The conversion of murundu fields into agricultural areas impacts soil attributes after several years of cultivation

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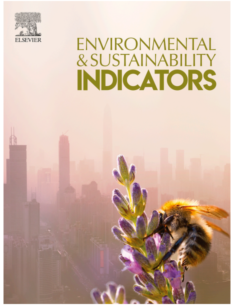

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THE CONVERSION OF MURUNDU FIELDS INTO AGRICULTURAL AREAS IMPACTS SOIL ATTRIBUTES AFTER SEVERAL YEARS OF CULTIVATION

Marisângela Viana Barbosa[a], Aline Oliveira Silva[a], Jessé Valentim dos Santos[a], Douglas Siqueira Freitas[b], Flávia Louzeiro de Aguiar Santiago[a], Flávia Reis Sales[a], Jordana Luísa de Castro[a], Juliana Volpi Emrich Pinto[a], Luciane Reis Sales[a], Nury Mariel Lutgarda Cazon Tapias[a], Olavo Augusto Arquimed Lopes de Sá[a], Raquel Milagros Rodríguez-Rodríguez[c], Thiago Palhares Farias[d], Amanda Azarias Guimarães[a], Marcia Rufuni[a], Paula Rose de Almeida Ribeiro[e], Fatima Maria de Souza Moreira[a]. Marco Aurélio Carbone Carneiro[a]*

[a]Setor de Biologia, Microbiologia e Processos Biológicos do Solo, Departamento de Ciência do Solo, Universidade Federal de Lavras (UFLA), Lavras, MG, Brazil.
[b]Universidade do Estado de Minas Gerais - UEMG, Minas Gerais, Brasil.
[c]Departamento de Biofertilizantes y Nutrición de las Plantas, Instituto Nacional de Ciencias Agrícolas, Mayabeque, Cuba.
[d]Instituto Federal de Educação, Ciência e Tecnologia do Maranhão - IF, Maranhão, Brasil.
[e]Universidade do Estado da Bahia - UNEB, Bahia, Brasil.

*Corresponding author: Universidade Federal de Lavras, Departamento de Ciência do Solo. Caixa Postal 3037, CEP: 37.200-000, Lavras - MG, Brasil. marcocarbone@ufla.com.br

THE CONVERSION OF MURUNDU FIELDS INTO AGRICULTURAL AREAS IMPACTS SOIL ATTRIBUTES AFTER SEVERAL YEARS OF CULTIVATION

ABSTRACT

The effect of *Murundus Field* conversion into agricultural areas on the physical, chemical and biological attributes of soil have been evaluated in 3 no-till chronosequences. The study was conducted at reminiscent *Murundus Fields*, at 3 other adjacent areas that were converted into agricultural fields in the state of Goiás-Brazil, over 11 (PD11), 15 (PD15) and 18 (PD18) years under a no-till system, and at a control area that suffered no anthropic interference. For this study, the native area was subdivided into mound bases (BM) and tops (TM) of murundus, due to environmental contrast among these environments. The physico-chemical and biochemical soil attributes evaluated were: fertility, texture, organic carbon, microbial biomass carbon, basal respiration, metabolic and microbial quotients, urease activity and fluorescein diacetate hydrolysis. In addition to these, microbial group densities were determined, that will vary between: total heterotrophic bacteria (2.36×10^6 and 5.93×10^6), total fungi (2.90×10^3 and 9.30×10^3), total actinobacteria (2.26×10^4 and 8.92×10^4) phosphate solubilizers (2.80×10^3 and 4.20×10^3), cellulolytic organisms (7.99×10^4 and 1.18×10^4), associative nitrogen-fixing bacteria (1.25×10^4 and 4.24×10^4), nitrifiers (0.00 and 9.37×10^5) and ammonifiers (1.17×10^5 and 5.20×10^4). The conversion of native areas into no-till systems presented tall in areas with PD15 (184.2 $\mu g\ g^{-1}$) and PD18 (316.0 $\mu g\ g^{-1}$) microbial biomass carbon in relation to the control area, however, there was a reduction of actinobacteria density when comparing murundus mound bases to their tops, as well as reduced phosphate solubilizers, cellulolytic organisms and associative nitrogen-fixing bacteria. *Murundus Fields* conversions into agricultural areas alter soil microbiota, which may affect important functional processes, possibly aggravating the frailty of this phytophysiognomy.

Keywords: Soil use changes; different chronosequences, functional groups; microbial activity; biological indicators.

1. INTRODUCTION

The Cerrado is the second largest tropical biome in Brazil and in South America, responsible for housing about 5% of global diversity and 30% of the Brazilian flora and fauna, many of which species being endemic, and is considered a biodiversity hotspot (Myers et al., 2000; Françoso et al., 2015). Furthermore, the Cerrado stands out as a great agricultural frontier for the production of food, fibers and biofuels (Lopes and Guilherme, 2016). The region's agricultural expansion has led to the incorporation of areas with lesser agricultural aptitude, such as the "*Murundus Fields*", inserted in this scenario (Assis et al., 2014; Carneiro et al., 2015; Souza et al., 2016; Martins et al., 2020).

The "*Murundus Fields*" are phytophysiognomies associated to soils which contain strong hydromorphism that encompass millions of km^2 and occur throughout the whole world, ranging from the South and North Americas to Oceania and even Africa (Rahlao et al., 2008; Midgley, 2010; Cramer et al., 2012; Paulino et al., 2015; Tarnita et al., 2017; Souza et al., 2020). These environments perform numerous ecosystemic duties, primarily at maintaining water sources (Gomes Filho et al., 2014; Souza et al., 2016), and, moreover, their biodiversity is relevant in the fulfillment of ecological duties (Barnes et al., 2014; Krashevska et al., 2015, Wang et al., 2017).

To convert *Murundus Fields* into agriculturally productive areas, great structural modifications must be applied to the landscape, such as the construction of drainage channels, terrain leveling by destroying the mounds and soil acidity and fertility adjustments. All these changes alter the physical, chemical, and biological properties of the soil, with subsequent effects over biodiversity and biological functions, compromising the ecosystems' productivity and sustainability (Souza et al., 2016; Pontes et al., 2017; Sá et al., 2017; Silva et al., 2018; Souza et al., 2020; Martins et al., 2020; Teixeira et al., 2021).

