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LONG-TERM EFFECTS OF SWINE WASTEWATER AND MINERAL FERTILIZER ASSOCIATION ON SOIL MICROBIOTA

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ABSTRACT: Swine wastewater (SW) application in agricultural soils may affect its microbial community in a long term. The objective of this study was to evaluate prospective changes in soil bacterial community after eight years continuous application of swine wastewater. The wastewater doses tested were 0; 100; 200 and 300 m^3 ha^{-1}, being applied from the beginning of the experiment and with or without recommended fertilization. Three soil samples were taken from each plot for determinations of basal respiration, microbial biomass and metabolic quotient. We also performed DGGE analysis and made a correlation between soil chemical conditions and microbial activity. Microbial community underwent significant structural changes from swine wastewater applications. Higher SW doses (200 and 300 m^3 ha^{-1}) influenced significantly ($p < 0.05$) and benefitted certain bacteria groups.

KEYWORDS: pig manure, soil bacterial diversity, water reuse, soil microbial biomass, basal respiration.

EFEITOS DE LONGO PRAZO DA ASSOCIAÇÃO DE ÁGUA RESIDUÁRIA DA SUINOCULTURA E ADUBAÇÃO MINERAL SOBRE A MICROBIOTA DO SOLO

RESUMO: A aplicação de água residuária de suinocultura (ARS) em solos agrícolas pode gerar impactos na comunidade microbiana do solo, quando realizada em longo prazo. O objetivo deste trabalho foi avaliar possíveis alterações na comunidade bacteriana do solo após oito anos de aplicação contínua de água residuária de suinocultura. As doses de água residuária (0; 100; 200 e 300 m^3 ha^{-1}), aplicadas desde o início do experimento, foram combinadas com a presença ou a ausência de adubação recomendada. No solo, foram feitas três coletas em cada parcela para a determinação da respiração basal, biomassa microbiana e quociente metabólico, análise de DGGE, correlação entre as condições químicas do solo e a atividade microbiana na comunidade bacteriana do solo. Alterações significativas na estrutura da comunidade microbiana do solo, com a aplicação de água residuária de suinocultura, foram observadas. Doses maiores de ARS (200 e 300 m^3 ha^{-1}) influenciaram de forma significativa ($p \leq 0,05$) e privilegiaram a permanência de alguns grupos específicos de bactérias.

PALAVRAS-CHAVE: dejetos suínos, diversidade bacteriana do solo, reúso de água, biomassa microbiana do solo, respiração basal.

INTRODUCTION

Population growth and climate changes in the recent decades have prompted the search for new methods and techniques of minimizing consumption and optimizing use of water and other

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natural resources. Sustainable alternatives are needed, mainly in agriculture where water demand is much higher compared to industrial and urban sectors. Livestock wastewater application in farm irrigation contributes to the conservation of water resources, the long-term improvement of soil quality and the reduction of fertilization costs (SAMPAIO et al., 2010; MEDEIROS et al., 2011; MAGGI et al., 2013; BATISTA et al., 2014).

However, like any unconsolidated technique, the use of wastewater of animal origin may generate adverse effects on the physical, chemical and biological composition of the environment. Among the aforementioned factors, the biological one stands out in the context of animal and human health. Swine have rich gastrointestinal tract microbiota, and part of it is released into the environment through feces. Studies associated with microorganisms from these residues and the soil are complex and multifactorial, since the size, the activity, the structure and the diversity of microbial communities in the soil are affected by various biotic and abiotic factors (ROUSK et al. 2010; CUNHA et al., 2012).

The disposal of swine wastewater (SW) in agricultural soils can change the soil microbial community. Even with the decrease of most intestinal origin and pathogenic microorganisms, in short time, small subpopulations can adapt to the soil characteristics, and through ecological relations, among the various living organisms in the soil (ROGERS & SMITH, 2007; SANTOS & MEURER, 2012).

Recently, studies have evaluated the influence of the application of wastewater from different sources in the soil microbial community. The main works are limited to the provision of wastewater of human origin (SOUZA et al. 2011; SIMÕES et al, 2013; HEINZE et al, 2014.). The impact of SW application on soil microbiota is little studied and is restricted to quantitative assessment (FINOCCHIARO & KREMER, 2010; SILVA et al., 2010; LOURENTE et al., 2011; VIEIRA et al., 2011). On the other hand, studies with a wider range of quantitative and qualitative parameters are observed on meso- and macrofaunal soil populations (TESSARO et al., 2013; ALMEIDA et al., 2013). These studies demonstrate concrete indications that SW can provide great diversity of microorganisms in agricultural soils, changing the soil microbial communities significantly.

