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ORIGINAL RESEARCH

Impacts of different pig slurry application methods on soil quality indicators in a maize-soybean cropping sequence in the sub-humid pampas of Argentina

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Abstract

Purpose In Argentina, pig slurry (PS) is spread in surface with N losses in ammonia form. Different methods to decrease these emissions are available, but there is poor information about their impacts on the soil-plant system. The objective of this study was to compare the effects of different PS application methods on the soil quality in a maize-soybean cropping sequence.

Methods PS application methods were evaluated: acidified (AS), incorporated (IS), surface (SS), mineral fertilization (MF) and control (C). The experimental design was arranged in a randomized block with three replicates. Chemical parameters and microbiological parameters were determined. Also, grain yields and N uptake were measured.

Results IS caused increases in anaerobic nitrogen and basal respiration of soil on soybean. Treatments with PS and/or MF showed lower values in pH than C in both crops, and higher electrical conductivity only in maize. SS treatment showed higher Pe on soybean, indicating a maintenance of the P levels with respect to those in MF and C. The concentration of NO_3^- increased with MF in both crops. In maize, MF presented similar concentrations to AS and SS. IS increased grain yields of maize by 16 %, whereas SS and AS increased yields of soybean by 112% and 79%, respectively, compared to C.

Conclusion The different PS application methods had similar effects on most of the indicators of soil quality. In maize, IS and AS were more efficient in retaining N within the soil-plant system, whereas, in soybean, the SS led to higher yields.

Keywords Pig slurry incorporation, Pig slurry acidification, Mineral fertilization, Chemical soil, Microbiological soil, Yield crops

Introduction

The number of pig breeding farms in Argentina has increased greatly during the last years. This has made pig slurry (PS) disposal a major problem. A promising option to solve this problem is the use of PS applications as a source of crop nutrients because the soil can accept and process residues, contributing with recycling (Ratto and Giuffré 2011). With adequate use, PS can replace, either partially or totally, mineral fertilizers, increasing soil fertility for input of essential nutrients, such as nitrogen (N) and phosphorus (P), and improving the soil physical properties (Biau et al. 2012; Carrizo et al. 2014; Tlustoš et al. 2018).

The main problem associated with PS applications is the N losses in ammonia form (NH_3), which cause negative impacts on the environment (Martínez et al. 2017a; Damian et al. 2018). Since N losses are related to soil N dynamics and mineralization-immobilization processes, which can be affected by some management practices (Park et al. 2018), the impact of PS applications on soil quality depends on the application methods.

In Argentina, due to the predominance of no-tillage seeding systems, PS is spread mainly by surface broadcasting by splash plate applicator. With this meth-

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od, NH_3 volatilization losses can exceed 50% during the PS applications to the soil (Fangueiro et al. 2015a; Park et al. 2018). Other application methods are PS incorporation (Costa et al. 2014; Schröder et al. 2015) and PS acidification with sulfuric acid, both aimed to prevent N losses (Fangueiro et al. 2015a). PS incorporation reduces the exposure of the slurry to the air and increases the contact with the soil (Webb et al. 2010). In addition, there is higher reactivity of NH_3 with the H^+ ions present in the soil, which displaces the chemical equilibrium to the cationic form of NH_4^+, which is temporarily retained in the liquid and solid phases of the soil, preventing its transformation to N-NH_3 (Costa et al. 2014). In contrast, PS acidification is based on the equilibrium between dissolved NH_4^+ and the NH_3 present in the slurry (Fangueiro et al. 2015a). In addition, it leads to a minimization of volatilization of NH_3 and leaching of NO_3^-, and a modification in the N of the soil dynamics, increasing the contents of NH_4^+ by inhibiting nitrification (Fangueiro et al. 2016; Park et al. 2018).

The sustainability of management systems in the short and long term can be determined by monitoring the soil quality (Doran and Parkin 1994; Ferreras et al. 2009). This can be done by evaluating several soil chemical and microbiological indicators (Astier-Calderón et al. 2002). Some of these microbiological indicators, such as microbial biomass carbon (MBC), enzymatic activities, basal respiration (BR), and metabolic quotient (qCO_2), can be improved by the use of amendments (Balota et al. 2014; Sousa et al. 2014; Morales et al. 2016) and slurry applications (Liu et al. 2010; Biau et al. 2012; Yagüe et al. 2012). In addition, the different methods used to apply PS can produce changes in the soil microbial composition, which generally occur through changes in the mineralization-immobilization processes and in the microbial decomposition of the organic compounds of the slurry (Sørensen and Eriksen 2009; Fangueiro et al. 2015b, 2016), and thus increase the productive capacity of the soil (Meade et al. 2011; Martínez et al. 2017b; Plaza-Bonilla et al. 2017). PS application methods such as PS acidification or PS incorporation have shown greater availability of N-NH_3 than surface applications, which causes yield increases and N accumulation in plant biomass in different crops (Costa et al. 2014; Fangueiro et al. 2015b; Damian et al. 2018). However, these effects are closely related to the climatic and edaphological conditions of each location. Thus, and based on the fact that information about the effects of PS application on the Mollisol soils of the Pampean region of Argentina is scarce, the objective of the present study was to compare the effects of different PS application methods on the soil quality in a maize-soybean cropping sequence in the sub-humid region of Argentina.

Materials and methods

PS composition, soils and field experiment

The composition of the PS used in the experiment is shown in Table 1. Only PS from fattening pigs was used. PS was collected directly from the pig houses, and the same batch was used for all the treatments.

