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Impacts of different coffee systems on soil microbial populations at different altitudes in Villavicencio (Colombia)

Impactos de diferentes sistemas de café sobre las poblaciones microbiales del suelo a diferentes altitudes en Villavicencio (Colombia)

Carlos Hernando Colmenares P.¹, Amanda Silva P.¹, and Ángela María Mogollón O.¹

ABSTRACT

Coffee in Colombia has favorable characteristics for agroforestry practices; farmers traditionally grow coffee under various types of organic coffee systems, mainly dominated by associated systems coffee with shade leguminous tree species, making it one of the essential ecosystems for microorganisms life. This research was developed in the coffee region of Puente Abadía, Villavicencio, Colombia; the objective was to evaluate the heterotrophic aerobic microbial population in *Coffea arabica* var. Castilla of bacteria (BP), fungi (FP) and actinomycetes (AP), due to the combined effect of three factors. Factor A was related to two bioclimates zones: Low altitude zone <700 m a.s.l. (Z1) and high altitude zone >700 m a.s.l. (Z2); factor B corresponded to two coffee systems: coffee monoculture system (unshaded) (S1), associated coffee system (shaded) (S2) and control treatment of fruit monoculture system (S3). On the other hand, factor C referred to two sampling depth, corresponding to a 0-20 cm depth (D1) and 20-30 cm depth (D2). Significant differences ($P \leq 0.05$) between the $Z2 \times S2 \times D2$ factor interaction caused the highest (BP) and (AP); (AP) also was positively influenced by the $Z2 \times S2 \times D1$, $Z1 \times S2 \times D2$ and $Z1 \times S2 \times D1$ interactions ($P \leq 0.05$). The (FP) was affected positively by the $Z1 \times S2 \times D2$ and $Z1 \times S2 \times D1$ interactions. The (S2) (shade) had comparative advantages for the soil microbial population, as compared with (S1) and (S3).

Key words: Actinomycete, bacteria, *Coffea arabica* L., fungi, soil quality, soil diversity.

RESUMEN

El café en Colombia presenta favorables características para prácticas agroforestales, los agricultores tradicionalmente cultivan el café bajo diferentes tipos de sistemas de café orgánico, principalmente dominados por sistemas de café asociado bajo sombra con especies de árboles tipo leguminoso, siendo uno de los ecosistemas importantes para la vida de microorganismos. Esta investigación fue desarrollada en la región cafetera de Puente Abadía, Villavicencio, Colombia. El objetivo fue evaluar la población de organismos heterotróficos aeróbicos en *Coffea arabica* var. Castilla de bacterias (BP), hongos (FP) y actinomicetos (AP), debido al efecto combinado de tres factores. El factor A fue relacionado con dos zonas bioclimáticas: zona de baja altitud < 700 msnm (Z1) y zona de alta altitud >700 msnm (Z2); el factor B correspondió a dos sistemas de café siendo que fue sistema de monocultivo sin sombra (S1) y sistema asociado con sombra (S2), y un tratamiento control de sistema de frutales (S3). Por otra parte, el factor C fue referido a dos profundidades de muestreo, correspondiendo a una profundidad de 10-20 cm (D1) y a una profundidad de 20 a 30 cm (D2). Diferencias significativas ($P \leq 0.05$) entre la interacción de factores $Z2 \times S2 \times D2$, causó las más altas de BP, pero también de AP; a su vez la variable actinomicetos también fue positivamente influenciada por las interacciones $Z2 \times S2 \times D1$, $Z1 \times S2 \times D2$ y $Z1 \times S2 \times D1$ ($P \leq 0.05$). Las poblaciones de hongos (FP) fueron afectados positivamente por las interacciones $Z1 \times S2 \times D2$ y $Z1 \times S2 \times D1$. El sistema con sombra (S2) tuvo ventajas comparativas sobre la población de microorganismos del suelo comparado con S1 y S3.

Palabras clave: Actinomiceto, bacteria, calidad del suelo, *Coffea arabica* L., hongo, diversidad del suelo.