Studies with greater quantitative and qualitative extent require complex techniques in the evaluation of soil microbial composition. In this sense, basal respiration, microbial biomass and molecular tools such as Electrophoresis in Denaturing Gradient Gel (DGGE) stand out (MILTNER et al. 2011; VAN ELSAS et al, 2012). Basal respiration assesses the metabolic activity and the environmental stress of soil microorganisms. Microbial biomass allows for the observation of quantitative changes in the microbial community, and is related to the natural decomposition and nutrient cycling. While the molecular DGGE analysis is useful in identifying changes in the composition of soil microbial communities.

The application of SW associated with mineral fertilizer in agriculture can change the soil microbial community. This study aimed to evaluate the quantitative effects (basal respiration, microbial biomass and metabolic quotient) and qualitative (DGGE, correlation between soil chemical conditions and microbial activity) in the soil bacterial community from the SW application associated with mineral fertilization.

MATERIAL AND METHODS

Characterization of the experimental area and sample collection

The study was conducted in an experimental area in the city of Cascavel, Paraná, located at 24º 48’ South Latitude and 53º 26' West Longitude and altitude of 760m. The climate is humid subtropical (*Cfa*), with an average annual temperature of 21°C. It presents higher temperatures in February, with an average of 28.5 °C and lower in July, with an average of 13.3 °C. The average annual rainfall is 1,900 mm in the months from December to February with longer period of rain and range from 500 to 600 mm (IAPAR, 2014).

The experimental area, organized into 24 plots of 1.60 m^2 has a history of eight years of SW application. The treatments in factorial scheme associate SW and mineral fertilizer (MF); they were installed in 2006 and used until the data collection in 2013, totaling 18 production cycles. During this period, rotation of soybean-corn-oat crops was used annually. SW was applied once prior to sowing. From the 1st to the 6th production cycle, SW was collected in a stabilization lagoon, from the 7th to the 13th cycle, it was collected at the biodigester exit and from the 14th to the 18th cycle, prior to the biodigester entry. The effect of MF (N: P: K) in the formulation 0:20:20, respectively, was evaluated simultaneously with the application of SW.

Treatments were determined by the combination of SW levels (0, 100, 200 and 300 $m^3\ h^{-1}$) and MF [Absence (A) and Presence (P) with three replicates]. Treatments were named as follows: A-SW-0; A-SW-100; A-SW-200; A-SW-300; P-SW-0; P-SW-100; P-SW-200; P-SW-300.

After the 18th production cycle, during the soybean crop, three samples of 200 g of soil at 0-20 cm depth were collected in each plot. The collection was carried out 10 days after application of SW with the help of the Dutch auger. Samples were homogenized and packaged in plastic bags; identified, refrigerated and sent for analysis. Analyses of soil microbial biomass (SMB) and basal respiration (BR) were performed in IAPAR-Londrina and the analysis of soil microbial communities by DGGE technique in EMBRAPA-Londrina. The physical and chemical parameters were determined in Agrilab-Botucatu laboratory.

Soil chemical and physical parameters

The soil of the experimental area was classified as Oxisol (EMBRAPA, 2006). Soil samples were collected and analyzed for each lysimeter according RAIJ et al. (2001). The results obtained were used to characterize the soil of the study area.

The mean values of the soil chemical characteristics are given in Table 1 for each treatment.

TABLE 1. Soil physico-chemical characterization for the evaluated plots.