Table 1 Composition of the pig slurry applied (mean ± standard deviation, n=3)

Characteristic	Unit	2014	2015
Dry matter (DM)	%	1.28±0.06	5.14±0.42
Organic matter (OM)	% DM	61.46±0.18	67.04±1.12
Ashes	% DM	38.54±0.18	29.29±1.12
Total N	$g\ l^{-1}$	2.98±0.09	5.32±0.11
NH_4^+-N	$g\ l^{-1}$	1.10±0.14	3.19±0.08
pH		6.17±0.06	7.27±0.06
Electrical conductivity	$dS\ m^{-1}$	13.15±0.10	28.97±0.69
Phosphorus	$mg\ l^{-1}$	435.83±20.70	279.17*
N:P relation		6	19
Doses	$m^3\ ha^{-1}$	100	50

* Data without repetition

The field experiment was conducted in the experimental field of the National Institute of Agricultural Technology (INTA), in the Southeast of Córdoba Province, in Argentina (32°42´44.65´´S, 62°05´46.07´´W) between 2014 and 2016 in a maize-soybean crop sequence. The soil is a Typic Argiudoll (USDA classification), with silty loam texture (25% clay, 69% silt and 5.4% sand), pH 6.02, electrical conductivity (EC) 0.09 $dS\ m^{-1}$, soil organic N (SON) 1.39 $g\ kg^1$, soil organic carbon (SOC) 15,20 $g\ kg^{-1}$ and extractable phosphorus (Pe) 29 $mg\ kg^{-1}$. The site is characterized by a temperate sub-humid climate with an average annual rainfall of about 894 mm and an average annual temperature of 16.9 ºC (INTA 1978). Other climatic parameters during the period are presented in Fig. 1.

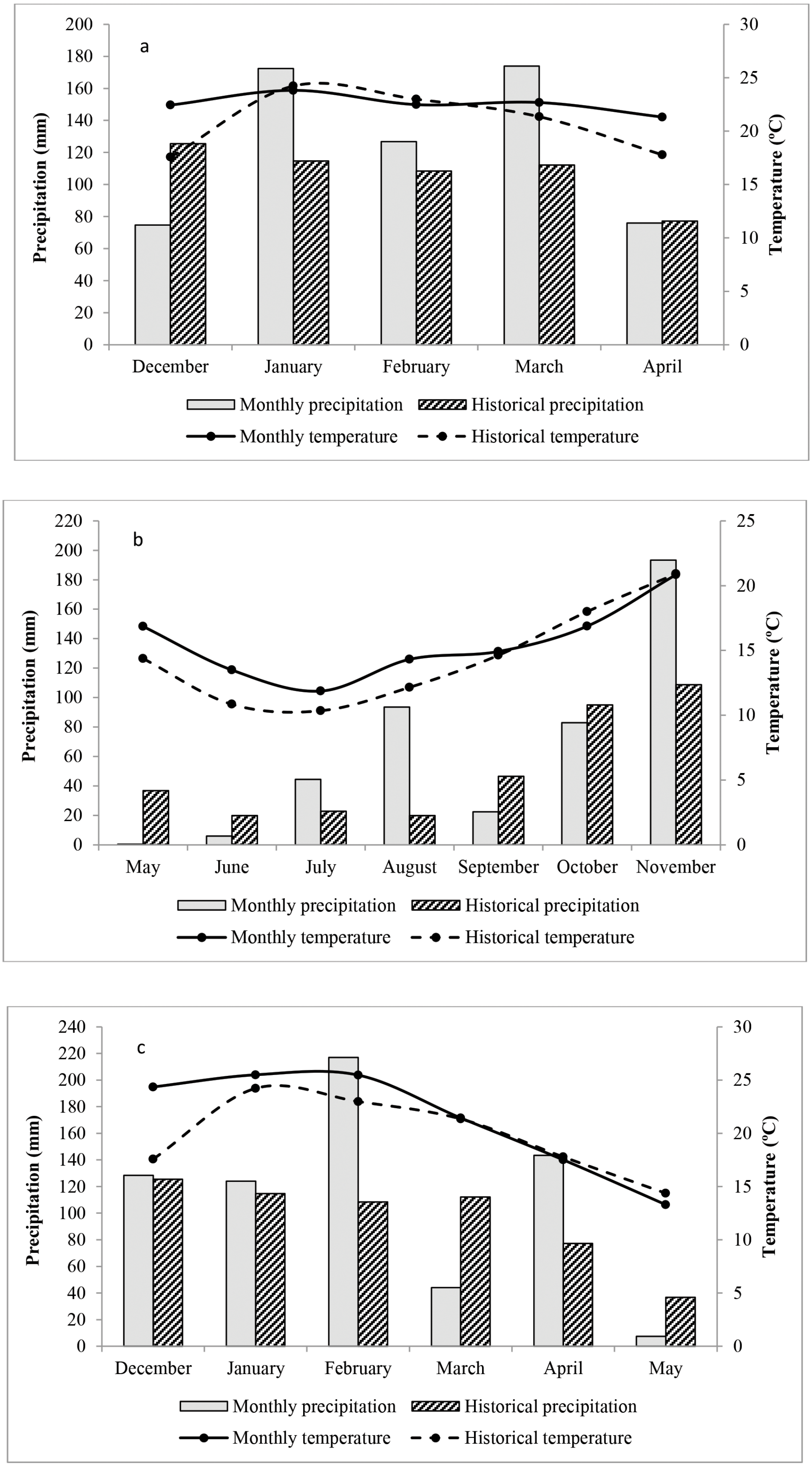

Fig. 1 Monthly precipitation and air temperature at the experimental site: a) maize (2014-2015), b) fallow (2015) and c) soybean (2015-2016)

Five PS application methods (treatments) were evaluated: acidified PS (AS), incorporated PS (IS), surface PS (SS), mineral N fertilization (MF) and control (C). The experimental design was arranged in a randomized block with three replicates. The plot dimensions were 5 x 8 m (40 m^2), separated by a corridor of 4 m to minimize interactions between plots. The PS was applied by spreading, from a tank, two days before the sowing of maize and thirty days before the sowing of soybean. The nutrient quantities supplied by pig slurry and nutritional need of the maize and soybean crops are shown in Table 2.

