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Indicadores de calidad del suelo en cafetales con aplicación de técnicas de agricultura ecológica en San Francisco, Cundinamarca, Colombia

Soil quality indicators in coffee plantations managed with ecological farming techniques in San Francisco, Cundinamarca, Colombia

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Abstract

Ecological farming (EF) is a sustainable agricultural production system that promotes customized technologies tailored to the specific needs of each agroecosystem. By optimizing available resources, EF aims to enhance the quality of cultivated soil. The objective of this research was to assess the impact of scheduled EF techniques, including biofertilizers, liquid and solid humus, effective microorganisms, phosphites, composted chicken manure, polycultures, and green fertilizers, on soil properties in coffee plantations of varying ages in San Francisco, Cundinamarca, Colombia. Samples were collected from coffee plantations of different ages (less than one year old, two, and four years) bimonthly over a 10-month period. Various soil quality indicators were evaluated, including physicochemical parameters (pH, gravimetric moisture, cation exchange capacity (CEC), organic carbon (OC), total nitrogen, and available phosphorus), abundance of functional groups of microorganisms (phosphate solubilizers, cellulolytics, and nitrogen fixers), and enzymatic activities (urease, protease, acid and alkaline phosphatase, and β-glucosidase). The results showed significant changes in soil quality indicators as a result of the application of EF techniques. These changes included a notable increase in carbon (from 9.0 % to 17.4 %), nitrogen (from 0.4 % to 0.9 %), and phosphorus content (from 9.3 to 40.4 ppm), as well as a substantial increase in the abundance of phosphate-solubilizing fungi (from 6.0 to 9.0 CFU/g) and cellulolytic fungi (from 5.6 to 9.2 CFU/g). Conversely, significant decreases were observed in soil pH (from 5.5 to 5.1) and β-glucosidase activity (from 55.8 to 10.7 μg N g^{-1} dm $2h^{-1}$), indicating shifts in nutrient dynamics and biological processes in response to ecological management.

Keywords: Enzymatic activity, functional groups of microorganisms, soil health, soil macronutrients.

Resumen

La agricultura ecológica (EF) es un sistema de producción agrícola sostenible que promueve tecnologías personalizadas adaptadas a las necesidades específicas de cada agroecosistema. Mediante la optimización de los recursos disponibles, la agricultura ecológica pretende mejorar la calidad del suelo cultivado. El objetivo de esta investigación fue evaluar el impacto de técnicas programadas de agricultura ecológica (incluyendo biofertilizantes, humus líquido y sólido, microorganismos efectivos, fosfitos, gallinaza compostada, policultivos y abonos verdes) en las características del suelo en cafetales de diferentes edades en San Francisco, Cundinamarca, Colombia. Se recolectaron bimensualmente muestras de cafetales de diferentes edades (menos de un año, dos y cuatro años), durante un período de 10 meses. Con base en estas muestras, se evaluaron varios indicadores de calidad del suelo, incluyendo parámetros fisicoquímicos (pH, humedad gravimétrica, capacidad de intercambio catiónico (CIC), carbono orgánico, nitrógeno total y fósforo disponible), abundancia de grupos funcionales de microorganismos (solubilizadores de fosfato, celulolíticos y fijadores de nitrógeno) y actividades enzimáticas (ureasa, proteasa, fosfatasa ácida y alcalina y β-glucosidasa). Los resultados evidenciaron cambios significativos en el comportamiento de los indicadores de calidad del suelo tras la aplicación de las técnicas de agricultura ecológica. Estos cambios incluyen un aumento notable en el contenido de carbono (de 9.0 % a 17.4 %,) nitrógeno (de 0.4 % a 0.9 %) y fósforo (de 9.3 a 40.4 ppm), así como un incremento considerable en la abundancia de hongos solubilizadores de fosfato (de 6.0 a 9.0 UFC/g) y hongos celulolíticos (de 5.6 a 9.2 UFC/g). En contraste, se observaron disminuciones significativas en el pH del suelo (de 5.5 a 5.1) y en la actividad de la enzima β-glucosidasa (de 55.8 a 10.7 μg N · g^{-1} dm · $2h^{-1}$), lo cual indica un cambio en la dinámica de nutrientes y los procesos biológicos en respuesta al manejo ecológico.

Palabras claves: actividad enzimática, grupos funcionales de microorganismos, macronutrientes del suelo, salud del suelo.