	Water		g dm^{-3}				mg dm^{-3}		mmolc dm^{-3}
Plots	pH	OM	NO_3	N inorg	N	N org	P	S	CTC
A-SW-0	7.1	30.6	26.60	41.77	931.00	889.00	3.91	1.23	128.67
P-SW-0	7.2	29.0	28.93	46.43	1113.00	1066.67	12.31	4.20	122.80
A-SW-100	7.1	36.4	46.43	61.60	1183.00	1121.33	8.13	4.20	134.76
P-SW-100;	6.5	29.7	58.10	95.43	1022.23	926.67	9.23	10.50	111.50
A-SW-200	6.7	33.5	41.77	61.60	1365.00	1303.33	11.62	3.38	118.30
P-SW-200	6.3	30.0	30.10	42.93	1012.67	952.67	23.17	12.63	114.85
A-SW-300	6.5	33.8	34.77	47.60	1003.33	955.33	23.36	5.89	125.57
P-SW-300	6.5	32.6	2.43	46.43	1110.67	1064.00	34.83	11.51	124.76

	mmolc dm^{-3}						mg dm^{-3}				
Plots	Al	H+Al	Na	Ca	Mg	K	Mn	Cu	Fe	Zn	B
A-SW-0	0.00	11.99	4.25	73.48	42.86	0.34	84.67	4.53	31.90	2.91	0.14
P-SW-0	0.00	4.16	4.25	74.46	42.57	1.61	57.67	4.36	12.86	2.89	0.15
A-SW-100	0.00	10.62	4.50	77.64	45.85	0.64	68.33	4.98	11.43	18.93	0.21
P-SW-100;	1.04	15.23	5.00	62.51	31.74	2.02	43.00	3.69	11.90	8.20	0.21
A-SW-200	0.00	14.16	3.88	65.98	36.32	1.84	52.33	7.47	26.19	23.87	0.32
P-SW-200	3.33	28.01	4.13	56.24	27.75	2.85	58.33	7.24	20.95	17.29	0.33
A-SW-300	0.63	20.81	4.50	66.67	35.26	2.84	60.33	7.29	12.86	27.33	0.39
P-SW-300	0.83	23.83	4.38	64.23	33.49	3.21	55.33	7.96	11.90	25.71	0.39

Basal respiration (BR), Soil microbial biomass (SMB) and Metabolic Ratio (qCO_2).

The evaluation of BR was determined from the release of CO_2 in the non-fumigated samples after 10 days of incubation (JENKINSON & POWLSON, 1976).

The determination of the SMB C content was performed by extracting fumigation due to the recent application of organic matter present in the SW (VANCE et al., 1987). The SMB C was calculated by subtracting the fumigated and non-fumigated samples, using a correction factor of 0.33. Values were expressed in micrograms of SMB C per dry gram of soil.

The metabolic quotient (qCO_2) was determined by the ratio between the BR and the SMB (ANDERSON & DOMSH, 2010).

Diversity of the soil bacterial community

The extraction of total soil DNA was performed in samples of 0.25 g of soil using the *Ultraclean Soil DNA Kit*, according to the manufacturer's specifications (Mobio Laboratories, Inc., California, USA). Two amplification reactions of the soil bacterial DNA were performed. The first amplifying the region 16S rDNA and the second amplifies the internal hypervariable region V3.

The first reaction consisted on the amplification of the soil bacteria DNA with the fD1 universal primer (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3), which codes the region of 16S rDNA with about 1500 bp (WEISBURG et al., 1991). The amplification reaction was carried out according to SILVA et al. (2013).

The second amplification reaction occurred using 1 µL (~20 ng) of the product from the first amplification reaction as template. The primers used were F-968 (5´CGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGGAACGCGAAGAACCTTAC-3), with the GC clamp and R-1401 5´-GCGTGTGTACAAGACCC-3) (NÜBEL et al., 1996). The amplification reaction was carried out according to SILVA et al. (2013). Gels were stained with ethidium bromide (0.3 µg m L^{-1}) and visualized with UV light.

The analysis of bacterial community by DGGE was performed by the D-Code System (Bio-Rad, Hercules, CA, USA) using 25 µL of the final PCR product for each replicate of each treatment. It used 6% polyacrylamide gel (w/ v) with gradient ranging from 35 to 55% of denaturing solution (7 M urea and 40% (v/ v) formamide). The electrophoresis was performed with 0.5X TAE Buffer, first with a pre-run at 60 °C and 100 V for 1 hour and then at a constant voltage of 100 V for 16 hours. Then the gels were stained with ethidium bromide and photographed under UV light.

A standard mixture of soil bacteria was prepared in laboratory, which consisted of equal proportions of *Klebsiella* sp, *Enterobacter aerogenes, Bacillus subtilis, Pantoea* sp., *Enterobacter* sp., *Paenibacillus polimyxa, Klebsiella variicola* and *Rhizobium*. The bacterial mixture was applied in three columns of each gel.

