AGRONOMY (AGRONOMIA)

Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.18, n.4, e3373, 2023
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v18i4a3373 - Protocol 3373
Subm. 20/07/2023 • App. 14/12/2023 • Pub. 29/12/2023

The effect of ecological farming techniques on nitrogen cycling microorganisms in soils with coffee plantations

Johan Alexander Bonilla Bernal[1], Andrés Felipe Calderón Cifuentes[1], Lizeth Manuela Avellaneda-Torres[2]*,

[1] Universidad Libre, Bogotá, Colombia. E-mail: johana-bonillab@unilibre.edu.co; andresf-calderonc@unilibre.edu.co
[2] Universidad Nacional de Colombia, Facultad de Ciencias, Departamento de Química, Bogotá, Colombia. E-mail: lmavellanedat@unal.edu.co

ABSTRACT: Ecological agriculture promotes an interactive system for the sustainability of soil and agroecosystems, leveraging and optimizing available resources to improve the quality of the cultivated soil. This research aims at evaluating the physical, chemical, and microorganism changes in the nitrogen cycle of the soil after applying ecological farming techniques such as liquid humus, solid humus, efficient microorganisms, biol, poultry manure, phosphites, polyculture stimulation and green manures, in coffee plantations of different ages. For this evaluation, we collected soil samples in plots cultivated with zero-, two-, and four-year-old coffee trees during months zero, four, and ten. We determined the physical and chemical parameters and abundance of microorganisms associated with the nitrogen cycle (ammonifiers (AMO), proteolytic (PRO), ammonium oxidizing (BOA), nitrite oxidizing (BON) and denitrifiers (DEN). The results showed statistically significant changes in soil organic carbon, nitrogen, and phosphorus content as a result of applying ecological farming techniques, which were applied sequentially and evaluated as a set over time. These statistically significant differences occurred in the different months evaluated and in month 10 in contrast to month zero of application of techniques. However, no statistically significant changes were found in the abundance of microorganisms in the nitrogen cycle. In addition, direct relationships were obtained between variables such as P and %OC; pH and DEN; and BON and AMO. It can be concluded that although the application of organic farming techniques improves the physical and chemical properties of soils, there are no statistically significant differences in the abundance of nitrogen cycle microorganisms.

Key words: Ammonifiers; ammonium oxidizers; denitrifiers; nitrite oxidizers; proteolytic enzymes

Efecto de técnicas de agricultura ecológica en microorganismo del ciclo del nitrógeno en suelos bajo cultivo de café

RESUMEN: La agricultura ecológica promueve un sistema interactivo para la sostenibilidad del suelo y los agroecosistemas, aprovechando y optimizando los recursos disponibles para mejorar la calidad del suelo cultivado. La presente investigación tuvo objetivo evaluar los cambios físicos, químicos y en los microorganismos del ciclo del nitrógeno del suelo tras la aplicación de técnicas de agricultura ecológica como humus líquido, humus sólido, microorganismos eficientes, biol, gallinaza, fosfitos, estimulo del policultivos y abonos verdes, en cafetales de diferentes edades. Para dicha evaluación se realizaron muestreos durante los meses cero, cuatro y diez en parcelas de cafetales de cero, dos y cuatro años de siembra. Se determinaron parámetros fisicoquímicos y abundancia de microorganismos asociados al ciclo del nitrógeno (amonificantes (AMO), proteolíticos (PRO), oxidantes de amonio (BOA), oxidantes de nitrito (BON) y desnitrificantes (DEN). Los resultados evidenciaron cambios estadísticamente significativos producto de la aplicación de las técnicas de agricultura ecológica en el contenido de carbono orgánico, nitrógeno y fósforo del suelo. Estas diferencias estadísticamente significativas se dieron en los diferentes meses evaluados y en el mes 10 en contraste con el mes cero de aplicación de las técnicas. No obstante, no se encontraron cambios estadísticamente significativos en la abundancia de los microorganismos del ciclo del nitrógeno. Se obtuvieron relaciones directas entre variables como P y %OC; pH y DEN; BON y AMO. Se puede concluir que si bien la aplicación de técnicas de agricultura orgánica mejora las propiedades físicas y químicas de los suelos, no existen diferencias estadísticamente significativas en la abundancia de microorganismos del ciclo del nitrógeno.

Palabras clave: Amonificantes; oxidadores de amonio; desnitrificantes; oxidadores de nitrito; enzimas proteolíticas

* Lizeth Manuela Avellaneda Torres - E-mail: lmavellanedat@unal.edu.co (Corresponding author)
Associate Editor: Mário de Andrade Lira Júnior

Introduction

Soils have diverse functionalities. One of the most important is food production, mainly through conventional agriculture. Conventional agriculture is understood as the model characterized by the predominance of monoculture, the use of fertilizers with high nutrient potential, a high level of mechanization and intensive use of external agricultural inputs (fertilizers and agrochemicals for pest, disease, and weed control) (Landini & Beramendi, 2020). Green Revolution can be defined as the increase in crop production by using chemically synthesized fertilizers, pesticides and high-yielding crop varieties (Bins, 2020).

Thus, agroecology arises as a response to the environmental impacts generated by the Green Revolution to rebuild and/or strengthen the functional biodiversity of agroecosystems, intending to improve the interactions among its components and achieving a flow of goods and services that is compatible with the interests of current and future generations, through healthy and nutritious food production systems that are economically viable, ecologically adequate, and socially fairer (Sarandón, 2020). Agroecology is used for the application of principles that favor natural processes and biological interactions.

