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Multivariate Tools for Evaluating the Use of Organic Fertilizers on Soil Microbial Properties and Maize Yield

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Abstract

The global demand for protein led to the increase of animal production in the world and, mainly, in Brazil. As a consequence, there was an increase in the amount of waste produced, and the need to seek alternatives for its sustainable use. Microbial indicators and multivariate tools can assist in the proper measurement of the impact of the use of this waste on the soil. This study aimed to: 1) measure the effect of the application of organic fertilizers of animal origin in the no-tillage system on soil microbial attributes and its relationship with maize yield; 2) evaluate the potential of separation/discrimination of the different sources of organic fertilizers based on yield and soil microbial and chemical-physical attributes, using multivariate tools. Treatments consisted of annual application of: poultry manure (PM), liquid swine manure (LSM), poultry manure compost (PMC), swine manure compost (SMC), cattle manure compost (CMC) and control (C), without fertilization. Organic fertilizers promoted higher values of microbial biomass (MB) and MBC:TOC ratio in treatments CMC, SMC and PM in the first sampling season (E_1), followed by PM, LSM and PMC in the second sampling period (E_2). The data show that PM promoted microbial growth in both seasons, with higher metabolic efficiency increasing maize yield by 30% in relation to the treatment with the second highest production, PMC. Multivariate analysis techniques prove to be important tools to study soil quality indicators in systems which use organic fertilizers.

Keywords: Animal waste, Microbial attributes, No-tillage, Soil quality

1. Introduction

The large number of animals in a small territorial area is a marked characteristic of the Brazilian animal production system, especially in southern Brazil, which consequently leads to a large production of waste, often without adequate disposal. Animals such as pigs and cattle can produce about 5 to 10% of their live weight in waste per day, whereas birds use only 40% of the food ingested (Konzen & Alvarenga, 2005).

One of the main alternatives for the use of animal wastes is their application in agriculture as organic fertilizer, an option repeatedly used by farmers for disposal in areas of grain and pasture production (Basso et al., 2012). The application of organic fertilizers increases the organic matter content of the soil, leading to alterations in its physical and chemical properties, as well as improvements in the microbial attributes (Kallenbach & Grandy 2011; Giacometti et al., 2013; Scherer & Spagnollo, 2014).

Maintenance or improvement of soil quality has been pointed as one of the main concerns of the scientific community and the soil microbial attributes, such as microbial biomass (MB), microbial respiration (CO_2-C) and relationships such as the metabolic quotient (qCO_2) and the ratio between microbial biomass carbon and total organic carbon (MBC:TOC) are important indicators of soil quality (Zornoza et al., 2015; Zago et al., 2019). Soil microbial biomass represents is a small proportion of soil organic matter (SOM) responsible for in terms of both nutrient cycling and estimation of soil use and management capacity for plant growth (Araujo et al., 2012; Xu et al., 2018). Therefore, the behavior of the microbial population depends on the quality, type and quantity of the animal waste applied to the soil, as well as on the frequency of its application.

Studies indicate that the application of swine waste alters the microbial activity of the soil, while promoting lower carbon loss and increase in its microbial biomass (Sousa et al. 2014; Da Silva et al., 2015), as well as the poultry manure, which increases microbial activity compared to the use of chemical fertilization (Da Silva et al., 2009). Authors such as Müller et al. (2014), studying different sources of organic fertilizers, found that the use of poultry and cattle manure increased soil microbial biomass, with lower microbial activity in the treatments using poultry and swine manure compost.

Thus, obtaining a large amount of information on the systems that use organic fertilizers and integrating this information is as important as its adequate and profitable exploitation. For this, univariate statistical models become less sensitive in biological systems, due to the particularities of the treatments, besides not considering the interactions between the chemical, physical and biological factors (Maluche-Baretta et al., 2006; Rauber et al., 2018), so the potential of information generated in research studies is often not used. Multivariate statistical analysis, in turn, allows detecting and describing structural, spatial and temporal patterns in biological communities, and formulating hypotheses based on the numerous biotic and abiotic factors that interfere with such characteristics (Baretta et al., 2005).

Thus, seeking to evaluate microbial attributes in subtropical soil typically used for the application of animal waste (17 years of application), as fertilizer source for grain and pasture production, this study aimed to: 1) measure the effect of the application of organic fertilizers of animal origin in no-tillage system on soil microbial attributes, and its relationship with maize yield; and 2) evaluate the potential for separation/discrimination of treatments with different sources of organic fertilizers based on microbial and environmental attributes (chemical and physical) of the soil, and yield variables, using multivariate analysis tools.

2. Material and Methods

2.1 Study Area

The study was carried out in an experimental area belonging to the Santa Catarina State Agricultural Research and Rural Extension Enterprise (EPAGRI), in the municipality of Chapecó-SC, Brazil (05°18’87” S latitude and 38º16’62” W longitude, average altitude of 679 m). The climate of the region is subtropical (*Cfa*) according to the Köppen’s classification, with hot summers, rare frosts and tendency of rainfall concentration in the summer months, without a defined dry season (Alvarez et al., 2014). The climatic data recorded during the period of the experiment are shown in supplementary data.

The experimental area is characterized by a long-term study, conducted since 2003 in no-tillage system under crop rotation with annual sowing of maize (*Zea mays*) or common beans (*Phaseolus vulgaris*) and cultivation of species for soil cover such as forage radish (*Raphanus sativus* L.), black oat (*Avena strigosa*) and velvet bean (*Mucuna pruriens* (L.) DC) in the autumn-winter in succession. The soil is classified as a LATOSSOLO VERMELHO Distroférrico típico (Oxisol) (EMBRAPA 2013). Exceptionally, in the agricultural year 2015/2016, maize was cultivated prior to black oats. The evaluations carried out in the experimental area occurred in the cycle of two plant species, black oat in the winter cycle (2016) and maize in the summer cycle (2017), due to the crop rotation system in the area.