Introduction

Worldwide, coffee systems are important natural resources that need to be preserved (Souza *et al.*, 2012; Evizal *et al.*, 2012; Srivastava *et al.*, 2014; Cardona and Sadeghiankh, 2005).

Soil microorganisms control the functioning of coffee ecosystems through decomposition and nutrient cycling,

which in turn play a vital role in microbial conversion of complex organic compounds into simple inorganic compounds (Evizal *et al.*, 2012; Nigussie and Kissi, 2012), so it could improve its productive capacity (Haggar *et al.*, 2011; Evizal *et al.*, 2012; Bagyaraj *et al.*, 2015; Salgado *et al.*, 2006).

It has been found that the bioclimatic characteristics of a region can influence the dynamics of soil microorganisms (Criquet *et al.*, 2004; Srivastava *et al.*, 2014). Generally,

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bacteria are the dominant group followed by actinomycetes and fungi are the least dominant among the three groups of microorganisms (Bagyaraj *et al.*, 2015). Srivastava *et al.* (2014) reported higher microbial populations in a sub-humid (moist) zone than in sub-humid (dry) and semi-arid zones.

On the other hand, depending on the coffee systems, the type can result in different substrate availabilities that affect the establishment of different microbial groups. Studies have revealed that the agroforestry coffee systems are more effective in promoting soil microbial population than conventional monoculture (unshaded) coffee systems (Evizal *et al.*, 2012; Notaro *et al.*, 2014; Bagyaraj *et al.*, 2015).

The denomination of associated coffee production mainly involves agroforestry based systems, where coffee grows beneath various shade trees (mainly tree legumes), and are well suited for sustainable production compared with conventional (unshaded) coffee monoculture systems (Salgado *et al.*, 2006; Hagggar *et al.*, 2011; Nigussie and Kissi, 2012; Bagyaraj *et al.*, 2015).

Agroforestry coffee systems can increase microbial populations and soil nutrient availability that are characterized by a complex and diverse interrelationship among and between the microorganisms (Evizal *et al.*, 2012; Souza *et al.*, 2012); affecting directly the beneficial soil populations of fungi, bacteria and actinomycetes (Beer *et al.*, 1998).

The result of the vertical distribution of bacteria, fungi and actinomycetes in coffee systems was observed by Notaro *et al.* (2014). The surface soils (0-30 cm) recorded higher bacteria (41.3%), actinomycetes (38.4%) and fungi (26.1%) as compared to the subsurface horizons (Srivastava *et al.*, 2014).

Understanding that worldwide the coffee ecosystems need to be preserved, the aim of this study was to research the microbial population of bacteria (BP), fungi (FP) and actinomycete (AP) that can be explained by a combination of coffee system types, soil and climatic factors in Puente Abadia-Villavicencio (Colombia).

Methodology

The study site was in the Puente Abadia region, which is part of Villavicencio (4°08'33" N, 73°37'46" W), Colombia. The area is a tropical rainforest (Holdridge, 1947). The research factors were as follows: Two bioclimatic zones were

defined with cartography in Puente Abadia, Villavicencio municipality corresponding to factor A.

Low zone of Puente Abadia (Z1): This area is characterized by an altitude of < 700 m a.s.l., temperature >24°C, humidity of 76%, average annual rainfall of 2,080 mm, located on the alluvial plain of the Guaitiquia River, which is less susceptible to erosion problems.

High zone of Puente Abadia (Z2): This area is characterized by an altitude > 700 m a.s.l., temperature 18-20°C, humidity of 85%, average annual rainfall of 3,663 mm, slopes greater than 30% and high susceptibility to erosion, soils characterized by layers of silt alluvium.

Two types of coffee production used in organic coffee producing systems, monoculture coffee system (40%) and associated coffee system (60%), were found in both zones, together with a monoculture fruit system, that was next to the production fields which constituted a control. The system types were as follows:

Coffee monoculture system (S1): The cultivation of coffee was the conventional type (unshaded), the variety is the *Coffea arabica* 'Castilla', with a spacing of 2.5 m between plants, made up of six-year-old coffee trees. Conventional tillage only in the first year.

