

Soil enzymatic activity and chemical attributes after continuous and interrupted application of pig slurry

Deisi Navroski¹, Arnaldo Colozzi Filho², Graziela Moraes de Cesare Barbosa², Adônis Moreira³

¹ Universidade Estadual de Londrina, Londrina, PR, Brasil. E-mail: deisinavroski@gmail.com (ORCID: 0000-0003-0427-7288)

² Instituto Agronômico do Paraná, Londrina, PR, Brasil. E-mail: acolozzi@iapar.br (ORCID: 0000-0002-9306-6724); graziela_barbosa@iapar.br (ORCID: 0000-0003-2116-4736)

³ Embrapa Soja, Londrina, PR, Brasil. E-mail: adonis.moreira@embrapa.br (ORCID: 0000-0003-4023-5990)

ABSTRACT: The objective of this work was to evaluate enzymatic activities and chemical attributes of a soil under continuous application for 28 years and after interruption for 5 and 12 months of pig slurry (PS). The soil received doses of 0, 30 and 60 m³ ha⁻¹ year⁻¹ of PS since 1988, and in 2016, the plots were divided in half and there was interruption of the application of PS in half plot, generating a 2 × 2 factorial scheme, being two doses of PS (30 and 60 m³ ha⁻¹ year⁻¹) and two application conditions (continuous or interrupted). The available P, total organic carbon (TOC), pH, Al³⁺, H⁺ + Al³⁺, Ca²⁺, Mg²⁺ and K⁺ and activity of the enzymes arylsulfatase (AR), β-glycosidase (β-G), acid (AP) and basic (BP) phosphatase, hydrolysis of fluorescein diacetate (FDA) of soil were assessed. The content of P, Mg²⁺ and H⁺ + Al³⁺ varied with the doses of PS applied, but not with the interruption of the applications. The continuous application of PS increased the activity of the enzymes AR, β-G and BP, whereas FDA and AP were not influenced by the interruption of the applications. The interruption of PS application for up to one year did not change the chemical attributes of the soil, but reduces the activity of the enzymes AR, β-G and BP.

Key words: animal manure; biogeochemical cycles; microbial activity; organic carbon

Atividade enzimática e atributos químicos do solo após aplicação contínua e interrompida de dejetos líquidos de suínos

RESUMO: O objetivo deste trabalho foi avaliar atividades enzimáticas e atributos químicos de um solo sob aplicação contínua por 28 anos e após interrupção por 5 e 12 meses de dejetos líquidos de suínos (DLS). O solo recebeu doses de 0, 30 e 60 m³ ha⁻¹ ano⁻¹ de DLS desde 1988, e em 2016, as parcelas foram divididas ao meio e houve a interrupção da aplicação de DLS em meia parcela, gerando o esquema fatorial 2 × 2 sendo duas doses de DLS (30 e 60 m³ ha⁻¹ ano⁻¹) e duas condições de aplicação (contínua ou interrompida). Avaliou-se no solo o P disponível, carbono orgânico total (COT), pH, Al³⁺, H⁺ + Al³⁺, Ca²⁺, Mg²⁺, K⁺ e a atividade das enzimas arilsulfatase (AR), β-glicosidase (β-G), fosfatase ácida (FA) e básica (FB), hidrólise do diacetato de fluoresceína (FDA). O teor de P, Mg²⁺ e H⁺ + Al³⁺ variaram com as doses de DLS aplicado, mas não com a interrupção das aplicações. A aplicação contínua de DLS aumentou a atividade das enzimas AR, β-G e FB, enquanto FDA e FA não foram influenciadas pela interrupção das aplicações. A interrupção da aplicação de DLS por até um ano não provocou alteração nos atributos químicos do solo, mas reduz a atividade das enzimas AR, β-G e FB.

Palavras-chave: dejetos animal; ciclos biogeoquímicos; atividade microbiana; carbono orgânico

Introduction

Brazil slaughtered 43.19 million swines in 2017 (IBGE, 2018), remaining as the fourth largest pork producer in the world (ABPA, 2017). Associated with this intense production of swines comes the generation of waste that often exceeds its economic use and, as a treatment form, ends up being constantly applied at high doses in the soil of a same area, generating an environmental burden.

The recycling of pig slurry (PS) as organic fertilizer in agricultural areas, is commonly employed in Brazil and, when used properly, promotes improvements in both the soil chemical attributes and the microbial activity (Plaza et al., 2004; Couto et al., 2013; Lourenzi et al., 2016). However, when the usage is continuous in the same area at higher doses and several times a year, the PS represents a factor of environmental risk. Literature data show that, in general, doses over $90 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ are harmful not only for affecting the microbial community but also for the accumulation of heavy metals, nitrates and phosphorus, which can contaminate water and soil through leaching or surface runoff (Lalande et al., 2000, Balota et al., 2011, Balota et al., 2012, Oliveira et al., 2012).

From the effects mentioned above, perhaps the main affected component is the soil microbiota, which is characterized by being the most sensitive to changes in the soil management (Araújo & Monteiro, 2007). One of the ways in evaluating the variation of the microbial activity regarding the PS application is through the quantification of the extracellular enzymes released by the microorganisms.