The changes in the use of these areas impact the structure of the environment's biological community, especially the microbial community in the soil, which is responsible for performing essential tasks that maintain ecosystems (Santos et al., 2015; D'Acunto et al., 2018; Pinto et al., 2020; Chatterjee et al., 2021; Tassano et al., 2021). The microorganisms in the soil are responsible for decomposition and nutrient cycling, aggregation and structuring, the formation of root symbiosis and several other processes that benefit plants and the environment. Soil microbiota are the first to be affected by use and handling, because they present great sensitivity towards environmental change, and can be utilized as soil quality indicators (Mendes et al., 2013; Assis et al., 2014; Silva et al., 2018; Souza et al., 2020; Carneiro et al., 2019; Aragão et al., 2020).

Although there are a few studies on the impact of converting Murundus Fields phytophysiognomy in areas into agricultural ones (Assis et al., 2014; Souza et al., 2016; Paulino et al., 2015; Carneiro et al., 2019; Martins et al., 2019), there is still a gap in the knowledge, as for the effects conversion has about the biology of soil, in the functional groups and communities of edaphic microorganisms, the physical and chemical characteristics of the soil. Especially for being an ecosystem fragile, whose equilibrium is regulated by the processes carried out by the biological diversity of the soil.

Therefore, this study aimed to assess whether the different chronosequences of the conversion of Campos de Murundus into agricultural areas affect the soil microbiota and the balance of the ecosystem. The study evaluated the effects that *Murundus Fields* being converted into agricultural areas had on the physical, chemical, and biological soil attributes, within three no-till chronosequence conversions.

2. MATERIALS AND METHODS

2.1 Experimental area description

The study was conducted in alluvial plains under *Murundus Fields* phytophysiognomy, in the Jataí county, Southwest Goiás - Brazil (17° 58' 20” S and 52° 04' 56” W). Whose tropical climate with a dry season in winter (Cw) Köppen-Geiger, with an annual temperature ranging from 30 °C and a minimum of 17 ºC, an annual relative humidity of 70% and accumulated rainfall of 1704 mm. The soil was classified as an Entisol (Petroplintic Tb Dystrophic Haplic Gleysols) (Survey Staff, 2014).

The study analyzed a *Murundus Fields* chronosequence conversion into agricultural areas, which used no-till systems during 11 (PD11), 15 (PD15) and 18 (PD18) years, apart from a control area that suffered no anthropic interference. The native area was subdivided into mound base (BM) and top (TM) according to the differences between natural soil drainage and vegetation structuring and composition (Paulino et al 2015). The agricultural areas were cultivated, annually, with the vegetable species of soy [*Glycine max* (L.) Merrill] - from November to March - and corn (*Zea mays* L.) - from April to August. These areas were maintained in fallow between August and November. In the native area, the vegetation is of the cerrado type (Neotropical Savanna), with grass-plots between the mounds (Figure 1).

The conversion processes began with the removal of vegetation followed by preparing the soil with plowing and harrowing. The leveling of the areas for cultivation was done via plow equipped with frontal and back-drag blades, followed by subsoiling, ground leveling, of the liming and fertilization, before the initial establishment of planting in the areas in no-till culture system.

2.2 Soil sampling and attribute evaluation

In each of the study areas, five samples of soil were made (n = 5). Each of these samples composed of soil was carried out at a depth of 0 - 10 cm, collected in parallel transects, spaced 50 m apart from each other. In each of the transects, five simple samples were taken, collected at a distance of 5 m from each other, assembled to form a composite sample. This same procedure was performed in all areas of the study. In all, twenty-five composite samples were collected, which were packed in sterile plastic bags and immediately stored at 4 ° C, until the analysis was carried out, and sent to the laboratory. In the laboratory, part of the samples was dried, sieved in a 2 mm mesh, and sent for analysis of physical and chemical attributes. Part of the samples was used for biological analysis of the soil.

The physical and chemical attributes determined were: pH in H_2O; P extracted by resin; K^+, Fe^{2+}, Zn^{2+}, Mn^{2+} and Cu^2+ extracted by the Mehlich-1 solution (Mehlich, 1953); Ca^{2+}, Mg^{2+} and Al^{3+} extracted by KCl 1 mol L^{-1} (Mehlich, 1953); potential acidity (H+Al) by SMP extractor (Shoemaker et al., 1961); texture by the Bouyoucos method (Boyoucos, 1951), (Table 1).

Organic carbon (Corg) was determined via the oxidation of potassium dichromate in the presence of H_2SO_4, followed by the back titration of the dichromate excess in a sample with ferrous ammonium sulfate and diphenylamine (1%) solution (Walkley and Black, 1934).

Microbial biomass carbon (MBC) was determined via the irradiation of a 20 g soil sample using a microwave oven with 120 V supply voltage (60 Hz), 2.450 MHz frequency and 1.35 KW power, during three minutes, in order to kill the microorganisms so that they released their cellular components (Islam and Weil, 1998). The carbon extraction via potassium sulfate (0.5 mol L^{-1}) was then done and followed by potassium dichromate oxidation in acidic medium (0.066 mol L^{-1}) and ferrous ammonia sulphate titration (0.033 mol L^{-1}) (Vance et al., 1987). The choice of microwave irradiation method to replace the use of chloroform occurred mainly due to the fact that it generates less waste, and is an efficient alternative as much as the use of chloroform.

Soil basal respiration (RBS) was estimated via CO_2 released from samples in soil incubated for three days, captured by a NaOH 0.5 mol L^{-1} solution and subsequently measured through titration with HCl (0.5 mol L^{-1}) using phenolphthalein (1%) as indicator (Alef, 1995). The metabolic quotient ($q$$CO_2$) was obtained by the RBS and MBC ratio (Anderson and Domsch, 1993). The microbial quotient (*q*Mic) was calculated by the CBM and Corg relation (Sparling, 1992).

Total soil enzymatic activity was determined via hydrolysis of fluorescein diacetate (FDA) in 2.5 g samples incubated at 37°C in conjunction with a fluorescein solution ($C_{20}H_{12}O_5$ 10 mg L^{-1}) in a sodium phosphate buffer (60 mmol L^{-1} a pH 7.0) for 24 hours, shaking at a speed of 50 rpm (Dick et al., 1996). The reaction was then interrupted with acetone (50%) and centrifuged for 5 minutes at 3.000 rpm; the supernatant was filtered and reading was done in a spectrophotometer at 490 nm.

