LAND USE IMPACT IN POPULATION DYNAMICS OF CULTIVABLE SOIL BACTERIA

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Recebido em: 15/08/2022 – Aprovado em: 15/09/2022 – Publicado em: 30/09/2022
DOI: 10.18677/EnciBio_2022C17

ABSTRACT

Bacteria are one of the main life forms present in soil and are directly affected by its characteristics. Each bacteria community has unique ecological and physiological properties. This makes it possible for them to carry out different processes that occur in soil, such as phosphate solubilization, biological nitrogen fixation, decomposition of organic matter and production of antibiotic compounds. The main goal of this study was to investigate how land use and anthropic activity can interfere in the population dynamics of soil-cultivable bacterial groups. Soil samples were collected from the Ubajara National Park, located in Ubajara, Ceará, Brazil. Sampling was carried out in order to contemplate three different land uses: preserved, conserved and agriculture. The microbial groups used in the research were phosphate solubilizers, nitrogen-fixing bacteria and actinobacteria. The abundance of each group was estimated by counting colonies in Petri dishes using the spread plate method. Serial dilutions of 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made for each soil sample collected. 0.1 ml of each dilution was spread on Pikovskaya agar medium, Burk's N-free medium and Casein Dextrose Agar culture medium. The plates were incubated at 28°C (± 2) and then colony counts were performed to assess the abundance of these bacteria. Results were expressed as colony forming units (CFU. g-1). Analyzes of variance followed by Tukey's test were performed. contemplating the plots and land uses. Agricultural activity interfered in the population dynamics of functional groups that can be cultivated in the soil.

KEYWORDS: Soil microbiota; Agriculture; Caatinga.

DINÂMICA POPULACIONAL DE GRUPOS BACTERIANOS CULTIVÁVEIS EM SOLO DA REGIÃO SEMIÁRIDA BRASILEIRA SOB DIFERENTES USOS

RESUMO

As bactérias constituem uma das principais formas de vida presentes no solo e são afetadas diretamente pelas características dele. Cada organismo dispõe de propriedades ecológicas e fisiológicas ímpares, possibilitando a realização de distintos processos que ocorrem no solo, como solubilização de fosfato, fixação biológica de nitrogênio, decomposição da matéria orgânica e produção de compostos antibióticos. O objetivo deste trabalho foi investigar como a atividade antrópica pode interferir na dinâmica populacional de grupos bacterianos cultiváveis de solo sobre diferentes usos. Foram coletadas amostras de solo do Parque Nacional de Ubajara, localizado no município de Ubajara. A amostragem foi realizada de modo a contemplar três usos de solo distintos: preservada, conservada e agricultura. Foram quantificados os grupos microbianos: solubilizadoras de fosfato, fixadoras de nitrogênio de vida livre e actinobactérias. A abundância de cada grupo funcional foi estimada pela contagem de colônias em placa de Petri através do método spread plate. Foi espalhado 0,1 ml de cada diluição no meio de ágar de Pikovskaya, meio livre de N de Burk e meio de cultivo Caseína Dextrose Ágar. As placas foram incubadas a 28° C (± 2) e então procedeu-se com as contagens das colônias para avaliar a abundância dessas bactérias. Os resultados foram expressos como unidades formadoras de colônias (UFC. g-1). Foram realizadas análises de variância seguidas pelo teste de Tukey. contemplando as parcelas e os usos do solo. A atividade agrícola interferiu na dinâmica populacional de grupos funcionais cultiváveis do solo.

PALAVRAS-CHAVE: Microbiota do solo; Agricultura; Caatinga.

INTRODUCTION

The soil ecosystem is the main promoter of terrestrial life. Microorganisms perform ecosystem services in soil that contribute to the maintenance of its quality, as well as to the different land uses that are employed in it (OSBURN; BARRET, 2020). However, the soil presents itself as a system with different characteristics that can interfere with the forms of life that inhabit it (VOS *et al.*, 2013). Bacteria are one of the main life forms present in the soil and are directly affected by soil and climate characteristics. Interferences in the life cycle of these bacteria can cause an imbalance in the various processes that occur simultaneously in the soil (ROCHA *et al.*, 2020).

