academicJournals

Vol. 11(49), pp. 5000-5007, 8 December, 2016
DOI: 10.5897/AJAR2016.11777
Article Number: CF11BBD62048
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Chemical and microbiological changes in a sandy soil with pig liquid waste application in Southern Brazil

Matos Maria Aparecida de[1,2], Colozzi Filho Arnaldo[2], Barbosa Graziela Moraes de Cesare[2,3], Caviglione João Henrique[2], Nogueira Marco Antônio[1,4], and Andrade Diva Souza[2,3]

[1]Universidade Estadual de Londrina, Doutorado em Agronomia, Londrina, PR, Brazil..
[2]Instituto Agronomia, Universidade Estadual de Londrina, Brazil.
[3]Mestrado em Agricultura Conservacionista, IAPAR, Brazil.
[4]Embrapa Soja, Cx. Postal 231, 86001-970 Londrina PR, Brazil.

Received 30 September, 2016; Accepted 8 November, 2016

Liquid residue from pig farming contains nutrients that can be used for the fertilization of cultivated soils. The aim of this study was to evaluate chemical and microbiological changes in a sandy soil under pasture with Bermuda Grass (C*ynodon* spp) that received doses of pig liquid waste (PLW). The experiment was conducted in Cianorte-PR, Brazil, in a Typic Hapludox soil with sandy texture. The treatments consisted of 30, 60 and 90 m^3 ha^{-1} yr^{-1} of PLW or chemical fertilizer (CF) applied for two years in a randomized block design, with three replications. Soil samples were taken at 0-10 cm, 10-20 cm and 20-40 cm layers, after three months of the second consecutive application of PLW in the second year, before grazing. PLW increased the concentrations of P, C and K at 10-20 and 20-40 cm soil depth, in addition to increasing the microbial biomass carbon and nitrogen and the population of rhizobia at 0-10 cm, in the treatment with 90 m^3 ha^{-1} yr^{-1}. PLW improved the chemical fertility at deeper soil layers and the biological fertility at 0-10 cm of a sandy soil under pasture.

Key words: Microbial biomass, organic fertilizer, phosphorus, potassium, rhizobia.

INTRODUCTION

Brazil is the fourth largest global pork producer and exporter, and Santa Catarina, Paraná and Rio Grande do Sul are the main producing states (MAPA, 2016), resulting in the production of pig liquid waste (PLW), which use as a source of nutrients can reduce the costs of agricultural production. However, we have to be aware of the possibility of negative effects of PLW on the soil and water quality due to nitrates, phosphates, salts, trace elements such as copper and zinc, xenobiotic compounds such as antibiotics, as well as potentially pathogenic organisms (Plaza et al., 2004; Scherer et al., 2010; Guardini et al., 2012).

Organic matter is a key component of soil fertility, affecting physical, chemical and biological properties. It includes microorganisms that act in the biogeochemical cycles of C, N, P, among others (Paul and Clark, 1996). Microorganisms are widely recognized to perform important processes in biogeochemical cycles and affect the functioning of natural ecosystems. In addition, the microbial community regulates the plant productivity by direct mechanisms, like symbiosis, and indirect effects on plant diversity through their influence on the availability of

*Corresponding Email: diva@iapar.br or 2013divaandrade@gmail.com.

Table 1. Chemical analysis of pig liquid waste (PLW) and soil at 0-20 cm depth before the installation of the experiment.

	DM	N	P	K	Ca	Mg	Al	Al+H	pH	C
PLW [1]	**-- g L^{-1} --**		**------------------------ g kg^{-1} ---------------------**							
	1.7	**31.3**	**31.6**	**71.2**	**39.8**	**17.1**	**-**	**-**	**-**	**-**
Soil [2]			**mg dm^{-3}**		**------------------- $cmol_c$ dm^{-3} ------------**					**g dm^{-3}**
	-	-	9.4	0.10	0.76	0.23	0.13	3.42	4.3	7.48

[1]DM, dry matter determined in the *in natura* residue; average of three repetitions. The nutrients in the PLW represent the total concentration determined in the DM. N determined by Kjeldahl method; P, K, Ca and Mg determined in nitric-perchloric extracts. [2]Nutrients and Al represent the available concentrations. K and P extracted by Melich-1; pH determined in $CaCl_2$ 0.01 mol L^{-1}; Ca and Mg extracted in KCl 1 mol L^{-1}, Al determined in SMP buffer; C: Walkley-Black method.

nutrients (Van Der Heijden et al., 2008). Even in agricultural soils, the microbial community plays a pivotal role (Paul and Clark, 1996).

Soil is usually the final recipient of wastes from human activity, where the biodegradable organic fraction is used as source of carbon and nutrients for microorganisms, which simultaneously carry out mineralization and immobilization processes. Most of the carbon added to the soil through waste returns to the atmosphere as CO_2, while the mineral fraction is immediately made available to plants or is partially immobilized in microbial biomass, which acts as a nutrient reserve (Paul and Clark, 1996). The microbial immobilization is important when the supply of nutrients is greater than the absorption and immobilization performed by plants, as it helps to prevent nutrient losses, as nitrogen by leaching of nitrate and phosphorus by fixation of phosphate, as they are temporarily protected in the microbial cells.