2.2 Cultural Practices and Experimental Design

The experiment has been conducted organically without the use of agrochemicals and soluble fertilizers, with a randomized block design in a factorial scheme (sources of waste x sampling period), six replicates in 18 m² plots (3.6 x 5.0 m), and a usable area for evaluation of 12 m². The treatments used are characterized by the annual application of organic fertilizers of animal origin, when the crops for grain production were planted, being the only source of nutrients applied to the soil: poultry manure (PM), liquid swine manure (LSM), poultry manure compost (PMC), swine manure compost (SMC), cattle manure compost (CMC) and a control (C) without fertilization.

Solid fertilizers were applied in the amount of 10 t ha^{-1} (dry basis) and the LSM at the dose of 60 m³ ha^{-1} for the maize crop, broadcast on soil surface one day prior to sowing. The average values of nutrients applied in each crop from different fertilizers are presented in Table 1.

Table 1. Mean values of pH, dry matter (DM) and nutrients in the organic materials used in maize fertilization along the 2016/2017 agricultural year

Fertilizers*	**pH**	**DM**	**N**	**P_2O_5**	**K_2O**	**Ca**	**Mg**	**Cu**	**Mn**	**Zn**
		%	.... g kg^{-1}					.... mg kg^{-1}		
PM	7.7	89.0	32.4	65.9	46.4	34.2	11.9	650	580	510
PMC	7.0	44.5	10.2	19.4	8.2	17.8	5.6	111	400	289
SMC	7.5	48.3	10.6	9.3	10.1	10.1	3.2	77	758	285
CMC	8.1	39.1	16.4	12.5	10.3	6.8	3.4	145	250	301
		%	.... g L^{-1}					.... mg L^{-1}		
LSM	7.0	1.30	3.64	1.81	2.31	0.61	0.83	53	25	36

*PM - poultry manure; PMC - poultry manure compost; SMC - swine manure compost; CMC - cattle manure compost; LSM - liquid swine manure. Results expressed on dry basis.

PMC and CMC were prepared in windrows by combining manure and bean/maize straw residues. The compost was prepared with the use of alternating layers of 15 to 20 cm height, composed of plant materials and manure, which were turned three times, reaching maturity on average after 120 days, as proposed by Kiehl (1985). The PM came from a broiler house with wood shavings for bedding, while the cattle manure came from an establishment with confined dairy cattle. The SMC was acquired by the Experimental Station of Epagri, produced on a composting platform with addition of liquid manure to the wood shavings and mechanical turning, as described in Scherer et al. (2009). The LSM was collected from a manure pit with anaerobic fermentation, stored for more than 40 days. More information can be obtained in Scherer & Spagnollo (2014).

Cover crops (black oat) were managed with cutting roller around 20 days before maize sowing and sown with a no-tillage seeder at spacing of 0.90 m and density of 62,500 seeds ha^{-1} (112 plants/plot). For pest control, especially cartridge caterpillar, a product based on *Bacillus thuringiensis* and Neem oil was used when necessary, and spontaneous plants were controlled with manual weeding.

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Two samples were collected in the experimental area - 2016/2017 season, at the end of the cover crop cycle (black oat), in September 2016, before applying the organic fertilizer (E_1) and after harvesting the summer crop (E_2), approximately 150 days after waste application. For microbiological and chemical analyses, a bucket auger was used to collect in the usable area of each plot five single samples in a cross transect, at 0.00-0.10 m depth. The single samples were homogenized to form composite sample, of which one fraction was used for chemical characterization and the remainder was placed in plastic bags and transported in polystyrene boxes with ice to the laboratory, where they were maintained at 2 ºC until analysis.

2.3 Soil Microbiological Characterization

Soil microbiological attributes were characterized using the samples kept under refrigeration, homogenized and sieved through 2-mm mesh, after removing plant residues. Microbial biomass carbon (MBC) was determined through the fumigation-extraction method (Vance et al., 1987). Soluble C content was determined by titration with 33.3 mmol L^{-1} ammonium ferrous sulfate hexahydrate ($Fe(NH_4)_2(SO_4)_2.6H_2O$), in the presence of the diphenylamine indicator (1%). MBC was calculated by dividing the difference between the carbon extracted from fumigated and non-fumigated soil samples by the factor Kec = 0.33, subsequently corrected for soil dry basis. Microbial activity was evaluated by the determination of basal respiration (CO_2-C) in 50 g of soil samples, placed in 500 mL bottles and incubated in laboratory for 15 days, at a temperature of 28 °C (Alef and Nannipieri 1995). The results of CO_2-C, MBC and TOC were used to calculate the values of metabolic quotient (qCO_2) and MBC:TOC ratio.

2.4 Soil Chemical and Physical Characterization

For soil chemical characterization, the samples were sent to the Soil Laboratory of EPAGRI/CEPAF/CHAPECÓ, and the standard methodology of the Official Network of Soil Analysis Laboratories (ROLAS), according to Tedesco et al. (1995), was used to determine pH, total organic carbon (TOC) and contents of macronutrients (P, K, Ca and Mg) and micronutrients (Cu and Zn) (Table 2).The soil was characterized for the physical attributes of soil bulk density (Ds) and total porosity (Pt), sampled at three points per plot using a volumetric ring, according to the methodology proposed by EMBRAPA (1997). To determine the volumetric moisture content (Uv) and soil resistance to penetration (RP), three measurements were performed close to the points collected for the chemical and microbiological variables, using an Electronic Soil Moisture Meter - HIDROFARM HMF 2030 and an Electronic Soil Compaction Meter - PenetroLOG® PLG1020, respectively, both from the FALKER brand (Table 2). The chemical and physical characterization of the area was used for evaluation in the multivariate model, which can be seen in the supplementary data.