The extracellular enzymes perform important functions in the biogeochemical cycles of the soil elements and have a close relationship with both the abiotic and biotic soil conditions, such as temperature, humidity, pH, TOC content, and nutrients, biomass, and microbial diversity (Balota et al. 2012, Li et al., 2018). The action of these enzymes in the soil occurs mainly due to hydrolysis reactions that can mineralize organic compounds, making them available for plants and microorganisms. Some examples are the phosphatases that catalyze the hydrolysis of esters and anhydrides from phosphoric acid, allowing mineralization of the organic P and arylsulfatase, promoting the hydrolysis of organic arylsulfate esters, acting on the organic S mineralization to SO_4^{2-} (Acosta-Martínez & Tabatabai, 2000). Moreover, in the C cycle some enzymes such as β -glycosidase degrade organic C by performing the hydrolysis of cellobiose (Araújo & Monteiro, 2007) and the lipases, esterases and proteases are related to the decomposition of the organic matter in the soil, and its activity can be evaluated through the determination of the hydrolysis of fluorescein diacetate (FDA) (Silva et al., 2015).

Several studies have been carried out in order to measure the enzymatic activity and chemical attributes of the soil under successive PS applications with different crop cultivations and climatic conditions (Couto et al., 2013, Silva et al., 2015, Li et al., 2018). However, few studies sought to answer what happens when the PS application is interrupted

after years of employment in the same area. This response is made necessary in relation to the scenario of the Brazilian pig farming that intensified the construction of biodigesters, thus the pig chain wastes are directed to the production of biogas with energy potential (thermal and electric) and generates stabilized waste before being used as fertilizer (Leitão & Silva, 2018).

In view of this, the hypothesis of this work was that the interruption in the PS application could reduce the contents of chemical elements and the enzymatic activity in response to the lower supply of organic substrates that are used by enzymes. The aim of this work was to evaluate the effects of the continuous application for 28 years and the interruption for 5 and 12 months of the PS application, on the chemical attributes and the enzymatic activity of a Dystroferic Red Latosol (Oxisol).

Materials and Methods

Area of study and sampling

The experiment was established in 1988 at the Experimental Station of the Paraná Institute of Agronomy (IAPAR), located in Palotina, State of Paraná ($24^{\circ}17' \text{ S}$, $53^{\circ}50' \text{ W}$). According to the Köppen classification, the climate of the region is the Cfa type (humid subtropical), with average annual temperature of 20° C and annual precipitation of 1800 mm (Caviglione et al., 2000). The soil is classified as a Dystroferic Red Latosol (Oxisol) (Santos et al., 2013) with 695.4 g kg^{-1} of clay content, 134.2 g kg^{-1} of silt and 170.4 g kg^{-1} of sand.

The experimental area consisted of plots with 5.0 m width by 9.0 m length (45 m^2), arranged in a randomized block design, with three replicates. The area was managed under no-tillage system (NTS) with soybean (*Glycine max* L.) or maize (*Zea mays* L.) rotation in the summer crop and with wheat (*Triticum sativum* Lam.) or oat (*Avena sativa* L.) in the winter crop. For 28 years, applications of the 30 and $60 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$ doses of PS occurred in a fractionated manner, that is to say, 50% of the dose before the summer crop and 50% before the winter crop, in addition to a control that never received the waste ($0 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$).

In 2016, the plots were divided (except the control), and the PS application in half the plot was interrupted, generating the 2×2 factorial scheme, with two PS doses (30 and $60 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$) and two application conditions (continuous or interrupted).

The applied PS was previously kept in manure for 70 days for partial fermentation and stirred for homogenization on the application day. The average chemical composition of the PS applied during the studied period presented 19.7 g L^{-1} of dry matter; 3.1 g L^{-1} of N; 2.7 g L^{-1} of P and 1.2 g L^{-1} of K.

Two soil samplings were carried out, one in the summer pre-cultivation (SPC) in October 2016 and the other in the winter pre-cultivation (WPC) in May 2017, corresponding to five and twelve months after the interruption of the PS application, respectively (Figure 1A). In the 10-day interval between the PS applications in half the plot until the soil

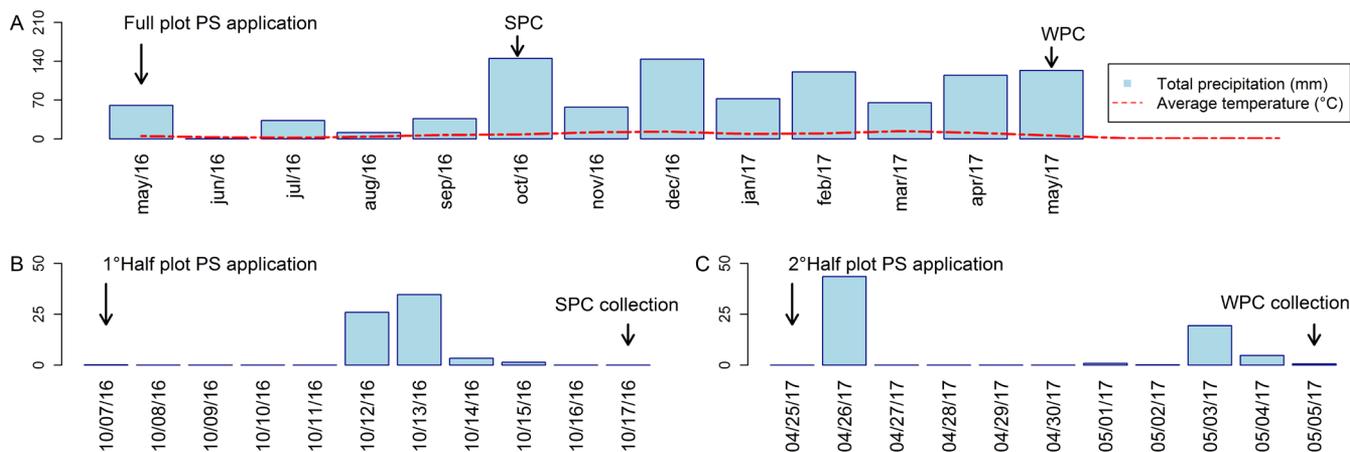


Figure 1. Total precipitation and average temperature during the experiment period. A: total period of the experiment; B: Interval corresponding to 10 days between the PS application and the SPC collection and C: interval corresponding 10 days between PS application and the WPC collection.