In general, agricultural studies on waste application focuses on agronomic and environmental changes mainly in clayey soil, due to its higher buffering capacity, whereas less frequent studies are conducted on sandy soils. In many cases, however, logistics involved in the transport of residues to be applied in clayey soil is unfeasible, which requires applying it in sandy soils close to the generating source. However, the environmental risks are higher and, therefore, careful monitoring is essential to reduce the risks of environmental degradation.

This work arose from the need to define safe doses of pig liquid waste in sandy soils of agricultural areas near the pig farmers in the state of Paraná, Brazil. At first, the main concern was about the effects of PLW on soil chemical and physical characteristics. Later, micro-biological and biochemical characteristics were included as they are pivotal components of the soil quality. This study aimed to evaluate the effects of PLW application on chemical and microbiological attributes of a sandy soil under pasture, compared with the use of chemical fertilizer.

MATERIALS AND METHODS

The study was developed in the second year of consecutive PLW application, in a field trial conducted in Cianorte, state of Paraná (PR), Brazil (23º 39' 28" S, 52º 42' 47" W), 490 m above sea level, humid subtropical climate (C*fa*) according to Köppen (Figure 1). The soil granulometric fraction is formed by 870 g kg^{-1} of sand, 90 g kg^{-1} of clay and 40 g kg^{-1} of silt, classified as Typic Haplustox (Soil Survey Staff, 2014; Santos, 2013).

The soil acidity was corrected with dolomitic limestone (20% of CaO and 20% of Mg) at 2.2 t ha^{-1} before the installation of the experiment. The four treatments consisted of three applications of PLW at doses of 30, 60 and 90 m^3 ha^{-1} yr^{-1}, and a control with only chemical fertilizer applied on the soil surface under pasture with Bermuda Grass (*Cynodon spp*). The experiment followed a randomized complete block design with three replications and plots of 10 m × 5 m, spaced 2 m apart. Doses of PLW were based on models to estimate the amount for each soil type, taking into account the texture, chemical properties and topography (Castro Filho et al., 2001). PLW was first applied in 2002 twice a year: half in early summer and half in early winter. The compositions of PLW and soil chemical properties at 0-20 cm are shown in Table 1. The control treatment with chemical fertilizer (CF) received 60 kg ha^{-1} of P_2O_5 (Triple Superphosphate), 60 kg ha^{-1} of K_2O (KCl) and the N recommended for group II pastures (eg., Bermuda grass), 80 kg ha^{-1} (urea). The fertilizers were divided in two applications a year, at the same time of PLW application, in the respective plots.

In October 2004, before grazing and three months after the application, 10 sub-samples were taken in each plot at 0-10 cm, 10-20 cm and 20-40 cm of soil depth using a Dutch type auger, and pooled to form a composite sample. For chemical analysis, samples were dried at 60°C and sieved (2 mm). Total organic C (TOC) was obtained by oxidation with $K_2Cr_2O_7$ in acid medium and conversion to the equivalent of organic matter (OM) by a factor of 1.724 (Van Bemmelen factor), pH ($CaCl_2$), H+Al (SMP - Shoemaker, McLean and Pratt - buffer), Ca and Mg (KCl), P and K (Mehlich) (Pavan et al., 1992). Microbiological analyzes were performed in field moist samples collected at 0-10 cm layer. Populations of ammonifying microorganisms (Amo) and rhizobia (Rhiz) nodulating common bean (*Phaseolus vulgaris L*.) were estimated by the most probable number (MPN) technique (Hungria and Araújo, 1994). Carbon (C-MB) and nitrogen (N-MB) in the soil microbial biomass were estimated by the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). The C-MB/TOC ratio was also calculated.

Statistical analysis

The analysis of variance (F-test, P <0.05) was performed using the software SISVAR v. 4.6 (Ferreira, 2011), with MPN data for ammonifyers and rhizobia transformed to Log_{10}. Once the effects of the treatments were observed, the averages were compared by Student's *t*-test (P<0.05). A principal component analysis (PCA) was performed using the results of chemical and microbiological

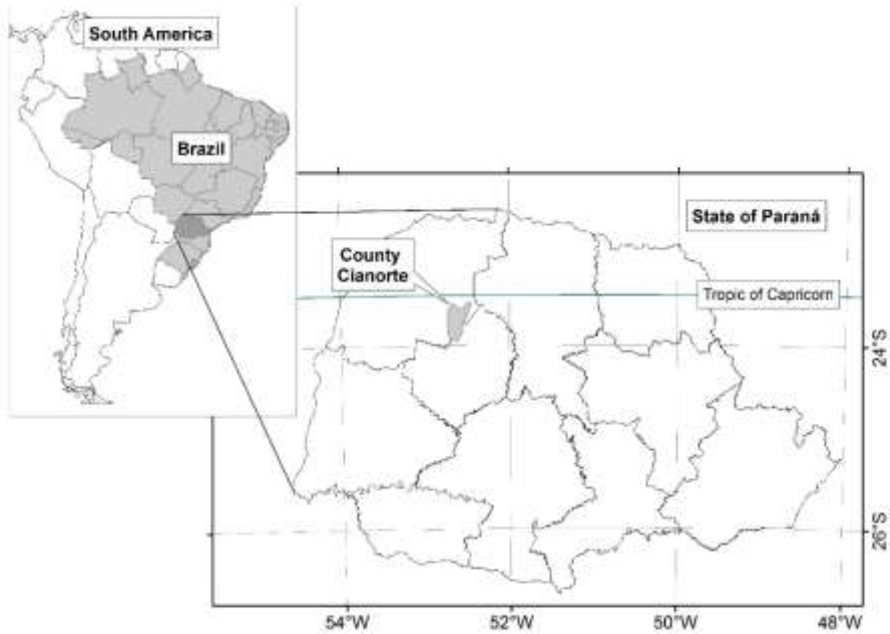

Figure 1. Location of the sampling site in Cianorte, State of Paraná, Southern Brazil.

soil properties at the 0-10 cm layer (Addinsolft, 2009).

