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**BIOTECHNOLOGY** 

# Microbial activity and carbon rates the soil in response to the application of potassium sources

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ABSTRACT. The continuous use of KCl may not be sustainable in the long term in agricultural systems. High doses used in crops accumulate in the soil and plants, hindering the metabolic processes of soil organisms. This study assessed the soil microbial activity in response to the application of K sources in banana crop and effects on microbial C. The experimental design was completely randomized with four K sources: potassium nitrate (KNO<sub>3</sub>), potassium chloride (KCl), potassium sulfate (K<sub>2</sub>SO<sub>4</sub>), and monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) at 200 mg kg<sup>-1</sup> of K<sub>2</sub>O, besides the control (without K) and combinations KCl:K<sub>2</sub>SO<sub>4</sub>. KCl application increased microbial activity 7 days after incubation, with gradual reduction over time. The isolated application of K<sub>2</sub>SO<sub>4</sub> and the combination KCl: K<sub>2</sub>SO<sub>4</sub> at the ratio 60: 40% increased total CO<sub>2</sub> released by the microbiota. K<sub>2</sub>SO<sub>4</sub> source had the highest microbial biomass C (MBC), as well as the 60: 40 combinations. Isolated application of K sources, especially with high chloride concentration, reduces the soil microbial activity and MBC.

**Keywords:** respirometry; salinity; microbial biomass C; potassium chloride.

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### Introduction

Intensive land use at the expense of population growth and food production in recent decades has led to the increase of degraded areas worldwide. Inadequate management of irrigation water and overuse of inorganic fertilizers, especially with high saline content, can lead to soil salinization, affecting the physical, chemical and biological characteristics of soils and consequently their biodiversity (Mavi & Marschner, 2013; Li et al., 2021). Approximately 7% of all land surface is salinized (Munns & Tester, 2008) and salinity reduces soil fertility, leading to desertification.

KCl is the most used K source worldwide, and knowledge of the potential damage caused its excessive use is of great relevance to crop management and soil microorganisms activity (Pereira, Santana, Megda, & Vieira-Megda, 2019; Yang et al., 2020), main responsible for the decomposition of organic waste, as well as soil nutrient and energy cycling (Jones et al., 2018; Silva-Sánchez, Soares, & Rousk, 2019). In Brazil, the state of Minas Gerais, for example, is the second largest banana producer in the country and, in this crop, recommended potassium (K) doses surpass 750 kg ha $^{-1}$  of  $K_2O$ .

The continuous and excessive use of high saline fertilizers, such as KCl, has hampered agricultural systems, mainly due to the Cl<sup>-</sup> in their composition (Megda, Mariano, Leite, Megda, & Trivelin, 2019). High saline doses generally used in crops accumulate in the soil, increasing the solution osmotic pressure, impairing the development of plants and living organisms in the soil. Therefore, other K sources, associated or not to KCl, could minimize the toxic effects of Cl<sup>-</sup> ion on microorganisms.

Thus, microbial biomass acts as a buffer in the soil, controlling nutrient availability through the mineralization and immobilization processes (Vieira-Megda et al., 2015; Mehnaz, Corneo, Keitel, & Dijkstra. 2019). According to (Rietz & Haynes, 2003), activity of exocellular enzymes involved in C ( $\beta$ -glycosidase) mineralization decreases exponentially with increasing salinity. Microbial biomass C, as well as other biological properties, is considered a soil quality indicator, as it represents the active and biodegradable fraction of OM and reflects changes in the medium and/or long terms (Geisseler & Scow, 2014; Liu et al., 2018; Leite et al., 2020).

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Awareness on excessive use of the KCl are crucial for sustainable soil management. Thus, total or partial replacement of KCl increases the soil biological activity, minimizing the biocidal effect of Cl<sup>-</sup> and influencing C cycling in the soil. This study assessed the microbial activity in the soil in response to the application of K sources and their effects on microbial carbon rates after banana cultivation in a greenhouse.

