

Responses of Soil Microbial Activity to Swine Manure Applications

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Abstract

The allocation of the large amount of swine waste from farms is an international concern. An efficient way of managing such waste is its use in farming. It is already known that the incorporation of organic waste into the soil significantly increases the microbial population. Therefore, the objective was to evaluate the impact of the use of swine manure on the soil microbiota in a Eutrophic Oxisol. The experiment was set up in a completely randomized design in a 6×4 factorial scheme (six concentrations of swine manure and four evaluation periods) with four replications. We evaluate the following characteristics: microbial respiration (C-CO₂), microbial biomass ($\mu\text{C g}^{-1}$ soil) and pH. A significant effect was found in the interaction between concentrations and time of incubation ($p < 0.05$) of swine manure on microbial activity in the soil. The amount of microbial carbon increased as a function of increased levels of liquid swine manure. No interaction was observed between concentrations and time of incubation for the pH. The evaluation of the isolated factors allowed to observe that the pH decreased as the doses of manure were incremented. Higher and lower pH values were found after 5 and 30 days of incubation. The application of liquid swine manure up to 6000 L ha^{-1} increases the release of CO₂ and carbon in the microbial biomass. The applications of liquid swine manure cause a gradual reduction in soil pH.

Keywords: swine waste, microbiota, microbial biomass, microbial carbon

1. Introduction

The demand for animal husbandry is directly proportional to the growth of the world's population (Safavi & Unnthorsson, 2017). The accelerated growth of swine rearing has caused an environmental issue due to the generation of large quantities of manure with high polluting potential (Veronese et al., 2019). To minimize this problem, one of the most rational forms of final disposal of these wastes is to use them as organic fertilizer (Miyazawa & Barbosa, 2015) since they are rich in nutrients necessary for plant growth. The incorporation of these wastes improves the physical, chemical and biological qualities of the soil as the diet currently supplied to pigs is highly concentrated in elements that are not completely utilized by the metabolism of the animals, therefore causing the excretion of more chemically concentrated waste (Rosov et al., 2020).

The search for farming practices that result in high productivity, but that also consider the various aspects related to environmental quality is a complex equation whose resolution cannot neglect the biotic components of the soil (El-Ramady et al., 2014), which has a close interrelation with the physical and chemical components (Delgado & Gomes, 2016). Thus, all factors that negatively affect microorganisms also cause the deterioration of the physical

and chemical properties of the soil (Pereira et al., 2013). Among the parameters used to characterize the adequate microbiological functioning of the soil, the determination of microbial activity through basal respiration, microbial biomass and pH stand out (Novak et al., 2017).

The amount of CO₂ released by the respiration of microorganisms is one of the most traditional and used methods in the assessment of the metabolic activity of the soil microbial population (Sabino et al., 2015). The estimation of microbial biomass can provide useful data on the changes that occur in the biological properties of soils, resulting from the types of the applied management (Semenov et al., 2018). Soils fertilized with organic waste tend to reduce the pH, due to the characteristic processes of organic matter degradation, releasing ions into the medium, therefore, acidifying it (Carmo et al., 2016). Thus, the authors of this paper aimed at evaluating the impact of the use of swine manure on the soil microbial activity in a Eutrophic Oxisol.

2. Material and Method

The experiment was carried out in the Microbiology and Chemistry laboratories of the State University of Montes Claros. The samples of Eutrophic Oxisol were collected in the city of Jaíba, Minas Gerais (MG). The samples were sieved in a 2 mm mesh and maintained at 60% of the soil's total water retention capacity. The swine manure used in the experiment was collected from a biodigester in the Jaíba Project region, in the municipality of Jaíba-MG. This was digested anaerobically at 56 °C for 30 days. The chemical composition of swine manure is described in table 1. The experiment was conducted in a 6 × 4 factorial design, with six different concentrations of swine manure: 0, 2000, 3000, 4000, 5000 and 6000 L ha⁻¹ and 4 evaluation periods (5, 10, 20 and 30 days), with four replications. Swine manure concentrations were determined according to the amount already applied to the soil by rural producers in the Jaíba region. It is thus adjusted, two concentrations upwards (5000 and 6000 L ha⁻¹) and two concentrations downwards (2000 and 3000 L ha⁻¹). The following characters were evaluated: microbial respiration (C-CO₂), microbial biomass and pH.