RESULTS AND DISCUSSION

Chemical Properties

The highest dose of PLW and the chemical fertilization resulted in higher P concentration in the topsoil, which decreased with depth. At the 0-10 cm layer, applications of PLW in doses from 30 to 90 m^3 ha^{-1} yr^{-1} resulted in lower P concentrations in the soil in relation to the chemical fertilizer. At the 10-20 cm layer, the addition of 90 m^3 ha^{-1} yr^{-1} significantly increased the P levels in relation to the other treatments. At 20-40 cm layer, there was a P increase with the addition of 60 or 90 m^3 ha^{-1} yr^{-1} of PLW compared with chemical fertilizer and 30 m^3 ha^{-1} yr^{-1} of PLW (Figure 2).

Increments of available P in the soil surface by the addition of PLW were observed by Scherer et al. (2010), with successive applications for 20 years from 30 to 60 m^3 ha^{-1} of PLW in Latossolo (Oxisol), Cambissolo (Inceptisols) and Neossolo (Entisols), containing 42, 42 and 36% of clay, respectively. Da Veiga et al. (2012) found smilar results in a Latossolo Vermelho Distroférrico (Hapludox) that received 50, 100 or 200 m^3 ha^{-1} yr^{-1} of PLW for nine years, as well as reported by other authors (Ceretta et al., 2010; Guardini et al., 2012). This result was expected because of the high content of P in the PLW, that is, 31.6 g kg^{-1} (Table 1).

The distribution of P along the soil layers have different mobility depending on the source, that is, CF or PLW. Greater mobility of P was observed in the soil profile in treatments with PLW. Taking the highest dose of PLW as example, the P concentration at 10-20 cm was 44% of the value found at the surface layer, whereas at 20-40 cm it was 37%. In turn, CF provided lower concentrations, both in absolute and relative figures, that is, only 18 and 8%, respectively, at the same layers (Figure 2).

P levels at 0-10 cm in treatments that received the highest dose of PLW or CF are above 42 mg dm^{-3}, considered high according to the Commission of Chemistry and Soil Fertility of the States of Rio Grande do Sul and Santa Catarina (CQFSESCRS, 2004), for a clay content of 9%. According to Ceretta et al. (2010), the soil P added via PLW is predominantly inorganic, especially $H_2PO_4^-$ and HPO_4^{2-}, and considerable amounts of P precipited with Mg^{2+}. This fact favors P losses by runoff and increases the risk of eutrophication of water resources (Scherer et al., 2010; Guardini et al., 2012). This requires special attention to conservation practices on soils intended to receive PLW. Moreover, the doses must be adjusted to the plants' nutrient requirement in order to avoid contamination of deep waters due to leaching.

The content of C and OM at 0-10 cm increased with doses of PLW and did not differ in relation to the CF treatment for doses 60 and 90 m^3 ha^{-1} yr^{-1} (Table 2). At 10-20 cm, the dose 90 m^3 ha^{-1} yr^{-1} significantly increased the C and OM contents in relation to the CF, while at 20-40 cm, the doses 60 and 90 m^3 ha^{-1} yr^{-1} resulted in higher levels. Conversely, the application of pig manure up to 200 m^3 ha^{-1} yr^{-1} for 9 years on an Oxisol, and 150 m^3 ha^{-1} yr^{-1} of pig slurry for 4 years on a Calcic Luvisol did not

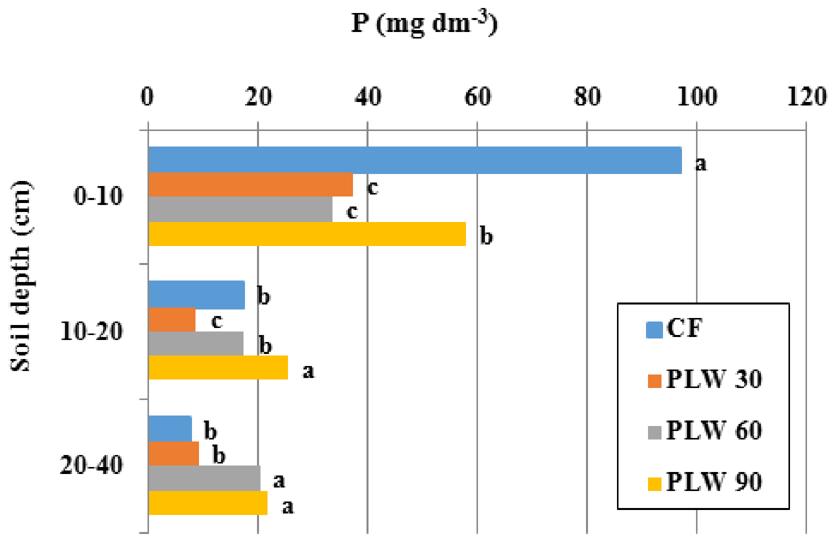

Figure 2. P levels at three depths of a Typic Haplustox soil under pasture, submitted to successive applications of doses of pig liquid waste (PLW) (30, 60 or 90 m^3 ha^{-1} yr^{-1} split in two applications) or chemical fertilizer (CF) (60 kg ha^{-1} of P_2O_5 as Triple Superphosphate, 60 kg ha^{-1} of K_2O as KCl, and 80 kg ha^{-1} of N as urea, split in two applications) in Cianorte-PR, Brazil. Coefficient of variation CV (%): 11.4% (0-10 cm), 17.4 (10-20 cm) and 28.9 (20-40 cm). Same letters in the column do not differ by Student's *t*-test (P <0.05).

