Microbiological and chemical attributes of a Hapludalf soil with swine manure fertilization

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Abstract – The objective of this work was to evaluate the microbiological and chemical attributes of a soil with a seven-year history of urea and swine manure application. In the period from October 2008 to October 2009, soil samples were collected in the 0–10 cm layer and were subjected to the treatments: control, without application of urea or manure; and with the application of urea, pig slurry, and deep pig litter in two doses, in order to supply one or two times the recommended N doses for the maize (*Zea mays*)/black oat (*Avena strigosa*) crop succession. The carbon of the microbial biomass (MB-C) and the basal respiration (C-CO$_2$) were analyzed, and the metabolic (qCO$_2$) and microbial quotient ($q$mic) were calculated with the obtained data. Organic matter, pH in water, available P and K, and exchangeable Ca and Mg were also determined. The application of twice the dose of deep pig litter increases the MB-C and C-CO$_2$ values. The $q$mic and qCO$_2$ are little affected by the application of swine manure. The application of twice the dose of deep pig litter increases the values of pH in water and the contents of available P and of exchangeable Ca and Mg in the soil.

Index terms: environmental quality, no-tillage, pig slurry, soil quality, swine production.

Atributos microbiológicos e químicos de um Argissolo adubado com esterco de suínos

Resumo – O objetivo deste trabalho foi avaliar os atributos microbiológicos e químicos de um solo com histórico de sete anos de aplicação de ureia e dejetos de suínos. No período de outubro de 2008 a outubro de 2009, foram coletadas amostras de solo na camada de 0–10 cm, as quais foram submetidas aos tratamentos: controle, sem aplicação de ureia ou dejetos; e com a aplicação de ureia, dejetos líquidos e cama sobreposta de suínos em duas doses, para suprir uma ou duas vezes a dose de N recomendada para a sucessão milho (*Zea mays*)/aveia-preta (*Avena strigosa*). O carbono da biomassa microbiana (C-BM) e a respiração basal (C-CO$_2$) foram analisados, e o coeficiente metabólico (qCO$_2$) e o microbiano ($q$mic) foram calculados com os dados obtidos. Também foram determinados matéria orgânica, pH em água, P e K disponíveis, e Ca e Mg trocáveis. A aplicação do dobro da dose de cama sobreposta de suínos aumenta os valores de C-BM e C-CO$_2$. O $q$mic e o qCO$_2$ são pouco afetados pela aplicação de dejetos de suínos. A aplicação do dobro da dose de cama sobreposta de suínos aumenta os valores de pH em água e os teores de P disponível e de Ca e Mg trocáveis no solo.

Termos para indexação: qualidade ambiental, plantio direto, esterco líquido suíno, qualidade do solo, suinocultura.

Introduction

Confined pig production generates liquid manure, from washing bays with water, and solid waste, such as deep litter (Jeppsson, 1999), generated by breeding pigs on organic material, which have been applied in no-tillage systems as a source of nutrients for crops. However, due to the large volume produced on swine raising properties and the small farm sizes, successive applications of manure are made on the same areas.

The application of manure, especially on degraded soils, can increase the accumulation of total organic carbon (TOC), particularly in the uppermost layers (Lourenzi et al., 2011). However, the speed of this increase is associated mainly with the composition,

the frequency, and the quantity of the manure applied (Guardini et al., 2012). The manure may also contribute to an increase in exchangeable aluminum uptake, especially in the humic and fulvic acid fraction of the organic matter (Ceretta et al., 2003), decreasing plant toxicity and increasing H^+ adsorption (Lourenzi et al., 2011), which may lead to an increase in the values of the pH in water. Since the manure retains calcium and magnesium, derived mostly from commercial animal feed, over the years, an increase is expected in the exchangeable contents of soils with a history of pig slurry or deep pig litter application (Lourenzi et al., 2011). Moreover, continued applications of manure may cause an increase in the phosphorus and potassium contents on the soil surface as well as the occupation of adsorption surfaces, which will consequently reduce adsorption energy of these nutrients, increasing their desorption and availability **(**Ceretta et al., 2010a, 2010b).