Table 1. Physicochemical composition of swine manure treated by anaerobic digestion (56 °C for 30 days)

Parameter	Values
pH	6.10±0.03
Turbidity (NTU-Nephelometric Turbidity Unit)	345.00
Total nitrogen (mg L ⁻¹)	496.00±7.00
Total phosphorus (mg L ⁻¹)	141.00±8.10
Total Carbon (mg L ⁻¹)	2376.7±229.00

Note. Average of four repetitions, ±standard deviation.

Five levels of manure were applied to each soil sample and homogenized using a glass stick. The soil microbial respiration was estimated by the amount of CO₂ released from the soil during 5, 10, 20 and 30 days of incubation, according to the methodology proposed by Mendonça and Matos (2005). The 50 g samples of Eutrophic Oxisol were placed in plastic containers of 2 dm³ at 28 °C and incubated aerobically in order to stimulate microbial activity. The moisture of the soil samples was adjusted to 60% of its total water retention capacity. The CO₂ produced was captured by 30 mL of a 0.5 mol L⁻¹ NaOH solution and quantified by titration with 0.25 mol L⁻¹ HCl, 10 mL of 0.05 mol L⁻¹ BaCl₂ was previously added and phenolphthalein at 1% was used as an indicator. Four titrations were performed after the beginning of the samples incubation in which the first was performed at 5 days, the second at 10 days, the third at 20 days and the fourth at 30 days. The microbial respiration data were expressed in mg C-CO₂ 100 cm⁻³ and calculated using the formula:

$$\text{C-CO}_2 \text{ (mg)} = (\text{B} - \text{V}) \times \text{M} \times 6 \times (\text{V}_1/\text{V}_2) \quad (1)$$

where, B = HCl volume in the blank (mL); V = Volume of HCl used in the sample (mL); M = Real HCl concentration (mol L⁻¹); 6 = Carbon atomic mass (12) divided by the number of moles of CO₂ that react with NaOH (2); V₁ = Total volume of NaOH used to capture CO₂ (mL); V₂ = Volume of NaOH used in the titration (mL).

The carbon in the microbial biomass was determined through irradiation-extraction according to the methodology proposed by Ferreira et al. (1999). Therefore, 40 g of soil were weighed in duplicates, one was irradiated in a microwave oven for 120 seconds to eliminate microorganisms and the other was not. After that, the duplicates were placed in 500 mL glass flasks for the carbon extraction process, with the addition of 50 mL of 0.5 mol L⁻¹ K₂SO₄ to each flask. The set was kept under stirring at 220 rpm for 30 minutes in a Tecnal-TE

420-Incubator orbital shaker. After this period, the samples were filtered on filter paper. In 500 mL glass flasks, a 25 mL aliquot of the filtered extract and 10 mL of $K_2Cr_2O_7$ extracting solution 0.167 mol L^{-1} and 20 mL of H_2SO_4 MW were added. The set was then manually homogenized for one minute and allowed to cool for 30 minutes. Next, 200 mL of H_2O and 10 mL of H_3PO_4 MW were added. After, three drops of the Diphenylamine indicator were added, and the excess of Potassium Dichromate was titrated with $Fe(NH_4)_2(SO_4)_2 \cdot H_2O$ 0.4 mol L^{-1} . The microbial carbon values were expressed in $\mu\text{g g}^{-1}$ of soil and calculated using the formula:

$$\text{Microbial carbon } (\mu\text{g g}^{-1} \text{ C in the soil}) = (\text{IC} - \text{NIC})/\text{Kc} \quad (2)$$

where, IC = irradiated carbon sample; NIC = not-irradiated carbon sample; Kc = 0.33 (correction factor proposed by Sparling and West (1988)).

The pH was determined according to the methodology described by Donagema et al. (2011) and performed in Tecnal® pH-meter. In 250 mL containers, 20 grams of soil and 25 mL of distilled water were placed. The containers were stirred at 220 rpm for 30 minutes on a Tecnal-TE 420-Incubator® orbital shaker, then subjected to rest for 60 minutes. Soon after, the electrodes were soaked in the suspension solution and the pH was read. The pH analysis was performed after 5, 10, 20 and 30 days of soil incubation. The data obtained in the evaluation of the experiment were subjected to tests of assumption of analysis of variance (Bartlett and Shapiro Wilk). The data were considered normal and were subjected to analysis of variance at 5% probability by the t test. When the interaction was significant ($p < 0.05$), it unfolded. The swine manure concentrations were subjected to regression analysis at 5% probability by the t test and the incubation days at the Tukey test at 5% probability. R Core Team software version 3.5 (2015) was performed.