# Material and methods

The experiment was conducted under aerobic incubation conditions temperature (27 to 28°C). Samples of *Typic Eutrudepts* (Soil Survey Staff, 2014) were collected in a banana experimental conducted in a greenhouse for 120 days. The soil chemical attributes before conducting the experiment in the greenhouse were analyzed according to the methodologies described in Teixeira, Donagema, Fontana, and Teixeira (2017): pH in water (5.4); organic matter (37 g kg<sup>-1</sup>) - colorimetric method; phosphorus (3.8) and K (150) - Mehlich 1 in mg dm<sup>-3</sup>; calcium (1.8), magnesium (0.7), H + Al (2.6) and aluminum (0.1) - KCl 1 mol L<sup>-1</sup> in cmol<sub>c</sub> dm<sup>-3</sup>; CTC (5.6), in cmol<sub>c</sub> dm<sup>-3</sup>; saturation of bases (53%). The cationic micronutrients were extracted by the Mehlich 1 method, as follows: Cu<sup>+2</sup> (0.4); Fe<sup>+2</sup> (19.2); Mn<sup>+2</sup> (85.9) and Zn<sup>+2</sup> (1.2), in mg dm<sup>-3</sup>. The sand, silt, and clay contents were respectively 42, 30 and 28 %.

The soil water retention capacity (WRC) was determined according to the methodology of Bremner & Shaw (1958). Subsequently, the saturation of bases was corrected to 70% with limestone application and the soils of the experimental were pre incubated at 50% of WRC for 30 days before the treatments for reestablishment of microbial biomass activity. To simulate field conditions, it was applied, equivalent to 750 kg ha<sup>-1</sup> year<sup>-1</sup> of  $K_2O$ , the same used by banana farmers in the region. Considering that the available K in the soil was 360 kg ha<sup>-1</sup> of  $K_2O$ , an additional 390 kg ha<sup>-1</sup> of  $K_2O$  was applied.

The greenhouse experimental design was completely randomized with four replications, consisting of nine treatments to provide 200 mg dm $^{-3}$  of  $K_2O$  in the forms of: potassium nitrate (KNO $_3$ ); potassium sulfate ( $K_2SO_4$ ); potassium chloride (KCl); monopotassium phosphate ( $K_2PO_4$ ); besides the combinations of KCl and  $K_2SO_4$  in the proportions of 80:20, 70:30, 60:40 and 50:50% of K, respectively, and a control plot (without K application). The sources were diluted in distilled water to raise the soil WRC to 60% and applied to soil surface. Depending on the treatment, Triple Superphosphate, elemental sulfur and Urea sources were applied to the soil to provide P, S and N, in order to maintain the nutritional balance between treatments.

The incubation experimental units were composed of containers containing 500 g of soil (at natural moisture) provides from the banana experiment and kept under aerobic incubation conditions for 45 days. To evaluate soil microbial activity, the evolved  $CO_2$  (mineralizable C) was quantified by the respirometry method, according to the methodology of Stotzky (1965) and Curl & Rodriguez-Kabana (1972). Carbon was extracted from the microbial biomass (MBC) using the microwave irradiation technique (Islam & Weil, 1998) and subsequently determined by chemical extraction and digestion (Raij, Andrade, Cantarella, & Quaggio, 2001). MBC concentrations were determined before incubation only for control to estimate initial soil content and at 45 days after incubation (dai) in all treatments. The chloride (Cl<sup>-</sup>) content was determined by the silver nitrate volumetric method (0.05 mol L<sup>-1</sup> AgNO<sub>3</sub>) in the presence of potassium chromate (5%  $K_2Cr_2O_4$ ) as indicator, according to methodology described in Teixeira et al. (2017).

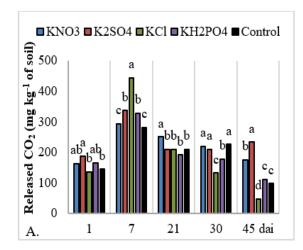
The metabolic quotient ( $qCO_2$ ) was obtained through the relationship between basal respiration and microbial carbon, representing the amount of  $CO_2$  released per unit of microbial biomass at a given time (Anderson & Domsch, 1993). The data were analyzed by comparing the potassium sources and the KCl:K<sub>2</sub>SO<sub>4</sub> combinations separately. The results were subjected to the analysis of variance using the F test, and when variation causes were significant, the Scott-Knott test was applied (p <0.05).