samples collection, there was a precipitation accumulation of 65 mm in the SPC collection and 69 mm in the WPC collection, according to Figure 1B and C.

The samples were composed of three soil subsamples in each of the three replicates from the treatment, with the aid of a straight shovel at the 0.00–0.10; 0.10–0.20 and 0.20–0.40 m depths.

Determination of soil chemical attributes

The chemical attributes of the soil were evaluated according to the described methodologies in Pavan et al. (1992). Initially, the soil was oven-dried at 60 °C for 24 hours, then milled and passed through a 2 mm sieve (air-dried fine soil – ADFS). The exchangeable Ca^{2+} , Mg^{2+} and Al^{3+} contents were extracted by 1.0 mol L^{-1} KCl, and then, afterwards, the exchangeable Ca^{2+} and Mg^{2+} were determined by the atomic absorption spectrophotometry and the Al^{3+} by titration with sodium hydroxide (1.0 mol NaOH L^{-1}). The extraction of available P and exchangeable K^+ was carried out with the Mehlich-1 extractive solution and quantified by spectrophotometry (630 nm) and flame photometry, respectively. For TOC, the soil was oxidized by potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in sulfuric medium with subsequent titration using ferrous sulfate (FeSO_4) in accordance with the Walkley-Black method. The pH was determined in suspension at 1:2.5 ratio (m:v) of soil and 0.01 mol L^{-1} CaCl_2 , and the potential acidity ($\text{H}^+ + \text{Al}^{3+}$) was estimated by pH after equilibration with the SMP buffer solution.

Determination of the extracellular enzymes activity in soil

The soil samples were homogenized and passed through a 4 mm mesh sieve and then stored at a temperature of 7 °C until evaluation. The determination of soil humidity was performed by the gravimetric method. The activity of the enzymes arylsulfatase (AR), acid (AP) and basic (BP) phosphatase, were all determined according to Tabatabai (1994). In the determination of arylsulfatase (AR), soil samples were incubated (1 hour at 37 °C) with potassium p-nitrophenyl sulfate substrate and, subsequently, the hydrolysis product

(p-nitrophenol) was read in a spectrophotometer at 400 nm. For phosphatases, the used substrate was p-nitrophenyl sodium phosphate with a modified universal buffer (MUB) (pH 5.5 for acid phosphatase and pH 11.0 for basic phosphatase) and read at 400 nm. The activity of β -glycosidase (β -G) was evaluated according the methodology of Eivazi & Tabatabai (1988) using p-nitrophenyl- β -D-glucopyranoside as substrate and subsequent colorimetric determination (410 nm). The hydrolysis of FDA was determined by the method described by Dick et al. (1996), using a solution of 2 mg mL^{-1} from fluorescein diacetate and incubating it with the soil under agitation for 20 min at 24 °C, and subsequently evaluated in a spectrophotometer at the wave length of 490 nm.

Statistical analysis

The data were tested as for the residues adequacy to the normal distribution by the Shapiro-Wilk test and the homoscedasticity by the Bartlett test. The variables that showed either absence of normality or homoscedasticity were transformed by the Box-Cox method and tested again. The enzymatic activity data were submitted to analysis of variance (ANOVA) according to a 2 × 2 factorial and when the interaction between the factors was significant, the dose effects and continuous or interrupted application were analyzed by the Tukey test ($p \leq 0.05$). The control treatment (0 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) and the continuous or interrupted doses were compared by Dunnett's test ($p \leq 0.05$). The chemical attributes of the soil were submitted to analysis of variance (ANOVA) and when significant, the means were compared by the Tukey test ($p \leq 0.05$). The relation between the enzymatic and chemical variables was calculated by Spearman correlation (r , $p \leq 0.05$). All statistical analyzes were performed using the R software (version 3.3.2).

Results and Discussion

Chemical attributes

The interruption of the PS application did not alter the soil chemical attributes. However, the available P content, Mg^{2+}

and potential acidity ($H^+ + Al^{3+}$) varied with the doses of PS applied (Table 1).

P content in the 0.00–0.10 m depth was significantly higher at the $60\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ dose in comparison to the $30\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ dose and the control (CON) in both the collections. In the 0.10–0.20 m depth there was difference only of the doses in relation to the control (Table 1). The PS has high P content, which is usually found in inorganic fractions (Ceretta et al., 2010). In this work, the mean P content in PS was 2.7 g L^{-1} , which is equivalent to approximately 2.7 kg of P at each m^3 of PS applied. Considering the cereal (corn/wheat/oats) and oleaginous (soybean) species cultivated in the area, the added P content exceeds the extraction capacity by the plants, therefore, part of it is accumulated mainly in the superficial layer of the soil.

