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Chemical and spectroscopic composition of humic substances in soil subjected to pig manure applications for ten years

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ABSTRACT: Application of pig manure (PM) in agriculture can influence the amount and composition of soil organic matter (SOM). This study evaluated the changes in contents and stock of C in chemical fractions of SOM and the chemical and spectroscopic composition of humic substances (HS) in a Typic Hapludult (*Argissolo Vermelho-Amarelo*) after ten years of PM application. Experimental area received 90 and 180 kg ha^{-1} of N in the form of pig slurry (PS90 and PS180) and pig deep litter (DL90 and DL180), in addition to the control, without application (SA). Soil samples were prepared, and the chemical fractioning of SOM, elemental analysis, and Fourier Transform Infrared Spectroscopy of HS were performed. Applications of PM favored the accumulation of C in soil (up to 53 %), and PS180 increased humic acids (HA) (up to 185 %), while applications of DL favored the increase of hydrophilic substances extracted with HCl and humin (HU) (up to 10 times and 60 %, respectively). Applications of PS180 and DL180 promoted an increase in the aromatic and carboxylic character of the HS, increasing cationic exchange capacity. Therefore, PM applications, especially with DL, contribute positively to C fixation in the soil and the chemical composition of organic material.

Keywords: organic matter, fulvic acid, humic acid, liquid pig manure, pig deep litter, FTIR.

INTRODUCTION

The volume of pig slurry (PS) produced is high in pig farms worldwide with intensive animal husbandry systems and can generate 8.6 L animal^{-1} day^{-1} in full-cycle production systems (Diesel et al., 2002). On the other hand, the pig deep litter system (DL) was proposed to reduce the volume of manure generated. In this system, pigs are bred on a material with a high C/N ratio, such as sawdust, generating a dry residue with a higher nutrient concentration at the end of breeding. The DL can be used as fertilizer when going through a composting process, allowing greater control of its use and facilitating its transport to areas more distant from where the waste is generated (Li et al., 2017).

Frequent applications of pig manure (PM) in liquid or solid form can increase the content of soil organic matter (SOM) because of the high C content in its composition and indirectly due to the increase in crop residues added to the soil by crops, resulting from fertilization, since these wastes have macro and micronutrients essential for crop development (Ferreira et al., 2021). However, the balance between C sequestration and loss in soils is strongly dependent on the physical, chemical, and biological soil properties, climatic conditions, and biochemical composition of organic compounds (Diacono and Montemurro, 2011). Adsorption of SOM in clay minerals and iron (Fe) oxides, as well as its occlusion inside micro aggregates, are examples of important mechanisms in the process of C stabilization in soil (Yan et al., 2013). Additionally, certain organic structures, such as C-alkyl and aromatic groups, and specific compounds, such as lignin and tannin, have lower biodegradability, favoring greater stability of SOM (Lützow et al., 2006; Zhou et al., 2010). Another mechanism of great importance for the stability of organic compounds is hydrophobicity, which reduces their surface hydration and, consequently, the accessibility of these compounds to microorganisms and enzymes (Lützow et al., 2006). Therefore, the nature of the added organic material and the management adopted in the soil can change the dynamics of the SOM and, consequently, its distribution and composition.

Chemical fractioning of SOM allows the separation of three main fractions that differ in their chemical composition, and therefore in their behavior in the soil (Hayes and Swift, 2018). The alkaline-soluble fraction is composed of functioned supramolecular aggregates that can be separated into humic acids (HA) and fulvic acids (FA). Humic acids are larger structures and insoluble in acid medium and can be defined with associations of compounds of greater hydrophobic character (polymethyle chains, fatty acids, and steroids) that are stabilized in neutral pH by hydrophobic dispersive forces (van der Waals interactions, π–π and CH–π bonds) (Piccolo, 2001). Fulvic acids are smaller and more carboxylated micellar structures than HA, consisting of associations of small hydrophilic molecules, such as polysaccharides, amino acids, and phenolic compounds, which remain soluble after acidification of the medium (Piccolo, 2001). Humin fraction (HU) is insoluble in aqueous media because it is hydrophobic (more lipid than other fractions), composed predominantly of aliphatic hydrocarbons, and/or due to the strong association with minerals (Hayes and Swift, 2018). Therefore, changes in the distribution of SOM chemical fractions may promote changes in their functionality and reveal the influence of management on SOM properties (Tejada et al., 2009).

Pig slurry and DL present different proportions of certain organic compounds in their composition due to the different chemical composition of the sources and the process of obtaining the bed (Provenzano et al., 2014; Wang et al., 2015). Infra-red spectra obtained with Fourier transform (FTIR) showed composting the solid phase of the PS reduced the intensity of the bands 2920-2930 cm^{-1} and 2851 cm^{-1} as a result of the decrease in aliphatic structures, and the band of 1560 cm^{-1}, by the biodegradation of amino acid chains (Provenzano et al., 2014). Also, during composting, the band intensity at 1640 cm^{-1} increased, which demonstrates the enrichment of aromatic structures in the material. Additionally, the composting of manure, such as from pigs, chickens, and cattle, promotes changes in the distribution of humic substances, increasing the levels of HA and reducing those of FA during this stabilization process (Wang et al., 2015). In addition to the increase in the degree of humification, these authors observed the C, N, S, and H levels tend to reduce during composting, with increased ash levels, and bovine manure presented the highest C levels and C/N ratio among the studied residues. In an incubation study, the applications of various sources of pig manure (PS, solid manure, and biocarbon) promoted different FTIR spectra of HS concerning the intensities of the identified groups (Yanardağ et al., 2015). This effect, in turn, varied depending on the soil type. Therefore, the addition of manure in the soil can change the distribution and composition of HS, and this effect can be differentiated depending on the form in which it was added and the type of soil. However, this information is scarce in sandy soils cultivated with annual crops in a conservation system with a long history of application of different kinds of manure in a subtropical climate.

This study aimed to evaluate the changes in the contents and stock of C in the chemical fractions of the SOM and the chemical and spectroscopic composition of humic substances in a Typic Hapludult (*Argissolo Vermelho-Amarelo Distrófico*) (Santos et al., 2018) located in the subtropical region, after ten years of successive applications of liquid manure and pig deep litter.

MATERIALS AND METHODS

Location and characterization of the study area

Soil samples used in the present study were collected in an experiment installed in 2002 in the Rio Cachorrinhos micro watershed, in the municipality of Braço do Norte, Santa Catarina, southern Brazil (Latitude 28° 14’ 20.7” S and Longitude 49° 13’ 55.5” W, altitude of 300 m). The climate is Cfa (humid subtropical climate), according to the Köppen classification system (Alvares et al., 2013), with average annual temperatures of 18.7 °C, with a maximum of 35 °C and a minimum of 0 °C. There is no defined dry season, with a trend of concentrated rainfall in the summer months and an annual average rainfall of 1,471 mm.

Cultivation system of the experiment was a no-till system (NTS), with a succession of oats/corn, using no herbicides, where only the corn ears were harvested, and the rest of the crops were kept in each plot. The soil of the experimental area was classified as Typic Hapludult soil (Soil Survey Staff, 2014) and, before the implementation of the experiment, presented the following properties in the 0.00-0.10 m layer: 19.4 g kg^{-1} total organic carbon (TOC); 5.1 pH measured in water (1:1); 5.5 SMP index; 19 mg dm^{-3} available P, and 130 mg dm^{-3} of exchangeable K (extracted by Mehlich-1); 0.8, 3.0 and 0.8 $cmol_c$ dm^{-3} of exchangeable Al, Ca, and Mg, respectively (extracted per KCl 1 mol L^{-1}); 7.7 $cmol_c$ dm^{-3} of H+Al; 11.9 and 4.9 $cmol_c$ dm^{-3} of CEC to pH7.0 and effective, respectively; and 34.8 and 16.2 % saturation by bases and by Al, respectively.

In December 2002, 6 Mg ha^{-1} of limestone (total neutralization relative power = 87.5 %) was applied to the soil surface, before installing the experiment, to raise the pH of the soil in water to 6.0. Subsequently, the treatments were installed: control treatment, with no application of fertilizers (SA); fertilization with liquid pig manure, equivalent to the recommendation of N (90 kg ha^{-1} yr^{-1}) for the corn crop (PS90); fertilization with liquid pig manure equivalent to twice the recommended dose of N (180 kg ha^{-1} yr^{-1}) for the corn crop (PS180); pig deep litter fertilization equivalent to the recommendation of N (90 kg ha^{-1} yr^{-1}) for the corn crop (DL90), and pig deep litter fertilization equivalent to twice the recommended dose of N (180 kg ha^{-1} yr^{-1}) for the corn crop (DL180) (CQFS-RS/SC, 2004).

The experimental design used was randomized blocks, with five treatments and three replicates, each block with dimensions of 4.5 × 42.0 m, separated from each other by a corridor one meter wide. Each treatment was formed by experimental units of 4.5 × 6.0 m (27 m^2). The liquid waste was collected in a full-cycle pig breeding system on the same property where the experiment was installed. Pig deep litter was obtained from the pig experimental field of Embrapa Swine and Poultry, located in the municipality of Concordia, Santa Catarina State, southern Brazil. Deep litter used in pig breeding was composed of sawdust substrate. The main characteristics of PS and DL are presented in table 1.

Table 1. Chemical characteristics and amount of nutrients added via pig slurry (PS) and pig deep litter (DL) during the experimental period (2002-2012)

Material	Dry matter	Nutrients contained in dry matter				Total amount added					
		C	N	P	K		Pig manure$^{(1)}$	C	N	P	K
	%	kg m^{-3}					m^3 ha^{-1}	Mg ha^{-1}			
PS	2.07	5.99	2.31	1.85	1.29	PS90	798.1	4.78	1.48	1.33	1.03
						PS180	1596.2	9.56	2.95	2.66	2.06
	%	g kg^{-1}					Mg ha^{-1}	Mg ha^{-1}			
DL	48.68	180.0	18.3	32.3	22.4	DL90	199.3	3.69	1.06	2.50	2.18
						DL180	398.6	7.38	2.13	5.01	4.35

(1) Volume (PS) or weight (DL) of pig manure applied during the experimental period.

The required amount of PS and DL to meet the demand for N for the oat/corn succession in each treatment, used from 2002 to 2012, was established according to the recommended dose for the crops (CQFS-RS/SC, 2004). Thus, the amount of PS90 and PS180 applied was defined by estimating the dry matter (DM), the concentration of N in the PS, and considering the mineralization of 80 % of the total N contained in the residue. The amount of DL90 and DL180 applied was calculated considering the concentration of N in the bed and the mineralization of 50 % of the total N contained in the residue.

