Journal of Agricultural Science; Vol. 11, No. 15; 2019
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Microbial Activity of a Plinthosol With Application of Thiamethoxam Insecticide and Biochar

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Received: May 30, 2019 Accepted: July 10, 2019 Online Published: September 15, 2019

doi:10.5539/jas.v11n15p52 URL: https://doi.org/10.5539/jas.v11n15p52

Abstract

Although thiamethoxam is an insecticide widely used in agriculture, its high mobility and persistence in the soil can result in contamination of groundwater and alteration in biogeochemical cycles. The objective of this study was to verify the effect of biochar, NPK fertilizer and thiamethoxam insecticide on soil microbial properties. The experiment was conducted in a randomized block design composed of the doses combination of mineral fertilizer NPK (0 and 300 kg ha^{-1} of the formulated 05-25-15), and biochar (0, 8, 16 and 32 t ha^{-1}) in the absence and presence of thiamethoxam. Deformed soil samples were collected in all plots in the 0 to 0.10 m layer to determine the activity of the enzymes: acid and alkaline phosphatase, beta glucosidase and urease, beyond the microbial biomass carbon (MBC), basal respiration rate (C-CO_2) and metabolic quotient (qCO_2). To compare soil microbiology before and after the application of thiamethoxam, multivariate statistical techniques were used. The application of biochar resulted in increased enzymatic activity of urease, acid phosphatase, increase of qCO_2 and basal respiration and reduction of MBC. In contrast, the application of the thiamethoxam insecticide suppressed the enzymatic activity of urease, acid phosphatase, resulting, however, in the elevation of alkaline phosphatase and basal respiration of the soil. Biochar application at doses greater than or equal to 16 t ha^{-1} resulted in elevation of qCO_2 and reduction of MBC, regardless of the absence or presence of NPK chemical fertilization. Biochar effect on soil microbiological attributes is less significant than the effect of thiamethoxam application.

Keywords: soil microbial biomass, enzymatic activity, soil quality

1. Introduction

Thiamethoxam insecticide is used worldwide in agriculture for the control of a wide variety of insect pests (Hilton, Jarvisa, & Ricketts, 2015). In Brazil, 7% of the insecticides used are from the neonicotinoid cluster, with thiamethoxam being one of the main commercialized molecules (IBAMA, 2017). The high demand for the use of this molecule in agricultural systems requires greater attention regarding its effects on the soil microbiota. This concern elapses from the physico-chemical characteristics of thiamethoxam (low sorption interaction, high solubility), which gives it, above all, high soil persistence (Hladik, Kolpin, & Kuivila, 2014). These aspects result in two main concerns with its application: i) high leaching potential and consequently subsurface water contamination; ii) deleterious effects on the soil microorganism and the ecological chain, resulting in toxic effects on several organisms, including humans.

Studies confirm the deleterious effect of thiamethoxam on the soil microbiota, such as toxicity to bacteria involved in the nitrogen cycle (Filimon et al., 2015), reduction in the activity of the urease, phosphatase and β-glucosidase enzymes (Jyot, Mandal, & Singh, 2015) and reduction of microbial biomass carbon (MBC) and basal soil respiration (Portillo, Scorza Junior Salton, Mendes, & Merchant, 2015).

Soil microorganisms have a role of great ecological and agricultural importance, working actively in the processes of genesis, nutrient cycling, decomposition of organic residues, synthesis of organic matter and degradation of organic contaminants (Kirchman, 2018; Mendes, Souza, & Reis Júnior, 2015). Therefore, it is necessary to study techniques to reduce the impact of agrochemicals on the soil microbiota. Due to its characteristics, mainly to the reactivity, the organic matter (OM) is the main component of the soil involved in the remediation of the contaminant potential of agrochemicals (Portilho et al., 2015; Petter et al., 2017). This fact, the OM also happens to be the main compartment of the soil to be improved from the point of view of handling techniques. However, in the tropics the maintenance of OM levels is hampered by high temperatures and precipitation, which requires alternative studies to maintain and/or increase soil carbon stocks. Given the high porosity, high molecular stability, the use of biochar is an alternative to improve carbon stocks in the soil, and can act as a source of nutrients and habitat for microorganisms (Li et al., 2019) and thereby minimize the harmful effects of thiamethoxam on the environment. In addition, although the biochar presents high molecular stability, after its application to the soil, processes of oxidation of the aromatic structures forming new electric charges and reactive functional clusters in the soil occur (Petter et al, 2017). This higher reactivity may represent an improvement in the retention of molecules as it occurs in organic matter (Schmidt et al., 2015).

The effect of biochar in the soil has been the subject of several studies that have shown beneficial effects on soil, such as increase fertility, water retention (Zhu et al., 2017), agrochemicals (Ali, Khan, Li, Zheng, & Yao, 2019; Hladik et al., 2014), increased microbial biomass (Lehmann et al., 2011), significant changes in the composition of the microbial community in clayey soils (Silva et al., 2018; Li, Liang, & Shangguan, 2017), increased enzymatic activities such as urease and β-glucosidase (Huang et al., 2017; Wang et al., 2017), increased nodulation of the root by nitrogen-fixing bacteria, nutrient cycling and carbon sequestration (Scheifele et al., 2017).

