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Phosphate fertilization and bioactivator influences on fractions of organic matter and soil microbial biomass

Influência da adubação fosfatada e bioativador nas frações da matéria orgânica e da biomassa microbiana do solo

Influencia de la fertilización fosfatada y bioactivador sobre las fracciones de materia orgánica y biomasa microbiana del suelo

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Abstract
Soil organic matter is considered a potential source of phosphorus (P) for plants because of biological cycling. Bioactivators are characterized as tools that can help soil microbial activity and the availability of P demanded by plants. The objective of this study was to evaluate the influence of the use of bioactivators and phosphate fertilization on the activity of soil microbial biomass and humified fractions of organic matter. The experimental design was completely randomized in a factorial scheme (6 × 2), with the first factor consisting of six doses of phosphorus applied to the soil (0, 30, 60, 90, 120, and 150 kg ha^{-1} of P_2O_5), using triple superphosphate as the source, and the second factor being the presence or absence of soil and plant bioactivator application, with four replications. The evaluated characteristics were soil microbial biomass carbon (SMBC), soil basal respiration (SBR), microbial biomass nitrogen (MBN), metabolic quotient (qCO_2), microbial quotient (q_{mic}), total organic carbon (TOC), fulvic acid fraction carbon (FAC), humic acid fraction carbon (HAC), and humin fraction carbon (HUMC). The use of soil and plant bioactivators and mineral phosphate fertilizer promoted changes in soil microbial activity. Increasing doses of phosphate fertilization promoted a reduction in the carbon characteristics of microbial biomass and basal soil respiration in the presence or absence of soil and plant bioactivators. Moreover, the use of bioactivator promoted higher averages for soil microbial biomass carbon, soil basal respiration, metabolic quotient, humic acid and humin and reduced the levels of microbial quotient, total organic carbon and fulvic acid.
Keywords: Phosphorus; Organic matter; Soil microbial biomass; Bioactivators.

Resumo
A matéria orgânica do solo é considerada uma fonte potencial de fósforo (P) às plantas em virtude da ciclagem biológica, em que microrganismos e raízes mineralizam o P orgânico por meio da síntese e exsudação de enzimas fosfatases. Os bioativadores são caracterizados como ferramentas que podem auxiliar a atividade microbiana do solo e na disponibilização de P demandado pelas plantas. Objetivou-se com o presente trabalho avaliar influência da

utilização de bioativador e adubação fosfatada na atividade da biomassa microbiana do solo e das frações humificadas da matéria orgânica. O delineamento experimental foi inteiramente aleatorizado em esquema fatorial (6x2), sendo o primeiro fator constituído de seis doses de fósforo aplicados ao solo (0, 30, 60, 90, 120 e 150 kg ha^{-1} de P_2O_5), utilizando-se como fonte o superfosfato triplo e o segundo fator da presença ou ausência da aplicação de bioativador de solo e planta, com quatro repetições. As características avaliadas foram carbono da biomassa microbiana (CBMS), respiração basal do solo (RBS), nitrogênio da biomassa microbiana (NBM), quociente metabólico (qCO_2), quociente microbiano (q_{mic}), carbono orgânico total (COT), carbono da fração ácido fúlvico (CAF), carbono da fração ácido húmico (CAH) e carbono da fração humina (CHUM). A utilização de bioativadores e adubação mineral fosfatada promoveram alterações na atividade microbiana do solo. Doses crescentes de adubação fosfatada promoveram redução nas características de carbono da biomassa microbiana e respiração basal do solo na presença ou ausência do bioativador de solo e planta. A utilização do bioativador promoveu maiores médias para as características CBMS, RBS, qCO_2, CAH e CHUM. A utilização do bioativador reduziu os teores de q_{mic}, COT e CAF.
Palavras-chave: Fósforo; Matéria orgânica; Biomassa microbiana do solo; Bioativadores.