Pig slurry and DL were the only sources of nutrients added to the soil surface in the NTS for the oat/corn succession throughout the experimental period. Pig slurry doses were divided and applied three times during the corn cultivation, as follows: 1st application at 15 days after sowing (DAS); 2nd application at 45 DAS; and 3rd application at 95 DAS. For the black oats crop, 30 and 60 kg N ha^{-1} were applied in PS90 and PS180, respectively, at 15 DAS. In total, 40 PS applications were carried out in ten years of experiment. For DL, ten applications were performed during the experimental period, only 15 to 30 days before the implementation of each corn crop. No DL was applied in the black oats cycles. Black oats were sown using a density of 120 kg ha^{-1} of seeds, while corn was grown with a row spacing of 0.90 m, with five plants m^{-1}, totaling approximately 55,555 plants ha^{-1}.

Collection and chemical characterization of the soil

A trench with 0.30 × 0.40 × 0.50 m dimensions was opened in the center of each experimental unit. Soil samples were collected using a cutting shovel and knives in the layers of 0.000-0.025, 0.025-0.050, 0.05-0.10, 0.10-0.20, and 0.20-0.40 m. Samples were air-dried, ground, and sifted through a 2.00 mm mesh sieve to obtain the fine air-dried soil (FADS).

Particle size composition determined in FADS (Claessen, 1997) was, on average, 269.4 g kg^{-1} of clay, 540.2 g kg^{-1} of sand, and 190.4 g kg^{-1} silt in the profile. Chemical characterization (Table 2) was determined according to Tedesco et al. (1995). Total organic carbon content (C_{Total}) was determined by the Walkley & Black method from the oxidation of wet organic matter with external heating, using a solution of potassium dichromate and sulfuric acid. Content of C_{total} was adjusted from a calibration curve with contents obtained by dry combustion (975 °C) using an elemental "Vario El" analyzer. Walkley & Black is a well-established method for determining organic carbon (OC), widely used in evaluations and studies in the last 50 years. However, this method presents as a major limitation the incomplete oxidation of OC and the inconsistency in the oxidation efficiency in different soils, influenced by the contents and quality of organic material, soil depth, soil texture, land-use and soil management. Therefore, a practical strategy to improve this method accuracy is applying a correction factor (Bahadori and Tofighi, 2017; Gessesse and Khamzina, 2018; Ramamoorthi and Meena, 2018).

Chemical fractionation of SOM

Soil organic matter chemical fractioning was performed according to the method described by Almeida et al. (2012). A volume of 30 mL of demineralized water was added in 1.0 g of soil in falcon tubes of 50 mL to remove particulate organic matter (POM) from the SOM, with a density <1.0 g cm^{-3}. The suspension was stirred in a horizontal agitator for 2 h and centrifuged at 1529 *g* for 10 min. The supernatant was filtered in a paper filter (0.45 µm), previously weighed, and the procedure was repeated three times. The POM contained in the filter was dried in a forced circulating air-drying oven at 60 °C and subsequently weighed. Subsequently, 30 mL of HCl 0.1 mol L^{-1} was added to the soil, and the suspension was stirred for 2 h. Later, the samples were centrifuged (10 min at 1529 *g*), and the supernatant was collected and reserved, repeating the procedure three times. The extract containing hydrophilic SOM and low molecular weight extractable with HCl solution (Potes et al., 2010) had its volume quantified, and an extract aliquot (20 mL) was stored.

Table 2. Chemical properties of Typic Hapludult soil without the application of manure (SA) and after ten years of applications with 90 and 180 kg ha^{-1} of N in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180)

Layer	pH(H_2O)(1)	N Total	K	P	Ca^{2+}	Mg^{2+}	Al^{3+}	Al+H	S(2)	CEC_{efet}	CEC_{pH7}	m(3)	V(4)
m		g kg^{-1}	mg kg^{-1}		cmol$_c$ dm^{-3}							%	
SA													
0.000-0.025	5.08	1.87	139.13	85.18	4.19	2.71	0.65	7.10	7.25	7.90	14.35	8.23	50.56
0.025-0.050	5.04	1.11	85.92	58.44	2.34	2.20	0.63	7.86	4.76	5.39	12.62	11.74	37.91
0.05-0.10	4.86	1.03	59.32	30.79	1.36	1.69	1.01	6.96	3.20	4.21	10.16	23.88	31.47
0.10-0.20	4.57	0.84	41.76	13.35	0.77	1.30	2.25	8.36	2.17	4.42	10.53	51.04	21.07
0.20-0.40	4.19	0.94	31.42	5.35	0.62	0.49	2.08	10.83	1.19	3.27	12.02	63.12	9.99
PS90													
0.000-0.025	5.14	1.67	272.40	516.04	4.40	2.06	0.12	7.94	7.16	7.27	15.10	1.59	47.44
0.025-0.050	5.28	1.26	196.15	393.33	4.88	2.11	0.03	8.03	7.49	7.52	15.52	0.45	48.29
0.05-0.10	5.25	0.78	155.35	284.59	1.23	1.61	0.17	7.35	3.23	3.40	10.58	4.86	30.69
0.10-0.20	5.27	0.65	67.17	79.80	1.14	1.22	0.10	5.78	2.53	2.63	8.31	3.65	30.52
0.20-0.40	4.75	0.69	47.32	14.67	1.03	1.00	0.10	7.20	2.15	2.25	9.35	4.52	23.13
PS180													
0.000-0.025	5.07	1.57	396.63	476.13	9.09	2.52	0.42	7.86	12.62	13.04	20.48	3.21	61.67
0.025-0.050	5.39	1.69	339.75	406.11	8.42	2.35	0.10	8.45	11.64	11.74	20.09	0.84	58.21
0.05-0.10	5.31	0.93	237.05	340.58	7.05	1.54	0.17	7.28	9.19	9.36	16.47	1.77	55.87
0.10-0.20	5.05	0.68	72.50	200.11	5.57	1.55	0.10	8.91	7.30	7.40	16.21	1.38	45.40
0.20-0.40	4.31	0.51	36.89	15.49	4.52	0.57	1.05	10.48	5.18	6.23	15.66	17.21	33.10
DL90													
0.000-0.025	5.72	2.48	610.23	615.93	9.64	2.71	0.23	6.30	13.91	14.14	20.21	1.70	68.33
0.025-0.050	5.94	1.69	463.36	542.12	12.26	2.40	0.13	5.25	15.84	15.98	21.09	0.82	75.06
0.05-0.10	5.96	1.18	376.53	348.66	8.77	1.51	0.02	4.86	11.25	11.27	16.10	0.15	69.94
0.10-0.20	6.17	0.80	305.44	193.94	9.97	1.17	0.13	4.08	11.92	12.05	16.00	1.08	74.43
0.20-0.40	5.40	0.71	163.44	32.31	7.75	0.72	0.20	5.37	8.89	9.09	14.26	2.22	62.30
DL180													
0.000-0.025	5.19	2.79	706.30	919.21	13.06	3.41	0.28	8.96	18.28	18.57	27.24	1.53	66.92
0.025-0.050	5.46	1.82	582.55	746.82	14.33	3.02	0.10	9.02	18.84	18.94	27.86	0.54	67.58
0.05-0.10	5.47	1.16	504.81	435.41	9.96	2.09	0.08	7.54	13.34	13.42	20.88	0.61	63.88
0.10-0.20	5.54	0.63	394.23	243.27	8.26	1.14	0.12	6.59	10.41	10.53	17.00	1.10	61.23
0.20-0.40	4.93	0.52	274.25	32.07	6.76	1.29	0.07	9.56	8.76	8.82	18.32	0.77	47.78

(1) pH in H_2O at a ratio of 1:1; (2) Sum of bases; (3) Saturation of $CEC_{efective}$ by Al; (4) $CEC_{ph7.0}$ base saturation.

Thirty milliliters of NaOH 0.5 mol L^{-1} were added to the soil, and the suspension stirred for 3 h and then centrifuged (10 min at 1529 *g*) to extract soluble humic substances (HS). This procedure was repeated until the supernatant became clear (three to five extractions). The total volume of the alkaline extract (EA) was measured, and an aliquot (5 mL) was stored at 4 °C. The remaining solution was acidified to pH 2.0 with HCl solution 4.0 mol L^{-1} and the suspension was kept at rest for 24 h for HA precipitation. Precipitated HA was separated from the FA by centrifugation (10 min at 1529 *g*), subjected to washing with distilled water, and dried in a forced circulating air-drying oven at 60 °C, obtaining the solid fraction of the HA. The volume of the solution containing FA was quantified and stored at 4 °C.

Carbon contents and stock in SOM fractions

Carbon content in the POM (C_{POM}) was obtained by dividing the POM content, which was determined gravimetrically, by 1.724 (Van Bemmelen factor). Carbon content in acid (C_{HCl}), alkaline (C_{EA}), and fulvic acids (C_{FA}) extracts was quantified by measuring absorbance at 580 nm (UV/Vis V1 spectrophotometer Model 600) after oxidation of C with potassium dichromate in an acidic medium at 60 °C for 4 h (Dick et al., 1998). Standard curve was elaborated from solutions with glucose in different concentrations (0, 5, 10, 20, 40, 80, 120, and 240 mg L^{-1} of C). The measured C in each separate fraction corresponded only to that fraction and related to its abundance.

Carbon content of humic acids (C_{HA}) and the humin fraction (C_{HU}) was calculated using equations 1 and 2, respectively. The HA/FA and (HA + FA)/HU ratios were calculated based on these data.

$$C_{HA} = C_{EA} - C_{FA} \qquad \text{Eq. 1}$$

$$C_{HU} = C_{Total} - (C_{POM} + C_{EA} + C_{HCl}) \qquad \text{Eq. 2}$$

Carbon stocks in the SOM chemical fractions were calculated by the “equivalent soil mass” (Ellert and Bettany, 1995). This method normalizes the densities of the layers of the treatments as a function of the density of the reference layer to equal its soil mass to the soil mass of the reference layer (SA treatment). Equation 3 was used for this method:

$$EC = [C] \times L \times d \times 10 \qquad \text{Eq. 3}$$

in which: [C] is the concentration of C in g kg^{-1}; L is the soil layer thickness, in meters; d is the soil layer density, in Mg m^{-3}. Sample density was obtained from Comin et al. (2013) from the same experimental area.