However, there is still a lack of studies using biochar in order to reduce the residual effect of pesticides in soil and its effect on long-term biological functions (Palansooriya et al., 2019). Aiming to fill this gap in the research, we propose in our studies to evaluate the use of biochar in the soil as a mitigating technique of the potential effect of the insecticide on the microbiological properties of the soil.

2. **Material and Methods**

2.1 Study Area

The experiment was conducted at Farm Estrela do Sul in Nova Xavantina, Mato Grosso, in the Central West region of Brazil (14°34′50″ S and 52°24′01″ W), with an average altitude of 310 m, and the region located in the 'Cerrado' biome. The climate of the region is hot and humid tropical type (Aw), according to the classification of Köppen-Geiger. The soil is classified according to the Brazilian system of soil classification (Santos et al., 2018) as a Dystrophic Haplic Plinthosol, sandy loam texture, with 763 g kg^{-1} of sand, 67 g kg^{-1} of silt and 170 g kg^{-1} of clay.

2.2 Characterization and Experimental Design of the Study Area

Before the implementation of the experiment the area was native forest until 1985, after it was used for grazing with *Urochloa brizantha* until 2008. The experimental design was randomized blocks in a 2 × 4 factorial scheme with three replications. The treatments consisted of the combination of two doses of NPK fertilizer 05-25-25 (0 kg ha^{-1} and 300 kg ha^{-1}) and four doses of charcoal (biochar) as a source of pyrogenic carbon (0 t ha^{-1}; 8 t ha^{-1}, 16 t ha^{-1} and 32 t ha^{-1}). Each plot was composed of nine soybean/maize lines with a length of 10 m, totaling 40.50 m^2, and the useful area for evaluations of 25.20 m^2.

Before being incorporated into the soil, the eucalyptus biochar was milled and passed through a 2 mm sieve. Its chemical composition is shown in Table 1. This material was applied to the soil only once in December 2008, being incorporated at a depth of 0.10 m by means of a rotary spade. After the incorporation, the experiment was conducted under no-tillage system.

Table 1. Elemental composition (total values) of the biochar used in the experiment

Element	Unit	Concentration
Total Nitrogen (N)		3.3
Phosphorus (P_2O_5 Citric acid)		0.14
Phosphorus (P_2O_5 total)		-
K_2O	g kg^{-1}	1.9
CaO		1.5
MgO		0.9
Sulfur (S)		-
Copper (Cu)		1.0
Zinc (Zn)		36.0
Molybdenum (Mo)	mg kg^{-1}	-
Cobalt (Co)		-
Boron (B)		-
Total carbon (C)		774.0
Humidity	g kg^{-1}	50.0
Total mineral material		-
C:N Ratio		234.5
Specific surface area	m^2 g^{-1}	41.2
Pore volume	cc g^{-1}	0.018
Pore diameter	µm	38.4
Density	g cm^{-3}	0.3
Pyrolysis temperature	°C	400-500

Source: Petter et al. (2012), and Carvalho et al. (2013).

Later, in the two subsequent harvests (2008/2009 and 2009/2010 harvests) after biochar application, rice (*Oryza sativa*) was cultivated in a conventional culture system, and after all the agricultural crops until the time of samples collection for this experiment soybean (*Glycine max*) was cultivated in no-tillage system on millet straw (*Pennisetum glaucum*) that was formed in all crops 45 days before soybean planting in the experimental area. Fertilization was repeated every year, using the same formulation and in the same amounts. To characterize soil fertility in the 2015/2016 harvest, four deformed soil samples were collected from each plot in 0 to 0.10 m layer. Thus, simple samples were mixed composing one single sample per plot (Table 2).

Table 2. Analysis for fertility purposes of a Plinthosol subjected to four doses of charcoal (biochar) as a source of pyrogenic carbon (0 t ha^{-1}; 8 t ha^{-1}, 16 t ha^{-1} and 32 t ha^{-1}) and two doses of NPK fertilizer 05-25-25 (0 kg ha^{-1} and 300 kg ha^{-1}) in the municipality of Nova Xavantina (MT) in the 2015/2016 harvest