3. Results

A significant effect was observed on the interaction between concentrations and incubation time ($p < 0.05$) of swine manure on microbial activity in the soil. The unfolding of the interaction between the dose level and the incubation periods allowed to observe that at doses 0, 2000, 4000 and 6000 L ha^{-1} , the highest values of basal respiration were observed on days 5, 10 and 20 of incubation (Table 2). At the doses of 3000 and 5000 L ha^{-1} , the highest value of basal respiration was found at 10 days of incubation. Regardless of the dose used, the lowest basal respiration values were found at 30 days of incubation (Table 2). All variables of the regressions in the four evaluation periods were significant at 5% probability by the T-test. The determination of the microbial activity of the soil by basal respiration allowed verifying the effect of the application of different concentrations of swine manure in four incubation periods (Figure 1).

Table 2. Basal respiration ($\text{mg C-CO}_2/100 \text{ cm}^3$) of Eutrophic Oxisol as a function of the incubation days and the applied doses of swine manure

Incubation days	Swine manure concentrations (L ha^{-1})					
	0	2000	3000	4000	5000	6000
5	102.23a	107.21ab	111.15b	114.75a	116.77b	120.26a
10	100.29a	108.79a	114.8a	116.44a	120.49a	121.50a
20	98.21a	104.96b	110.36b	114.07a	116.55b	120.15a
30	93.71b	97.31c	100.23c	106.76b	109.12c	111.71b
Coefficient of variation (%)	1.69					

Note. Means followed by the same letter in the column do not differ by Tukey's test at 5% probability.

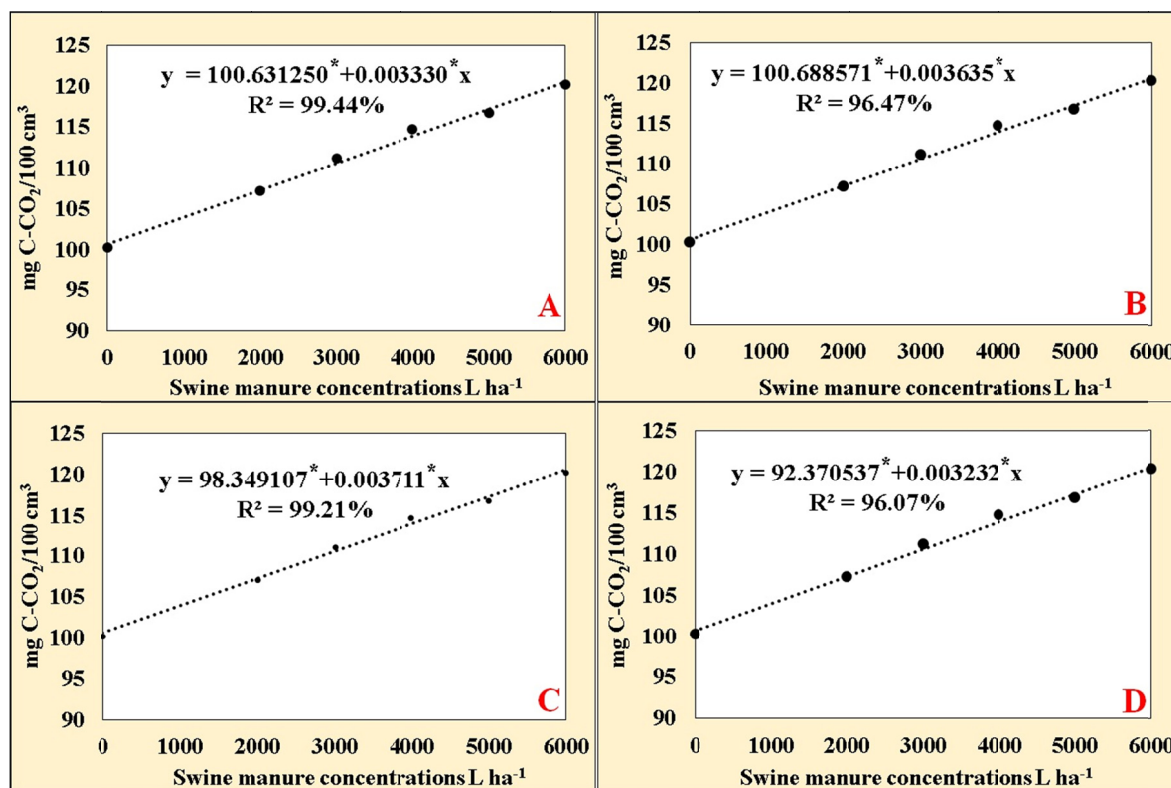


Figure 1. C-CO₂ release according to the concentrations applied and the evaluation periods. * Significant at 5% probability by the t test. Incubation periods 5 (A), 10 (B), 20 (C) and 30 (D) days