In addition, the application of pig slurry or deep pig litter may lead to modifications in the soil microbiological attributes, such as microbial biomass carbon (MB-C). In spite of representing the lowest fraction of TOC (2 to 3%), MB-C exhibits rapid cycling and responds significantly to seasonable fluctuations of moisture and temperature, and to the type of organic residue deposited (Balota et al., 2010). The high levels of labile C and low C:N ratio in pig slurries stimulate the soil microbial population, increasing their size and activity. However, organic residues containing potentially toxic chemical elements may negatively affect soil microorganisms (Berton et al., 2006). In this case, the MB-C may be useful for providing rapid information on changes in the soil organic properties.

One of the indicators most used for evaluating the activity of heterotrophic soil microbiota is basal respiration, which represents the CO_2 produced by the microbial biomass during the processes of decomposition and mineralization of the organic matter added to the soil (Moreira & Siqueira, 2006). High values of basal respiration usually indicate greater metabolic activity of the microorganisms, since the flow of CO_2 is related to the intensity of the catabolic processes; however, high values of basal respiration of microorganisms may also indicate the loss of soil organic matter, mainly in situations in which C balance is negative (Parkin et al., 1996).

The metabolic quotient (qCO_2) is an index obtained by the ratio between the basal respiration of the microbial activity and the MB-C, which is also considered as a sensitive indicator of alterations in the ecosystem (Anderson & Domsch, 1989). Changes in the qCO_2 reflect the organic matter status of the soil, the efficiency of microbial C conversion, and the losses and stabilization of organic C by the mineral fraction of the soil. Therefore, its value may indicate if C accumulation or loss is occurring in the soil.

The objective of this work was to evaluate the microbiological and chemical attributes of a soil with a seven-year history of urea and swine manure application.

Materials and Methods

The experiment was carried out on a pig farm near the municipality of Braço do Norte, in the south of the state of Santa Catarina, Brazil (28º14'20.7"S, 49º13'55.5"W, at 300 m of altitude). The climate of the region is subtropical humid (Cfa according to Köppen), with an annual average temperature of 18.7ºC and a mean annual rainfall of 1,471 mm. The soil is classified as a Typic Hapludalf (Soil Survey Staff, 1999). Before setting up the experiment, soil analysis indicated in the 0–10 cm layer: 330 g kg^{-1} of clay; 33 g kg^{-1} of organic matter; pH in water of 5.1; 19 mg dm^{-3} of available P and 130 mg dm^{-3} of available K (both extracted by Mehlich 1); 0.8, 3.0, and 0.8 cmol$_c$ dm^{-3} of exchangeable Al, Ca, and Mg, respectively (extracted by KCl 1 mol L^{-1}); CTC$_{pH\ 7.0}$ of 11.9 cmol$_c$ dm^{-3}; base saturation of 41.7% and Al saturation of 16.13%.

Soil samples were collected from a naturalized pasture area predominantly of *Paspalum notatum*, *P. plicatulum*, *Eryngium ciliatum*, and *Stylosanthes montevidensis*, with a history of sporadic application of pig slurry on the soil surface and of crop succession of maize (*Zea mays*) and black oat (*Avena strigosa*). In December 2002, 6.0 Mg ha^{-1} of limestone were applied (PRNT = 87.5%) on the soil surface, without incorporation, to raise pH in water up to 6.0 (Recomendações de adubação e de calagem para os Estados do Rio Grande do Sul e Santa Catarina, 1994). In January 2003, the pasture was desiccated. The five treatments were: control; U90 and U180, annual fertilization with urea at rates of 90 and 180 kg ha^{-1} N; PS90 and PS180, pig slurry at rates of 90 and

180 kg ha^{-1} N; and DL90 and DL180, annual fertilization with pig deep-litter at rates of 90 and 180 kg ha^{-1} N. The recommended quantity for N in pig slurry and deep litter was 90 kg ha^{-1} per year for maize and black oat succession in the no-till system, according to the Comissão de Fertilidade do Solo (Recomendações de adubação e de calagem para os Estados do Rio Grande do Sul e Santa Catarina, 1994). To calculate doses of manure, the total N content of each kind of manure was used, the residual effect of these materials on N availability in the second year was not considered, and doses of 90 and 180 kg N were maintained over the years. Urea and manure were the only sources of nutrients added to the crop succession over the years. A randomized complete block design was used, with three replicates, and each experimental plot was 27 m^2 (4.5 m width x 6.0 m length).