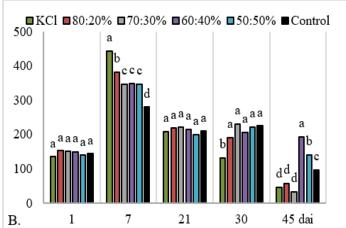
#### Results and discussion

The application of K influenced the microbial activity in the soil 1 day after incubation (dai) (Figure 1A). The  $K_2SO_4$  promoted microbial respiration of 187 mg kg<sup>-1</sup> of  $CO_2$ , differing from KCl and control, which had the lowest  $CO_2$  rates of 135 and 144 mg kg<sup>-1</sup>, respectively. At 7 dai, microbial respiration increased for all treatments, when the highest biological activity was observed for KCl of 444 mg kg<sup>-1</sup> of  $CO_2$ . At 30 and 45 days, for the other sources and control, the lowest averages of 132 and 47.2 mg kg<sup>-1</sup> were observed, respectively. These values represent 30 and 10% of the rate at 7 dai, respectively. At 45 dai, the  $K_2SO_4$  provided the greatest microbial activity in the soil, followed by KNO<sub>3</sub>, with respiration of 232 and 175.5 mg kg<sup>-1</sup> of  $CO_2$  respectively (Figure 1A).

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The KCl:  $K_2SO_4$  combinations showed no difference at 1 dai. At 7 dai, the KCl treatment provided the highest biological respiration of 444 mg kg<sup>-1</sup> of  $CO_2$  (Figure 1B), with a reduction in the microbial activity at 21, 30 and 45 dai of 208, 132, and 47 mg of  $CO_2$  kg<sup>-1</sup>, respectively. At the end of the experimental period, treatments with the lowest KCl proportion presented the highest rates of  $CO_2$  released. The combination of 60:40% KCl: $K_2SO_4$  provided respiratory rate of 193.5 mg kg<sup>-1</sup> the soil, while combinations 100:0, 80:20, and 70:30% provided the lowest  $CO_2$  emissions, an average of 46 mg kg<sup>-1</sup> of  $CO_2$ , even differing from control 97.5 mg kg<sup>-1</sup> (Figure 1B). The soil microbial respiration for most treatments, especially those containing higher KCl doses, showed a gradual reduction over time for K sources (Figure 1A) and KCl: $K_2SO_4$  combinations (Figure 1B).





**Figure 1**. Soil microbial activity in response to the application of potassium sources (A) and KCl:K<sub>2</sub>SO<sub>4</sub> combinations (B) at a dose of 200 mg dm<sup>-3</sup> of K<sub>2</sub>O at 1, 7, 21, 30 and 45 days after incubation (dai). 80:20% corresponds to the percentage of KCl and K<sub>2</sub>SO<sub>4</sub>, respectively, and so on. Equal letters do not differ from each other by the Scott-Knott test (p <0.05).

At 1 dai, KCl presented the highest availability of Cl<sup>-</sup> ion in the soil, 0.38 cmol<sub>c</sub> kg<sup>-1</sup> (Figure 2A), equals to KH<sub>2</sub>PO<sub>4</sub> at 7 dai, both at 0.37 cmol<sub>c</sub> kg<sup>-1</sup>. At 45 dai, KCl presented an average of 0.44 cmol<sub>c</sub> kg<sup>-1</sup> of Cl<sup>-</sup>, differing from the treatments, including the control, which had an average of 0.22 cmol<sub>c</sub> kg<sup>-1</sup> of Cl<sup>-</sup>. Regarding the KCl:K<sub>2</sub>SO<sub>4</sub> combinations, the control showed less availability at 1 dai, 0.34 cmol<sub>c</sub> kg<sup>-1</sup>, in relation to the other treatments, which did not differ from each other and presented on average 0.39 cmol<sub>c</sub> kg<sup>-1</sup> of Cl<sup>-</sup>. At 7 dai, the 100% KCl treatment and the 80:20% combination showed the highest Cl<sup>-</sup> levels, 0.36 and 0.34 cmol<sub>c</sub> kg<sup>-1</sup>, respectively, differing from treatments 60:40, 50:50% and control, which presented on average of 0.28 cmol<sub>c</sub> kg<sup>-1</sup> de Cl<sup>-</sup> (Figure 2B). At 45 dai, the concentration of Cl<sup>-</sup> in the soil solution for KCl remained higher than in the other treatments, with a content of 0.44 cmol<sub>c</sub> kg<sup>-1</sup>.