Introduction

Conventional agriculture has become a major contributing factor to the current environmental crisis due to its reliance on monoculture and high-input techniques promoted by the capitalist system. These practices have led to the degradation of natural resources (Chávez-Caiza and Burbano-Rodríguez, 2021) and have had significant negative social and economic consequences (Oteros-Rozas *et al.*, 2019). A clear example is the Green Revolution, which aimed to standardize and increase agricultural production but ultimately resulted in reduced energy efficiency, a decline in soil productivity, and increased dependence on agrochemicals (Chávez-Caiza and Burbano-Rodríguez, 2021). In response to these outcomes, many farmers are actively seeking sustainable alternatives. Therefore, it is crucial to develop and evaluate ecological agricultural techniques that enhance productivity without the adverse effects associated with conventional practices.

In this regard, ecological farming (EF) offers an alternative to conventional agriculture by prioritizing biodiversity conservation and promoting natural renewal processes and ecosystem services to enhance and regulate agricultural production (Chávez-Caiza and Burbano-Rodríguez, 2021). EF is a comprehensive science that draws knowledge from multiple disciplines; however, it still faces challenges, including social and political issues associated with this model (Domené-Painenao and Herrera, 2022). In this context, Moneva Roca (2020) highlights the potential and responsibility of agroecology in promoting sustainable agriculture to meet the Sustainable Development Goals proposed by the UN. However, further research is required to examine the interaction between EF techniques and various components of agroecosystems, as well as their effects on soil and quality indicators.

Soil quality indicators provide information about changes in soil properties resulting from its manipulation and management. These indicators are not only essential to conserve natural ecosystems but also provide valuable information on the state of soil, as well as the impact of land use on biodiversity (Basantes Cárdenas and Lemache Rivera, 2023). Soil is a complex, living, heterogeneous, and dynamic system composed of physical, chemical, and biological components, along with their interactions (Ortega Rodríguez, 2023). Therefore, it is necessary not only to analyze the physicochemical properties of the soil but also to evaluate the abundance of microorganisms and the enzymatic activities involved in the cycles of the three primary macronutrients essential for plant growth: carbon, nitrogen, and phosphorus (C, N, and P, respectively).

Physical indicators are properties associated with the size, arrangement, and organization of soil particles, while chemical indicators reflect the chemical conditions that affect soil-plant interactions, water quality, buffering capacity, and the availability of water and nutrients for both plants and microorganisms (Nuñez-Peñaloza *et al.*, 2023). The abundance of microorganisms influences nutrient availability in the soil, as they contribute to the energy flow within this living system. Furthermore, microorganisms serve as integral indicators of soil health and respond rapidly to changes in soil management due to their adaptability to environmental conditions (Tajik *et al.*, 2020). Enzymatic activity plays a fundamental biochemical role in organic matter decomposition in soil, since it is closely related to physical, chemical, and biological properties of soil and is essential for catalyzing reactions necessary for the life microorganisms, stabilizing soil structure, and contributing to the biogeochemical cycling of nutrients (C, N, and P) (Vélez *et al.*, 2024).

Research on EF plays a pivotal role in promoting and expanding sustainable agriculture (Intini *et al.*, 2019), while also advancing knowledge and generating results through a holistic approach (Domené-Painenao and Herrera, 2022), which requires a two-way learning process. Therefore, considering the interest of farmers from San Francisco, Cundinamarca (Colombia), in more sustainable agriculture techniques, particularly regarding coffee production—the region's primary agricultural product (Rocha Aldana, 2017)—, this study aims to evaluate the response of soil quality indicators (pH, gravimetric moisture, cation exchange capacity (CEC), organic carbon (OC) content, total nitrogen, and available phosphorus) associated with the C, N, and P cycles, before and during the implementation of EF techniques tailored to the needs and conditions of existing coffee plantations of varying ages. Based on this premise, the working hypothesis is that the scheduled implementation of ecological farming practices induces statistically significant changes in soil quality indicators — including physicochemical properties, microbial functional groups, and enzymatic activity— in coffee plantations of different ages in the region of San Francisco in Cundinamarca.