Urease activity was estimated from 5 g of soil samples that were incubated for two hours at 37°C in the presence of toluene, THAM buffer (0.05 mol L^{-1}, pH 9.0) and a urea solution (0.2 mol L^{-1}) (Tabatabai and Bremner, 1972). The reaction was then interrupted with the addition of KCl-Ag_2SO_4 (2.5 mol L^{-1}, 100 mg L^{-1}), the solution was shaken (150 rpm) for 30 minutes and filtered, and the ammonium was determined by microdistillation (Keeney and Nelson, 1982).

The quantification of functional groups in the soil was done by plate counting or the most probable number after the inoculation of soil suspensions obtained via serial dilution of 10 g of soil in 90 mL of sterile saline solution (0.85% NaCl), followed by the inoculation of 0.1 mL aliquots in different culture mediums. For the quantifying of total heterotrophic bacteria, a nutrient culture agar medium with 5.0 g of peptone, 3.0 g of meat extract, 1.0 g of yeast extract, 5.0 g of glucose, 1 L of distilled water, 15 g of agar, in pH 6.8 was added to a cycloheximide solution at 0.03%. For total fungi, a Martin medium (Martin, 1950) was utilized with 10 g of glucose, 5.0 g of peptone, 0.5 g of yeast extract, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of rose bengal, 1 L of distilled water, 15 g of agar and a streptomycin solution at 0.03%. The quantifying of total actinobacteria was determined in an SCN medium (Wollum, 1982) comprised of 10 g of glucose, 0.3 g of casein, 2.0 g of KNO_3, 2.0 g of NaCl, 2.0 g of K_2HPO_4, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.04 g of $CaCl_2$, 0.01 g of $FeSO_4.7H_2O$, 1 L of distilled water, 15 g of agar and in a pH of 7.2, with nystatin 50 mg mL^{-1}, cycloheximide 50 mg mL^{-1} and 0.035 g of bengal rose.

For the phosphate solubilizers microorganisms two culture mediums were utilized: the NBRIP (Nautiyal, 1999) and the GES (Sylvester-Bradley et al., 1982). The most probable number (NMP) of ammonifiers was according to Sarathchandra (1978). The cellulolytic NMP was according to the methodology of Parkinson et al. (1971). The quantifying of nitrifiers was according to the methodology described by Schimidt and Belser (1982). Free-living N_2 fixing bacteria were evaluated by using two culture mediums: FAM (FixFAM) - described by Magalhães and Döbereiner (1984) that favors *Azospirillum amazonense* and the NFb (NFB) medium that favors *A. lipoferum*, *A. brasilense*, *A. irakense* and *A. halopraeferans*, as described by Döbereiner et al. (1995). Both mediums can favor the growth of other N_2 fixing bacteria (Pinto et al., 2020).

2.3 Statistical analysis

The data was subdued to normality analysis, and then the normals were submitted to variance analysis (ANOVA) and the Scott-Knott test (5%) through the SISVAR statistical program (Ferreira, 2011). The soil attributes were submitted to the Pearson correlation test using the R software (ggplot2, ggcorrplot and corrplot packages) (R Development Core Team, 2011). A principal component analysis (PCA) was made using the R software (vegan and gbbiplot software) in order to observe the spatial planning of the variables that belong to the evaluated areas; eigenvalues larger than 0.5 were considered to indicate a strong association between the main components.

3. RESULTS

3.1 Physical and chemical soil attributes

Attribute analysis was done according to Alvarez et al. (1999). Soil pH values did not present significant differences ($p > 0.05$) and varied between 4.10 and 5.49, which is considered high acidity, in their native environments, and even intermediate acidity on PD11 (Table 1). P, Ca, Mg and K values were lower in the native areas in comparison to the cultivated ones, the lowest values being attributed to TM (Table 1), which were between low and intermediate. 5), BM (Al: 8.15 mmol$_c$ dm^{-3}; H+Al: 55.6 mmol$_c$ dm^{-3}) and TM (Al: 8.88 mmol$_c$ dm^{-3}; H+Al: 59.9 mmol$_c$ dm^{-3}) (Table 1), showing active and high potential acidity in native areas (Alvarez et al., 1999). The Fe content varied between 49.2 mg dm^{-3} and 79.6, 21 mg dm^{-3}, with lower values in PD11. The Mn content, however, varied between 0.5 mg dm^{-3} and 1.21 mg dm^{-3}, with larger values in PD11. Zn presented the largest values in PD15 (Table 1). All the evaluated areas presented a clayey texture, although they had larger clay content in cultivated areas significant (Table 1). Total organic carbon (COT) varied between 17.21g kg^{-1} and 22.03 g kg^{-1}, with larger values in BM, TM and PD11 (Table 2).

3.2 Biochemical soil attributes

Microbial carbon biomass (MBC), microbial quotient (*q*Mic), metabolic quotient (*q*CO$_2$) and soil basal respiration (RBS) results are shown in Table 2. It has been observed that the CBM and *q*Mic values were higher in PD15 and PD18 ($p < 0.05$), varying, respectively, between 184.2 μg g^{-1} to 316.0 μg g^{-1} and 0.93 % to 1.77 %. The RBS, however, varied between 8.57 to 8.82 mg C-CO$_2$ g^{-1}, being higher in native areas (Table 2). There were no significant differences ($p < 0.05$) for the *q*CO$_2$ among the evaluated areas (Table 2).

The enzymatic activity results are presented in Fig. 2. Urease activity was bigger in the native areas (BM and TM), having a 60% reduction comparatively to the control area (BM) and the PD11 cultivation (Fig. 2A). On the other hand, there were no significant differences for the fluorescein diacetate (FDA) hydrolysis ($p > 0.05$) (Fig. 2B).