Resumen
La materia orgánica del suelo se considera una fuente potencial de fósforo (P) para las plantas debido al ciclo biológico, en el que los microorganismos y las raíces mineralizan el P orgánico a través de la síntesis y exudación de enzimas fosfatasa. Los bioactivadores se caracterizan por ser herramientas que pueden ayudar a la actividad microbiana del suelo y a la disponibilidad de P demandado por las plantas. El objetivo del presente trabajo fue evaluar la influencia del uso de bioactivadores y fertilización fosfatada sobre la actividad de la biomasa microbiana del suelo y las fracciones humificadas de la materia orgánica. El diseño experimental fue completamente aleatorio en esquema factorial (6x2), con el primer factor compuesto por seis dosis de fósforo aplicadas al suelo (0, 30, 60, 90, 120 y 150 kg ha^{-1} de P_2O_5), utilizando como la fuente fue superfosfato triple y el segundo factor fue la presencia o ausencia de suelo y aplicación de bioactivador vegetal, con cuatro repeticiones. Las características evaluadas fueron carbono de biomasa microbiana (CBMS), respiración basal del suelo (RBS), nitrógeno de biomasa microbiana (NBM), cociente metabólico (qCO_2), cociente microbiano (q_{mic}), carbono orgánico total (TOC), carbono ácido fúlvico (CAF), carbono de la fracción de ácido húmico (CAH) y carbono de la fracción de humina (CHUM). El uso de bioactivadores y la fertilización con fosfato mineral promovieron cambios en la actividad microbiana del suelo. Las dosis crecientes de fertilización con fosfato promovieron una reducción en las características de carbono de la biomasa microbiana y la respiración basal del suelo en presencia o ausencia del bioactivador del suelo y la planta. El uso del bioactivador promovió promedios superiores para las características CBMS, RBS, qCO_2, CAH y CHUM. El uso del bioactivador redujo los niveles de q_{mic}, TOC y CAF.
Palabras clave: Fósforo; Materia orgánica; Biomasa microbiana del suelo; Bioactivadores.

1. Introduction

Soil organic matter (SOM) is considered a potential source of phosphorus (P) for plants because of biological cycling. Bioactivators are characterized as tools that can help soil microbial activity and the availability of P demanded by plants, acting on the microbial population responsible for the decomposition of organic compounds, nutrient cycling, and the flow of energy in the soil (Araújo et al., 2019; Agostinho et al., 2017). Furthermore, it is considered more sensitive to changes in soil quality, as it has the ability to respond much faster to changes in soil management and use (Almeida et al., 2016).

Soil organic matter is mostly formed by humic substances, a product of the biological transformation of organic residues to stabilized forms of compounds that are relatively refractory to biological decomposition (Cavalcanti et al., 2017; Huang & Hardie, 2009). Based on their solubility, humic substances can be classified into fulvic acids, humic acids, and humines (Caron et al., 2015). The relative distribution of humified fractions can be used as an indicator of the quality of organic matter (Danchenko et al., 2020; Maia & Parron, 2015; Novotny et al., 2020; Savarese el al., 2021).

SOM becomes an important source of P for plants, when microorganisms and roots are able to mineralize organic P through the synthesis and exudation of phosphatase enzymes. The use of organic P as a source of P for plants can be maximized when plant residues are slowly decomposed to synchronize P availability and plant growth (Etesami et al., 2021; Cardoso & Andreote, 2016).

In tropical soils, the dynamics of phosphorus (P) are associated with environmental factors that control the activity of microorganisms, which immobilize inorganic P and mineralize organic P, and physicochemical reactions such as adsorption,

desorption, and precipitation (Martinez et al., 2021; Bünemann, 2015; Novais & Smyth, 1999). In these soils, the adsorption process reduces the amount of inorganic P owing to the high binding stability of this nutrient to solid soil particles, resulting in low levels of P in the solution, thereby limiting agricultural production (Peluco et al., 2015).

The development of practices that provide greater availability of P for plants can lead to a reduction for phosphate fertilizers applied to the soil, resulting in economic and environmental advantages because these fertilizers are produced from non-renewable mineral reserves. According to Nava and Gris (2014), a technology that has been developed to improve the nutritional efficiency of plants is bioactivation, which promotes the increase in the biological activity of soils and plants, generating a biological balance through increasing the number of naturally present microorganisms, nutrient cycling, explored soil area, and the biological control of pathogens.

According to Aparecido de Souza et al. (2017), bentonite-based soil bioactivator powder intensifies and accelerates biological activities in cropping systems and plant photosynthesis. Their use does not replace the fertilization of macro- and micronutrients but can complement the nutrition required by crops, improving the absorption of immobilized nutrients.