Biochar	pH	P	K	Ca	Mg	H+Al	Al	SB	CEC	V%	OM
t ha^{-1}		---- mg dm^{-3} ----		---------- $cmol_c$ dm^{-3} ----------							g dm^{-3}
No fertilization											
0	3.82	18.4	63.0	0.37	0.18	4.35	0.87	0.73	5.08	14.2	11.5
8	3.80	10.2	61.7	0.25	0.12	4.40	1.06	0.53	4.93	10.6	10.5
16	3.85	14.78	57.5	0.37	0.16	4.40	0.83	0.65	5.05	13.3	10.6
32	3.80	17.73	64.8	0.42	0.20	4.93	1.03	0.78	5.30	13.7	10.7
300 kg ha^{-1} of NPK											
0	3.78	24.40	68.5	0.29	0.12	4.35	1.01	0.58	4.93	12.6	11.5
8	3.89	35.35	67.5	0.65	0.37	4.03	0.73	1.13	5.15	19.1	13.5
16	3.88	53.05	65.0	0.49	0.16	4.45	0.80	0.80	5.25	15.4	11.8
32	3.85	35.38	80.0	0.56	0.23	4.58	1.02	1.00	5.58	17.9	10.9

Note. pH at $CaCl_2$; P and K determined by Mehlich-1; Ca, Mg and Al exchangeable extracted by KCl; H + Al extracted by calcium acetate; SB: sum of bases ;CEC: cation exchange capacity at pH 7; V%: soil base saturation; OM: soil organic matter determined by sodium dichromate.

2.3 Soil microbiological Analyzes

After eight years of biochar incorporation, 2018, deformed soil samples were collected with the aid of a Dutch auger in 0 to 0.10 m layer to determine their microbiological attributes. The collection was carried out when the soybean crop was in full bloom. During the collection the samples were packed in polystyrene boxes containing ice to maintain the temperature until dispatch to the laboratory.

After the first sampling, the thiamethoxam insecticide (record dose 105 g ha^{-1} of active) was applied with pressurized CO_2 pump throughout the experimental area (soybean crop). After 48 hours of application, a new soil sample was taken to determine its microbiological attributes, as described in the first collection. Soil moisture was in the same condition as the first collection.

The activities of four soil enzymes were determined: β-glucosidase, acid phosphatase, alkaline phosphatase according to the methods described by Tabatabai (1994), and urease by the method of Kandeler and Gerber (1988). These methods are based on the colorimetric determination of p-nitrophenol (yellow color) formed after the addition of colorless substrates specific to each enzyme evaluated.

For each soil sample, three analytical replicates were performed in the laboratory. The soil enzymatic activity was expressed in μg p-nitrophenol released per gram of dry soil per hour. For the determination of β-glucosidase, phosphatases (acid and alkaline), and urease, the respective substrates were used p-nitrophenol-β-D-Glucopyranoside 0.05 M (PNG 0.05 M), nitrophenol phosphate 0.05 M (PNP 0.05 M) and urea solution Absorbance readings ranged from 400 nm to β-glucosidase, 490 nm to acid phosphatases, 400 nm to alkaline phosphatase, and 690 nm to urease.

The soil basal respiration rate ($C\text{-}CO_2$) was determined by the method described in Anderson and Domsch (1993), and microbial biomass carbon (MBC) by the fumigation-extraction method described by Vance et al. (1987) and Brookes et al. (1985). With data from the biological analyzes, the metabolic quotient (qCO_2) was determined. The qCO_2 is the amount of $C\text{-}CO_2$ produced by unit of soil microbial biomass per unit time (mg $C\text{-}CO_2$ mg^{-1} MBC $hour^{-1}$) (Anderson & Domsch, 1993).

2.4 Statistical Analysis

Residual normality and variances homogeneity among treatments were confirmed by the Shapiro Wilk and Levene tests, respectively. To compare soil microbiological attributes before and after the application of thiamethoxam insecticide, the data were standardized to have mean 0 and variance 1, followed by performing the following multivariate statistical methods: hierarchical cluster analysis, k-means and main components.

A hierarchical cluster analysis was performed only for the insecticide factors and biochar doses, calculating the Euclidean distance between the "accesses" or plots, for the set of 7 variables, and using the Ward algorithm to obtain similar accesses clusters. The result of the analysis was presented in graphical form (dendrogram), which assisted in the identification of the clusters.

The identification of accesses in the clusters was also performed by the k-means analysis (Hair et al., 2009), which belongs to the class of methods of non-hierarchical and unsupervised clusters. In the clusters analysis by k-means, a multivariate analysis of the variables between the established clusters was performed.

Two principal component analyzes were performed: i) with biochar and insecticide factors, and ii) with biochar, insecticide and fertilization factors.

3. Results

3.1 Hierarchical Analysis

In order to define the number of clusters by dendrogram it is considered "jumps" or expressive variations in the distance of connection between the accesses. Among the Euclidean distances from 6 to 9, there was an expressive separation of clusters allowing the definition of 4 clusters (Figure 1). The accesses grouped in clusters 1 and 2 represent the soil without the application of thiamethoxam insecticide, however, it is verified that the biochar doses of 16 and 32 t ha^{-1} modified the microbiological attributes of the soil when compared with the doses of 0 and 8 t ha^{-1}. After applying thiamethoxam insecticide in the soil, it was verified that only 32 t ha^{-1} dose of biochar was able to alter the soil microbiological attributes, isolating the accesses in cluster 4.