Associated coffee system (S2): The cultivation of coffee had been associated type (shaded), the plants responsible for the shading are banana (*Musa paradisiaca*), guamo (*Inga* spp.), legumes (*Leucaena leucocephala*) and native trees of the region, even citrus; made up of six-year-old coffee trees. Without planning shading with coffee trees. Zero tillage.

Monoculture fruit system (S3): This area was investigated for comparative purposes and served as the control treatment. The area was made up of guamo (*Inga* spp.), citrus plants (*Citrus sinensis* and *Citrus latifolia*), guava (*Psidium guajava*) and other fruit trees typically found in the zone. In addition, systems of the same age as S1 and S2 were selected.

In all evaluated systems predominate the application of organic matter, formed by the pulp of *C. arabica* and residues of *Musa paradisiaca* (banana), being the major constituents of organic amendments, as implemented in most coffee areas of Colombia (Cardona and Sadeghiankh, 2005). Farmers usually do not apply agrochemicals in the S2 and S3 systems.

In each zone, three farms were selected including the three researched systems (S1, S2, S3); in 1,000 m² sub-areas, 10 soil sub-samples were extracted from the first 0-20 cm (D1) and 20-30 cm of depth (D2), which were mixed until obtaining a homogeneous sample of the soil, this procedure was performed in triplicate for microbiological and physico-chemical soil analysis purposes (Tab. 1). The D1 and D2 corresponding to factor C.

Statistical analysis corresponded to a factorial design (2 zones x 3 systems x 2 depth) in a random arrangement unrestrictedly, with three replications for a total of 36 experiment units, composite soil samples were stored in a deep freezer to control microbial activities for soil dilution, plating and biological analysis. A microbial analysis was carried out within 48 to 72 h.

The variables analyzed were bacteria, fungi and actinomycetes population (BP, FP, AP), determined by serial dilution plate count method (Notaro *et al.*, 2014). Soil dilution (10⁻¹) prepared by 10 g of soil sample was transferred into 90 mL sterile water and mixing the solution for few seconds. Then, 1 mL of dilution was transferred into 9 mL of sterile

peptonized water test tube (10⁻²) and a soil serial dilution was prepared, up to 10⁻⁵.

Spread 0.1 mL of diluted soil suspension from each serial dilution (10⁻⁶ for Bacteria, 10⁻⁴ for fungi, 10⁻⁵ for actinomycetes) on different selective media, nutrient agar (NA) medium for bacteria, potato dextrose agar (PDA) medium for fungi and starch ammonia (SA) medium for actinomycetes and the plates in triplicate were incubated at optimum temperature between 25°C and 30°C at the duration of 5-7 d for fungi, 1-2 d for bacteria, 12-14 d for actinomycetes. For determine fungal growth was added chloramphenicol 30 mg L⁻¹. The colony forming units are expressed as CFU 10ⁿ/g of soil on a moisture basis.

The number of microorganisms were used in the following formula: Population = number of colonies × dilution factor × 10. Expressed as colony forming units per gram of soil (CFU/g soil) (Notaro *et al.*, 2014).

All the experimental data were analyzed using INFOSTAT. The variations among microbial population by effect of impact of different coffee systems were analyzed by one

TABLE 1. Physico-chemical characterization of soil for coffee producing systems, at different depths and two bioclimatic zones of Puente Abadia-Villavicencio (Colombia).