Purification of samples and spectroscopic and elemental characterization

Humic acid samples were purified by treatment with 30 mL of HF/HCl 5 % solution (v/v). The suspensions were stirred for 2 h and centrifuged (10 min at 1529 *g*), repeating this procedure five times. The remaining mass (purified HA) was washed with distilled water by mechanical stirring for 30 min and the suspension was centrifuged (5 min at 1100 *g*). This procedure was repeated five times. Next, the purified HA were dried in a forced circulating air-drying oven at 60 °C (Dick et al., 2005). The solutions containing the FA fraction were purified by the passage in XAD-8 resin previously acidified with HCl 0.1 mol L^{-1}. After adding the FA to the column, it was eluted with NaOH 0.1 mol L^{-1}. The eluate was acidified to pH 2.0 with HCl 0.1 mol L^{-1} and kept under stirring for 3 h to ensure protonation of the carboxylic groups. In the end, the solutions were dialyzed in membranes (cut-off of 1000 Da, SUPELCO) until the negative test for chloride with $AgNO_3$ concentrated solution, and freeze-dried. Purification of the HU fraction was carried out by treatment with 10 % HF solution (30 mL) and stirring for 2 h. Then, the samples were centrifuged (10 min at 2500 rpm), and the supernatant was discarded. This procedure was repeated five times. The concentrated HU was washed with distilled water three times (30 mL and 30 min of mechanical stirring) and subsequently dried at 60 °C.

A few layers of the treatments were selected to obtain the infrared spectra with Fourier transform (FTIR) and elemental analysis, as follows: HA samples from the 0.000-0.025 m layer, purified and not purified from the SA, PS180, and DL180 treatments; purified FA samples from the 0.000-0.025 m layer of the SA, PS90, PS180, DL90, and DL180 treatments; and purified HU samples from the 0.00-0.025 and 0.10-0.20 m layers of the SA, PS180, and DL180 treatments.

The FTIR spectra were obtained in KBR tablets (dried at 105 °C) in the sample: KBR ratio of 1:100, in the spectral range of 4000-400 cm^{-1}, 32 scans, and resolution of 4 cm^{-1} (Shimadzu FTIR 8300). The absorption bands were assigned according to Tan (1996).

Relative intensities (RI) of the main absorption bands were calculated according to Gerzabek et al. (2006), dividing the intensity of a given peak (e.g., around 2920, 1715, 1640, 1545, and 1070 cm^{-1}) by the sum of the intensities of all the peaks considered, and multiplying by 100 %.

Ash contents (Tz) of the purified samples were determined gravimetrically after heating in a muffle at 550 °C for 4 h. Contents of C, N, and H of the fractions were determined by elemental analysis (Fisons Instrument elemental analyzer model EA 1108). Oxigen content was calculated by difference (Equation 4).

$$O\ (\%) = 100 - (C + N + H + Tz)\% \qquad \text{Eq. 4}$$

Contents of C, N, H, and O were corrected for their percentage considering only the volatile solids (VS) (Equation 5) from the humic fractions, i.e., for an ash-free base.

$$SV = M_{FH} - Mz \qquad \text{Eq. 5}$$

in which M_{FH} is the mass of the humic fractions (FA, HA, and HU) before muffle heating and Mz is the mass (Ash) of the respective fractions after drying.

Atomic ratios C/N, O/C, and H/C were calculated using equations 6, 7, and 8, with the corrected percentages of C, N, H, and O, respectively.

$$\text{Atomic ratios } \frac{C}{N} = \frac{\left(\dfrac{C\%}{\text{Atomic mass of } C}\right)}{\left(\dfrac{N\%}{\text{Atomic mass of } N}\right)} \qquad \text{Eq. 6}$$

$$\text{Atomic ratios } \frac{O}{C} = \frac{\left(\dfrac{O\%}{\text{Atomic mass of } O}\right)}{\left(\dfrac{C\%}{\text{Atomic mass of } C}\right)} \qquad \text{Eq. 7}$$

$$\text{Atomic ratios } \frac{H}{C} = \frac{\left(\dfrac{H\%}{\text{Atomic mass of } H}\right)}{\left(\dfrac{C\%}{\text{Atomic mass of } C}\right)} \qquad \text{Eq. 8}$$

Statistical analysis

Longitudinal nature of this research likely violates the essential assumption of independent residuals in the ANOVA due to the sampling at different layers and the analysis of fractions from the same sample. Ignoring this essential premise of ANOVA is a common practice in the agricultural sciences and should be refrained from. To address this issue, the model used was longitudinal data analysis via the implementation of ANOVA with repeated measurements. In this analysis, the depths and organic matter fractions were treated as repeated measures.

All the C content and C stock data were submitted to repeated measures analysis of variance (ANOVA). Normality and homoscedasticity of the residuals were tested. A few variables failed to meet the criteria in these tests, and standard data transformations such as logarithmic or square root functions were not successful.

In fact, the violation of these premisses was caused by a small number of outliers (5 instances out of 450, around 1 % of the total). However, these outliers degraded the normality and homoscedasticity of the residuals. Consequently, Partial Least Square Regression was used to estimate these five instances from the remaining dataset. In this way, the full data covariance is taken into account, and all relevant information is included in the estimation of this missing data.

Considering this is a field experiment with several analytical fractionation steps, we consider only 1 % of outliers is acceptable. After replacing these outliers, the residues were normally distributed according to the Jarque-Bera test of normality and homogeneously distributed according to Bartlett's test for homogeneity of variances. When statistical significance was detected, the means were compared by Duncan's test at $p = 0.05$.

RESULTS AND DISCUSSION

C content and stock in SOM fractions

Applications of PS and DL promoted similar changes in the contents (Table 3) and stocks of C (Table 4) in the soil and in the different fractions of the SOM when compared with SA and, therefore, will be discussed jointly. However, the contents and stocks of C presented a distinct behavior in the profile. While the C_{total} in soil and fractions were higher in the most superficial layers (0.00-0.025 and 0.0025-0.050 m) the reverse occurred with C stocks, where the 0.10-0.20 and 0.20-0.40 m layers presented the highest results. In this sense, it was observed depth had an exponential effect on the contents of total C and SOM fractions (POM, FA, HA and HU), except for HCl fraction, which presented poorly adjusted regression curves. Despite the lower C levels in the lower layers, the higher soil volume in these layers resulted in higher stocks (Table 4).

Content of C_{total} up to the depth of 0.10 m of soils fertilized with PS180, DL90, and DL180 was greater than in the SA soil (Table 3). However, in the soil under PS90, the content of C_{total} in the 0.000-0.025 m layer was lower than in SA. In the 0.10-0.20 m layer, only DL180 had higher C_{total} content than the SA soil. There was no difference in the contents in the 0.20-0.40 m layer. A similar result was obtained for the C stock (Table 4). This increase of C_{total} up to 0.10 m can be related to the higher production of dry matter of the crops (Table 5) due to the application of fertilizers that promotes the accumulation of nutrients in the surface layers in the NTS. The combined effect of the addition of residues on the soil surface, associated with the use of PM, contributes to the addition of C in the soil (Lourenzi et al., 2011; Ferreira et al., 2021). However, the decrease in C_{total} in PS90 may be the result of the "priming effect", where the addition of organic material rich in nutrients and with labile compounds favored the mineralization of plant residues and the natural C of the soil (Angers et al., 2010). Thus, this lower dose of PS, which has a lower value for the C/N and C/P ratios than DL (Table 1), may have promoted greater mineralization of SOM (Kuzyakov et al., 2000; Kuzyakov and Bol, 2006; Kuzyakov, 2010).

Fertilizers application did not promote changes in C_{POM} levels compared to SA soil (Table 3). However, C_{POM} contents in PS180, DL90 and DL180 were 43, 32 and 46 % higher, respectively, compared to the SA soil, in the 0.000-0.025 m layer. Additionally, in DL180 and PS180 soils, the C_{POM} content was higher in the 0.000-0.025 m layer compared to the

Table 3. Carbon content in the chemical fractions of soil organic matter without fertilization (SA) and with 90 and 180 kg ha^{-1} of N in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180)

Layer	SA	PS90	PS180	DL90	DL180
m	mg g^{-1}				
C_{Total}					
0.00-0.025	42.45 ± 1.03 aD	39.93 ± 0.82 aE	49.02 ± 1.20 aC	52.59 ± 1.45 aB	65.33 ± 0.61 aA
0.025-0.05	30.95 ± 0.90 bC	28.68 ± 1.06 bC	40.31 ± 1.78 bB	42.64 ± 1.30 bAB	43.01 ± 0.82 bA
0.05-0.10	21.71 ± 0.44 cC	19.91 ± 0.71 cC	26.43 ± 0.75 cAB	27.19 ± 0.61 cA	24.84 ± 1.24 cB
0.10-0.20	17.01 ± 0.62 dBC	15.85 ± 0.59 dC	17.82 ± 1.55 dB	19.15 ± 0.66 dA	17.64 ± 0.43 dAB
0.20-0.40	13.15 ± 0.68 eAB	12.55 ± 1.32 eB	14.01 ± 0.52 eA	13.58 ± 0.56 eAB	13.34 ± 0.61 eAB
C_{POM}					
0.00-0.025	2.45 ± 0.27 aA[1]	2.36 ± 0.41 aA	3.51 ± 0.42 aA	3.23 ± 0.58 aA	3.58 ± 0.27 aA
0.025-0.05	2.31 ± 0.45 aA	2.24 ± 0.32 aA	1.83 ± 0.24 bA	2.07 ± 0.30 abA	1.78 ± 0.39 bA
0.05-0.10	1.61 ± 0.06 aA	1.62 ± 0.20 aA	2.09 ± 0.12 bA	2.18 ± 0.32 abA	1.56 ± 0.10 bA
0.10-0.20	1.61 ± 0.18 aA	1.21 ± 0.30 aA	1.89 ± 0.03 bA	1.86 ± 0.21 abA	1.65 ± 0.15 bA
0.20-0.40	1.12 ± 0.30 aA	1.17 ± 0.31 aA	1.31 ± 0.46 bA	1.72 ± 0.58 bA	1.43 ± 0.27 bA
C_{HCl}					
0.00-0.025	0.84 ± 0.21 aABC	0.69 ± 0.16 aBC	0.27 ± 0.07 aC	1.82 ± 0.02 aAB	2.01 ± 0.08 aA
0.025-0.05	0.70 ± 0.12 aB	0.59 ± 0.06 aB	0.50 ± 0.08 aB	1.94 ± 0.18 aA	2.13 ± 0.32 aA
0.05-0.10	0.86 ± 0.14 aAB	0.65 ± 0.05 aAB	0.34 ± 0.01 aB	1.82 ± 0.06 aA	1.81 ± 0.10 aA
0.10-0.20	0.23 ± 0.05 aC	0.76 ± 0.29 aBC	0.80 ± 0.05 aBC	2.06 ± 0.03 aA	1.78 ± 0.12 aAB
0.20-0.40	0.16 ± 0.11 aB	0.74 ± 0.12 aAB	0.81 ± 0.09 aAB	1.73 ± 0.06 aA	1.64 ± 0.06 aA
C_{FA}					
0.00-0.025	3.23 ± 0.08 aB	3.95 ± 0.03 aAB	3.99 ± 1.55 aA	3.48 ± 0.08 aB	2.68 ± 0.34 aB
0.025-0.05	2.64 ± 0.10 abBC	3.22 ± 0.06 aAB	3.80 ± 1.22 aA	2.10 ± 0.17 abBC	1.71 ± 0.11 abC
0.05-0.10	1.78 ± 0.08 bcA	1.77 ± 0.25 bA	1.70 ± 0,12 bA	0.94 ± 0.16 bcA	1.14 ± 0.14 bcA
0.10-0.20	1.13 ± 0.41 cA	1.09 ± 0.23 bA	1.30 ± 0.12 bA	0.53 ± 0.16 cA	0.55 ± 0.21 bcA
0.20-0.40	0.27 ± 0.15 dA	0.62 ± 0.08 bA	0.63 ± 0.18 bA	0.44 ± 0.15 cA	0.25 ± 0.02 cA
C_{HA}					
0.00-0.025	5.56 ± 0.49 aB	3.37 ± 0.36 aC	9.03 ± 0.29 aA	5.85 ± 0.31 aB	8.84 ± 0.86 aA
0.025-0.05	5.53 ± 0.65 aB	2.18 ± 0.62 abC	8.12 ± 0.44 aA	6.07 ± 0.19 aB	5.63 ± 0.45 bB
0.05-0.10	3.30 ± 0.42 bB	1.74 ± 0.27 bcC	6.31 ± 1.12 bA	3.06 ± 0.13 bB	3.48 ± 0.31 cB
0.10-0.20	2.81 ± 0.37 bA	0.71 ± 0.54 cB	2.64 ± 1.02 cA	2.12 ± 0.12 bA	2.67 ± 0.14 cA
0.20-0.40	0.68 ± 0.02 cB	0.34 ± 0.29 cB	2.22 ± 0.94 cA	2.85 ± 0.09 bA	2.75 ± 0.13 cA
C_{HU}					
0.00-0.025	30.38 ± 1.14 aC	29.55 ± 0.35 aC	30.21 ± 2.10 aC	38.51 ± 1.05 aB	48.21 ± 1.68 aA
0.025-0.05	19.76 ± 0.98 bD	20.69 ± 1.65 bD	26.06 ± 3.03 aC	30.46 ± 1.23 bB	31.76 ± 1.24 bA
0.05-0.10	14.17 ± 0.07 cC	14.13 ± 1.22 cC	15.99 ± 0.79 bB	19.19 ± 0.89 cA	16.85 ± 1.46 cB
0.10-0.20	11.23 ± 0.69 dB	12.09 ± 0.99 dAB	11.70 ± 1.80 cAB	12.59 ± 1.11 dA	11.00 ± 0.37 dB
0.20-0.40	11.45 ± 0.74 dA	9.69 ± 1.29 eB	9.05 ± 0.63 dB	6.84 ± 1.09 eC	6.65 ± 0.66 eC