Twenty-eight applications of urea and pig slurry were made during the experimental period from 2002 to 2009 (four applications per year during the growing of maize and black oat). Urea was applied on the vegetative stage (V1), elongation stage (V6), and at tasseling in the maize crop, and at the beginning of tillering in the black oat crop. Doses of 90 and 180 kg N of pig slurry were divided into four equal doses over the maize and black oat crops: before maize sowing; 51 and 95 days after maize sowing; and 15 days after black oat sowing. In the same period (2002–2009), seven applications of deep litter were made, each one on the soil surface around 15 to 30 days before maize sowing. Maize was sown annually in November in rows with 50,000 plants per hectare, whereas black oat was broadcast seeded every year in April/May at a quantity of 90 kg of seeds per hectare.

In each plot, ten soil subsamples were collected at the 0–10 cm layer on 10/17/2008, 12/18/2008, 2/18/2009, 3/23/2009, 6/4/2009, 8/21/2009, and 10/12/2009. After collection, the samples were taken to the laboratory and were passed through a 2 mm mesh sieve. Plant fragments were removed manually and soil moisture was adjusted to 60% field capacity with the addition of distilled water. After that, soil samples were stored in a refrigerator at 4°C for ten days. Subsequently, the MB-C was analyzed in triplicate, subdivided into six subsamples of 30 g, of which three were subjected to radiation in a microwave oven to cause cell lysis. The samples were subjected to microwave radiation for 2 min at 1.62x10^5 J (Ferreira

et al., 1999). Then, the organic C samples (radiated and non-radiated) were extracted with a solution of potassium sulfate (0.5 mol L^{-1} K$_2$SO$_4$), oxidized by a solution of potassium dichromate (0.066 mol L^{-1} K$_2$Cr$_2$O$_7$), and titrated with a solution of ammonium ferrous sulfate [0.033 mol L^{-1} FeSO$_4$ (NH$_4$) 2SO$_4$.6H$_2$O]. The coefficient (Kc) used in the calculation to determine the MB-C was 0.33. Soil basal respiration (BR) was determined according to Jenkinson & Powlson (1976). The qCO$_2$ was obtained through the ratio BR / MB-C (Anderson & Domsch, 1993). The $q$mic was obtained by the ratio MB-C / TOC (Anderson & Domsch, 1989), in which the soil TOC was determined by the wet oxidation method for organic matter (Walkley-Black) (Tedesco et al., 1995).

In October 2008 and 2009, soil was collected from the 0–10 cm layer. After collection, soil samples were air-dried, passed through a 2 mm mesh sieve, and subjected to TOC analysis. The following were calculated with the obtained data: organic matter content (Donagema et al., 2011); pH in water (1:1), Ca and Mg exchangeable contents (KCl 1 mol L^{-1} extractor); available P and K (Mehlich 1 extractor) (Tedesco et al., 1995).

The results of the microbiological and chemical attributes were tested as to normality by the Kolmogorov-Smirnov method and subjected to analysis of variance. When significant, mean values of chemical attributes were compared by Tukey's test, at 5% probability, and mean values of microbiological attributes were compared by Duncan's test, at 5% probability. All the attributes were subjected to principal component analysis (PCA), in which the microbiological attributes of the soil collected on 10/17/2008, 12/18/2008, 2/18/2009, and 3/23/2009 represented the maize cycle, and of the soil collected on 6/4/2009, 8/21/2009, and 10/12/2009 characterized the black oat cycle. The soil chemical attributes, collected in October 2008 and 2009, corresponded to the maize and black oat cycle, respectively. To reduce discrepancy among the data, the quantitative values were normalized through the equation: [(value - minimum value)/maximum value - minimum value) x 2] - 1.