Although there are numerous studies on phosphate fertilization and organic matter fractions (Araújo et al., 2019; Ashraf et al., 2021; Bünemann, 2015; Ebeling et al., 2011; McGonigle & Turner, 2017; Souza et al., 2016), the use of bioactivators and their contribution to Cerrado soils are still quite limited. Therefore, the objective of this study was to evaluate the influence of the use of bioactivators and phosphate fertilization on the activity of soil microbial biomass and humified fractions of organic matter.

2. Methodology

2.1 Experimental location and description

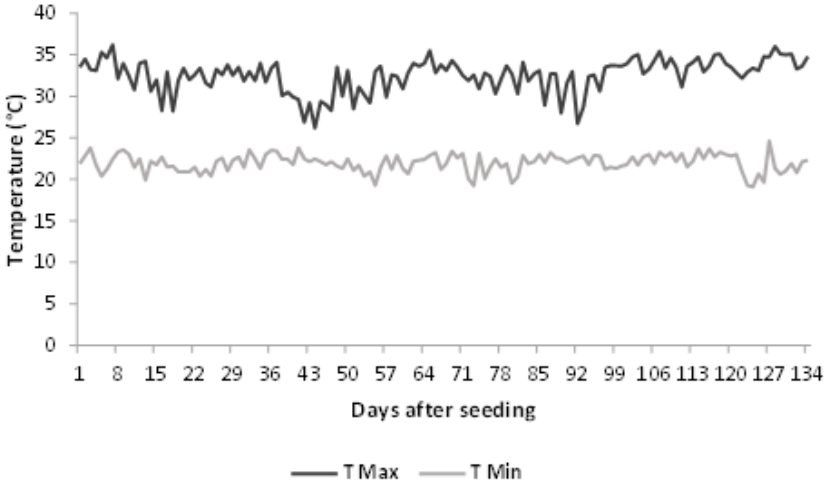
The experiment was carried out in the south of the State of Tocantins, in a greenhouse, at the experimental station of the Federal University of Tocantins, Campus de Gurupi, located at latitude 11°43'45"S and longitude 49°04'07" W, at an altitude of 280 m, between January and May 2017. A deep, acidic, dystrophic Red-Yellow Latosol with a sandy loam texture (Embrapa, 2018) collected in an area previously cultivated with soybean (*Glycine max* L.) was used, whose soil chemical properties were as follows: $pH_{(CaCl2)}$= 5.2; $P_{(meh)}$= 14.9 mg dm^{-3}; K=0.16 $cmol_c$ dm^{-3}; Ca=1.7 $cmol_c$ dm^{-3}; Mg= 0.7 $cmol_c$ dm^{-3}; H+Al= 2.20 $cmol_c$ dm^{-3}; Al^{3+} = 0.0 $cmol_c$ dm^{-3}; SB= 2.56 $cmol_c$ dm^{-3}; V= 54%; O.M.= 1.2%; sand= 75.2 dag kg^{-1}; silt= 5.0 dag kg^{-1}; and clay= 19.8 dag kg^{-1}. The temperature climatic data referring to the experiment conduction period can be found in Figure 1.

2.2 Experimental design and treatments

The experimental design was completely randomized in a factorial scheme (6 × 2), with the first factor consisting of six doses of phosphorus applied to the soil (0, 30, 60, 90, 120, and 150 kg ha^{-1} of P_2O_5), using triple superphosphate (41% of P_2O_5) as the source, and the second factor being the presence or absence of soil and plant bioactivator application, with four replications. The bioactivators used were powdered bentonite clay and calcium carbonate, which are recommended for soil and plant applications, respectively. The application of the bioactivator was carried out in three stages: one application of soil bioactivator (250 g ha^{-1}) applied to the soil 1 day before soybean sowing plus one application each of plant bioactivator (125 g ha^{-1}) in stages V4 and R1, according to the manufacturer's recommendations for the culture evaluated.

For each experimental unit, 10 L of soil was used, and soil moisture was maintained at 75% of the water holding capacity throughout the evaluation period. Soybean seeds (cultivar M8808 IPRO) were inoculated with *Bradyrhizobium* strains. Sowing fertilization was carried out according to the recommendations of soil analysis, and potassium chloride was used as a source of K. Cultural treatments were performed according to the needs of the culture.