The unfolding of the interaction between incubation periods within each dose level shows that at dose 0, regardless of the incubation period, the carbon of the microbial biomass remained the same (Table 3). Higher microbial carbon values were found at 20 days of incubation at the concentrations 2000, 3000, 4000, 5000 and 6000 L ha⁻¹ (Table 2), indicating greater metabolic activity in this period.

Table 3. Microbial carbon ($\mu\text{C g}^{-1}$ soil) of Eutrophic Oxisol as a function of the incubation days and the applied concentrations of swine manure

Incubation days	Swine manure concentrations (L ha ⁻¹)					
	0	2000	3000	4000	5000	6000
5	122.57a	163.12b	187.05b	270.25b	273.60bc	283.60b
10	138.439a	169.22b	180.87b	207.95b	285.21b	308.90b
20	119.82a	448.80a	445.52a	482.12a	477.50a	484.07a
30	101.82a	158.12b	152.50b	223.92b	230.47c	322.07b
Coefficient of variation (%)		11.07				

Note. Means followed by the same letter in the column do not differ by Tukey's test at 5% probability.

The interaction between concentrations and incubation days was significant for the variable response, the carbon in the microbial biomass ($p < 0.05$). The unfolding of the interaction between the dose within each level of the evaluation period allowed to observe a linear behavior for days 5 and 10 (Figure 2) and a quadratic behavior for days 20 and 30 (Figure 2). The regression parameters on days 5, 10, 20 were all significant at 5% probability by the t test ($p < 0.05$). Only the parameter β_1 of the regression equation on day 30 was not significant ($p > 0.05$). The derivation of the equations for the days 20 and 30 generated a maximum and a minimum point of 4,346.71 L ha⁻¹ and 990.25 L ha⁻¹, respectively.