Means followed by the same uppercase letter in each line and lowercase in the columns do not differ significantly by the Duncan test, $p<0.05$.

other layers. In contrast, the largest C_{POM} stocks were found in the 0.20-0.40 m layer in all treatments (Table 4). Changes in the C_{POM} are related to changes in soil management or use because the POM is considered a labile fraction, composed of particles of partially decomposed plant and animal residues, fungal hyphae, spores, root fragments, and seeds with recognizable anatomical structure, susceptible to attack by the soil microbial population (Liaudanskiene et al., 2013). The increase in the contents of C_{POM} in DL180 and PS 180 is a direct result of the addition of C by waste and cultural remains on the soil surface. These materials, deposited on the soil when they undergo decomposition

Table 4. Carbon stock in the chemical fractions of soil organic matter without fertilization (SA) and with 90 and 180 kg N ha^{-1} in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180)

Layer	SA		PS90		PS180		DL90		DL180	
	Mg ha^{-1}	%	Mg ha^{-1}	%	Mg ha^{-1}	%	Mg ha^{-1}	%	Mg ha^{-1}	%
m	C_{Total}									
0.00-0.025	12.49 dCD	12.9	11.73 dD	12.9	14.42 dBC	13.2	15.45 dB	13.9	19.22 cA	17.3
0.025-0.05	9.91 eB	10.2	9.18 eB	10.1	12.91 dA	11.8	13.65 eA	12.3	13.77 eA	12.4
0.05-0.10	14.74 cB	15.2	13.51 cB	14.9	17.94 cA	16.5	18.46 cA	16.6	16.86 dA	15.2
0.10-0.20	23.27 bBC	24.0	21.66 bC	23.9	24.86 bB	22.8	26.20 bA	23.5	24.12 bB	21.8
0.20-0.40	36.40 aB	37.6	34.66 aC	38.2	38.80 aA	35.6	37.57 aAB	33.7	36.92 aB	33.3
Sum	96.81	100.0	90.75	100.0	108.93	100.0	111.34	100.0	110.89	100.0
	C_{POM}									
0.00-0.025	0.72 bA	9.3	0.69 bA	9.3	1.03 bcA	11.1	0.95 bcA	9.2	1.06 bA	11.9
0.025-0.05	0.74 bA	9.5	0.72 bA	9.7	0.59 cA	6.4	0.66 cA	6.4	0.57 bA	6.4
0.05-0.10	1.09 bA	14.0	1.10 bA	14.9	1.42 bcA	15.4	1.48 bcA	14.3	1.06 bA	11.8
0.10-0.20	2.19 abA	28.0	1.65 abA	22.4	2.58 abA	28.0	2.54 bA	24.5	2.25 bA	25.2
0.20-0.40	3.08 aA	39.3	3.22 aA	43.7	3.61 aA	39.1	4.73 aA	45.6	3.98 aA	44.7
Sum	7.83	100.0	7.38	100.0	9.22	100.0	10.37	100.0	8.91	100.0
	C_{HCl}									
0.00-0.025	0.24 aA	13.1	0.20 bA	5.2	0.08 bA	2.2	0.54 cA	5.4	0.59 cA	6.2
0.025-0.05	0.22 aA	12.1	0.19 bA	4.9	0.16 bA	4.5	0.62 cA	6.2	0.68 bcA	7.2
0.05-0.10	0.62 aA	33.7	0.44 abA	11.4	0.23 bA	6.6	1.24 bcA	12.4	1.23 bcA	12.9
0.10-0.20	0.31 aB	16.9	1.04 abAB	26.6	0.85 abB	23.9	2.81 bA	28.2	2.43 bA	25.7
0.20-0.40	0.45 aC	24.1	2.03 aBC	52.0	2.22 aB	62.8	4.78 aA	47.9	4.54 aA	47.9
Sum	1.85	100.0	3.90	100.0	3.54	100.0	9.98	100.0	9.48	100.0
	C_{FA}									
0.00-0.025	0.95 aA	17.9	1.16 aA	17.8	1.50 aA	17.8	0.93 aA	22.2	0.79 aA	22.1
0.025-0.05	0.85 aA	16.0	0.98 aA	15.0	1.43 aA	15.0	0.67 aA	16.1	0.55 aA	15.4
0.05-0.10	1.21 aA	22.7	1.20 aA	18.4	1.15 aA	18.4	0.64 aA	15.3	0.78 aA	21.8
0.10-0.20	1.55 aA	29.3	1.48 aA	22.7	1.78 aA	22.7	0.72 aA	17.4	0.75 aA	21.2
0.20-0.40	0.75 aA	14.1	1.71 aA	26.1	1.75 aA	26.1	1.21 aA	29.0	0.69 aA	19.5
Sum	5.30	100.0	6.54	100.0	7.61	100.0	4.16	100.0	3.56	100.0
	C_{HA}									
0.00-0.025	1.64 bAB	14.4	0.99 aB	20.6	3.03 bcA	16.0	1.73 bAB	10.4	2.61 bcAB	13.5
0.025-0.05	1.77 bAB	15.5	0.70 aB	14.6	2.60 cA	13.7	1.94 bAB	11.8	1.80 cAB	9.3
0.05-0.10	2.24 abB	19.7	1.18 aB	24.7	4.29 abA	22.7	2.08 bB	12.6	2.36 bcAB	12.2
0.10-0.20	3.84 aA	33.8	0.98 aB	20.5	3.63 bcA	19.2	2.89 bA	17.5	3.66 bA	18.8
0.20-0.40	1.89 bC	16.6	0.93 aC	19.5	5.39 aB	28.4	7.89 aA	47.7	8.96 aA	46.2
Sum	11.38	100.0	4.78	100.0	18.94	100.0	16.53	100.0	19.39	100.0
	C_{HU}									
0.00-0.025	8.93 dC	12.4	8.69 cC	12.8	8.92 dC	12.9	11.31 dB	16.1	14.17 bA	20.5
0.025-0.05	6.33 eC	8.8	6.62 dBC	9.7	8.35 dAB	12.1	9.75 eA	13.9	10.16 cA	14.7
0.05-0.10	9.62 cB	13.4	9.58 cB	14.1	10.85 cB	15.7	13.03 cA	18.5	11.44 cAB	16.5
0.10-0.20	15.36 bAB	21.4	16.51 bAB	24.2	16.03 bAB	23.2	17.23 bA	24.5	15.04 bB	21.7
0.20-0.40	31.66 aA	44.0	26.77 aB	39.3	25.03 aC	36.2	18.97 aD	27.0	18.41 aD	26.6
Sum	71.91	100.0	68.18	100.0	69.17	100.0	70.29	100.0	69.22	100.0

Means followed by the same uppercase letter in each line and lowercase in the columns do not differ significantly by the Duncan test, $p<0.05$.

Table 5. Production of black oats dry matter and corn grain during ten years of swine manure application in a Typical Hapludult soil in Braço do Norte, SC, Brazil

Crops	Treatments				
	SA	PS90	PS180	DL90	DL180
	Mg ha^{-1}				
Black oats	6.96	8.81	9.29	9.44	9.96
Corn	2.36	4.39	4.86	4.83	5.89

PS90: pig slurry, at a dose equivalent to the recommended amount of N; PS180: pig slurry, at a dose equivalent to twice the recommended amount of N; DL90: pig deep-litter, at a dose equivalent to the recommended amount of N; DL180: pig deep-litter, at a dose equivalent to twice the recommended amount of N.

and transformation, originate POM, remaining in the soil for months or even years (Zhou et al., 2010). He et al. (2015) also found the use of pig manure for 17 years in soils cultivated with corn/wheat succession increased the content of C of the POM in the 0.00-0.20 m layer compared to mineral fertilization and control areas (without fertilization). The authors reported that the increase in C in the POM was due to the greater contribution of plant residues in the areas fertilized with pig manure and the greater microbial activity, which may have provided greater decomposition of plant residues for later POM formation.