In this context, different agroecological practices appear, such as solid humus, liquid humus, efficient microorganisms, biol, green manures, and polyculture; which promote a change of perspective to preserve a healthy environment (Sailema-Sailema et al., 2022).

These techniques improve the physical, chemical and biological conditions of the soil, hence the importance of nitrogen in plants and soil. As Stein & Klotz (2016) point out, a series of processes develop in the soil that play a fundamental role in the nitrogen cycle, such as: biological fixation, nitrification (oxidation of ammonium to nitrite and nitrate), assimilation, ammonification and denitrification (reduction of nitrate to molecular nitrogen, N2), including the two anaerobic processes (assimilation of nitrate to ammonium and anaerobic oxidation of ammonium). Nitrogen is present in various forms, and is transformed throughout the cycle by the action of ammonifying microorganisms (AMO), proteolytics (PRO), ammonia oxidizing bacteria (BOA), nitrite oxidizing bacteria (BON) and denitrifiers (DEN) (Cañón-Cortázar et al., 2012).

On the other hand, and taking into account the agronomic and economic importance of coffee in Colombia, it is considered a dynamic area in the regions whose income directly impacts the commercial and consumption actions of the municipalities that are dedicated to coffee production (Garzón, 2022). This is the case of Cundinamarca, one of the departments where coffee is cultivated in 69 of the 116 municipalities that comprise it, covering an area of approximately 30,142 ha (Carrero-Cañon, 2021)

It is in this context that this research was proposed, with the purpose of evaluating whether ecological farming techniques generate changes in the functional groups of the microorganisms of the nitrogen cycle (AMO, PRO, BOA, BON, and DEN) in coffee plantations in the municipality of San Francisco, Cundinamarca.

Materials and Methods

Description of the study area

The study area was located in the municipality of San Francisco - Cundinamarca, 55 km from Bogotá, in the province of Gualivá. This municipality on the western side of the Colombian Andes Mountains is located at coordinates 04° 58’ 38’’ N and 74° 1’ 32’’ W, at an altitude of 1500 m above sea level, and is characterized by its tropical climate.

Experimental design and soil sampling

In order to evaluate the microbiological indicators and to establish the physical and chemical parameters of the soils that had been intervened with ecological farming techniques, soil samples were collected during months zero, four, and ten, in three coffee plantations aged zero, two, and four years of seeding. The study had a duration of ten months, in which three soil samples were collected during each month of evaluation. Each sample was composed of nine sub-samples, collected in a zigzag pattern, from the first 20 cm of the soil, obtaining nine soil samples for each month of evaluation. Sampling was carried out during three months (month 0, 4, and 10 of applying ecological farming techniques) obtaining a total of 27 samples. These samples were labeled and stored at different temperatures depending on the analysis to be performed. For physical and chemical parameters analysis, the samples were stored at room temperature, while for analysis of microorganism abundance, they were stored at 4 °C. Samples were processed in the shortest possible time after collection.

Ecological farming techniques applied

The techniques used during the period of application of ecological farming were: 1. Solid humus: the solid organic waste generated on the farm was placed in plastic baskets in order to breed the Californian red worm (*Eisenia foetida*); a process that results in solid humus; 2. Liquid humus: the product of the process mentioned above was collected into plastic baskets to hold the liquid material processed by the worms; 3. Biopreparation of efficient microorganisms: nine parts of soil were mixed with one part of leaf litter and soil preparation from the nearest forest, mixed with rice semolina, water, and molasses. It was left to ferment for one month in an airtight container to ensure the reproduction of efficient microorganisms (Tencio, 2015); 4. Biol: it is made with chicha, cow dung, wood ash, milk, water, green leaves, yeast, and molasses. Everything is placed in a container in a liquid matter medium. The proportions are maintained as established in FONCODES (2014); 5. Use of poultry manure and phosphites obtained from local organic trade; 6. Planting of corn, beans, and yucca among the coffee trees in order to promote polyculture and functional diversification; 7. Green manure: red clover (*Trifolium pratense*) is sown randomly.

Soil physical and chemical parameters

In order to evaluate the physical and chemical parameters, we determined the pH (in water by the potentiometric method), gravimetric moisture (by the gravimetric method), organic carbon (by Walkley-Black), nitrogen (total nitrogen in soils by the modified Kjeldahl method), and phosphorus (available phosphorus in soils by the modified Bray - II method) (IGAC, 2006).

Abundance of functional groups of microorganisms associated with the nitrogen cycle