For the AS treatment, before its application, the PS was acidified with sulfuric acid until achieving a pH of 5.5-6 (Fangueiro et al. 2015a), whereas for the IS treatment, the PS was immediately incorporated by disking at 0–10 cm soil depth. For the MF treatment, the mineral fertilizer was applied at an N dose equivalent to that applied with PS, with granulate solid urea at 46%, at sowing. The C soil consisted of non-amended plots.

Maize (*Zea mays* L.) was sown in December 2014, at a density of 10 plants m^2 and 52 cm between rows, and was harvested in late April 2015. The variety La Tijereta triple pro, intermediate cycle, was used. Soybean (*Glycine max* L.) was sown in December 2015 at a density of 40 plants m^2 and 42 cm between rows, and was harvested in May 2016. The variety DM 4712 was used.

Table 2 Mean nutrients supplied by pig slurry (n=3) and nutritional need of the maize and soybean crops

Quantities supplied (kg ha^{-1})	Maize	Nutritional need (kg tn^{-1} grain)	Soybean	Nutritional need (kg tn^{-1} grain)
Dry matter	1280	-	2570	-
Organic matter	787	-	1723	-
Ashes	493	-	847	-
Total N	298	22	266	80
NH_4^+-N	110	-	160	-
Phosphorus	44	4	14	8

Analysis of chemical and microbiological indicators of the soil quality

For the analysis of chemical indicators of the soil quality, five soil samples (core diameter 2.5 cm) from a depth of 0-20 cm were randomly collected from each plot, to make one composite sample. Soil samples were air-dried, passed through 2-mm and 0.5-mm pore sieves, and the following parameters were determined: SOC, SON, Pe, pH, and EC. SOC was determined by the wet oxidation method for organic matter (Walkley-Black) (IRAM-SAGyP 29571-2 2011), SON by the micro-kjeldahl digestion method (IRAM-SAGPyA 29572 -1 2011), Pe by the Bray and Kurtz Nº 1 method (IRAM-SAGyp 29570-1 2010), and pH and EC were measured on a 1:2.5 soil:water suspension using a glass electrode InoLab pH 720 and multi-range HANNA, respectively (Rhoades 1996; IRAM 29410 1999). These chemical parameters were determined at the six-leaf stage (V6) in maize, and at the beginning of flowering (R1) in soybean.

For the analysis of the particulate organic matter (POM) and microbiological quality indicators (acid phosphatase (AP) activity, BR, anaerobic N (AN) and MBC), three soil composite samples, formed by ten soil samples (core diameter 2.5 cm) from a depth of 0-10 cm, were randomly collected from each plot at the physiological maturity of the crops. Before the analysis of AP and BR, fresh soils (field moisture) were sieved using a 2-mm mesh and stored at 4 °C. The soil moisture content was also gravimetrically analyzed in oven-dried soil samples taken from each sampling point, at 105 °C. AN and MBC were analyzed in dry soil.

Soil fractionation by particle size (106 µm) was conducted by the wet sieving method proposed by Cambardella and Elliott (1993) using a vibratory sieve shaker (FRITSCH, Analysette 3 Pro, Germany). AN was determined by anaerobic incubation at 40 ºC for 7 days (Echeverria et al. 2000), whereas MBC content was determined by the fumigation–extraction method (Vance et al. 1987); prior to the analysis, the samples were incubated for 16 h at 28 ºC. BR was determined by measuring the CO_2 produced in a 7-day incubation experiment, at 25 ºC, in which 30 g of each soil was placed in a hermetically sealed polyethylene flask with a vial containing 20 ml 0.1 M NaOH, treated with 0.1 M HCl (Jenkinson and Powlson 1976), and the qCO_2 was inferred directly by dividing the CO_2 by the MBC. The AP activity was determined according to the method proposed by Alef and Nannipieri (1995).

Soil NO_3^- levels were determined during the crop growing period (six-leaf stage for maize and beginning of flowering for soybean) and after harvesting (only for maize) in samples taken at five depths (0-20 cm, 20-40 cm, 40-60 cm, 60-100 cm and 100-150 cm). Five soil samples from a depth of 0-20 cm and three soil samples from a depth of 20-150 cm were randomly collected from each plot and then mixed to make a composite sample. Soil NO_3^- was extracted using phenoldisulfonic acid (Bremmer 1965) and determined by colorimetric by spectrophotometer SPECTRUM SP-1105.

Determination of the crop biomass, stem and grain N contents, and grain yield

Crop biomass was estimated at physiological maturity by hand cutting 1 m from two central rows of each plot ($1.04\ m^2$ in maize and $0.84\ m^2$ in soybean) to determine the DM content. Subsequently, the maize aerial biomass was divided in stalk and grain. Maize stems and total plants of soybean were dried at 60 °C (until constant weight), weighed, and milled. In maize, stem and grain total N contents were determined by a semi-micro kjeldahl modification (Bremmer 1996), whereas in soybean the grain N content was determined by near infrared spectroscopy from the concentration of protein using a FOSS Infratec 1241 Grain Analyzer.

Grain yields were determined by harvesting two complete central rows of each crop ($5.2\ m^2$ in maize plots and $4.20\ m^2$ in soybean plots). Grains were taken from each plot to determine moisture content and to adjust grain yield to 14.5% moisture in maize and to 13.5% moisture in soybean. The values of each parameter analyzed were transformed to kg ha^{-1}.

Statistical analysis

The effects of each PS application method tested in maize and soybean were determined by analysis of variance using the Mixed Model in the Infostat Professional software (Di Rienzo et al. 2017). The PS application methods were set as fixed factors and the replications as a random effect. Means were separated by LSD Fisher test ($p<0.05$). Since soil attributes are known to differ with depth, depth was not included in the statistical model for the analysis of NO_3^- data, and individual statistical analyses were performed for each depth.