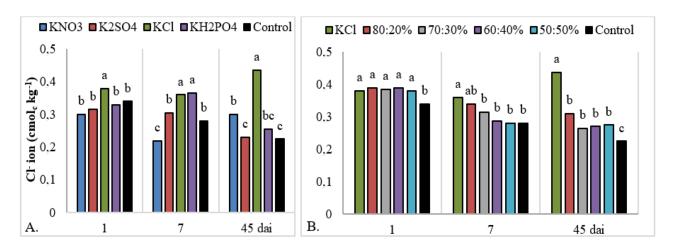
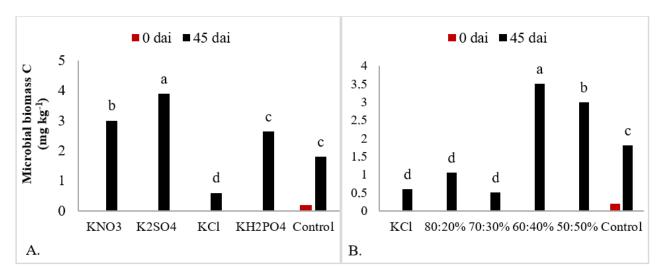


Figure 2. Chloride (Cl<sup>-</sup>) content in soil in response to the application of potassium sources (A) and KCl: $K_2SO_4$  (B) combinations at 200 mg dm<sup>-3</sup> of  $K_2O$  at 1, 7 and 45 days after incubation (dai). 80:20% corresponds to the percentage of KCl and  $K_2SO_4$ , respectively, and so on. Equal letters do not differ from each other by the Scott-Knott test (p <0.05).

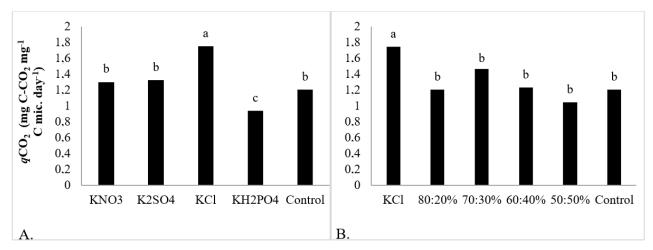
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The initial soil MBC content estimated by the control was 0.22 mg kg<sup>-1</sup> C and MBC increased significantly in all treatments after 45 dai (Figure 3). Regarding the K sources, KCl presented the lowest average of 0.6 mg kg<sup>-1</sup>. For K<sub>2</sub>SO<sub>4</sub>, MBC displayed greater concentration of 3.9 mg kg<sup>-1</sup>, followed by KNO<sub>3</sub> of 3.0 mg kg<sup>-1</sup>, KH<sub>2</sub>PO<sub>4</sub> of 2.6 mg kg<sup>-1</sup>, and control of 1.8 mg kg<sup>-1</sup> of soil C (Figure 3A). Regarding combinations of KCl:K<sub>2</sub>SO<sub>4</sub>, the treatment 80:20% showed the highest MBC of 3.5 cmol<sub>c</sub> kg<sup>-1</sup>, followed by the combination of 50: 50% with 3 cmol<sub>c</sub> kg<sup>-1</sup> and control with 1.8 cmol<sub>c</sub> kg<sup>-1</sup> of soil C. The lowest averages were observed for the treatment of 100% KCl with 0.6 mg kg<sup>-1</sup> of soil C and combinations 80:20 and 70:30%, with 1.1 and 0.5 mg kg<sup>-1</sup> of soil C, respectively (Figure 3B).



**Figure 3.** Carbon of the microbial biomass in response to the application of potassium sources (A) and KCl:K<sub>2</sub>SO<sub>4</sub> combinations (B) at a dose of 200 mg dm<sup>-3</sup> of K<sub>2</sub>O at 0 and 45 days after incubation (dai). 80:20% corresponds to the percentage of KCl and K<sub>2</sub>SO<sub>4</sub>, respectively, and so on. Equal letters do not differ from each other by the Scott-Knott test (p <0.05).

Considering the K sources, KCl presented higher  $q\text{CO}_2$  of 1.7 mg C-CO<sub>2</sub> mg<sup>-1</sup> C mic. day<sup>-1</sup> (Figure 4A). Regarding the KCl:K<sub>2</sub>SO<sub>4</sub> combinations, the treatment with 100% KCl presented the highest  $q\text{CO}_2$  of 1.7 mg C-CO<sub>2</sub> mg<sup>-1</sup> C mic. day<sup>-1</sup>, differing from the other sources (Figure 4B).