From the analysis of hierarchical cluster, it was evident that the application of the thiamethoxam insecticide associated to biochar incorporation altered the microbiological attributes of the soil. However, this analysis alone does not allow the visualization of these changes between the established clusters.

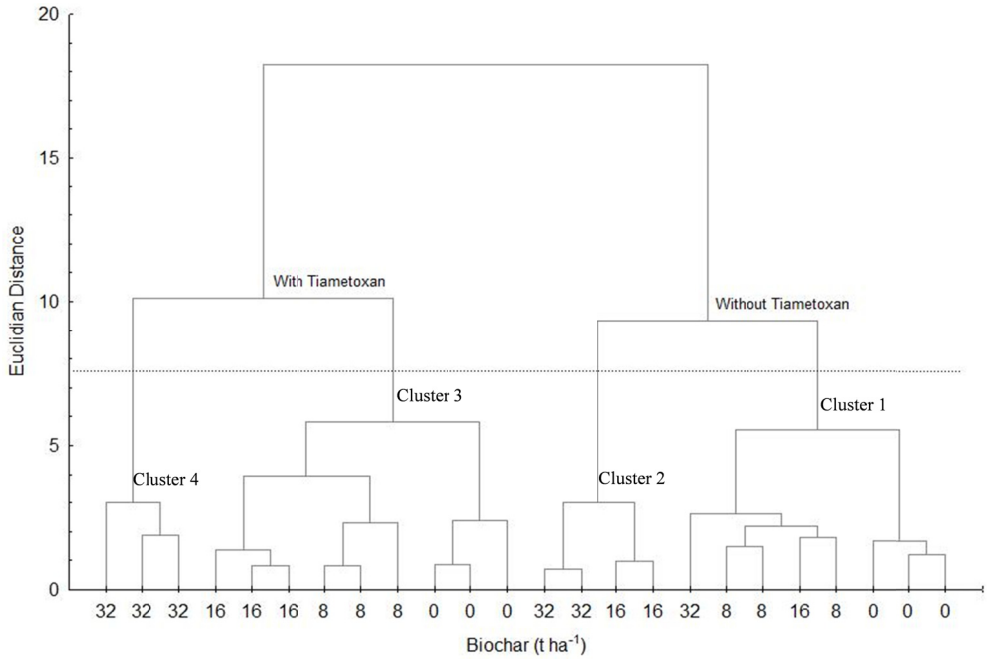

Figure 1. Dendrogram resulting from the hierarchical clusters analysis showing the formation of clusters according to acidic and alkaline phosphatase, β-glucosidase, urease, microbial biomass carbon, C-CO_2: basal respiration and qCO_2: metabolic quotient of a Plinthosol subjected to biochar doses

3.2 K-means Analysis

To characterize the clusters formed in the dendogram, the non-hierarchical k-means analysis was performed, considering four clusters established in the dendogram. By means of multivariate analysis of variance (MANOVA), it was possible to verify that all attributes of the soil presented differences of means among the four clusters (Table 3), confirming appropriate cutting height of the dendrogram for cluster definition.

Table 3. Analysis of variance for microbiological attributes of a Plinthosol submitted to biochar doses among the clusters formed by the non-hierarchical analysis of k-means clusters

Variables	Sum of squares among clusters	Degrees of freedom	Sum of squares among clusters	Degrees of freedom	Fc	Prob
Acid P	19.92	3	3.07	20	43.13	<0.001
Alkaline P	15.27	3	7.72	20	13.18	<0.001
β-Gluco	12.19	3	10.80	20	7.52	<0.01
Urease	16.55	3	6.44	20	17.13	<0.001
MBC	15.53	3	7.46	20	13.86	<0.001
C-CO_2	15.49	3	7.50	20	13.75	<0.001
qCO_2	13.89	3	9.10	20	10.17	<0.001

Note. Acid P: acid phosphatase; Alkaline P: alkaline phosphatase; β-Gluco: Beta glucosidase; MBC: microbial biomass carbon; C-CO_2: basal respiration; qCO_2: metabolic quotient; Fc: value of F calculated; Prob: probability of obtaining a value of $F \geq Fc$.

Clusters 1 and 2 represent the microbiological attributes of the soil without the application of thiamethoxan (Figure 1). Within cluster 1 it is verified that the incorporation of 8 t ha^{-1} of biochar was not sufficient to modify the microbiological attributes of the soil. Likewise, in cluster 2, the doses of 16 and 32 t ha^{-1} also did not present differences for the attributes of the studied soil. The difference between clusters 1 and 2 can be verified by the centroids of the K-means analysis and the standard errors of the standardized means of each attribute (Figure 2). Therefore, it can be inferred that in the absence of insecticide in the soil, doses equal to or greater than 16 t ha^{-1} resulted in a significant increase of β-glucosidase, C-CO_2 and qCO_2, and also reduced MBC.