Previous studies have reported a linear increase in the available P content due to the increasing doses of PS, which may represent a potential risk for contamination of the surface water by P carriage through rainfall (Ceretta et al., Balota et al., 2014, Lourenzi et al., 2016).

The TOC contents did not show significant differences in the sampled depths (Table 1), corroborating the results of Balota et al. (2014) and Couto et al. (2013), who also did not observe alteration in the TOC content. The effect absence in the TOC content is possibly due to the high N content and low C:N ratio (mean of 3.1 g L^{-1}), which, according to Plaza et al. (2004) may stimulate microbial metabolism and limit TOC accumulation even with higher doses of PS.

At the 0.20–0.40 m depth during SPC collection, the $60\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ dose resulted in lower potential acidity (Table 1). This is attributed to the fact that PS is slightly alkaline (Miyazawa & Barbosa, 2015), providing a favorable condition for the base saturation increase. Similar result was obtained by Lourenzi et al. (2016), who studied the chemical attributes changes related to the acidity of the soil subjected to PS applications and observed that the contents of $H^+ + Al^{3+}$ did not differ in the 0.00–0.08 m depth.

The Mg^{2+} content in the soil increased with the PS application, mainly in the layer of 0.00–0.10 m depth (Table 1). This increase is attributed to the large amount of magnesium and other bases present in the PS composition that when applied to the soil are manifested as exchangeable cations, such as it happened for the Mg^{2+} . This result is in agreement with Couto et al. (2013), which also found an increase in the content of the available exchangeable P, Ca and Mg in the soil after the application of pig slurry.

Enzymatic activity

The PS (interrupted and continuous) application factor changed the activity of the enzymes arylsulfatase (AR), β -glycosidase (β -G) and basic phosphatase (BP), while for FDA only the dose factor (60 and $30\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$) had a significant effect.

In the SPC (summer pre-cultivation) collection there was interaction between the factors for arylsulfatase (AR) activity in 0.20–0.40 m depth. In this case, the continuous

Table 1. Chemical attributes of a Dystroferic Red Latosol (Oxisol) under continuous or interrupted application of pig slurry (PS).

Treat.	Depth (m)					
	0.00-0.10		0.10-0.20		0.20-0.40	
	SPC	WPC	SPC	WPC	SPC	WPC
P (mg dm ⁻³)						
CON	15.3 c	15.4 c	7.3 b	6.8 b	3.1 a	2.3 a
30IN	57.5 bc	56.5 bc	22.9 ab	32.0 a	7.5 a	3.8 a
30CO	48.7 bc	66.3 b	27.1 a	35.7 a	8.8 a	7.7 a
60IN	87.0 ab	125.1 a	37.3 a	59.4 a	9.3 a	9.2 a
60CO	104.8 a	124.1 a	38.1 a	49.8 a	9.4 a	7.4 a
pH (CaCl ₂)						
CON	4.4 a	5.1 a	4.4 a	4.8 a	4.6 a	5.1 a
30IN	4.5 a	5.1 a	4.5 a	4.9 a	4.7 a	5.2 a
30CO	4.4 a	5.2 a	4.5 a	4.9 a	4.7 a	5.1 a
60IN	4.5 a	5.1 a	4.6 a	4.8 a	4.9 a	5.3 a
60CO	4.7 a	5.1 a	4.6 a	4.7 a	4.8 a	5.2 a
Al ³⁺ (cmol _c dm ⁻³)						
CON	0.5 a	0.13 a	0.4 a	0.5 a	0.2 a	0.13 a
30IN	0.3 a	0.08 a	0.3 a	0.2 a	0.2 a	0.04 a
30CO	0.3 a	0.04 a	0.3 a	0.3 a	0.2 a	0.10 a
60IN	0.4 a	0.09 a	0.3 a	0.3 a	0.1 a	0.00 a
60CO	0.3 a	0.04 a	0.3 a	0.4 a	0.1 a	0.00 a
H ⁺ +Al ³⁺ (cmol _c dm ⁻³)						
CON	6.7 a	5.4 a	6.2 a	5.9 a	5.2 ab	4.6 a
30IN	6.9 a	5.6 a	6.2 a	5.8 a	5.3 ab	4.3 a
30CO	7.0 a	5.5 a	6.4 a	5.9 a	5.8 a	4.7 a
60IN	7.0 a	5.9 a	6.4 a	6.1 a	5.0 b	4.3 a
60CO	6.4 a	5.8 a	6.2 a	6.4 a	5.0 b	4.2 a
Ca ²⁺ (cmol _c dm ⁻³)						
CON	3.2 a	3.9 a	3.1 a	3.3 a	3.4 a	4.0 a
30IN	3.3 a	3.9 a	3.0 a	3.5 a	3.3 a	3.9 a
30CO	3.1 a	4.6 a	3.2 a	3.6 a	3.3 a	3.6 a
60IN	3.3 a	4.2 a	3.1 a	3.4 a	3.4 a	3.8 a
60CO	3.5 a	4.1 a	2.9 a	3.1 a	3.1 a	3.5 a
TOC (mg dm ⁻³)						
CON	17.6 a	19.3 a	11.9 a	12.7 a	9.3 a	8.7 a
30IN	18.7 a	18.7 a	13.1 a	12.2 a	9.2 a	7.8 a
30CO	18.0 a	20.9 a	13.4 a	14.0 a	10.1 a	9.3 a
60IN	18.5 a	19.7 a	12.3 a	13.6 a	9.8 a	8.7 a
60CO	18.0 a	20.0 a	11.9 a	12.7 a	8.2 a	8.2 a
Mg ²⁺ (cmol _c dm ⁻³)						
CON	1.0 c	1.8 b	0.8 b	1.0 b	1.0 a	1.0 b
30IN	1.5 ab	2.2 ab	1.2 ab	1.7 a	1.2 a	1.3 a
30CO	1.3 bc	2.6 a	1.2 ab	1.5 a	1.1 a	1.3 a
60IN	1.6 ab	2.5 ab	1.4 a	1.7 a	1.3 a	1.6 a
60CO	1.8 a	2.8 a	1.4 a	1.8 a	1.3 a	1.5 a
K ⁺ (cmol _c dm ⁻³)						
CON	0.4 a	0.5 a	0.3 a	0.3 a	0.3 a	0.2 a
30IN	0.8 a	0.6 a	0.6 a	0.4 a	0.4 a	0.3 a
30CO	0.7 a	0.7 a	0.5 a	0.5 a	0.4 a	0.3 a
60IN	0.8 a	0.8 a	0.7 a	0.7 a	0.5 a	0.4 a
60CO	0.9 a	0.7 a	0.6 a	0.6 a	0.5 a	0.4 a