Systems	pH	Sand ¹ (%)	Silt ¹ (%)	Clay ¹ (%)	SOM (%) ²	P ³ (mg kg ⁻¹)	CDC ⁴ (%)	Ca ⁵	Mg ⁵	K ⁵	Al ⁶
Low altitude (Z1)											
0-20 cm (D1)											
S1 ⁷	4.5	35	55	20	6.22	10.80	14.90	1.80	0.40	0.20	1.70
S2 ⁸	4.5	23	61	16	6.43	22.5	4.88	5.40	0.85	0.40	0.80
S3 ⁹	5	50	50	25	4.64	7.80	20.0	2.85	0.80	0.14	0.25
20-30 cm (D2)											
S1	4.5	30	50	20	5.59	6.20	28.25	4.30	0.50	0.16	0.50
S2	5	8	66	26	6.00	21.20	8.50	5.60	0.90	0.41	0.85
S3	5	10	63	27	5.22	5.70	29.60	4.80	0.40	0.21	0.75
High altitude (Z2)											
0-20 cm (D1)											
S1	5	42	50	8	10.74	26.60	30.09	0.70	0.25	0.21	0.40
S2	4	40	52	8	17.44	28.50	12.25	10.05	1.30	0.40	0.70
S3	4.5	37	55	8	11.57	17.80	12.27	16.59	0.91	0.24	1.95
20-30 cm (D2)											
S1	4	40	50	10	10.76	16.50	46.70	2.80	0.90	0.23	1.90
S2	5	20	53	27	14.75	22.0	9.88	5.60	6.20	0.22	0.35
S3	5	31	44	25	10.44	19.50	8.04	2.50	0.85	0.21	1.20

¹Textural Bouyucos method; ²soil organic matter, Walkley Black method; ³Bray II method; ⁴clay dispersion coefficient, comparison between bouyucos method and method without dispersant; ⁵extraction by Ammonium Acetate method 0.1 N; ⁶extraction by KCl method 0.1 N; ⁷monoculture coffee system (unshaded); ⁸associated coffee system (shade); ⁹monoculture fruit system (control treatment).

way ANOVA test. The treatment means were compared using Fisher's test (comparison of means least significant difference LSD) at 5% level of confidence.

Results and discussion

Bacterial population (BP)

The BP was significantly higher ($P \leq 0.05$) in S2, 98.0×10^6 CFU/g, than in S1 and S3, accounting for 51.0×10^6 CFU/g and 9.57×10^6 CFU/g, respectively (Fig. 1). It demonstrated that (BP) in associated coffee system (shaded) has a role in nutrient cycling and soil fertility through biochemical processes which preserve the source of soil mineral nutrients (Garcia *et al.*, 2010; Evizal *et al.*, 2012; Bagyaraj *et al.*, 2015).

On the other hand, these differences in (BP) in (S1) and (S3) may be driven, in part, by differences in agriculture intensification and anthropogenic activities that affected

the soil quality, as demonstrated also by Ademir *et al.* (2009) (Fig. 1).

Additionally, (BP) showed significant variation due to sampling depth ($P < 0.05$), it was higher in (D2) than in (D1) accounting for 71.18×10^6 CFU/g compared with 40.44×10^6 CFU/g (Fig. 1). This response coincided with the results obtained by Srivastava *et al.* (2014), while the maximum microbial communities were restricted at 0-30 cm depth (surface horizon).

Higher populations of heterotrophic (BP) in (S2) can be attributed to their higher tolerance and adaptation to (Z2), accounting for 141.73×10^6 CFU/g (Fig. 2) probably can be due to the availability of soil for C accumulation occasioned in high humidity and low temperature of the site, as stated by Bardgett *et al.* (2005), as suggested also by Criquet *et al.* (2004). The (BP) was more or less same in humid and sub-humid dry bioclimates humid zones directly influences