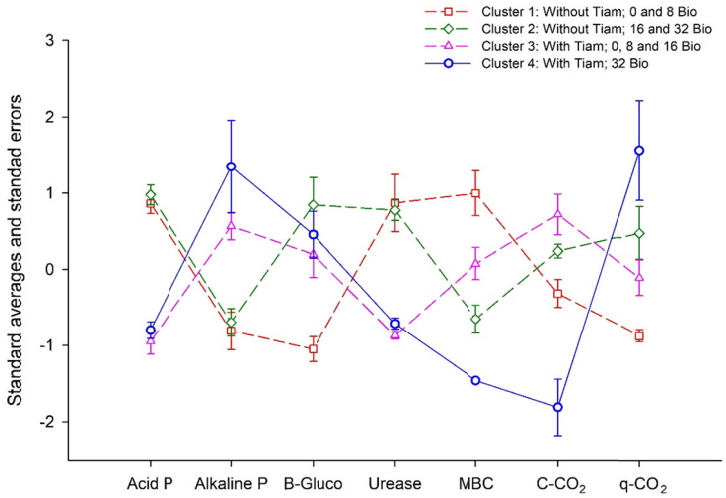

Figure 2. Standardized means of microbiological attributes of a Plinthosol submitted to biochar doses and clusters by non-hierarchical k-means analysis. Acid P: acid phosphatase; Alkaline P: alkaline phosphatase; β-Gluco: Beta glucosidase; MBC: microbial biomass carbon; C-CO_2: basal respiration and qCO_2: metabolic quotient

For the soil condition with insecticide application, observed in clusters 3 and 4, differences in soil attributes were verified only at the dose of 32 t ha^{-1}, occurring increase of qCO_2 and reduction in MBC and C-CO_2 values when compared to cluster 3.These results evidenced that the application of biochar did not attenuate the deleterious effects of thiamethoxam on the soil microbiota, do not confirming our initial hypothesis.

By the method of k-means analysis (Figure 2), it is generally verified that the main changes imposed on the soil by the application of the insecticide were the elevation of alkaline phosphatase and reduction of acid phosphatase and urease.

3.4 Principal Component Analysis

3.4.1 In the Absence of NPK Fertilizer

In order to evaluate the importance of variables in the separation of the four clusters formed in the hierarchical and not-hierarchical analysis, it was carried out the principal components analysis (PC's). The components with eigenvalues greater than or equal to 1 were selected, with only three principal components defined. The eigenvalues found were 3.01, 1.61 and 1.00 for the principal components as one (PC1), two (PC2) and three (PC3), respectively. With these three components it was possible to explain 80.25% of all data variability.

These PCs were constructed by combining the eigenvectors, which are values that represent the weight of each attribute in the components (Silva et al., 2015). In order to select the significant variables for analysis of principal components, we considered only eigenvectors with correlation greater than or equal to 0.5 according to the criteria proposed by Coelho (2003). All soil attributes showed correlations above 0.50 in some of the three components. Only the variable C-CO_2 did not present significant correlation in PC1 and PC2, being isolated in PC3.

The biplot plot of PC1 versus PC2 shows that the application of the insecticide actually altered the microbiology of Plinthosol (Figure 3), confirming the statements made according to the dendrogram and the k-means analysis. Accessions without thiamethoxam application on the soil showed a positive correlation with urease, acid phosphatase and MBC. However, within this cluster it is verified that the biochar incorporation to the soil promoted increase of the urease and acid phosphatase, as well as the reduction of the MBC.

In contrast, the cluster formed by the accesses with application of insecticide negative correlation presentation with urease and acid phosphatase positive with β-glucosidase, alkaline phosphatase and qCO_2 (Figure 3). Within this cluster it is verified that biochar incorporation also provided reduction of MBC and increase of qCO_2.

As the PC3 explained only 14.26% of the total data variability, being represented only by the C-CO_2 attribute the biplot graph was not presented. What can be observed is that the separation of the large clusters according to the application of the insecticide was maintained as a function of PC1. However, there was a confounding among the accesses due to the biochar doses within each large cluster. It was possible to only infer that the accesses with the

presence of thiamethoxam associated with 32 t ha^{-1} dose of biochar had lower values of C-CO_2 compared to the other accessions.