Results and discussion

Soil chemical properties

The results regarding the soil chemical properties (SOC, SON, Pe, pH and EC) are shown in Table 3.

SOC and SON

In the three treatments with PS applications (AS, IS and SS), the SOC and SON reserves remained stable in both crops with respect to the C treatment, with a slight tendency to increase. This may be due to the inputs of DM and OM provided by the PS application prior to the sowing of the crops. In addition, it should be noted that the maize crop preceded that of soybean, so the increase in both variables in the second year may also respond to greater input of plant residues from maize. Similar trends in SOC and SON after PS applications have been shown in previous short- and mid-term studies (Biau et al. 2012; Comin et al. 2013; Bócoli et al. 2016; Morales et al. 2016; Park et al. 2018).

Pe concentration

Regarding Pe, in maize, which received a single PS application, the Pe concentration remained stable in all the treatments. In contrast, in soybean, which received two consecutive applications, the Pe concentration in the SS treatment was greater, indicating a maintenance of the P levels with respect to those in MF and C. In addition, the Pe in SS did not differ from that in IS or AS, whereas the Pe concentration in these two treatments did not differ from that in MF, but did differ from that in C ($p\leq0.05$). This indicates that the crops extracted P and that this was not replaced by MF or C. In previous studies, several authors reported accumulation of P after PS applications, and linked it directly to the high amount of P added with the PS. However, in the present study, the total amounts applied were only 58 kg P ha^{-1}. In turn, it should be noted that the PS used in this work had higher N:P ratios than other slurries used by other authors (Plaza et al. 2004; Balota et al. 2010; Lourenzi et al. 2013), reducing the risk of over-application of P when doses are calculated based on the N requirements.

Soil pH

The pH was the chemical indicator most sensitive to the PS applications and MF. This parameter was decreased both by PS applications and by MF. In maize, the highest decrease in pH was caused by MF, AS and SS, with the different PS application methods showing similar pH values. In soybean, the highest decrease was caused by the MF treatment ($p\leq0.05$) and the different PS applications did not change the pH. The main cause of this acidification was the production of H+ ions due to the hydrolysis and oxidation of the NH_4^+ present in the PS and in the urea used in the MF treatment (Divito et al. 2011).

Soil EC

Soil EC increased only in maize with PS applications and MF ($p\leq0.05$). IS caused a greater EC increase than C, but did not differentiate from that observed with the SS and MF treatments ($p\leq0.05$). Several authors have shown that PS applications increase EC, and attributed this to the addition of soluble salts that come from the pig diet and are present in the PS (Liu et al. 1998; Plaza et al. 2004; Pegoraro et al. 2014; Saviozzi et al. 1997). On the other hand, the increase in EC caused MF may be attributed to the rapid nitrification of the soil with the addition of N, finally presenting high NO_3^- content (Wienhold 2005). The fact that EC increased only in maize may respond to the fact that the samples were collected 42 days after the PS and urea applications, whereas in soybean, the samples were collected 72 days after the PS and urea applications, which could result in a transient modification of the EC. In addition, the precipitation regime may have influenced the results obtained (Fig. 1), as rainwater can generate a displacement of salts below the sampling depth (Hao and Chang 2003).

POM and microbiological properties

The effects of the different treatments on POM and microbiological soil quality indicators (AN, BR, AP activity, MBC and qCO_2) are summarized in Table 4. Most of the parameters evaluated showed no significant differences among the treatments, except AN and BR in soybean.

POM, AN and BR

AN is an N indicator potentially mineralizable by microorganisms, whereas BR estimates the microbial activity in general. In soybean, IS led to an increase in these two indicators with respect to C. These increases are consistent with previous studies that showed that the continued addition of slurry had a significant impact on the soil respiration and biological activity in general (Liu et al. 2010; Biau et al. 2012). This may be due either to a direct effect of the OM contribution or to an indirect effect as a result of a higher contribution of C through the plant biomass added by the maize crop sown the previous year. Similar results have been reported by other authors who showed that PS applications either maintained or increased POM and the mineralizable N indicator (Wienhold 2005; Balota et al. 2010; Biau et al. 2012; Yagüe et al. 2012).

AP activity

AP activity is a potential index of organic P mineralization. Although this activity is inhibited by high concentrations of P, in the present study, we observed a tendency of AP to increase with the PS applications, although without significant differences. Balota et al. (2014) and Tiecher et al. (2017) also reported an increase in AP with PS applications and with high doses of added P, and Tiecher et al. (2017) attributed this response to a higher biological activity of both plants and microorganisms and a protection of the enzyme by the soil organic matter (SOM).

Table 3 Effect of three different PS application methods and mineral fertilization on chemical soil quality indicators (0-20 cm) in maize (six-leaf stage) and soybean (beginning of flowering) crops

Crops	Treatments	SOC (g kg^{-1})		SON (g kg^{-1})		Pe (mg kg^{-1})		pH		EC (dS m^{-1})	
Maize	IS	14.77	a	1.37	a	28.33	a	5.98	b	0.11	b
	AS	15.22	a	1.42	a	29.33	a	5.87	bc	0.14	a
	SS	16.67	a	1.51	a	35.00	a	5.89	bc	0.13	ab
	MF	15.41	a	1.42	a	27.33	a	5.79	c	0.14	ab
	C	14.52	a	1.39	a	28.67	a	6.18	a	0.07	c
Soybean	IS	16.14	a	1.31	a	30.67	ab	6.03	a	0.10	a
	AS	15.53	a	1.23	a	29.33	ab	6.00	a	0.09	a
	SS	15.76	a	1.28	a	35.67	a	5.97	a	0.11	a
	MF	15.41	a	1.25	a	22.33	bc	5.73	b	0.12	a
	C	14.64	a	1.19	a	21.33	c	6.03	a	0.09	a

IS: Incorporated Slurry, AS: Acidification Slurry; SS: Surface Slurry, MF: Mineral Fertilization, C: Control. Soil Organic Carbon (SOC), Soil Organic Nitrogen (SON), extractable Phosphorus (Pe), Electrical Conductivity (EC). Different letters indicate significant differences between treatments in each crop ($p \leq 0.05$).