Figure 1. Daily average values of maximum and minimum temperatures (ºC), occurred during the conducting the experiment in Gurupi-TO, Brazil. The data were obtained from the automatic weather station of Gurupi-TO (A019) of the National Institute of Meteorol.



Source: National Institute of Meteorol – INMET

2.3 Data sampling and measurement

Soil samples were collected when more than 80% of the plants were in the R5 stage with the aid of a probe, and three simple samples were extracted to form a composite. The samples were then air dried, crushed, and passed through a 2.00-mm mesh sieve.

Total organic carbon (TOC) was determined according to the procedure of Embrapa (2011): oxidation of organic matter by potassium dichromate (0.2 mol L^{-1}) in a sulfuric medium and titration with ferrous ammonium sulfate (0.1 mol L^{-1}). For nitrogen (N) quantification, the samples were subjected to sulfuric digestion with subsequent Kjeldahl distillation (Tedesco et al., 1995).

The humic substances were fractionated using the differential solubility technique, separating the humic acid (FHA), fulvic acid (FFA), and humin (HUM) fractions according to the concepts established by the International Society of Humic Substances (Swift, 1996) and adapted by Benites et al. (2003). NaOH (0.1 mol L^{-1}) was used as the extractor. The C levels of each fraction was quantified via wet oxidation (Yeomans & Bremner, 1988) and N levels via sulfuric digestion followed by Kjeldahl distillation (Tedesco et al., 1995).

Microbial biomass C (MBC) and N (MBN) were estimated using the fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). After fumigation of the soil, MBC and MBN were extracted from the samples using K_2SO_4 at a ratio of 1:4 (soil:solution) under agitation for 30 min. Afterward, the samples were allowed to rest for another 30 min, and the supernatant was filtered through qualitative paper. Analysis of C and N in the extracts was performed using a total organic C analyzer equipped with a kit for N analysis (Shimadzu TOC-LCPH, Shimadzu, Kyoto, Japan). Basal soil respiration was estimated by the amount of C-CO_2 released over a 7-day period (Jenkinson & Powlson, 1976). CO_2 was captured in 10 mL of 0.5 mol L^{-1} NaOH, and after the incubation period, titration was performed with 0.5 mol L^{-1} HCl. The metabolic quotient (qCO_2) was calculated according to Anderson and Domsch (1989) using the ratio between basal respiration and MBC.

2.4 Data analysis

The experimental data were subjected to an analysis of variance (ANOVA). The means of the treatments with (activator) and without (control) a bioactivator were compared using Tukey's test at 5% probability. The effects of triple superphosphate doses were evaluated by a regression analysis using the computer application SISVAR (Ferreira, 2011).

3. Results

3.1 Effect of phosphate fertilization and bioactivator on soil microbial activity

The results of the ANOVA are presented in Table 1, where a significant effect of the interaction is verified for the characteristics of soil microbial biomass carbon (SMBC) and soil basal respiration (SBR), indicating the dependence of the factors; therefore, one factor is split into another. Significant effects are also observed for the factor involving the use of bioactivators for the microbial biomass carbon, basal respiration, metabolic quotient, microbial quotient, TOC, carbon of the fulvic acid fraction, carbon of the humic acid fraction and carbon of the humin fraction (Table 1).