Means followed by the same letter in the column for the same depth did not differ significantly by the Tukey test ($p \geq 0.05$). CON= control, $0\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$; 30IN= portion of the $30\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ interrupted application dose; 30CO= portion of the $30\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ continuous application dose; 60IN= portion of the $60\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ interrupted application dose; 60CO= portion of the $60\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ continuous application dose. SPC= collection carried out at 5 months after the interruption, before the summer crop. WPC= collection carried out at 12 months after interruption, before winter crop.

PS application of $60\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ promoted twice activity as much as the same interrupted dose (Table 2) and in the unfolding of the continuous application, the $60\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ dose presented higher activity for arylsulfatase (AR) than the $30\text{ m}^3\text{ ha}^{-1}\text{ year}^{-1}$ dose (Table 2).

Table 2. Arylsulfatase activity (AR) in a Dystroferic Red Latosol with continuous or interrupted application of pig slurry (PS) in summer pre-cultivation (SPC) and winter pre-cultivation (WPC).

Dose (D)	SPC			WPC		
	Arylsulfatase (AR) - $\mu\text{g PNP g}^{-1} \text{hour}^{-1}$					
	Application (A)		\bar{x} (D)	Application (A)		\bar{x} (D)
Interrupted	Continuous	Interrupted		Continuous		
0.00-0.10 m						
30 $\text{m}^3 \text{ha}^{-1}$	27.2	22.9	25.0 a	14.6 B b	21.6 A a	18.1
60 $\text{m}^3 \text{ha}^{-1}$	25.1	26.0	25.5 a	23.3 A a	20.7 A a	22.0
\bar{x} (A)	26.1 A	24.5 A		17.7	22.5	
0 $\text{m}^3 \text{ha}^{-1}$	20.7			15.8		
CV (%)	7.4			10.9		
0.10-0.20 m						
30 $\text{m}^3 \text{ha}^{-1}$	18.5	19.4	18.9 a	11.7	11.2	11.4 a
60 $\text{m}^3 \text{ha}^{-1}$	20.6	14.0	17.3 a	11.8	12.2	12.0 a
\bar{x} (A)	19.55 A	16.7 A		11.7 A	11.7 A	
0 $\text{m}^3 \text{ha}^{-1}$	17.1			12.2		
CV (%)	20.8			19.6		
0.20-0.40 m						
30 $\text{m}^3 \text{ha}^{-1}$	14.9 A a	15.5 A b	15.2	6.8	4.4	5.6 a
60 $\text{m}^3 \text{ha}^{-1}$	11.1 B a	26.7 A a	18.9	5.8	6.8	6.3 a
\bar{x} (A)	13.0	21.1		6.4 A	5.6 A	
0 $\text{m}^3 \text{ha}^{-1}$	11.1			7.5		
CV (%)	31.5			30.4		

Capital letters in the same line compare the application factor (Continuous or Interrupted); lower case letters in the column compare the dose factor (30 or 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) by the Tukey test ($p \leq 0.05$) and the asterisk (*) indicates a significant difference of the control treatment (0 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) from the others by the Dunnett's test ($p \leq 0.05$).

In the WPC (winter pre-cultivation) collection, arylsulfatase (AR) activity also decreased in response to the interruption of PS application at the 0.00–0.10 m depth. In this case the continuous 30 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose resulted in higher activity than the same interrupted dose (Table 2). Moreover, the unfolding of the application factor showed that the activity of this enzyme at the 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ interrupted dose was higher than the 30 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ interrupted dose (Table 2).

The synthesis of arylsulfatase by microorganisms is mainly controlled by the carbon and sulfur contents in the environment, so when there is reduction in the SOM content there is also a decrease in arylsulfatase activity since SOM is the main stock of sulfate esters that are substrates of this enzyme (Silva et al., 2012). This was confirmed by the significant correlation of arylsulfatase with the TOC content ($r = 0.63$, $p \leq 0.05$).

Lalande et al. (2000) and Balota et al. (2014) also observed that the application of PS had a positive effect on arylsulfatase, besides stimulating the activity of other enzymes such as acid and basic phosphatases, dehydrogenase and urease.