Applications of DL increased the content and stock of C_{HCl} in 0.10-0.40 m layer and C_{HCl} contents in 0.025-0.050 m layer to the SA soil (Tables 3 and 4). Considering SOM extracted with dilute HCl solution may be related to microbial activity because they are composed of hydrophilic and low molecular weight structures (Potes et al., 2010), it is inferred the addition of overlapping bed led to an environment of greater microbial activity when compared to other treatments, mainly in the soil subsurface layers. These C_{HCl} results are according to Couto et al. (2013) and Morales et al. (2016), who observed higher microbial biomass in soils fertilized with DL, in research conducted in the same experimental area of the present study. However, according to Huang et al. (2006) and Provenzano et al. (2014), the initial composting stages are characterized by the fast degradation of compounds of easy degradability, resulting in an organic material of greater aromatic character at the end of the process. This strengthens the hypothesis that the largest stocks of C_{HCl} present in soils with DL is a direct result of the decomposition of organic compounds regularly added to the soil by successive additions of DL. Additionally, higher stocks in the 0.20-0.40 m layer demonstrate the greater solubility of the compounds present in this fraction, which is favored by the low clay contents in the soil (Lützow et al., 2006; Kramer et al., 2012). The PS180 treatment only showed an increase in the C stock of the deepest soil layer (0.20-0.40 m) (Table 4), which may result from the migration of these hydrophilic compounds along with the soil profile.

Applications of PS and DL did not change the stocks of C_{FA} for the SA soil (Table 4). Additionally, there was no change in stocks at depth. However, PS180 applications increased C_{FA} contents in the most superficial soil layers (0.00-0.05 m) compared to the SA soil and DL applications (Table 3). This result may be related to the nature of the organic fertilizers applied. Because they undergo no stabilization process, such as composting or biodigestion, the PS has more labile structures and more hydrophilic character (Provenzano et al., 2014), which would contribute to the FA fraction.

Applications of PS180 elevated the contents of C_{HA} in the 0.00-0.025 and 0.025-0.050 m and the contents and stock of C_{HA} in the 5-10 and 20-40 cm layers compared to the SA soil (Tables 3 and 4). The addition of DL180 raised C_{HA} contents in the 0.000-0.025 and 0.20-0.40 m layers, and DL90 in the 0.20-0.40 m layer. Application of DL additionally promoted the highest C_{HA} stocks in the 0.20-0.40 m layer. In contrast, fertilizations with PS90 reduced the levels of C_{HA} to the depth of 0.20 m and stock in the 0.10-0.20 m layer. The absence or little changes in FA and the increase in HA with PS180 and DL180 show the formation of larger supramolecular structures when applying considerable amounts of fertilizers to the soil.

Applications of DL90 and DL180 increased C_{HU} contents to a depth of 0.10 m, relative to the SA soil (Table 3). A similar result was obtained for the C_{HU} stock, up to a depth of 0.05 m (Table 4). The PS90 achieved more prominent results at greater depths (0.20 m for content, and 0.10 m for stock). However, there was a decrease in the contents and stock of C_{HU} in the 0.20-0.40 m layer with the addition of PS and DL compared to the SA soil. The most outstanding levels and stocks of HU, especially in soils with DL, indicate a considerable amount of SOM is associated with soil minerals, which is an important mechanism to protect organic compounds in the soil. However, the reduction of this fraction in the deeper layer also reveals the effects of PM applications are much more significant in the more superficial layers (Rodrigues et al., 2021). Thus, other strategies should be applied to promote the greater accumulation of C in-depth in these no-till systems with oats and corn succession.

Regarding C contents and stocks in the soil and SOM fractions, it is worth highlighting the Walkley & Black method, due to incomplete oxidation of organic carbon (OC), can lead to underestimated results (Bahadori and Tofighi, 2017; Gessesse and Khamzina, 2018). Some studies indicate the Walkley & Black method can oxidize between 60 and 80 % of soil organic carbon, others with even lower values, between 48 and 58 % (Ramamoorthi and Meena, 2018; Kumar et al., 2019), requiring an adjustment with data obtained from dry oxidation via automated analyzer, with correction factors that can vary from 1.25 to 1.85, but depending on the soil properties, these correction factors can reach even higher values (Shamrikova et al., 2023). Variations in correction factors are related to the ability of the Walkley & Black method to oxidize organic carbon depending on soil properties and the temperature and oxidation time in the method (Kumar et al., 2019; Roper et al., 2019). Therefore, specific correction factors can be observed depending on the soil type, management system, vegetation and soil depth, promoting more appropriate adjustments (Bahadori and Tofighi, 2017). Therefore, the results obtained in the present study, although meticulously analyzed, must also be interpreted with caution, as the determination of C with the Walkley & Black method, even with the adjustments made, may have been underestimated, including in the evaluations carried out in the different layers. In the future, it would be interesting to carry out similar assessments, determining organic carbon in the soil only with more precise methods, with dry oxidation via automated analyzer.

Humic fractions distribution

Considering the distribution of fractions in the SA treatment (Figure 1), most of the C is present in the HU fraction, representing approximately 70 % of the total composition up to the depth of 0.20 m and more than 80 % in the 0.20-040 m layer. The large share of the HU fraction in the stock of C_{total} in the soil can be a favorable characteristic since the HU may be partly associated with the mineral fraction of the soil, being protected by the aggregates (Kalisz et al., 2010; Guimarães et al., 2013). There was a decrease in the proportion of C_{HU} and a concomitant increase in the C_{HA} fraction up to the depth of 0.40 m in the PS180 treatment. An inverse behavior was observed for PS90, mainly up to a depth of 0.20 m. There was a general increase in the proportion of C_{HU} up to 0.10 m of depth, with a decrease in the C_{FA} and C_{HA} fraction for the treatments with DL compared to the SA treatment. There is a comparatively decrease in the proportion of C_{FA} and C_{HU} and a relevant increase of C_{HCl} and C_{POM} and C_{HA} between 0.10 and 0.40 m in soils fertilized with DL compared to SA soil. These results indicate the addition of fertilizers containing pig manure in the NTS, in addition to increasing C levels, changes the humification dynamics up to at least 0.40 m.

In the SA, PS180, DL90, and DL180 treatments, the HA/FA ratio is greater than 1, indicating the more stable character of HS due to the predominance of larger supramolecular structures (Table 6) (Campos et al., 2010; Lüdtke et al., 2016). Increased HA/FA ratio was also observed in samples of Haplic Acrisol grown with lettuce at 64 days after application of pig manure composted with sawdust that had been previously acidified (Lüdtke et al., 2016).

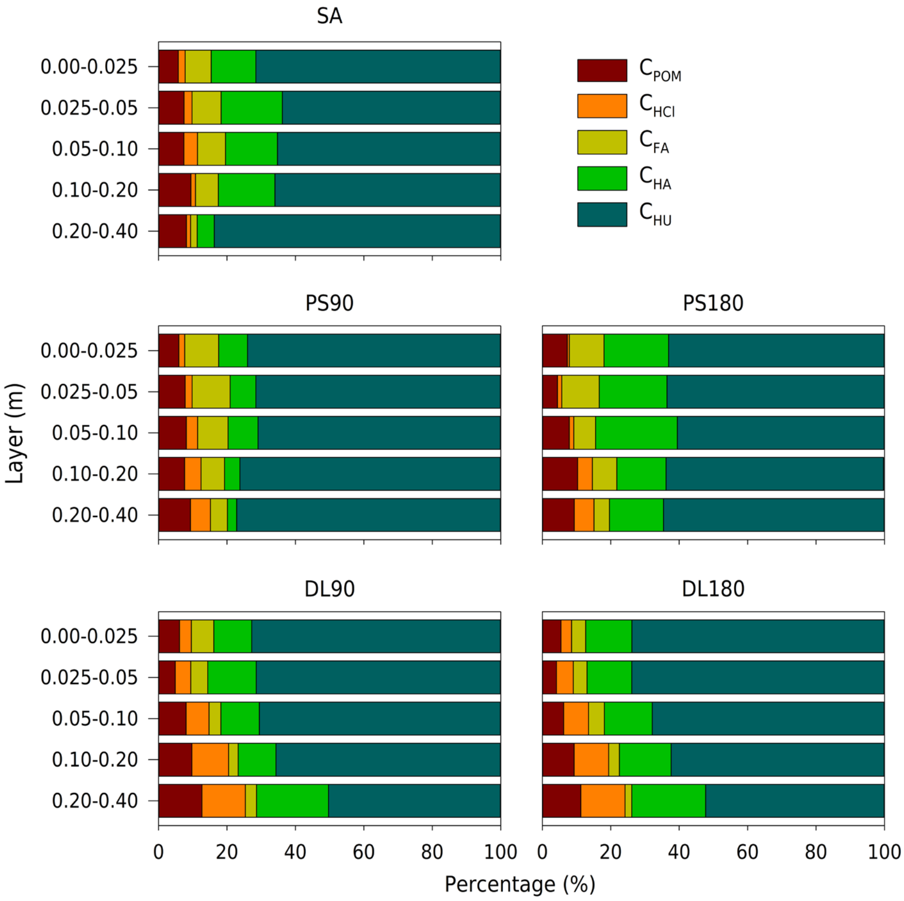

Figure 1. Distributions of the chemical fractions of SOM without fertilization (SA) and with 90 and 180 kg ha^{-1} of N in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180).

Table 6. Relations between the chemical fractions of soil organic matter with and without 90 and 180 kg ha^{-1} of N fertilization (SA) in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180)

Layer	SA	PS90	PS180	DL90	DL180
m					
			C_{HA}/C_{FA}		
0.00-0.025	1.72	0.85	1.85	1.68	3.29
0.025-0.05	2.09	0.68	1.81	2.89	3.29
0.05-0.10	1.86	0.98	3.72	3.27	3.05
0.10-0.20	2.48	0.65	2.02	3.99	4.87
0.20-0.40	2.55	0.54	3.50	6.49	11.01
			$C_{HA}+C_{FA}/C_{HU}$		
0.00-0.025	0.29	0.25	0.46	0.24	0.24
0.025-0.05	0.41	0.26	0.48	0.27	0.23
0.05-0.10	0.36	0.25	0.50	0.21	0.27
0.10-0.20	0.35	0.15	0.34	0.21	0.29
0.20-0.40	0.08	0.10	0.32	0.48	0.45

HA: Humic Acid; FA: Fulvic Acid; HU: Humin.

Lower values of the HA/FA ratio were observed in soils fertilized with PS90, which is mainly due to the decrease in HA (Table 3). Due to its smaller supramolecular size and greater functionalization, the FA may eventually promote greater mobility of nutrients linked to its structure when changing the supramolecular aggregate (Piccolo, 2001). This fact can become problematic due to the high levels of Cu and Zn in the soil, added by pig manure, which can present greater mobility when complexed by FA (Violante et al., 2010; Hattab et al., 2014).