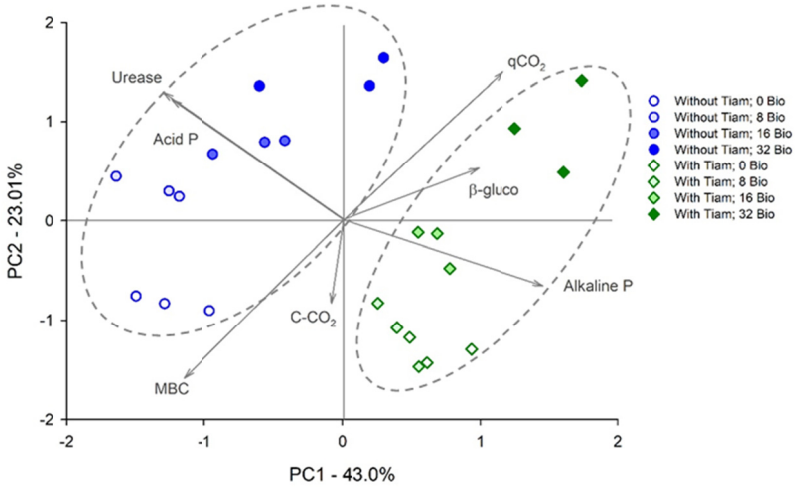

Figure 3. Principal component analysis of a microbiological attributes of a Plinthosol subjected to biochar doses in the absence of fertilization. Acid P: acid phosphatase; Alkaline P: alkaline phosphatase; β-Gluco: Beta glucosidase; MBC: microbial biomass carbon; C-CO_2: basal respiration and qCO_2: metabolic quotient. Without Tiam: without application of the thiamethoxam insecticide. With Tiam: soil submitted to the application of thiamethoxam insecticide. Bio: biochar doses in t ha^{-1}

3.4.2 In the Presence of NPK Fertilizer (300 kg ha^{-1})

With the previous analyzes it was possible to evaluate the consequences of thiamethoxam application on the soil microbiological attributes and its interaction with biochar doses. However, the analyzed plots were not fertilized, leaving doubts as to whether the results found would be similar in fertilized areas. Due to this, another analysis of principal components was performed with the factors such as insecticide, biochar doses and soil fertilization (Figure 4). Only the first two principal components (PCs) were considered. The first principal component (PC1) had eigenvalue of 3.05, and the second (PC2) eigenvalue of 1.53. These two PCs explained more than 65.4% all of variability of the soil microbiological attributes. The greatest variability was retained in the first principal component (PC1), summarizing in one axis of the biplot graph 43.5% of all variability found in the experiment (Figure 4).

The microbiological attributes that presented significance for the separation of the clusters in PC1 were acid and alkaline phosphatase, urease and basal respiration, with eigenvectors or correlations ≥ 0.50, which according to Silva et al. (2015) are highly significant variables. For PC2 only MBC, qCO_2 and β-glucosidase had correlations above 0.50, these variables being responsible for the separation of clusters on the y axis of the biplot graph (Figure 4).

The biplot graph shows the formation of 4 clusters, one in each quadrant (Figure 4). Clusters 1 and 2 are composed of accessions or plots of the experiment in which the soil received application of thiamethoxam insecticide. By analyzing only PC1, it can be verified that the accessions of these clusters showed a high correlation with alkaline phosphatase and basal respiration. Clusters 3 and 4, however, presented a high correlation with acid phosphatase and urease.

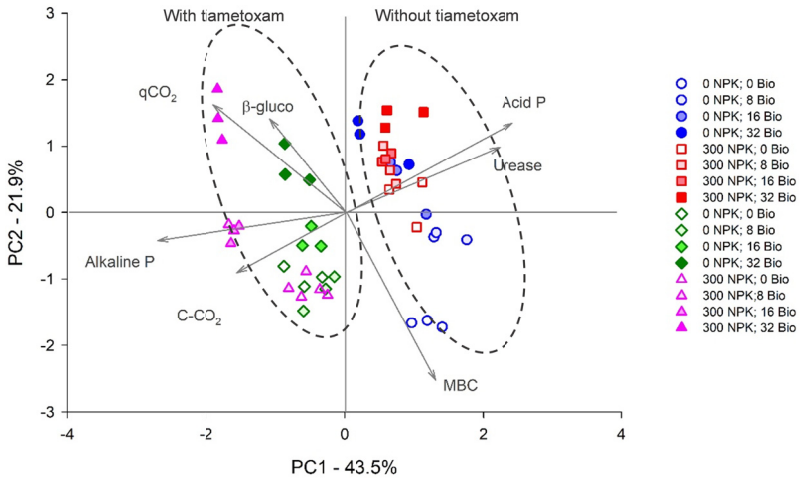

Figure 4. Principal component analysis of microbiological attributes of a Plinthosol subjected to biochar doses and fertilizer (NPK). Acid P: acid phosphatase; Alkaline P: alkaline phosphatase; β-Gluco: Beta glucosidase; MBC: microbial biomass carbon; C-CO_2: basal respiration and qCO_2: metabolic quotient. NPK: 0 or 300 kg ha-^{1} of the formulated 05-25-25, Bio: biochar doses in t ha^{-1}

With this, we can affirm that the soil without the presence of thiamethoxam insecticide presented high values for acid phosphatase and urease, being these soil attributes suppressed with the application of thiamethoxam, resulting in elevation of alkaline phosphatase and basal respiration.