MBC and qCO_2

MBC and qCO_2 did not present a clear trend among the treatments evaluated. Several authors have reported increases in MBC in soils with PS applications, compared to MF and a control situation (Yagüe et al. 2012; Balota et al. 2014; Yanardağ et al. 2017). These differences can be attributed to the compositions of the slurries used, and to the different soil conditions, because these authors applied more organic carbon and other degradable organic residues that stimulate the growth and activity of local soil microorganisms. In addition, Yanardağ et al. (2017) emphasized that changes in these indicators by the addition of organic compounds are more sensitive in soils with low SOC.

Soil N content

The NO_3^- content was increased by both MF and PS applications, relative to C, differing according to the application method, moments and depths evaluated (Fig. 2, 3). These increases may be attributed to a rapid nitrification of the NH_4^+ present in the PS and the transformed NH_4^+ of the urea, as well as to the type of soil and management. Aita and Giacomini (2008) also reported rapid increases in the NO_3^- content after PS applications, and confirmed that the N-NH_4^+ present in the PS was rapidly nitrified. Aita et al. (2007) also verified that, 20 days after PS application, virtually all the 130 kg ha^{-1} of the N-NH_4^+ present in the PS had oxidized to NO_3^-.

Soil N content in the maize crop

In the maize crop, after the first application of PS and MF, the NO_3^- content increased at all the depths evaluated. When analyzing the distribution of NO_3^- in the soil profile, we observed that the increase in NO_3^- content in the surface horizon was immediately accompanied by a transfer to the lower horizons. In the MF treatment, the NO_3^- content was highest up to 100 cm depth, whereas in the different PS application treatments, the NO_3^- content varied according to the depth evaluated. At 0-20 cm, the NO_3^- content in AS was similar to that in MF, whereas, at 20-40 cm, both the NO_3^- content in AS and the NO_3^- content in SS were similar to those in MF. At 40-60 cm, SS continued to present high NO_3^- content, whereas at 60-100 cm all the PS application methods presented NO_3^- contents similar to those in MF, differing from the C situation. At higher depth, the trend remained, with the SS and MF treatments decreasing the NO_3^- content to a lesser extent.

Table 4 Effect of three different PS application methods and mineral fertilization on particulate organic matter and microbiological soil quality indicators (0-10 cm) in maize (2015) and soybean (2016) crops at physiological maturity

Crops	Treatments	POM (mg kg^{-1})		AN (mg kg^{-1})		AP (mg kg^{-1})		BR (mg kg^{-1})		MBC (mg kg^{-1})		qCO_2	
Maize	IS	11.13	a	90.07	a	1026.33	a	327.40	a	122.81	a	2.66	a
	AS	9.95	a	99.63	a	1114.96	a	302.20	a	166.67	a	1.91	a
	SS	10.51	a	91.23	a	1132.00	a	288.70	a	100.88	a	2.85	a
	MF	8.77	a	85.87	a	1099.74	a	327.10	a	122.81	a	2.67	a
	C	11.79	a	99.63	a	983.00	a	387.50	a	127.19	a	3.25	a
Soybean	IS	10.85	a	131.45	a	1262.22	a	306.60	a	213.45	a	1.52	a
	AS	9.89	a	114.64	b	1174.40	a	281.40	ab	254.38	a	1.19	a
	SS	10.65	a	113.17	b	1262.89	a	276.50	ab	195.91	a	1.48	a
	MF	9.34	a	103.06	b	1141.60	a	181.30	b	181.29	a	1.11	a
	C	9.12	a	114.72	b	1115.93	a	187.50	b	264.62	a	0.72	a

IS: Incorporated Slurry, AS: Acidification Slurry; SS: Surface Slurry, MF: Mineral Fertilization, C: Control. Particulate Organic Matter (POM), Anaerobic Nitrogen (AN), Acid Phosphatase (AP), Basal Respiration (BR), Microbial Biomass Carbon (MBC), Metabolic quotient (qCO_2). Different letters indicate significant differences between treatments in each crop ($p \leq 0.05$).

The different PS application methods had similar NO_3^- contents up to 100 cm depth. At the deepest depth evaluated (100-150 cm), SS presented higher NO_3^- contents, whereas AS and IS did not differentiate from each other (Fig. 2 A). At physiological maturity, significant increases in NO_3^- were observed only at 20-40 and 40-60 cm depth, being greater for IS at the former depth and greater for IS and MF at the latter depth (Fig. 2 B).

Soil N content in the soybean crop

In soybean, after the second consecutive year of PS application, in stage R1, the NO_3^- content was highest in MF, differing from that in the PS applications at the first two depths evaluated and being equal to that in SS at 40-60 cm. This response may be due to the fact that MF was applied at the time of sowing, 30 days after PS application. Regarding the different PS application methods, at the first depth evaluated, they did not differ from each other, while at 20-40 and 40-60 cm, SS showed greater NO_3^- content than IS but did not differ from AS (Fig. 3).

Comparative analysis of the Soil N content

In both crops, the soil NO_3^- content was higher in the MF treatment than in the PS applications. This may be due to differences in the availability of N, which varies depending on whether mineral or slurry fertilizers are used. When mineral fertilizers are applied, the N is available to be used by crops immediately (Salazar Martínez Lagos et al. 2015), whereas, when slurry fertilizers are applied, the availability of N is affected by different processes. A fraction of the NH_4^+ present in the PS can be immobilized by microorganisms, which is initially associated with the easily mineralizable SOM fraction, consisting mainly of cells and residues of microbial cells.