3.3 Soil microorganism functional groups

Time and cultivation did not significantly influence the density of total heterotrophic bacteria, NFB nitrogen-fixers and ammonifying microorganisms (Table 3). Actinobacteria density varied between 2.26 x10^4 UFC g^{-1} and 8.92 x10^4 UFC g^{-1} of soil, with largest values observed in TM. Total fungi presented the lowest values in TM, not differing between uses (Table 3). Phosphate solubilizers in both mediums (NBRIP and GES) presented higher density in the BM control area, as was also observed for the cellulolytic microorganisms, nitrogen-fixers in FAM medium and nitrifiers ($p < 0.05$) (Table 3).

3.4 Pearson Correlation

The correlations between the physical, chemical, biochemical, and biological soil attributes were presented in Fig. 2. COT presented positive correlations with RB ($r = 0.4$; $p < 0.05$), NBRIP ($r = 0.5$; $p < 0.05$); Al ($r = 0.4$; $p < 0.05$); silt ($r = 0.6$; $p < 0.05$) and sand ($r = 0.5$; $p < 0.05$), and negative correlation with nitrifiers, P and B ($r = -0.5$; $p > 0.05$), Cu ($r=-0.4$; $p > 0.05$), Zn ($r = -0.1$; $p > 0.05$) and clay ($r = -0.7$; $p > 0.05$), (Fig. 2). CBM presented positive correlation with total fungi, nitrifiers, pH and P, K, Ca, Mg, B, Mn, Cu, Zn, and clay contents (Fig 2). RBS correlated positively with urease ($r = 0.5$; $p < 0.05$), Al ($r = 0.6$; $p < 0.05$) and silt ($r = 0.4$; $p < 0.05$) activities and negatively with pH ($r = -0.6$; $p < 0.05$), P ($r= -0.7$; $p<0.05$), Ca and B ($r=-0.5$; $p < 0.05$), Mg and Zn ($r = -0.6$; $p < 0.05$) and clay ($r = -0.4$; $p < 0.05$) (Fig. 3). Total bacteria correlated positively with fixers in the FAM medium, ammonifiers and silt contents (r =

0.5; $p < 0.05$) as well as with the solubilizers in both culture mediums (r = 0.4; $p < 0.05$). The total fungi correlated more strongly with solubilizers in the GES medium (r = 0.6; $p < 0.05$) and with B contents (r = 0.4; $p < 0.05$) contents. Among the functional groups, just fixers in the NFB medium correlated with the solubilizers in the GES (r = 0.5; $p < 0.05$), and the ones in the FAM medium with the solubilizers in both mediums (r = 0.6; $p < 0.05$). The solubilizers correlated among themselves (r = 0.5; $p < 0.05$) (Fig. 3). The pH correlated strongly with P, K, Ca, Mg, B, Mn, Zn.

3.5 Principal component analysis

In the PCA it was observed that the components (PC1 and PC2) explained together 56.43% of the variations in the biological, biochemical, physical, and chemical characteristics of the soil in the areas that were studied (Fig. 4). The spatial distribution of data showed the formation of two large groups. Group 1 assembles the cultivated areas (PD11, PD15 and PD18), primarily due to the differences in the chemical attributes of the soil (pH, P, K, Ca, Mg), in the clay content and in the concentration of nitrifying microorganisms (Fig. 4) The cultivated areas formed individual subgroups inside group 1 (Explain 1a, 1b and 1c). Group 2 corresponded to the native area (Fig. 3). Inside this group, the subgroup 2a was formed, composed of the mound bases (BM), which was influenced primarily by the content of COT, silt, sand, solubilizers in Nbrip, cellulolytics and ammonifiers. And the subgroup 2b, which was composed of the top of the mounds (TM), was separated by the influence of urease, RB and Al contents (Fig. 4). The attributes that positively correlated the most to the PC1 were COT, RB, phosphate solubilizers in Nbrip, urease, Al, silt, and sand, and negatively, pH, P, K, Ca, Mg, B, Mn, Zn, and clay. With the PC2, however, the positively correlated were bacteria, fungi, microorganisms in the FAM medium and phosphate solubilizers and the negatively correlated were actinobacteria and Cu.

4. DISCUSSION

Murundus Field conversion into agricultural areas, even in conservative systems with no-tilling affected and modified soil attributes, especially biological and biochemical ones. In the present study, In the present study, the conversion of native areas to agriculture caused changes in the biological soil attributes, smaller respiration, urease activities and functional group density has been. These results show the impacts of anthropogenic interference on the balance and functionality of the biological communities of the soil. these alterations were also observed in other studies in the phytophysiognomy of *Murundus Fields*, converted to agricultural areas (Assis et al., 2014; Martins et al., 2019; Paulino et al., 2015) converted to no-tillage areas, which verified a reduction in the activity and composition of microbial communities in cultivated areas, when compared to native areas. In general, biological attributes were sensitive in demonstrating changes in soil use, confirming their efficacy as soil quality indicators (Sá et al., 2017; Silva et al., 2018; Souza et al., 2020; Pinto et al., 2020; Aragão et al., 2020; Martins et al., 2020; Tassano et al., 2021).

The changes observed in the chemical characteristics of the soil are attributed primarily to soil handling and the time it takes to implement a no-till system, related to soil correction practices such as liming and use of fertilizers, which explain the high Ca, Mg and K values in the cultivated areas, whilst the native areas presented the higher active acidity (Al) and potential activity (H+Al) values (Table 1). Commonly, pH is one of the main abiotic factors which regulate the soil microbial community and activities (D'Acunto et al., 2018), however, it was observed in this study that by correlating soil pH to its biological attributes, it was verified that the pH did not have an influence over the activities of most of the functional groups that were analyzed. This was one of the main factors that influenced cultivated area distribution and the content of nutrients available in the soil, verified in the PCA.

COT content in the soil decreased with cultivation time in the agricultural areas, observing greater reductions for no-till 15 and 18 years, which indicates that even areas with almost 20 conservative years of cultivation system were not efficient in reestablishing C accumulation in the soil greater than that of the native areas. Martins et al. (2019) observed the gradual reduction in soil COT content in *Murundus Fields* converted into no-till areas, which indicates the impact the conversion has, revealing cultivation condition improvement needs to supply a higher carbon rate to the system.

