4) are those with a more outstanding contribution to the average value of 3.25% for Dim 1 and Dim 2 as Fe (leaf) > N (leaf) > Cu > K (soil) > mesophilic bacteria > nitrifying bacteria > actinomycetes > CEC > K (leaf) > Ca > denitrifying bacteria > Na > P (soil) > Zn > P (leaf) > fungus.

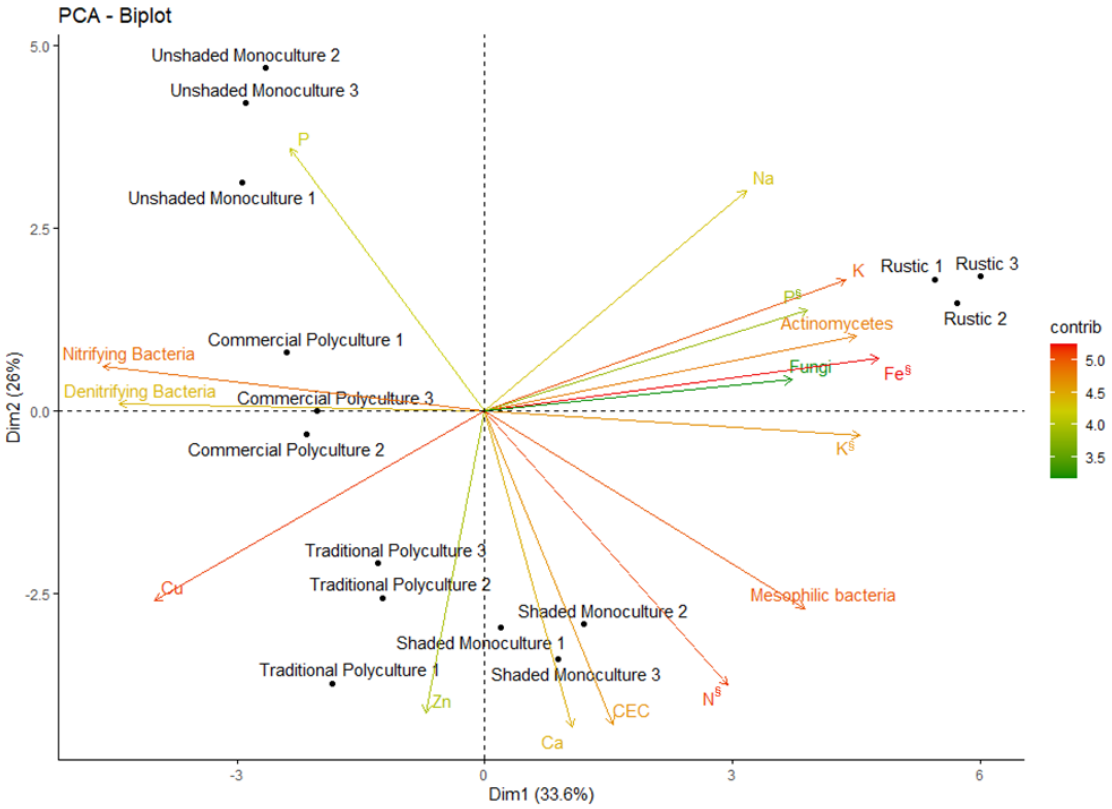

Figure 4. Principal component analysis (PCA) for leaf nutrients, physical, chemical and biological properties of soil. §: Nutrients in *Coffea* spp leaf. CEC: Cation exchange capacity.

In the cultivation system of *Coffea* spp rustic, it is influenced by Fe (leaf) > K (soil) > actinomycetes > K (leaf) > Na > P (leaf) > fungus. Shaded monoculture showed a relationship with the variables of N (leaf) > mesophilic bacteria > CEC > Ca. Commercial polyculture and unshaded monoculture

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systems presented a relationship with nitrifying bacteria > denitrifying bacteria > P (soil), and traditional polyculture was only related to Cu > Zn.

For the *Coffea* spp rustic cultivation system, the variables with the most significant contribution are actinomycetes (8.10%), fungi (5.55%), mesophilic bacteria (5.99%), and macroelements such as K (7.63%). Furthermore, this system favors the assimilation of nutrients such as K (8.27%), P (6.11%), and Fe (9.11%) in the leaf.

The shaded monoculture of *Coffea* spp is influenced by the CEC (9.50%) due to the amount of Ca (9.66%) found in the soil. It also favors the availability of nutrients such as N (7.28 %).

In traditional polyculture, the micronutrients Cu (6.39%) and Zn (8.82%) are found in a higher proportion and present a negative relationship with Na (4.70%), which may mean competition for Cu.

The commercial polyculture and unshaded monoculture systems presented similar behavior. The high participation of nitrifying (8.57%) and denitrifying (7.82%) bacterial populations related to the loss of N and availability of P (6.70%) in the soil. Fungi and actinomycetes compete for nutrients, and Fe and K are unavailable for *Coffea* spp plants.

4. Discussion

The conservation of the *Coffea* spp crop in Mexico is susceptible since it suffers from diseases (rust, cercosporiosis, phoma leaf spot, and bacterial blight) and pests [22]. The different cultivation systems, including rustic, unshaded monoculture, shaded monoculture, commercial polyculture, and traditional polyculture, have negatively affected the harvesting of coffee cherries [3]. Some factors, such as climate, soil fertility, plant nutrition, diseases and pests, can reduce the crop yield and grain quality of *Coffea* spp [23].

