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# Quantification of the population of microorganisms in different management systems and land use

#### Quantificação da população de microrganismos em diferentes sistemas de manejo e do uso do solo

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#### ABSTRACT

Quantitative evaluations of the microbial density of soils are important to understand the ecological relationships that occur and to identify factors that influence the microbiological balance. The objective was to quantify soil microorganisms in three different areas and use management. The work was carried out at the Federal Institute of Roraima, in three areas: gliricidia, orange and banana, orange and ipê consortium and forest area. Soil samples were collected at depths of 0-10 and 10-20 cm. The total microbiota was evaluated by the surface plating technique and quantified by colony forming units per gram of soil (CFU g soil<sup>-1</sup>). Plates were incubated at 28°C for 48 and 96 hours. Ammonifying, cellulolytic and actinobacteria organisms were quantified by soil suspension, using specific culture media. The results showed that the area of the intercropping gliricidia + orange and banana has a higher population of microorganisms, proving to be an alternative for intercropping systems with agricultural crops, providing greater biodiversity, in addition to providing nitrogen for the plants.

Keywords: *Glyricidia*; Population; Depth; Unit trainer in Cologne.

#### **RESUMO**

Avaliações quantitativas da densidade microbiana dos solos é importante para entender as relações ecológicas que ocorrem e identificar fatores que influênciam no equilíbrio microbiológico. Objetivou-se quantificar os microrganismos do solo em três diferentes áreas e manejo de uso. O trabalho conduzido no Instituto Federal de Roraima, em três áreas: consórcio gliricídia, laranja e banana, laranja e ipê e área de mata. Coletou-se amostras de solo nas profundidades de 0-10 e 10-20 cm. A microbiota total foi avaliada pela técnica de plaqueamento em superfície e quantificada por unidades formadoras de colônias por grama de solo (UFC g solo<sup>-1</sup>). As placas foram incubadas a 28 °C por 48 e 96 horas. Os organismos amonificadores, celulolíticos e actinobactérias foram quantificados por suspensão do solo, utilizando meios de cultura específicos. Os resultados demostraram que a área do consórcio gliricídia + laranja e banana apresenta maior população de microrganismos, mostrando ser uma alternativa para sistemas de consórcios com culturas agrícolas, proporcionando uma maior biodiversidade, além de fornecer nitrogênio para as plantas.

Palavras-chave: Gliricídia; População; Profundidade; Unidade Formadora de Colônia.

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#### **INTRODUCTION**

Soil is a fundamental natural resource for agricultural production, and its quality is formed by chemical factors, physical, biological and ecological, and can be modified in favor of better quality. Soil quality is a property sustainability of cultures, also influencing the health of plants, animals and consequently in human beings (Melo *et al.*, 2017). Soil is a naturally diverse habitat, with highly complex biological communities in which are different forms of microorganisms, both eukaryotes and prokaryotes, that interact in an environment dynamic and in state of balance (Carrer Filho, 2002).

The populations of microorganisms in the soil coexist in an ecological balance that can be significantly influenced by the cultivated species, by soil disturbance, application of inputs and by predominant climatic factors, especially temperature and humidity (Mathew *et al.*, 2012; Jacobsen & Hjelmsø, 2014). The decomposition of matter organic, the nitrification, fixation of N<sub>2</sub> atmospheric, the aggregation of ground and the production in compounds able in interfere in the development of other organisms are processes mostly governed by soil microorganisms (Nair & Ngouajio, 2012).

The quantity and diversity of microorganisms in the soil is enormous. Microbial biomass is directly and indirectly by microbiological and biochemical processes. They play important roles in biogeochemical cycles and in the functioning of ecosystems (Bell *et al.*, 2005). In addition to these environmental "functions", microorganisms and their derivatives have great biotechnological potential, such as bioinoculants for agricultural production, biological control, bioremediation, production of drugs, such as antibiotics, enzymes, dyes and other chemical substances. However the magnitude of microbial biodiversity is still poorly known and consequently the potential to be explored, since, We know only 1% (Moreira & Siqueira, 2006).

Considering that the microorganisms constitute great indicator of biological conditions from soil, Besidesits effect on agricultural productivity, knowledge of soil management and vegetation cover on the microbial population (Ruegger & Tauk-Tornisielo, 2004).