Table 2. Chemical characteristics at three soil layers of a Typic Haplustox under pasture, submitted to successive applications of doses of pig liquid waste (PLW) or chemical fertilizer (CF) in Cianorte-PR, Brazil.

Treatments	C	OM	pH	H+Al	Ca	Mg	K
	g kg^{-1}	g kg^{-1}	$CaCl_2$	------------------cmolc dm^{-3} ---------------------			
				Depth: 0-10 cm			
CF[1]	18.3^{ab}	31.5^{ab}	5.5^{a}	2.8^{a}	2.8^{a}	1.6^{ab}	0.17^{c}
30 PLW[2]	15.0^{c}	25.8^{c}	5.3^{a}	2.9^{a}	1.6^{b}	1.0^{c}	0.30^{b}
60 PLW	17.2^{ab}	29.6^{ab}	5.2^{a}	2.9^{a}	1.8^{b}	1.2^{bc}	0.37^{ab}
90 PLW	24.5^{a}	42.1^{a}	5.6^{a}	2.7^{a}	2.5^{a}	1.8^{a}	0.44^{a}
CV (%)	22.7	22.7	5.8	8.2	16.1	15.8	21.0
				Depth: 10-20 cm			
CF	8.1^{b}	14.0^{b}	5.0^{a}	2.9^{a}	1.3^{a}	1.0^{ab}	0.11^{c}
30 PLW	8.1^{b}	13.9^{b}	4.9^{a}	2.9^{a}	1.0^{a}	0.8^{b}	0.17^{bc}
60 PLW	11.7^{ab}	20.2^{ab}	4.9^{a}	3.0^{a}	1.4^{a}	0.9^{ab}	0.19^{ab}
90 PLW	12.6^{a}	21.7^{a}	5.4^{a}	2.7^{a}	1.4^{a}	1.3^{a}	0.25^{a}
CV (%)	20.8	20.8	8.5	12.0	17.0	17.0	22.0
				Depth: 20-40 cm			
CF	6.8^{b}	11.7^{b}	5.0^{a}	2.9^{a}	1.0^{a}	0.6^{a}	0.07^{b}
30 PLW	8.4^{ab}	14.5^{ab}	5.0^{a}	2.9^{a}	1.1^{a}	0.7^{a}	0.11^{ab}
60 PLW	11.2^{a}	19.3^{a}	5.0^{a}	2.9^{a}	1.3^{a}	0.8^{a}	0.15^{a}
90 PLW	10.3^{a}	17.6^{a}	5.1^{a}	2.8^{a}	0.9^{a}	0.8^{a}	0.17^{a}
CV (%)	17.4	17.4	6.8	13.7	23.5	16.3	29.5

Average of three replications. K extracted by Melich-1; pH determined in $CaCl_2$ 0.01 mol L^{-1}; Ca and Mg extracted in KCl 1 mol L^{-1}, Al determined by SMP buffer; C: Walkley-Black and OM: organic matter (carbon × 1.724). Same letters in the column do not differ by Student's *t*-test (P <0.05). [1]Chemical fertilizer (60 kg ha^{-1} of P_2O_5 as Triple Superphosphate, 60 kg ha^{-1} of K_2O as KCl, and 80 kg ha^{-1} of N as urea, split in two applications). [2]Pig liquid waste (30, 60 or 90 m^3 ha^{-1} yr^{-1} split in two applications). CV: coefficient of variation.

Table 3. Microbiological characteristics at 0-10 cm of soil depth in a Typic Haplustox under pasture, submitted to successive applications of doses of pig liquid waste (PLW) and chemical fertilizer (CF) in Cianorte-PR, Brazil.

Treatments	C-MB[1]	N-MB[2]	C-MB/TOC[3]	Amo[4]	Rhiz[5]
	---------- $\mu g\ g^{-1}$ of soil ----------		%	------ Log_{10} MPN g^{-1} -----	
CF[6]	260[b]	52[b]	1.51[a]	7.7[a]	3.5[b]
30 PLW[7]	213[b]	68[b]	1.43[a]	7.8[a]	3.9[b]
60 PLW	262[b]	58[b]	1.51[a]	7.7[a]	2.8[b]
90 PLW	351[a]	87[a]	1.47[a]	7.7[a]	4.7[a]
CV (%)	15.2	12.6	23.0	5.6	16.8

Average of three replications. Same letters in the column do not differ by Student's *t*-test ($P < 0.05$). [1]Carbon microbial biomass; [2]Nitrogen microbial biomass; [3]Ratio between microbial biomass carbon and total soil organic carbon; [4]Population of ammonifying microorganisms; [5]Population of rhizobia nodulating common bean. [6]Chemical fertilizer (60 kg ha^{-1} of P_2O_5 as Triple Superphosphate, 60 kg ha^{-1} of K_2O as KCl, and 80 kg ha^{-1} of N as urea, split in two applications). [7]Pig liquid waste (30, 60 or 90 $m^3\ ha^{-1}\ yr^{-1}$ split in two applications). CV: coefficient of variation.

change the soil OM (Da Veiga et al., 2012; Plaza et al., 2004). Differences in climate, soil type, management and crops may reflect different accumulations of OM in the soil profile due to PLW applications.

