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# ANALYSIS OF SOIL QUALITY BIOINDICATORS IN GRAIN CULTIVATION SYSTEMS (SOYBEAN AND CORN) IN THE REGION OF SANTARÉM, MOJUÍ DOS CAMPOS AND BELTERRA, PA

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## ABSTRACT
The aim of this work was to analyze the biological indicators of the soil in areas where grain, soy and corn are grown in the region of Santarém, Mojuí dos Campos and Belterra Pará. The analyses were carried out using BioAS technology, developed by EMBRAPA Cerrado. Through enzyme activity, it is possible to access the soil memory associated with the non-living fraction accumulated in the protected soil, through absorption in clay particles and organic matter. Soil samples were collected from four areas: Tapajós National Forest - FLONA (very clayey texture), Novo Horizonte Farm (very clayey texture) and two areas on Texa Farm (clayey and medium clayey texture). Samples were collected at depths of 0 to 10 and 10 to 20 cm at the end of the region's rainy season. The samples were packaged, identified and sent to the EMBRAPA Amazônia Oriental Laboratory in Belém, where enzymatic analyses of arylsulfatase and β-glucosidase were carried out. The data obtained was subjected to descriptive statistics, analysis of variance ($p<0.05$) and Tukey's test ($p<0.05$), using SISVAR statistical software and Multivariate Principal Component Analysis (PCA) was carried out using R Software. The activity of the β-glucosidase enzyme was significantly higher in the Fazenda Novo Horizonte area, favored by the practice of no-till farming and the annual increase in organic matter, and the activity of arylsulfatase was higher in the FLONA area, stimulated by the constant deposition of organic matter from the forest. Studies are needed to elucidate the low activity of β-glucosidase in the FLONA area.

**Keywords:** Soil Quality Indicators. Microbiology. Enzyme Activity. Soil memory.

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## INTRODUCTION

The soil has a very close involvement and relationship with the micro-organisms that make up the biological part of the soil and is made up of around 70% roots and animal remains, which directly affect the chemical and physical components of the soil [2]. They have an influence on various parameters relating to the sustainable use of agricultural systems, environmental systems and the planet's ecology [6]. The importance of biological components in the health and maintenance of agricultural production that can supply food to humanity in quality and quantity has increasingly raised debate in all areas of the agricultural sciences [12]. Currently, healthy soils are considered to have an adequate balance of fertility related to chemical and physical concepts such as pH, N, P, K, erosion, among others, but a healthy soil must have these concepts and one more, that of being biologically active, productive, capable of storing water, promoting the degradation of synthetic products and sequestering carbon.

Bioanalysis is developed through the soil's ability to establish and protect enzymes that are related to the ability to store and stabilize Organic Matter (OM) and other associated substances. Thus, through enzymatic activities, it is possible to access the soil's memory, which is associated with the non-living fraction that accumulates in the soil, protected through adsorption on clay particles and OM [18].

Among the enzymatic activities of the soil, the enzymes β-Glycosidase and Arylsulfatase are commonly used as biochemical indicators of the edaphic environment because they are highly sensitive to the changes caused by management systems under agroecosystems. In addition, the close relationship between the dynamics of MOS and enzymes in the soil justifies the choice of this parameter for assessing the quality, productivity and sustainability of agricultural production [10].

BioAS is the technology used to study soil bioindicators. It has been developed by EMBRAPA Cerrado for several years and, in 2020, was launched for public use [9] [10]. Its objective is to evaluate bioindicators in grain, soybean and corn growing areas in the municipalities of Santarém, Mojuí dos Campos and Belterra Pará, accessing the soil's memory in order to assess future management decisions. The importance of carrying out bioanalysis on soil samples in the region is that it allows us to access the memory and obtain a complete assessment of soil health. This is an aspect that goes unnoticed in conventional soil analysis.