In particular, plants constitute a true microbial ecosystem. In these host plants, different niches are busy by the microorganisms, such as at surfaces of roots and sheets (at epiphytes), or then, these colonizing the interior of various plant tissues. Bacteria that live inside plants can be divided into two groups, based on their relationship with the host, associative and symbiotic (Souza, 2005). Among the bacteria associated the species

vegetable, at bacteria fasteners in nitrogen are considered as a from groups in larger importance at tropical agriculture by your association with at plants legumes or not.

Previati *et al.* (2012), working with isolation and quantification of bacterial and actinomycete populations present in the soil, concluded that the count of microorganisms in the soil, despite being viewed with reservations, helps to understand the processes that occur in it and can serve as an indicator of the impact of different anthropic activities. Cherubim *et al.* (2015), mentions that the need to analyze the quality of the soil, a minimum set of indicators encompassing characteristics physical, chemicals and biological are used. At the however, per to be the part most live and most active gives matter organic of ground andbecause it acts in important biochemical processes, studies show that biological indicators to detect changes that occur in the soil as a result of its use and management (Stocker *et al.*, 2017).

The identification and quantification of the diversity of microorganisms present in a soil can be evaluated as biological parameters indicative of ecological stresses and the general health of the flora and fauna of the place local (Van Bruggen & Semenov, 2000). Thus, the objective was to evaluate the population of microorganisms present in the soil in three different forms of use and management.

### MATERIAL AND METHODS

The study was carried out at the Federal Institute of Science and Technology of Roraima - *Campus* Novo Paraíso, in the municipality of Caracaraí, Roraima, at geographic coordinates N 01°14'51.6" and W 60°28'20.4", altitude of 105 m, seasonal tropical climate – Aw, according to the Köppen classification. The soil in the area was classified as Argisolic Dystrophic Red Latosol (Embrapa, 2013). The treatments consisted of three areas with different management and use: area in intercropping system (gliricidia, orange and banana), area with intercropping of orange and forest essence (Ipê) and the forest area. Each area of 1600m<sup>2</sup> was divided into five plots, of which each one was subdivided into two subplots, in which soil samples were collected in the rhizosphere of the plants, at depths of 0 – 10 and 10 -20 cm. The samples were packed in sterile and dark plastic bags, properly identified, and stored in a thermal box and later in a cold chamber (4 °C).

The active soil microbiota was evaluated by the surface plating technique, in triplicate, using specific growth media for each microorganism. The soil samples were homogenized and sieved, then 10 g of soil was removed from each sample, carefully ground in a sterilized grail, then transferred to a 250 mL erlenmeyer flask containing 90 mL of saline solution (NaCl 0.85%), shaken. for thirty minutes, then transferred 1 mL of this solution to a sterile test tube containing 9 mL of saline solution (NaCl 0.85%), homogenized in a vortex mixer, in decimal serial dilution as mentioned by Neder, (1992). Then 0.1 mL of this dilution was transferred to Petri dishes in triplicate containing YMA culture medium. The plates were taken to a growth oven at 28 °C. After 48 and 96 hours, the colony forming units were counted.

Quantification was obtained by colony forming units per gram of soil (CFU g soil  $^{-1}$  average of counts x selected dilution x 10), as cited by Dionísio *et al.*, (2016). The results of the repetitions were used to calculation of averages and error standard, using the Microsoft software Office Excel 2010 (Microsoft).

Phenotypic characterization was performed through the morphology of colonies in solid culture medium 79 (Fred & Waskman 1928), containing bromothymol blue (0.5%) and incubated at 28 °C. The characterization started from the appearance of at least three isolated colonies (Moreira *et al.*, (2013). The characteristics evaluated were: growth in days (very fast - up to 1 day, fast - 2 to 3 days, intermediate - 4 to 3 days). 5 days, slow 6 to 10 days, very slow >10 days) and change in the pH of the medium (neutral, acidic and alkaline).

At assessments of features morphological From fungi were performed The leave of cultivation From isolated in quiteof BDA + Stroptomycin culture, incubated at 28°C for 8 days (Ferreira, 2017).. The characteristics were evaluated: texture of the colony (cottony, velvety, granular, powdery and creamy), colony shape (circular, irregular, filamentous, rhizoid, punctiform and spindle), colony elevation (flat, elevated, convex, pulvinate, umbiculate and crateriform), colony edge (whole, wavy, lobed, irregular, filamentous and coiled), colony surface (rough, verrucous and umbiculate) and colony color (cream, white and yellow).