Biological nitrogen fixation (BFN) is one of the most important ecosystem functions performed by bacterial groups and is performed by diazotrophic bacteria. Biological nitrogen fixation makes atmospheric N available to plants and its importance is evidenced by the high amount of this nutrient required by plants and by its largest fraction in the soil being in organic form. Phosphate solubilization are also important to the environment and is performed by phosphate-solubilizing microorganisms. Actinobacteria are abundant in the soil and perform various functions that promote plant growth (CAVALCANTE *et al.*, 2022).

Phosphate solubilizing bacteria also act in the transformation of immobilized P into forms in which it can be absorbed by plants through the release of organic acids. Some microorganisms have the ability to solubilize phosphate, however, bacteria have a greater ability to perform this function and provide a greater amount of P (RAWAT *et al.*, 2022). The content of this macronutrient in the plant is directly linked

to its good development and the quality of seeds and fruits, thus evidencing the importance of these microorganisms (SUI *et al.*, 2022).

The actinobacteria phylum corresponds to one of the largest taxonomic units of the Bacteria domain. They are present in environments with different characteristics (ARAUJO *et al.*, 2020). Actinobacteria act in the decomposition of recalcitrant materials, production of enzymes, antibiotics and phytohormones, making these bacteria strong promoters of plant growth by direct and indirect methods (CAVALCANTE *et al.*, 2022).

Several factors are known to affect the abundance, composition and activity of microbial communities in the soil such as edaphic factors (soil type, texture, moisture, pH, nutrient availability) and land management practices (anthropic activity) (HEROLD *et al.*, 2014). Although plant community responses to environmental and anthropogenic disturbances have been well studied, microbial responses still remain poorly understood despite their critical importance for ecosystem functioning (LEFF *et al.*, 2015).

Thus, it becomes important to understand how terrestrial microbial communities will respond to disturbances in order to understand the long-term consequences of land management to sustain ecosystem services as well as to understand how the effect of different stressors can alter communities (SINGH *et al.*, 2015). The main goal of this research was to investigate how anthropic activity can interfere in the population dynamics of bacterial groups cultivable in soil under different uses.

MATERIAL AND METHODS

Study Area

The Ubajara National Park (UNP) is located in the Municipality of Ubajara, in the State of Ceará (Brazil). It has an area of 6,288 ha in the Serra da Ibiapaba. UNP is located in Brazilian semi-arid region, with an altitude ranging from 400 to 900 m above the sea. The soil collected comes from areas below 500 m, where the average annual rainfall is 943 mm and the average temperature is 28°C (FLORES *et al.*, 2017).

Sampling

The soil sampling was carried out in order to contemplate three different land uses: preserved, conserved and agriculture (Table 1). Three transects were adopted starting from inside the Conservation Unit (UNP) towards the outer areas. In each transect, samples were collected in five plots, one inside the park and four in the external areas.

TABLE 1 - Description of land uses adopted.

Land use	Description	Sample ID	Number of samples
Preserved	Forest conserved in an advanced stage of succession within the Conservation Unit.	PRE50, PRE60 PRE70,	3
Conserved	Forest conserved in an advanced stage of succession outside the Conservation Unit.	A51, A55, A61, A64, A71, A74	6
Agriculture	Agricultural cultivation areas	C52, C57, C62, C65, C72, C75	6

Source: Authors

Each soil sample was collected at a depth of 0-20 cm and were composed of five subsamples of 300 g of soil, 20 m apart of each other and the collections were made in a zigzag path. The samples were packed in identified plastic bags, kept in styrofoam boxes and sent to the Laboratório de Microbiologia Ambiental (LAMAB) of the Biology Department of the Federal University of Ceará (UFC) for further analysis. Soil and bacteria collections within the UC were authorized according to the National Council for Scientific and Technological Development (CNPQ) with the Project: CNPq/ICMBio/FAPs No. 18/2017.