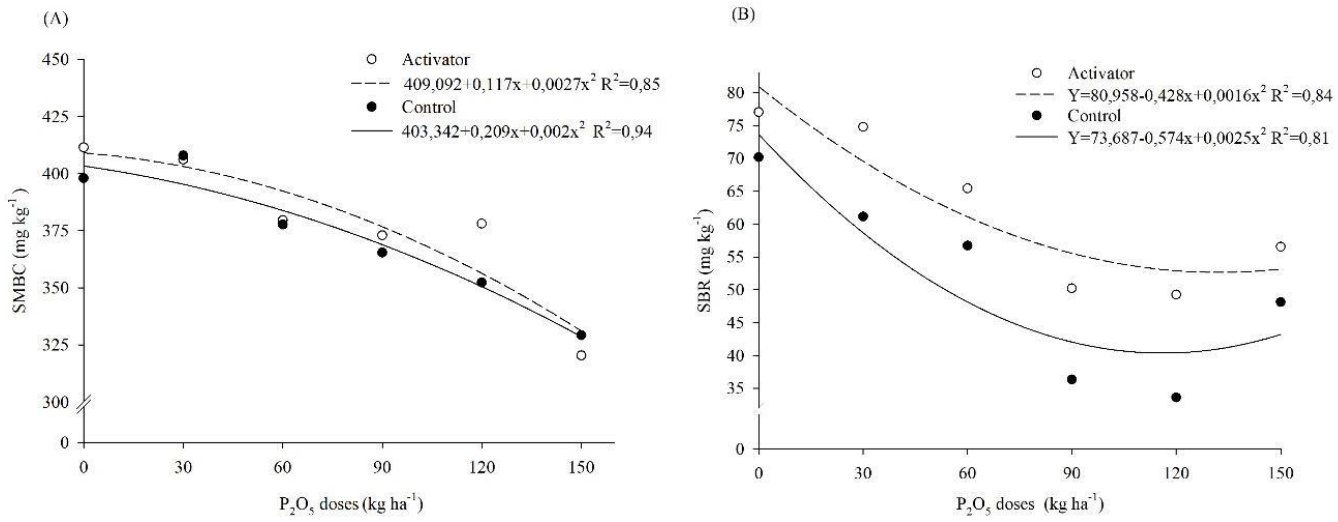
Table 1. Summary of analysis of variance of soil microbial biomass carbon (SMBC), soil basal respiration (SBR), microbial biomass nitrogen (MBN), metabolic quotient (qCO_2), microbial quotient (q_{mic}), total organic carbon (TOC), carbon of the fulvic acid fraction (FAC), carbon of the humic acid fraction (HAC), and carbon of the humin fraction (HUMC) in soybean plants submitted to six doses of super triple phosphate (D) in the absence and presence of the application of soil and plant bioactivators (B). Gurupi, TO, 2018.

Source of Variation	DF	Mean squares								
		SMBC	SBR	MBN	qCO_2	q_{mic}	TOC	FAC	HAC	HUMC
Doses (D)	5	29929.78*	7582.48**	164.26^{ns}	0.09^{ns}	12.52^{ns}	0.52^{ns}	4.28^{ns}	9.32^{ns}	21.41^{ns}
Bioactivador (B)	1	6493.94*	1144.13**	897.90^{ns}	0.12*	4.57**	3.68*	84.46**	120.38**	323.52**
D x B	5	2014.18**	21.82*	136.78^{ns}	0.12^{ns}	9.37^{ns}	1.52^{ns}	8.93^{ns}	29.31^{ns}	86.41^{ns}
Rep	2	293.17	14.37	6.93	0.0006	0.29	0.89	0.11	0.64	3.72
Residue	22	208.12	6.63	2.73	0.0005	0.11	0.27	0.46	0.61	2.6
CV (%)		8.05	9.75	15.65	12.58	6.56	7.33	13.06	10.32	13.41
Overall Average		342.42	54.81	10.56	0.18	4.99	7.12	5.21	7.59	12.03

^{ns}Not significant; **Significant at P≤0.01; *Significant at P≤0.05 by the F test. Source: Authors.

Regarding the SMBC and SBR characteristics, quadratic responses were observed as a function of the application of increasing doses of super triple phosphate. Significant effects were observed for SMBC and SBR in relation to bioactivator use. This suggests that mineral fertilization possibly influenced the activity of SMB, since when the bioactivator was used, a reduction in SMB was noted as doses were increased. Furthermore, the highest value (412.89 mg kg^{-1}) was obtained at the dose of 21.67 kg ha^{-1}, in the absence of product application, and the maximum response (419.72 mg kg^{-1}) was obtained at a dose of 52.25 kg ha^{-1} (Figure 2A).

Figure 2. Soil microbial biomass carbon (A) and soil basal respiration (B) as a function of increasing doses of phosphate fertilization with absence and presence of soil and plant bioactivator application. Gurupi, TO, Brazil, 2018.



Source: Authors.