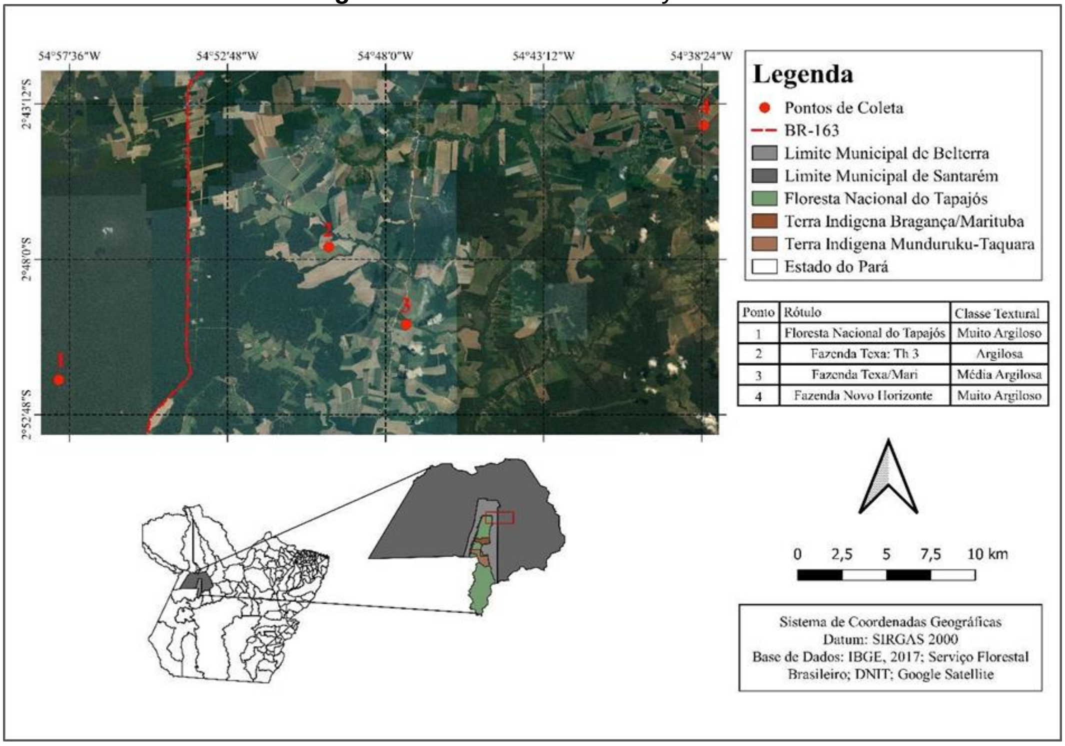
The aim of this study was to analyze the biological indicators of soil quality in soybean and corn growing areas in the region of Santarém, Mojuí dos Campos and Belterra/PA.

## MATERIAL AND METHODS

The study was carried out in agricultural production areas in the municipalities of Santarém, Mojuí dos Campos and Belterra, in the west of the state of Pará, in the lower Amazon region and in the Tapajós National Forest. According to the Köppen classification. The state of Pará is under the influence of three climatic types (Af, Am and Aw) and the driest month has an average rainfall of less than 60 mm, for the Am and Aw types and Af is equal to or greater than 60 mm. Annual rainfall varies from 2,000 mm to 3,000 mm, distributed irregularly, with a short dry period. However, the current month draws attention to the high spatial variability of the climate in the state. The climate in the three municipalities is very close to each other and falls into the Am4 classification, since the driest month has an average rainfall of less than 60 mm and the average annual rainfall is between 1,500 mm and 2,000 mm. The dry period extends from approximately July to November [13].

The study was conducted on two rural properties and in the Tapajós National Forest, the latter being considered the absolute reference area (Figure 1). The FLONA is a Conservation Unit created by Decree in 1974 and is intended primarily for the sustainable exploitation of forest resources and scientific research. The study area is located at latitude -2.86199190416132 and longitude -54.9653728473364. The soil texture was classified as Very Clayey and the soil was collected at depths of 0 to 10, the diagnostic layer for microbiological analysis, and 10 to 20, an important layer in determining the storage and cycling of nutrients.