When microorganisms die and their residues decompose, part of the immobilized N is mineralized again (Terrero et al. 2018). Also, there may be a temporary immobilization of NH_4^+ in the interlaminar spaces

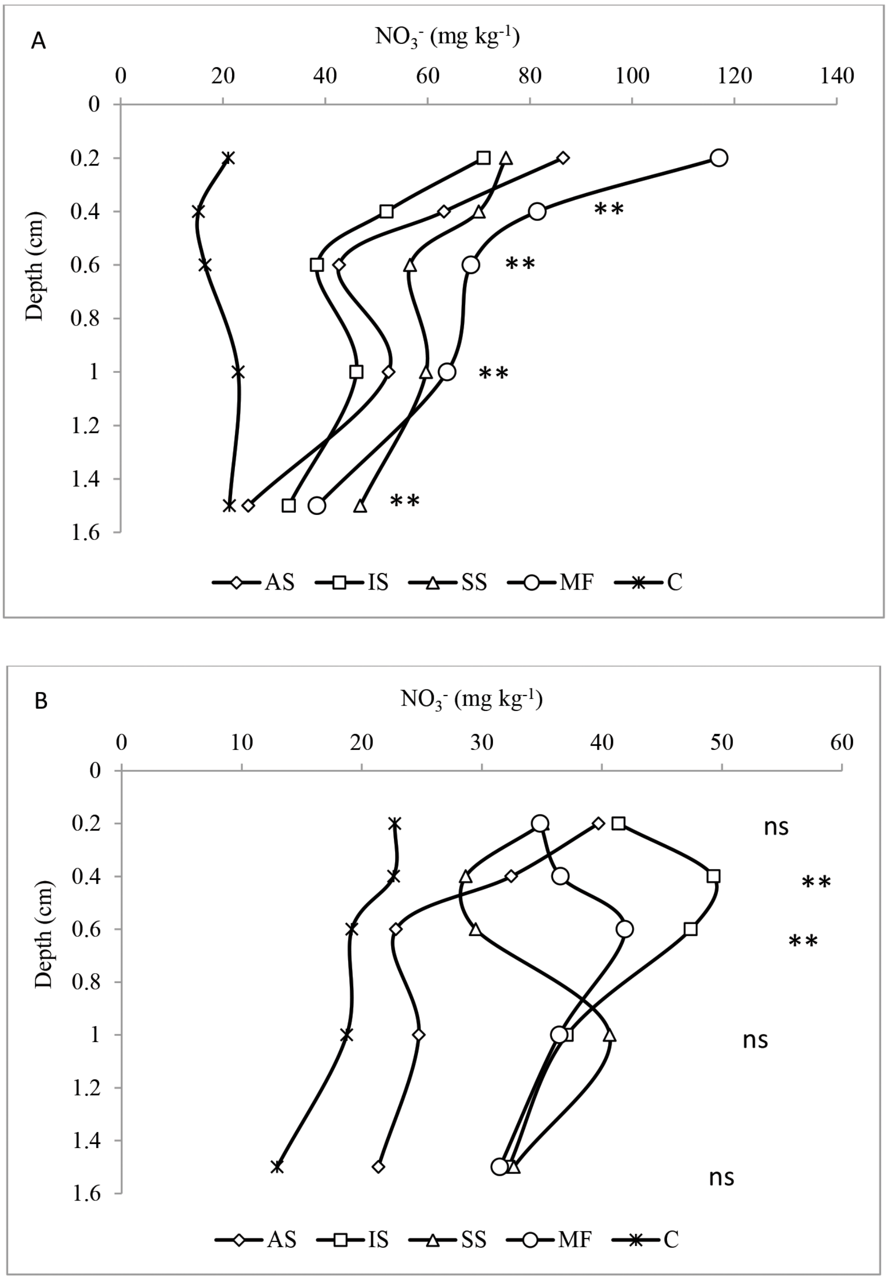

Fig. 2 Distribution of soil nitrate (NO_3^-) in the soil profile for the different treatments applied to maize at A) the six-leaf stage and B) physiological maturity. **Indicates statistical differences between treatments within each depth ($p \leq 0.001$); ns: no statistical differences between treatments within each depth

of clay minerals (Daudén and Quílez 2004). Sørensen and Amato (2002) have shown that the net immobilization of N after PS application is significantly higher than that after MF. These immobilization-mineralization processes complicate the prediction of the fertilizer value of the slurry, because it can release N in the years following application (Sieling et al. 2014).

In the present study, the different PS application methods evaluated showed differences in the N immobilization-mineralization processes. In general, when the PS was applied superficially (SS treatment), the NO_3^- content increased rapidly with respect to the C situation, similar to that observed in the MF treatment, whereas, when the PS was incorporated into the soil (IS treatment) or previously acidified (AS treatment), nitrification was slower, presenting more gradual variations in the soil NO_3^- content. In the case of IS, this may be because when the PS is mixed with the soil, a high proportion of microorganisms related to the decomposition of the PS are protected in the soil matrix. Consequently, a larger amount of N is retained in the microbial biomass and then released during the crop growing cycle and in subsequent years. This contributes to a long-term accumulation of SON in the mid- or long term, which can be released more slowly throughout the growing cycle (Sørensen and Amato 2002; Costa et al. 2014; Sieling et al. 2014).