The interruption of PS application for 5 months (SPC collection) also promoted significant effects on basic phosphatase (BP) activity. Although the interaction was significant only at the 0.20-0.40 m depth, it is noted that it followed the same behavior in the three depths, in which the continuous application of the 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose provided better conditions for this enzyme activity (Table 3). The WPC collection though, both the interruption and dose factors had no effect on the activity of basic phosphatase (BP) (Table 3).

The activity of acid phosphatase (AP) both in soil samples and for all depths did not vary in relation to the treatments

(Table 4). As it was with the other studied enzymes, the phosphatases are related to the TOC (Silva et al., 2012) and to the biogeochemical cycle to which it belongs, in this case with phosphorus. In this study, the P content was high (Table 1) and according to Balota et al. (2014) the production of acid phosphatase (AP) is higher when the available P content reaches critical levels for plant and microbial growth.

The activity of β -glycosidase (β -G) at five months after the interruption (SPC collection) was not altered. However, at 12 months (WPC collection) β -glycosidase (β -G) activity decreased in the 0.00–0.10 m depth, with the continuous 30 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose being higher than the discontinued 30 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose (Table 5). The unfolding of the application factor showed that the interruption differed itself in the dose factor levels, with the interrupted 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose higher than the interrupted 30 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose (Table 5) showing a directly proportional relation where the higher PS dose promoted greater soil microbial activity.

The alteration in the activity of the extracellular enzymes in the soil due to the PS application seen above may be a response to the greater capacity of the nutrients immobilization by the microbial community since the addition of organic compounds increases the supply of substrates readily available, like carbohydrates, for microorganisms which produce the majority of soil enzymes (Balota et al., 2011).

The hydrolysis of fluorescein diacetate (FDA) did not show difference in the SPC collection for any of the factors, but in the WPC collection there was a dose factor effect on its activity (Table 6). At the 0.00–0.10 and 0.10–0.20 m depths of the WPC collection there was an increase in the FDA activity

Table 3. Basic phosphatase activity (BP) in a Dystroferric Red Latosol with continuous or interrupted application of pig slurry (PS) in summer pre-cultivation (SPC) and winter pre-cultivation (WPC).

Dose (D)	SPC			WPC		
	Basic phosphatase (BP) - $\mu\text{g PNP g}^{-1} \text{hour}^{-1}$					
	Application (A)		\bar{x} (D)	Application (A)		\bar{x} (D)
Interrupted	Continuous	Interrupted		Continuous		
0.00-0.10 m						
30 $\text{m}^3 \text{ha}^{-1}$	32.3*	49.6	40.9 a	39.9	38.5	39.2 a
60 $\text{m}^3 \text{ha}^{-1}$	41.2	58.2	49.7 a	63.8	42.0	52.9 a
\bar{x} (A)	36.8 B	53.9 A		51.8 A	40.3 A	
0 $\text{m}^3 \text{ha}^{-1}$	59.6			45.2		
CV (%)	18.1			25.1		
0.10-0.20 m						
30 $\text{m}^3 \text{ha}^{-1}$	27.2	34.8	31.0 b	27.5	21.8	24.6 a
60 $\text{m}^3 \text{ha}^{-1}$	32.6	57.3*	45.0 a	20.9	24.4	22.6 a
\bar{x} (A)	29.9 B	46.1 A		24.2 A	23.1 A	
0 $\text{m}^3 \text{ha}^{-1}$	39.6			32.5		
CV (%)	16.5			14.4		
0.20-0.40 m						
30 $\text{m}^3 \text{ha}^{-1}$	33.4 A a	40.1 A b	36.75	32.9	31.5	32.2 a
60 $\text{m}^3 \text{ha}^{-1}$	32.2 B a	55.0* A a	43.6	32.6	23.7	28.1 a
\bar{x} (A)	32.8	47.55		32.7 A	27.6 A	
0 $\text{m}^3 \text{ha}^{-1}$	34.2			31.8		
CV (%)	12.8			16.9		

Capital letters in the same line compare the application factor (Continuous or Interrupted); lower case letters in the column compare the dose factor (30 or 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) by the Tukey test ($p \leq 0.05$) and the asterisk (*) indicates a significant difference of the control treatment (0 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) from the others by the Dunnett's test ($p \leq 0.05$).

Table 4. Acid phosphatase activity (AP) in a Dystroferric Red Latosol with continuous or interrupted application of pig slurry (PS) in summer pre-cultivation (SPC) and winter pre-cultivation (WPC).

Dose (D)	SPC			WPC		
	Acid phosphatase (FA) - $\mu\text{g PNP g}^{-1} \text{hour}^{-1}$					
	Application (A)		\bar{x} (D)	Application (A)		\bar{x} (D)
Interrupted	Continuous	Interrupted		Continuous		
0.00-0.10 m						
30 $\text{m}^3 \text{ha}^{-1}$	272.3	313.2	292.8 a	208.2	187.6	197.9 a
60 $\text{m}^3 \text{ha}^{-1}$	258.2	279.5	268.9 a	211.2	205	208.1 a
\bar{x} (A)	265.3 A	296.4 A		209.7 A	196.3 A	
0 $\text{m}^3 \text{ha}^{-1}$	294.7			220.0		
CV (%)	17.2			11.8		
0.10-0.20 m						
30 $\text{m}^3 \text{ha}^{-1}$	160.7	166	163.3 a	82.1	79.4	80.7 a
60 $\text{m}^3 \text{ha}^{-1}$	146.4	150.1	148.2 a	81.3	72.7*	77.0 a
\bar{x} (A)	153.5 A	158.0 A		81.7 A	76.1 A	
0 $\text{m}^3 \text{ha}^{-1}$	167.1			114.4		
CV (%)	7.9			13.8		
0.20-0.40 m						
30 $\text{m}^3 \text{ha}^{-1}$	131.1	150.1	140.6 a	49.5	43.8	46.6 a
60 $\text{m}^3 \text{ha}^{-1}$	158.9	138.1	148.5 a	45.0	30.2*	37.6 a
\bar{x} (A)	145.0 A	144.1 A		47.2 A	37.0 A	
0 $\text{m}^3 \text{ha}^{-1}$	132.5			65.5		
CV (%)	26.4			19.9		