**Figure 1.** Location of the study areas.




**Source:** the authors

The two properties selected for the study were Fazenda Texa and Fazenda Novo Horizonte, located in the municipality of Santarém, approximately 3 km from each other. At Fazenda Texa, two plots were selected, the first (Th 03), classified as clay-textured soil, covering 100 hectares. Before being acquired by the current owner, the area was cultivated with pasture and was in a process of degradation. In 2019, the area was prepared for the cultivation of soybeans and corn (annual crops). The area was prepared using conventional tillage to grow grains. In the same year, the area was limed with 3 tons of lime per hectare and 600 kg of NPK in the 4-20-30 formulation. The following year, the land was fertilized again with 500 kg of NPK 5-25-25 and, in 2021, it was limed again with 3 tons of limestone per hectare and fertilized with 500 kg of NPK 4-20-30.

The second plot on Fazenda Texa (Th 02) was classified as having medium loamy soil, with an area of 80 hectares. Similar to Th 03, this area was previously cultivated with pasture in a state of degradation and was traditionally prepared for the cultivation of soybeans and corn in a no-till system. The liming and fertilization of the area were similar to those used in the previous area.

The plot chosen at Fazenda Novo Horizonte has an area of 50 hectares and the soil was classified as Very Clayey. The area was opened in 2010 to grow soybeans and corn. It was previously an area of secondary forest that was cleared for preparation. In the first year of cultivation, 3 tons of lime and 500 tons of NPK 4-25-30 were added. Since then, 1 t ha-1 of limestone and 1.5 t ha-1 of road litter have been added every two harvests. In 2020, 1.6 t ha-1 of agricultural gypsum was incorporated and forage grasses were planted to provide cover and increase organic matter in the soil, in addition to the straw from the crop itself produced in a no-till system. In 2021, a new fertilization was carried out with 250 kg of NPK 5-20-30 and the forage was planted in the off-season.

The productivity data from the study areas showed that the Novo Horizonte farm stands out in terms of average productivity over the last 3 years of cultivation. Corn yields were, on average, 1280 kg higher than on the Texa farm with clay-textured soil and 1450 kg higher than on the Texa farm with medium-clay-textured soil. As for soybean yields, the Novo Horizonte farm produced 550 and 800 kg more than the Texa farm areas.

The Novo Horizonte farm area had the highest levels of clay in the soil, which directly influences the availability of nutrients, CEC (Cation Exchange Capacity) and water retention in the soil, favoring the development of crops. In addition, the specific surface area in clay soils is greater than in sandier soils, which provides a suitable environment for the development of microorganisms. The CEC and the dominant type of charge in the soil (+, - or without charge) directly affect the interaction between microorganisms and the soil [11].

**Table 1.** Average productivity of the study areas.

| **Area** | **Soil type** | **Productivity* Soybean** | **Productivity* Milho** | **Clay content ($g\ kg^{-1}$)** |
|---|---|---|---|---|
| N. Horiz. | Very clayey | 3.600 | 6.000 | 845,60 |
| Texa1 | Clayey | 3.050 | 4.720 | 585,00 |
| Texa2 | Medium clay | 2.800 | 4.550 | 195,00 |

*Average productivity of the last 3 years of cultivation (2022, 2021, 2020).

The sampling methodology used followed the recommendations of Mendes et al. (2018) for analyzing the parameters used by BioAS technology. This collection methodology was adopted to facilitate the analysis process for producers, so that different collections are not required for fertility and bioindicator analyses. In this way, the same sample can be prepared and used for both analyses. An interpretation table (Table 1) was developed, based on BioAS technology, exclusively for samples collected after harvest and dried in the air (Mendes et al., 2018). The CLOVER CRM mobile app, developed by DATACOPER, was used to collect the coordinates of the analyzed areas.