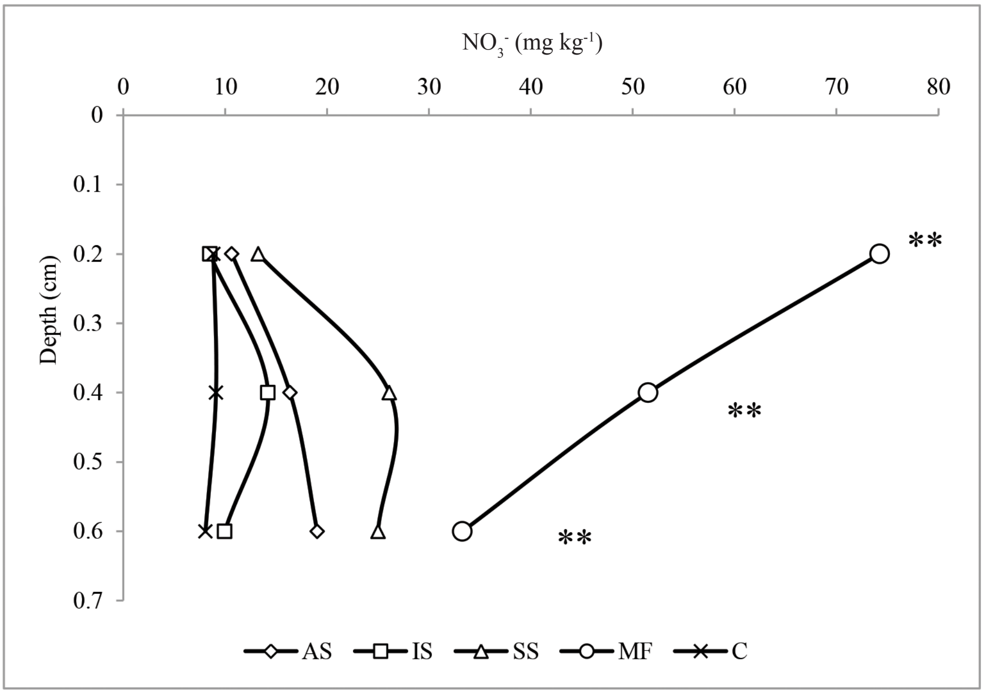

Fig. 3 Distribution of soil nitrate (NO_3^-) in the soil profile at the beginning of flowering in soybean for the different treatments applied to soybean. ** Indicates statistical differences between treatments within each depth ($p \leq 0.001$)

Sørensen and Amato (2002) reported that the initial immobilization of the N of the PS was higher when the PS was incorporated into the soil, with N recovery rates of 41-45% two years after application. Fangueiro et al. (2016) also reported that PS acidification causes delays in N-NH_4^+ nitrification, decreasing N-NO_3^- losses. This allows us to conclude that a proportion of the N immobilized with IS and AS may have been remineralized later. This delay in nitrification is important to make the moments of N offer coincide with those of higher requirement by the crop, and in this way make a more efficient management of N. Thus, we may state that when PS is superficially applied (SS treatment),the nitrification process is faster than with the other PS application methods.

These results show that the potential NO_3^- losses by leaching in the MF treatment are similar to or greater than those in the PS treatments. This is consistent with that reported by Plaza-Bonilla et al. (2017), who found that the availability of N is slightly lower with PS applications than with MF. These authors concluded that the release of the N applied with the PS would represent a useful mechanism to become synchronized with the crop requirements.

Crop yield and N uptake

The PS application methods modified the N immobilization-mineralization processes in the soil, and therefore the production and absorption capacity by the crops. When the PS was incorporated into the soil (IS treatment), maize achieved increases in grain yield and aerial biomass of 16% and 24.6%, respectively, with respect to C ($p\leq0.05$). In addition, the IS treatment did not differ from AS and MF ($p>0.05$) (Table 5). The percentage of N in the grain showed no statistical differences between treatments ($p>0.05$), whereas the N absorbed in the grain exhibited the same behavior as grain yield. On the other hand, the concentration of N in biomass was higher in the MF, SS and AS treatments than in C and IS ($p\leq0.05$). This led to an increase of 89.7% in the N absorbed in the biomass when MF was used, compared to C ($p\leq0.05$). In addition, the PS application methods did not differ from MF. Total N uptake (grain + aerial biomass) varied from 179 to 235 kg N ha^{-1}, with no statistical differences between treatments, and with a recovery of the N applied that ranged from 68 to 79% (Table 5).

In the second year, in the soybean crop, yield behavior was different. The SS and AS treatments increased yields by 112% and 79%, respectively, compared to C ($p\leq0.05$). Like in maize, the concentration of N in the soybean grain was not affected by the MF treatment or PS application ($p>0.05$). However, the N absorbed in the grain exhibited the same behavior as yield. The aerial biomass generated was not significantly modified, but its N concentration was increased when FM was applied ($p\leq0.05$). In contrast, the N absorbed by the biomass exhibited the same behavior between treatments, with total N uptake being higher in SS than in C ($p\leq0.05$) (Table 5).

The PS application had a positive impact on both crops, but the impact of the different application methods varied depending on the crop. In maize, IS showed the best response in terms of production (biomass and yield), with increases similar to those obtained with MF. In contrast, in soybean, IS presented the highest yield, with values higher than those obtained with MF (Table 5). These differences can respond to different factors, such as the type of crop, the composition of the slurry used each time, the time of application, the climatic conditions of each year, and the soil type.

The increase in maize yield and biomass when the PS was incorporated (IS treatment) may be due to a better synchronization between the release and demand of N. In addition, the IS presented greater ability to translocate N from the aerial biomass to the grain, achieving greater grain N uptake, which was then reflected in yield. This may be because when the PS is mixed with the soil, as in the IS treatment, the N immobilization-mineralization process is modified. In addition, Sørensen and Amato (2002) demonstrated that when the PS is incorporated, a high proportion of microorganisms related to the decomposition of the slurry are protected in the soil matrix. Thus, a higher amount of N is retained in the microbial biomass and then released during the growing cycle, and in subsequent years. These results may be due to a decrease in the N losses by volatilization, which is more evident in summer crops with high N requirement. Therefore, the IS application method retained the N within the system more effectively for better use by maize.