Handling employed in the area, from the implantation of no-till via mechanical enhancements to the soil, fertilizer, and corrective usage, as well as the cultivation of different vegetable species (soy and corn, mainly) from the native vegetation favored the selection of more adapted microbiota, leading to changes, losses, or reductions of some functional soil groups. As observed in the group reduction of NBRIP, GES, cellulolytic, FAM in the BM native area, actino in the TM native area and total fungi increase in the native TM area, with no changes to ammonifiers, total bacteria and NFB. Areas cultivated with 10 and 15 years presented higher MBC than the values found in the control area and the no-till 11 years, indicating that the systems of agricultural handling led to MBC increases. Contrary to our results, Martins et al. (2019) observed that the conversion of native areas into agricultural ones led to great CBM reduction, which gradually restored itself in 50% of the handling time. Higher MBC in native areas is justified by the superior diversity of compounds exudated by the rhizosphere, as well as the constant deposit of different residues in the soil litter surface, which helps maintain temperature and humidity in adequate levels (Martins et al., 2019; Silva et al., 2018; Teixeira et al., 2021).

When observing the different microbial groups, it can be verified that in the natural environments, primarily the murundus bases, were the areas that presented the highest phosphate solubilizer (NBRIP and GES), Celul and FAM densities; and the murundus tops were the ones with greater actinobacteria density (Table 3). The NFB and ammonifier densities, however, did not differ among the areas that were studied, which indicates the adaptability of the microorganisms in these groups that have the conversion of natural areas into agricultural ones. In the cultivated areas, actinobacteria and GES densities increased with the no-till 11 < no-till 5 < no-till 18 years cultivation times. In general, soil use altered soil microorganism group densities in the *Murundus Fields* areas, decreasing due to the conversion of the fields into agricultural areas.

FDA was not affected by the conversion of *Murundus Fields* into agricultural areas. FDA activity in an indirect measure of soil heterotrophic activity, relating itself to organic matter decomposition (Ciarkowska et al., 2014; Muscolo et al., 2015), indicating microbial activity, nutrient availability, and soil basal respiration (Medeiros et al., 2015). However, soil basal respiration (RBS) was sensitive at indicating higher activities in native areas where there is a greater availability of exudates and easily decomposable compounds that can stimulate the decomposition activity of the soil, which was verified in this study by greater CO_2 emission in native environments. Bigger RBS did not indicate stress in the evaluated systems of soil use, because there were no significant differences for the qCO_2 among the areas that were studied. qCO_2 is an efficient indicator when it comes to soil stress, by the relation between how much C is emitted as CO_2 and how much is absorbed and immobilized in microbial biomass (Anderson and Domsch, 1993). *q*Mic, however, was close to 1%, which is relatively low, indicating that the MBC contributed lightly to the soil COT composition (Jenkinson and Ladd 1981; Smith and Paul 1990).

Urease activity was reduced in the cultivated areas up to 65.7% when compared to the native areas. The handling in the agricultural areas, mainly fertilizing, can contribute to this decrease in urease activity. Urease is an enzyme that is synthesized by soil microorganisms and is responsible for the hydrolysis of urea, CO_2 and NH_3, with wide natural occurrence (Kandeler and Gerber, 1988), a very important enzyme in the N cycle, sensitive to the decrease in exudate compound diversity in the rhizosphere and to the quantity and quality of material that is deposited in the soil (Silva et al., 2018), which makes it sensitive in indicating changes in the handling system deployed in the soil. The high correlation between fertility attributes was already expected because the soil had previously been handled by cultivation with liming and fertilizing, due to the intense productive demand for commodities produced in the Brazilian Cerrado.

PCA shows the cultivated area grouping independently of cultivation time (Fig. 4). The groups corresponding to the BM and TM native environments, which were in proximity, primarily due to the Al, sand, silt and Fe contents, urease activities and RBS demonstrated how the functionality of *Murundus Fields* soils is more elevated than the one in cultivated areas. It is possible to observe the relations between the functional cellulolytic and ammonifier groups in the BM area, the use of a liquid culture medium can favor the organisms that come from environments with reducing conditions such as the ones found in Murundus bases. In the Murundus tops, however, the respiration activity can be related to a higher oxygenation condition for the metabolism of aerobic heterotrophic organisms.

MBC and nitrifier group increases in the cultivated areas may also be related to higher clay contents, which favors the creation of microsites that offer protection to the microorganisms, as well as increasing water retention (Stotzky, 1972). However, the different cultivation chronosequences negatively affected the functional organism groups.

Although no-tilling is considered a conservative system, the incorporation of areas and the agricultural expansion in the native Cerrado generates preoccupations with the potential loss of soil biological diversity, as observed by Assis et al. (2014), Carneiro et al. (2019), Souza et al. (2020) and Martins et al. (2020), primarily in areas with elevated frailty and low resilience, such as *Murundus Fields*. These areas exert higher contributions to environmental factors, such as the maintenance of the water cycle in the microbasin, than to agricultural production. Therefore, it is extremely important to maintain the *Murundus Fields* areas as permanent preservation areas due to all their functions in the ecosystemic services, as after the conversion into agricultural systems, there is no return to previous structures and functions that are naturally found in *Murundus Fields*.

5. CONCLUSION

Physical, chemical and biological soil attributes evaluated were notoriously altered by the conversion of *Murundus Fields* into agricultural areas, even though the conservative no-till planting system was adopted.

The most sensitive biological soil use attributes after *Murundus Fields* conversions via no-tilling were urease, actinobacteria, phosphate solubilizers, fungi, bacteria, nitrogen-fixers, and soil basal respiration. The most sensitive physical and chemical attributes were Ca, Mg, Al, B, P, silt, pH, clay, K and Zn.

Despite the conversion of native *Murundus Fields* into no-till agricultural production systems affects key functional groups of the biological community differently, such as fungi, nitrifiers and phosphate solubilizers, new conversions of *Murundus Fields* are not recommended.

Based on these results and discussion, the authors of this study position themselves unfavorable for the conversion of *Murundus Fields* into agricultural areas, which is in conformity with current legislation.