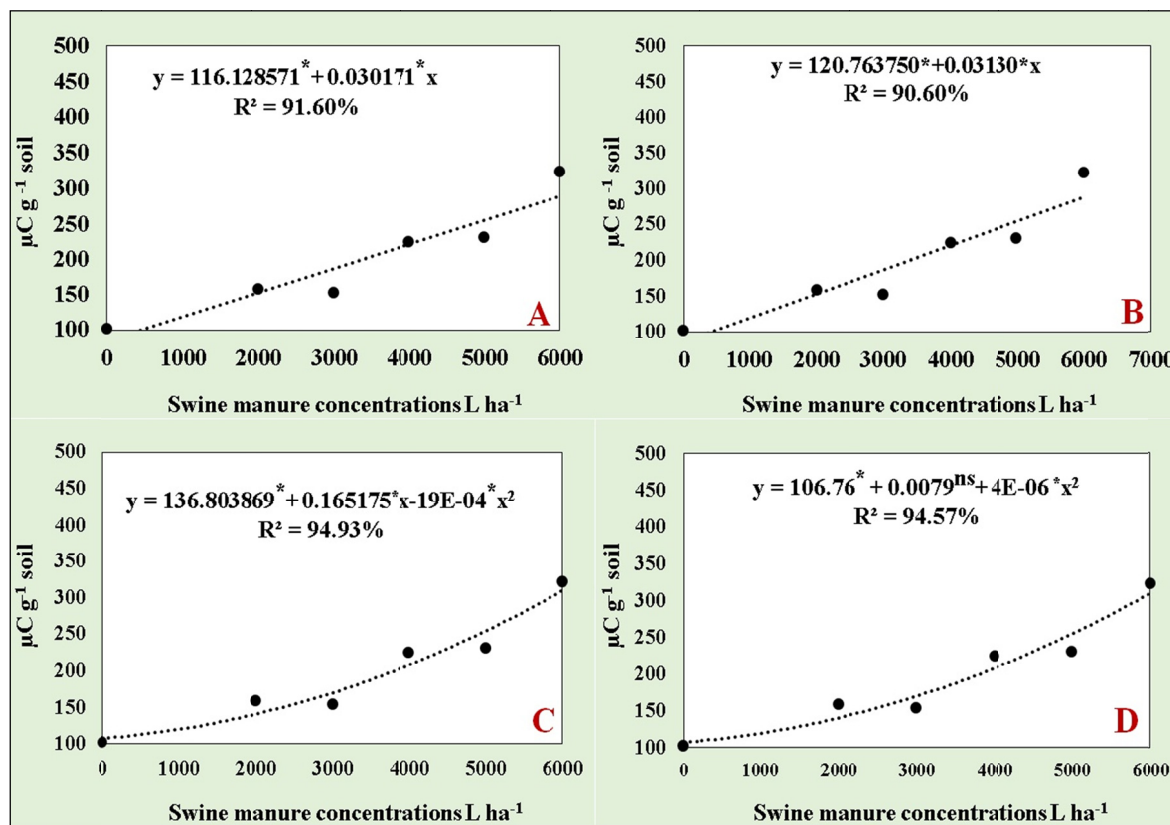


Figure 2. Microbial carbon ($\mu\text{C g}^{-1}$ soil) as a function of the concentrations applied and the evaluation periods. * significant and ^{ns} not-significant at 5% probability by the T test. Incubation periods 5 (A), 10 (B), 20 (C) and 30 (D) days

No significant interaction was observed between the applied concentrations and the days of incubation ($p > 0.05$). The evaluation of the isolated factors showed that the pH decreased as the concentrations increased (Figure 3A). The lowest pH value was found at 6000 L ha^{-1} . The highest and lowest pH values were observed at 5 and 30 days of incubation (Figure 3B).

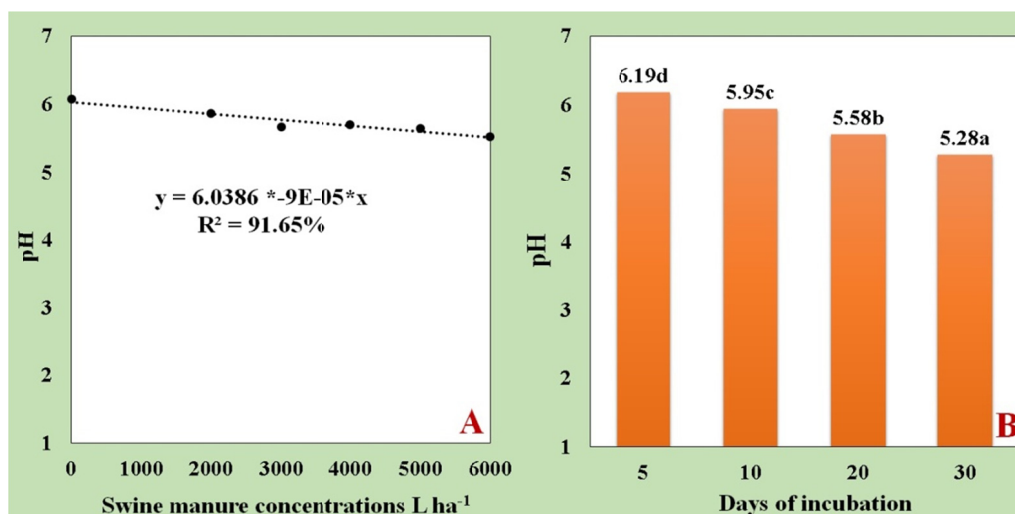


Figure 3. Hydrogen potential (pH) as a function of the different concentrations of swine manure. Means followed by the same letter in the bar do not differ by Tukey's test at 5% probability. Coefficient of variation 2.64%

4. Discussion

The residues organic in the soil activate physiological responses of microorganisms, which are shown on the performance of the microbial community (Grenni et al., 2018). These physiological effects can be assessed using variables such as basal respiration and microbial biomass, since they display the metabolic processes involved in the growth and reproduction of these organisms (Anderson & Domsch, 2010). The increase in the concentration of swine manure resulted in the rise for CO₂ (Figure 1). The linear model equations show an increase for CO₂ due to the increase in the levels of liquid swine manure. In all concentrations of swine manure, the C-CO₂ values were higher than in the control treatment. The increase in the amount of mineralizable carbon is explained by the increase and availability of organic matter in the soil, which improves the environment for the development of microbial flora (Quadro et al., 2011).

Basal respiration is an indicator of soil quality and the available organic carbon for heterotrophic microorganisms. In addition, the greater the amount of CO₂ released per unit of soil weight, the greater the amount of assimilable substrate for the growth of microbial biomass (Oliveira et al., 2016). The concentration of residue organic applied to the soil influences the amount of nutrients and mainly of the organic carbon available to the soil microbiota. Silva et al. (2015) found that the concentration of 200 m³ ha⁻¹ swine liquid manure applied to the soil resulted in higher microbial respiration values.