In furtherance of determining the microorganisms in the nitrogen cycle, we used the most probable number (MPN) method, in which the absence or presence of the microorganisms is estimated by means of a series of dilutions. Each procedure was performed in triplicate, and each replicate used five tubes per dilution, making dilutions from -1 to -10 in order to find the adequate reading dilution. The base to be used for PRO and BOA was Winogradsky's salts solution at a rate of 50 mL L^{-1} and the modified trace element solution $MgCl_2.6H_2O$ (2.5 g), $MnCl_2.4H_2O$ (0.6 g), NaCl (1.0 g), $FeCl_2.4H_2O$ (0.1 g), $CoCl_2.6H_2O$ (0.1 g), $AlCl_3$ (0.01 g), H_3BO_3 (0.01 g), $Na_2MoO_4.2H_2O$ (0.01 g), $CuCl_2.2H_2O$ (0.01 g), $CaCl_2.2H_2O$ (0.1 g), $ZnCl_2$ (0.1 g) at the rate of 1 mL L^{-1} (Flórez-Zapata & Uribe-Vélez, 2011). For PRO, gelatin (30 g L^{-1}) was used as the sole source of C and N with an incubation time of 15 days at 28 °C. For BOA, $CaCO_3$ (1 g L^{-1}) was added as a source of C and $(NH_4)_2SO_4$ (0.5 g L^{-1}) as a source of N, with an incubation time of 30 days at 28 °C. In order to determine BON, we used KNO_2 (0.006 g L^{-1}), K_2HPO_4 (g L^{-1}), $FeSO_4$-$7H_2O$ (0.0 g L^{-1}), NaCl (0.3 g L^{-1}), $MgSO_4.7H_2O$ (0.1 g L^{-1}), $CaCO_3$ (1 g L^{-1}), and $CaCl_2$ (0.3 g L^{-1}), with an incubation time of 4 days at 28 °C. For AMO, bacteriological peptone (2 g L^{-1}) was used as culture medium and sole source of C and N, with an incubation time of 1 day at 30 °C. Finally, a mixture of two stock solutions, A and B, was used as culture medium for DEN, with the following composition: Solution A: KNO_3(1 g L^{-1}), $C_4H_8N_2O_3$ (1 g L^{-1}) and $C_2H_2Br_2O$ - S 1 % (5 mL); and Solution B: $Na_3C_6H_5O_7$ (8. 5 g L^{-1}), KH_2PO_4 (1 g L^{-1}), $MgSO_4$ (1 g L^{-1}), $CaCl_2$- $6H_2O$ (0.2 g L^{-1}), and $FeCl_3$- $6H_2O$ (0.05 g L^{-1}), with an incubation time of 3 days at 28 °C (IGAC, 2006).

The results were interpreted according to the most probable number table (Argüello-Navarro et al., 2016) to determine the colony forming units (CFU g^{-1}).

Statistical analysis

The assumption of homogeneity of variances was verified using the Levene test, and the Bartlett and Fligner-Killeen test. Once this assumption was verified, the Kruskal-Wallis test was performed (R Core Team, 2021). Finally, a principal component analysis (PCA) was carried out in order to characterize the variables and find the different relationships among them, and the relationship they have with the different years and months. The analyses were performed with the statistical software R version 4.1.1 (R Core Team, 2021).

Results

Figure 1 presents the physical and chemical results for soils as a function of the months of application of ecological farming techniques in coffee plantations aged zero, two, and four months old. In the case of pH (Figure 1A), no statistically significant changes were found, so it remained constant during the period of application of ecological farming techniques, regardless of the age of the coffee plantation. Figure 1B corresponds to gravimetric humidity, which does not show significant changes and remains constant as ecological farming techniques are applied. Figure 1C represents the results of organic carbon and shows a statistically significant increase as ecological farming techniques were applied to coffee plantations of the three ages. Figure 1D presents the percentage of nitrogen in the soil, showing changes in coffee plantations aged two and four years old, and the significant increases between months zero and ten of the application of ecological farming techniques. Figure 1E corresponds to the amount in parts per million of phosphorus present in the soil, which shows statistically significant increases as the months of application of the ecological farming techniques for the different coffee plantations elapsed.

The results of abundance of microorganisms in nitrogen cycle during the months of application of ecological farming techniques are shown in Figure 2. There were no statistically significant changes For PRO (Figure 2A) in relation to either the application of ecological farming techniques or the age of the coffee plantations. There is a significant decrease for BOA (Figure 2B) in the coffee trees aged zero and four years old, while in the coffee trees aged two years old, there is an increasing trend during the months of application of ecological farming techniques. There are no statistically significant changes for BON and DEN (Figure 2C and 2D) in relation to either the application of ecological farming techniques or the age of the coffee plantations. No statistically significant changes were observed for AMO (Figure 2E), remaining constant during the application of ecological farming techniques.

Principal components analysis (PCA)

Figure 3 shows the principal components analysis (accumulated variance 60.0 %) for the different variables under study. Among the direct relationships, we found %OC, %N, and P(ppm) with an increase, especially in month 10 of application of ecological farming techniques for the three coffee plantations under study. There was also a relationship between BON and AMO microorganisms, which increased in coffee plantations aged two years old. This is followed by a relationship between pH and DEN microorganisms, which is accentuated in month zero of application of ecological farming techniques. Besides, Figure 3 presents parameters with inverse relationship such as P with BON and AMO.