The sampling depth should be from 0 to 10 cm because it is the layer with the greatest microbial and enzymatic activity, serving as a diagnostic layer of the soil's biological quality. However, in the complete BioAS, which involves detecting the conditions not only of cycling, but also of nutrient supply storage in the soil, as well as the determinations of the chemical biological quality indices and these calculated in aggregate, a second soil sample should be analyzed, usually in the 0 to 20 cm layer.

The analysis of the β-glucosidase enzyme was carried out by colorimetric determination of the p-nitrophenol that is released by the β-glucosidases in the soil when it is incubated with a solution of p-nitrophenyl-β-D-glycopyranoside. To do this, the soil was sieved through a 4 mm sieve and all the coarser pieces of tissues and plants were removed with tweezers in order to eliminate possible interference from organic residues. The reading is made on a spectrophotometer at 420 nm [17] [4].

Arylsulfatase analysis was carried out by colorimetric determination of p-nitrophenol released by the enzymes from the soil when incubated with buffered p-nitrophenol sulfate solution. The soil was sieved through a 4 mm sieve and all the coarser pieces of tissue and plants were removed with tweezers in order to eliminate possible interference from organic residues. The intensity of the yellow coloration was determined using a spectrophotometer at 410 nm [17] [4].

The data obtained was subjected to descriptive statistics, analysis of variance ($p<0.05$) and Tukey's test ($p<0.05$) using SISVAR statistical software [6]. In addition, a multivariate principal component analysis (PCA) of the data was carried out using R software [15] to obtain a visual representation of the main characteristics and distribution of the soil's biological attributes in the different areas.

To construct the Principal Components graph, the data from the bioindicators analyzed at both depths was used to construct a visualization that took into account the entire initial layer reached by the roots of annual crops such as soybeans and corn. Location/texture was defined as a qualitative variable in order to visualize the distribution pattern of the vector loads of the results. In this way, it is possible to visualize the relationship between the collection site and the better or worse results in relation to the bioindicators.

## RESULTS AND DISCUSSION

Lopes [8] and Mendes [9] evaluated soil quality parameters and defined some interpretation classes based on enzyme activity. The interpretation classes are identified by the colors red (low activity), yellow (moderate activity) and green (adequate activity). These interpretation classes were constructed using regression models based on nutrient calibration systems and represent the current state of the soil in terms of its enzymatic activity.

Arylsulphatase activity was found to be adequate in all the areas and depths studied, although they differed according to Tukey's test, with the FLONA area showing the highest activity values for this enzyme (324.81), more than double that of the other areas (Table 2). Arylsulfatase activity was also statistically different on the Novo Horizonte farm (very clayey soil), which was lower than the FLONA results, but higher than the results from the areas on the Texa farm. The result for the FLONA was expected as it is an area of native forest and little anthropized. The high amount of organic matter from the decomposition of animal and plant remains contributes to the high biological activity of this soil, which promotes an enzymatic residual reflected in the results observed. Similarly, the Novo Horizonte farm stands out due to its history of previously being an area of secondary forest and, after it was opened up to annual crops, it was always managed using soil cover, no-till farming and an increase in organic matter with the annual application of poultry litter [9] [8] [10].

**Table 2.** Averages and Tukey's test ($p<0,05$) of the values of enzime activity (µg of p-nitrophenol $g^{-1}$ of soil) and interpretation classes of the soil quality bioindicators for the different areas and soil textures analyzed. (Should be colored)

| Depth (cm) | Location | Texture | β-glucosidase | Arylsulfatase |
|---|---|---|---|---|
| 0 a 10 | Tapajós FLONA | Very clayey | 37.82 b | 324.81 a |
| | Novo Horizonte | Very clayey | 83.47 a | 137.18 b |
| | Texa I | Clayey | 43.98 b | 96.64 c |
| | Texa II | Medium clay | 39.74 b | 112.54 bc |
| 10 a 20 | Tapajós FLONA | Very clayey | 21.26 c | 277.16 a |
| | Novo Horizonte | Very clayey | 50.14 a | 154.09 b |
| | Texa I | Clayey | 31.37 b | 112.86 bc |
| | Texa II | Medium clay | 31.96 b | 101.85 c |