Mineral fertilization also influences the characteristic SBR. In general, a tendency toward a reduction in SBR was noted as the dose of phosphate fertilization was increased, regardless of the use of bioactivators. With the application of bioactivators, the dose of 133.75 kg ha^{-1} was the one that presented the lowest response (52.33 mg kg^{-1}), and in the absence of the product, the dose of 114.8 kg ha^{-1} showed the lowest response (40.74 mg kg^{-1}) (Figure 2B).

Table 2. Means of soil microbial biomass carbon (SMBC) and soil basal respiration (SBR) characteristics in six doses of phosphate fertilization, with (activator) and without (control) application of soil and plant bioactivators. Gurupi, TO, 2018.

Doses (kg ha^{-1})	SMBC (mg kg^{-1})		Overall Average	SBR (mg kg^{-1} hora^{-1})		Overall Average
	Activador	Control		Activador	Control	
0	411.49 a	397.98 b	404.73	77.07 a	70.20 b	73.63
75	406.17 a	407.92 a	407.04	74.80 a	61.16 b	67.98
150	379.64 a	377.71 a	378.67	65.43 a	56.73 b	61.08
225	372.97 a	365.48 a	369.22	50.23 a	36.34 b	43.28
300	378.12 a	352.28 b	365.20	49.25 a	33.61 b	41.43
373	320.34 b	329.17 a	324.75	56.56 a	48.13 b	52.34
Overall Average	378.12	371.76		62.22	51.03	
CV (%)	8.05			9.75		

Means followed by the same letters in the column do not differ from each other by Tukey's test (P≤0.05). Source: Authors.

Basal respiration expresses the state of microbial metabolism in the soil, where the more CO2 released into the environment, the greater the microbial activity. Thus, high respiration rates can indicate both an adverse condition and adequate levels of microbial activity in the soil (Islam & Weil, 2000).

As for the splitting of the interaction (D*B), the absence of bioactivators resulted in a lower average SMBC when there was no supplementation with triple superphosphate. At a dose of 300 kg ha^{-1} of mineral fertilizer, significant differences were observed between the use and non-use of the product. In general, a tendency toward a reduction in this characteristic was noted as the dose of the phosphate source was increased, regardless of the use of bioactivators. A similar behavior was observed for the SBR trait, where a reduction in SBR was observed as the fertilizer doses increased in the absence or presence of bioactivators (Table 2).

Regarding qCO_2, q_{mic}, TOC, FAC, HAC, and HUMC, a significant difference was observed between the application and non-application of the bioactivator on the evaluated characteristics. The use of the product contributed to the highest values of qCO_2, HAC, and HUMC, with 0.24 mg C-$CO_2 g^{-1}$, 9.42 g kg^{-1}, and 15.03 g kg^{-1}, respectively. In the absence of the product, q_{mic}, TOC, and FAC presented higher averages (Table 3).

The qCO_2 represents the specific respiration of microbial biomass, where the elevation of this index indicates that the microbial population is possibly consuming large amounts of C for its maintenance and if the soil system cannot replace the carbon lost by respiration, the microbial biomass will probably decline (Anderson & Domsch, 2010). The high consumption of C can be seen when, with the use of bioactivator, the levels of FAC and TOC are reduced, and concomitantly greater presence of HUM are diagnosed. The carbon in the humin fraction is resistant to biological degradation, unlike FAC, which are more easily solubilized by the microbial population, contributing to the humification of organic matter (Ebeling et al., 2011; Menezes et al., 2017).

The microbial quotient (q_{mic}), which expresses the contribution of carbon from microbial biomass to the total organic carbon of the soil, showed a significant difference between the evaluated systems, with a higher index in plants that did not receive bioactivators (Table 3).

Table 3. Microbial biomass nitrogen (MBN), metabolic quotient (qCO_2), microbial quotient (q_{mic}), total organic carbon (TOC), and carbon contents in the fulvic acid (FAC), humic acid (HAC), and humin (HUMC) fractions in soil with (activator) and without (control) bioactivator application.

Bioactivador	MBN	qCO_2	q_{mic}	TOC	FAC	HAC	HUMC
	mg kg^{-1}	mg C-$CO_2 g^{-1}$ CBM h^{-1}	mg g^{-1}	g kg^{-1}			
Activator	15.56 a	0.24 a	0.463 b	6.80 b	3.68 b	9.42 a	15.03 a
Control	15.55 a	0.12 b	0.534 a	7.43 a	6.74 a	5.76 b	9.04 b

Means followed by the same letter in the column do not differ statistically from each other by Tukey's test ($P \leq 0.05$). Source: Authors.