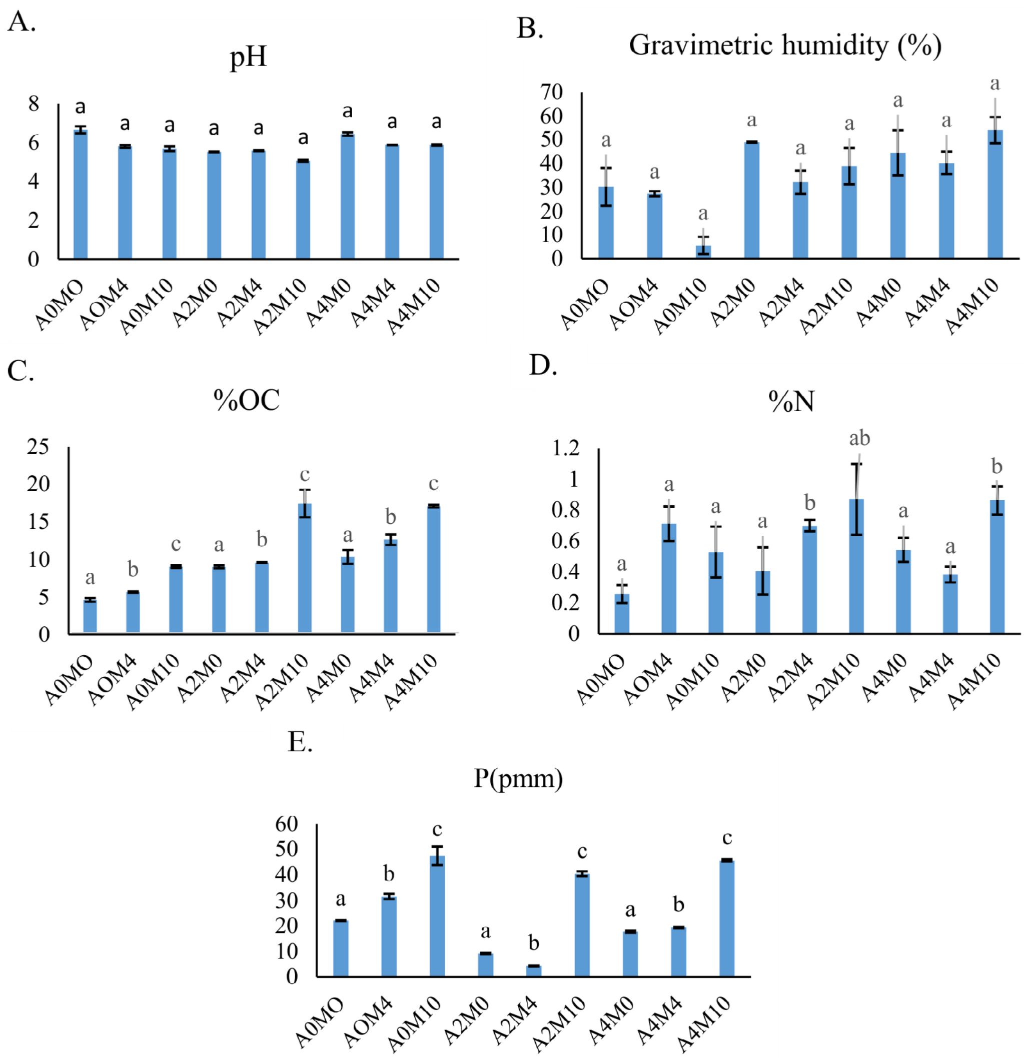

Figure 1. Abundance of physical and chemical parameters in soils under coffee plantations of different ages with the application of ecological farming techniques. On the horizontal axis, the number followed by letter A represents the age of the coffee plantation and the number followed by letter M represents the month of application of ecological farming techniques. Different letters in each parameter show statistically significant differences; equal letters in each parameter show that there were no statistically significant differences.

Discussion

The dynamics of the physical, chemical and biological processes of soils give rise to an endless number of natural interactions in which there is no unique and direct relationship between the functions of the main components, but rather they can occur simultaneously and relevantly depending on the particular attributes of the soil to be studied. This corresponds to an integrated vision of each of the organic farming techniques applied in the different coffee plantations under study. It also shows the evolution or transition in the responses of the physical and chemical parameters and functional groups of microorganisms in the soil nitrogen cycle.

According to the results in the present investigation, the application of ecological farming techniques contributes a considerable amount of ecological matter, which significantly increases the content of C, N, and P, as shown in Figures 1C, 1D, and 1E. In this sense, Martínez et al. (2008) mention that the increase in the percentage of carbon corresponds to the organic fertilizers applied during the study period; and that when ecological farming techniques are used, the availability of macronutrients is higher. In addition, these authors relate this interaction to mineralization in the decomposition stage, which acts as the main source of phosphorus. This source increases porosity levels, contributing to the improvement and effective growth of roots.

Likewise, Trinidad-Santos & Velasco (2016) established the previous relationship with the organic matter content in the medium and long term. Thus, we can see that the older the coffee plantation, the higher the percentage of organic matter (Figure 1C) and phosphorus available in the soil (Figure 1E). When periodic organic fertilizers are used, N levels tend to increase in relation to organic matter and an availability between nitrogen and phosphorus is exposed, which requires a better functioning of the nitrogen cycle