Materials and methods

Description of the research location

The study was conducted in the municipality of San Francisco, located in northwestern Cundinamarca, in the central region of Colombia. The municipality is situated on the western slope of the Eastern Range of the Andes, with its main town located at coordinates 04°58'38'' N, 74°1'32'' W, at an elevation of 1500 m a. s. l. It is a tropical region, whose climate is determined mainly by variations in altitude, the topography of the landforms, and two rainy and two dry seasons interspersed throughout the year: one rainfall period from February to May, and a second one of more intense rainfall between September and November (Rocha Aldana, 2017).

According to IGAC (2017), the studied soils are classified as Humic Dystrudepts and Typic Hapludands, which, according to Rodríguez Albarracín (2017), are associated with mountain soils characterized by crest-type landforms and intercalations of classic silty clay rocks and carbonates in some areas, along with volcanic ash deposits, variable parent material, and differing pH levels.

Experimental design and soil sampling

Soil samples were taken bimonthly (at months zero, two, four, six, eight, and ten) from three coffee plantations of different ages: a recently established coffee plantation less than one year old (referred to as C0A), a two-year-old plantation (C2A), and a four-year-old plantation (C4A). The same ecological farming techniques were uniformly applied to all plots, with plantation age serving as the basis for analysis over a ten-month observation and application period.

Three samples were drawn from each plot, each composed of 12 soil sub-samples taken in a zigzag pattern at a depth of 0-0.20 m—a layer corresponding to the zone with the highest concentration of active coffee roots and the most intense biological and chemical activity. Samples were then homogenized, labeled, and stored at different temperatures according to their intended analysis: samples for physicochemical analysis were kept at room temperature, those for microorganism abundance at 4 °C, and those for enzymatic activity at -20 °C. The samples were processed within 24 hours after collection. Altogether, there were three soil samples for every land plot (C0A, C2A, and C4A), over six sampling times (zero, two, four, six, eight, and ten months), for a total of 54 soil samples.

The ecological farming techniques applied during the study were: 1. solid humus produced through composting with Californian red worms (*Eisenia foetida*) in plastic bins, using organic waste generated on the farm (Román *et al.*, 2013); 2. liquid humus generated from the same process as the previous one; 3. biopreparation of effective microorganisms applied in solid form by mixing nine parts of soil with one part of the solid preparation: leaf litter and soil collected from the surroundings of the farm and mixed with rice semolina, water and molasses, fermented in an airtight container for one month (Tencio, 2015); 4. composted chicken manure acquired in the local market; 5. phosphites (bone meal) acquired in the local market; 6. liquid biofertilizer (biol) prepared with chicha, cow dung, wood ash, milk, water, green leaves, yeast, and molasses, following the proportions indicated in FONCODES (2014); 7. corn (*Zea mays*), beans (*Phaseolus vulgaris*), and yucca (*Manihot esculenta*), which were also sown among the coffee plants to promote polyculture and functional diversification; and 8. red clover (*Trifolium pratense*) seeds sown randomly to serve as green manure.

Table 1 summarizes the periods during which each technique was applied, corresponding to the sampling months. All techniques were applied simultaneously and uniformly across the plots. The species sown around the coffee crops, intended to accompany the main crop and improve soil conditions, remained in place throughout the study period. Inputs were applied superficially through manual incorporation.

Physicochemical soil parameters

The physicochemical parameters evaluated in the samples included the following, according to IGAC (2006): pH in water (1:1 soil-to-water ratio) measured by the potentiometric method (Bernal Figueroa and Forero Ulloa, 2014); gravimetric moisture content determined by the gravimetric method (Shukla *et al.*, 2014); cation exchange capacity (CEC) assessed by the ammonium acetate 1N and neutral method;

Table 1. Ecological agriculture techniques applied per month during the study

Month	EF technique	Sampling
0	Initial sampling After sampling seeds were sown randomly to serve as green manure (*Trifolium pratense*) Polyculture sowing (*Zea mays, Phaseolus vulgaris, Manihot esculenta*)	Yes
1	Solid humus Composted chicken manure	No
2	Liquid humus Solid humus Liquid humus	Yes
3	Composted chicken manure Biopreparation of effective microorganisms Liquid humus	No
4	Biopreparation of effective microorganisms Composted chicken manure Solid humus	Yes
5	Liquid humus Biopreparation of effective microorganisms Composted chicken manure Liquid biofertilizer	No
6	Liquid humus Solid humus Phosphites	Yes
7	Liquid biofertilizer Composted chicken manure Liquid humus	No
8	Biopreparation of effective microorganisms Phosphites	Yes
9	Liquid biofertilizer	No
10	Phosphites	Yes

organic carbon quantified by wet digestion using the Walkley-Black method (Vela *et al.*, 2012); total nitrogen measured by the modified Kjeldahl method (Jarquín-Sánchez *et al.*, 2011); and available phosphorus determined by the modified Bray II method (García Galvis and Ballesteros, 2006).