The quantification of the number of ammonifiers was used 10 g of soil sample mixed in saline solution and prepared from the dilution 10<sup>-1</sup> successively to 10<sup>-5</sup>. Then 1mL of each dilution was transferred to a test tube. containing quite in culture specific for ammonifiers by technique in Sarathchandra (1978). For The dilution, were Three test

tubes were used, containing 4 mL of culture medium and autoclaved for 20 minutes. It was later 1 ml of the soil suspensions were added to each tube; and kept for 5 days in an acclimatized room (28 °C). In the presence of ammonifiers was determined by the color change (from orange to pink), which were noted as positive. (presence) and those without color change as negative (absence) of ammonifying microorganisms. The determination of most likely number (MPN) was performed by counting the tubes that presented the occurrence of ammonia, and the calculation was carried out by the use from the probability table of occurrence (Sarathchandra, 1978).

The quantification of cellulolytic microorganisms was used 10g of soil sample diluted in saline solution (NaCl 0.85%), the serial dilutions were from  $10^{-1}$  to  $10^{-3}$ . 1.0 mL of the soil suspension was transferred to test tubes containing 9.0 mL of liquid medium for cellulolytic organisms, according to the method of Bose (1963). A strip of sterile filter paper measuring 7.0 x 1.0 cm was placed in each tube, 2.0 cm above the level of the medium. The cultures were incubated in a B.O.D oven at 28 °C for 37 days. The estimation of the number of viable cells was made using the most likely number table method described by Sarathchandra (1978).

For the quantification of actinobacteria, the surface seeding method, proposed by Arifuzzamanet *et al.* (2010). Serial dilutions were from  $10^{-2}$  to  $10^{-6}$ , 0.1 ml of the soil suspension was transferred to Petri dishes containing casein-dextrose-agar culture medium (Clark, 1965). The cultures were incubated in a B.O.D oven at 28 °C for 7 days. After this period, the dilutions that presented between 30 and 300 colonies were selected and quantified and the result expressed in CFU g soil<sup>-1</sup> (Dionisio *et al.*, 2016).

### **RESULTS AND DISCUSSION**

You results obtained (Table 1), it is observed what you factors time in incubation and depth in collect, influenced the number of colony forming units (CFU g soil-<sup>1</sup>). The highest counts were obtained after 96h in incubation and at depth of 0 - 10 cm, independent of system of management and use of ground.

Table 1. Population in microorganisms (total, cellulite, ammonifiers and actinobacteria)in the areas of study.

Area Collect	Depth	рН	total microorganisms		collulito	ammonifiers	actinohacteria
			48h	96h	cenunic	annionners	actinobacteria

	of collection of soil (cm)		CFU g solo -1							
Glyricidia	0 -10	6.13	2.4 x 10 <sup>4</sup>	$3.0 \times 10^{4}$	11.0 x 10 <sup>3</sup>	14.0 x 10 <sup>4</sup>	3.6 X 10 <sup>3</sup>			
+ orange	10 - 20	6.07	2.9 x 10 <sup>4</sup>	$3.5 \times 10^{4}$	2.5 x 10 <sup>3</sup>	14.0 x 10 <sup>4</sup>	3.1 X 10 <sup>3</sup>			
+ banana										
Orange +	0 - 10	6.00	2.2 x 10 <sup>6</sup>	2.4 x 10 <sup>6</sup>	$0.4 \times 10^{-3}$	14.0 x 10 <sup>4</sup>	3.0 X 10 <sup>3</sup>			
Ipe	10 - 20	6.09	2.1 x 10 <sup>6</sup>	1.7 x 10 <sup>6</sup>	0.35 x 10 <sup>-3</sup>	11.0 x 10 <sup>4</sup>	*			
Woods	0 - 10	4.57	*	6.8 x 10 <sup>5</sup>	11.0 x 10 <sup>3</sup>	*	*			
	10_20	4 76	$2.2 \times 10^{6}$	$2.2 \times 10^{6}$	$11.0 \times 10^{-3}$	$0.07 \times 10^{4}$	*			

\* there was not growth

Analysis performed at the IFRR Soil and Plant Laboratory, Caracaraí-RR, 2021. Source: authors (2023)

In relation to soil pH, the areas with gliricidia, orange and banana and orange and ipê showed similar pH (Table 1). This fact is due to the management of acidity correction with limestone for orange cultivation. pH is one of the attributes soil physicochemicals most described for influencing the different microbial communities that inhabit soils. You exact mechanisms of how this parameter influences microbial communities are not yet well described, but it is known that pH values affect the solubility of minerals in the soil, thus affecting the availability of nutrients. The Microbial diversity is directly related to a set of abiotic factors, one of which is pH and biotic. The interaction between these factors directly influences the ecology, activity and population dynamics of microorganisms in the soil (Moreira & Siqueira, 2006; Silva *et al.*, 2021).