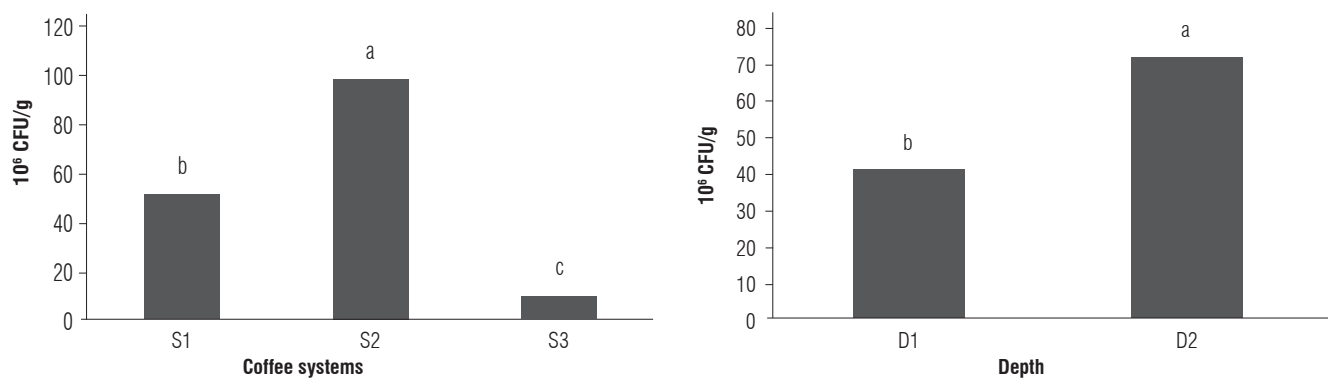


FIGURE 1. Soil bacterial population in coffee producing systems and depth of soil in Puente Abadia, Villavicencio, 2015. Means with a common letter are not significantly different ($P \leq 0.05$). LSD due to systems = 25.61×10^6 CFU/g. LSD due to depth = 20.91×10^6 .

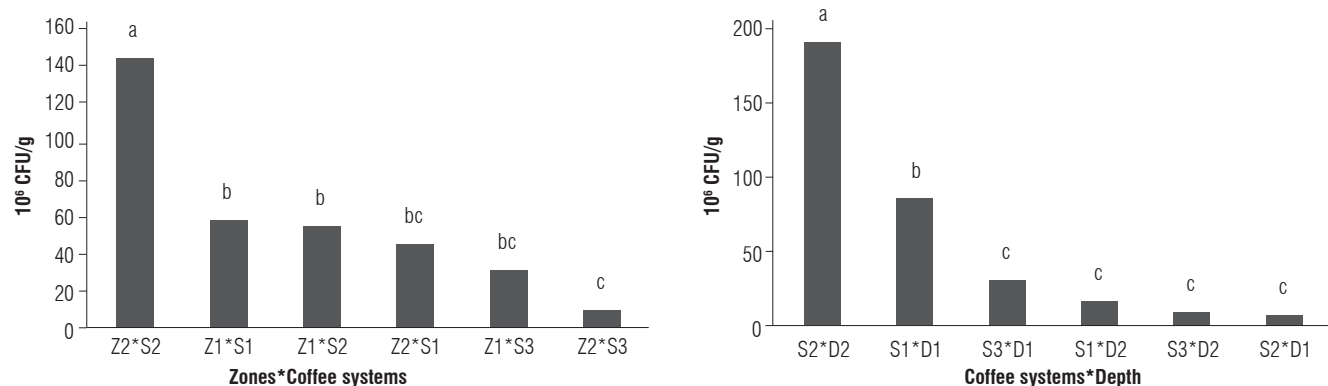


FIGURE 2. Soil bacterial population for factor interaction producing coffee systems (S1 monoculture coffee system, S2: associated coffee system, and S3: control treatment) altitude (Z1: low zone and Z2: high zone) and sample depth (D1: 0-20 cm and D2: 20-30 cm) in Puente Abadia, Villavicencio, 2015. Means with a common letter are not significantly different ($P \leq 0.05$). LSD for zone*systems = 20.91×10^6 CFU/g; LSD for systems*depth = 36.21×10^6 CFU/g.

higher soil humid for movement and nutrient acquisition of (BP) through of soil profile (Srivastava *et al.* 2014).

The maximum (BP) in (S2) was restricted to (D2) (188.0×10^6 CFU/g), the (BP) of this study is similar to that (BP) found by Muhammed *et al.* (2015) in a culture with different coffee systems. However, a similar concept of sustainability of the systems was applied. The low amount of (BP) found in the (S2) at the 0-20 cm depth (D1) (7.07×10^6 CFU/g) was similar when compared to data found by Notaro *et al.* (2014) in biodynamic coffee plantations (BS) at 0-10 cm depth (8.1×10^6 CFU/g).

The (BP) had a decline in (S1) and (S3) with an increase in D1 ($P \leq 0.05$) (Fig. 2), possibly related with the deficiency of soil nutrients through of soil profile in more simplified coffee production systems (unshaded), as demonstrated by Notaro *et al.* (2014).