In general, lower values of the (HA+FA)/HU ratio were observed in soils with DL and PS90 application compared to the SA treatment, up to a depth of 0.20 m (Table 6), indicating these treatments lead to lower functionalization of SOM compared to the SA. However, the elevation of this ratio in the last evaluated layer (0.20-0.40 m) in DL90 and DL180 may be related to the illuviation of organic C (Campos et al., 2010; Guimarães et al., 2013), in addition to favoring the formation of the HA fraction to HU (Tables 4 and 5). The PS180 treatment presented the highest values for HA + FA/HU up to the depth of 0.10 m, indicating the application of liquid waste at this dose leads to the formation of a more functionalized SOM and, therefore, with greater contribution to CEC.

Chemical composition of the humic fractions evaluated by FTIR and elemental spectroscopy

Levels of C and H in FA in SA were lower and the levels of O were higher than those obtained in PS180 and DL180 in the 0.000-0.025 m layer (Table 7). The C/N interface followed the order PS180>DL180>SA. The DL180 applications increased the H/C ratio and reduced the O/C ratio of the FA, revealing a more aliphatic FA character, with less presence of oxygenated groups. This result agrees with those obtained by Plaza et al. (2003), who observed high doses of PS favor functionalized FA less (and therefore lower O/C ratio) and aliphatic more. This result may be due to the preferential decomposition of more labile compounds during composting, as already reported in manure composting studies (Huang et al., 2006; Marcato et al., 2009; Wang et al., 2015). Therefore, the addition of DL would promote increased aromaticity in FA. Thus, the higher concentrations of O and the lower H/C ratio can also be attributed to the higher polysaccharides concentration in FA in SA (Provenzano et al. 2014).

Elemental composition of HA in the 0.000-0.025 m layer was less affected by treatment than FA (Table 7). Fertilizations with PS180 and DL180 promoted a decrease in the contents of C and H and an increase in O. The HA in DL180 presented a higher O/C ratio than in the FA, indicating comparatively greater functionalization of this fraction. Huang et al. (2006) also observed a higher O/C ratio and lower H/C ratio of HA compared to FA after 64 days of composting pig manure with sawdust. The authors attribute this result to the increase of groups containing O, such as carboxylic, ketonic (C=O), and phenolic and alcoholic (OH), and relative enrichment of unsaturated and aromatic structures. Additionally, these authors observed increased total acidity (COOH and phenolic OH) of HA during composting, strengthening the hypothesis of increased reactivity of this fraction.

As for the HU fraction, the elemental composition data varied little between the treatments, indicating this fraction is the least affected by the treatments (Table 7). However, when evaluating the HU data between the 0.000-0.025 and 0.10-0.20 m layers, the H/C ratio decreases in-depth in SA, indicating an increase in aromaticity, as is usually observed in soils. This decrease also occurred in the PS180 and DL180 treatments but was less pronounced. On the other hand, the increase in the O/C ratio in-depth, indicating an increase in oxygen groups, was more significant in these treatments than in SA. These results suggest treatments with pig manure led to the formation of humin fraction less hydrophobic in-depth than in SA, which may be related to the migration of hydrophilic soluble compounds as evidenced by data from C_{HCl} already discussed earlier.

Table 7. Element compositions and anatomic ratios between the chemical fractions of soil organic matter, corrected to ash-free basis, in soil with and without 90 and 180 kg ha^{-1} of N fertilization (SA) in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180)

Treatments	Layer	C	H	N	O	C/N	H/C	O/C
	m	————		%	————			
		FA						
SA	0.00-0.025	41.2	5.4	4.0	49.4	12.16	1.58	0.90
PS180	0.00-0.025	47.3	7.1	3.5	42.1	15.70	1.79	0.67
DL180	0.00-0.025	51.5	7.9	4.3	36.4	14.12	1.84	0.53
		HA						
SA	0.00-0.025	50.2	6.4	4.7	38.7	12.38	1.53	0.58
PS180	0.00-0.025	48.5	6.0	4.4	41.1	12.77	1.47	0.64
DL180	0.00-0.025	48.6	6.1	4.8	40.5	11.85	1.51	0.62
		HU						
SA	0.00-0.025	45.5	6.8	3.7	44.0	14.22	1.79	0.73
PS180	0.00-0.025	45.4	6.8	3.6	44.2	14.74	1.80	0.73
DL180	0.00-0.025	45.0	6.4	2.8	45.8	18.49	1.71	0.76
SA	0.10-0.20	45.2	5.4	3.6	45.8	14.53	1.43	0.76
PS180	0.10-0.20	42.6	6.2	3.3	47.9	15.18	1.76	0.84
DL180	0.10-0.20	40.3	5.5	3.2	51.1	14.77	1.63	0.95

HA: Humic Acid; FA Fulvic Acid; HU: Humin.

The FTIR spectra of FA, HA, and HU of all treatments presented a similar pattern (Figure 2) and the main absorption bands identified and their respective assignments were: bands in 3441-3396 cm^{-1}, assigned to the stretching vibrations of OH group (of carboxyl, phenol, water, and alcohol); two bands in the 2920 and 2850 cm^{-1} region, referring to the aliphatic C-H stretch; band in 1722-1706 cm^{-1}, due to the stretching vibrations of C=O bonds of the carboxyl groups, ketones, and aldehydes; band in the 1652-1635 cm^{-1} region, related to the structural vibrations of aromatic C=C stretch, asymmetric vibrations of COO groups, deformation vibrations of adsorbed water molecules, and N-H groups of amides (amide I); bands around 1560-1516 cm^{-1}, attributed to the N-H deformation, C-N stretch (amide II), and COO and C=C bonds; bands in 1462-1418 cm^{-1}, due to aliphatic C-H deformation, N-H stretch bands, and C-N stretch; bands in the 1386-1374 cm^{-1} region, referring to a complex of several groups, such as the aliphatic C-H, stretching COO of deprotonated carboxylic acids (carboxylate), and C-OH of phenolic compounds; bands around 1268-1222 cm^{-1}, attributed to the C-O stretch and OH deformation of carboxylic groups; two bands in the 1164-1110 cm^{-1} and 1096-1057 cm^{-1} regions, referring to the polysaccharides C-O stretch, and refers to the oscillation of the C-O bonds, including those present in the epoxy and alkoxy groups; and a band in 1044-1026 cm^{-1}, attributed to the Si-O vibration of inorganic material that has not been removed by purification with HF (Tan, 1996; Pérez et al., 2004; Shulga et al., 2017; Silva et al., 2020; Machado et al., 2020; Song et al., 2021).

Addition of PS180 and DL to the FA of the 0.000-0.025 m layer altered the relative intensities of the functional groups compared to the SA soil (Table 8). The greatest values of IR_{2920} and/or IR_{1640} and IR_{1545} and lower value of IR_{1070} in these treatments indicate a relative increase in amine/amide structures and aromatic and/or aliphatic groups and a decrease in carbohydrate structures with the addition of pig manure. Comparing these results with the data in table 7, it is inferred the higher C/N value in FA is determined mainly by the more relevant increase in aromatic groups (DL180) or aliphatic structures (PS180) than nitrogen structures. Compared to FA of SA, the higher H/C ratio of PS180 FA (Table 7) is due to its higher aliphaticity (IR_{2920}= 13.4), while for the FA of DL180, the higher value of H/C may be due to the greater presence of carboxylic groups (IR_{1715}= 22.3) and amines (IR_{1545}= 15.2).

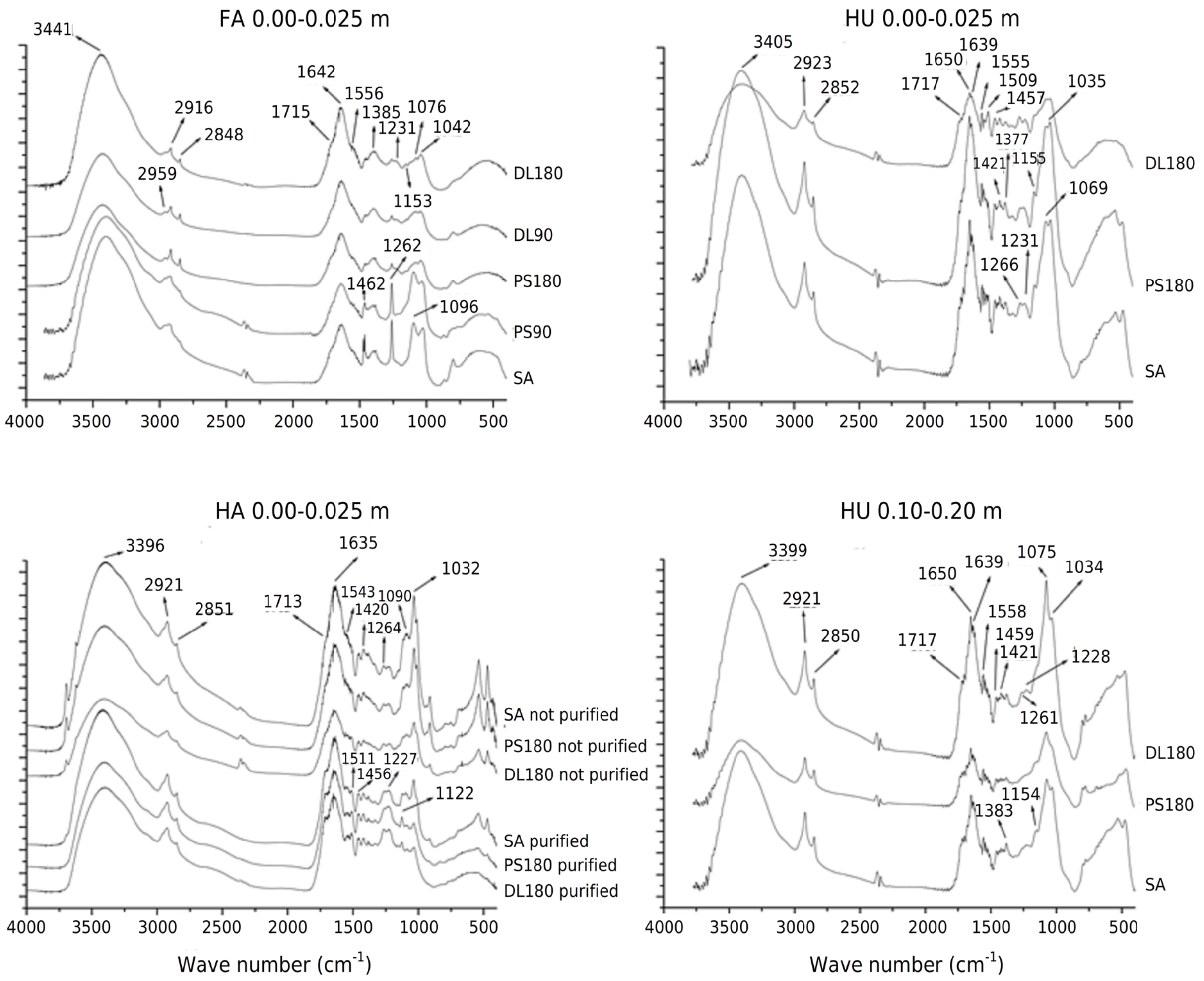

Figure 2. FTIR spectra of fulvic (FA), humic (HA), and humin (HU) acids in soil without fertilization (SA) and with 90 and 180 kg ha^{-1} of N in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180).

