Different pig slurry application methods modify the soil quality and increase the productivity of winter wheat (*Triticum aestivum* L.) Crop (Córdoba, Argentina)

Diferentes métodos de aplicação de dejectos líquidos de suínos modificam a qualidade do solo e aumentam a produtividade da cultura de trigo de inverno (*Triticum aestivum* L.) (Córdoba, Argentina)

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Vanesa Romina Pegoraro
Doctora en Ciencias Agropecuarias por la Universidad Nacional de Córdoba / Facultad de Ciencias Agropecuarias
Institución: Instituto Nacional de Tecnología Agropecuaria, Área Suelo, Grupo Gestión Ambiental
Dirección: Ruta Nº 12 km 3, Marcos Juárez, Córdoba, Argentina
Correo electrónico: pegoraro.vanesa@inta.gob.ar; vanesapegoraro@gmail.com

Leticia Mir
M. Sc. en Ciencias Agropecuarias de la Universidad Nacional de Córdoba/ Facultad de Ciencias Agropecuarias
Institución: Instituto Nacional de Tecnología Agropecuaria, Área Mejoramiento Vegetal, Laboratorio de Calidad Industrial de Cereales y Oleaginosas.
Dirección: Ruta Nº 12 km 3, Marcos Juárez, Córdoba, Argentina
Correo electrónico: mir.leticia@inta.gob.ar

Martina Briacchi
Lic. en Recursos Naturales de la Universidad de Rosario/ Facultad de Ciencias Agrarias
Institución: Universidad de Rosario, Cátedra de Manejo de Tierras
Dirección: Campo Experimental Villarino, Zavalla, Santa Fe, Argentina.
Correo electrónico: briacchimartina@gmail.com

Omar Bachmeier
Doctor en Ciencias Químicas de la Universidad Nacional de Córdoba/ Facultad de Ciencias Químicas
Institución: Universidad Nacional de Córdoba. Facultad de Ciencias Agropecuarias. Cátedra de Edafología.
Dirección: Ing. Agr. Félix Aldo Marrone 746 - Ciudad Universitaria, Ciudad de Córdoba, Argentina.
Correo electrónico: obachmei@agro.unc.edu.ar

Jimena Ortiz
Doctoranda en Ciencias Agropecuarias por la Universidad Nacional de Córdoba / Facultad de Ciencias Agropecuarias
Institución: Instituto Nacional de Tecnología Agropecuaria, Área Suelo, Grupo Gestión Ambiental
Dirección: Ruta Nº 12 km 3, Marcos Juárez, Córdoba, Argentina
Correo electrónico: ortiz.jimena@inta.gob.ar

Claudio Lorenzon
Bioquímico por la Universidad Nacional de Córdoba / Facultad de Ciencias Químicas
Institución: Instituto Nacional de Tecnología Agropecuaria, Área Suelo, Grupo Fertilidad de Suelos
Dirección: Ruta Nº 12 km 3, Marcos Juárez, Córdoba, Argentina
Correo electrónico: lorenzon.claudio@inta.gob.ar

Susana Hang
Doctora en Ciencias Agropecuarias de la Universidad Nacional de Córdoba/ Facultad de Ciencias Agropecuarias
Institución: Universidad Nacional de Córdoba. Facultad de Ciencias Agropecuarias. Cátedra de Edafología
Dirección: Ing. Agr. Félix Aldo Marrone 746, Ciudad Universitaria, Ciudad de Córdoba, Argentina
Correo electrónico: shang@agro.unc.edu.ar

Marta Susana Zubillaga
Doctora Toxicología de Suelos/ Universidad de Buenos Aires/ Facultad de Ciencias Veterinaria.
Doctora Ciencias y Tecnología Ambiental/ Universidade Da Coruña/ España
Institución: Universidad de Buenos Aires. Facultad de Agronomía
Cátedra de Fertilidad de Suelos.
Dirección: Av. San Martin 4453, Buenos Aires, Argentina
Correo electrónico: zubillag@agro.uba.ar

ABSTRACT
In Argentina, slurry surface application is the technique most used, although with high risk of ammonia losses. These emissions can be reduced by using different application methods, but their impacts on the soil-plant system have been little explored. Thus, this study aimed to evaluate the effects of different pig slurry application methods: acidified slurry (AS), incorporated slurry (IS), and surface slurry (SS) on the soil quality and productivity of a wheat crop, as compared with mineral fertilization (MF) and control (C). IS caused an increase in acid phosphatase values, whereas SS presented the lowest metabolic quotient values of all methods and high values of fluorescein diacetate hydrolysis. Treatments with pig slurry and MF showed significantly lower pH values than C. SS led to a rapid increase in NO_3^- concentration, whereas AS and IS showed slower nitrification values than SS. Depending on the availability of NO_3^-, N uptake and aerial biomass showed a similar behavior. Wheat yield increased up to 57% with pig slurry application, without significant differences between the different application methods used. However, MF reached the highest yield value. We conclude that pig slurry application, mainly surface application, improves soil quality and increases wheat production.

Keywords: manure, chemical soil, microbiological soil, nitrogen dynamics.

RESUMEN
Na Argentina, a aplicação superficial de dejecto líquido é a técnica mais utilizada, embora com alto risco de perdas de amônia. Essas emissões podem ser reduzidas por meio de diferentes métodos de aplicação, mas seus impactos no sistema solo-planta têm sido pouco explorados. Assim, este trabalho teve como objetivo avaliar os efeitos de diferentes métodos de aplicação de dejetos líquidos de suínos: dejetos acidificados (AS), dejetos incorporados (IS) e dejetos superficiais (SS) na qualidade do solo e a produtividade de uma cultura de trigo, em comparação com a fertilização mineral (MF) e controle (C). IS causou aumento nos valores de fosfatase ácida, enquanto SS apresentou os menores valores de quociente metabólico de todos os métodos e altos valores de

hidrólise de diacetato de fluoresceína. Os tratamentos com dejecto líquido de suino e MF mostraram valores de pH significativamente mais baixos do que C. SS levou a um rápido aumento na concentração de NO3-, enquanto AS e IS mostraram valores de nitrificação mais lentos do que SS. Dependendo da disponibilidade de NO3-, a absorção de N e a biomassa aérea mostraram um comportamento semelhante. O rendimento do trigo aumentou até 57% com a aplicação de dejetos líquido de suínos, sem diferenças significativas entre os diferentes métodos de aplicação usados. No entanto, MF atingiu o valor de rendimento mais alto. Concluímos que a aplicação de dejetos líquido de suínos, principalmente na superfície, melhora a qualidade do solo e aumenta a produção de trigo.