**Source:** The authors

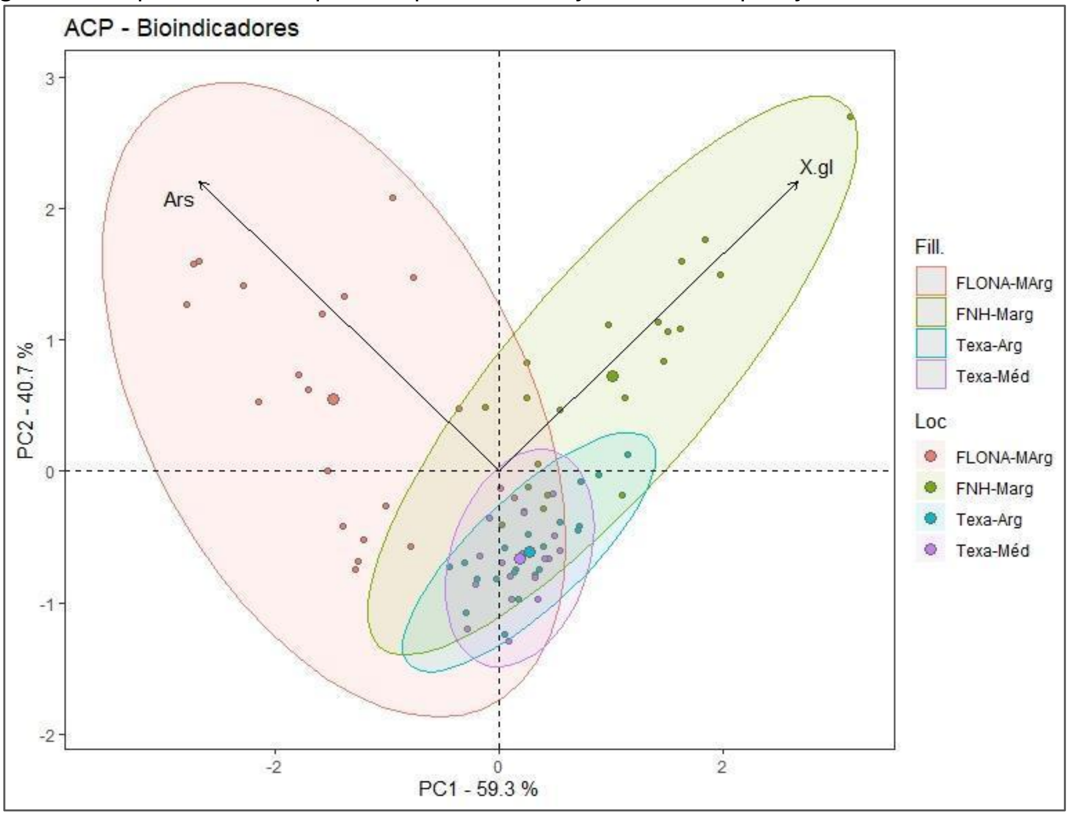
It can be seen that the enzyme activity values of Arilsulfatase were lower in the Texa Farm areas, regardless of soil class, than the soils in the FLONA and Novo Horizonte Farm areas (Table 2). Bearing in mind that the areas were previously occupied by pastures in a stage of degradation and were converted into annual plantations, it is clear that proper management is important to guarantee structural, chemical and biological conditions to support the management of annual crops [10] [16].

It is important to note that the β-glucosidase activity was categorized as regular, being higher than all the other areas, including the FLONA area. This result shows that management based on no-till farming and the annual addition of organic matter are techniques that significantly increase soil quality [14] [9].