In the case of HA, the applications of manure little influenced the relative intensities of HA (Table 8). The highest value of IR$_{1715}$ in HA of DL180 and PS180 may be related to the respective lower O/C value (Table 7), indicating a decrease in the functionalization of HA after applying pig manure. The decrease in the ratio and reduction of IR$_{1070}$ in the PS180 and DL180 treatments and the reduction of IR$_{2920}$ in DL180 may suggest HA in these fertilized soils have a higher proportion of carboxylic groups, especially in DL180. Additionally, it is possible to observe the process of purification of the HA samples promoted the reduction of IR$_{1070}$ and increased IR$_{2920}$ and IR$_{1715}$ in all treatments. Purification promoted the reduction of IR$_{1545}$ in soils with PS180 and DL180. The sharpest decrease in IR$_{1070}$, influenced by the reduction of the band around 1030, demonstrates the purification process contributed significantly to the removal of mineral components from the soil, identified by the presence of silicon, but not completely. It was evident that some HA and HU samples, especially without purification, presented bands close to 1070 cm^{-1} with narrow and highlighted peaks, related to the presence of minerals, such as kaolinite (Figure 2).

The spectra of the HU fraction presented a very intense band around 1034-1070 cm^{-1}, which is probably due to the presence of Si structures that have not been removed by treatment with HF 10 %. The high ash content obtained in this fraction (from 18 to 65 %) confirms the presence of inorganic structures. As a result, the relative intensities were not calculated. All the HU spectra presented the bands of the aliphatic groups (2920-2860 cm^{-1}) with good resolution, confirming the aliphatic character of this fraction comparable to that of HA and FA identified by the H/C ratio data (Table 7).

Table 8. Relative intensity (IR) of FTIR bands of the fractions of soil organic matter with and without 90 and 180 kg ha^{-1} of N fertilization (SA) in the form of liquid manure (PS90 and PS180) and pig deep litter (DL90 and DL180)

Treatments	Layer	Fraction	IR_{2920}	IR_{1715}	IR_{1640}	IR_{1545}	IR_{1070}
	m				%		
		FA					
SA	0.00-0.025	Purified	8.2	17.4	28.8	11.4	34.2
PS90	0.00-0.025	Purified	8.6	15.2	26.7	11.0	38.6
PS180	0.00-0.025	Purified	13.4	19.7	35.3	13.9	17.6
DL90	0.00-0.025	Purified	10.3	17.3	37.9	16.9	17.7
DL180	0.00-0.025	Purified	7.1	22.3	40.1	15.2	15.2
		HA					
SA	0.00-0.025	Purified	10.0	25.5	36.0	12.1	16.3
SA	0.00-0.025	Not purified	8.4	20.9	35.3	11.2	24.2
PS180	0.00-0.025	Purified	10.1	28.8	35.8	12.5	12.8
PS180	0.00-0.025	Not purified	7.1	21.8	35.3	14.3	21.4
DL180	0.00-0.025	Purified	9.3	29.5	35.8	13.4	11.9
DL180	0.00-0.025	Not purified	6.4	21.8	35.0	16.2	20.5
		HU					
SA	0.00-0.025	Purified	12.5	14.8	26.7	13.4	32.6
PS180	0.00-0.025	Purified	13.2	14.5	28.2	14.0	30.1
DL180	0.00-0.025	Purified	11.2	19.0	26.5	15.0	28.3
SA	0.10-0.20	Purified	14.4	14.9	24.9	11.9	34.0
PS180	0.10-0.20	Purified	14.5	14.8	22.1	13.1	35.5
DL180	0.10-0.20	Purified	11.5	14.7	25.1	12.3	36.4

CONCLUSIONS

Applications of pig slurry (PS) and pig deep litter (DL) promoted significant effects on the soil organic matter (SOM) of the most superficial layers, which is attributed to the surface applications and the non-revolving of the soil. Applications with pig slurry fertilization equivalent to the recommendation of N (90 kg ha^{-1} yr^{-1}) for the corn crop (PS90) promoted a negative priming effect, reducing the content of carbon (C) in the soil and in the chemical fractions of SOM, especially humic acids (HA). However, a contrary effect is observed with the addition of pig slurry fertilization equivalent to twice the recommended dose of N (180 kg ha^{-1} yr^{-1}) for the corn crop (PS180) treatment, with an increase in the SOM and HA levels. Additionally, DL applications in both doses evaluated promoted the highest levels and total stocks of C in the soil and favored the concentration of C in the humin (HU) fraction. The addition of DL also increased the C content in acid extracts (C_{HCl}) in the soil, which may indicate a more constant microbiological activity in these treatments. The presence of hydrophobic groups and their interaction with soil mineral components may also have contributed to the increase of this fraction in DL90 and DL180.

Elemental and spectroscopic analyses of humic substances (HS) revealed fulvic acids (FA), HA, and HU in the soil with no application of fertilizers (SA) have a more aliphatic character and higher concentrations of polysaccharides than in soils with the addition of pig manure, indicating fractions with a lower degree of stabilization. However, in soils fertilized with PS180 and DL180, the HS presented a higher degree of humification, which was proven by the HA/FA and HA+FA/HU ratios, especially in the more superficial layers. Among the humic fractions, the FA was the most affected in their chemical composition by the treatments, where the addition of manure promoted increased aromaticity, functionality, and/or aliphaticity and decreased polysaccharide structures. These results suggest this chemical fraction of the SOM can be used as an indicator of the quality of the SOM due to the addition of pig manure. However, more studies are needed to confirm this hypothesis.

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COMPETING INTERESTS

The authors report there are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the first author, [LB], upon reasonable request.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://www.rbcsjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-48-e0230126/1806-9657-rbcs-48-e0230126-suppl01.pdf.

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REFERENCES

Almeida HC, Dick DP, Bertotto FL, Chitarra GS. Distribution of chemical compartments of soil organic matter and C stocks of a Cambisol from south Brazil as affected by Pinus afforestation. Quim Nova. 2012;35:1329-35. https://doi.org/10.1590/S0100-40422012000700009

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JDM, Sparovek G. Köppen's climate classification map for Brazil. Meteorol Z. 2013;22:711-28. https://doi.org/10.1127/0941-2948/2013/0507

Angers DA, Chantigny MH, MacDonald JD, Rochette P, Côté D. Differential retention of carbon, nitrogen and phosphorus in grassland soil profiles with long-term manure application. Nutr Cycl Agroecosyst. 2010;86:225-9. https://doi.org/10.1007/s10705-009-9286-3

Bahadori M, Tofighi H. Investigation of soil organic carbon recovery by the Walkley-Black method under diverse vegetation systems. Soil Sci. 2017;182:101-06. https://doi.org/10.1097/SS.0000000000000201

Campos JRDR, Silva AC, Vasconcellos LL, Silva DV, Romão RV, Silva EDB, Grazziotti PH. Pedochronology and development of peat bog in the environmental protection area pau-de-fruta-Diamantina, Brazil. Rev Bras Cienc Solo. 2010;34:1965-75. https://doi.org/10.1590/S0100-06832010000600021

Claessen MEC. Manual de métodos de análise de solo. 2. ed. Rio de Janeiro: Embrapa Solos; 1997.

Comin JJ, Loss A, Veiga M, Guardini R, Schmitt DE, Oliveira PAV, Belli Filho P, Couto RR, Benedet L, Müller Júnior V, Brunetto G. Physical properties and organic carbon content of a Typic Hapludult soil fertilised with pig slurry and pig litter in a no-tillage system. Soil Res. 2013;51:459-70. https://doi.org/10.1071/SR13130

Comissão de Química e Fertilidade do Solo - CQFS-RS/SC. Manual de calagem e adubação para os Estados do Rio Grande do Sul e de Santa Catarina. 10. ed. Porto Alegre: Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul; 2004.

Couto RDR, Comin JJ, Soares CRFS, Belli Filho P, Benedet L, Moraes MPD, Brunetto G, Beber CL. Microbiological and chemical attributes of a Hapludalf soil with swine manure fertilization. Pesq Agropec Bras. 2013;48:774-82. https://doi.org/10.1590/S0100-204X2013000700010

Diacono M, Montemurro F. Long-term effects of organic amendments on soil fertility. In: Lichtfouse E, Hamelin M, Navarrete M, Debaeke P, editors. Sustainable Agriculture. Dordrecht: Springer; 2011. v. 2. p. 761-86. https://doi.org/10.1051/agro:2009040

Dick DP, Gonçalves CN, Dalmolin RSD, Knicker H, Klamt E, Kögel-Knabner I, Simões ML, Martin-Neto L. Characteristics of soil organic matter of different Brazilian Ferralsols under native vegetation as a function of soil depth. Geoderma. 2005;124:319-33. https://doi.org/10.1016/j.geoderma.2004.05.008

Dick DP, Gomes J, Rosinha PB. Caracterização de substâncias húmicas extraídas de solos e de lodo orgânico. Rev Bras Cienc Solo. 1998;22:603-11. https://doi.org/10.1590/S0100-06831998000400005

Diesel R, Miranda C, Perdomo C. Coletânea de tecnologias sobre dejetos suínos. Porto Alegre: Emater, Concórdia: Embrapa Suínos e Aves; 2002. (Bipers, 14).