No effect of the application of PLW was observed in relation to soil pH and potential acidity at any layer (Table 2). However, Ca increased at the higher dose at 0-10 cm, compared with 30 and 60 mg $ha^{-1}\ yr^{-1}$, without differ from CF. Similar behavior was observed for Mg at 0-10 and 10-20 cm. The use of PLW at doses of 60 and 90 $m^3\ ha^{-1}\ yr^{-1}$ increased K concentration in relation to CF. Although the levels decreased with soil depth, this effect was observed up to 20-40 cm. These results are consistent with several studies that demonstrated higher levels of K at the topsoil with texture ranging from 15 to 42% of clay, and receiving up to 200 $m^3\ ha^{-1}\ yr^{-1}$ of PLW (Scherer et al., 2010; Guardini et al., 2012).

Microbiological characteristics

The application 90 $m^3\ ha^{-1}\ yr^{-1}$ of PLW resulted in significant increases in C-MB, N-MB and the population of rhizobia (Rhiz) nodulating of common bean. However, there was no effect on the C-MB/TOC ratio and in the population of ammonifying microorganisms (Amo) (Table 3).

In the present study, only two years of implementation of the trial were enough to observe increases by 35 and 67% in the C-MB and N-MB in soil, which reached 351 µg C g^{-1} and 87 µg N g^{-1}, respectively. This increase was probably due to the increased content of organic matter caused by the application of PLW, as the C-MB/TOC ratio remained unchanged, ranging from 1.43 to 1.51%, with no significant differences between treatments. Increases in C-MB and N-MB were observed in Neossolo with 19% clay after four years of applications (Plaza et al., 2004). Moreover, the application of PLW also contains microbial biomass that can be incorporated into the soil (Plaza et al., 2007; Zornoza et al., 2013).

These results for microbial biomass are in agreement with works on applications of PLW in soil, where values for C-MB are between ~37 and ~570 µg g^{-1} (Deng et al., 2006; Zornoza et al., 2013) and N-MB ranges between ~30 to ~120 µg g^{-1} (Deng et al., 2006). Differences in values in soil microbial biomass depend on the PLW and on soil and environmental characteristics, such as the contents of C and N in manure and in soil, temperature and moisture regimes, etc.

In general, the levels of soil organic matter resulting from application of PLW did not differ significantly between treatments with and without PLW (Hernandez et al., 2007; Plaza et al., 2007). Slight variations in soil organic matter in sandy soil and in clay soil receiving 90-300 $m^3\ ha^{-1}\ yr^{-1}$ was enough to stimulate the soil microbial carbon by 36 to 68%, between 340 and 572 µg C-MB g^{-1}, in periods ranging from 14 days to 5 years (Hernandez et al., 2007; Plaza et al., 2007). It is possible that effects of PLW application are more noticeable in soils with low initial levels of organic matter as in the present study (12.9 g dm^{-3} before the applications). Studies of Zornoza et al. (2013) showed that soils receiving the same dose of PLW but with different contents of organic matter (4.1 or 21.1 g kg^{-1}) resulted in similar levels of C-MB. However, in soils with higher content of organic matter (57.0 g kg^{-1}), Morales et al. (2016) found no effect even after the application of 209 kg N as PLW $ha^{-1}\ yr^{-1}$ for fourteen years. Thus, in soils with low initial content of organic matter, organic carbon added via pig manure provides a source of energy and organic and inorganic nutrients to the soil microorganisms and boosts the microbial biomass.

In this study, microbial biomass was a sensitive biological indicator with significant responses to organic matter changes in the soil, as a result of C and N inputs with the application of PLW in a short period. However, these effects may be temporary due to the presence of readily degradable compounds, associated to a lower C/N ratio and low stability of the organic matter added (Guerrero et al., 2007; Hernandez et al., 2007). In this regard, relatively high doses are required to keep the MB values significantly higher than the control soil (Guerrero et al., 2007) and depend on the continuous C input over time.

Compared with the treatment with chemical fertilizer, the application of 90 m^3 ha^{-1} of PLW stimulated almost 16 times the population of rhizobia that nodulates common bean living saprophytically in the soil (Table 3). Although growing in a soil without a legume host, such microorganisms also act in the biogeochemical cycles, besides being plant growth promoters. Thus, its increase in soil is considered beneficial, although not directly benefiting the pasture with *Cynodon* sp.