Abundance of microorganisms

To evaluate the abundance of microorganisms associated to the C, N, and P cycles, the sowing and plate counting methods were applied on different media types, according to Fernández *et al.* (2020). To quantify colony-forming units (CFU) of phosphate-solubilizing bacteria and fungi, researchers followed an adapted version of Sundara and Sinha's method (1963), incubating bacterial cultures at 28 °C for 40 hours and fungal cultures at 20 °C for six days. The count of cellulolytic microorganisms (bacteria and fungi) was conducted using a medium with 1 % carboxymethylcellulose as the sole carbon source (Avellaneda-Torres *et al.*, 2014, with bacteria incubated at 28 °C for 48 hours and fungi at 20 °C for six days. Finally, a modified version of Rennie's (1981) selective medium lacking nitrogen was used to evaluate nitrogen-fixing bacterial populations, incubated at 28 °C for 48 hours (Avellaneda-Torres *et al.*, 2020).

Enzymatic activity

To determine the enzymatic activity, enzymes associated with the C, N, and P cycles were analyzed. For those related to nitrogen, urease and protease activities were evaluated. Urease activity was measured by the colorimetric determination of ammonia released from samples incubated with urea solution at 37 °C for two hours. Protease activity was assessed using casein as the substrate, with soil samples incubated at 50 °C and pH 8.1 for two hours. For phosphorus-related enzymes (acid and alkaline phosphatase), activity was measured by determining the amount of p-nitrophenol released after the incubation of soil samples with a p-nitrophenyl phosphate solution at 37 °C for one hour. Finally, β-Glucosidase activity, corresponding to carbon-related enzymes, was assessed by measuring the p-nitrophenol released after incubation of soil with a p-nitrophenyl glycoside solution under the same conditions (37 °C for one hour). All procedures followed the methodology described by Avellaneda-Torres *et al.* (2018).

Statistical analysis

The results obtained for each parameter were subjected both to univariate and multivariate statistical analyses to compare values and evaluate their evolution over the ten-month observation period. These tests were used to evaluate whether the hypotheses derived from the research questions were supported. As part of the analysis, the assumptions of normality and homogeneity of variances were verified. The Kolmogorov-Smirnov test was used to assess normality, while Bartlett's test was used to evaluate homogeneity of variances. To determine significant differences among treatments, Kruskal-Wallis tests were applied, followed by post-hoc analysis using the Mann-Whitney test. All statistical tests were performed using the stats package in R, and the Principal Component Analysis (PCA) was conducted using the factoextra package (Kassambara, 2017). All analyses were performed using R statistical software, version 3.5.3. (R Core Team, 2021).

Results and discussion

According to Núñez *et al.* (2021), the dynamic and interactive nature of soil processes prevents a single, direct relationship between functions and indicators, as multiple properties can influence several attributes simultaneously. Consequently, each EF technique used, despite having a specific function, can impact multiple soil indicators. These variations provide a comprehensive perspective on soil development, which is essential for understanding its appropriate management.

This research aimed to determine whether the application of EF techniques significantly affects soil quality. Each parameter was analyzed using univariate and multivariate statistical methods, including PCA, to identify tendencies and statistically significant changes ($p<0.05$). Significant differences are denoted by superscript letters in the tables and explained in the corresponding footnotes.

The results from Table 2 show increases in organic carbon (OC), total nitrogen (N), and available phosphorus (P) throughout then ten months of EF-technique application. Organic carbon increased progressively in all three plantations (in C0A from 4.6 % to 9.0 %, in C2A from 9.0 % to 17.4 %, and in C4A from 10.3 % to 17.1 %). Similarly, total nitrogen increased from 0.3 % to 0.5 % in C0A, from 0.4 % to 0.9 % in C2A, and from 0.5 % to 0.9 % in C4A. Available phosphorus also showed significant increases: from 22.1 ppm to 47.5 ppm in C04, from 9.3 ppm to 40.4 ppm in C2A, and from 17.8 ppm to 45.7 ppm in C4A. All increases were statistically significant and may be attributed to fertilization with organic amendments, which is consistent with the findings of Yan *et al.* (2018). Additionally, the initial values of OC show that older coffee plantations tend to have higher OC content.