There are reports of soil's physical and chemical characteristics in relation to plant nutrient deficiency, such as the availability of organic matter, water, texture and pH, to mention a few [24,25]. Nutrients are essential in several metabolic pathways that involve defense mechanisms [26], so it is essential to quantify soil nutrients in the study area.

Soil health results from the interactions between physical, chemical, and biological properties that determine its function [27,28]. In the cultivation of *Coffea* spp, the nutrients that are most demanded are N and K, followed by Ca, P, Mg, S, Fe, Mn, Zn, Cu, and B [29] (pp. 45-47). However, the nutritional requirements of the *Coffea* spp crop can change depending on factors such as variety, yield, plant age, and crop management [30].

In the study site, the rustic cropping system presented high amounts of K in the soil (0.76 cmol kg^{-1}), considered high according to the Official Mexican Standard NOM-021-RECNAT-2001 (>0.6 cmol kg^{-1}) [16] (p. 35) and the recommended dose of the culture (> 0.4 cmol kg^{-1}) [31]. There is high participation of actinomycete populations (0.83 x10^6 CFU g^{-1} soil), fungi (0.59 x10^6 CFU g^{-1} soil) and mesophilic bacteria (30.16 x10^6 cells g^{-1} soil) [32] involved in the mobilization of K towards the plant (17297.22 mg kg^{-1}) for resistance processes against fungal diseases and photosynthesis [33,34]. The amounts of K in the rustic cropping system are in the optimal production range (15800-21499.99 mg kg^{-1}) [29] (p. 175).

On the other hand, this rustic system presented the most significant amount of assimilable P (660.01 mg kg^{-1}). However, the P is below the optimal range of the crop (1400 -2000 mg kg^{-1}), so the plant is deficient and can negatively affect yield [29,35] (p. 175). The rustic system also presented the highest concentration of Fe (271.24 mg kg^{-1}) in the leaf, which is involved in photosynthesis processes in productive stage crops [37] for an optimal range of 54-121 mg kg^{-1} [29] (p. 175). Therefore, it can be inferred that rustic system, the soil´s acidity favors the assimilation of Fe.

Other quantified micronutrients, such as Cu, are present in all cropping systems in amounts of 1.05 to 3.45 mg kg^{-1}, suitable for this soil type (1.0–3.0 mg kg^{-1}) [16,31] (p. 40). Cu can be related to plant respiration and photosynthesis, carbohydrate and N metabolism, antioxidant activity and lignification processes [36]. The presence of Na and K in the soil compete with Cu in the edaphic system of the *Coffea* spp.

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The CEC can affect the availability of nutrients in the soil for the plant since it influences the capacity of the soil to retain cations such as Ca, as observed in the shaded monoculture system, where the value of the CEC (18.40 cmol kg^{-1}) is considered medium (15-25 cmol kg^{-1}) [16,31] (p. 35). Also, this cultivation system presented the highest concentration of N by the plant (2.98%), which is considered high according to the recommended dose of the crop (2.36-2.78%) [29] (p.175).

In modified cultivation systems (commercial polyculture and unshaded monoculture), P is low in availability for mesophilic bacterial populations and in the cultivation of *Coffea* spp. N loss is also due to nitrification and denitrification processes documented in other intensive farming systems [38]. P in all cultivation systems is considered deficient according to the recommended dose for *Coffea* spp (10 mg kg^{-1}) [31].

Small producers of *Coffea* spp present the need to change crop management to diversify their production and ensure food sovereignty [39]. Carrying out this diversification under agroecological principles can reduce the use of inputs, increase crop yields, promote sustainability, and conserve biodiversity and soil health [40,41]. However, diversified agroecosystems such as the rustic system do not imply that it is the most productive. There is compensation for its ecosystem services, such as preserving soil health and biodiversity, CO_2 capture, improving the landscape, resilience to climate change, water conservation, and maintenance of biogeochemical cycles, mainly [40,42,43].

5. Conclusions

The different cultivation systems of *Coffea* spp in a cooperative society in Puebla, Mexico, indicated that the rustic cultivation system stands out for conserving soil health due to its relationship with different properties such as the availability of K, Fe, P, and the participation of different microbial populations (mesophilic bacteria, fungi and actinomycetes) that influence the maintenance of the culture of *Coffea* spp. The rustic system presented low participation of bacterial populations in denitrification processes. This can be attributed to the rustic system's minimal intervention in the forest ecosystem. Only *Coffea* spp is introduced, and the rest of the plant layer is preserved in its entirety. This minimal modification allows the conservation of soil health and the fulfillment of its functions in the ecosystem, such as diversification of production, resilience against external factors, and maintaining long-term soil fertility. In addition, by guaranteeing the health of the soil, benefits can be obtained in the plant's nutritional status.

The rest of the coffee system (traditional polyculture, commercial polyculture, unshaded monoculture and shaded monoculture) presented a K deficiency mainly in the soil.

Systems conserved under agroecological management can become a sustainable ecosystem for small producers of *Coffea* spp in third-world countries.

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