Capital letters in the same line compare the application factor (Continuous or Interrupted); lower case letters in the column compare the dose factor (30 or 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) by the Tukey test ($p \leq 0.05$) and the asterisk (*) indicates a significant difference of the control treatment (0 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$) from the others by the Dunnett's test ($p \leq 0.05$).

under the 60 $\text{m}^3 \text{ha}^{-1} \text{year}^{-1}$ dose (Table 6). Silva et al. (2015) also observed increased FDA activity in an area that received PS for 14 years.

The β -glycosidase (β -G) and the FDA hydrolysis are closely related to the carbon cycle and their increase in the highest PS dose may have occurred in response to the availability of carbon substrates. This was confirmed by their significant

correlation with soil TOC content (Table 7). The β -glycosidase (β -G) is one of the extracellular enzymes responsible for the hydrolysis of cellobiose, which is one of the steps in the process of SOM decomposition, as it occurs in the PS degradation by microorganisms (Acosta-Martínez & Tabatabai, 2000).

In the two collections, the activity of all enzymes (AR, β -G, AP, BP and FDA) decreased with increasing soil depth (Tables 2,

Table 5. β -glycosidase activity (β -G) in a Dystroferic Red Latosol with continuous or interrupted application of pig slurry (PS) in summer pre-cultivation (SPC) and winter pre-cultivation (WPC).

Dose (D)	SPC			WPC		
	β -glycosidase (β -G) - $\mu\text{g PNP g}^{-1} \text{hour}^{-1}$					
	Application (A)		\bar{x} (D)	Application (A)		\bar{x} (D)
Interrupted	Continuous	Interrupted		Continuous		
0.00-0.10 m						
30 m ³ ha ⁻¹	103.3	100.5	101.9 a	113.4 B b	181.8 A a	147.6
60 m ³ ha ⁻¹	87.3	107.9	97.6 a	166.8 A a	170.5 A a	168.6
\bar{x} (A)	95.3 A	104.2 A		140.1	176.2	
0 m ³ ha ⁻¹	97.6			132.4		
CV (%)	14.5			11.7		
0.10-0.20 m						
30 m ³ ha ⁻¹	37.4	36.5	36.9 a	41.9	41.9	41.9 a
60 m ³ ha ⁻¹	42.5	41.6	42.0 a	46.9	51.3	49.1 a
\bar{x} (A)	39.9 A	39.0 A		44.4 A	46.6 A	
0 m ³ ha ⁻¹	38.5			42.4		
CV (%)	17.8			18.2		
0.20-0.40 m						
30 m ³ ha ⁻¹	32.7	34.4	33.5 a	24.5	25.4	25.0 b
60 m ³ ha ⁻¹	37.4	35.9	36.6 a	32.5	37.5	35.0 a
\bar{x} (A)	35.0 A	35.1 A		28.5 A	31.4 A	
0 m ³ ha ⁻¹	27.9			26.0		
CV (%)	24.4			15.9		

Capital letters in the same line compare the application factor (Continuous or Interrupted); lower case letters in the column compare the dose factor (30 or 60 m³ ha⁻¹ year⁻¹) by the Tukey test ($p \leq 0.05$) and the asterisk (*) indicates a significant difference of the control treatment (0 m³ ha⁻¹ year⁻¹) from the others by the Dunnett's test ($p \leq 0.05$).

Table 6. Hydrolysis of fluorescein diacetate (FDA) in a Dystroferic Red Latosol with continuous or interrupted application of pig slurry (PS) in summer pre-cultivation (SPC) and winter pre-cultivation (WPC).

Dose (D)	SPC			WPC		
	Hydrolysis of fluorescein diacetate (FDA) - $\mu\text{g PNP g}^{-1} \text{hour}^{-1}$					
	Application (A)		\bar{x} (D)	Application (A)		\bar{x} (D)
Interrupted	Continuous	Interrupted		Continuous		
0.00-0.10 m						
30 m ³ ha ⁻¹	65.3*	95.9	80.6 a	193.1	212.4	202.8 b
60 m ³ ha ⁻¹	67.7*	69.2*	68.4 a	235.7	236.2	235.9 a
\bar{x} (A)	66.5 A	82.6 A		214.4 A	224.3 A	
0 m ³ ha ⁻¹	120.2			218.7		
CV (%)	22.6			9.5		
0.10-0.20 m						
30 m ³ ha ⁻¹	45.6*	49.9	47.7 a	133.6	143.6	138.6 b
60 m ³ ha ⁻¹	43.1*	47.2	45.1 a	185.2	161.4	173.3 a
\bar{x} (A)	44.3 A	48.5 A		159.4 A	152.5 A	
0 m ³ ha ⁻¹	69.0			124.5		
CV (%)	15.2			10.3		
0.20-0.40 m						
30 m ³ ha ⁻¹	44.8	40.2*	42.5 a	110.6	124.3	117.4 a
60 m ³ ha ⁻¹	36.7	30.5*	33.6 a	118.4	115.2	116.8 a
\bar{x} (A)	40.7 A	35.4 A		114.5 A	119.8 A	
0 m ³ ha ⁻¹	59.9			110.3		
CV (%)	17.9			15.9		