Palabras claves: esterco, solo químico, solo microbiológico, dinâmica do nitrogênio.

1 INTRODUCTION

Argentina has a stock of more than 5 million pigs distributed mainly in the Humid Pampas, with 61% of the national production being concentrated in the provinces of Buenos Aires, Córdoba and Santa Fe (SENASA 2017). Extensive pig farming, which is characterized by low levels of environmental impact, is the prevailing production system in the country. However, in the last years, the use of partial or total pig confinement systems has increased significantly (Iglesias and Ghezan 2013). The intensification of the activity is generating high amounts of residues which, depending on their management, may be either an important source of fertilizers or a potential environmental hazard (Boitt et al. 2018).

An option for the management of pig slurry is its use as a nutrient source for crops (Wilson et al. 2020), since the soil can take up and process residues, thereby contributing to their cycling. In Argentina, where no-till is the most widely used system, surface application is the most frequently used technique for pig slurry application (Pegoraro et al. 2020a; Pegoraro et al. 2020b). However, the main problem associated with the use of pig slurry as a nitrogen (N) fertilization strategy is the loss of N as ammonia (NH_3) due to volatilization (Erdmann et al. 2020). Therefore, to control these losses, it is essential to investigate other techniques that improve the efficiency of application and reduce its environmental impact. Application options include incorporation to the soil (Lourenzi et al. 2014; Schröder et al. 2015) and acidification with sulfuric acid, both of which aim to prevent loss through volatilization (Fangueiro et al. 2015; Park et al. 2018).

The benefits of pig slurry application on soil physical chemical and biological properties have been frequently reported (Biau et al. 2012; Liu et al. 2010; Yagüe et al. 2012). These benefits include an increase in the ecosystem services provided by the soil, such as nutrient cycling and availability, soil structure maintenance, prevention of damage by erosion, and food resource provision. However, the use of pig slurry as fertilizer requires cautious management, since it has been demonstrated that its application alters mineralization, immobilization and soil N denitrification (Burger and Venterea 2008). These effects are closely related to the climatic and edaphic conditions of each site. Hence, this study aimed to evaluate the effects of different pig slurry application methods on soil quality and their potential benefits for wheat production. The study was conducted on Mollisols of the Pampas of Argentina, a region for which there is little information about the effects of pig slurry application.

2 MATERIALS AND METHODS

Pig slurry composition, soil and field experiment

The composition of the pig slurry used in the present study and the amount of nutrients supplied to the soil are shown in Table 1. Only slurry from fattening pigs was used. Pig slurry was collected directly from the pig houses, and the same batch was used for all the treatments.

Table 1. Composition of the pig slurry used in the present study (mean ± standard deviation, n=3) and amount of nutrients supplied to the soil.

Characteristic	Unit	Values	Quantities supplied (kg ha^{-1})
Dry matter (DM)	%	2.05±0.01	515
Organic matter	% DM	57.62±1.03	297
Ashes	% DM	42.38±1.03	218
Total N	g l^{-1}	5.08±0.07	127
NH_4^+-N	g l^{-1}	4.1±0.22	103
pH		7.47±0.08	-
Electrical conductivity	dS m^{-1}	31.73±0.29	-
Phosphorus	mg l^{-1}	43.33±1.53	1
N:P ratio		95	-
Doses	m^3 ha^{-1}	25	

The field experiment was conducted on a wheat crop in an experimental field of the National Institute of Agricultural Technology (INTA), in the southeast of Córdoba province, Argentina (32°42´44.65´´S, 62°05´46.07´´W) in 2016. Other climatic parameters of the study period are presented in Figure 1.

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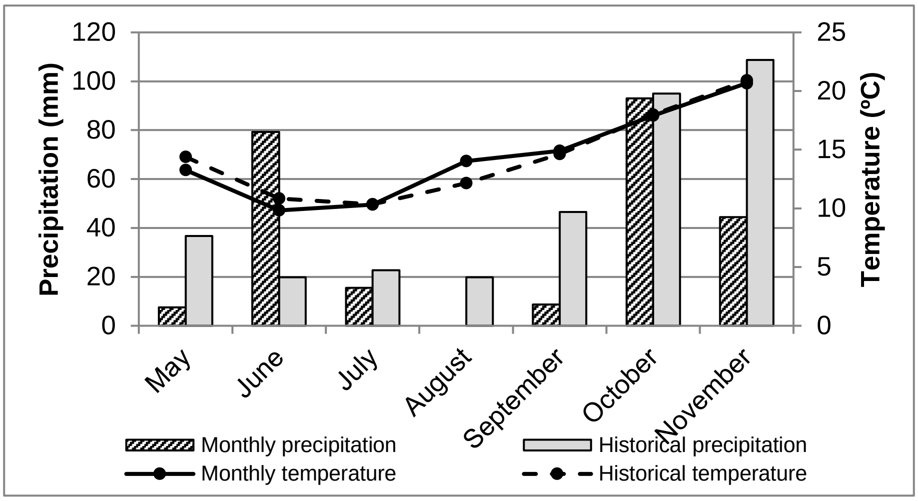
Figure 1. Monthly precipitation and air temperature recorded in the experimental site during the wheat crop growth cycle and historical monthly averages. Data from the weather station of the Experimental Station Marcos Juárez of the National Institute of Agricultural Technology (Córdoba, Argentina).



Five pig slurry application methods (treatments) were evaluated: acidified slurry (AS), incorporated slurry (IS), surface slurry (SS), mineral N fertilization (MF) and control (C). The experimental design was arranged in a randomized block with three replicates. The experimental unit consisted of a 40-m^2 plot (5 m wide x 8 m long, located at 4-m intervals to minimize interactions between plots). The pig slurry was applied by spreading, from a tank, nine days before wheat sowing.

For the AS treatment, before its application, the pig slurry was acidified with sulfuric acid until a pH of 5.5-6 was achieved (Fangueiro et al. 2015a), whereas for the IS treatment, the pig slurry 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 pig slurry, with granular solid urea at 46%, at sowing. The C soil consisted of non-amended plots.

Wheat was sown at a density of 280 plants m^2 and 17 cm between rows, in June 2016, and harvested in late December 2016. The variety Algarrobo Don Mario, intermediate cycle, was used.