Analyzing PC2, that is, only the separation of clusters in the y-axis, is verified that clusters 1 and 3 showed high correlation with qCO_2 and β-glucosidase. Clusters 2 and 4, however, had a high correlation with MBC. This separation was not performed due to the application of the thiamethoxam insecticide, but due to biochar doses to the soil with thiamethoxam application (clusters 1 and 2) and biochar and NPK doses to the soil without insecticide application (clusters 3 and 4). Cluster 1 was formed by accesses that received the highest biochar dose (32 t ha^{-1}) independent of fertilization, cluster 2 formed predominantly by accessions that received lower biochar doses (0, 8 and 16 t ha^{-1}). Cluster 3 consists of accessions, most of which received the highest biochar doses (16 and 32 t ha^{-1}) without fertilizers and all samples with application of 300 kg ha^{-1} of NPK, and cluster 4 formed predominantly by accessions that received the lowest biochar doses (0 and 8 t ha^{-1}) without fertilizer.

Due to this, it can be stated that independently of the insecticide and NPK application, biochar application at doses greater than or equal to 16 t ha^{-1} resulted in elevation of qCO_2 and reduction of MBC of Plinthosol. The same occurred with application of 300 kg ha^{-1} of NPK independent of biochar dose resulted in elevation of qCO_2 and reduction of MBC of Plinthosol.

The changes imposed by biochar and fertilization application (PC2) on soil microbiological attributes are less significant than the effect of thiamethoxam application (PC1).

4. Discussion

4.1 Biochar in Soil Microbial Properties

Even after eight years of biochar incorporation it was observed an increase of urease and acid phosphatase enzymes of Plinthosol (Figures 2 and 3). Huang et al. (2017) observed increased activity of specific enzymes related to the use of N in the soil in the presence of biochar. Li, Song, Singh, and Wang (2019) also investigated the increase in microbial activity and improvements in soil properties with the release of nutrients from biochar.

Possible explanations for our results would be due to: i) the biochar serving as a substrate of N (N-biochar) released slowly to the soil, resulting in an increase of the total nitrogen as shown in the studies of Petter et al. (2016). The results presented by the authors correspond to biochar application effect after five years of its incorporation into the same Plinthosol of this experiment and six years in an Oxisol. With this, we can affirm that the results verified by Petter et al. (2016) still persist in the soil even after eight years of biochar application. Although not completely elucidated, biochar may promote greater interaction with native organic matter of the soil, generating a positive priming effect, which would result in greater availability of nutrients (*e.g.*, nitrogen and phosphorus) in the soil, especially in sandy soils and of low fertility, thus justifying in part the effect on acid phosphatase and urease. This fact was verified by Wang et al. (2016), in which a 20% increase in OM

mineralization was observed with the application of biochar in sandy soils; the biochar serves as micro-habitat for the soil microbiota through its pores that protect the colonies of fungi and bacteria from natural predators, thereby the enzymes remain for a longer period in the soil (Petter et al., 2018; Scheifele et al., 2017; Pietikäinen, Kiikkilä, & Fritze, 2003). Furthermore, biochar porosity increases the water availability, as noted in the study of Petter et al. (2016), and Carvalho et al. (2013) in the same soil of our study. In periods of scarcity, this water retention in the biochar pores can promote greater survival of microorganisms (Junna, Bingchen, & Gang, 2014), especially during drought periods.

The decrease of the microbial biomass carbon (MBC) and increase of qCO_2 demonstrates that the soil microbiota was under stress. Some studies report that MBC is closely related to the C/N ratio and soil organic carbon (SOC) (Li et al., 2017), decreasing significantly after biochar application biochar (Dempster, Gleenson, Solaiman, Jones, & Murphyet, 2012; Santos, Madari, & Tsai, 2013). Our results indicate that biochar application in the soil reduces MBC proportionally to the applied doses (Table 3). There are several possible reasons that would explain these results: i) the high molecular stability of polycondensed aromatic structures of the biochar carbon formed by the slow pyrolysis at high temperature, resulting in recalcitrant C and more resistant to degradation by microorganisms (Pietikäinen et al., 2019; Chintala et al., 2015; Farrel et al., 2013). Although, the biochar applied in this experiment contained C-labile, after eight years of its application, it was practically no longer observed. This effect was verified in a three-year study after biochar application and incorporation in this same soil and experiment, where it was characterized that there still was C-labile (oxidizable) from biochar available (Petter et al., 2016). The origin of this C-labile would be related to the condensable compounds formed in the biochar pyrolysis process; ii) with the permanence of the biochar to the soil, the oxidation of the biochar carbon-labile associated to a possible positive priming effect in the native OM may have contributed to the reduction of C-labile and increase of the C-recalcitrant in the soil, resulting in an organic matrix with high C: N ratio. This effect would result in greater difficulty of gain for MBC or even MBC loss, as verified in the present study; iii) no less important, the long residence time of biochar in the soil can induce changes in the mineralization rate of the SOC as suggested by Li et al. (2018), especially carbon from recent soil inputs. This may be related to the modification of the microorganisms' abundance related to the C and N cycle of the soil, observed by Xu et al (2014).