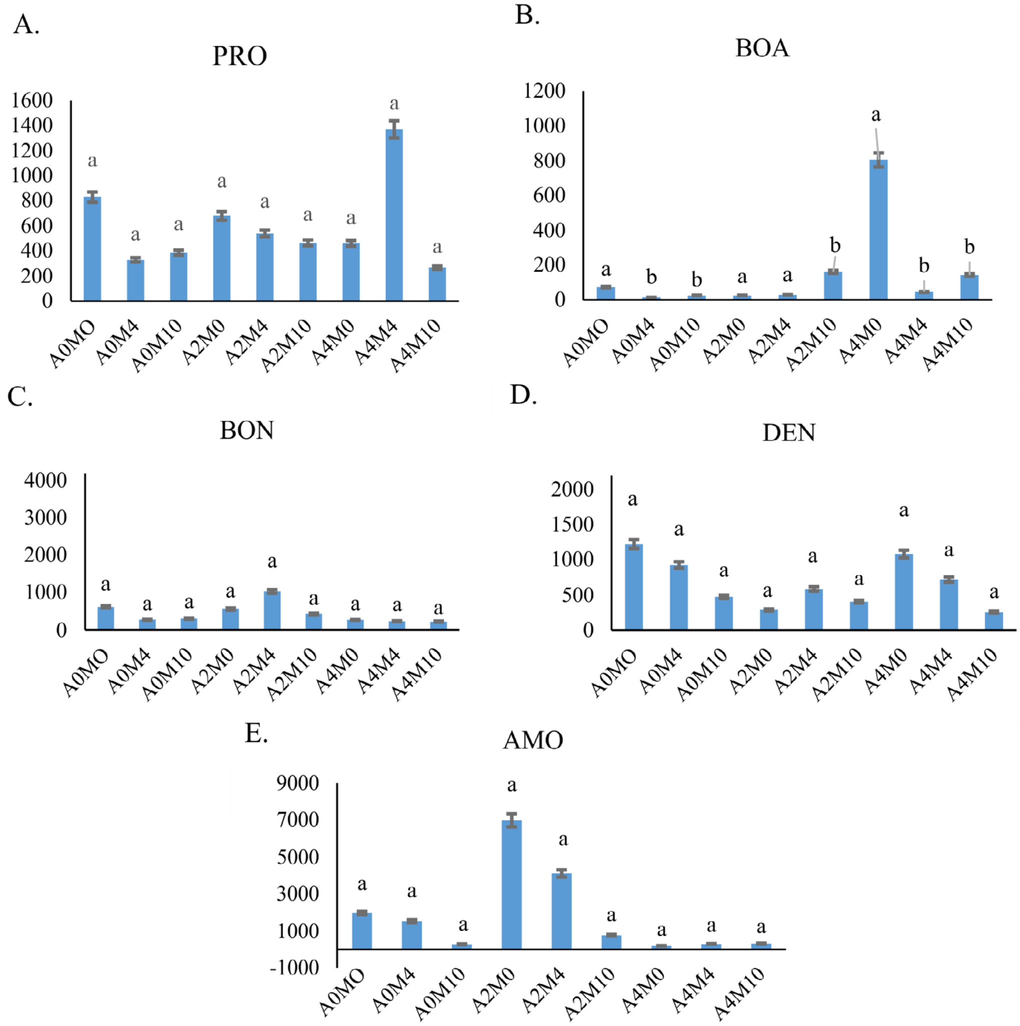

Figure 2. Abundance of microorganisms associated with the nitrogen cycle in soils under coffee plantations of different ages with the application of ecological farming techniques. The number followed by letter A represents the age of the coffee plantation and the number followed by letter M represents the month of application of ecological farming techniques. Different letters in each parameter show statistically significant differences; equal letters in each parameter show that there were no statistically significant differences.

in the soil, due to the organic matter content that favors and increases the mineralization of organic phosphorus, efficiently contributing to the absorption of phosphorus in the plant (Caballero et al., 2018). This relationship can be observed in Figure 3, where the %CO, %N and P obtained a strong relationship with the oldest coffee plantations. Salinas & Hernández (2008) state that this interaction is a fundamental determinant for plant growth; the older a coffee plantation is, the greater its volume, growth, and absorption of the main macronutrients.

On the other hand, Figure 2B shows two changes that take place as a function of ammonia oxidizing bacteria (BOA). These relationships are established as time goes by with the application of ecological farming techniques, first in a characteristic decrease in coffee plantations aged zero and four years old. According to Ferguson (1998), these decreases correspond to low assimilation in the ammonium to the nitrite transformation stage. Additionally, it represents an increase in BOA for the two-year-old coffee plantation, where the nitrite conversion rate is strongly linked to a nitrogen growth curve over time. This interaction is demonstrated in Figure 1D, where we obtained significant increases as the period of application of ecological farming techniques progressed for coffee plantations aged two years old. This establishes the need for longer sampling times to observe long-term behavior; however, these decreases and increases had relationships to demonstrate what Ferguson (1998) tells us about the timing of application of ecological farming techniques, and that ammonium to nitrite conversions should be studied further over a medium to long term period to observe the abundance of such microorganisms.

Figure 3 shows a direct relationship between denitrifying microorganisms (DEN) and pH during month zero of application of organic farming techniques. This behavior is due to the fact that pH is a fundamental factor in the abundance of DEN microorganisms. According to Chen et al. (2023), the DEN community are more likely to inhabit environments with neutral and alkaline conditions, while the