Results and Discussion

No significant differences were observed among treatments for MB-C in the soil collected in October

2008 (Table 1). In December 2008, the MB-C content in the soil subjected to the application of twice the dose of deep pig litter (DL180) was greater than in the control. In February 2009, the MB-C in the soil subjected to the application of DL180 was greater than in the soil of the other treatments. The MB-C also showed highest contents in the soil of the DL180 treatment in March, differing from the control, U90, U180, and DL 90 treatments. However, in June 2009, the soil of the treatments PS180, DL90, and DL180 presented the highest MB-C contents, which were higher than in the soil of the U90 treatment. In August and October 2009, the soil of DL180 treatment once more showed the highest values of MB-C, differing from the control, U90, U180, and PS90 treatments on the next-to-last date of assessment and from the PS180 and U90 treatments on the last date. The U90 treatment presented the lowest values on these last two dates of soil collection.

Although the MB-C values found are lower than those usually reported in the literature (Guerrero et al., 2007), they are in accordance with studies carried out in Brazil (Santos et al., 2012) and other countries (Liu et al., 2013). The differences in MB-C values can be explained by the fact that the soil microbial biomass is highly variable, dependent on environmental conditions such as the soil type, climate, and culture (Moreira & Siqueira, 2006).

The highest MB-C content in the soil subjected to the application of DL180 on most of the dates may be attributed to the application of 30 kg ha^{-1} TOC throughout the seven experimental years, a quantity greater than the 15, 10, and 20 kg ha^{-1} per year of TOC added to the soil on the DL90, PS90, and PS180 treatments, respectively. The greater MB-C content in the soil may also be a result of the more recalcitrant forms of C in the deep pig litter than in the pig slurry (Giacomini & Aita, 2008), which is easily decomposable and, therefore, has slower mineralization and, consequently, lower increase in the solution or even in the microbial biomass (Gessner et al., 2010). Furthermore, the carbon increase of the microbial biomass in the PS180 treatment may be associated with the application of the manure in the soil, which contained, in average, 30, 8, and 27 kg ha^{-1} N, P and K, respectively, and was carried out 15 days before soil collection. This result corroborates those obtained by Chu et al. (2007), who also found higher MB-C values in the soil with the application of organic fertilizer than with chemical fertilization with N, P, and K.

Similar results were found for MB-C in the soil of the control, U90, and U180 treatments. The highest

Table 1. Carbon contents in the microbial biomass and basal respiration values in the 0–10 cm layer in soil subjected to the application of urea and swine manure for a period of seven years[1].

Treatment[2]	October 2008	December 2008	February 2009	March 2009	June 2009	August 2009	October 2009
	Microbial biomass carbon (mg kg^{-1} of dry soil)						
Control	53Aa	47ABd	54Ab	54Ab	53Aab	37Bbc	59Aabc
U90	63Aa	62Abc	55ABb	53ABb	36BCb	32Cc	40BCc
U180	55Aa	57Abcd	51ABb	54Ab	58Aab	38Bbc	55Aabc
PS90	40Da	56BCbcd	53BCb	67Aab	49CDab	41Dbc	61ABabc
PS180	44Ba	57Abcd	56ABb	63ABab	62ABa	48ABab	51ABbc
DL90	45Ba	52ABcd	55ABb	56ABb	63ABa	44Babc	66Aab
DL180	59ABa	75ABa	71ABa	71ABa	61ABa	56Ba	77Aa
	Basal respiration (C-CO$_2$, mg kg^{-1} per hour)						
Control	0.81ABa	0.74Bb	1.08Ac	1.10Aa	0.91ABa	0.91ABa	0.67Bab
U90	0.70BCa	0.46Cb	0.98ABc	0.89ABa	1.10Aa	1.01ABa	0.42Cb
U180	0.70Ba	0.83ABb	0.97ABc	0.85ABa	1.44Aa	0.98ABa	0.84ABab
PS90	0.88Aa	0.84Aab	0.95Ac	1.12Aa	1.03Aa	1.12Aa	0.76Aab
PS180	0.80Aa	0.81Aab	1.00Ac	0.80Aa	0.92Aa	0.86Aa	0.84Aab
DL90	0.75Ba	0.76Bab	1.45Ab	0.89Ba	1.07ABa	1.12ABa	0.79Bab
DL180	0.78Ba	1.36ABa	1.98Aa	1.00Ba	1.23Ba	1.08Ba	1.40ABa