Analysis of chemical and microbiological indicators of soil quality

For the analysis of chemical indicators of soil quality, five soil samples (core diameter 2.5 cm) from a depth of 0-20 cm were randomly collected from each plot and a composite sample was performed. 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 and Black) (IRAM-SAGPyA 29571-2 2011), SON by the micro-Kjeldahl digestion method (IRAM-SAGPyA 29572 -1 2011) and Pe by the Bray and Kurtz Nº 1 method (IRAM-SAGyP 29570-1 2010). 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 (IRAM 29410 1999; Rhoades 1996). These chemical parameters were determined at anthesis.

Particulate organic matter (POM) and microbiological quality indicators (fluorescein diacetate hydrolysis (FDA), acid phosphatase activity (AP), basal respiration (BR), anaerobic N (AN) and microbial biomass carbon (MBC)) were analyzed using three soil composite samples. These samples were composed of 10 soil samples (core diameter 2.5 cm) randomly collected at a depth of 0-10 cm from each plot at crop physiological maturity. Before the analysis of AP activity and BR, fresh soils (field moisture) were sieved using a 2-mm mesh and stored at 4 °C. 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 (POM) by particle size (106 μm) was conducted by the wet-sieving method proposed by Cambardella and Eliott (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). Before analysis, samples were incubated at 28 ºC for 16 h. BR was determined by measuring the CO_2 produced in a 7-day incubation experiment at 25 ºC. Briefly, 30 g of each soil sample 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 metabolic quotient (qCO_2) was inferred directly by dividing the CO_2 by the MBC. The AP activity and FDA were determined according to the method proposed by Alef and Nannipieri (1995).

Soil NO_3^- levels were determined at three crop growth stages: tillering, anthesis, and physiological maturity, i.e. growth states 29, 65 and 91, according to (Zadoks, Chang, and Konzak 1974). At tillering, samples were taken at three depths (0-20, 20-40 and 40-60 cm), whereas at anthesis and physiological maturity, samples were taken at five depths (0-20, 20-40, 40-60, 60-100 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 the colorimetric method using a spectrophotometer SPECTRUM SP-1105.

Determination of crop biomass, N content in stem and grain, and grain yield

During soil sampling, crop aerial biomass samples were also collected. At the first two stages (tillering and anthesis), aerial biomass was obtained by manual harvesting plants from 0.50 m of each of three contiguous rows (0.255 m^2). At physiological maturity, 2 m of two rows (0.680 m^2) were sampled to determine dry matter (DM), N uptake and yield. To determine DM, the aerial biomass was dried in an oven at 60 °C until constant weight. Finally, the samples were crushed to 0.5 mm for analysis. To determine N concentration in aerial biomass, the modified semi-micro kjeldahl method was used (Bremmer 1996). The results are expressed as kg ha^{-1}.

The aerial biomass samples collected at physiological maturity were threshed in a static thresher to separate the straw and chaff from the grain. Then, the grains obtained were weighed and humidity was determined by near infrared spectrometry for the determination of yield (FOSS Infratec 1241 Grain Analyzer). Grains were affected by a commercial humidity of 13.5. The N percentage in grain was calculated from the relationship between % crude protein and the conversion factor from protein to N (5.7). The results are expressed as kg ha^{-1}.

Statistical analysis

The effects of each pig slurry application method were determined by analysis of variance using the Mixed Model in the Infostat Professional software (Di Rienzo et al. 2017). The pig slurry application methods were set as fixed factors and the replications as a random effect. Means were separated by LSD Fisher test ($p \leq 0.05$). 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.

3 RESULTS AND DISCUSSION

Soil chemical properties

The results regarding the soil chemical properties (SOC, NOS, Pe, pH and EC) are shown in Table 2.

SOC and SON:

SON and SON showed changes according to the pig slurry application method used, with lower values in SS than in IS, AS and MF ($p \leq 0.05$), being significant only SON, but with no differences from C (Table 2). These responses may be due to a delay in the nitrification process of the N applied due to the application of AS and IS, causing a temporary increase in SON compared to SS (Damian et al. 2018; Fangueiro et al. 2016).

Pe concentration:

Regarding Pe, the lack of effects on the surface horizon ($p > 0.05$) may be due to the high N:P ratio of the slurry applied. Therefore, the P supplied at the rate used was low (Table 2). Similar results have been previously reported in (Pegoraro et al. 2020a) in a corn-soybean cropping system, where no changes were detected in Pe concentration in the first crop (corn). Other works have reported increases in soil P content after application of pig slurry at higher rates or in long periods of time (Balota et al. 2010; Lourenzi et al. 2013).

Soil pH and EC:

The most sensitive soil chemical indicator of the effects of pig slurry application and MF was pH, which was lower than in C ($p \leq 0.05$) (Table 2). This reduction is attributed to the production of H^+ ions as a result of hydrolysis and oxidation of the NH_4^+ present in the slurry and in urea (Divito et al. 2011). Similar results have been previously reported by Da Veiga et al. (2012) after continuous application of pig slurry for 9 years. Therefore, continuous application of pig slurry or nitrogen fertilizers at high rates may result in soil acidification, which may require remediation strategies, such as liming, in the long term. Regarding EC, no changes were detected at the time of sampling, which may respond to a washing of salts to sub-surface horizons, due to rainfall (Figure 1).

Table 2. Effects of three pig slurry application methods and mineral fertilization on chemical soil quality indicators (0-20 cm) in wheat crop (anthesis stage) (mean ± standard error, n=3).

Treatments	SOC (g kg)			SON (g kg)			Pe (mg kg)			pH			EC (dS m)		
IS	14.25	±0.42	a	1.13	±0.04	a	23.67	±3.76	a	6.22	±0.14	b	0.08	±0.003	a
AS	14.58	±1.05	a	1.16	±0.06	a	21.67	±5.24	a	6.30	±0.12	b	0.08	±0.010	a
SS	13.11	±0.15	a	1.09	±0.05	b	24.33	±1.33	a	6.18	±0.12	b	0.07	±0.000	a
MF	13.23	±0.61	a	1.14	±0.04	a	20.00	±0.58	a	6.36	±0.10	b	0.08	±0.010	a
C	14.14	±1.15	a	1.12	±0.04	ab	17.67	±2.96	a	6.44	±0.11	a	0.08	±0.003	a

IS: Incorporated slurry, AS: acidified slurry, SS: surface slurry, MF: mineral fertilization, C: control. SOC: Soil organic carbon, SON: soil organic nitrogen, Pe: extractable phosphorus, pH: soil reaction, CE: electrical conductivity EC. Different letters indicate significant differences (p≤0.05).