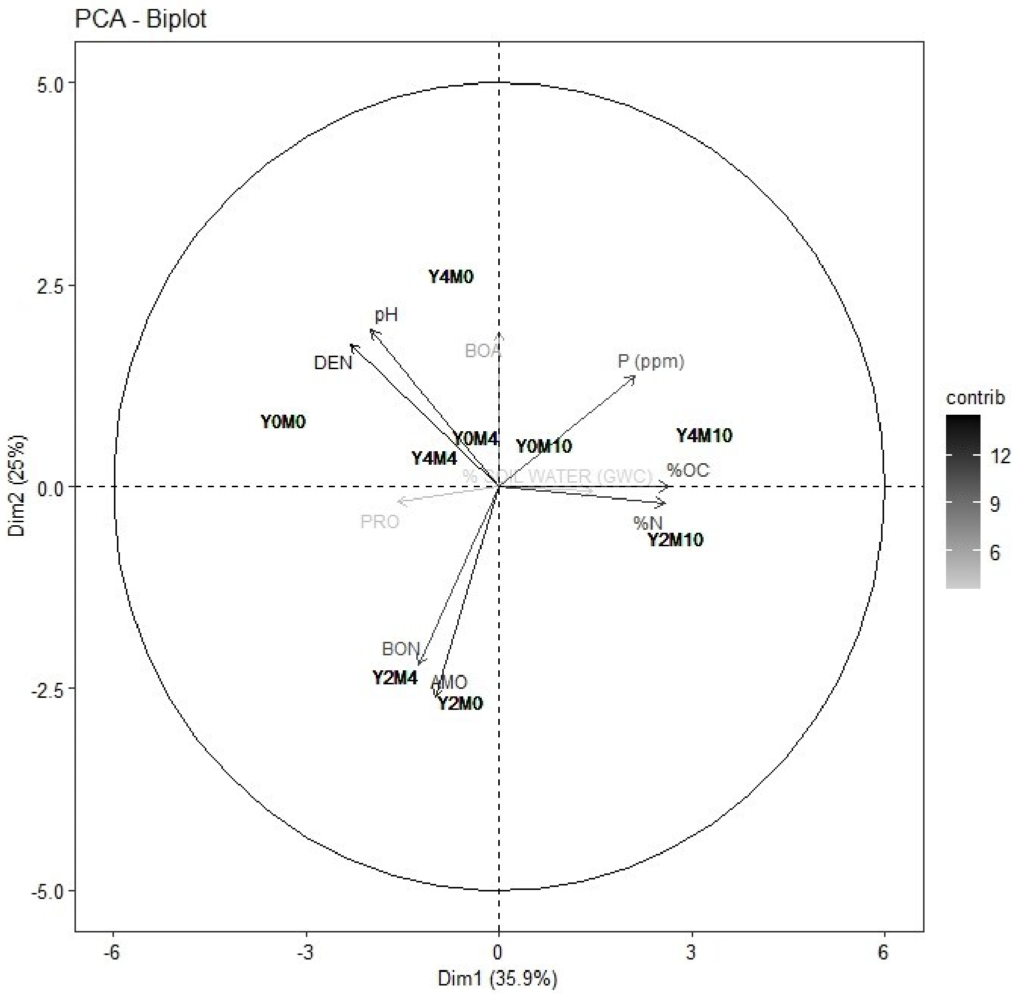

Figure 3. PCA graph of physical, chemical, and microbiological parameters in relation to the application of ecological farming techniques during the study period.

soil under study is slightly acid and ranges between 5 and 6 on the pH scale. Despite not finding statistically significant changes in pH (Figure 1A) and DEN microorganisms (Figure 2D), we observed that their microbial abundance did not change significantly, possibly because the soil was not at optimal pH levels for the correct development of DEN colony formation; In addition, it is possible to notice that the development of heterotrophic microorganisms is low due to abiotic factors, such as N availability, pH, organic carbon, structure and oxygen concentrations, which greatly affect soil denitrification (Qian et al., 2019). This interaction between DEN and pH was inversely proportional to the amount of organic matter available in the soil. Gerke (2022) states that soil organic matter not only improves the bioavailability of soil nutrients, but also affects soil fertility. Notwithstanding, Labrador (2008) also states that the application of ecological farming techniques impacts pH because they regulate the soil, keeping it at static levels, causing some organisms and nutrients not to develop efficiently.

According to the ACP, AMO and BON have a direct relationship. This coincides with Caballero et al. (2018) on the dynamics of the nitrogen cycle for the aforementioned microorganisms, which corresponds to the fact that they occur simultaneously in the stage of mineralization and release of organic compounds, thanks to the fixation of atmospheric nitrogen to give way to a beneficial enzymatic activity for the plant.

Despite statistically significant increases in physical and chemical parameters during the application of ecological farming techniques such as C, N, and P, we found that, in general, the functional groups of nitrogen cycle microorganisms did not significantly change their abundance with the application of ecological farming techniques. This reflects, according to Bollmann & Conrad (1998), that the microorganisms of the nitrogen cycle have low microbial activity at a given time and under certain conditions and then, over time, become fully active, so that longer study and sampling periods should be established in the application of ecological farming techniques. This author also states that microbial activities can occur in the short term for some microorganisms, but this depends on climatic conditions and the type of organic fertilizer applied to the crop or plot under study.

As opposed to the outcomes of this study, Caballero et al. (2018) stated that enzymatic activities change during the application of ecological farming techniques, and this directly influences the microorganisms of the nitrogen cycle (PRO, BOA, AMO, DEN, and BON). It should be noted that the study by Caballero et al. (2018) was conducted in coffee plantations that had more than ten years of application of ecological farming techniques, while the period of application in this study was ten months, directly influencing the long-term time for the abundance of these microorganisms. The results of the present study are similar to those reported by Rayo & Avellaneda-Torres (2022), who stated that ecological farming practices, such as the application of mycorrhizae, have a significant importance on soil physical and chemical parameters, while no statistically significant differences were reported for the abundance of microorganisms in the nitrogen cycle for a ten-month evaluation period.