The favorable response to PS applications in maize is consistent with results from other studies, such as those of Sartor et al. (2012), who reported that maize production increased linearly with PS doses, with a PS application of 60 m^{3} ha^{-1} achieving higher yield than

Table 5 Effect of three PS application methods and mineral fertilization on grain yield, biomass at maturity and N uptake in maize (2015) and soybean (2016) crops

Crops	Treatments	Yield (kg ha^{-1})		Biomass maturity (kg ha^{-1})		Grain N (%)		Biomass N (%)		Grain N uptake (kg ha^{-1})		Biomass N uptake (kg ha^{-1})		Total N uptake (kg ha^{-1})	
Maize	IS	13722	a	24147	a	1.23	a	0.63	b	168	a	67	ab	235	a
	AS	12818	ab	20729	ab	1.26	a	0.69	a	161	ab	55	ab	216	a
	SS	11318	b	20717	b	1.23	a	0.66	a	139	b	62	ab	202	a
	MF	12501	ab	22670	ab	1.22	a	0.73	a	153	ab	74	a	226	a
	C	11789	b	19378	b	1.18	a	0.51	b	140	b	39	b	179	a
Soybean	IS	1962	bc	8439	a	7.18	a	2.65	b	141	bc	222	a	362	b
	AS	2782	ab	7449	a	7.24	a	2.55	b	201	ab	192	a	393	ab
	SS	3302	a	7763	a	7.04	a	3	ab	234	a	233	a	467	a
	MF	2264	bc	8308	a	7.12	a	3.01	a	161	bc	255	a	417	ab
	C	1556	c	6612	a	7.16	a	2.79	b	111	c	187	a	298	b

IS: Incorporated Slurry, AS: Acidification Slurry; SS: Surface Slurry, MF: Mineral Fertilization, C: Control. Different letters indicate significant differences between treatments in each crop ($p\leq0.05$).

that achieved with MF. These authors attributed this higher yield to an improvement in soil fertility. On the other hand, Schröder et al. (2015) found that maize DM production for silage responded positively to PS application combined with phosphate fertilizer, whereas Carrizo et al. (2014) reported that doses of 60 and 120 kg N ha^{-1} applied with the PS resulted in higher grain production and total aerial biomass than the control treatment. In turn, also in a maize crop, Martínez et al. (2017a) obtained increases in grain yield, aerial biomass and N uptake, both with single PS applications or combined with MF, with respect to the control treatment.

In soybean, on the other hand, the highest production was obtained with the SS treatment. This response could be more related to the P contributed by the PS and its permanence in the soil (Table 5). These results are similar to those reported in other studies, where soybean yield was increased or maintained with PS applications, with respect to C and MF. By using PS applications of 112 and 224 kg N ha^{-1}, Woli et al. (2013) found increases in five out of eight sites evaluated, and no decreases compared with the C treatment. In turn, Rocha Junior et al. (2017) reported that, with PS applications of 100 m^3 ha^{-1}, yield showed a tendency to increase (13%) with respect to a dose of 25 m^3 ha^{-1}, but without significant differences. On the other hand, Maggi et al. (2013) found that PS applications led to yields similar to those of mineral fertilization.

Helmers et al. (2008) and Woli et al. (2013) have emphasized that the response of soybean yield to PS applications is not solely due to the provision of N. Among other causes, they have suggested residual effects, such as the contribution of OM and other nutrients, increased microbial activity, concentrations of substances that mimic the regulatory effects of crop growth, and other unknown factors present in the PS. In addition, Schmidt et al. (2000) pointed out that although PS has not traditionally been applied to this crop, there is the possibility of a favorable agronomic response.

As seen, most previous studies on the agronomic use of PS have evaluated the response of crops such as maize and soybean to increasing PS doses and have compared PS applications with mineral fertilizers, but few have evaluated the differential effects of different PS application methods. Some studies, however, have been made in pastures. Costa et al. (2014), for example, found that the PS application method influenced the pasture yield. These authors obtained yield increases of 45% with PS surface application, 62% with PS incorporation at 0.05 m depth, and 77% with PS incorporation at 0.1 m depth. They also found that the incorporation of PS into the soil at both depths led to higher DM yields and that this increase was proportional to the depth of application. In another study in pastures, Groot et al. (2007) found that PS injection resulted in higher recovery of N (42%) than PS surface application (26%), whereas, in an oat field where cattle slurry was applied, Fangueiro et al. (2015c) reported increases in biomass production and absorbed N, with respect to a control, but without differences between the different application methods (acidified, surface and incorporated slurries). Finally, in a ryegrass (*Lolium perenne* L.) crop, Park et al. (2018) obtained less absorption of N by applying acidified slurry, and attributing this to an inhibition of the NH_4^+ applied with the PS.

The differences in the results obtained by different authors may be associated with the heterogeneity of the slurries. In addition, the response of soil quality indicators and crops may be influenced by other factors such as the agronomic management practices, the crop type, the doses used, the time and depth of sampling, and the soil type. In addition, local soil and climatic conditions, as well as biotic factors, are also determining factors that act on the processes of mineralization and humification of nutrients and SOM.

Conclusion

The different PS application methods studied (AS, IS and SS) had similar effects on most of the chemical and microbiological indicators of soil quality here evaluated. In general, PS applications caused increases in SOC, SON, POM, NO_3^-, Pe and AP, relative to the MF and C treatments. However, they also led to an increase in EC and a decrease in pH. Therefore, these latter indicators should be monitored in the mid- and long term, to avoid negative impacts on the soil quality.

Regarding the different application methods, AN and BR were increased by IS only in the soybean crop and were able to respond to the increased contribution of plant residues resulting from the increased production of aerial maize biomass with such treatment. In maize, IS and AS were more efficient in retaining N within the soil-plant system, whereas, in soybean, the SS led to higher yields. This response of soybean may be due to a compensation of the N losses by volatilization exerted by the biological fixation of N and to the fact that soybean responded to the maintenance of P in the soil by the contribution made by the PS.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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