All images found were normalized by band identification of the standard bacteria mix in the reference columns, keeping the positions of the reference bands. After this stage, via SPADE (Species Prediction and Diversity Estimation) the DGGE images were transformed in biodiversity indexes of microorganisms: indexes Shannon (H), *Evenness* (E) and abundance (ACE) (Abundance-based Coverage Estimator).

Data analysis

Considering that the experimental area has as factors SW (0, 100, 200 and 300 m^3 ha^{-1}) and MF [Absence (A) and presence (P)], which provided a design in a 4x2 factorial scheme, with three blocks, exploratory analysis of quantitative parameters were performed (SMB, BR and qCO_2), by the Shapiro-Wilk normality test. Later, the data were submitted to variance analysis by the Tukey test at 5% probability.

For qualitative parameters from the DGGE results and consequent determination of biodiversity indexes (H, E and ACE), we used similar analysis as applied for quantitative variables, i.e. a 4x2 factorial scheme of the field experiment.

The UPGMA algorithm and the Jaccard coefficient (J) with a 5% tolerance were used in similarity analysis between band profiles by DGGE. In order to evaluate the soil parameters that most influence the microbial community, Spearman correlations were estimated among the parameters measured with the plot soil chemical properties.

RESULTS AND DISCUSSION

Soil bacterial communities were evaluated for quantitative factors such as BR, SMB and qCO_2 and qualitative as biodiversity by the DGGE technique, correlation between the soil physical and chemical parameters and the bacterial community and indexes of Shannon (H), Evenness (E) and abundance (ACE). Results show that the application of SW changed the microbial activity and the bacterial diversity of soil qualitatively and quantitatively, according to the dose applied.

Quantitative factors (BR, SMB and qCO_2)

It is observed in Table 2 that only the SW factor showed effects, with high significance levels in BR, SMB and qCO_2. The SW dose of 100 m^3 ha^{-1} stood out with higher values of BR and qCO_2, which represent the live dynamic by microorganisms' respiration. SMB estimated from the soil C was increasing in the face of SW doses, following a similar pattern to carbon (C) accumulation over the eight years with SW application in all 18-production cycles of the experimental area.

TABLE 2. Variance analysis (p-value) and mean test for the qualitative parameters of soil microorganisms in relation to the SW and MF.

SW and MF	BR	SMB	qCO_2
0	2.078 c	243.133 b	8.633 c
100	4.976 a	350.017 a	14.221 a
200	4.369 b	384.817 a	11.408 b
300	4.356 b	378.083 a	11.610 b
A	3.968 A	347.179 A	11.218 A
P	3.921 A	330.179 A	11.718 A
SW	0.000*	0.000*	0.000*
MF	0.741	0.225	0.316
SWxMF	0.238	0.330	0.409
CV (%)	8.71	9.35	10.31

Means followed by the same lowercase letters in the column show no significant differences in the Tukey test at 5% significance level. Means followed by the same capital letters in the column show no significant differences in the Tukey test at 5% significance level.

* means with significant effects on the Tukey test at 5% significance

BR: Basal Respiration; SMB: Microbial Biomass; qCO_2: Metabolic Ratio

A: absence of mineral fertilization; P: presence of mineral fertilizer; CV: coefficient of variation; BR expressed in µg C -CO_2 g^{-1} soil h^{-1}; CBM µg C-BMS g soil $^{-1}$; qCO_2 in mg C-CO_2 g^{-1} C-BMS. h^{-1}

Temperature and nutrients are important factors that can influence soil microbial activity (SUGIHARA et al. 2010). Average soil temperature analyzed at the time of collection was 28 ºC. Most soil microorganisms belong to the mesophilic group; therefore, the temperature factor had no negative effect on the metabolism of microorganisms. Nutrients from application of higher SW doses may have contributed to similarity in the analyzed variables between microorganisms at rates of 200 and 300 m^3 ha^{-1} of SW. Important nutrients that act as enzyme cofactors of respiratory processes such as Zn, Cu and Mn, showed increasing values proportional to SW doses accumulating in the soil during the experiment. The greater availability at doses of 200 and 300 m^3 ha^{-1} (Table 1) allowed the metabolic stability of microorganisms in relation to the evaluated parameters.