The (BP) had a significant difference among the various factors ($P \leq 0.05$). The (Z2) cultivated with coffee under the shade of fruit trees (S2), resulted in a high (BP) at a 20-30 cm depth (D2) (280×10^6 CFU/g).

The increase in (BP) may be explained by a combination of high additions of SOM in the (S2) in surface soil, mulching with fruit and coffee residues, leaves, nourishes the rhizosphere and positively influences the (BP), making this an important practice for maintaining high levels of soil carbon (Cardona and Sadeghiankh, 2005), improving the soil fertility and soil microbial activity, as supported by Beer *et al.* (1998); Notaro *et al.* (2014); Bagyaraj *et al.* (2015).

But also, (BP) may benefit more from elevated precipitation and soil moisture, because they are more abundant in surface soils (Srivastava *et al.* 2014).

Actinomycetes population (AP)

The (AP) increased from (S3) to (S2) accounting for 11.80×10^5 CFU/g; (S3) and (S1) were statistically similar ($P \leq 0.05$) accounting for 1.60 and 1.90×10^5 CFU/g ($P < 0.05$), respectively (Fig. 3). This study proved that (S2) increased the (AP) as demonstrated by Bagyaraj *et al.* (2015). Biological indicators are extremely sensitive when attempting to detect changes in soil quality, as showed in (S3) and (S1) (Fig. 3).

The increase in (AP) of (S2) in Z1 and Z2 can be attributed to their higher tolerance and adaptation to wide variations of climate and soil in tropical conditions, accounting for 12.27 and 11.3×10^5 CFU/g respectively, with equal significance ($P \leq 0.05$) (Fig. 4) which was different to conditions found in temperate zones (Srivastava *et al.*, 2014).

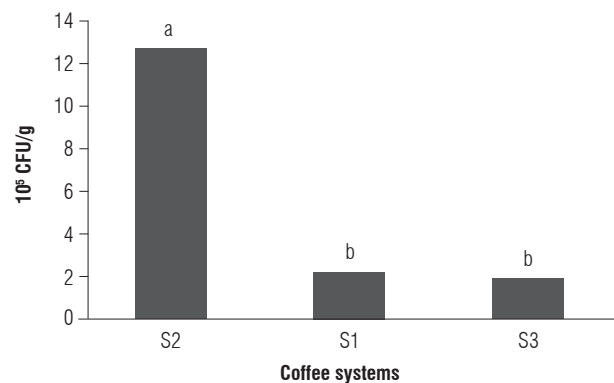


FIGURE 3. Actinomycetes population of soil in producing coffee systems in Puente Abadia, Villavicencio, 2015. S1: monoculture coffee system, S2: associated coffee system, and S3: control treatment. Means with a common letter are not significantly different ($P \leq 0.05$). LSD due to systems = 3.46×10^5 CFU/g.

Reduction in soil organic matter (SOM) in monoculture systems regardless of the area in which it is, may have had a negative effect on (AP) as showed for the Z1*S3, Z1*S1, Z2*S1, Z2*S3 interactions that recorded the lowest count of 2.87 , 1.93 , 1.53 and 1.27×10^5 CFU/g, respectively, with no statistically differences ($P < 0.05$) (Fig. 4).

The content of (AP) in the (S2) at (D1) and (D2) was not different (13.93 and 9.67×10^5 CFU/g) ($P < 0.05$) (Fig. 4). On the other hand, the S3*D2, S1*D1, S3*D1, S1*D2 interactions can affect the establishment of (AP) (3 , 1.87 , 1.40 and 1.33×10^5 CFU/g), respectively (Fig. 4). The surface soils recorded higher (AP) compared to the subsurface horizons (Srivastava *et al.* 2014; Hartmann *et al.* 2009).

The results of (AP) in the Z1*S2*D1 and Z2*S2*D2 interactions responsible for 16.40 , 11.47 , 11.47 and 11.20×10^5 CFU/g, respectively, with no significant difference ($P > 0.05$) between them, but different to the other interactions ($P \leq 0.05$) which demonstrate the similarity of (S2) between the areas and soil depths studied.