POM and microbiological indicators

The effects of the different treatments on POM and microbiological soil quality (AN, BR, AP, FDA, MBC and qCO_2) are summarized in Table 3.

POM and AN:

No statistical differences in POM and AN were observed among treatments. However, absolute values tended to increase slightly with pig slurry applications with respect to MF and C ($p>0.05$) (Table 3). Our results are consistent with previous works reporting that pig slurry applications either maintained or increased POM and potentially mineralizable N (Pegoraro et al. 2020a; Yagüe et al. 2012).

Enzyme activity:

Regarding general enzyme activity (FDA), the only pig slurry application method with higher activity than that of C was SS ($p\leq0.05$), with no statistical differences from IS or MF ($p>0.05$). In turn, FDA was lower in AS than in IS and SS, but did not differ from C or MF ($p\leq0.05$) (Table 3). This result may be attributed to the activity of several enzymes, which may be affected by differences in nutrient availability and inhibition of microbial activity caused by pig slurry acidification (Roboredo et al. 2012; Sørensen and Eriksen 2009). On the other hand, AP showed a slight increase with slurry applications, with no differences among methods, and with IS being the only treatment differing from MF and C ($p\leq0.05$) (Table 3). Regarding this enzyme, a previous study by (Biau et al. 2012) showed that the application of slurry on maize (Zea mays) crop caused a 40% increase in its activity. This response may be due to the slurry supply of labile nutrients to the soil, which can increase the synthesis of this enzyme (Da Silva et al. 2015). A significant and positive correlation between the activity of this enzyme and Pe was observed ($R=0.54$; $p\leq0.05$). Regarding this issue, Olander and Vitousek (2000) indicated that under low nutrient supply, enzymes are induced and nutrients are mineralized, whereas under high nutrient supply, enzymes are suppressed and mineralization ceases.

MBC, BR and qCO_2:

No significant changes in MBC or BR were detected among treatments (Table 3). Since these indicators alone are not good predictors of good environmental practices, in these situations, qCO_2 is used. qCO_2 indicates the efficiency of soil microbial populations in using organic carbon compounds. Soil stress and alterations may reduce microbial efficiency and increase qCO_2 because the microbial population needs to spend more energy in maintenance (Da Silva et al. 2015; Plaza et al. 2004). In the present study, the lowest energy expenditure was recorded in SS ($p \leq 0.05$) (Table 3). Our results agree with findings of Liu et al. (2010), who applied pig manure combined with a mineral fertilizer and obtained a lower qCO_2 than in the control treatment. In addition, the lack of differences between IS or AS and C is due to the changes generated by these slurry application methods (AS or IS) in soil microbial composition and in the processes of mineralization-immobilization of the organic compounds supplied with the slurry (Fangueiro et al. 2016; Sørensen and Eriksen 2009). The lack of differences between MF and C may be attributed to the fact that the addition of fertilizers that only contribute N is not enough to meet the nutritional needs of soil microorganisms.

Table 3. Effects of three different pig slurry application methods and mineral fertilization on particulate organic matter and microbiological soil quality indicators (0-10 cm) in wheat crop at physiological maturity (mean ± standard error, n=3).

Treatments	POM (mg kg)			AN (mg kg)			FDA (μg fluor $g^{-1}h^{-1}$)			AP (mg kg)			BR (mg kg^{-1})			MBC (mg kg)			qCO_2		
IS	16.94	±1.57	a	81.4	±1.12	a	134.04	±6.39	ab	970.99	±53.32	a	308.26	±9.81	a	204.68	±42.55	a	2.28	±0.26	a
AS	16.49	±1.38	a	79.63	±0.54	a	98.23	±11.61	c	960.75	±43.82	ab	285.74	±11.36	a	192.99	±63.91	a	2.26	±0.44	a
SS	17.26	±0.31	a	81.44	±1.40	a	142.49	±12.07	a	969.85	±24.64	ab	225.56	±88.64	a	216.38	±49.77	a	0.98	±0.17	b
MF	15.72	±167	a	78.34	±1.62	a	120.60	±9.62	abc	935.54	±53.87	b	290.82	±66.30	a	172.51	±13.95	a	2.43	±0.67	a
C	15.25	±2.21	a	77.02	±2.95	a	111.09	±2.58	bc	940.96	±52.67	b	252.82	±14.54	a	220.76	±28.80	a	2.07	±0.62	a

IS: Incorporated slurry, AS: acidified slurry, SS: surface slurry, MF: mineral fertilization, C: control. POM: Particulate organic matter, AN: anaerobic nitrogen, FDA: fluorescein diacetate hydrolysis, AP: acid phosphatase, BR: basal respiration, MCR: microbial biomass carbon, qCO_2: metabolic quotient. Different letters indicate significant differences between treatments ($p \leq 0.05$).

NO_3^- concentrations

The responses of NO_3^- concentrations to pig slurry application depended on the application methods timing of evaluation, and depth studied (Figure 2). At tillering, NO_3^- concentration was highest under MF, followed by SS, with MF differing significantly from the remaining treatments up to 40 cm in depth. At 40-60 cm in depth, NO_3^- concentrations were highest under MF, but the existence of a wide variation in the data did not allow us to detect statistical differences ($p>0.05$). In turn, no differences were detected among the pig slurry application methods (Figure 2A) or between the different methods and C. At anthesis, pig slurry and MF treatments did not show increases at the surface level (0-20 cm) as compared to C. At the remaining depths, SS had the highest NO_3^- concentrations, with statistical differences at 20-40, 60-100, and 100-150 cm from the remaining treatments at the upper depth, and from C and MF at the last two depths analyzed. In the two lower strata, the different slurry application methods had similar NO_3^- concentration (Figure 2B).

Finally, at physiological maturity, treatments tended to have similar results at most of the depths evaluated. However, AS had lower concentrations at 0-20 and 100-150 cm than IS and SS, and similar to those of C and MF, whereas IS exhibited a higher NO_3^- content at 100-150 cm than C, MF and AS, with no differences from SS (Figure 2C).

N uptake

N uptake by aerial biomass at different phonological stages depended on the pig slurry application methods and on the time of evaluation. At tillering, N uptake was higher under SS and MF than in C ($p\leq0.05$). The remaining pig slurry application methods did not differ among them or from MF ($p>0.05$). At anthesis, MF and SS showed the same trend, with higher N uptake, whereas IS and AS had similar N uptake to that of C. Finally, at physiological maturity, the highest values of N uptake were recorded under MF, whereas the pig slurry application methods did not differ between them, but did differ from C ($p\leq0.05$) (Figure 3).