4.2Thiamethoxam in Soil Microbial Properties

The reduction of urease and acid phosphatase enzymatic activity may be directly related to the toxicity of the insecticide on the soil microbiota, corroborating the results of Filimon et al. (2015), which verified the decrease of urease and acid phosphatase in the presence of thiamethoxam and its toxic effect on the bacteria involved in the nitrogen cycle. It is well known in the literature (Moreira & Siqueira, 2006) the perception of the sensitivity of soil nitrifying bacteria to agrochemicals as herbicides, fungicides and insecticides.

On the other hand, the increase of enzymatic activity of β-glucosidase and alkaline phosphatase with the application of thiamethoxam may be related to the soil microbiota modification selecting through selective pressure microorganisms capable of producing these enzymes, as well as providing energy in their degradation. A study by Myresiotis, Vryza, and Papadopoulou-Mourkidou (2012) showed that the increasing of bacterial growth resulted in greater degradation of thiamethoxam. The β-glucosidase enzyme is essential for carbon degradation to generate energy for the microorganisms through the catalysis of cellobiose hydrolysis into two glucose molecules (Adetunji et al., 2017). Thus, the selective microbiota possibly acted on the degradation of thiamethoxam resulting in momentarily high levels of β-glucosidase.

Thiamethoxam degradation in soil primarily involves bacterial activity of *Pseudomonas* sp genre, resulting in metabolites of the 'magic-nitro' cluster (=N-NO_2) and subsequently transformed into metabolites such as nitroguanidine, desnitro/guanidine (THX-II) and urea (THX-III) (Pandey et al., 2009). According to these authors, 'magic-nitro' clusters (=N-NO_2) can be converted to bacterial enzymes in a nonspecific way, which would explain in part the increase in the activity of β-glucosidase and acid phosphatase even in momentary events after the application of thiamethoxam.

It is noticeable while on one hand biochar provides increased enzymatic activity of urease and acid phosphatase, on the other hand, thiamethoxam provides precisely the reduction of the activity of such enzymes, showing that biochar little attenuates the deleterious effect of this molecule on soil microbial activity. This fact is further confirmed by the reduction of MBC, increase of qCO_2 and C-CO_2 with the application of both. These results reinforce the need for long-term studies after application of biochar on microbial activity in agricultural areas submitted to the intensive use of agrochemicals, since they have high sorption interaction with biochar, especially polar pesticides. Thus, this greater sorptive interaction could provide greater availability of access of

microorganisms to these molecules and provide long-term significant change in the community and microbial activity, whose results may alter the biogeochemical cycles of nutrients in the soil and the growth and development patterns of the plants.

4.3. NPK fertilizer in Soil Microbial Properties

The increase of qCO_2 and reduction of the MBC of Plinthosol in the presence of chemical fertilization, independent of biochar dose seems to be related to the adaptation of microorganisms to the soil environment with Biochar + NPK. These results confirm the observations of recent studies in which high doses of biochar (> 16 t ha^{-1}) provided lower biodiversity when compared to control soil (Santos, 2013), reduction of enzyme activity and microbial abundance, besides alter the microbial community structure (Huang et al., 2017). The reports justify the hypothesis above, since it was expected that, with the application of chemical fertilization, there would be higher MBC due to the greater contribution of vegetal residues.

Thus, it seems that the biochar interferes in the soil organic matter dynamics and this on the microbial activity in the presence of chemical fertilization under three main points: i) lower biodiversity and alteration of microbial community structure; ii) priming effect on organic carbon derived from plant residues as previously discussed, a fact that would reduce the effect of the higher contribution of labile carbon on plant residues on MBC; iii) presence of inhibitor substances (ethylene, phenolic compounds) of soil microbial processes in the presence of high biochar doses (Deenik, McClellana, Ueharaa, Antal, & Campbell, 2010; Spokas et al., 2010).

5. Conclusions

Microbial properties of Plinthosol showed different responses after eight years of biochar incorporation. The increase of biochar doses resulted in an increase in the production of urease and acid phosphatase enzyme, increase of qCO_2 and basal respiration and reduction of MBC. The application of biochar in larger doses than or equal to 16 t ha^{-1} resulted in elevation of qCO^2 and reduction of MBC.

The application of thiamethoxam insecticide suppressed the enzymatic activity of urease and acid phosphatase, resulting in elevation of alkaline phosphatase and reduction of basal respiration of the soil.

The application of thiamethoxam insecticide led to more significant modifications on the soil microbiota than biochar.

The application of biochar in the soil did not attenuate the negative effects of thiamethoxam on the soil microbiota.

The results of the present study suggest that the application of biochar in the soil may result after long term in significant transformations in the soil microbiota, either through the selection of microorganisms or the alteration of microbial and enzymatic activity.

Acknowledgements

We would like to thank the State Department of Education of Mato Grosso (Secretaria de Estado de Educação de Mato Grosso-SEDUC) for the release and financial support and the Foundation for Research Support of Mato Grosso (Fundação de Amparo à Pesquisa de Mato Grosso-FAPEMAT) for the first author's doctoral scholarship. We also thank the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq) by the "Productivity scholarship PQ1" for the second author and financial support for the biochar project coordinated by Fabiano André Petter.

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