Thus, the microbial behavior of the nitrogen cycle depends on the application time of organic farming techniques, finding that these communities are activated for long periods of time. This is because the availability of this limiting nutrient depends on various nitrogen transformation reactions carried out by complex networks of metabolically versatile microorganisms (Kuypers et al., 2018). On the other hand, Cañón-Cortázar et al. (2012) report significant changes in the abundance of the functional groups of the nitrogen cycle (PRO, AMO, BOA, BON, and DEN), mainly associated with climatic conditions. For this case study, Cañón-Cortázar et al. (2012) made a relationship with paramo soils subjected to potato cultivation and livestock breeding, in which these factors were also found to greatly decrease the abundance of microorganisms in nitrogen cycle after cultivation periods of approximately 50 years.

Some microorganisms are not culturable because of their behaviors; likewise, environmental factors also play a role in microbial development, as not all microorganisms are culturable under laboratory conditions. Argüello-Navarro et al. (2016) demonstrate in their study that such behaviors are reflected according to environmental conditions, and that despite being a realistic method to determine the microorganisms that are present, it also limits the determination of other microorganisms that are more susceptible to changes before the culture media; thus, the results did not demonstrate the effects on the microorganisms of the nitrogen cycle during the application of ecological farming techniques. Similarly, the various microbial transformations are driven by soil microbial activity rather than the number of microorganisms involved in these transformations (Caballero et al., 2018). That being said, it could be one of the limiting factors of the method used for this study.

Conclusions

The analysis of the different physical, chemical, and microbiological parameters established that four of the ten parameters under study, including %OC, %N, and P, showed statistically significant differences, thus affirming that the application of ecological farming techniques has a positive impact on the moisture and chemical parameters of the soil.

On the other hand, the abundance of microorganisms in the nitrogen cycle (PRO, AMO, BOA, BON, and DEN) did not present statistically significant changes due to the application of ecological farming techniques during the ten months of the study. Direct relationships were obtained between P and %OC, pH, and DEN, and a correlation was observed in PCA for BON and AMO.

It can be concluded that although the application of organic farming techniques improves the physical and chemical properties of soils, there are no statistically significant differences in the abundance of nitrogen cycle microorganisms.

Acknowledgments

The authors would like to thank Mrs. Mercedes Zambrano, Mr. Julián Castellanos, Mileidy Reyes, and Mariana Calderón for their support in the development of this research.

Compliance with Ethical Standards

Author contributions: Conceptualization: LZAT; Data Curation: LZAT, JABB, AFCC; Formal Analysis: LZAT, JABB, AFCC; Funding Acquisition: LZAT; Investigation: LZAT, JABB, AFCC; Methodology: LZAT; Project Administration: LZAT; Resources: LZAT; Software: LZAT; Supervision: LZAT; Validation: LZAT, JABB, AFCC; Visualization: LZAT, JABB, AFCC; Writing – Original Draft: LZAT, JABB, AFCC; Writing – Review & Editing: LZAT, JABB, AFCC.

Conflict of interest: The authors declare that they have no conflicts of interest (professional or economic) that could influence the article.

Funding source: The Universidad Libre and the Universidad Nacional de Colombia.

Literature Cited

Argüello-Navarro, A. Z.; Madiedo-Soler, N.; Moreno-Rozo, L. Y. Cuantificación de bacterias diazótrofas aisladas de suelos cacaoteros (*Theobroma cacao L.*), por la técnica de Número Más Probable (NMP). Revista Colombiana de Biotecnología, v. 18, n. 2, p. 40-47, 2016. https://doi.org/10.15446/rev.colomb.biote.v18n2.47678.

Bins, H. La revolución verde, la agricultura sostenible y la seguridad alimentaria. Monteverde: Monteverde Institute, 2020. 7p. (Research Trial). https://digitalcommons.usf.edu/community_health/145. 13 Jul. 2023.

Bollmann, A.; Conrad, R. Influence of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. Global Change Biology, v. 4, n. 4, p. 387-396, 1998. https://doi.org/10.1046/j.1365-2486.1998.00161.x.

Caballero, J. J.; Mejía, K. B.; Avellaneda-Torres, L. M. Effect of ecological and conventional managements on soil enzymatic activities in coffee agroecosystems. Pesquisa Agropecuária Tropical, v. 48, n. 4, p. 420-428, 2018. https://doi.org/10.1590/1983-40632018v4852373.

Cañón-Cortázar, R. G., Avellaneda-Torres, L. M.; Torres-Rojas, E. Microorganismos asociados al ciclo del nitrógeno en suelos bajo tres sistemas de uso: cultivo de papa, ganadería y paramo, en el parque Los Nevados, Colombia. Acta Agronómica, v. 61, n. 4, p. 371-379, 2012. http://www.scielo.org.co/pdf/acag/v61n4/v61n4a10.pdf . 13 Jul. 2023.

Carrero-Cañon, J. S. Diagnóstico de la situación actual y perspectivas al mercado internacional del café en el departamento de Cundinamarca. Bogotá: Universidad Antonio Nariño. 2021. 64p. Degree Thesis. http://repositorio.uan.edu.co/handle/123456789/6501. 13 Jul. 2023.

Chen, Z.; Zhang, S.; Li, Y.; Wang, Y. Characteristics of denitrification activity, functional genes, and denitrifying community composition in the composting process of kitchen and garden waste. Bioresource Technology, v. 381, p. 129-137, 2023. https://doi.org/10.1016/j.biortech.2023.129137.