Figure 2. Distribution of nitrate (NO_3^-) in the soil profile for the different treatments applied to wheat crop at A) tillering stage, B) anthesis stage and C) physiological maturity. IS: Incorporated slurry, AS: acidified slurry, SS: surface slurry, MF: mineral fertilization, C: control. Bars denote mean standard errors.

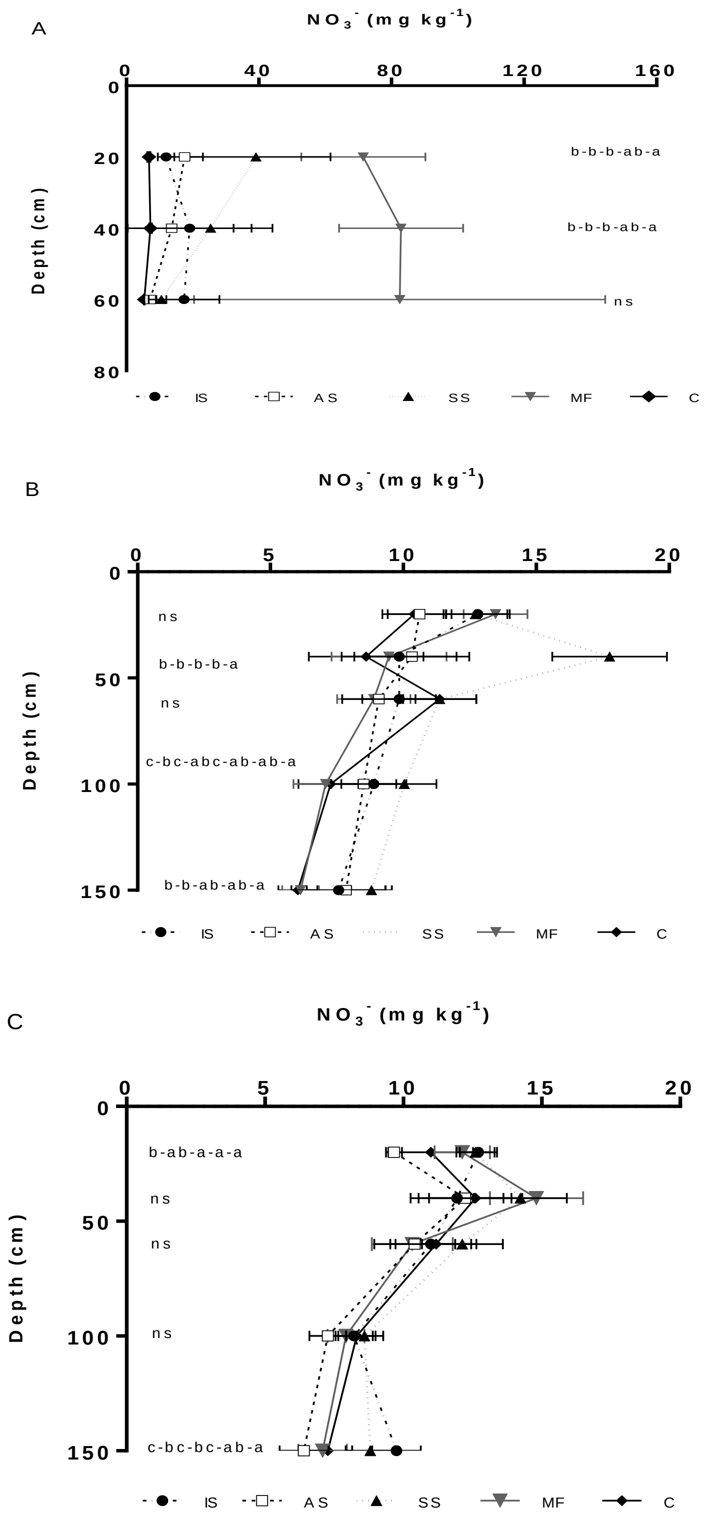
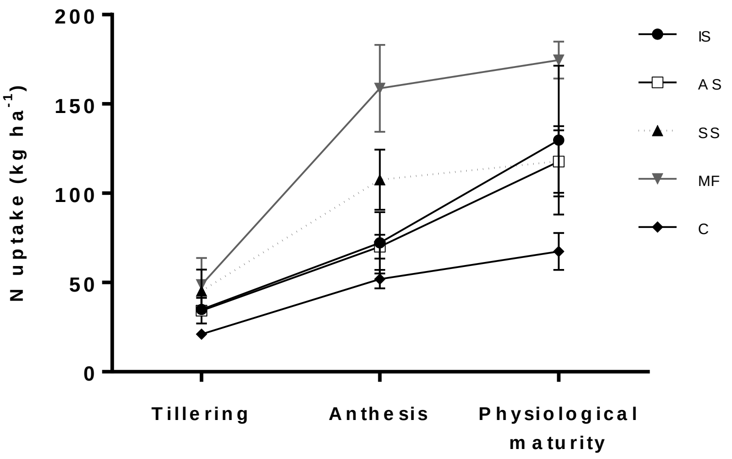


Figure 3. Nitrogen uptake (kg ha^{-1}) by wheat crop at tillering, anthesis and physiological maturity stages under different treatments. IS: Incorporated slurry, AS: acidification slurry, SS: surface slurry, MF: mineral fertilization, C: control (Bars denote mean standard errors).



Relationship between NO_3^- and N uptake

The crop response of N uptake to changes in NO_3^- concentration in the soil varied with the moment of evaluation and the application methods (Figures 2 and 3). A positive and significant correlation was observed between total N uptake and NO_3^- concentrations at tillering ($R=0.51$; $p\leq0.05$) and anthesis ($R=0.64$; $p\leq 0.01$).

SS caused a rapid increase in N uptake at the earliest crop stages with respect to C. In contrast, in IS and AS, N uptake increased significantly towards the end of the crop cycle with respect to C. Although N uptake at physiological maturity did not differ among pig slurry application methods, it increased more slowly under IS and AS. On the other hand, IS and AS did not show variations in soil NO_3^- concentration among the stages evaluated, whereas SS had the highest NO_3^- concentrations up to anthesis, subsequently decreasing towards physiological maturity. This result might be due to the slurry application technique, which modifies the N immobilization-mineralization process in the soil and, therefore, the crop capacity for N production and uptake. The more gradual variation in soil NO_3^- content and N uptake by the crop found in IS and AS is due to a slower nitrification process associated with these two management practices. Regarding IS, when the slurry is mixed with the soil, a high proportion of microorganisms associated with slurry decomposition is protected in the soil matrix; consequently, a high amount of N is retained in the microbial biomass, being released during the crop cycle and in subsequent years (2-3 years) (Hernández et al. 2013).

Slurry acidification has also been found to cause a delay in nitrification (Fangueiro et al. 2016). Thus, a proportion of the immobilized N under IS and AS may have been remineralized, generating a higher N uptake after anthesis than under the other treatments, whereas under SS, the

N immobilization process was slower, probably because of a reduced contact with the soil and, therefore, a faster nitrification process.

Differences between slurry application methods and MF were also observed. MF reached the highest NO_3^- concentrations in the soil and the highest increase in N uptake in most of the wheat crop stages evaluated. This result may be due to differences in N availability, which varies with the use of mineral fertilizers or organic amendments. When mineral fertilizers are applied, N is immediately available to crops (Salazar Martínez Lagos et al. 2015). By contrast, when slurries are applied, different processes, such as temporal immobilization of NH_4^+ in the clay mineral interlayers (Daudén and Quílez 2004) or the presence of microorganisms (Terrero et al. 2018), interfere with N availability. Sørensen and Amato (2002) showed that net immobilization of N after slurry application is significantly higher than after MF. These immobilization-mineralization processes hinder prediction of the fertilizing value of the slurry, since it can release N some years after application (Sieling et al. 2014). On the other hand, the higher NO_3^- concentration at tillering obtained with MF shows a potential for N-NO_3^- losses by leaching similar to or higher than that with slurry due to the high precipitations recorded, mainly in June (Figure 1). This result shows that, when the slurry is used correctly, the amounts of leached NO_3^- are lower than or similar to those leached with mineral fertilizers.

Yield

Wheat yield responded similarly to N uptake, with both variables showing a significant and positive correlation ($R=0.97$; $p \leq 0.01$). Yield increase was 86% under MF, and 57, 56 and 50% under IS, AS and SS, respectively, with respect to C ($p \leq 0.05$). No statistical differences were observed between MF and IS or AS ($p>0.05$), or among pig slurry applications ($p>0.05$) (Table 4). Regarding N percentage in wheat grain, MF showed the highest values, with an increase of 0.41%, followed by pig slurry applications, with increases of 0.26, 0.18 and 0.25% for IS, AS and SS, respectively, with respect to C ($p \leq 0.05$), but without statistical differences among them (Table 4).

Table 4. Effects of three pig slurry application methods and mineral fertilization on grain yield, biomass at maturity and N uptake in wheat crops (mean ± standard error, n=3).

Treatments	Yield ($kg\ ha^{-1}$)			N in grain (%)			Aerial biomass ($kg\ ha^{-1}$)			N in biomass (%)		
IS	6662	±866	ab	1.59	±0.10	b	13902	±2132	ab	0.3	±0.02	b
AS	6614	±323	ab	1.51	±0.07	b	13874	±387	ab	0.24	±0.01	c
SS	6385	±528	b	1.58	±0.02	b	13357	±1146	b	0.25	±0.01	bc
MF	7908	±104	a	1.74	±0.02	a	16469	±356	a	0.43	±0.04	a
C	4243	±288	c	1.33	±0.02	c	8551	±687	c	0.25	±0.01	bc

IS: Incorporated slurry, AS: acidified slurry, SS: surface slurry, MF: mineral fertilization, C: control. Different letters indicate significant differences between treatments ($p \leq 0.05$)

Our results are not consistent with those of Kai et al. (2008), who reported significant increases in wheat yield with application of acidified slurry compared to a non-acidified slurry. On the other hand, Damian et al. (2018) obtained higher dry matter production and grain yield with injected pig slurry than when using surface application or MF. These authors attributed the results to a higher synchronization between N release from slurry and N uptake by crops, due to a reduction of N losses by volatilization and a slower nitrification process. Regarding the differences found between pig slurry application methods and MF, wheat responded to higher NO_3^- concentrations in soils treated with MF. This treatment showed the highest increase in N uptake at all the stages evaluated (tillering, anthesis and physiological maturity). Biau et al. (2012) suggested the use of slurry induces nutrient immobilization and further release, improving soil quality. By contrast, studies conducted by Sartor et al., (2012) showed a linear increase in wheat yield at high pig slurry rates, with 30 $m^3\ ha^{-1}$ being, in general, the rate that produced higher yield than MF for all the years evaluated. Likewise, Sieling et al. (2014) found higher grain yield with pig slurry applications than with MF. This response may be due to a residual effect of the continuous slurry applications, which cause the mineralization of the organic fraction of the slurry (Cela et al. 2011).

In a previous study evaluating the same treatments on a maize-soybean sequence, we reported that, in maize, IS and AS were the most efficient treatments in retaining N within the plant-soil system, whereas SS increased soybean yield (Pegoraro et al. 2020a). This response of the soybean crop may be attributed to the fact that N losses by volatilization are offset by N biological fixation and a response to P maintenance in the soil by the contribution of pig slurry. These previous results are not consistent with our present findings, since the different methods of pig slurry application in wheat resulted in similar N retention. These findings show that winter climatic conditions favor the reduction of N losses by volatilization.

In a study involving oat (Avena strigosa) crop, Fangueiro et al. (2015b) reported increases in biomass production and N uptake with the application of cattle slurry with respect to the control, with no differences among application methods (acidified, surface and incorporated slurry). In contrast, in a ryegrass (Lolium perenne) crop treated with acidified slurry, Park et al. (2018) obtained lower N uptake and attributed this result to inhibition of the NH_4^+ supplied by the pig slurry.

Differences in results among research works may be associated with the heterogeneity of the slurries. In addition, other factors, such as agronomic management practices, type of crop, rates used, timing and depth of sampling, and soil type, influence the response of indicators of soil and crop quality. Furthermore, local edaphic and climatic conditions as well as biotic factors are determinant factors operating on nutrient mineralization and humification and SOM.

4 CONCLUSIONS

The application of pig slurry, especially surface application, improves soil quality, mainly microbiological indicators. Surface slurry application improved soil enzyme activity while reducing the metabolic quotient. In turn, the different pig slurry application methods induced changes in soil N dynamics and, consequently, in N uptake by the crop. The incorporated and acidified applications resulted in slower N release, whereas the surface application resulted in the highest N uptake and availability during the earliest crop stages. Therefore, improvements in soil quality increased the soil capacity for crop production, regardless of the application technique used. However, further studies should evaluate the residual effects of the agronomic use of pig slurry.

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REFERENCES

Alef K, Nannipieri P (1995) Methods in applied soil microbiology and biochemistry. Academic Press, London, England

Balota EL, Machineski O, Priscila E, Truber V (2010) Soil carbon and nitrogen mineralization caused by pig slurry application under different soil tillage systems. Pesqui Agropecu Bras 45:515–521. https://doi.org/http://dx.doi.org/10.1590/S0100-204X2010000500011

Biau A, Santiveri F, Mijangos I, Lloveras J (2012) The impact of organic and mineral fertilizers on soil quality parameters and the productivity of irrigated maize crops in semiarid regions. Eur J Soil Biol 53:56–61. https://doi.org/https://doi.org/10.1016/j.ejsobi.2012.08.008

Boitt G, Schmitt DE, Gatiboni LC, Wakelin SA, Black A, Sacomori W, Cassol PC, Condron LM. (2018) Fate of phosphorus applied to soil in pig slurry under cropping in southern Brazil. Geoderma 321:164–172

Bremmer J. (1965) Inorganic forms of nitrogen. In: Evans D, White J, Ensminger L, Clark F (eds) Methods of soil analysis. Part 2. Chemical and microbiological properties. Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA, pp 1179–1237

Bremmer J (1996) Nitrogen-Total. In: Sparks D, Page A, Loeppert R, Soltanpour P, Tabatabai M, Johnston C, Summer M (eds) Methods of soil analysis: Chemical methods. Part 3. Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA, pp 1085–1121

Burger M, Venterea RT (2008) Nitrogen immobilization and mineralization kinetics of cattle, hog, and turkey manure applied to soil. Soil Sci Soc Am J 72:1570–1579. https://doi.org/10.2136/sssaj2007.0118

Cambardella C, Eliott E (1993) Methods for physical separation and characterization of soil organic matter fractions. Geoderma 56:449–457. https://doi.org/https://doi.org/10.1016/B978-0-444-81490-6.50036-4

Cela S, Santiveri F, Lloveras J (2011) Residual effects of pig slurry and mineral nitrogen fertilizer on irrigated wheat. Eur J Agron 34:257–262

Da Silva D, Antoniolli ZI, Jacques Seminoti RJ, De Oliveira Silveira A, Amaro da Silva DA, Rache MM, Gomes Passos VH, Da Silva BR (2015) Indicadores microbiológicos de solo em pastagem com aplicação sucessiva de dejetos de suínos. Rev Bras Ciência do Solo 39:1585–1594

Da Veiga M, Pandolfo CM, Balbinot Junior AA, Spagnollo E (2012) Chemical attributes of a hapludox soil after nine years of pig slurry application. Pesqui Agropecu Bras 47:1766–1773. https://doi.org/10.1590/S0100-204X2012001200013

Damian JM, Muraro DS, Basso CJ, Da Silva AN, Pinto MAB, Santi AL (2018) Strategies for fertilization with pig and cattle slurry in wheat crop. Rev Bras Eng Agrícola e Ambient 261–266. https://doi.org/http://dx.doi.org/10.4067/S0718-58392014000300009

Daudén A, Quílez D (2004) Pig slurry versus mineral fertilization on corn yield and nitrate leaching in a Mediterranean irrigated environment. Eur J Agron 21:7–19. https://doi.org/https://doi.org/10.1016/S1161-0301(03)00056-X

Di Rienzo J, Casanoves F, Balzarini M, Gonzalez L, Tablada M, Robledo C (2017) InfoStat versión 2017. Grup. InfoStat, FCA. Univ. Nac. Córdoba

Divito GA, Rozas HRS, Echeverria HE, Studdert GA, Wyngaard N (2011) Long term nitrogen fertilization: Soil property changes in an Argentinean Pampas soil under no tillage. Soil Tillage Res 114:117–126. https://doi.org/https://doi.org/10.1016/j.still.2011.04.005

Echeverria HE, San Martin NF, Bergonzi R (2000) Metodos rápidos de estimación de nitrógeno potencialmente mineralizable en suelos. Cienc del Suelo 18:9–16

Erdmann LF, Cassol PC, Sacomori W, Dall'orsolleta DJ, Montovani A (2020) Incorporation in soil and addition of enzyme inhibitor as a way to increase the efficiency of pig slurry and mineral fertilizer. Rev Bras Cienc do Solo 44:. https://doi.org/10.36783/18069657rbcs20190103

Fangueiro D, Hjorth M, Gioelli F (2015a) Acidification of animal slurry– a review. J Environ Manage 149:46–56. https://doi.org/https://doi.org/10.1016/j.jenvman.2014.10.001

Fangueiro D, Surgy S, Fraga I, Cabral F, Coutinho J (2015b) Band application of treated cattle slurry as an alternative to slurry injection: Implications for gaseous emissions, soil quality, and plant growth. Agric Ecosyst Environ 211:102–111. https://doi.org/10.1016/j.agee.2015.06.003

Fangueiro D, Surgy S, Fraga I, Monteiro FG, Cabral F,Coutinho J (2016) Acidification of animal slurry affects the nitrogen dynamics after soil application. Geoderma 281:30–38. https://doi.org/https://doi.org/10.1016/j.geoderma.2016.06.036

Hernández D, Polo A, Plaza C (2013) Long-term effects of pig slurry on barley yield and N use efficiency under semiarid Mediterranean conditions. Eur J Agron 44:78–86

Iglesias DH, Ghezan G (2013) Análisis de la cadena de carne porcina en Argentina. Instituto Nacional de Tecnología Agropecuaria, Buenos Aires

IRAM-SAGPyA 29572 -1 (2011) Determinación de nitrógeno en suelo por el método Kjeldahl modificado. Parte 1 - Escala semi-micro

IRAM-SAGyP 29570-1 (2010) Determinación de fósforo extraíble en suelos. Parte 1 - Método Bray Kurtz 1 modificado (Extracción con solución de fluoruro de amonio - ácido clorhídrico). 19

IRAM-SAGyP 29571-2 (2011) Determinación de materia orgánica en suelos. Parte 2 - Determinación de carbono orgánico oxidable por mezcla oxidante fuerte, escala semi-micro. IRAM 15

IRAM 29410 (1999) Determinación del pH. IRAM 14

Jenkinson D, Powlson D (1976) The effects of biocidal treatments on metabolism in soil-V: A methods for measuring soil biomass. Soil Biol Biochem 8:209–213. https://doi.org/https://doi.org/10.1016/0038-0717(76)90005-5

Kai P, Pedersen P, Jensen JE, Hansen MN, Sommer SG (2008) A whole-farm assessment of the efficacy of slurry acidification in reducing ammonia emissions. Eur J Agron 28:148–154

Liu E, Yan C, Mei X, He W, Bing SH, Ding L, Liu Q, Liu S, Fan T (2010) Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. Geoderma 158:173–180. https://doi.org/https://doi.org/10.1016/j.geoderma.2010.04.029

Lourenzi CR, Ceretta CA, Brunetto G, Girotto E, Tiecher TL, Vieira RCB, Cancian A, Ferreira PAA (2014) Dejeto líquido de suínos e o acúmulo de nutrientes e matéria seca e a produção de grãos de culturas diversificadas. Rev Bras Cienc do Solo 38:949–958. https://doi.org/10.1590/S0100-06832014000300027

Lourenzi CR, Ceretta CA, Souza Da Silva L, Girotto E, Lorensini F, Tiecher TL, De Conti L, Trentin G, Brunetto G (2013) Nutrients in soil layers under no-tillage after successive pig slurry applications. Rev Bras Ciência do Solo 37:157–167. https://doi.org/http://dx.doi.org/10.1590/S0100-06832013000100016

Olander LP, Vitousek PM (2000) Regulation of soil phosphatase and chitinase activity by N and P availability. Biogeochemistry 49:175–190

Park SH, Lee BR, Jung KH, Kim TH (2018) Acidification of pig slurry effects on ammonia and nitrous oxide emissions, nitrate leaching, and perennial ryegrass regrowth as estimated by15N-urea flux. Asian-Australasian J Anim Sci 31:457–466. https://doi.org/https://doi.org/10.5713/ajas.17.0556

Pegoraro V, Cazorla C, Bachmeier O, Baigorria T, Boccolini M, Ortiz J, Lorenzon C, Hang S, Zubillaga M (2020a) Impacts of different pig slurry application methods on soil quality indicators in a maize-soybean cropping sequence in the sub-humid pampas of Argentina. Int J Recycl Org Waste Agric 9:259–272. https://doi.org/10.30486/IJROWA.2020.674424

Pegoraro V, Cazorla C, Masino A, Alladio M, Cottura G, Bachmeier O, Hang S, Muñoz S, Zubillaga MS (2020b) Diagnóstico del manejo de los efluentes porcinos generados en la provincia de Córdoba. Rev Investig Agropecu 46:362–370

Plaza C, Hernández D, García-Gil JC, Polo A (2004) Microbial activity in pig slurry-amended soils under semiarid conditions. Soil Biol Biochem 36:1577–1585. https://doi.org/https://doi.org/10.1016/j.soilbio.2004.07.017

Rhoades JD (1996) Electrical conductivity and total dissolved solids. In: Sparks D, Page A, Helmke P, et al. (eds) In Methods of Soil Analysis: Chemical Methods. Part 3. Soil Science Society of America and American Society of Agronomy, Madison, Wisconsin, USA, pp 417–435

Roboredo M, Fangueiro D, Lage S, Coutinho J (2012) Phosphorus dynamics in soils amended with acidified pig slurry and derived solid fraction. Geoderma 189–190:328–333

Salazar Martínez Lagos F, Alfaro M, Rosas M, Rampoldi E, Macías F (2015) Factores edafoclimáticos que afectan las emisiones de amoniaco por aplicación de urea y purín de lechería en Andosoles. Chil J Agric Anim Sci, ex Agro-Ciencia 30:15–28

Sartor LR, Assmann AL, Assmann TS, Bigolin PE, Miyazawa M, Carvalho PC (2012) Effect of swine residue rates on corn, common bean, soybean and wheat yield. Rev Bras Ciência do Solo 36:661–669. https://doi.org/http://dx.doi.org/10.1590/S0100-06832012000200035

Schröder JJ, Vermeulen GD, Van der Schoot JR, Van Dijk W, Huijsmans JFM, Meuffels GJHM, Van der Schans DA (2015) Maize yields benefit from injected manure positioned in bands. Eur J Agron 64:29–36. https://doi.org/https://doi.org/10.1016/j.eja.2014.12.011

SENASA (2017) Informes y estadísticas. http://www.senasa.gob.ar/cadena-animal/porcinos/informacion/informes-y-estadisticas#tramites. Accessed 9 Nov 2017

Sieling K, Ni K, Kage H (2014) Application of pig slurry- First year and residual effects on yield and N balance. Eur J Agron 59:13–21. https://doi.org/https://doi.org/10.1016/j.eja.2014.05.003

Sørensen P, Amato M (2002) Remineralisation and residual effects of N after application of pig slurry to soil. Eur J Agron 16:81–95. https://doi.org/https://doi.org/10.1016/S1161-0301(01)00119-8

Sørensen P, Eriksen J (2009) Effects of slurry acidification with sulphuric acid combined with aeration on the turnover and plant availability of nitrogen. Agric Ecosyst Environ 131:240–246. https://doi.org/https://doi.org/10.1016/j.agee.2009.01.024

Terrero MA, Faz Á, Ondoño S, Muñoz MÁ (2018) Impacts of raw and purified pig slurry on carbon and nitrogen contents in Mediterranean agricultural soil. In: Muñoz, M., y Zorzona R (ed) Soil management and climate change: Effects on organic carbon, nitrogen dynamics, and greenhouse gas emissions. pp 207–219

Vance E, Brookes PC, Jenkinson D (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707. https://doi.org/https://doi.org/10.1016/0038-0717(87)90052-6

Wilson ML, Niraula S, Cortus EL (2020) Nutrient Characteristics of Swine Manure and Wastewater. Anim Manure Prod Charact Environ Concerns, Manag 89–113. https://doi.org/10.2134/asaspecpub67.c6

Yagüe MR, Bosch-Serra ÁD, Antúnez M, Boixadera J (2012) Pig slurry and mineral fertilization strategies' effects on soil quality: Macroaggregate stability and organic matter fractions. Sci Total Environ 438:218–224. https://doi.org/https://doi.org/10.1016/j.scitotenv.2012.08.063

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421