[1] Mean values followed by the equal letters, lowercase in the columns and uppercase in the lines, do not differ by Duncan's test, at 5% probability. [2]U90, mineral nitrogen fertilization at recommended level; U180, mineral nitrogen fertilization two times the recommended level; PS90, pig slurry at recommended level; PS180, pig slurry two times the recommended level; DL90, deep pig litter at recommended level; DL180, deep pig litter two times the recommended level.

contents were observed from October 2008 to June 2009, and the lowest in August 2009 (Table 1). The soil of the treatments with the application of deep pig litter at one and two times the recommended amount presented the highest contents in October 2009. The lowest MB-C content was registered in August 2009 in all the treatments, with the exception of PS180, in which it was observed in October 2008.

The biomass captured organic C derived from the mineralization of C that was more easily decomposed from pig slurry (PS90 and PS180), including C of the soil subjected to the application of urea (U90 and U180). In the soil of these two treatments, it is probable that part of the C was derived from the crop residue of maize and, especially, from black oats in decomposition on the soil surface. That is because, throughout the years, dry matter production of maize and black oat did not differ among the treatments. However, in the treatments with the application of deep pig litter, material with a high C:N ratio is provided to the soil microbial biomass, with C being highly recalcitrant. This makes an immediate colonization of the microbial biomass impossible in this substrate.

It is important to note that the microbial biomass first degrades the substrates recently added to the soil, or part of them, which contain a source of labile C, like that of the pig slurry. In the second phase, there is an increase of the biomass activity and of the consumption of the remaining substrates, which are more utilizable than those normally present in the soil. Finally, the substrates reach a state similar to the initial one of the soil, and there is the decline of microbial activity and of the biomass.

The basal respiration values in December 2008, February, and October 2009 were higher in the soil of the DL180 treatment (Table 1). In February 2009, the soil of the DL90 treatment (deep pig litter at recommended level) presented a basal respiration value lower than that observed in the soil of the DL180 treatment. However, these values were higher than those verified in the soil of the other treatments (control, U90, U180, PS90, PS180), which did not differ on any collection date. A possible explanation is that deep pig litter normally has a high C:N ratio and high lignin contents, which retards its decomposition, increasing colonization by specialized microorganisms equipped with more complex enzymatic systems capable of degrading molecules of greater weight,

probably polymerized (Gessner et al., 2010). As the microorganisms release enzymes for the initial rupture of those molecules, other less specialized microorganisms find conditions for proliferation, which may be reflected in higher breathing values. These results are similar to those obtained by Ros et al. (2007), who reported higher basal respiration values in a soil subjected to the application of deep pig litter from November 2002 to May 2003 than in the soil with the application of pig slurry.

Throughout the collection dates, the basal respiration values oscillated in the soil in most of the treatments, with the exception of PS90 and PS180 (Table 1). In the soil of the control treatment, the higher value of CO_2 released by the microbial biomass was observed in February and March 2009, which may be explained by the higher air temperatures at this time of the year. As for the treatments U90 and U180, the greatest value was observed in June 2009. However, a reduction in the basal respiration value from the control soil and U90 treatments was registered in December 2008 and October 2009; whereas in the U180 treatment, the lowest value was observed in October 2008.