Table 2. Soil chemical and physical attributes in the 0.00-0.10 m in the treatments: control (C), cattle manure compost (CMC), poultry manure (PM), poultry manure compost (PMC), liquid swine manure (LSM) and swine manure compost (SMC)

Fertilizers*	TOC	pH	H+Al	P	K	Ca	Mg	CEC	V	Cu	Zn	Ds	Uv	Pt	RP
	g kg^{-1}		cmol$_c$ dm^{-3}	--- mg dm^{-3} ---		--- cmol$_c$ dm^{-3} ---			%	-- mg dm^{-3}--		g cm^{-3}	-- cm^3 cm^{-3} --		MPa
----------------------- Before fertilizer application (E$_1$) -----------------------															
C	18.17	6.70	2.36	8.56	68.00	8.28	5.00	15.82	84.55	3.04	2.64	1.40	0.16	0.469	2.66
PM	18.66	6.97	2.00	68.63	229.33	10.62	4.05	17.25	88.32	3.13	13.70	1.34	0.16	0.497	2.75
LSM	18.66	6.70	2.60	30.22	80.00	8.40	4.62	15.82	83.12	4.60	10.67	1.36	0.20	0.478	2.64
PMC	19.14	7.00	1.98	86.18	120.00	11.88	4.58	18.73	89.44	3.25	22.85	1.34	0.16	0.497	2.31
SMC	19.62	6.85	2.10	79.38	206.00	10.13	4.68	17.50	87.52	4.62	62.75	1.40	0.15	0.487	2.36
CMC	19.62	6.64	2.08	49.34	164.80	9.50	4.94	16.63	87.49	2.86	12.50	1.41	0.16	0.487	2.33
-------------------------- After fertilizer application (E$_2$) ---------------------															
C	17.59	6.35	2.14	10.78	70.67	6.32	3.32	11.95	82.12	3.78	3.05	1.36	0.14	0.528	3.74
PM	19.14	6.70	1.72	71.63	383.00	8.63	2.82	14.16	87.64	4.30	20.45	1.29	0.15	0.537	2.77
LSM	18.27	6.45	1.99	20.93	71.33	7.03	3.55	12.75	84.42	5.70	10.02	1.35	0.15	0.517	2.50
PMC	18.85	6.72	1.68	109.48	198.00	9.45	3.13	14.78	88.58	3.68	22.73	1.29	0.16	0.539	3.09
SMC	21.56	6.45	2.24	44.17	236.67	8.40	3.83	15.09	85.17	4.95	40.03	1.31	0.14	0.532	3.01
CMC	19.91	6.45	1.97	37.28	175.33	7.30	3.93	13.64	85.38	3.68	16.03	1.34	0.15	0.524	2.78

Ds = soil bulk density, Uv = volumetric moisture, Pt = Total porosity and RP = resistance to root penetration.

2.5 Plant Yield Evaluations

The following yield variables were also obtained: number of plants (NP), Number of ears (NE) and thousand-grain weight (TGW). After harvesting, the ears were husked and threshed manually, and the grains were dried in an oven until the standard moisture content of 13%. Then, the thousand-grain weight was quantified and grain yield per hectare was determined (Y).

2.6 Data Analysis

Microbiological and yield data were subjected to analysis of variance (Two-way and One-way ANOVA, respectively) and, when significant differences were found in the F test, the means were compared by the Scott-Knott test ($P < 0.05$).

Data were subjected to a multivariate analysis, canonical discriminant analysis (CDA), to identify the most relevant microbiological attributes for separating the treatments studied. The CDA was also used to compare the types of fertilization studied, and the means comparison test was performed with the values of the homogenized canonical coefficients (HCC), in the different canonical functions, using the LSD test ($P < 0.05$), according to Cruz-Castillo et al. (1994) and Maluche-Baretta et al. (2006). Canonical correlation analysis (CCA) between the microbiological and chemical attributes evaluated was also performed.

3. Results and Discussion

3.1 Evaluation of Microbial Attributes

The mean values found varied in the treatments and evaluation periods from 122.03 to 311.90 mg kg^{-1} for microbial biomass carbon (MBC) (F = 25.75; $P < 0.001$), from 0.60 to 1.64% for the ratio between microbial biomass carbon and total organic carbon (MBC:TOC) (F = 23.85; $P < 0.001$), from 0.29 to 0.47 mg g^{-1} of soil per day for basal soil respiration (CO_2-C) (F =

2.69; $P < 0.05$), and from 1.43 to 3.56 mg CO_2-C g^{-1} mg^{-1} soil for the metabolic quotient (qCO_2) (F = 14.27; $P < 0.001$) (Table 3).

These values are within the expected range found in the literature, especially considering the use of waste in soils of tropical regions (Couto et al., 2013; Balota et al., 2014; Da Silva et al., 2015). For the first evaluation period (E_1), microbial biomass increased in the areas that received fertilizers cattle manure compost (CMC), swine manure compost (SMC) and poultry manure (PM), which did not differ from one another, and the lowest values were found with liquid swine manure (LSM), control (C) and poultry manure compost (PMC) (Table 4). Compared to the control treatment, the manures promoted increases in microbial biomass of 40.8% (PM), 60.6% (SMC) and 62.9% (CMC), evidencing the potential of using animal waste to promote microbial growth, providing nutrients for the subsequent crop.