The highest amount of colony forming units (CFU g soil<sup>-1</sup>) presented in soil with intercropped plants with gliricídia (*Gliricidia sepium* (Jacq.) Steud.), is explained by this legume, proves to be a viable alternative for the supply of nitrogen to fruit species, given their ability to biologically fix the nutrient (Espindola *et al.*, 2006).

According to Paula et al. (2015), the high biomass rates and short half-life of gliricídia biomass help, in the long term, to increase soil fertility and nutrient availability for crops that are interspersed in the planting area. Thus, it favored the increase in the population density of microorganisms in the area with the presence of gliricídia. For Barreto and Fernandes (2001), gliricídia is capable of increasing the productivity of agricultural crops to which it is associated, by improving the chemical, physical and biological characteristics of the soil, especially in the more superficial layers.

In the soil of the orange and ipê intercropping area, there was a greater amount of CFU g soil-<sup>1</sup> at a depth of 0- 10cm (Table 1), corroborating with Tortora; Funke; Case, (2016), who state that the population is greater in the few centimeters from the soil

surface, and declines rapidly with depth. Biological activity is highly concentrated in the first layers of ground, at depth in between 1 The 30 cm. in these layers, O component biological occupies one fractionin any less what 0.5 % of volume total of ground and represents any less what 10 % gives matter organic. Being compound mainly per microorganisms what perform various functions essential for O operation of ground. You Microorganisms break down organic matter, release nutrients in forms available to plants, and degrade substances toxic (Kennedy & Doran, 2002).

In the phenotypic characterization of the isolates, in the area with the presence of gliricidia, orange and banana, there was a predominance of isolates that alkalized the culture medium (83%) and of rapid growth (100%) (Figures 1 and 2). Regarding the consistency of the mucus, there was a predominance of aqueous, 15 isolated (83.3%), followed by dry (11.2%) and viscous (5.5%). As for the color of the colonies, 16 (88.9%) were white and up to 1 mm in diameter, 2 (11.1%) were cream.

In the soil of the area with orange and ipê (without gliricidia), the predominance of isolates that acidified the medium was observed. of culture, of rapid growth and consistency of watery mucus (75%), the remaining 25% of the isolates from this area showed reaction alkaline, growth much fast (Figure 1 and 2) and consistency of mucus dry. All you isolated of this area had a smooth edge, diameter of 1 mm and white color. The soil of this area presented three isolates with mucus production scarce, all you others presented production in mucus (little to moderate).

In the forest area, there was a predominance of isolates that acidified the medium (100%), with very high growth. fast (68.75%), fast (31.25%), with watery consistency, edge smooth, diameter in 1 mm and color white (Figure 1 and 2).

Figure 1. Time in growth of isolated us many different management and use of the soil.



Some strains of rhizobia, especially the fast-growing ones, present colonies with a very gummy appearance, mainly on plates with carbon-rich culture medium (eg, YM). In low-carbon culture media, the bacteria tend to produce colonies droughts (Araújo, 1993).

According to Araújo (1993), colonies are generally round, ranging from flat to conical, or even dome-shaped. The margins of colonies are usually smooth. When colonies grow under the surface of the medium they acquire the characteristic shape of biconvex lenses. Colonies can be opaque white, or milky, and even translucent, some can produce pink or yellow colonies. Showing that this work presented similar characteristics to the literature.

The pH is one of the edaphic attributes that limits the presence of microorganisms in the soil (Brockwell *et al.*, 1991). Giongo *et al.* (2007), verified when evaluating the environmental factors of the soil that affected the diversity of populations of Bradyrhizobium spp., isolated from soybean nodules, that the soil pH was the main characteristic that affected the diversity of populations, and that less diversity was found in soils with more acidic pH. This characteristic relates to the results obtained in this work, in which the microorganisms isolated from the soil of the forest area, with pH ranging from 4.57 to 4.76 (Table 1), presented acidic reactions (Figure 2).

Figure 2. Reaction of pH alteration in YMA medium containing bromothymol blue of the isolates in the different handling and use of the ground.