**Figure 4.** Metabolic quotient (qCO<sub>2</sub>) at 45 days after incubation in response to the application of potassium sources (A) and KCl:K<sub>2</sub>SO<sub>4</sub> combinations (B) at a dose of 200 mg dm<sup>-3</sup> of K<sub>2</sub>O. 80:20% corresponds to the percentage of KCl and K<sub>2</sub>SO<sub>4</sub> respectively, and so on. Equal letters do not differ from each other by the Scott-Knott test (p <0.05).

The excess of salts in the soil has a short- and medium-term effect on microbial biomass, as observed at 21 dai, reaching its peak at 45 dai. The negative effect of salinity, caused by the application of fertilizers on soil microorganisms is mainly attributed to the increase in osmotic potential. However, in the case of KCl, some studies have shown that the Cl<sup>-</sup> and its derivatives in the soil have a strong oxidizing action, constituting a potent biocide (Chen & Wong, 2004; Souri, 2010). Megda, Mariano, Leite, Megda, and Trivelin (2014) evaluated the Cl<sup>-</sup> potential of KCl and ammonium chloride (NH<sub>4</sub>Cl) and found a depressive effect of chloride

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on microbial activity. Some studies have also reported the gradual reduction in the soil microbial activity time observed for most treatments, especially those containing higher KCl doses (Yuan, Li, Liu, Gao, & Zhang, 2007; Mavi & Marschner, 2013; Geisseler & Scow, 2014).

The higher microbial activity provided by the treatments of  $K_2SO_4$  source and 60:40% KCl: $K_2SO_4$  combination, considering the  $CO_2$  released during 45 days, may be related to the lower solubility of  $K_2SO_4$  soon after its application. Considering the temperature of  $20^{\circ}$ C,  $K_2SO_4$  shows solubility of 110,  $KH_2PO_4$  of 230,  $KNO_3$  of 320, and KCl of 337 g L<sup>-1</sup> (Trani & Trani, 2011). The lower solubility of  $K_2SO_4$  can initially minimize the salinity effect caused by the exclusive application of KCl. Additionally, the high KCl salt index of 116, compared to the other sources of 46, 74, and 8 for  $K_2SO_4$ ,  $KNO_3$ , and  $KH_2PO_4$ , respectively, is an aggravating factor and can trigger plasmolysis of microbial cells through water loss, leading to cell death.

The higher availability of  $Cl^-$  in the soil for the KCl treatment at 1 dai is attributed to the higher chlorine content, on average 47% of its formulation, in addition to its high solubility as previously mentioned. After 7 dai,  $KH_2PO_4$  showed  $Cl^-$  content similar to that found for the KCl source. Possibly, the presence of phosphate anion in its constitution, whose attraction force due to the soil colloids is higher than that of  $Cl^-$ , was capable of occupying positive charges that were initially occupied by  $Cl^-$ , making it readily available in the soil. The control treatment initially presented 0.34 cmol<sub>c</sub> kg<sup>-1</sup> of  $Cl^-$ , corresponding to 120 mg kg<sup>-1</sup> or 241 kg ha<sup>-1</sup> of  $Cl^-$ . Thus, the content of  $Cl^-$  available in the soil may have been increased by the displacement caused by the anion  $H_2PO_4^-$  in the exchange complex.

At 45 dai, the KCl source showed an increase in the Cl $^-$  content in the soil solution compared to the initial period, which was the treatment with the highest available Cl $^-$  content of 0.43 cmolc kg $^{-1}$ . This increase represents 21.3 mg kg $^{-1}$  of soil in relation to the initial period (1 dai). Cl $^-$  and Na $^+$  are the main ions responsible for increasing electrical conductivity (EC) of the soil solution. The EC of fertilizers before the installation of the experiment was 1.7 for KCl, 1.4 for K<sub>2</sub>SO<sub>4</sub>, 1.3 for KNO<sub>3</sub>, and 0.7 mS cm $^{-1}$  for KH<sub>2</sub>PO<sub>4</sub>. Higher EC of KCl in relation to the other K sources worsens its biocidal potential in the soil.