Table 3. Microbial biomass carbon (MBC), ratio between microbial biomass carbon and total organic carbon (MBC:TOC), basal respiration (CO_2-C) and metabolic quotient (qCO_2) in relation to the organic fertilizers: cattle manure compost (CMC), poultry manure compost (PMC), poultry manure (PM), swine manure compost (SMC), liquid swine manure (LSM) and control (C), in different sampling periods

Period	**C**	**PM**	**LSM**	**PMC**	**SMC**	**CMC**
			MBC (mg kg^{-1} soil)			
E_1*	134.05 bA	188.80 aB	148.46 bB	122.03 bB	215.29 aA	218.36 aA
E_2	148.69 cA	311.90 aA	239.84 bA	214.73 bA	140.37 cB	117.28 cB
C.V. (%)	17.58					
			MBC:TOC ratio (%)			
E_1	0.74 bA	1.01 aB	0.79 bB	0.64 bB	1.10 aA	1.11 aA
E_2	0.85 cA	1.64 aA	1.32 bA	1.17 bA	0.66 dB	0.60 dB
C.V. (%)	23.85					
			CO_2-C (mg g^{-1} day)			
E_1	0.47 aA	0.46 aA	0.45 aA	0.47 aA	0.45 aA	0.46 aA
E_2	0.29 bB	0.43 aA	0.39 aB	0.40 aB	0.38 aB	0.38 aB
C.V. (%)	12.66					
			qCO_2 (mg CO_2-C mg^{-1} soil)			
E_1	3.56 aA	2.48 aA	3.16 aA	3.86 aA	2.18 bA	2.14 bB
E_2	1.97 bB	1.43 bB	1.65 bB	1.91 bB	2.89 aA	3.39 aA
C.V. (%)	24.02					

*E_1 – Sampling period 1: black oat cycle, before organic fertilizer application; E_2 – Sampling period 2: maize crop fertilized with the organic fertilizers. Means followed by the same letter, lowercase in the rows and uppercase in the columns, do not differ by the Scott-Knott test ($P < 0.05$). C.V. = Coefficient of variation.

The application of organic fertilizer in the second sampling period (E_2) increased microbial biomass with the use of PM, which was statistically superior to the other treatments, followed by LSM and PMC, which did not differ from each other. The lowest MBC values were found in the treatments C, SMC and CMC (Table 3). As in E_1, the treatments stimulated microbial growth compared to the control, increasing it by 109.8% (PM), 61.3% (LSM) and 44.4% (PMC). The treatments SMC and CMC, which promoted microbial growth in E_1, caused reductions of 5.6 and 21.1% in microbial biomass in the period, respectively. There was no difference between sampling periods for the control treatment, which may be attributed to the absence of the cumulative residual effect of the organic fertilizers applied to the soil.

MBC is an important ecological indicator as it is responsible for the decomposition and mineralization of plant and animal residues, working as a reservoir of nutrients and energy for the soil (Araujo et al., 2012). Compost and fertilizers of animal origin often offer high concentrations of microbial C and N, promoting the entry of these elements into the soil (Diacono & Montemurro, 2010). Hence, different types of management, types of soil and characteristics of each soil may favor or reduce soil microbial activity, depending on the environmental condition and the interrelationship of the factors in the environment (Da Silva et al., 2015).

In order to understand the behavior of the response of microbial biomass to organic alterations in different types of soil, cultivation and geography, Kallenbach & Grandy (2011) conducted a meta-analysis evaluating 296 scientific articles published between the years 1990 and 2010. The authors concluded that the use of cattle manure promoted the best response, with increments of 58% in MBC compared to the other sources used, and also concluded that, on average, the addition of wastes contributes to the increase of 36% in MBC and quantities smaller than 5 t ha^{-1} result in small increments.

Organic composts and solid manure have higher content of nutrients in the organic form, with less immediate effect, but with higher efficacy in their response over time (Hartz et al., 2000; Scherer & Spagnollo, 2014), which partly explains the observed values of MBC in the treatments CMC, SMC and PM in E_1, and the reduction of MBC in the treatments CMC and SMC in E_2.

In E_2, the reduction of microbial biomass under CMC and SMC may represent characteristics of the waste itself and of the previous crop. Studies show that microbial biomass is directly affected by the quantity and quality of plant species (Belo et al., 2012; Da Silva et al., 2015; Moreira et al., 2016), influencing the response of microbial biomass. Oat has a high C:N ratio, which causes a reduction in microbial activity with greater energy expenditure on residue decomposition. When associated with the use of a more stable organic source, such as organic composts, which are the poorest wastes in nutrients, especially N, and richest wastes in C compounds, of difficult degradation (Scherer & Spagnollo, 2014), it can have a suppressing effect on the biomass of microorganisms.

PM has a low C:N ratio, as well as LSM, promoting the increase of microbial activity (Tremblay et al., 2010). Likewise, compared to solid manures and organic composts, LSM has higher capacity for immediate availability of nutrient to the soil (Scherer & Spagnollo, 2014).

For the MBC:TOC ratio, there was an effect of the sampling period in all treatments, except for the control, and in the first period the behavior of the treatments was identical for MBC, reflecting the effect of organic fertilization in promoting greater proportion of microbial carbon (MBC) in comparison to total organic carbon (TOC). For the organic fertilizers CMC and SMC, the highest ratio was obtained in the period prior to their application, whereas in the control (C) no difference was observed between the sampling periods (Table 3).

The MBC:TOC ratio represents the quality of the organic matter present in soil and expresses the efficiency of the microbial biomass in using its C and accumulating it as biomass, representing a labile reserve of nutrients for the soil (Dadalto et al., 2015). High MBC values are directly related to higher temporary immobilization of nutrients and, consequently, lower propensity to nutrient losses in the soil-plant system (Ferreira et al., 2016). Under adequate conditions, the microbial biomass represents approximately 1 to 4% of soil TOC, while values below 1% may indicate the presence of limiting factors for microbial growth (Jenkinson & Ladd, 1981).

The lower MBC:TOC ratio found in SMC and CMC is related to greater stability of this wastes and its greater C:N ratio, which affect their decomposition rate and energy expenditure in the process. The ease of decomposition of the wastes with lower C:N ratio, such as PM and LSM, leads to higher MBC:TOC ratio, as observed in E_2 under the effect of the application of the waste.

For the microbial activity measured through soil basal respiration, the highest CO_2-C emissions occurred in the period prior to organic fertilizer application (E_1), while the lowest values were found in the sampling performed after fertilizer application (E_2). All the evaluated treatments showed this behavior, except for the AE, in which there was no significant difference between the two sampling periods. In E_2, all treatments were superior to the control (Table 3).