According to GAMA-RODRIGUES (2008), SW increases the amount of microorganisms in the soil, affecting the dumpsite of this type of manure. GUERRERO et al. (2007) observed a significant decrease in microbial activity days after SW application, including values close to areas without SW application. Moreover, repetitive SW application can lead to stability in metabolic rates of microorganisms present in the soil, indicating residual effects at higher concentrations, probably caused by the accumulation of organic matter, as observed in this study and by VIEIRA et al. (2004). Stability can be associated with the addition of labile compounds present in SW, which increase the amount of available or partially usable resources for microorganisms.

CO_2 flow is correlated with the intensity of catabolic processes; therefore, an increase in BR indicates a greater metabolic activity of the microorganisms. The increase in BR rates in the plots with SW may represent metabolic alteration of soil microbiota due to the application of SW and relate directly to increased microbial biomass (CATTELAN & VIDOR, 1990).

Behavior similar to BR was also found for qCO_2. There was an increase in qCO_2 in treatments with 100-m^3 ha^{-1} SW with subsequent decrease for the treatments with 200 and 300-m^3 ha^{-1} SW. The increase in BR and qCO_2 could indicate physiological stress condition with microorganisms increasing the release of CO_2 due to its intense metabolic activity for metabolism maintenance (ANDERSON & DOMSCH, 2010). The qCO_2 result demonstrates the adaptation SMB to conditions greater organic matter availability in the soil treatments with levels of 200 and 300 m^3 ha^{-1} of SW.

SMB results show significant differences only in the presence of SW when compared to the plots with no application. There were no differences between the different SW concentrations. These results demonstrate a metabolic adaptation of microorganisms at smaller SW concentrations, followed by metabolic increase together with biomass increase, demonstrating balance in the system without stress conditions that induce the decrease in the efficiency of microorganisms (ISLAM & WEIL, 2000).

Diversity of soil bacterial community

The DGGE technique allowed detecting the diversity of the soil bacterial community, including those not cultivable, by the differences between intensities and band positions. Figure 1 shows only the bands of the first repetition, because high similarity (99.9%) was found between treatment repetitions. The analysis of Figure 1 suggests the presence of different groups, with some dominant communities represented by bands of greater intensity.

Bacterial DNA profiles are different between the plots with and without SW, in spite of evidencing communities in common between treatments. MF did not interfere with bacterial diversity.