When organic fertilizers are used regularly, there is greater availability of macronutrients such as nitrogen and phosphorus due to the medium and long-term increase in the content of organic matter (OM), which positively affects nutrient availability in the soil

(Velasco Orea *et al.*, 2023). Approximately 98 % of the N present in soil originates from organic compounds; therefore, the nitrogen content is closely linked to the soil's OM content (Quisimalin *et al.*, 2024).

In line with this, a comparison of the initial and final nitrogen content in the soils revealed significant increases across all coffee plantations, despite some fluctuations over time, which may be related to seasonal climate dynamics in the study place. During periods with higher precipitation, such as in September (month 6), nitrogen losses due to leaching are more likely, particularly in younger coffee plantations where root systems are less developed. Mastrocicco *et al.* (2019) demonstrated that intense rainfall events significantly increase nitrogen leaching, particularly in soils with limited plant uptake. This may explain the temporary decreases in total nitrogen observed in this study, followed by recoveries during drier or more moderate rainfall periods, when mineralization of organic matter resumes and nutrient retention improves.

The addition of OM may increase plants' phosphorus uptake by decreasing the apparent density and increasing the porosity, which in turn enhances root development and their nutrient intake efficiency. This may also contribute to the observed changes in available phosphorus, which showed a significant increase across all coffee plantations as the application of EF techniques went on, with similar behavior observed regardless of plantation age. In this sense, Guillén *et al.* (2020) explain that the organic phosphorus pool in soil increases due to OM, and some compounds can mineralize during their decomposition, having a significant influence on the availability of P in soil for future crops.

Regarding macronutrients, Álvarez Moreno (2019) affirms that there is a positive correlation between nitrogen and phosphorus, where nitrogen may play a critical role in the assimilation of phosphorus, enhancing plant absorption capacity. This fact may explain the increase in the values of these two macronutrients in the present study.

The physicochemical indicators also made it possible to compare the three coffee plantations of different ages. This comparison revealed that both OC and CEC levels were generally higher in older plantations, confirming the findings of Calixto and Kheffinir (2014), who reported a positive correlation between soil development and the longevity of perennial crops. In this study, the average CEC in the youngest plantation (C0A) was 27.6 cmol(c)·kg^{-1}, while values in older plantations reached 42.0 cmol(c)·kg^{-1} (in C2A), and 45.5 cmol(c)·kg^{-1} (in C4A). Similary, OC values in C0A peaked at 9.0 %, whereas they reached 17.4 % and 17.1 % in C2A and C4A, respectively. These results highlight the relationship between plantation age and the accumulation of organic matter and key soil fertility indicators.

In contrast, pH values showed a statistically significant decrease in all plots (from 6.7 to 5.7 in C0A, from 5.5 to 5.1 in C2A, and from 6.4 to 5.9 in C4A) throughout the months of application of the EF techniques. Beltrán (2024) states that the high content of OM in soils leads to greater presence

Table 2. Physicochemical parameters for the three coffee plantations

CP	Month	pH	θg	CEC	OC	Nitrogen	Phosphorus
C0A	0	6.7a	30.3ac	27.0ac	4.6a	0.3a	22.1a
	2	5.9b	46.9b	35.0b	4.8a	0.5b	21.9a
	4	5.8c	27.4c	28.1c	5.6b	0.7b	31.5b
	6	5.7c	32.3a	22.2d	5.6b	0.3a	34.4c
	8	5.3d	48.9b	25.6a	7.7c	0.6b	34.8c
	10	5.7c	29.7ac	25.6a	9.0d	0.5b	47.5d
C2A	0	5.5a	49.0ab	35.5c	9.0a	0.4a	9.3a
	2	5.5a	67.1c	39.3ab	7.9b	0.9b	10.9a
	4	5.6b	32.2b	38.6ab	9.6c	0.7c	4.3b
	6	5.5ab	45.7a	42.0b	8.6ab	0.4a	20.3c
	8	5.8c	88.1d	41.8ab	11.4d	1.0b	42.1d
	10	5.1d	38.9ab	38.0a	17.4e	0.9bc	40.4e
C4A	0	6.4c	44.6ab	45.4a	10.3a	0.5a	17.8c
	2	6.0d	81.0c	32.5d	11.9a	0.8b	30.1a
	4	5.9a	40.3b	45.5ab	12.7a	0.4c	19.4d
	6	5.8b	56.9a	38.3c	13.5a	1.3d	27.8b
	8	5.9ab	65.6d	37.9c	13.6a	0.4ac	28.6ab
	10	5.9ab	54.1a	48.0b	17.1a	0.9b	45.7e

*Within each property, non-matching letters denote significant statistical differences (*p*>0.05).