The microbial activity rates reflect practices of land use and management, increasing or decreasing the carbon stocks in soil (Sousa et al., 2014). The increase in these values is related to the disturbances imposed to the soil and microbial populations, but high values can indicate situations of both disturbance and high level of system productivity (Duarte et al., 2014; Dadalto et al., 2015). However, the application of organic fertilizers, regardless of the source, promoted greater activity compared to the control (E_2), which may result in greater cycling of nutrients and, consequently, in their availability to the crop.

For the metabolic quotient (qCO_2), the highest values were found in E_1 sampling for all treatments evaluated, except CMC, which had the highest value after fertilizer application (E_2) (Table 3). For SMC, there was no difference between the evaluated periods (Table 3). The qCO_2 represents the efficiency of the community in decomposing the organic material with lower losses of C and greater accumulation in its biomass. High values of qCO_2 are indicative of environmental stress, which may indicate that this microbial population is under adverse or stressful conditions (Anderson & Domsch, 1993).

Despite the CO_2-C losses occurring after management, due to the composition of the waste, the composts CMC and SMC promoted increase of C content in the soil which received their application (Table 3). This result was observed by Scherer & Spagnollo (2014), when evaluating soil chemical properties and the yields of maize and beans obtained in the same experimental area. The authors found that only the treatments with CMC, PMC and SMC increased soil organic matter content in the superficial layer (up to 5 cm), while the treatments PM and LSM did not differ from the control.

3.2 Maize Yield

The use of organic fertilizer sources increased the number of ears (F = 4.64; $P < 0.01$), thousand-grain weight (F = 6.29; $P < 0.001$) and yield per hectare (F = 6.19; $P < 0.001$) of all treatments compared to the control. Among the sources of fertilizer, use of poultry manure (PM) stands out as it differed from the others. The sources cattle manure compost (CMC), poultry manure compost (PMC), liquid swine manure (LSM) and swine manure compost (SMC) did not differ but were superior to the control for the variables number of ears (NE), thousand-grain weight (TGW) and yield (Y) (Table 4).

Table 4. Number of plants per hectare (NP), number of ears per hectare (NE), thousand-grain weight (TGW) and yield per hectare (Y) of maize in relation to the organic fertilizers: cattle manure compost (CMC), poultry manure compost (PMC), poultry manure (PM), swine manure compost (SMC), liquid swine manure (LSM) and control (C), in different sampling periods

	NP ha^{-1}	**NE ha^{-1}**	**TGW (g)**	**Y (Kg ha^{-1})**
C	36,46^{ns}	34,58 c	203.56 c	2,785.87 c
CMC	38,00	46,17 b	618.62 b	8,842.40 b
PM	36,80	60,00 a	981.78 a	13,635.90 a
PMC	38,33	48,17 b	664.54 b	9,473.66 b
LSM	38,54	46,46 b	676.05 b	8,997.15 b
SMC	37,36	43,61 b	543.74 b	7,552.01 b
C.V. (%)	4.24	18.13	39.00	39.00

Means followed by the same letter, lowercase in the rows and uppercase in the columns, do not differ by the Scott-Knott test ($P < 0.05$). C.V. = Coefficient of variation. ^{ns} Not significant.

The results obtained by Scherer & Spagnollo (2014) demonstrated efficiency in the use of organic fertilizers for maize yield, differing from the control. However, for the period evaluated by these authors, the treatments PM and LSM did not differ, but differed from the others.

As in the study proposed by the above-mentioned authors, the results obtained in the present study for the control are particularly due to the absence of nutrient entry in the system, accompanied by the low availability of some nutrients such as P, which is below the critical level (Table 2), according to the Committee on Soil Chemistry and Fertility RS/SC (CSCF – RS/SC, 2016). A study conducted by Novakowiski et al. (2013), using poultry manure, in winter crops (oats and ryegrass) and in summer crop (maize), found that the highest maize yield in areas without grazing in the winter was reached with the use of 9 t ha^{-1}.

The response observed by the application of AE may be linked to its lower C:N ratio, compared to the other solid fertilizers used (Tremblay et al., 2010), and higher N concentration (Table 2). Thus, it results in a faster release of nutrients to short-cycle crops such as maize, with high N requirements in the initial growth periods, especially under conditions where the previous crops is a grass (oat), which has high C:N ratio. For Scherer & Spagnollo (2014), the higher efficiency of the fertilizers PM and LSM in maize yield is due to the higher concentration and availability of N present in these fertilizers.

Amaral-Filho et al. (2005), evaluating the influence of row spacing, population densities and N fertilization on maize nutrition and yield, found that the increase in N rates caused a linear increase in the number of grains per ear, 1000-grain weight, grain yield and protein content in the grains. Grasses in general demand large amounts of N and, because it is the maize crop, substantial amounts of this element are necessary for high yields (Gagnon et al., 2012), especially in the initial growth stages, in which the production potential is defined.

Another important point to emphasize is the accumulation of nutrients in soil promoted by the fertilization with PM, now focusing on K (Table 2). This element is one of the essential macronutrients and performs several functions in crop development, growth and yield formation, serving as a cofactor for more than 40 enzymes in different metabolic pathways, regulating cellular osmotic pressure and stomatal movements (Zhao et al., 2016). In a study conducted by Parente et al. (2016) evaluating the immediate efficiency of K in second-crop maize and the possible residual effect on soybean crop cultivated in succession in a no-tillage system, these authors observed a quadratic effect of K fertilization on the thousand-grain weight.

The results found for the yield variables in the maize crop show similar behavior to that of soil microbiological quality variables in relation to the treatments, since the highest values of MBC, CO_2-C and MBC:TOC were also observed in the treatment with PM (Figure 1). The increase of variables such as MBC and CO_2-C due to the use of PM may have led to greater nutrient cycling (Araujo et al., 2012) and subsequently release of these nutrients to maize plants in their initial growth period. It should be pointed out that no soluble sources of nutrients were used in the experiment, so soil biological activity was fundamental for the dynamics of nutrients in soil.