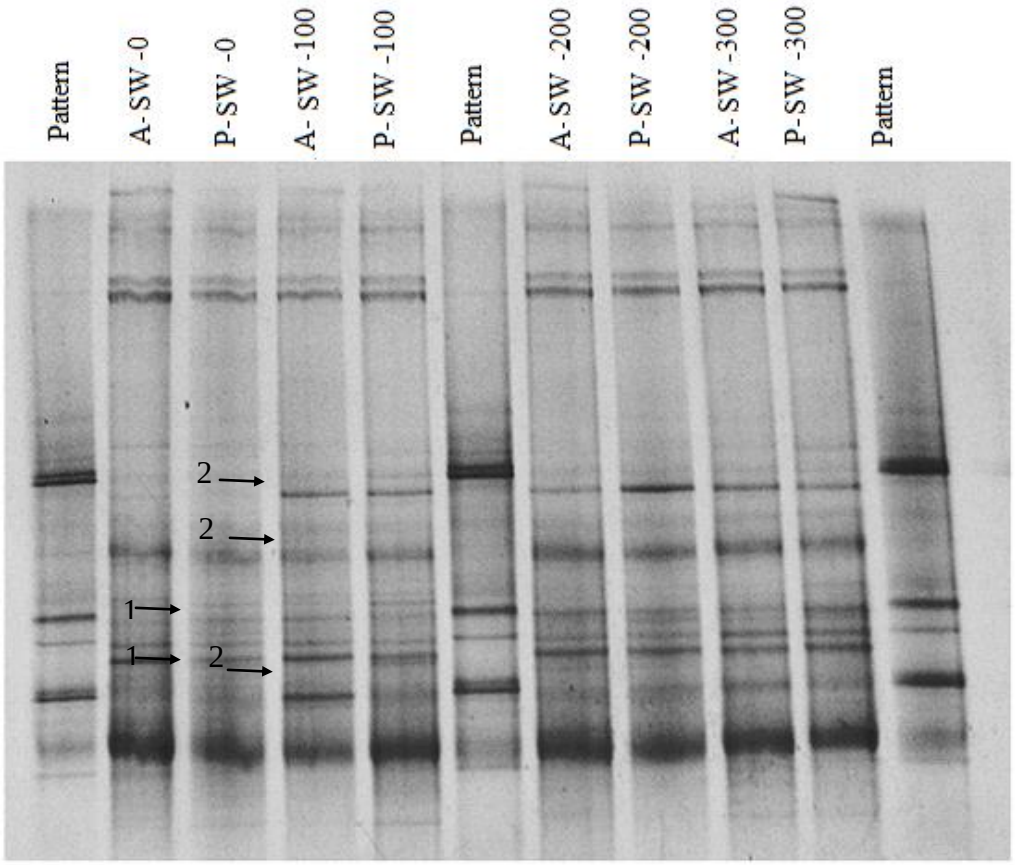

FIGURE 1. PCR-DGGE profiles of the soil bacterial communities obtained in treatments of the first repetition.

Plots which have not received SW had higher bacterial diversity, with bands different from the others (arrow 1). However, treatments that received SW presented more dominant communities, represented by thick bands (arrow 2). Thus, SW contributes with the permanence of dominant groups in soils. Although undergoing anaerobic treatment, SW carries a great number of microorganisms such as those of origin in the gastrointestinal animal tract. Thus, the different contribution of microorganisms shall induce the technique user to have greater care and environmental impact studies in agriculture using SW.

A soil with high organic matter content tends to maintain a more stable microbial population throughout the year; probably due to the abundance of ecological niches, with the heterogeneity of carbon sources (DE FEDE et al., 2001; GRAYSTON et al., 2001). The biodiversity changed by SW is a risk to soil quality, for the reduction of microbial diversity can be an important indicator of maintenance loss of biochemical functions in the ecosystem and, therefore, soil quality. The dominance of some species of microorganisms does not seem to be as important as the maintenance of diversity, because dominance reflects most immediately on short-term microbial fluctuation, while diversity reveals the balance between various organisms and functional domains in the soil (LAVELLE, 2000).

Besides the DGGE technique, most traditional indicators as H and ACE indexes also indicate greater biodiversity and number of species in plots where there was no SW application (Table 3). In plots without SW, the higher value of Evenness index (E) also indicates equal distribution between the different species present in the soil, less dominant than those observed in the plots with SW were, corroborating thus with the DGGE analysis. In this sense, HIDRI et al. (2010) demonstrated that the SW, when altering physico-chemical characteristics, also changes the bacterial community. An important observation by the authors, which was also seen in this study, is that higher concentrations SW associated with a prolonged period can significantly change ($p < 0.05$) the soil native bacterial structure.

TABLE 3. Diversity indexes[1] of bacterial communities in soil samples under different SW and MF concentrations.

Bacterial Diversity	Treatments							
	A-SW-0	P-SW-0	A-SW-100	P-SW-100	A-SW-200	P-SW-200	A-SW-300	P-SW-300
H	2.817±0.095a	2.883±0.091a	2.590±0.085b	2.595±0.086b	2.623±0.087b	2.615±0.090b	2.654± 0.092b	2.663 ±0.086b
ACE	42.4±19.1a	45.6±20.2a	19.8±3.5b	20.0±3.7b	24.4±6.7b	27.0±6.4b	24.5±7.8b	22.4 ±4.4b
Total bands	19	20	15	15	17	17	16	16
E	0.96	0.96	0.95	0.95	0.93	0.93	0.95	0.95

[1]Values obtained ± standard deviation. The means followed by the same lowercase letters on the line do not differ significantly in the Tukey test at 5% significance level.

The clustering results corroborate other techniques and the biodiversity assessment indexes (DGGE, H, ACE and E). Two main groups were formed (G1 and G2); with 81%, similarity under different SW doses (Figure 2). Group 1 (A-SW-0, P-SW-0) and group 2 (A-SW-100, P-SW 100, A-SW-200, P-SW-100, A-SW-300 and P-SW-300 respectively include treatments without SW and with SW.

Two subgroups with 91% similarity have been observed within group 2. The first represented by treatments A-SW-100 and P- SW-100. The second similarity with over 94% was represented by treatments A-SW-200, A-SW-300, P-SW-200 and P-SW-300. At each SW concentration, the MF factor showed no difference (100% similarity).