4. Discussion

4.1 Effect of phosphate fertilization and bioactivator on soil microbial activity

The use of the bioactivator promoted stimuli to biomass and microbial activity (C-CO_2), possibly impacting on greater carbon (C) mineralization of soil organic matter. SMBC acts as a substrate for soil enzyme activity during mineralization process and help in physical stabilization of C (Tabatabai, 1994). Higher C mineralization rates due to increased availability of easily decomposable carbon leads to enhanced nutrients availability in addition to increased TOC (Bera et al., 2018; Saikia et al., 2020).

Evaluating the effect of bioactivators on soil microbial activity, Antonilli et al. (2016) highlighted that, even without showing a significant difference, the application of the bioactivator compared to the control expressed an increase in the C of the soil microbial biomass, highlighting that the use of the technology instigates the microbial activity of the soil.

Indeed soil organic matter decomposition depends on microbial activity of soil and enzymatic activity, which determines the release and availability of soil nutrients (Tejada & Gonzalez, 2006; Sharma et al., 2020) Soil organic C plays a key role as a precursor for enzyme synthesis via increased microbial activity (Benbi at al., 2015).

It is noted that, in general, with increasing doses of mineral fertilization, they concomitantly resulted in a decrease in microbial activity, impacting the CBMS and RBS characteristics. Mineral fertilization normally acts negatively on microbial biomass due to the lack of a C source, and in high doses, mineral fertilization negatively affects the microbiota, probably due to soil salinization, decrease in soil pH and lower rhizodeposition (Goyal et al., 1992; McCarty & Meisinger, 1997; Luo et al., 2015).

SMBC, a labile and biologically active fraction of SOM, is sensitive when diagnosing soil alterations and identifying significant differences between the treatments studied. According to Cardoso and Andreote (2016), SMBC plays a fundamental role in the productivity and maintenance of ecosystems as it acts as a catalyst for important chemical transformations in the soil. Furthermore, as a labile component of SOM, SMBC constitutes a reservoir of nutrients available to plants and is influenced by biotic and abiotic conditions. This influence was probably stimulated by the use of the bioactivator as a treatment. As it constitutes most of the active fraction of organic matter, it becomes more sensitive to changes in SOM caused by soil management and cultivation practices (Franzluebbers et al., 1996).

The use of bioactivators showed a higher average SBR in relation to treatments that did not receive the same application, indicating that the product may accelerate the process of mineralization of organic matter. Soil respiration, which reflects the activity of a major part of the soil microbial community, in geral, was steadily decreasing by the increasing rate of applied fertilizer mineral. This characteristic indicates the decomposition capacity of the organic material by heterotrophic microorganisms, as it involves measuring the metabolic functions in which CO2 production occurs (Malý et al., 2009; Silva et al., 2021). Soil respiration is considered critical for C exchange within ecosystem and may therefore, determine whether a system is a source or sink for C (Singh & Benbi, 2020; Van Hees at al., 2005).

Higher CO2 release is generally associated with higher biological activity, which in turn is directly related to the amount of labile C in the soil (Alvaro-Fuentes at al., 2009). The qCO2 has been a sensitive indicator for estimating the influence of cultivation on soil biological activity and substrate quality (Schnurer et al., 1985; Haney et al., 2008; Muñoz-Rojas et al., 2016; Sharma et al., 2020). These microbial parameters indicate how efficiently the microbial biomass utilizes substrate C for biosynthesis, rather than for the maintenance of soil respiration (Van Hees et al., 2005).

The qCO_2 represents the specific respiration of the microbial biomass, and high values indicate that the microbial population consumes large amounts of C for its maintenance under stressful conditions and if the soil system cannot replenish the carbon which is lost through respiration, microbial biomass must decline (Anderson & Domsch, 2010). The use of bioactivators favored higher averages, indicating a greater expenditure of energy for the maintenance of the microbial community, which may be an indication of stress, since the greater activity of the microbial population in the soil increased the rates of qCO_2, however this activity did not increase the availability of TOC available in the soil. Under stressful conditions, microorganisms need to consume more substrate for survival, resulting in higher qCO_2 values (Cheng at al., 2013; Pfeiffer et al., 2001; Fidelis et al., 2016).