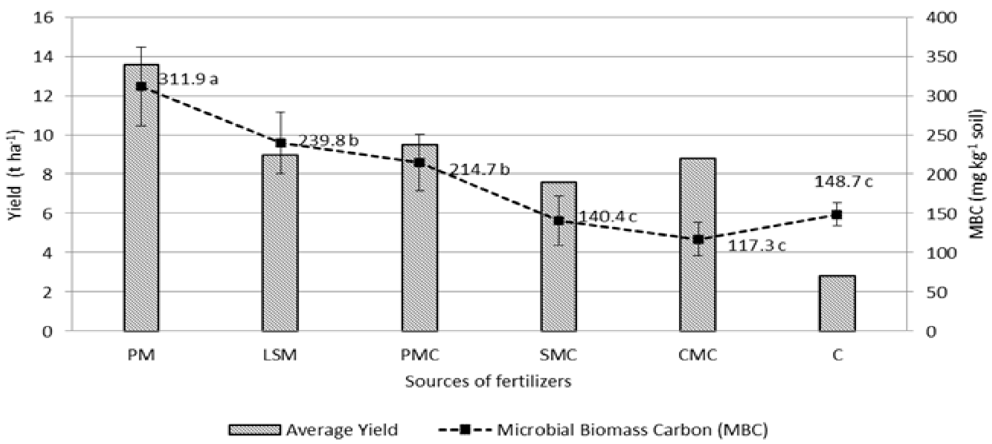

Figure 1. Relationship between average maize yield data (t ha^{-1}) (bars) and microbial biomass carbon data (lines)

The present study demonstrates that the use of PM, LSM and PMC as sources of fertilizer promotes microbial growth more efficiently, leading to higher accumulation of carbon in the biomass of microorganisms, with lower losses of CO_2 by the system, when evaluated in a period of up to 180 days after their application (E_2). Regarding the yields of the summer crop, all treatments caused increase in maize yield compared to the control, especially the fertilization with poultry litter, which in addition to promoting greater yield, allows a greater microbial growth.

A study conducted by Mendes et al. (2019) reported important results, evaluating microbial indicators with crop yield in the Cerrado region. The results showed positive correlations of the microbial biomass carbon (0.48%**) with soil organic carbon content (SOC) and of SOC (0.77%**) with the accumulated relative yields of the crops (soybean and maize). In another study conducted by the same research team, Lopes et al. (2013) found correlations of 0.62*** and 0.79*** of MBC and CO_2-C with the accumulated relative yields of the crops. The MBC values found for the highest yield obtained by this study are within an average range according to Mendes et al. (2019), varying from 246 to 415 mg C kg^{-1} soil. However, the edaphoclimatic differences of each site can promote changes in the indicator values.

3.3 Canonical Discriminant Analysis (CDA)

The canonical discriminant functions 1 and 2 (CDF_1 and CDF_2) showed canonical correlations of 94.7 and 92.2%, respectively, demonstrating association between the studied attributes and the use of the different sources of manure as fertilizers, besides demonstrating that CDA is recommended for this type of study. The multivariate statistical test of Wilks' Lambda indicated significant difference between the treatments studied (P < 0.0001) for microbial attributes and explanatory variables, present in these functions, where CDF_1 explained 42.4% of data variability, while CDF_2 explained 27.4% of the remaining variability (Figure 2).

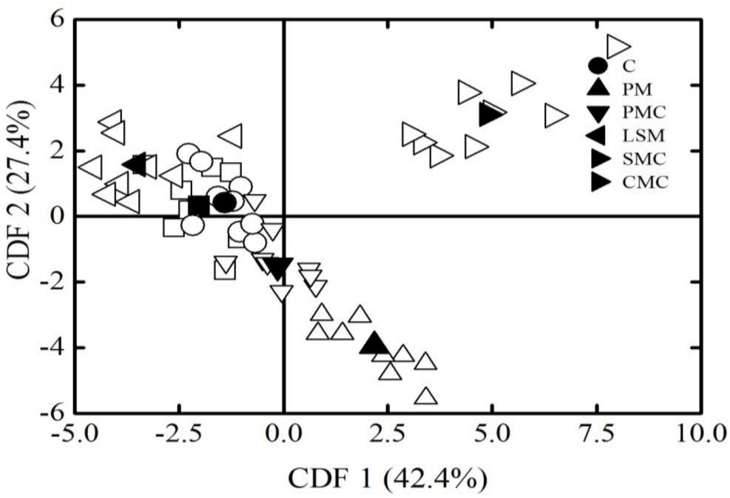

Figure 2. Relationship between the first and second canonical discriminant function (CDF_1 and CDF_2) on the means (centroids) of the standardized canonical coefficients (SCC) referring to microbial, physical, chemical and yield attributes, discriminating the different

sources of fertilizer, regardless of sampling period effect

The standardized canonical coefficients (SCC) showed, within the CDF_1, that the studied wastes are different from one another (Table 5); CDF_1 separates the treatment of SMC, with higher values of SCC, followed by the treatment PM, which were separated from the others with the lowest (negative) values of SCC (Figure 2). There was no discrimination between the treatments C and CMC, which were farther away from the others.

Table 5. Analysis of average variance of the standardized canonical coefficients (SCC) of the first canonical discriminant function (CDF_1 and CDF_2) referring to the values of microbial, physical, chemical and yield attributes, discriminating the different sources of organic fertilizers, regardless of sampling period

	C	**PM**	**LSM**	**PMC**	**SMC**	**CMC**	**C.V. (%)**
CDF1	-2.02 d	2.18 b	-3.5 e	-0.14 c	4.88 a	-1.41 d	2.09

Means followed by the same lowercase letter in the row do not differ at 0.05 significance level by the LSD test. C.V.: Coefficient of variation.

The contribution of the studied attributes to separating the different sources of organic fertilizers can be verified through the values of the parallel discriminant ratio (PDR) (Table 6), which were obtained by multiplying the values of the standardized canonical coefficients (SCC) and of correlation (R) and are more adequate to discriminate the areas, showing that the studied attributes were efficient to promote the separation between treatments, as sensitive indicators.