θg: gravimetric humidity; CEC: cation exchange capacity (in cmol(c)·kg^{-1}); OC: organic carbon (in %); N: nitrogen (in %); P: phosphorus (in ppm).

of carboxyl groups, which increases the density of deionized sites and promotes the release of H+ ions. This release of hydronium ions from decomposing OM causes a decrease in pH, therefore, the decrease in pH values observed in this study can be attributed to the application of organic fertilizers. This can be seen in the PCA, which demonstrates an inverse relationship between soil pH and the levels of OC, N, and P. Also, when comparing the results with the literature, it is observed that the initial pH values were above the optimal range recommended for coffee cultivation, which, according to Martinez *et al.* (2019), range between 5.0 and 5.4. Throughout the period of EF technique application, soil tended to move toward this optimal range.

With regard to the biological indicators (Table 3), the results for phosphate-solubilizing bacteria in the soils did not show a consistent trend, and no significant differences were observed between the initial and tenth month of EF technique application. However, phosphate-solubilizing fungi (PSF) increased from 6.0 to 9.0 log CFU/g in C2A, and cellulolytic fungi (CF) increased from 5.6 to 9.2 log CFU/g in C4A; both increases were statistically significant. This finding aligns with the research conducted by Fernández *et al.* (2020), who established a correlation between the abundance of cellulolytic fungi and the application of organic matter (OM). Additionally, the increases in phosphate-solubilizing fungi support the findings of Rahmann *et al.* (2017), who demonstrated that the use of organic inputs positively stimulates microbial activity and promotes the mobilization of phosphorus in the soil. The authors further argue that EF techniques enhance various indicators of the biological phosphorus cycle, and that the incorporation of OM, along with the preservation of microorganisms associated with this macronutrient, helps prevent phosphorus from remaining in inorganic forms that are inaccessible to plants.

Furthermore, the increase in phosphate-solubilizing fungi may be attributed to decreased pH values, consistent with the findings of Moratto *et al.* (2005), who reported that the strains exhibiting the highest phosphorus solubilization also recorded lower pH values. They concluded that the production of organic acids is the primary mechanism employed by these microorganisms for the solubilization of phosphates, and that pH is inversely related to the amount of phosphorus solubilized by phosphate-solubilizing fungi. On the other hand, cellulolytic bacteria showed no single trend in relation to the time of the technique application; in contrast, cellulolytic fungi showed an increasing statistically significant tendency throughout the months of application of EF techniques, which is corroborated by Fernández *et al.* (2020), who, based on the results of their research, suggest that the application of organic matter and biological preparations favors the proliferation of cellulolytic fungi.

Table 3. Microorganisms count in the three coffee plantations

CP	Month*	PSB	PSF	CB	CF	NB
C0A	0	6.6a	6.4b	6.9b	6.7b	10.0a
	2	6.0b	6.1c	7.3c	8.8a	8.6b
	4	7.9c	8.8a	6.4a	8.9a	7.9c
	6	7.5d	8.7a	5.2d	8.7c	8.7b
	8	7.0e	5.1d	8.3e	9.6d	7.5d
	10	5.7f	9.2e	6.5a	9.8e	10.0a
C2A	0	7.2a	6.0a	6.5b	7.8c	9.7a
	2	5.9b	7.1b	6.3c	5.1d	6.6b
	4	7.8c	8.6c	5.4a	7.7a	7.8c
	6	6.4d	8.7d	6.1a	7.6a	9.5d
	8	4.8e	5.7e	7.8d	9.2b	7.2e
	10	7.4a	9.0f	7.5e	9.5b	9.8f
C4A	0	7.1a	6.0a	7.8c	5.6a	9.8b
	2	5.1b	6.2a	8.1a	5.9b	5.5c
	4	7.7c	7.7b	5.2b	7.3c	7.8d
	6	6.4d	8.6c	5.2b	6.4d	9.5a
	8	7.0a	4.9d	8.0a	9.5e	9.6e
	10	7.0a	8.8e	5.8d	9.2f	9.5a