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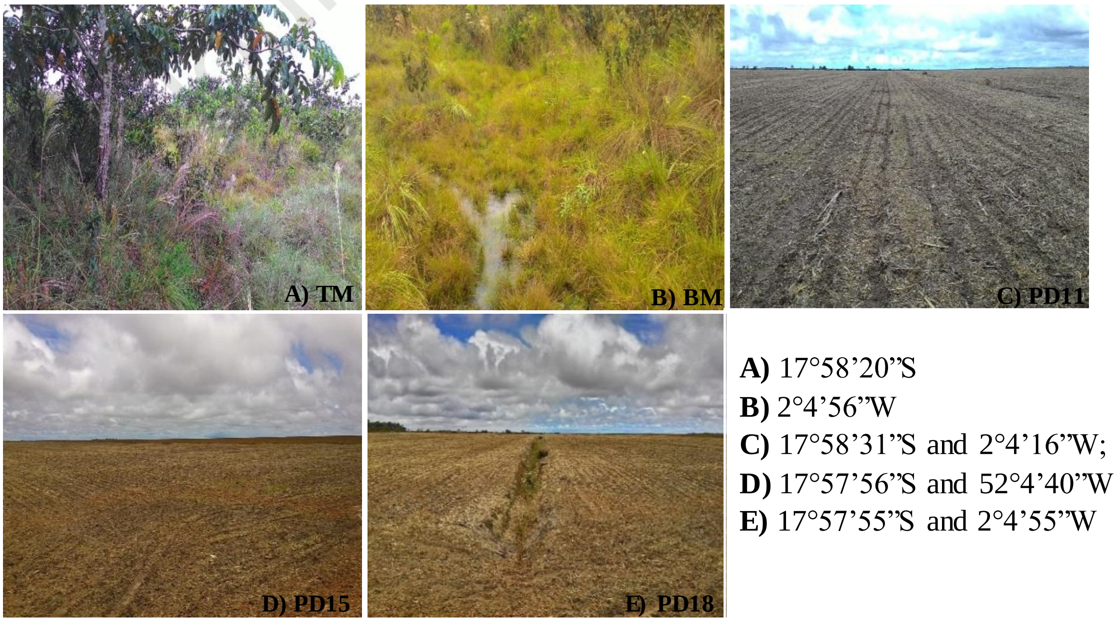
TABLE AND FIGURE LIST



A) 17°58’20’’S
B) 2°4’56’’W
C) 17°58’31’’S and 2°4’16’’W;
D) 17°57’56’’S and 52°4’40’’W;
E) 17°57’55’’S and 2°4’55’’W

Fig. 1. Area description **A** - Microrelief murundu fields correspondent to the top of the mounds (TM), and **B** - Microrelief murundu fields correspondent to the base of the mounds (BM) (153 ha

- Native Cerrado); **C** - Area cultivated for 11 years with no-tilling (PD 11) in soy/corn succession (45.9 ha); **D** - Area cultivated for 15 years with no-tilling (PD 15) in soy/corn succession (98.7 ha); **E** - Area cultivated for 18 years with no-tilling (PD 18) in soy/corn succession (49.9 ha).

Table 1. Chemical and physical soil attributes in the non-anthropized murundu field - mound base (BM) and mound top (TM) - and in the adjacent areas converted into agricultural cultivations under no-tilling (PD) in different chronosequences (PD 11, PD 15 and PD 18 years).

Physical and chemical characteristics	**PD18**	**PD15**	**PD11**	**BM**	**TM**
pH (CaCl$_2$)	4.79ns	5.23ns	5.49ns	4.28ns	4.10ns
K (mg dm^{-3})	1.49b	1.68b	2.53a	0.19c	<0.70c
P (mg dm^{-3})	46.7a	66.8a	56.3a	6.70b	7.90b
Ca (mmol$_c$ dm^{-3})	20.1c	28.9b	45.8a	0.30d	0.10d
Mg (mmol$_c$ dm^{-3})	6.4b	10.3a	11.6a	<2.50c	<2.50c
Al (mmol$_c$ dm^{-3})	1.12b	0.36b	0.0b	8.15a	8.88a
H+Al (mmol$_c$ dm^{-3})	36.8b	31.4b	21.9c	55.6a	59.9a
MO (g dm^{-3})	29.6ns	32.6ns	34.2ns	37.9ns	34.2ns
CTC (mmol$_c$ dm^{-3})	64.85b	72.28a	81.83a	56.95b	60.72b
Fe (mg dm^{-3})	79.6a	79.2a	49.2b	72.7a	73a
B (mg dm^{-3})	31.4b	21.9b	55.6a	59.9a	0.197c
Mn (mg dm^{-3})	0.68b	0.56b	1.21a	<0.5c	<0.5c
Cu (mg dm^{-3})	72.28a	81.83a	56.95b	60.72b	84a
Zn (mg dm^{-3})	2.37b	3.47a	2.46b	0.11c	0.16c
Clay (g kg^{-1})	676.3a	650.9a	664.8a	550.8c	601.9b
Silt (g kg^{-1})	117.4c	137.7c	133.1c	212.8a	174.7b
Sand (g kg^{-1})	206.3ns	211.4ns	202.1ns	236.4ns	223.4ns

Means followed by the same letter do not differ statistically through the Scott-Knott test ($p < 0.05$).

Table 2. Total organic carbon (COT), microbial biomass carbon (MBC), microbial quotient (*q*Mic), metabolic quotient (*q*CO$_2$) and basal respiration (RBS) of soil cultivated via no-till (PD) in different chronosequences (PD11, PD15 and PD18 years) in natural soils situated under microrelief murundu fields (BM - mound base and TM - mound top).

Land use	COT	CBM	*q*Mic	*q*CO$_2$	SBR
	g kg^{-1}	μg g^{-1}	%	μg C-CO$_2$ μg CBM h^{-1}	mg C-CO$_2$ g^{-1} soil dry h^{-1}
PD11	19.88a	215.2b	0.96b	0.032ns	6.51b
PD15	18.95b	316.0a	1.62a	0.024ns	5.92b
PD18	17.21b	301.2a	1.77a	0.024ns	6.45b
BM	22.03a	202.3b	0.92b	0.045ns	8.82a
TM	19.88a	184.2b	0.93b	0.042ns	8.57a

Means followed by the same letter do not differ statistically through the Scott-Knott test ($p < 0.05$).