Table 6. Parallel discriminant ratio (PDR) for the canonical discriminant functions 1 and 2 (CDF_1 and CDF_2), referring to the microbial attributes (Response variables), chemical-physical attributes (Environmental variables) and production attributes, regardless of organic fertilization used and sampling period

Variables	CDF_1 (42.4%)	CDF_2 (27.4%)
	PDR	PDR
	Response variables	
MBC*	0.16	0.35
CO_2-C	-0.02	-0.01
qCO_2	0.05	0.02
MBC:TOC	-0.15	-0.45
	Environmental variables	
TOC	0.04	0.21
pH	0.03	0.06
P	-0.12	-0.04

K	0.32	0.16
Ca	0.01	0.15
Mg	0.01	0.26
H+Al	0.00	0.01
Zn	0.69	0.21
Cu	-0.01	-0.04
Ds	-0.00	0.06
Uv	0.00	0.02
Pt	0.01	-0.04
RP	-0.00	0.00
CL	0.00	-0.00
Production variables		
NP	-0.01	0.01
NE	0.02	0.02
TGW	0.34	0.83
Y	-0.38	-0.81

*MBC = Microbial biomass carbon; CO_2-C = basal respiration; qCO_2 = metabolic quotient; MBC:TOC = ratio between microbial biomass carbon and total organic carbon; TOC = Total organic carbon; pH = Hydrogen potential; P = Phosphorus; K = Potassium; Ca = Calcium; Mg = Magnesium; H+Al = Hydrogen+Aluminum; Zn = Zinc; Cu = Copper; Ds = soil bulk density, Uv = volumetric moisture, Pt = Total porosity and RP = resistance to root penetration; CL = Clay; NP = Number of plants; NE = Number of ears; TGW = Thousand-grain weight; Y= yield per hectare.

In the CDF_1, the environmental attributes Zn and K (respectively, 0.69 and 0.31), the yield variable thousand-grain weight (TGW; 0.34) and the microbial attributes MBC (0.16) and qCO_2 (0.05) had the highest positive values, indicating that almost all the separations between treatments are explained by the difference in the values found for these variables, with greater discrimination power for the Zn contents found in the treatments. However, with less contribution to the separation of treatments, the CDF_2 allowed listing a higher number of evaluated attributes accounting for the differences between treatments, which were the ones with highest positive values of PDR (TGW = 0.83; MBC = 0.35; Mg = 0.26; Zn = 0.21; TOC = 0.20; K = 0.16; Ca = 0.15; Ds = 0.06 and pH = 0.06) (Table 6).

According to Baretta et al. (2010), the CDA for the selection of indicator values uses the following PDR values as reference: ≤ 0.03 (low), 0.04 to 0.09 (medium), 0.10 to 0.20 (good), 0.21 to 0.41 (very good), from 0.42 to 0.80 (great) and > 0.81 (excellent). Hence, the Zn contents in soil under waste application are classified as a great indicator. Likewise, for the study in question, the chemical and yield attributes are classified in the CDF_1 as very good indicators, while MBC is a good indicator. For the CDF_2, the thousand-grain weight (TGW) and MBC would be, respectively, excellent and very good indicators. Therefore, the higher the positive values of PDR, the greater the effect of separation between the areas, while negative values mean similarity between the fertilization systems for this attribute.

According to Balota et al. (2012), soil preparation and application of different doses of liquid swine manure influence the C, N and P contents of the microbial biomass and its activity has good sensitivity in detecting alterations in soil, due to management. The effect of the applications of organic compost derived from liquid swine manure and wood shavings causes alterations in soil chemical attributes, especially in the superficial layers, with greater emphasis to increase of soil pH and P, K, Cu and Zn contents (Lourenzi et al., 2016). According to Andrade (2015), long periods of application of swine and poultry manures increased the Zn contents in the areas that received waste for a longer time.

Sources of manure such as that of cattle stand out in several aspects, including organic matter percentages from 30 to 58% (Carneiro et al., 2016). These wastes act on soil fertility by increasing the organic matter, cation exchange capacity and reducing the contents of exchangeable aluminum. Besides improving many soil attributes, the application of organic wastes also increases phosphorus availability in the soil.

The variation of microbiological attributes is visible through the variation of the chemical attributes and these results confirm a higher sensitivity of microbial attributes to the chemical variations observed in the studied treatments, indicating that barely visible chemical changes in soil may affect the microbial attributes and their relationship with the soil. Changes in microbial biomass may provide an early indication of short-term modifications in total organic carbon (Ge et al., 2010) and in the dynamics of nutrient availability to plants.

Studies show that the higher the MBC value, the higher the temporary immobilization of nutrients, hence lower loss in the soil-plant system (Müller, 2014). MBC is important because it affects the physical and chemical properties of the soil and quantitatively represents the labile component of soil organic matter, being an indicator of great sensitivity to evaluate changes in the soil (Dionísio et al., 2016).

In the CDF_1, the chemical variables Zn and K had the highest values of PDR, which demonstrates that they are directly associated, influence the response of microbial attributes, as in the case of MBC, and, from this parameter, related to crop yield variables such as thousand-grain weight (TGW). For the area, the average maize yields were 14 t ha^{-1} in PM, 9.4 t ha^{-1} in PMC, 8.9 t ha^{-1} in LSM, 8.8 t ha^{-1} in CMC, 7.5 t ha^{-1} in SMC and 2.8 t ha^{-1} in the control. These values are extremely high and were also found by Scherer and Spagnollo (2014).

3.4 Canonical Correlation Analysis (CCA)

From the obtained data, five canonical correlations were studied, relating the microbiological attributes (MBC, TOC, CO_2-C and qCO_2) with the chemical and physical environmental variables of the treatments (pH, P, K, Ca, Mg, Cu, Zn, H+Al, Ds, Uv, Pt and RP). The first canonical correlation between the attributes was 0.70 ($P < 0.0001$), and 58% of the variation in the scores of the first canonical variable of the microbial attributes was explained by the scores of the chemical and physical attributes (Figure 3). The other correlations were not significant ($P > 0.05$) and, therefore, will not be explained.