*Month of application of EF techniques. PSB: phosphate solubilizing bacteria; PSF: phosphate solubilizing fungi; CB: cellulolytic bacteria; CF: cellulolytic fungi; NB: nitrogen fixing bacteria. The units of the microorganism counts are in log (CFU/g). Within each property, non-matching letters denote significant statistical differences (p>0.05)

Regarding the results of the enzymatic activity (Table 4), a statistically significant decrease was observed only in β-glucosidase activity over the ten-month period. In the youngest plantation (C0A), β-G dropped from 55.8 to 10.7 µg PNP · g^ (-1) dm · h^ (-1), while in C2A, it dropped from 40.8 to 10.2 µg PNP · g^ (-1) dm · h^ (-1), and in C4A, from 30.9 to 6.3 µg PNP · g^ (-1) dm · h^ (-1). This trend was statistically significant (p > 0.05) and was further supported by the multivariate analysis (Figure 1), which highlights the relationship between this enzyme and soil pH. These results are consisted with Naranjo Pérez (2019), who stated that soil acidity can influence the enzymatic activity, and that, in general, enzymatic activity increases when soil acidity decreases—that is, as pH increases.

Multivariate analysis

The principal component analysis (PCA) integrates data from all parameters analyzed across the three plots, covering the period from month zero to month ten of EF technique application. The cumulative variance of the PCA is 71.1 % (Figure 1), indicating a low level of stress in the analysis. The PCA shows that, by the last month of application of the EF techniques, there is an increase in the levels of phosphorus, organic carbon, cellulite fungi, and nitrogen. This trend is inversely related to the behavior of soil pH, highlighting significant changes in soil indicators after the application of EF techniques.

Table 4. Enzyme activity in the three coffee plantations

CP	Month	P	U	Ac. P	Ak. P	β-G
C0A	0	4.5ab	2.36ac	82.2a	45.8a	55.8a
	2	1.5b	5.88b	82.2a	139.0b	55.5abcd
	4	1.1abc	2.08ac	36.9ab	42.6ab	2.5c
	6	0.0c	8.84cb	71.2ab	107.8b	5.1bc
	8	7.8a	4.01b	39.8ab	59.1ab	20.1d
	10	8.1a	3.05a	149.7a	79.9ab	10.7b
C2A	0	2.6ab	6.42ab	86.9abc	145.0ac	40.8e
	2	1.4ab	4.00b	346b	38.8bcd	14.3abcd
	4	2.0ab	1.65a	339.6d	472.9e	2.5b
	6	4.9a	8.33ab	179.6ac	132.4c	14.7c
	8	1.5ab	2.93b	30.3b	17.8d	19.4d
	10	0.8b	7.86ab	129.9ac	82.5abc	10.2a
C4A	0	7.3a	5.61c	43.7ab	34.0a	30.9c
	2	1.5b	5.69a	20.1b	22.5a	16.0d
	4	2.0b	3.21d	83.0abc	88.7b	7.5a
	6	1.1b	1.27ab	132.6c	53.4ab	7.1ab
	8	6.3a	1.25b	77.7ac	28.2a	8.8a
	10	6.3a	1.60b	57.5ab	30.2a	6.3b b

P: protease activity (in µg tyr • g^ (-1) dm • 2h^ (-1)); U: urease activity (in µg N • g^ (-1) dm • 2h^ (-1)); Ac. P: acid phosphatase; Ak. P: alkaline phosphatase; β-G: β-Glucosidase. The activity of the last three enzymes is expressed in µg PNP • g^ (-1) dm • h^ (-1). Within each property, non-matching letters denote significant statistical differences (*p*>0.05)

Other inverse relationships were observed between cellulolytic bacteria and gravimetric humidity, and solubilizing bacteria and alkaline phosphatase activity. Direct correlations were found between cellulolytic bacteria and gravimetric humidity, phosphorus and nitrogen, the enzymatic activity of acid phosphatase and urease, and cellulolytic fungi and organic carbon.

From this analysis, it is possible to establish a correlation between the decrease in pH and the increase in cellulolytic fungi. This relationship is reinforced by the fact that fungi have a greater capacity to proliferate at acidic pH levels, whereas bacteria exhibit a lower tolerance to such conditions (pH = 6-7.5). Therefore, they develop better in soils with neutral and basic pH values (Rojas Rivero, 2024). This is also confirmed in Table 2, where initial measurements showed higher bacterial counts than fungi for a functional group; however, over time, fungi became predominant.