According to Morales et al., (2016), the high values of basal respiration point to a greater metabolic activity of microorganisms as the CO₂ flow correlates with the intensity of the catabolic processes. Aerobic decomposition of organic residues occurs in biochemical redox reactions (Hullebusch et al., 2019) through which the microorganisms obtain energy using part of the carbon in the substrate in catabolic reactions while CO₂ is released (Podmirseg et al., 2019). The evolution of CO₂ as a measure of respiration, then, represents a measure of total decomposition (Londoño, 2012).

Firstly, microbial biomass degrades the substrates recently added to the soil, or part of them (Ali et al., 2018). In addition, after that, the metabolic activity and consumption of the remaining substrates increases (Bonner et al., 2018). In the final stage, the substrate reaches a status similar to the initial one of the soils, and there is a decline in microbial activity and biomass (Cortes-Tolalpa et al., 2018), therefore, reducing basal respiration, which was observed in this study at 30 days of incubation.

There was an increase in carbon due to the increase in swine liquid manure concentrations in the evaluated period. Microbial biomass is the total mass of microorganisms in a particular volume of soil or even the living component of the organic matter according to Miltner et al. (2012). Biomass is responsible for a large part of the biological activity of the soil, this is the main source of enzymes responsible for the biochemical transformations that occur there (Singh et al., 2014).

Balota et al. (2012) when working with the concentrations of 0, 30, 60 and 120 m³ ha⁻¹, also saw significant increases for biomass as the volume of swine manure applied to the soil increased. Studies evaluating microbial biomass in soils fertilized with swine manure have shown increases in the carbon content of microbial biomass in relation to soils without fertilization (Morales et al., 2016). These changes occur through the addition of microorganisms by means of manure, which contribute to the increase in carbon in microbial biomass (Li et al., 2018). Moreover, the addition of low molecular-weight compounds and easy transport across the cell membrane increases the number of resources available or partially utilized by the microorganisms, which can increase in quantity due to the favorable condition that the manure creates temporarily (Londoño, 2012).

Microbial biomass has the ability to mineralize and to immobilize nutrients. In addition, the characterization of microbial communities, as well as their cycles, can be useful for understanding and explaining changes of these organisms, of the nutrient cycling and decomposition and mineralization processes (Cardoso et al., 2013). Microbial biomass be as the central compartment in the carbon cycle, where it is associated with the processes of decomposition of plant residues until the cycling of the constituents of the organic matter (Cronan, 2018).

Currently, an immense range of organic compounds it is used to support the demand for nutrients by crops such as sewage sludge, sludge from tanneries, oil residues, animal waste, among others (Zhang et al., 2017). Because microbial biomass uses such organic wastes as a source of nutrients, it directly affected when applied to the soil (Teutscherova et al., 2017). Tavares et al. (2019) when working with swine manure (25, 50, 100 and 200 m³ ha⁻¹) application in cerrado soils in Brazil, concluded that the application of this residue promoted the mineralization of organic matter, resulting in the formation of humic substances and improvement in the degree of soil structure.

The fluctuation of soil pH caused by the metabolic activity of microorganisms is dependent on the type of the substrate metabolized. The addition of carbohydrate reduces the initial pH by producing acidic and CO₂

metabolites (Song et al., 2018). The application of nitrogen fertilizers has an acidic residual effect caused by the nitrification reaction. The decomposition of organic matter can decrease the pH of the soil due to the formation of organic acids in the first stage of the decomposition of the anaerobic phase (Santos et al., 2015), which justifies the pH 6.08 at concentration 0 and more acid in other concentrations (2000, 3000, 4000, 5000 and 6000 L ha⁻¹).

The soil microbial components have been being the indicators of the dynamics of organic matter due to their rapid turnover (Cagnarini et al., 2019). In this context, the parameters evaluated in this experiment showed microbial activity, when these are offered a lesser or greater amount of nutrients. As well as the period in which this source of nutrients is available in the soil, affecting its dynamics.

5. Conclusion

The application of liquid swine manure up to the dose of 6000 L ha⁻¹ increases the release of CO₂ and the carbon in the microbial biomass at days 5, 10, 20 and 30 of incubation. The applications of liquid swine manure cause a gradual reduction in soil pH.

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