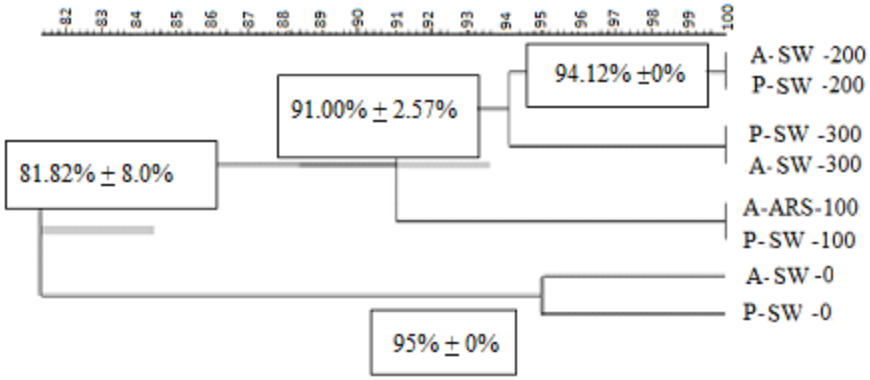

FIGURE 2. Genetic similarity dendrogram of soil bacterial communities under different doses of SW and MF. The Jaccard similarity coefficient (5% tolerance) and cluster analysis by the unweighted pair-group analysis (UPGMA).

Correlation between soil characteristics and quantitative factors (BR, SMB and qCO_2)

From the Spearman correlation analysis with 5% significance between BR, SMB and qCO_2 with soil physical and chemical parameters, it was possible to identify that the BR has a positive correlation with OM (0.52) and Zn (0.47) and negatively with Mn (-0.45) and Fe (-0.42). SMB achieved positive correlation with Zn (0.73), OM (0.57), Cu (0.57), N (0.43) P (0.42) and N-org (0.41). Only one positive correlation of S (0.49) was observed with qCO_2.

The positive correlation between OM and BR with SMB was expected, since SW provides microorganisms and nutrients in organic matter. The microorganisms increase represents an increase in SMB, as well as the nutrients favoring increased metabolism, and hence the respiratory rate (GAMA-RODRIGUES et al., 2008).

Considering that Cu, Zn and Mn act as cofactors of respiratory enzymes such as superoxide dismutase, it is possible to infer that the positive correlation between Zn and BR and SMB indicates that SW provides this element for microorganisms, which increases metabolic activity and growth rate. On the other hand, the positive Cu correlation only with SMB indicates that such element in the SW did not affect the breathing rate, but contributed to the proliferation of microorganisms. The positive correlation only with SMB was also observed with P, a key element for microbial growth,

for participating in the formation of nucleic acids and the synthesis of organic high-energy compounds, such as adenosine triphosphate.

The negative correlation between BR with Mn and with Fe was possibly due to significant concentration of Mn element in SW (40 mg^{-1} dm^{-3} a 100 mg^{-1} dm^{-3}) and Fe in the soil (7 mg^{-1} dm^{-3} a 68 mg^{-1} dm^{-3}), which can adversely affect the energy metabolism of microorganisms via oxidative stress and cell death (FARINA et al., 2013).

The positive correlation of S with qCO_2 indicates that the increase in this element can increase metabolic efficiency of microorganisms. The S content in the soil was due to the presence of MF together with the SW. Fertilizers formulated with lower concentration of N, P and K, as the one used in this study, contain approximately 5% of S, simultaneously contributing to the SW in this nutrient availability in soil. S comprises enzymes and coenzymes, participates in the carbohydrate metabolism, and aids in fixing N in free and symbiotic forms, thus its availability associated with various nutrients present in SW contributes to increasing the metabolic rate of the soil microorganisms.

CONCLUSIONS

The use of SW in the long term interfered significantly in the amount and quality of soil microorganisms, while mineral fertilization did not induce any significant effect on the microbial population.

The elements Zn, Cu, P and N were important for the maintenance and growth of microbial population, however, Manganese and Iron negatively affected the microbial population.

The prolonged use of SW, especially in higher doses (200 and 300 m^3 ha^{-1}), induced lesser diversity of microorganism groups in the soil and consolidated the permanence of certain groups of the bacterial community.

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