The highest concentration of Cl<sup>-</sup> at 45 dai for the KCl source (Figure 4) corroborates the data presented for the soil microbial activity, where lower microbial activity was observed in the same period (Figure 1). Chen & Wong (2004) emphasize that Cl<sup>-</sup> and its derivatives have strong oxidizing and biocidal action in the soil and could considerably reduce the microbial population. Besides, excessive accumulation of soluble salts in plants and microorganisms may be caused by the difficulty of water absorption, inducing a water stress condition, by the toxicity of specific ions, such as Na<sup>+</sup> and Cl<sup>-</sup> (+Rietz & Haynes, 2003). Shah and Shah (2011) evaluated the increased salinity in 30 soil samples and found a decrease in N of microbial biomass, N mineralization, nitrification, and CO<sub>2</sub> evolution rate with increasing EC.

The lower MBC value at 45 dai for the KCl treatment, in relation to other sources and control, reinforces the biocidal effect of highly saline fertilizers on soil microbial activity (Figure 4). Leite et al. (2020) evaluated the efficiency of revegetation on the regeneration of saline soil in a Brazilian semiarid area and showed that the cultivation of the saline soil increased microbial biomass carbon and mycorrhizal colonization.

The results also reiterate that the association of KCl with other K sources of lower salinity, such as  $K_2SO_4$ , mitigates the toxic effect of KCl. The combination 60:40% KCl: K2SO4 presented six-fold more MBC when compared to the application of 100% KCl. Tripathi et al. (2005) evaluated the salinity effect on microbial and biochemical parameters of salt-affected soils in a coastal region of India and found negative influence on microbial biomass C and soil basal respiration. Tripathi et al. (2005) found similar results and reported that C of the soil microbial biomass decreased, on average, from 391 mg kg<sup>-1</sup> in soil with EC < 4.0 dS m<sup>-1</sup> to 209 mg kg<sup>-1</sup> in soil with EC > 12 dS m<sup>-1</sup>. Pereira el al. (2019) evaluated the application of KCl doses associated to banana plant residues on soil and Cl<sup>-</sup> availability and reported that the high litter accumulation associated to the application of KCl doses above 200 mg dm<sup>-3</sup> reduced the soil microbial activity, which is aggravated over time.

The metabolic quotient (qCO<sub>2</sub>), used as a soil quality indicator, shows that as microbial biomass becomes efficient to use energy sources available, less carbon is lost as CO<sub>2</sub> by respiration and is immobilized in the microbial tissue (Anderson & Domsch, 1993). Thus, lower qCO<sub>2</sub> represents a stable microbial biomass or a less disturbed environment. The higher qCO<sub>2</sub> observed for KCl source indicated lower use of mineralizable C and consequently greater disturbance of the environment. This result corroborates the toxic effect of salinity on the soil microbial activity caused by KCl excess. As previously mentioned, low qCO<sub>2</sub> indicates energy savings and supposedly reflects a more stable environment or closer to its equilibrium; however, estimation of qCO<sub>2</sub> involves the soil respiration rate and must be analyzed within each context, according to (Islam & Weil, 2000). Bezerra, Lacerda, Sousa, Gomes, and Mendes Filho (2010) studied the effects of saline on the

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microbial biomass activity and concluded that higher salinity indices reduced soil basal respiration and coefficient microbial metabolism.

According to some authors, the lower evolution of  $C-CO_2$  may be indicative of greater efficiency in the use of soil resources (Sakamoto & Oba, 1994), as long as the same microbial biomass is kept, which was not observed in our study. Thus, the reduction in the microbial population was attributed to the toxic effect of KCl at high doses.

# Conclusion

- 1. In the medium term, the application of K<sub>2</sub>SO<sub>4</sub> or in combination 60: 40% KCl: K<sub>2</sub>SO<sub>4</sub> resulted in higher microbial activity.
- 2. The K application increases C of microbial biomass, especially for source  $K_2SO_4$  and combination  $KCl:K_2SO_4$  60:40%, favoring the association of these nutrients in soil biological processes.
- 3. Excess of Cl<sup>-</sup> in the soil from high KCl dose is a potential inhibitor of microbial activity affecting C rates in the medium term.
- 4. The joint application of KCl with K<sub>2</sub>SO<sub>4</sub> presented lower metabolic quotients, indicating less disturbance to soil microbiota.

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