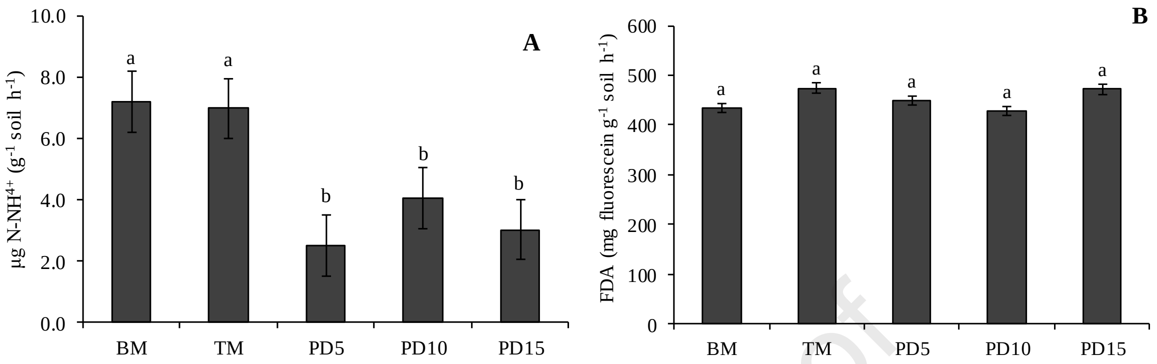


Fig. 2. Specific enzymatic activity (A) urease and (B) FDA fluorescein diacetate hydrolase in soils cultivated via no-till in three chronosequences (PD11, PD15 and PD18 yeats) and in soils native to the microrelief murundu fields (BM - mound base and TM - mound top). Means followed by the same letter do not differ statistically through the Scott-Knott test ($p < 0.05$).

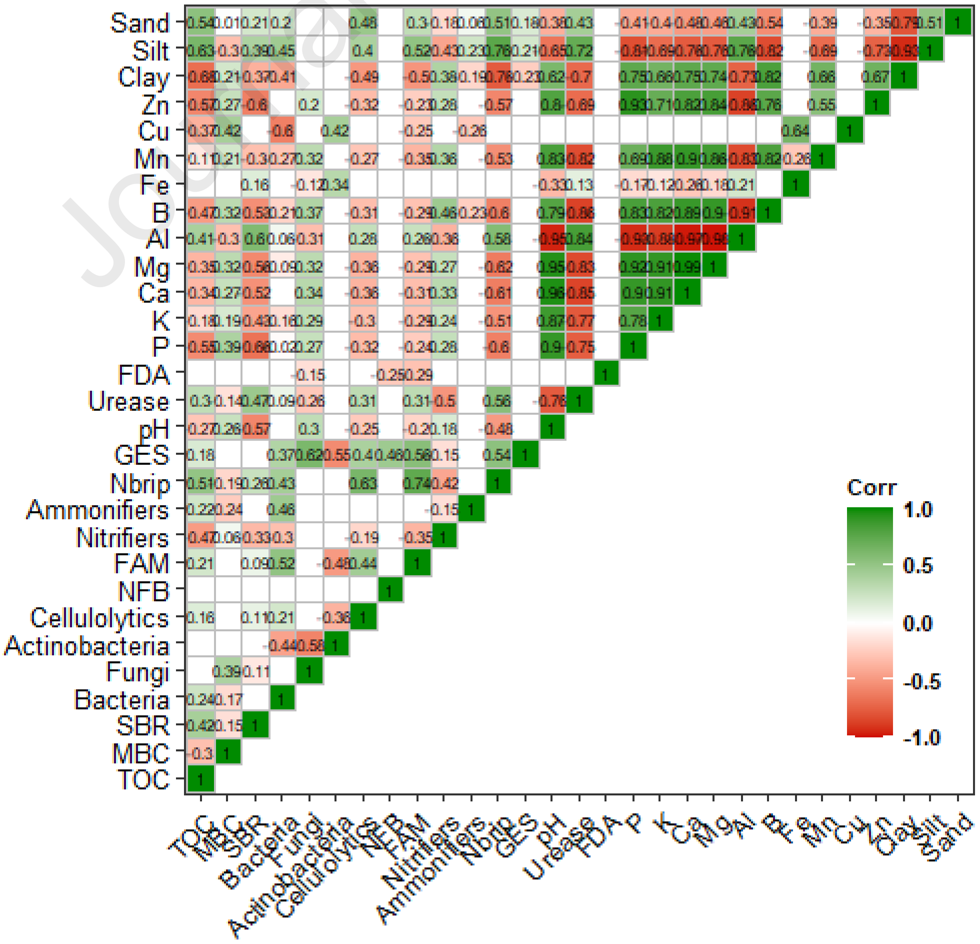


Fig. 3. Pearson Correlation ($p < 0.05$) between physical, chemical and biochemical attributes of soil cultivated under no-till planting (PD), in the chronosequences PD 5, PD10 and PD15 years and in a non cultivated native area situated under the microrelief murundu fields. Microbial biomass carbon (MBC); soil basal respiration (RBS); urease; fluorescein diacetate hydrolysis (FDA); total bacteria; total actinobacteria; total fungi; nitrogen-fixing bacteria (NFB and FAM); nitrifiers; ammonifiers; phosphate solubilizers (Nbrip and GES).

Table 3. Functional group density: total heterotrophic bacteria (Bact), actinobacteria (Actno), total fungi (Fungi), phosphate solubilizers (NBRIP and GES), cellulolytics (Celul), nitrogen-fixers in the FAM culture medium, nitrogen-fixers (BFN), nitrifiers (Nitrif) and ammonifiers (amonif) in the soil cultivated under no-till planting in different chronosequences (PD11, PD15 and PD18 years) and in natural soils situated under murundu fields (BM - mound base and TM - mound top).