In the characterization of the fungi isolated from the area of the intercropping gliricidia, orange and banana, a predominance of irregular fungi, with raised colonies, wavy edges, umbiculate surface; while, in the area of the orange consortium and ipê, there was a predominance of irregular fungi, with flat colonies, entire edges, umbiculate surface. already in the area of forest, there was a predominance of irregular fungi, with high colonies, wavy edges, umbiculate surface. Only in area soil of woods that no presented edges irregular and surface wrinkled

The results obtained in the study regarding the morphological characteristics were similar to those mentioned by Pelczar *et al.* (1980), who mention that in appropriate culture media, colonies vary in size, texture and edges. Per for example, some colonies are smooth, others wrinkled; some are flat, others raised; some have full edges, others have irregular or filamentous edges. Young colonies have a consistency comparable to that of a paste. thick, the which after O aging, becomes most thick and most dry and they can produce pigments.

The presence of ammonifying microorganisms was similar in the two areas managed in consortium (Table 1). Being that, in the area of the forest it presented a lower value. According to Bandick and Dick (1999), the microbiota is in love with the greatest diversity floristic and for the roof vegetable, that provides larger accumulation in organic matter, providing larger the amount in nutrients for O development gives community microbial. This would lead the forest area to have a soil more favorable to these organisms, but not only the cover makes these organisms opportune, but also other factors that can affect them in their absence. Showing that the soil with the consortium of gliricidia, orange and banana, provided a favorable environment, since in this area the applied managements, pruning of gliricidia plants and pseudostem of banana trees, provides a greater vegetal cover of the soil, consequently the accumulation of organic matter in it.

The cellulosic population was observed in the three areas evaluated (Table 1). In the forest area, it obtained higher values, regardless of the depth of collection. The intercropping area with gliricidia, orange and banana had value equal to the area of forest at a depth of 0-10cm and the area of the orange and ipê intercropping (without gliricidia) was the one with the lowest population of cellulite microorganisms. In stable ecosystems, as in soil under forest, there is a trend of the community microbial to be larger numerically in terms in diversity (Cardoso, 1992). The presence in bacteria cellulolytic at the soil, play similar roles in terms of soil availability of cellulose substrate, provide carbon sources for will improve fertility from soil and keep balance in nutrients through gives decomposition in cellulose (Yang *et al.*, 2014).

For Ramos *et al.* (2012), this bacterial group is strongly influenced by the vegetation cover of the place, thus, the results obtained in the work are justified, demonstrating that the areas with greater diversity and vegetation cover, the area of the gliricidia consortium, orange and banana and of woods, larger population of microorganisms at the ground.

Results obtained by Melonni *et al.* (2001), working with soil under riparian forest and cerrado fields, verified the presence of cellulosics was higher in the soil under forest, however, they did not detect significant differences between them, corroborating with you results obtained in this study.

The number of actinobacteria was higher in the area with gliricidia, orange and banana, with similar values in the two areas. depths. In the area with orange and ipê (without gliricidia) only in the depth of 0 - 10 cm showed a number of colony forming units (CFU g soil <sup>-1</sup>) with values close to the area with gliricidia, orange and banana (Table 1). Actinobacteria, due to their wide distribution in the soil, play an important role in the degradation of organic material, is also used as indicator of quality of ground (Mangamuri *et al.*, 2014).

Second Arifuzzaman *et al.* (2010), the population in actinobacteria It is affected for the localization geographic, temperature and soil type, pH, organic matter content,

type of cultivation, aeration and humidity. pH is a determining factor for most species, being optimal between 6.5 and 8.0 and limiting for most species at 5.5. The absence of these organisms at the ground gives area Woods, then it has a pH below of wanted per these species.

# CONCLUSION

The area intercropped with gliricidia, orange and banana has a higher population of microorganisms (total, cellulolytics, ammonifiers and actinobacteria), showing to be one plant alternative for systems in consortium agroecological, providing a greater amount of microorganism in the soil, improving the cycling of nutrients in the system, beyond to help in supply in nitrogen to at species consortium.

The presence of the legume gliricidia, associated with the management of plant pruning, was crucial to increase the population of microorganisms at the ground.

Works involving the study of population of soil microorganisms, in agricultural systems intercropped in the Amazonia must be developed, for better use of the resources available in the rural properties of this region, there is View The scarcity in resources financial by the farmers relatives. perspectives as others plants agricultural and legumes native are parameters what they can to be analyzed and come to add in this study.

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