Capital letters in the same line compare the application factor (Continuous or Interrupted); lower case letters in the column compare the dose factor (30 or 60 m³ ha⁻¹ year⁻¹) by the Tukey test ($p \leq 0.05$) and the asterisk (*) indicates a significant difference of the control treatment (0 m³ ha⁻¹ year⁻¹) from the others by the Dunnett's test ($p \leq 0.05$).

3, 4, 5 and 6). This decrease may be related to the lower labile carbon content and lower availability of O₂ in deepness, which decreases the number of aerobic microorganisms in relation to the superficial layer. Moreover, the precipitation volume in the 10 days prior to the two collections was high (Figure 1B and C), thus, the saturation of water in the soil creates temporary anaerobic conditions that can affect growth and microbial activity.

Confirming this, Li et al. (2018) verified that the enzymatic activity decreased in response to the high humidity of the soil due to (1) changes in the microbial community (2) decrease in the enzyme production itself and (3) increase in inhibitory factors, such as the presence of free metal ions.

When there was a significant correlation of the enzymes with the chemical attributes, it was positive for most cases

Tabela 7. Spearman Correlations ($r, p \leq 0.05$) between enzymatic and chemical variables of a Dystroferric Red Latosol (Oxisol) under PS application. Data referring to all studied treatments and depths.

Variables	AR	β -G	AP	BP	FDA	P	TOC	pH	Al ³⁺	H ⁺ +Al ³⁺	Ca ²⁺	Mg ²⁺	K ⁺
AR	1	-	-	-	-	-	-	-	-	-	-	-	-
β -G	0.69*	1	-	-	-	-	-	-	-	-	-	-	-
AP	0.99*	0.68*	1	-	-	-	-	-	-	-	-	-	-
BP	0.44*	0.31*	0.55*	1	-	-	-	-	-	-	-	-	-
FDA	-0.18	0.45*	-0.04	-0.07	1	-	-	-	-	-	-	-	-
P	0.57*	0.78*	0.55*	0.14	0.32*	1	-	-	-	-	-	-	-
TOC	0.63*	0.87*	0.74*	0.27	0.45*	0.79*	1	-	-	-	-	-	-
pH	-0.38*	-0.01	-0.38*	-0.15	0.53*	-0.14	-0.06	1	-	-	-	-	-
Al ³⁺	0.17	-0.01	0.17	-0.03	-0.26	0.18	0.06	-0.86*	1	-	-	-	-
H ⁺ +Al ³⁺	0.50*	0.44*	0.53*	0.17	-0.10	0.57*	0.51*	-0.79*	0.79*	1	-	-	-
Ca ²⁺	-0.01	0.23	0.01	-0.05	0.48*	0.05	0.27	0.73*	-0.69*	-0.45*	1	-	-
Mg ²⁺	0.2	0.63*	0.17	0.02	0.57*	0.72*	0.55*	0.42*	-0.30*	0.01	0.38	1	-
K ⁺	0.67*	0.74*	0.60*	0.24	0.15	0.85*	0.75*	-0.10	0.02	0.45*	0.12	0.63*	1

AR= arylsulfatase; β -G= β -Glycosidase; AP= acid phosphatase; BP= basic phosphatase; FDA= hydrolysis of fluorescein diacetate; P= available phosphorus; TOC= total organic carbon; pH= potential of hydrogen; Al= exchangeable aluminum; H⁺+Al³⁺= potential acidity; Ca= exchangeable calcium; Mg= exchangeable magnesium; K= exchangeable potassium.

(Table 7), as for example, all enzymes, except BP, had a significant correlation with TOC (Table 7), showing that the PS provides substrates for all enzymes. This is in agreement with works found in the literature that suggest that the changes in the SOM content by the use of organic fertilization, like the PS increases the enzymatic activity in the soil (Li et al., 2018; Balota et al., 2014; Plaza et al., 2004).

In addition to providing substrates, organic colloids protect these enzymes (AR, β -G, AP, BP and FDA) from degradation by proteolytic enzymes, contributing to the maintenance of their catalytic capacity (Huang et al., 2009; Li et al., 2018).

In both collections, higher enzymatic activities were observed in the highest amount of applied PS (up to 60 m³ ha⁻¹ year⁻¹, maximum dose of this work), due to the supply of substrates with the PS addition. However, the increase in enzyme activity will also depend on the metabolic ability of microbial communities in using the supplied substrates. Thus, new studies still must be carried out in order to understand as well the change in the structure of microbial communities after interruption of the PS application.

Conclusions

The interruption of the pig slurry (PS) application in the soil for up to 12 months does not reduce the content of total organic carbon (TOC) and the other soil chemical attributes, so these were not good indicators of soil management changes in the short term.

The activity of the enzymes arylsulfatase, β -glycosidase and basic phosphatase reduces with the interruption of the PS application in response to the lower supply of organic substrates caused by the interruption of the pig slurry application in the soil.

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