In the soil of the treatments with the application of deep pig litter, at one and two times the recommended dose of N, a peak in the release of CO_2 was observed in February 2009, and basal respiration reached its highest values. This can be attributed to the fact that deep pig litter was applied soon after the first soil collection (October 2008). This material has larger particles, with a high C:N ratio and greater surface for bacterial colonization and reaction than in the other treatments (Masse et al., 2005). This delays the microbial attack at first, and the degradation is slower, occurring for a longer period of time, which helps to explain the greater CO_2 emissions after approximately 90 days from the application of organic matter in the soil of these treatments. Moreover, the increase in air temperature in February 2009 may have assisted microbial biomass in the decomposition of the deep pig litter and in the release of CO_2 to the atmosphere (Hoyle et al., 2006). However, with the application of pig slurry, which has low C:N ratio and small-sized particles, the microbial attack, its degradation, and the CO_2 emissions occur rapidly, in a matter of days (Chantigny et al., 2004), explaining in part why there was no oscillation of the basal respiration values in the DL90 and DL180 treatments.

The $q$mic values did not differ among treatments, with mean value of 0.32%. The $q$mic expresses how much of the soil organic C is contained in the microbial biomass, showing the efficiency of the microorganisms in immobilizing it. The $q$mic values generally ranged from 0.2 to 6% of the soil organic C. Low values of the $q$mic may be a result of a stress factor to which the microbiota is subjected to (Jakelaitis et al., 2008), making it unable to use readily available C derived from soil organic matter or organic residue, which may hinder the use of C by the microbial biomass. With the application of increasing doses of swine manure in Oxisol under no-tillage, Balota et al. (2012) verified that the $q$mic ranged from 1.2 to 2% of TOC and that the values increased up to a dose of 60 m³ ha⁻¹; however, a slight decrease was observed in the $q$mic values with the application of a dose of 120 m³ ha⁻¹ of swine manure.

The qCO$_2$ values did not differ among the treatments, with mean value of 18.7 μg C-CO$_2$ g⁻¹ MB-C per hour. This index represents the quantity of C released in the form of CO$_2$ per unit of microbial biomass at a determined time. When high, these values indicate microbial communities in initial stages of development or derived from some type of metabolic stress (Anderson & Domsch, 1993). This shows that the microbial community present in the soil of the assessed treatments was stabilized. Therefore, the C used by the microbial biomass was equally distributed in the treatments, agreeing with the results obtained by Guerrero et al. (2007) with the application of deep pig litter and pig slurry in a limestone soil in Spain.

No significant differences were observed in organic matter contents among the treatments in the soil collected in October 2008 and 2009 (Table 2). It is important to note that even in the control treatment, which did not receive the application of manure throughout the experiment, the organic matter contents remained similar to those of the other treatments with the application of urea (U90 and U180), pig slurry (PS90 and PS180), and deep pig litter (DL90 and DL180). Normally, the TOC content in soil is expected to increase with added organic wastes with high C:N ratio, such as, deep litter. However, this was not evaluated in the present work, which could be explained by the deposition of maize and black oat waste in the soil surface over the years. In a long-term experiment in northeast China, Kou et al. (2012) observed that the treatment under soybean cultivation, with the application of 23 kg ha⁻¹ of horse manure and the removal of crop residues from the soil surface, showed the same levels of TOC in the soil as the control treatment (natural field), where crop residues were kept on the soil surface. According to Manfogoya

Table 2. Organic matter, pH in water, available P and K, exchangeable Ca and Mg in the 0–10 cm layer in a soil with a history of application of urea and swine manure for a period of seven years[1].

Treatment[2]	Organic matter (g kg⁻¹)	pH in water	Available P (mg dm⁻³)	Available K (mg dm⁻³)	Exchangeable Ca (cmol$_c$ dm⁻³)	Exchangeable Mg (cmol$_c$ dm⁻³)
			October 2008			
Control	15.6a	5.5a	9.9b	123.3b	4.1b	1.4b
U90	16.2a	5.3a	6.8b	158.7ab	4.1b	1.4b
U180	15.0a	5.4a	8.4b	125.3b	4.2b	1.4b
PS90	16.8a	5.5a	14.4b	183.3ab	4.3ab	2.1ab
PS180	16.2a	5.1a	22.9b	167.3ab	4.2ab	1.9ab
DL90	16.8a	5.9a	35.3b	279.3ab	5.5ab	1.8ab
DL180	19.1a	6.2a	81.0a	388.0a	6.7a	2.8a
			October 2009			
Control	14.5a	5.8ab	10.0b	128bc	3.3bc	1.2b
U90	15.0a	5.2b	31.0b	182abc	2.4c	1.3b
U180	14.5a	5.4ab	15.6b	111c	2.5c	1.2b
PS90	15.6a	6.0ab	59.4ab	145abc	3.8abc	1.6ab
PS180	15.0a	5.5ab	82.0ab	178abc	3.1bc	1.6ab
DL90	16.2a	6.1ab	135.5a	313ab	4.7ab	1.9ab
DL180	17.4a	6.4a	130.7a	334a	5.4a	2.4a