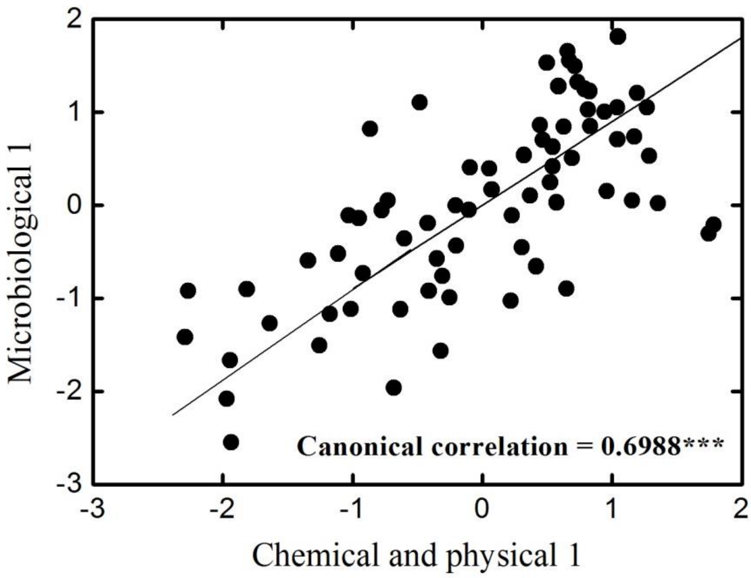

Figure 3. Canonical Correlation Analysis (CCA) between microbial and chemical-physical attributes for the different fertilizers tested, regardless of treatment

The microbiological canonical variable 1 ($MBCV_1$) had the highest values of standardized canonical coefficients (SCC) and canonical correlation (CC) for the microbial attributes CO_2-C (1.56 and 0.85, respectively) and MBC (-3.84 and -0.16, respectively) (Table 7). The negative signs of MBC and positive signs of CO_2-C indicate that areas with lower values of MBC had higher values of CO_2-C. The opposite signs found in the values of SCC and CC for TOC, qCO_2 and MBC:TOC ratio indicate that these parameters, despite the high values of SCC (MBC:TOC and qCO_2) had a suppressing aspect and do not contribute to the discrimination of the studied areas.

Table 7. Standardized canonical coefficients (SCC) and canonical correlation (CC) for the microbial attributes of the microbiological canonical variables 1 ($MBCV_1$) and chemical and physical canonical variables 1 ($CPCV_1$) of the soil, regardless of treatment

Attribute	SCC	CC
	$MBCV_1$	
MBC	-3.84	-0.16
TOC*	0.44	-0.21
CO_2-C	1.56	0.85
qCO_2	-1.32	0.41
MBC:TOC	2.70	-0.12
	$CPCV_1$	

pH	0.71	0.69
P	-0.04	0.12
K	0.05	-0.03
Ca	-0.05	0.54
Mg	-0.18	0.48
H+Al	0.44	0.32
Cu	-0.04	-0.47
Zn	0.24	0.04
Ds	-0.62	0.27
Uv	-0.03	0.08
Pt	-0.76	-0.46
RP	-0.16	-0.28
Clay	0.32	0.50

*Chemical attribute of microbiological influence

The chemical and physical canonical variable 1 ($CPCV_1$) had the highest values of standardized canonical coefficients (SCC) and canonical correlation (CC) for the attributes pH (0.71 and 0.69) and Pt (-0.76 and -0.46). The attributes H+Al (0.44 and 0.32) and clay content (0.32 and 0.50) showed a lower relative importance, while the other attributes contributed little to the first canonical correlation with the studied microbiological attributes (Table 7).

The canonical correlation data show that there is a correlation between the microbiological, chemical and physical attributes and that this correlation occurs mainly between the microbiological attributes CO_2-C and MBC and the values of pH and Pt, reinforcing the sensitivity of soil microbiota to the adequate conditions of a favorable chemical and physical environment.

For Balota (2017), controlling soil acidity is of great importance, because it affects the availability of nutrients, cation exchange capacity (CEC) and biological activity and development. In addition, under conditions of low pH there may be toxicity of Al^{3+} and Mn^{+2}, one of the main factors compromising plant growth. Cherubin et al. (2015), evaluating soil management systems (no-tillage, chisel plowing and minimum tillage) associated with the use of pig slurry and mineral fertilization, observed that, regardless of the management system and fertilizer used, there were increments in the values of soil bulk density (Ds) and resistance to penetration (RP), with a consequent reduction of macroporosity and total porosity (Pt) in most treatments, compared to the native forest. These results indicate that agricultural systems under soil management for grain production cause major changes in soil physical attributes, resulting in the compaction of the superficial layers.

4. Conclusion

The study shows that the different organic fertilizers affect microbial biomass and activity, differing between the periods of evaluation, and poultry manure (PM), liquid swine manure (LSM) and poultry manure compost (PMC) promote microbial growth more efficiently. As observed in the present study, and already found in a previous study, all tested sources promoted increments in maize yield, especially poultry litter, and the average yields obtained confirm that organic production systems can obtain yields that are equivalent to or even higher than those found in systems that use soluble fertilizers. The use of multivariate analysis techniques proved to be an important tool in the selection of soil quality indicator attributes in systems of fertilization with animal wastes, establishing relationships between microbial, chemical, physical and maize yield attributes. The canonical discriminant analysis (CDA) showed that the indicators with higher sensitivity for discriminating the areas in terms of the application of animal waste are related to the MBC, as the microbial attribute of greatest contribution, whereas the attributes Zn, K, Mg, Ca and pH stood out among the chemical attributes, as well as the physical attribute soil bulk density (Ds) and thousand-grain weight (TGW).

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