Land use	Bact	Actino	Fung	NBRIP	GES	Celul	FAM	NFB	Nitrif	Amonif
	----------------------------UFC g^{-1} of soil----------------------------					-----------------------------NMP $g^{-1}$of soil------------------------				
PD11	4.78 x 10^{6} ns	3.00 x 10^{4} d	1.04 x 10^{4} a	2.80 x 10^{3} b	4.60 x 10^{3} c	5.75 x 10^{3} b	1.83 x 10^{4} b	3.28 x 10^{3} ns	9.37 x 10^{5} a	1.21 x 10^{5} ns
PD15	5.70 x 10^{6} ns	5.12 x 10^{4} c	9.30 x 10^{3} a	4.20 x 10^{3} b	6.60 x 10^{3} b	1.18 x10^{4} b	3.90 x 10^{4} b	1.25 x 10^{4} ns	1.60 x 10^{4} b	1.17 x 10^{5} ns
PD18	2.36 x 10^{6} ns	6.32 x 10^{4} b	1.16 x 10^{4} a	1.06 x 10^{4} b	8.40 x 10^{3} b	7.99 x 10^{4} b	3.93 x 10^{4} b	4.24 x 10^{4} ns	1.40 x 10^{6} a	5.20 x 10^{4} ns
BM	5.93 x 10^{6} ns	2.26 x 10^{4} d	1.24 x 10^{4} a	1.80 x 10^{5} a	1.08 x 10^{4} a	2.63 x 10^{5} a	1.09 x 10^{5} a	3.46 x 10^{4} ns	0.00 c	1.17 x 10^{5} ns
TM	3.00 x 10^{6} ns	8.92 x 10^{4} a	2.90 x 10^{3} b	2.40 x 10^{4} b	2.00 x 10^{3} d	5.78 x 10^{3} b	1.20 x 10^{4} b	3.50 x 10^{3} ns	4.93 x 10^{5} b	1.16 x 10^{5} ns

Means followed by the same letter do not differ statistically through the Scott-Knott test ($p < 0.05$).

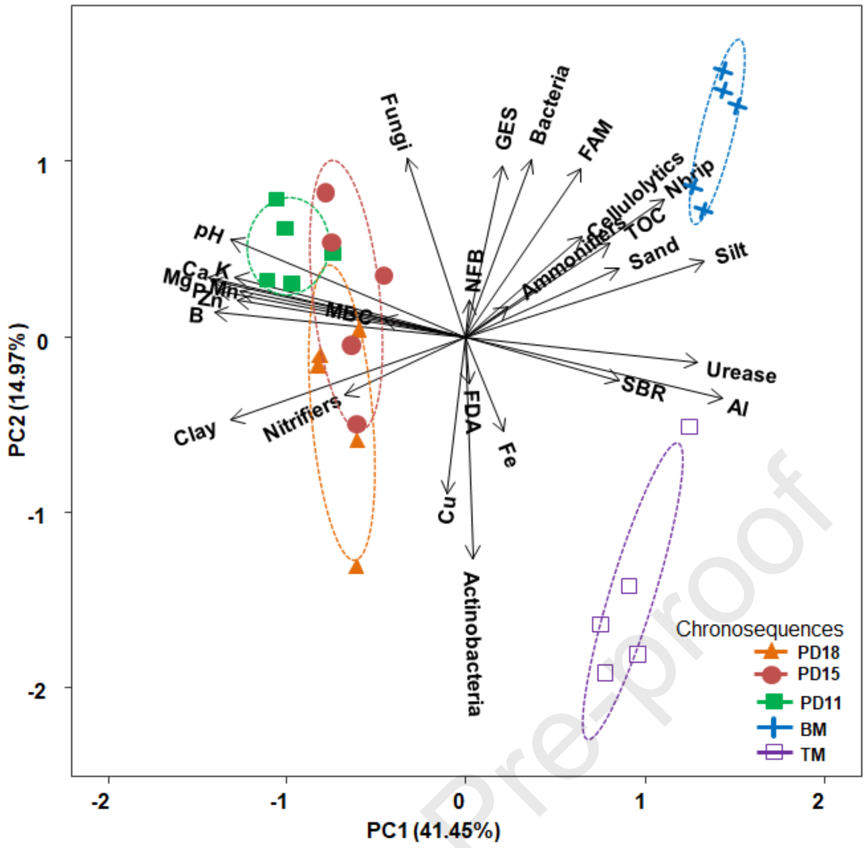


Fig. 4. Principal component analysis between physical, chemical, biochemical and biological attributes of no-till soil (PD) in different chronosequences (PD11, PD15 and PD18 years) and in a natural area not cultivated under microrelief murundu fields (BM - mound base and TM - mound top). Microbial biomass carbon (MBC); Soil basal respiration (RBS); Urease; fluorescein diacetate hydrolysis (FDA); Total heterotrophic bacteria; Actinobacteria; Total Fungi; Nitrogen-fixing bacteria (NFB and FAM); Nitrifiers; Ammonifiers; Phosphate solubilizers (Nbrip and GES).

Table S1: Eigenvalues of principal component analysis between physical, chemical, biochemical and biological attributes of no-till (PD) soil in different agricultural chronosequences (PD11, PD15 and PD18 years) and in a natural non cultivated area situated under microrelief murundu fields (BM - mound bases and TM - mound tops). Microbial biomass carbon (MBC); Soil basal respiration (RBS); Urease; fluorescein diacetate hydrolysis (FDA); Total bacteria; Total actinobacteria; Total Fungi; Nitrogen-fixing bacteria (NFB and FAM); Nitrifiers; Ammonifiers;

Phosphate solubilizers (Nbrip and GES). The indicators with highest explanation proportions are in bold (> 0.6).

	PC1	**PC2**
Eigenvalue	12.02	4.34
Explained Proportion	41.45	14.97
Cumulative Proportion	41.45	56.43
Scores		
COT	0.51	0.34
MBC	-0.29	0.07
RB	0.54	-0.16
Bacteria	0.23	**0.64**
Fungi	-0.21	0.65
Actinobacteria	0.02	**-0.80**
Cellulolytics	0.41	0.37
NFB	0.01	0.13
FAM	0.41	**0.61**
Nitrifiers	-0.43	-0.21
Ammonifiers	0.14	0.11
Nbrip	**0.70**	0.50
GES	0.13	**0.62**
pH	**-0.84**	0.35
Urease	**0.82**	-0.09
FDA	0.01	-0.17
P	**-0.88**	0.17
K	**-0.82**	0.22
Ca	**-0.91**	0.22
Mg	**-0.91**	0.21
Al	**0.91**	-0.22
B	**-0.89**	0.09
Fe	0.13	-0.34
Mn	**-0.80**	0.16
Cu	-0.07	-0.57
Zn	**-0.81**	0.13

Clay	**-0.83**	-0.30
Silt	**0.85**	0.27
Sand	0.54	0.25

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: