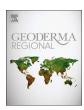
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# Temporal variation and critical limits of microbial indicators in oxisols in the Cerrado, Brazil



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#### ABSTRACT

In this study, the temporal variation of microbial biomass carbon (MBC), arylsulfatase, acid phosphatase and  $\beta$ -glucosidase was evaluated, over a 5-years period, comparing it with soil organic carbon (SOC) and Mehlich-P, in cultivated clayey Oxisols, under NT and conventional-tillage (CT) management systems. We also determined the extent of the temporal variation in the critical levels (CLs) for these microbial indicators. Soil samples (0 to 10 cm depth) were collected at the flowering stage, in 2011, 2013 and 2015 from a set of 13 treatments under NT and 8 treatments under CT. Regardless of the management system, MBC, arylsulfatase and acid phosphatase were the most stable microbial variables. Consistent increases in  $\beta$ -glucosidase activity over time were observed in the treatments under NT, and are likely related to its abiontic accumulation. New CLs for these microbial indicators, in the clayey Cerrado oxisols, were generated with the multi-year data set. In addition to the temporal variation, the new CLs, specific for NT and CT, represent an advance since they also consider the differences in soil biological functioning present in these management systems, at the flowering stage.

#### 1. Introduction

The Cerrado region covers an area of approximately 2.04 million square kilometers, of the Brazilian territory. From a region considered unfit for farming in the early 1970's, the Cerrado is responsible today for about 55 and 60% of the national production of beef and grain, respectively (CONAB, 2016), and has become one of the largest agricultural frontiers in the world, with approximately 17.4million ha under annual crops (MMA, 2015). Within this large extension of land, a broad variety of environmental conditions occurs, but the most widespread is tropical climate, with a rainy season that varies from 3 to 8 months and where annual rainfall ranges from 500 mm in the northeast to 2300 mm in the sub-Amazonian areas (Silva et al., 2008). The most optimal growing conditions for plants and soil microorganisms in these ecosystems occur after the first rains in September/October, which also is the beginning of the planting date for annual grain crops.

During the past 40 years, Cerrados agriculture has moved faster towards sustainability, with the widespread adoptions of no-tillage (Freitas and Landers, 2014) and more recently of integrated crop-live-stock systems (Cordeiro et al., 2015), which have promoted increased interest in soil health assessments (Balota et al., 2004; Hungria et al., 2009; Mendes et al., 2015; Mendes, 2016). As in other parts of the world, in Brazil the metrics for soil quality in agroecosystems also have been discussed (Lehman et al., 2015; Lopes et al., 2013; Stott et al., 2010; Mendes et al., 2015). Although we still lack a consensus regarding the minimum data set to evaluate soil quality in Brazilian agroecosystems, it is clear the need for indicators related to the soil biological functioning in soil quality indexes (Balota et al., 2004; Hungria et al., 2009; Peixoto et al., 2010; Lopes et al., 2013; Mendes et al., 2015).

The interpretation of individual values of microbial indicators independent of a comparative control or treatment is one of the major challenges in soil quality assessments using microbial indicators (Dick,

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1992; Trasar-Cepeda et al., 1998; Gil-Sotres et al., 2005; Biswas et al., 2017). As part of the efforts to incorporate soil microbial analyses in commercial routine soil analyses in Brazil, Lopes et al. (2013), reported the critical levels (CLs) (low, moderate and adequate) for MBC,  $\beta$ -glucosidase, acid phosphatase, and arylsulfatase for the clayey Oxisols of the Brazilian Cerrado generating the first interpretation tables for microbial indicators in these soils, which cover an area of 40 million ha. The definition of the CLs for these bioindicators was based on the mathematical expressions of their relationships with the soil organic carbon (SOC) and the relative cumulative yields (RCY) of corn and soybean. However, these interpretation tables have primarily been developed with data collected in one single-year and, did not consider how these microbial indicators respond to different tillage systems.

Unlike chemical indicators of soil fertility, the biological component of soil is strongly influenced by climatic factors such as soil moisture and temperature, generating temporal variation patterns. Microbial variables that vary widely in short periods makes calibration and interpretation difficult (Knight and Dick, 2004). For this reason, small standard deviations and low variation over time are some of the requirements needed for the use of microbiological parameters in soil quality monitoring (Tscherko and Kandeler, 1997). Seasonal (intraannual) fluctuations in microbiological attributes are directly linked to substrate quality, quantity and availability, in the different seasons of the year, and vary according to the ecosystem assessed, the parameters evaluated and soil properties (McGill et al., 1986; Rastin et al., 1988; Kramer and Green, 2000; Wick et al., 2002; Cleveland et al., 2004; Boerner et al., 2005; Bell et al., 2008; Mendes et al., 2012; McDaniel et al., 2013; Baldrian et al., 2013; Kotroczó et al., 2014; Pathan et al., 2017). The interannual variation, in soil biological activity as well as the variation over time in monitoring studies of microbiological attributes have also been reported in the literature (Kandeler et al., 1999; Liu et al., 2010; Acosta-Martínez et al., 2011; Edwards and Jefferies, 2013; Kotroczó et al., 2014; Singh and Goshal, 2010; Mendes et al., 2012; McDaniel et al., 2013). The advent of issues related to climate change, has evidenced the importance of long-term (multi-year) data sets, which are important for a better understanding of the relative sensitivity of soil biological parameters to changing biotic and abiotic drivers (Gutknecht et al., 2010, 2012; Baldrian et al., 2013;. Henry, 2013; Moyes and Bowling, 2013; Slaughter et al., 2015; Romero-Olivares et al., 2017).