Similar populations of rhizobia in soil that received wastes as organic fertilizers have been found (Zengeni et al., 2006; Kimiti and Odee, 2010). In general, the cowpea cultivation in sandy soil that received animal manure, application of P and a combination of both resulted in a population of rhizobia ranging from 2.7 to 4.3 Log_{10} MPN g^{-1} (Kimiti and Odee, 2010). Increases of 100 times in the rhizobia population were observed soils with 20 to 30% clay, from 10^1 CFU g^{-1} in the control to 10^3 CFU g^{-1} in the treatment that received 10 t ha^{-1} of manure and cultivated with soybean for two years (Zengeni et al., 2006). The application of PLW provided a favorable environment for the population of rhizobia due to increases in soil organic matter (Plaza et al., 2007), in addition to the supply of nutrients and improvement of soil fertility (Zengeni et al., 2006; Kimiti and Odee, 2010).

Although moderate doses of PLW may favor the soil chemical and biological fertility, excessive doses of PLW for long periods may accumulate high concentrations of heavy metals in the soil, such as copper, zinc and other elements. Therefore, there is a great environmental concern about the disposal of PLW in soil that might lead to accumulation of metals and other elements, and could have negative impacts on soil microorganisms. Excess of metals may impair the activity, the survival, growth and N fixation capacity of rhizobia, and change populations in relation to metal tolerance (Chaudri et al., 1992; Tindwa et al., 2014).

Regarding metal tolerance, strains of *Rhizobium leguminosarum* biovar *trifolii* isolated from root nodules of clover grown in sandy soil contaminated with heavy metals (Zn, Cu, Ni and Cd) were more tolerant to those metals, thus allowing their survival, but lost their ability to fix nitrogen with *Trifolium repens*. On the other hand, strains originating from non-contaminated soil can fix nitrogen, but due to intolerance to metals, they are not able to survive in contaminated soil (Chaudri et al., 1992).

The absence of adverse effects of a higher dose of PLW on the population of rhizobia in the soil of the present work probably indicates low input of toxic elements because of the short period of two years and four applications of PLW on soil under pasture.

Furthermore, it should be considered that the presence of vegetation provides greater protection to rhizobia, reducing the availability of metals and consequently its toxicity (Renella et al., 2007).

In theory, the physiology of microbial groups related to the nitrogen cycle such as ammonifyers and nitrobacteria are responsible for controlling ammonification and nitrification processes, respectively (Jiang et al., 2015). The ammonifying microorganisms are represented by various prokaryotes, algae (Cyanophyceae) and fungi using nitrogenous organic compounds as source of carbon and N, releasing ammonia, which in part volatilizes to the atmosphere. In the soil solution, ammonia is converted into ammonium ion in the presence of H^+, which may be adsorbed on negative charges of soil or converted into amino acids by plants and microorganisms. It may be microbiologically oxidized to nitrate in the nitrification process and thereafter follow denitrification (Paul and Clark, 1996). In the present work, the density of ammonifyers was not affected by the treatments, averaging 7.7 Log_{10} MPN g^{-1} (Table 3). The lack of effect of treatments on the population of ammonifyers is probably related to the low C/N ratio of PLW. As it is a source of easily degradable carbon, we must consider the time between the PLW application and the soil sampling. The elapsing time might have been enough to recover any eventually transient effect on the ammonifying population.

Ammonifying microorganisms play important role in the soil biological fertility and its capacity to supply nitrogen to the soil microbiota and plants (Acea and Carballas, 1996). Therefore, management practices that maintain the population of ammonifying microorganisms is important to keep the soil health and its role in the nitrogen cycling (Aparna et al., 2014). As seen in the present work, the application of PLW was not harmful to the soil ammonifying microorganisms.

Principal component analysis

Principal component analysis (PCA) correlates the set of chemical and microbiological soil attributes to treatments (Figure 3), where factors 1 and 2 accounted for 86% of the total variability.

The factor 1 was more related to the variables C, pH, H+Al, Ca, Mg, N-MB and the rhizobial population, explaining 55% of the variability. The factor 2 was more associated to the variables P, K, C-MB, C-MB/ TOC and the population of ammonifyers, explaining 31% (Figure 3). The factor 1 was associated with the doses of PLW 30 (24%), 60 (16%) and 90 (56%) m^3 ha^{-1} yr^{-1}, whereas the factor 2 was associated with the CF (54%).

The CF treatment influenced the values of P, Ca, Mg and the C-MB/TOC ratio. The dose of 90 m^3 ha^{-1} yr^{-1} was more related to the variables C, pH, K, C-MB, N-MB and the rhizobia population. The dose of 30 m^3 h^{-1} yr^{-1} showed effects on the population of ammonifying microorganisms, whereas the dose 60 m^3 h^{-1} yr^{-1} only