Soil texture may be an important factor in the results obtained in this study, since clay particles form aggregates more easily, which may be important for maintaining enzymatic activity [7]. The average productivity of the last 3 years of cultivation was higher in the Novo Horizonte farm area for both the soybean and corn crops. The soybean crop was 550 and 800 kg higher than the other areas and the corn crop was 1280 and 1450 kg higher. On the Novo Horizonte farm, the cultivated area was previously secondary forest, which in itself represents a greater contribution of organic matter and greater soil protection. Mendes [9] and Lopes [8] highlight the importance of minimally adequate management for improving soil bioindicators and their consequent influence on the area's productivity. Added to this is the annual increase in organic matter (road litter) and the use of the no-till method, which increases the amount of organic matter and protects the soil against erosion and water loss.

According to the Principal Components Analysis (Figure 6), the variables analyzed explain 100% of the total variation in the study and also have the same individual contribution and are not correlated. This shows that both enzymes studied and used in BioAS technology are relevant to the study of soil biological quality [9] [10]. Analyzing the qualitative factors assigned (Location/texture), it can be seen that the FLONA and Fazenda Novo Horizonte areas stood out in different ways. The FLONA data is more clustered and related to the activity of Arylsulfatase, an enzyme involved in the sulfur cycle. The FLONA area had the highest values for the activity of this enzyme due to the better soil conditions for nutrient cycling. On the other hand, Fazenda Novo Horizonte stood out for its β-glucosidase activity, which was higher than the Texa farm areas combined and more than 2 times higher than in the Tapajós National Forest area. This analysis corroborates the hypothesis that soil management with cover crops (no-till) and the frequent addition of organic matter (cattle litter) promoted an increase in enzymatic activity and, consequently, improved soil quality [8] [9] [10].

**Figure 2.** Graph of the Principal Component Analisys of the soil quality bioindicators evaluated.




**Source:** The authors

β-glucosidase acts in the final phase of cellulose breakdown and is responsible for hydrolyzing the β-D-glucosidic bonds of a wide range of compounds, including disaccharides and short-chain oligosaccharides, releasing glucose molecules from their non-reducing ends [1]. Thus, the action of this enzyme secreted by microorganisms is essential for them to obtain the glucose that will be used in their metabolism.

The high activity of β-glucosidase in the Novo Horizonte farm area can be attributed to the high clay content, which favors the interaction of microorganisms with soil colloids [11] combined with the organic matter content from years of cultivation in a no-till system, which improves the environment for carbon cycling as it offers a larger interaction area with both soil clay and OM [11] [1]. The low activity of β-glucosidase in the FLONA area may be related to the enzyme's tolerance to glucose, since there may be an inhibitory effect of glucose on the catalytic activity of β-glucosidase [3]. However, more studies are needed to assess the reasons why the activity of this enzyme was lower than expected, so that management practices can be optimized to obtain maximum enzymatic efficiency.

## CONCLUSIONS

1. The activity of β-glucosidase was significantly higher in the Fazenda Novo Horizonte area, demonstrating that the no-till system and the addition of organic matter favored biological activity in this soil.
2. Arylsulfatase activity was higher in the FLONA and Novo Horizonte farm areas compared to the Texa farm areas due to the deposition of organic matter (FLONA) and management with no-till farming and annual organic fertilization (Fazenda Novo Horizonte).
3. Further studies are needed to ascertain the reasons for the low β-glucosidase activity in the FLONA and Fazenda Texa areas.
4. The analysis of enzymatic activity, especially β-glucosidase and arylsulfatase, using the BioAS methodology, proved to be suitable for assessing the quality and productivity of the soils analyzed, since the results showed that soils with higher enzymatic activity were more productive.
5. Principal Component Analysis showed that the enzymes analyzed make the same contribution to the variations in the study.

## CONFLICT INTERESTS

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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