The lower value observed in the treatments with bioactivators may reflect the lower use of carbon by the soil microbiota, since, in soils with low availability or quality of substrate or even another limiting factor, the microbial biomass cannot fully utilize organic C, thereby decreasing q_{mic} values (Anderson & Domsch, 1989). Gómez-Muñoz et al., (2017)

reported that microbial biomass became more efficient when less C-CO_2 was lost to the atmosphere, and a higher rate of carbon was incorporated into the microbial biomass, resulting in lower qCO_2 values.

Low values of q_{mic} can be caused by circumstances in which the microbiota is under stress. It can even indicate a lower reserve of organic compounds in these areas, since q_{mic} reflects the percentage of total organic carbon reserves in the soil, causing the microbial biomass to be unable to efficiently utilize organic C (Antisari et al., 2021; Cai et al., 2019).

TOC is directly linked to the chemical, physical, and biological attributes of the soil, as it is dependent on the decomposition, mineralization, and humification of organic matter present in the soil (Liu et al., 2020). In this study, TOC presented low levels, which may have occurred due to the great soil disturbance, since the experiment was conducted in pots, resulting in losses of this element. Moreover, a lower accumulation of TOC was noted in the treatments that received the application of the product, showing the possibility of this bioactivator acting primarily on more labile organic matter fractions, since there was an increase in recalcitrant fractions with the use of product.

According to Rosset et al. (2016), more recalcitrant fractions of SOM, such as humic substances, are the least responsive to changes in land use but have the longest mean residence times in the soil, which would allow greater storage of these elements in the ecosystem. Additionally, Kotzé et al. (2016) showed that the residence time of humic substances in the soil is determined by their recalcitrance, soil use, and associated agricultural practices. As the present study showed no change in the climatic conditions and in the management adopted, decomposition rate and the speed of chemical reactions might have increased, possibly by the participation of the bioactivator in the microbial activity, resulting in faster mineralization of the SOM, leading to different TOC contents in the treatments evaluated.

Regarding the chemical fractionation of organic matter, the treatments that received the application of the bioactivator showed differences between the humic fractions, with a greater predominance of the humin fraction (HUMC) to the detriment of the humic acid (HAC) and fulvic (FAC) fractions. The higher C content of the humin fraction indicated that the bioactivators provided a stimulus for greater microbial activity, contributing to the humification of organic matter. The predominance of this fraction can be attributed to its low solubility and resistance to biological degradation due to the formation of metal complexes (Ebeling et al., 2011; Menezes et al., 2017).

The humin fraction is less prone to change with management practices, because it is the most stable and recalcitrant fraction (Stevenson, 1994), however, it appears that the bioactivator was active in this portion of the SOM fractions, since a higher concentration of C in the humin fraction of the soil surface layer could be related to intensive microbial activity and consequently a higher SOM decomposition rate (Guimarães et al., 2013)

The predominance of the humic acid (HA) fraction over the fulvic acid (FA) fraction may be the result of intense humification and rapid mineralization of large amounts of organic material (Caron et al., 2015). Changes in the fulvic acid fraction are expected since it is less stable and more mobile (Christensen et al., 1998). The lower percentage of FA may have been caused by microbial activity that uses nutrients from aliphatic chains of poorly condensed organic molecules, such as those that occur in this fraction, in its metabolism (Stroud et al., 2007). According to Rosa et al. (2017), under favorable conditions, biological activity increases, resulting in greater mineralization and promotion of the synthesis of a more condensed substance, which could justify the increase in humic fractions.

5. Conclusions

The use of soil and plant bioactivators and mineral phosphate fertilizer promoted changes in soil microbial activity. Increasing doses of phosphate fertilization promoted a reduction in the carbon characteristics of microbial biomass and basal soil respiration in the presence or absence of soil and plant bioactivators. Moreover, the use of bioactivator promoted higher averages for soil microbial biomass carbon (SMBC), soil basal respiration (SBR), metabolic quotient (qCO_2), humic acid

(HAC), and humin (HUMC) and reduced the levels of microbial quotient (qmic), total organic carbon (TOC) and fulvic acid (FAC).

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