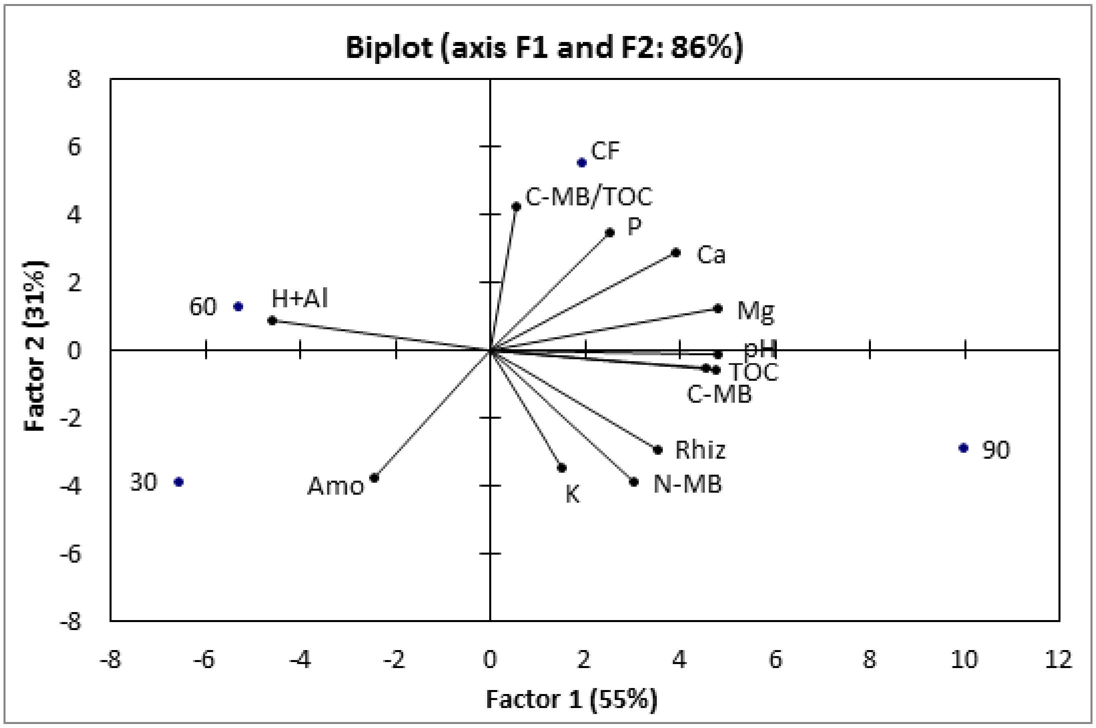

Figure 3. Principal component analysis based on eight chemical and five microbiological attributes at 0-10 cm of depth in a Typic Haplustox soil under pasture, submitted to application of three doses of pig liquid waste (PLW) and chemical fertilizer (CF) in Cianorte-PR, Brazil. CF: Chemical fertilizer (60 kg ha^{-1} of P_2O_5 as Triple Superphosphate, 60 kg ha^{-1} of K_2O as KCl, and 80 kg ha^{-1} of N as urea, split in two applications); PLW: Pig liquid waste (30, 60 or 90 m^3 ha^{-1} yr^{-1} split in two applications); C-MB: microbial biomass carbon; N-MB nitrogen microbial biomass; TOC: total organic carbon; C-MB/TOC (%): carbon microbial biomass in relation to the total organic carbon; Amo: ammonifying microorganisms; Rhiz: *Rhizobium* sp.

presented a relation with the potential acidity. The dose 90 m^3 ha^{-1} yr^{-1} was associated with microbiological variables, K, total carbon and pH, which are related to the availability of nutrients in the soil. Soil pH is directly correlated with the availability of nutrients and also influences the microbial activity.

Conclusions

Applications of pig liquid waste in soil increase C, P, and K levels, especially at deeper soil layers compared with chemical fertilization.

Applications of pig liquid waste for two years change nutrients and soil microbiota, contributing to improve the chemical and microbiological properties of a sandy Typic Hapludox soil under pasture.

Overall, the absence of negative effects of pig liquid waste on the soil populations of rhizobia and ammonifying microorganisms suggests low concentration of available harmful elements in the residue.

Finally, the application of pig liquid waste to pastures on sandy soils with original low levels of soil organic matter seems promising as final destination of residues and improvement of soil chemical and biological fertility. However, the doses must be criteriously established and the soil monitoring must be done to minimize risks of soil and water degradation.

Conflict of interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The study was partially supported by project Parana 12 Meses (32.02.53.00.00-4). D.S. Andrade and M.A. Nogueira are Fundação Araucária/Paraná and CNPq scholars, respectively. Special thanks to Dr. Celso Castro Filho (in memoriam) who installed the field experiment.

REFERENCES

Acea MJ, Carballas T (1996). Microbial response to organic amendments in a forest soil. Bioresource Technol. 57(2):193-199.
Addinsolft SARL (2009). XLSTAT for Windows.

Aparna K, Pasha MA, Rao DLN, Krishnaraj PU (2014). Organic amendments as ecosystem engineers: Microbial, biochemical and genomic evidence of soil health improvement in a tropical arid zone field site. Ecol. Eng. 71:268-277.

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17:837-842.

Castro Filho C, Caviglione JH, Costa MAT (2001). Estimativa Preliminar do volume de chorume suíno a ser aplicado nos solos do Paraná. Londrina-PR. Anais. Congresso Brasileiro de Ciência Do Solo. Londrina. Anais, Londrina-PR, SBCS.

Ceretta CA, Lorensini F, Brunetto G, Girotto E, Gatiboni LC, Lourenzi CR, Tiecher TL, De Conti L, Trentin G, Miotto A (2010). Phosphorus fractions in soil after sucessive pig slurry applications in no-tillage system. Pesqui. Agropec. Bras. 45(6):593-602.

Chaudri AM, McGrath SP, Giller KE (1992). Metal tolerance of isolates of *Rhizobium leguminosarum* biovar *trifolii* from soil contaminated by past applications of sewage sludge. Soil Biol. Bioch. 24(2):83-88.