The increase in (AP) under associated coffee systems may be explained by a combination of system types than can markedly benefit the activity of soil microorganisms and their diversity (Evizal *et al.*, 2012; Notaro *et al.*, 2014) and climatic factors (Bagyaraj *et al.*, 2015).

Fungus population (FP)

Our (S2) maintained an almost consistent (FP) (39.50×10^4 CFU/g) than (S1) (13.83×10^4 CFU/g), but the lowest (FP) count was recorded in (S3) accounting for 4.50×10^4 CFU/g (Fig. 5). All of the systems were statistically different ($P \leq 0.05$). Various coffee management systems can result in different substrate availabilities that affect the establishment of a fungal population (Notaro *et al.*, 2014).

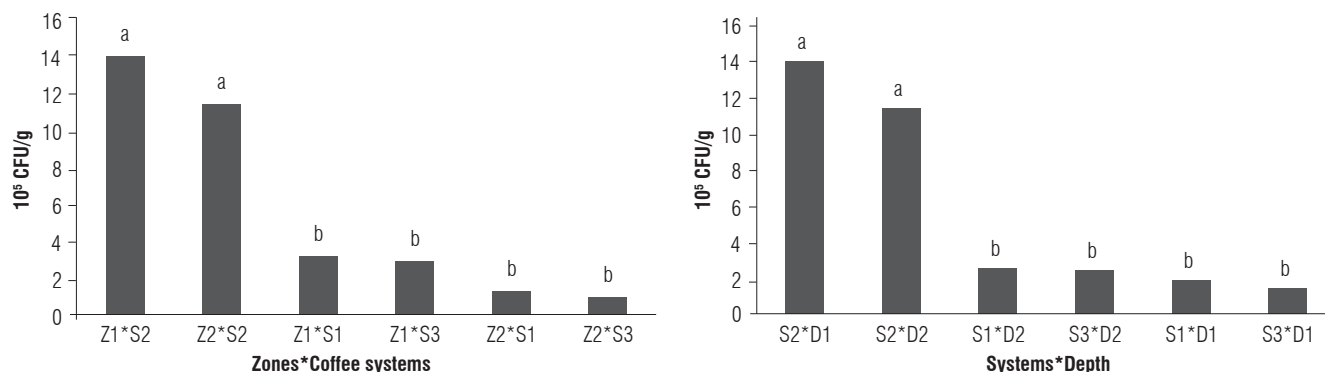


FIGURE 4 Actinomycetes population of soil for factors interaction producing coffee systems (S1 monoculture coffee system, S2: associated coffee system, and S3: control treatment) altitude (Z1: low zone and Z2: high zone) and sample depth (D1: 0-20 cm and D2: 20-30 cm) in Puente Abadia, Villavicencio, 2015. Means with a common letter are not significantly different ($P \leq 0.05$). LSD for zone*systems interaction = 4.89×10^5 CFU/g. LSD for systems*depth interaction = 4.89×10^5 CFU/g.

A higher (FP) was observed in (S2) of the Z1 and Z2 (43 and 36×10^4 CFU/g) respectively (Fig. 6), indicating a similar trend with (AP). The lowest (FP) was observed in (S3) of Z2 and Z1, but equal to the (S1) of the (Z1), in turn this did not have a significant difference with the (S1) of the

(Z2) ($P \leq 0.05$) (Fig. 6). Our results do not corroborate with the findings of Srivastava *et al.* (2014) and Mogollón and Martínez (2009); due to the fact that (FP) was more tolerant to variations of weather conditions.

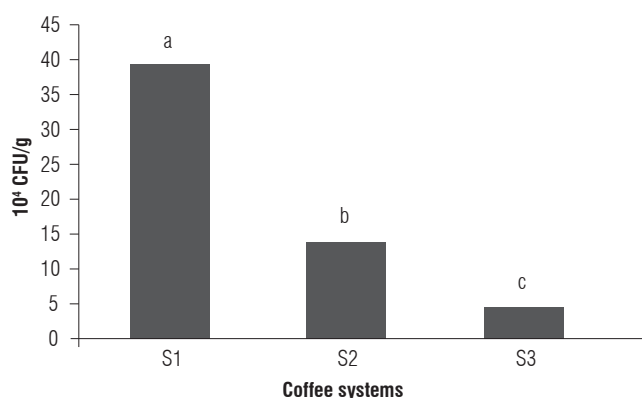


FIGURE 5 Fungal population of soil in producing coffee systems in Puente Abadia, Villavicencio, 2015. Means with a common letter are not significantly different ($P \leq 0.05$). LSD due to systems = 6.81×10^4 CFU/g.