Ferguson, S. J. Nitrogen Cycle enzymology. Current Opinion in Chemical Biology, v. 2, n. 2, p. 182-193, 1998. https://doi.org/10.1016/s1367-5931(98)80059-8.

Flórez-Zapata, N.; Uribe-Vélez, D. Biological and Physicochemical Parameters Related to the Nitrogen Cycle in the Rhizospheric Soil of Native Potato (*Solanum phureja*) Crops of Colombia. Applied and Environmental Soil Science, v. 2011, p.1-10, 2011. https://doi.org/10.1155/2011/847940.

Fondo de Cooperación para el Desarrollo Social - FONCODES. Producción y uso de abonos orgánicos: biol, compost y humus. Lima: MIDIS, 2014. https://draapurimac.gob.pe/sites/default/files/revistas/Producci%C3%B3n%20y%20uso%20de%20abonos%20org%C3%A1nicos_%20biol,%20compost%20y%20humus.pdf. 14 Jul. 2023.

Garzón, M. A. Estrategias técnicas para el cultivo de café en Colombia dirigidas a pequeños productores. Bogotá: UNAD, 2022. 51p. Degree Thesis. https://repository.unad.edu.co/handle/10596/50044. 14 Jul. 2023.

Gerke, J. The central role of soil organic matter in soil fertility and carbon storage. Soil Systems, v. 6, n. 2, p. 33, 2022. https://www.mdpi.com/2571-8789/6/2/33

Instituto Geográfico Agustín Codazzi – IGAC. Métodos analíticos del laboratorio de suelos. 6.ed. Bogotá: IGAC, 2006. 648p.

Kuypers, M.; Marchant, H; Kartal, B. La red microbiana del ciclo del nitrógeno. Nature Reviews Microbiology, v. 16, n. 5, p. 263-276, 2018. https://doi.org/10.1038/nrmicro.2018.9.

Labrador, J. Manejo del suelo en los sistemas agrícolas de producción ecológica. Madrid: SEAE, 2008. https://www.ecoagricultor.com/wp-content/uploads/2013/12/manual-manejo-del-suelo-en-agricultura-ecologica.pdf. 14 Jul. 2023.

Landini, F.; Beramendi, M. ¿Agroecología o agricultura convencional moderna? Posicionamientos de extensionistas rurales argentinos. Revista de Investigaciones Agropecuarias, v. 46, n. 3, p. 352-361, 2020. http://www.scielo.org.ar/pdf/ria/v46n3/0325-8718-RIA-46-03-00352.pdf. 14 Jul. 2023.

Martínez, E.; Fuentes, J. P.; Acevedo, E. Carbono orgánico y propiedades del suelo. Revista de la ciencia del suelo y nutrición vegetal, v. 8, n. 1, p. 68-96, 2008. https://doi.org/10.4067/s0718-27912008000100006.

Qian, W.; Ma, B.; Li, X.; Zhang, Q.; Peng, Y. Long-term effect of pH on denitrification: High pH benefits achieving partial-denitrification. Bioresource Technology, v. 278, p. 444-449, 2019. https://doi.org/10.1016/j.biortech.2019.01.105.

R Core Team. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2021. https://www.r-project.org/index.html. 11 Jul. 2023.

Rayo, C. L.; Avellaneda-Torres, L. M. Effects of applying arbuscular mycorrhizal fungi to nitrogen cycle microorganisms in soil with coffee plantations. Ciencia y Tecnología Agropecuaria, v. 23, n. 3, 2022. https://doi.org/10.21930/rcta.vol23_num3_art:2342.

Sailema-Sailema, E. W.; Siza-Saquinga, A. I.; Guamán-Mendoza, A. R.; Acosta-Velarde, J. I.; Vásconez-Núñez, D. C.; Tello-Oquendo, F. M. Uso de residuos orgánicos de hojas de mora para la producción de biol en la sierra ecuatoriana. Polo del Conocimiento, v.7, n.5, p.1439-1467, 2022. https://www.polodelconocimiento.com/ojs/index.php/es/article/view/4038/9434.14 Jul. 2023.

Salinas, Z.; Hernández, P (Eds.). Guía para el diseño de proyectos MDL forestales y de bioenergía. Serie Técnica. Manual Técnico no°83. Turrialba: Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), 2008. 236p. https://repositorio.catie.ac.cr/handle/11554/2259. 14 Jul. 2023.

Sarandón, S. J. Biodiversidad, agroecología y agricultura sustentable. 1.ed. La Plata: EDULP, 2020, 430p. https://doi.org/10.35537/10915/109141 .

Stein, L. Y.; Klotz, M. G. The nitrogen cycle. Current Biology, v. 26, n. 3, p. R94-R98, 2016. https://doi.org/10.1016/j.cub.2015.12.021.

Tencio, R. Reproducción y aplicación de los microorganismos de montaña (MM) en la actividad agrícola y pecuaria. San José (Costa Rica): Agriculture and Livestock Ministery (MAG), 2015. 6p. https://www.mag.go.cr/bibliotecavirtual/AV-1847.pdf. 14 Jul. 2023.

Trinidad-Santos, A.; Velazco, J. Importancia de la materia orgánica en el suelo. Agro Productividad, v.9, n.8, p.52-56, 2016. https://revista-agroproductividad.org/index.php/agroproductividad/article/view/802. 14 Jul. 2023.