CQFSESCRS (2004). Comissão de Química e Fertilidade do Solo dos Estados de Santa Catarina e Rio Grande do Sul. Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. (S.B. de C. do S.C. de Q. e F. do Solo, Ed.) (10th ed.). Porto Alegre. Brazil. Available at: <http://www.sbcs-rs.org.br/docs/manual_de_adubacao _2004_versao_internet.pdf>

Da Veiga M, Pandolfo CM, Balbinot Junior AA, Spagnollo E (2012). Chemical attributes of a hapludox soil after nine years of pig slurry application. Pesqu. Agropec. Bras. 47(12):1766-1773.

Deng SP, Parham JA, Hattey JA, Babu D (2006). Animal manure and anhydrous ammonia amendment alter microbial carbon use efficiency, microbial biomass, and activities of dehydrogenase and amidohydrolases in semiarid agroecosystems. Appl. Soil Ecol. 33(3):258-268.

Ferreira DF (2011). Sisvar: um sistema computacional de análise estatística. Ciênc. Agrotec. 35(6):1049-1042.

Guardini R, Comin JJ, dos Santos DR, Gatiboni LC, Tiecher T, Schmitt D, Bender MA, Belli Filho P, Oliveira PAV, Brunetto G (2012). Phosphorus accumulation and pollution potential in a Hapludult fertilized with pig manure. Rev. Bras. Ciênc. Solo. 36(4):1333-1342.

Guerrero C, Moral R, Gomez I, Zornoza R, Arcenegui V (2007). Microbial biomass and activity of an agricultural soil amended with the solid phase of pig slurries. Bioresour. Technol. 98(17):3259-3264.

Hernandez D, Fernandez JM, Plaza C, Polo A (2007). Water-soluble organic matter and biological activity of a degraded soil amended with pig slurry. Sci. Total Environ. 378(1-2):101-103.

Hungria M, Araújo RS (1994). Manual de Métodos Empregados em Estudos de Microbiologia Agrícola. Brasilia, DF, Brazil. Embrapa-Serviço de Produção e Informação 542p.

Jiang J, Liu X, Huang Y, Huang H (2015). Inoculation with nitrogen turnover bacterial agent appropriately increasing nitrogen and promoting maturity in pig manure composting. Waste Manage. 39:78-85.

Kimiti JM, Odee DW (2010). Integrated soil fertility management enhances population and effectiveness of indigenous cowpea rhizobia in semi-arid eastern Kenya. Appl. Soil Ecol. 45(3):304-309.

MAPA-Ministério da Agricultura, P e AS (2016). Suínos. Available at: http://www.agricultura.gov.br/animal/especies/suinos.

Morales D, Oliveira MP, Taffe BL, Comin J, Soares CR, Lovato P (2016). Response of soil microbiota to nine-year application of swine manure and urea. Ciênc. Rural 46(2):260-266.

Paul EA, Clark FE (1996). Soil microbiology and biochemistry. San Diego, Calif., USA: Academic Press 2:340.

Pavan MA, Bloch MF, Zempulski HDC, Miyazawa M, Zocoler DC (1992). Manual de análise química do solo e controle de qualidade. Londrina, Brazil, IAPAR-circular 76.

Plaza C, Garcia-Gil JC, Polo A (2007). Microbial activity in pig slurry-amended soils under aerobic incubation. Biodegradation 18:159-165.

Plaza C, Hernandez D, Garcia-Gil JC, Polo A (2004). Microbial activity in pig slurry-amended soils under semiarid conditions. Soil Biol. Biochem. 36(10):1577-1585.

Renella G, Chaudri AM, Falloon CM, Landi L, Nannipieri P, Brookes PC (2007). Effects of Cd, Zn, or both on soil microbial biomass and activity in a clay loam soil. Biol. Fertil. Soils. 43(6):751-758.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Cunha TJF, Oliveira JB (2013). Sistema Brasileiro de Classificação de Solos. Brasília, DF: EMBRAPA - Centro Nacional de Pesquisa de Solos. 3:353.

Scherer EE, Nesi CN, Massotti Z (2010). Atributos químicos do solo influenciados por sucessivas aplicações de dejetos suínos em áreas agrícolas de Santa Catarina. Rev. Bras. Cienc. Solo 34:1375-1383.

Soil Survey Staff (2014). Illustrated guide to soil taxonomy. U.S. Department of Agriculture, Natural Resources Conservation Service, National Soil Surveyn Center, Lincoln, Nebraska 552 p.

Tindwa H, Semu E, Msumali GP (2014). Effects of elevated copper levels on biological nitrogen fixation and occurrence of rhizobia in a Tanzanian coffee-cropped soil. J. Agric. Sci. Applications. 3(1):13-19.

Vance ED, Brookes PC, Jenkinson DS (1987). An extraction method for measuring microbial biomass C. Soil Biol. Biochem. 19(6):703-707.

Van Der Heijden MGA, Bardgett RD, Van Straalen NM (2008). The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11(3):296-310.

Zengeni R, Mpepereki S, Giller KE (2006). Manure and soil properties affect survival and persistence of soyabean nodulating rhizobia in smallholder soils of Zimbabwe. Appl. Soil Ecol. 32(2):232-242.

Zornoza R, Faz A, Carmona DM, Acosta JA, Martínez-Martínez S, Vreng A (2013). Carbon mineralization, microbial activity and metal dynamics in tailing ponds amended with pig slurry and marble waste. Chemosphere 90(10):2606-2613.