[1]Mean values followed by equal letters, lowercase in the columns, do not differ by Tukey's test, at 5% probability. [2]U90, mineral nitrogen fertilization at recommended level; U180, mineral nitrogen fertilization two times the recommended level; PS90, pig slurry at recommended level; PS180, pig slurry two times the recommended level; DL90, deep pig litter at recommended level; DL180, deep pig litter two times the recommended level.

et al. (1997), annual additions of approximately 7 to 10 Mg ha^{-1} residues with a high C:N ratio, such as tissue oat, may maintain TOC and, therefore, organic matter over the years. In the control treatment, only 6.5 Mg ha^{-1} of oat residues were added annually, excluding residue inputs for maize.

Soil pH values did not differ among treatments in October 2008. However, in October 2009, the soil pH value was higher in the DL180 treatment than in the U90 treatment and, in general, than in the other treatments. This occurred because deep pig litter is composed by a higher CaCO$_3$ content, derived from animal feed, due to a higher percentage of dry matter in relation to pig slurry (Whalen et al., 2000). In addition, the decomposition of deep pig litter and of the crop residues deposited on the soil surface throughout the years released organic acids in the soil, increasing the adsorption of H$^+$ and Al^{+3}, which may promote the increase in pH values and the reduction of the exchangeable Al^{+3} contents (Lourenzi et al., 2011).

The greatest P content available in the soil collected in October 2008 was observed in the treatment with the application of twice the dose of deep pig litter (DL180) (Table 2). In the soil collected in October 2009, the greatest contents were usually observed in the PS90, PS180, and, especially, the DL90 and DL180 treatments. Higher P contents are available in soils with the application of manure, which already contain the element in their composition, and the concentration is higher in residues with greater dry matter content, such as deep pig litter, which is reflected in higher accumulation in the soil (Guardini et al., 2012).

The higher exchangeable Ca and Mg contents in the soil collected in October 2008 and 2009 were obtained in the PS90, PS180, DL90, and DL180 treatments. This is attributed to the Ca and the Mg contained in the swine manure and in the deep pig litter, which are mineralized when added to the soil.

The principal component analyzis showed, through the relation between principal component 1 (CP1, factor 1) and principal component 2 (CP2, factor 2), that there was a separation between the treatments in the 2008/2009 maize crop season (Figure 1 A) and in the 2009 black oat crop season (Figure 1 B). In the maize cycle, the DL90, DL180, and PS180 treatments were separated by the CP1, which explains 57.09% of the results, and differed from the other treatments (control, U90, U180, and PS90).