Regarding management practices such as no-tillage (NT) and conventional-tillage (CT), it is known that they influence the soil environment and therefore the habitat of soil microorganisms in different ways (Johnson and Hoyt, 1999; Martens, 2001; Kaschuk et al., 2010). A recent meta-analysis utilizing 139 observations from 62 studies from around the world; confirmed that NT promotes larger microbial communities and greater enzymatic activity (Zuber and Villamil, 2016). In the Cerrados region, NT has been adopted on most large-scale mechanized farms where direct seeding without tillage operations allows farmers to plant earlier in the season. Nowadays, it is estimated that about 11 million ha of cropland are cultivated under NT systems in the Cerrado (FEBRAPD, 2017).

Taking advantage of the unique opportunity offered by three well-managed and carefully documented long-term field experiments, in the present study we considered: i) the importance of climatic effects on soil microbiological processes and of multi-year microbial data sets covering periods of interannual variability and ii) the fact that the soil biological functioning is different under CT and NT systems. Our objective was to monitor the temporal variation of MBC, arylsulfatase, acid phosphatase and  $\beta$ -glucosidase over a 5-year period in a set of 13 treatments under NT and 8 treatments under CT, in long-term field experiments. We also evaluated the temporal variation of SOC and Mehlich-P, two parameters frequently used in routine soil chemical analysis. The knowledge of the magnitude of changes over time in the MBC, arylsulfatase, acid phosphatase and  $\beta$ -glucosidase activities, under different management systems, is important not only to quantify

how temporal variations influence these parameters, but also for calibration and interpretation studies. Therefore, the other objective of this study was to determine the CLs for these microbial indicators in clayey Cerrado Oxisols, under NT and CT, using a multi-year data set, with soil samplings in 2011, 2013 and 2015.

#### 2. Materials and methods

#### 2.1. Study site

The study area was located at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), Centro de Pesquisa Agropecuária dos Cerrados (Embrapa Cerrados), close to the city of Planaltina, DF, Brazil (15°35′30″S and 47°42′00″W, at an altitude 1175 m). The soil is a very fine, mixed isothermic Rhodic Haplustox (a Typic Dystrophic Red Latosol, according to the Brazilian soil classification system). According to the Köppen classification, the regional climate is Cwa, which corresponds to a typical savanna climate with 1500 mm of mean annual precipitation and two well-defined seasons (dry from May to September and rainy from October to April). The maximum and minimum yearly average temperatures are 26.4 °C and 15.9 °C, respectively.

A set of twenty-one treatments from three long-term field experiments were selected for the study, based on their historical cumulative grain yields of soybean [Glycine max (L.) Merr], and corn (Zea mays L.). These experiments (randomized complete block with three replicates) were designed to study the management of P fertilizers using various sources, doses and placement methods (Fig. S1). The set of 21 treatments included 13 treatments under NT and 8 treatments under CT. These treatments were selected because the different P-fertilization strategies resulted in different crop yields and residue production, which ended up modulating the SOC levels in the soil.

Experiment I was initiated in 1994 and experiments II and III in 1999. Cumulative grain yields (CGY) ranging from 3490 to 128,623 kg ha<sup>-1</sup> were recorded in these 21 treatments in 2015, during a 21-year period for experiment I and a 16-year period for experiments II and III (Table 1). Granular triple superphosphate was the only P source used in the 21 treatments selected for the present study. Crops were planted in November. A variable defined as the relative cumulative yield (RCY) was calculated to express the cumulative yields of corn and soybean as a percentage of the greatest cumulative yield obtained in each of the three experiments. This relative variable was used to express the area's productivity using historical data from these three experiments, which had different durations and rotations. A detailed description of these three long-term experiments is reported in Lopes et al. (2013). The chemical analyses of the soil samples collected in these areas, in 2015, at a depth of 0 to 10 cm, are presented in Table 1.

#### 2.2. Soil sampling and preparation

The soil samples were collected in January 2011, January 2013 and February 2015 at the corn flowering stage, at a depth of 0 to 10 cm. For the January 2011 sampling, in each plot of the 21 selected treatments, soil samples were collected in four different points to form a composite sample. At each point 7 soil cores were collected equidistantly using a soil probe (5-cm diameter); the soil cores were collected perpendicular to the planting row, with one core positioned in the middle of the planting row and the other 3 positioned on each side of the inter-row spacing. The sampling strategies at these three long-term experiments had to be reconciled to meet the needs of several research groups and as consequence, in the years 2013 and 2015 the soil sampling protocol was modified in order to minimize the impact at the experimental plots. Although the sampling protocol was altered, we kept the same proportion of samples collected in the middle of the planting row (3 samples) and on each side of the inter-row space (17 samples). These sub-samples were homogenized in large plastic bags, transported to the laboratory and sieved through a 4-mm sieve. At each sampling (2011;

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