Soil chemical analysis

Soil samples were evaluated for chemical characteristics, such as pH, phosphorus, total nitrogen, total organic carbon according to Teixeira *et al.* (2017).

Quantification of Bacterial Groups

Phosphate solubilizers, nitrogen fixing bacteria and actinobacteria were the microbial groups quantified. The abundance of each group was estimated by counting colonies in a Petri dish using the spread plate inoculation technique. The quantification procedures were performed in triplicate with three replications, making a database of 1620 observations. Serial dilutions were made for each analyzed area, initially dispersing 10 g of soil in 90 ml of sterile saline solution and later agitated at 120 rpm for 30 min. Thus, 1 ml of this suspension was diluted in 9 ml of sterile saline, making the 10^{-2} dilution. Following the same method, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were made for each collected soil sample.

Phosphate solubilizing bacteria count

Phosphate solubilizing bacteria were isolated from each soil sample by serial dilution by the spread plate method. For each dilution, 0.1 ml was spread on Pikovskaya agar (PVK) medium containing insoluble tricalcium phosphate and bromophenol blue to facilitate the visualization of the halos, then incubated at 28 °C (± 2) for seven days (PIKOVSKAYA, 1948; PRASAD *et al.*,2021). After incubation, colonies that showed halo zones were considered as phosphate solubilizers and colony counts were performed to assess the diversity and abundance of these bacteria. Results were expressed in colony forming units (Log CFU. g^{-1}).

Nitrogen-fixing bacteria count

Nitrogen-fixing bacteria were determined using a nitrogen-free culture medium Burk's medium with the composition: 10 g glucose, 0.41 g KH_2PO_4, 0.52 g K_2HPO_4, 0.05 g Na_2SO_4, 0.2 g $CaCl_2$, 0.1 g $MgSO_4$ $7H_2O$, 0.005 g of $FeSO_4$ $7H_2O$, 0.0025 g of Na_2MoO_4 $2H_2O$, 1.8 g of agar for semi-solid medium and 15 g of agar for solid medium, dissolved in 1 liter of distilled water (WILSON; KNIGHT, 1952; RÍOS-RUIZ *et al.*, 2020). The pH of the medium was adjusted to 7, with a variation of 0.1 and sterilized at 121°C for 15 minutes. Each dilution was spread on Petri dishes containing Burk's medium. The bacteria were incubated for seven days at 28°C (± 2) and the colonies were counted to assess the abundance of them. Results were expressed as colony forming units (Log CFU. g^{-1}).

Actinobacteria count

Each dilution was spread on the Casein Dextrose Agar (CDA) culture medium with the following composition per liter: K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, glucose 10 g, casein 0.2 g, nystatin 0.05 mg and pH ranging between 6.5 and 6.6 (KUSTER;

WILLIAMS, 1964; ARIFUZZAMAN *et al.*, 2010). The bacteria were incubated for seven days at 28°C (± 2) and then colony counts were performed to assess the diversity and abundance of these bacteria. Results were expressed as colony forming units (Log CFU. g-1).

Statistical Analysis

The abundance of bacterial groups was determined by counting colonies on Petri dishes. The quantitative data obtained by chemical analysis of the soil and colony count were submitted to the normality test and the Levene test. Analyzes of variance were performed ($p<0.05$) followed by Tukey's test, contemplating the soil samples and land uses. Data analysis was performed in PAST software and graphs were plotted in Microsoft Excel 2016.

RESULTS AND DISCUSSION

Soil chemical analysis

Table 2 presents the ANOVA results considering the chemical parameters of the soil. It is observed that the soil carbon content was the only one that showed a significant difference between land uses ($p<0.05$). The nitrogen and phosphorus content showed no significant difference.

TABLE 2 - Means of soil chemical parameters in relation to different land uses (g/Kg).

	Carbon	Nitrogen	Phosphorus
Preserved	43,30[a]	13,95*	15,40*
Conserved	27,39[b]	8,80*	7,39*
Agriculture	24,03[b]	10,62*	16,27*

Means with the same letter do not differ from each other by the Tukey test (5% significance).
* No significant difference. Source: Authors

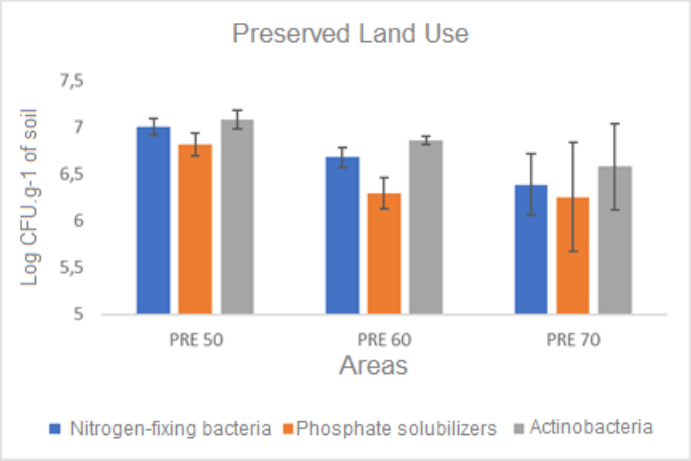
Abundance of bacterial groups in the preserved areas

The highest mean values of abundance were found in soils in the preserved area, as shown in Graph 1, with the exception of the group of phosphate solubilizing bacteria. Actinobacteria had the highest mean value of abundance among the groups with 6.80 Log CFU. g^{-1}. Considering the samples, the actinobacteria from the PRE 50 (preserved) area stood out for their highest abundance value 7.08 Log CFU. g^{-1}. On the other hand, the lowest value was found in the PRE 70 area with 6.57 Log CFU. g^{-1}.

Nitrogen-fixing bacteria expressed a mean abundance value of 6.64 Log CFU. g^{-1} and standard deviation of 0.33. In such bacterial group the PRE 50 area had the highest abundance value with 7.00 Log CFU. g^{-1}. PRE 70 area showed the lowest abundance with 6.38 Log CFU. g^{-1}.

The phosphate solubilizers obtained the lowest mean abundance value in this land use with 6.42 Log CFU. g^{-1} and standard deviation of 0.45. As in the other groups, the PRE 50 area had the highest value with 6.81 Log CFU. g^{-1}. The lowest abundance value was found in the PRE 70 area with 6.25 Log CFU. g^{-1}.

Graph 1 - Mean values of the bacterial abundance in the of preserved land use;



Source: Authors

Abundance of bacterial groups in agricultural areas

Agricultural areas also had their highest mean abundance values for the actinobacteria group as in the preserved area (Graph 2). In the soil samples belonging to this land use, the actinobacteria had an average abundance of 6.97 Log CFU. g^{-1} and standard deviation of 0.48. The highlight was the A61 area, as it presented the highest abundance with 7.42 Log CFU. g^{-1} of soil. The lowest value was verified in the A55 area with 6.33 Log CFU. g^{-1} of soil.

The average abundance of nitrogen-fixing was 6.76 Log CFU. g^{-1} with a standard deviation of 0.56. For that bacterial group, the area that presented the highest abundance average was A64 with 7.31 Log CFU. g^{-1} of soil. The lowest abundance result was found in area A55 with 5.84 Log CFU. g^{-1} of soil.

The group of phosphate solubilizing bacteria was the one with the lowest average abundance in the analyzed soil samples, similarly to the preserved area, with 6.31 Log CFU. g^{-1} and standard deviation of 0.58. Area A64 had the highest mean abundance result with 7.07 Log CFU. g^{-1} of soil. The lowest result was obtained in the area A71 with 5.58 Log CFU. g^{-1} of soil.

Abundance of bacterial groups in the conserved area

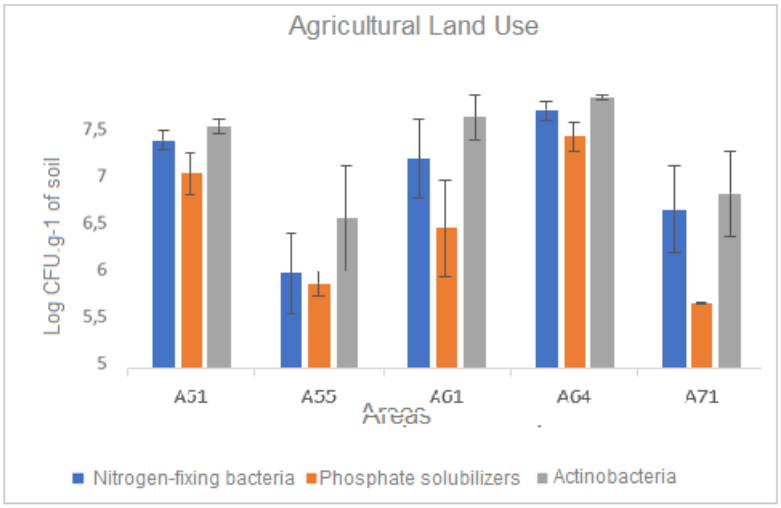
The conserved area also presented a higher *mean* abundance value for the actinobacteria group with a value of 6.66 Log CFU. g^{-1} of soil and standard deviation of 0.36 as shown in Graph 3. For this group of bacteria, area C57 had the highest abundance with 7.10 Log CFU. g^{-1} of soil. The lowest result was found in the area C52 with 6.22 Log CFU. g^{-1} of soil.

Nitrogen-fixing bacteria were the second group with the highest average abundance in the conserved area, repeating the pattern observed in the other land uses. The mean abundance for the group was 6.42 Log CFU. g-1 of soil and standard deviation of 0.41. The highest abundance was found in plot C57, with 6.91 Log CFU. g-1 of soil. The plot C52 showed lower abundance, with 6.04 Log CFU. g-1 of soil.

The group of phosphate solubilizing bacteria presented the lowest average abundance in the conserved area with a value of 6.12 Log CFU. g^{-1} of soil and standard deviation of 0.43. The area C65 showed the highest abundance value with

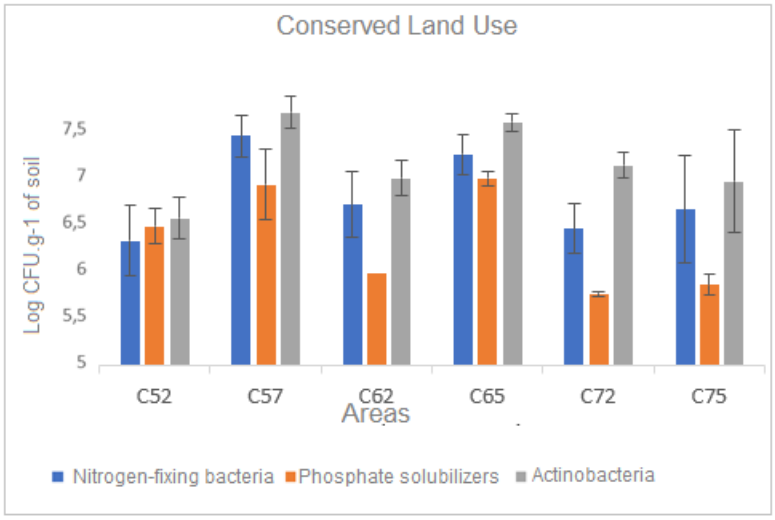
6.56 Log CFU. g^{-1} of soil and area C72 showed the lowest abundance value with 5.59 Log CFU. g^{-1} of soil.

Graph 2 - Mean values of bacterial abundance in agricultural land use.



Source: Authors

Graph 3 - Mean values of bacterial abundance in conserved land use.



Source: Authors

According to the statistical analysis, the studied soil samples showed a significant difference ($p<0.05$) considering the different land uses and the different bacterial groups. The results of ANOVA and Tukey's test are shown in Table 3.

TABLE 3 - Results of ANOVA and Tukey's test considering the bacterial groups abundance and land uses.

	Actinobacteria	Nitrogen-fixing	Phosphate solubilizing
Preserved	PRE50^{ab}	PRE50^{a}	PRE50*
	PRE60^{b}	PRE60^{b}	PRE60*
	PRE70^{b}	PRE70^{c}	PRE70*
Agriculture	A64^{a}	A64^{ab}	A64^{ab}

	A74[a]	A74[ab]	A51[bc]
	A61[a]	A51[b]	A61[bc]
	A51[a]	A61[b]	A55[bc]
	A71[b]	A71[c]	A71[c]
	A55[b]	A55[d]	
Conserved	C57[ab]	C65[a]	C57[a]
	C65[ab]	C57[a]	C65[ab]
	C62[bc]	C52[ab]	C72[bc]
	C72[bc]	C75[b]	C62[bc]
	C75[bc]	C72[b]	C75[c]
	C52[c]		C52[c]

Source: Authors

Some soil samples are repeated at the top of the list of each land use for presenting the highest abundance. Furthermore, it is possible to observe that there was no significant difference for the group of phosphate solubilizing bacteria present in the preserved area. The areas present different abundances according to the statistical analysis. There was a lot of variation in the results, which is due to several specific factors of each soil sample.

Actinobacteria had the highest average abundance. This group of bacteria has a high capacity to degrade residues present in the soil and use them as a source of carbon. Its high adaptability can explain its greater abundance in different land uses, including anthropic ones (agricultural areas). Other studies report these bacterial group as the most abundant in the soil (ARAUJO *et al.*, 2020; LEE *et al.*, 2020).

The preserved area represents a soil rich in organic matter due to the recurrent deposition of plant and animal remains, causing the soil to have a higher content of nitrogen (N) and organic carbon (OC). This makes the preserved land use soil highly conducive to the permanence of these bacteria (PASTORE *et al.*,2020). Also, the landscape suffers less anthropic influence in that land use since it is protected by law and data obtained in this use serve as a reference (control) for other land uses.

The abundance of actinobacteria that live in the soil is also affected by climate as the abundance of some genera is enhanced in dry climate regions. Dry, sandy, alkaline or neutral soils favor the maintenance of the life of actinobacteria (ZANG *et al.*,2022). This is quite relevant to the results obtained, as the sampled region has a dry climate.

Nitrogen-fixing bacteria showed higher abundance in agricultural soils. Microbial communities play a fundamental role in providing nutrients for plant growth, including N in these soils (JIA *et al.*,2020). However, the availability of N in the soil acts as a regulator of the biological nitrogen fixation process. Thus, areas with agricultural land use may have a higher average abundance of nitrogen-fixing bacteria due to their lower availability of N when compared to the preserved land use (RUDNIK *et al.*, 1997).

Soil characteristics such as vegetation cover, humidity, location in a protected area or in a semi-arid region may be factors of little influence on the survival of nitrogen-fixing bacteria. This is due to the greater influence of physical-chemical components of the soils on the survival of these microorganisms (OLIVEIRA *et al.*, 2004).

The highest average abundance of phosphate solubilizing bacteria was found in the preserved area. Results like this are scarce in the literature and further studies are needed for this group of bacteria in conservation areas. The results found in this study may be due to the set of factors that an undisturbed area can provide, including

greater deposition of organic compounds in the soil and less variation in temperature and humidity, which favor the activity of microorganisms (CONTE *et al.*, 2002).

The influence of anthropic activity was evidenced in this study as the abundance of the microbial groups studied showed a different pattern between land uses. Such changes in the population pattern of these microorganisms may reflect the disturbances caused by human activity that has the potential to cause imbalances in the dynamics of these groups that can ultimately affect their functions in the soil.

CONCLUSION

Agricultural activity interfered in the population dynamics of cultivable soil bacterial groups. Nitrogen-fixing bacteria, phosphate solubilizers and actinobacteria from agricultural cultivation areas showed differences in their abundance when compared to other uses.

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