Finally, the influence of EF techniques on soil quality is evident, as shown by the significant increases in soil carbon, nitrogen, and phosphorus content, along with the abundance of phosphate-solubilizing and cellulolytic fungi. A notable decrease in the pH and β-glucosidase activity was also observed. While the reduction in pH suggests an environment favorable to certain microbial processes

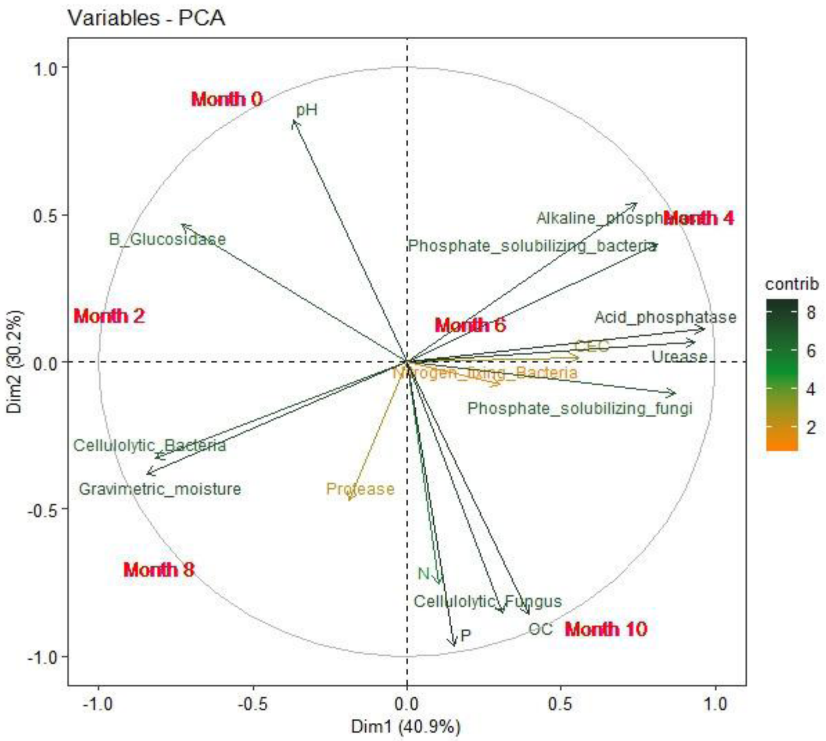

Figure 1. Principal component analysis for the three coffee plantations.
N: nitrogen; P: phosphorus; OC: organic carbon; CEC: cation exchange capacity.

and organic matter mineralization, it is important to note that it could also lead to the loss of essential base cations, potentially affecting long-term soil fertility. These findings highlight how the correct and consistent use of ecological farming resources produces significant changes in soil quality, reflecting a shift in nutrient dynamics due to the replacement of agrochemicals with ecological inputs.

Conclusions

The research findings revealed that the application of EF techniques positively influenced various parameters of agricultural soil quality, including organic carbon, available phosphorus, total nitrogen, and the abundance of phosphate-solubilizing and cellulolytic fungi. Additionally, a significant trend was observed in β-glucosidase activity. These improvements occurred regardless of the age of the coffee plantation, suggesting that EF techniques can offer broad benefits across different stages of crop establishment.

Based on the study, it can be concluded that EF techniques effectively modify microbial and enzymatic activities in the soil. However, predicting their response to different stimuli remains challenging due to the complexity of their nature and their interactions within the soil environment.

In summary, this research provides evidence of the positive impact of EF techniques on agricultural soil quality parameters. The improvements observed in organic carbon, available phosphorus, total nitrogen, and the abundance of phosphate-solubilizing and cellulolytic fungi, as well as the decrease in β-glucosidase activity and soil pH, highlight the complex nature of soil biochemical responses to organic amendments. While microbial abundance and nutrient availability were enhanced, enzymatic activity showed greater variability, reflecting the influence of pH, organic matter quality, and environmental conditions.

This research contributes to the understanding of how EF techniques influence the biological and chemical functioning of agricultural soils, while also emphasizing the complexity of soil systems. It also highlights the need for further investigation to better understand their behavior under varying conditions.

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