Soil fungi can extend hyphae through air-filled pore spaces to access moisture and nutrients and can translocate these resources to water and nutrient-limited cells within their mycelia network (Muleta *et al.*, 2007; Srivastava *et al.*, 2014).

Comparatively, (S2), which are better managed, are rich in (FP) showing more diversity through the soil profile (39.67 and 39.63×10^4 CFU/g) indicate a significant difference than in the poorly managed systems (S1) and (S3) ($P < 0.01$) (Figure 6).

The (S1) and (S3) require different adaptation strategies of (FP) to soil edaphic factors with emphasis on the rhizosphere; biological indicators are extremely sensitive when

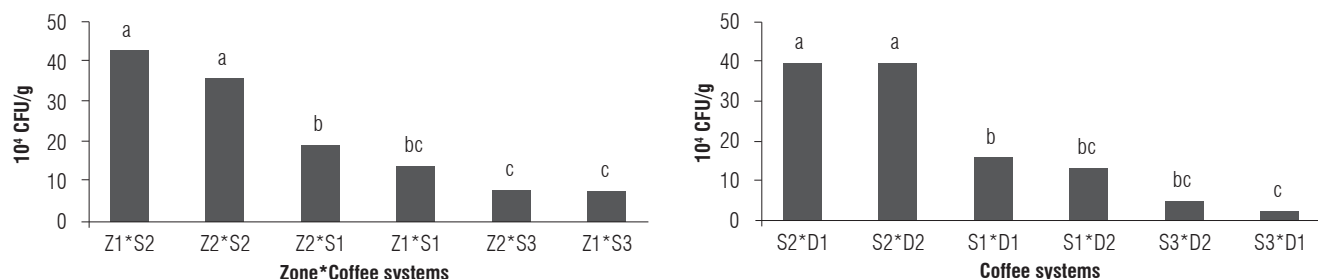


FIGURE 6 Fungal population of soil for factors interaction producing coffee systems (S1 monoculture coffee system, S2: associated coffee system, and S3: control treatment) altitude (Z1: low zone and Z2: high zone) and sample depth (D1: 0-20 cm and D2: 20-30 cm) in Puente Abadia, Villavicencio, 2015. Means with a common letter are not significantly different ($P \leq 0.05$). LSD for zone*systems interaction = 9.63×10^4 CFU/g. LSD for systems*depth interaction = 9.63×10^4 CFU/g.

attempting to detect changes in soil quality (Bardgett *et al.*, 2005; Muleta *et al.*, 2007). Reduction in soil carbon, may have had a negative effect on (FP).

The Z2*S2*D2 and Z1*S2*D1 interactions had a higher (FP) accounting for 46.67 and 39.33 x 10⁴ CFU/g, respectively; confirming that there has been a growing interest in the (S2). (S2) is based on principles of diversification, recycling, biological processes and the imitation of natural habitats, as demonstrated by Beer *et al.* (1998); Notaro *et al.* (2014); Evizal *et al.* (2012); Garcia *et al.* (2010) and Cardona and Sadeghiankh (2005).

Conclusions

The data so generated on microbial population of (S1), (S2) and (S3) can be considered part of the soil information for monitoring the soil quality and sustainability of coffee systems in Puente Abadia, Villavicencio.

The (Z2) in Puente Abadia are zones that are highly susceptible to environmental degradation (erosion), and can be used for the (S2) because it potentiated a greater number of (BP), and (AP) to a 20-30 cm depth (D2); that stimulates growth of (S2) in (Z1), which generated major (AP) and (FP) at both depths evaluated.

Agroforestry can be an interesting alternative for improving soil quality in marginal coffee areas in Colombia, due to the increase in soil microbial activity.

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