The microbiological attributes that most contributed to this separation were the qCO$_2$ and basal respiration; and, within the chemical attributes, the organic matter content, exchangeable Ca, exchangeable Mg, available
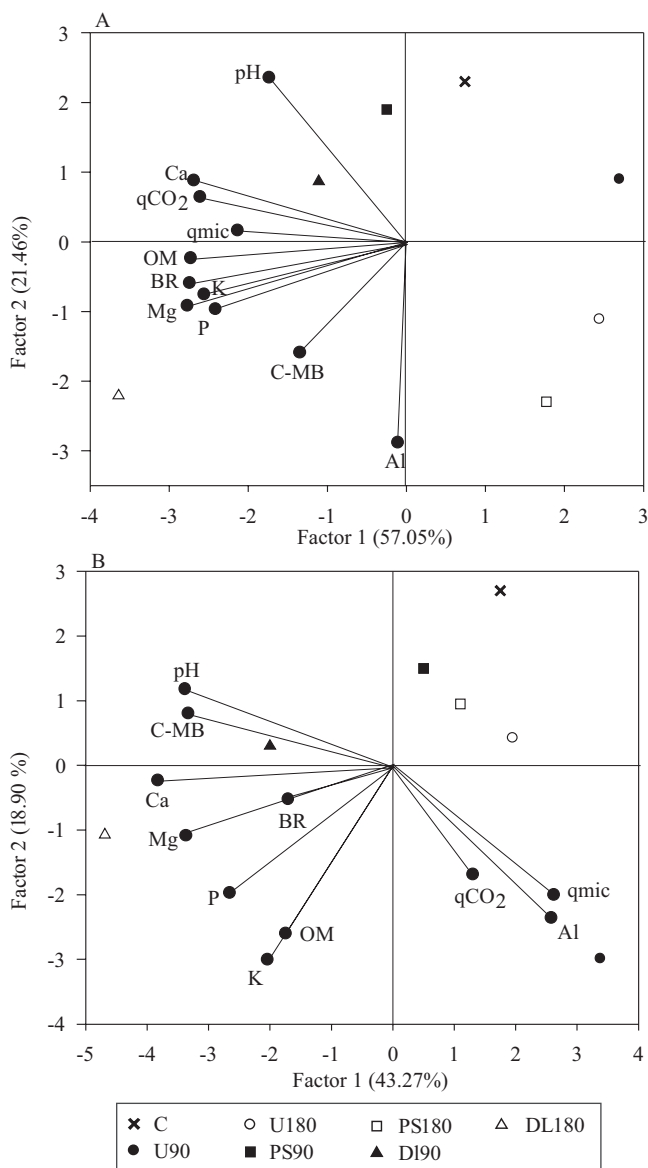

Figure 1. Relation between principal component 1 (factor 1) and principal component 2 (factor 2), during the 2008/2009 maize crop season (A) and the 2009 black oat crop season (B). Chemical and microbiological attributes: pH, pH in water; P, available phosphorus; K, available potassium; Ca, exchangeable calcium; Mg, exchangeable magnesium; OM, organic matter; Al, exchangeable aluminum; MB-C, microbial biomass carbon; BR, basal respiration; qCO$_2$, metabolic quotient; and $q$mic, microbial quotient.

P and K. That reflects a strong relation between soil organic matter and microbial activity, showing the effect caused by the addition of P and K, and by the C:N ratio of the fertilizers added to the soil, which may have affected the results. The microbial biomass needs C to maintain its metabolic activities, and, when there is a greater increase of readily mineralizable N than C, the biomass suffers some type of stress (Delbem et al., 2011), which explains the form of grouping adopted for the assessed treatments.

Factor 2 separates the U90, PS90, and DL90 treatments from the U180, PS180, and DL180 treatments, showing the effect of fertilization on the characteristics of the treatments. Soil microorganisms depend on other nutrients, such as available P and available K, for their survival, which have greater availability in the treatments with the application of twice the dose of manure; therefore, separating the evaluated treatments. This result is due to the fact that in the PCA of the maize crop (Figure 1 A), the biological (basal respiration, $q\mathrm{CO_2}$, and $q$mic) and the chemical attributes (exchangeable Ca, organic matter, available P and K, and exchangeable Mg) are found to be strongly related, showing the effect of large quantities of nutrients on microbial activity.

In the 2009 crop season of black oat, the DL90 and DL180 treatments were separated from the others by factor 1, which explains 43.27% of the results (Figure 1 B). This result is similar to those found in the 2008/2009 maize crop season (Figure 1 A), showing the strong effect of fertilization with deep pig litter on the characteristics of the treatments. The chemical attributes pH, available P and K, organic matter, exchangeable Ca and Mg, and the microbiological attribute MB-C were those that most contributed to these results.

Conclusions

1. The application of once or twice the dose of deep pig litter increases the carbon of the microbial biomass and the basal respiration of the soil.

2. The microbial and metabolic quotients are little affected by the application of swine manure.

3. The application of twice the dose of deep pig litter increases the values of pH in water and the contents of available P, Ca, and Mg in the soil.

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