Ellert BH, Bettany JR. Calculation of organic matter and nutrients stored in soils under contrasting management regimes. Can J Soil Sci. 1995;75:529-38. https://doi.org/10.4141/cjss95-075

Ferreira GW, Benedet L, Trapp T, Lima AP, Müller Júnior V, Loss A, Lourenzi CL, Comin JJ. Soil aggregation indexes and chemical and physical attributes of aggregates in a Typic Hapludult fertilized with swine manure and mineral fertilizer. Int J Recycl Org Waste Agric. 2021;10:1-17. https://doi.org/10.30486/IJROWA.2021.1896960.1051

Gerzabek MH, Antil RS, Kögel-Knabner I, Knicker H, Kirchmann H, Haberhauer G. How are soil use and management reflected by soil organic matter characteristics: a spectroscopic approach. Eur J Soil Sci. 2006;57:485-94. https://doi.org/10.1111/j.1365-2389.2006.00794.x

Gessesse TA, Khamzina A. How reliable is the Walkley-Black method for analyzing carbon-poor, semi-arid soils in Ethiopia? J Arid Environ. 2018;153:98-101. https://doi.org/10.1016/j.jaridenv.2018.01.008

Guimarães DV, Gonzaga MIS, Silva TO, Silva TL, Dias NS, Matias MIS. Soil organic matter pools and carbon fractions in soil under different land uses. Soil Till Res. 2013;126:177-82. https://doi.org/10.1016/j.still.2012.07.010

Hattab N, Soubrand M, Guégan R, Motelica-Heino M, Bourrat X, Faure O, Bouchardon JL. Effect of organic amendments on the mobility of trace elements in phytoremediated techno-soils: role of the humic substances. Environ Sci Pollut Res. 2014;21:10470-80. https://doi.org/10.1007/s11356-014-2959-8

Hayes MHB, Swift RS. An appreciation of the contribution of Frank Stevenson to the advancement of studies of soil organic matter and humic substances. J Soils Sediments. 2018;18:1212-31. https://doi.org/10.1007/s11368-016-1636-6

He YT, Zhang WJ, Xu MG, Tong XG, Sun FX, Wang JZ, Huang SM, Zhu P, He XH. Long-term combined chemical and manure fertilizations increase soil organic carbon and total nitrogen in aggregate fractions at three typical cropland soils in China. Sci Total Environ. 2015;532:635-44. https://doi.org/10.1016/j.scitotenv.2015.06.011

Huang GF, Wu QT, Wong JWC, Nagar BB. Transformation of organic matter during co-composting of pig manure with sawdust. Bioresource Technol. 2006;97:1834-42. https://doi.org/10.1016/j.biortech.2005.08.024

Kalisz B, Lachacz A, Glazewski R. Transformation of some organic matter components in organic soils exposed to drainage. Turk J Agric For. 2010;34:245-56. https://doi.org/10.3906/tar-0905-33

Kramer MG, Sanderman J, Chadwick OA, Chorover J, Vitousek PM. Long-term carbon storage through retention of dissolved aromatic acids by reactive particles in soil. Glob Chang Biol. 2012;18:2594-605. https://doi.org/10.1111/j.1365-2486.2012.02681.x

Kumar S, Ghotekar YS, Dadhwal VK. C-equivalent correction factor for soil organic carbon inventory by wet oxidation, dry combustion and loss on ignition methods in Himalayan region. J Earth Syst Sci. 2019;128:62. https://doi.org/10.1007/s12040-019-1086-9

Kuzyakov Y. Priming effects: interactions between living and dead organic matter. Soil Biol Biochem. 2010;42:1363-71. https://doi.org/10.1016/j.soilbio.2010.04.003

Kuzyakov Y, Bol R. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. Soil Biol Biochem. 2006;38:747-58. https://doi.org/10.1016/j.soilbio.2005.06.025

Kuzyakov Y, Friedel JK, Stahr K. Review of mechanisms and quantification of priming effects. Soil Biol Biochem. 2000;32:1485-98. https://doi.org/10.1016/S0038-0717(00)00084-5

Li J, Wang J, Wang F, Wang A, Yan P. Evaluation of gaseous concentrations, bacterial diversity and microbial quantity in different layers of deep litter system. Asian Austral J Anim Sci. 2017;30:275-83. https://doi.org/10.5713/ajas.16.0282

Liaudanskiene I, Slepetiene A, Velykis A, Satkus A. Distribution of organic carbon in humic and granulodensimetric fractions of soil as influenced by tillage and crop rotation. Est J Ecol. 2013;62:53-69. https://doi.org/10.3176/eco.2013.1.05

Lourenzi CR, Ceretta CA, Silva LSD, Trentin G, Girotto E, Lorensini F, Tiecher TT, Brunetto G. Soil chemical properties related to acidity under successive pig slurry application. Rev Bras Cienc Solo. 2011;35:1827-36. https://doi.org/10.1590/S0100-06832011000500037

Lüdtke AC, Dick DP, Aita C, Pavan B, Morosino L, Doneda A, Cantú RR. Organic matter and humic fractions of a Haplic Acrisol as affected by composted pig slurry. Quim Nova. 2016;39:14-8. https://doi.org/10.5935/0100-4042.20160001

Lützow MV, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions–a review. Eur J Soil Sci. 2006;57:426-45. https://doi.org/10.1111/j.1365-2389.2006.00809.x

Machado W, Franchini JC, de Fátima Guimarães M, Tavares Filho J. Spectroscopic characterization of humic and fulvic acids in soil aggregates, Brazil. Heliyon. 2020;6:e04078. https://doi.org/10.1016/j.heliyon.2020.e04078

Marcato CE, Mohtar R, Revel JC, Pouech P, Hafidi M, Guiresse M. Impact of anaerobic digestion on organic matter quality in pig slurry. Int Biodeter Biodegr. 2009;63:260-6. https://doi.org/10.1016/j.ibiod.2008.10.001

Morales D, Vargas MM, Oliveira MPD, Taffe BL, Comin J, Soares CR, Lovato P. Response of soil microbiota to nine-year application of swine manure and urea. Cienc Rural. 2016;46:260-6. https://doi.org/10.1590/0103-8478cr20140565

Pérez MG, Martin-Neto L, Saab SC, Novotny EH, Milori DMBP, Bagnato VS, Colnago LA, Melo WJ, Knicker H. Characterization of humic acids from a Brazilian Oxisol under different tillage systems by EPR, ^{13}C NMR, FTIR and fluorescence spectroscopy. Geoderma. 2004;118:181-90. https://doi.org/10.1016/S0016-7061(03)00192-7

Piccolo A. The supramolecular structure of humic substances. Soil Sci. 2001;166:810-32. https://doi.org/10.1097/00010694-200111000-00007

Plaza C, Senesi N, Polo A, Brunetti G, Garcıa-Gil JC, D'orazio V. Soil fulvic acid properties as a means to assess the use of pig slurry amendment. Soil Till Res. 2003;74:179-90. https://doi.org/10.1016/j.still.2003.07.002

Potes MDL, Dick DP, Dalmolin RSD, Knicker H, Rosa ASD. Matéria orgânica em Neossolo de altitude: Influência do manejo da pastagem na sua composição e teor. Rev Bras Cienc Solo. 2010;34:23-32. https://doi.org/10.1590/S0100-06832010000100003

Provenzano MR, Malerba AD, Pezzolla D, Gigliotti G. Chemical and spectroscopic characterization of organic matter during the anaerobic digestion and successive composting of pig slurry. Waste Manage. 2014;34:653-60. https://doi.org/10.1016/j.wasman.2013.12.001

Ramamoorthi V, Meena S. Quantification of soil organic carbon-Comparison of wet oxidation and dry combustion methods. Int J Curr Microbiol Appl Sci. 2018;7:146-54. https://doi.org/10.20546/ijcmas.2018.710.016

Rodrigues LAT, Giacomini SJ, Aita C, Lourenzi CR, Brunetto G, Bacca A, Ceretta CA. Short- and long-term effects of animal manures and mineral fertilizer on carbon stocks in subtropical soil under no-tillage. Geoderma. 2021;386:114913. https://doi.org/10.1016/j.geoderma.2020.114913

Roper WR, Robarge WP, Osmond DL, Heitman JL. Comparing four methods of measuring soil organic matter in North Carolina soils. Soil Sci Soc Am J. 2019;83:466-74. https://doi.org/10.2136/sssaj2018.03.0105

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Araujo Filho JC, Oliveira JB, Cunha TJF. Sistema brasileiro de classificação de solos. 5. ed rev ampl. Brasília, DF: Embrapa; 2018.

Shamrikova EV, Vanchikova EV, Lu-Lyan-Min EI, Kubik OS, Zhangurov EV. Which method to choose for measurement of organic and inorganic carbon content in carbonate-rich soils? Advantages and disadvantages of dry and wet chemistry. Catena. 2023;228:107151. https://doi.org/10.1016/j.catena.2023.107151

Shulga YM, Baskakov SA, Baskakova YV, Lobach AS, Volfkovich YM, Sosenkin VE, Shulga NY, Parkhomenko YN, Michtchenko A, Kumar Y. Hybrid porous carbon materials derived from composite of humic acid and graphene oxide. Micropor Mesopor Mat. 2017;245:24-30. https://doi.org/10.1016/j.micromeso.2017.02.061

Silva LS, Constantino IC, Bento LR, Tadini AM, Bisinoti MC, Boscolo M, Ferreira OP, Mounier S, Piccolo A, Spaccini R, Cornélio ML, Moreira AB. Humic extracts from hydrochar and Amazonian Anthrosol: Molecular features and metal binding properties using EEM-PARAFAC and 2D FTIR correlation analyses. Chemosphere. 2020;256:127110. https://doi.org/10.1016/j.chemosphere.2020.127110

Soil Survey Staff. Keys to soil taxonomy. 12th ed. Washington, DC: United States Department of Agriculture, Natural Resources Conservation Service; 2014.

Tan KH. Infrared spectroscopy. In: Tan HK, editor. Soil sampling, preparation and analysis. New York: Marcel Dekker; 1996. p. 278-98.

Song G, Hayes MH, Novotny EH. A two-year incubation study of transformations of crop residues into soil organic matter (SOM) and a procedure for the sequential isolation and the fractionation of components of SOM. Sci Total Environ. 2021;763:143034. https://doi.org/10.1016/j.scitotenv.2020.143034

Tedesco MJ, Volkweiss SJ, Bohmen H. Análises de solo, plantas e outros materiais. Porto Alegre, RS: Universidade Federal do Rio Grande do Sul; 1995.

Tejada M, Hernandez MT, Garcia C. Soil restoration using composted plant residues: Effects on soil properties. Soil Till Res. 2009;102:109-17. https://doi.org/10.1016/j.still.2008.08.004

Violante A, Cozzolino V, Perelomov L, Caporale AG, Pigna M. Mobility and bioavailability of heavy metals and metalloids in soil environments. J Soil Sci Plant Nutr. 2010;10:268-92. https://doi.org/10.4067/S0718-95162010000100005

Wang K, He C, You S, Liu W, Wang W, Zhang R, Qi H, Ren N. Transformation of organic matters in animal wastes during composting. J Hazard Mater. 2015;300:745-53. https://doi.org/10.1016/j.jhazmat.2015.08.016

Yan X, Zhou H, Zhu QH, Wang XF, Zhang YZ, Yu XC, Peng X. Carbon sequestration efficiency in paddy soil and upland soil under long-term fertilization in southern China. Soil Till Res. 2013;130:42-51. https://doi.org/10.1016/j.still.2013.01.013

Yanardağ İH, Zornoza R, Cano AF, Yanardağ AB, Mermut AR. Evaluation of carbon and nitrogen dynamics in different soil types amended with pig slurry, pig manure and its biochar by chemical and thermogravimetric analysis. Biol Fertil Soils. 2015;51:183-96. https://doi.org/10.1007/s00374-014-0962-3

Zhou P, Pan GX, Spaccini R, Piccolo A. Molecular changes in particulate organic matter (POM) in a typical Chinese paddy soil under different long-term fertilizer treatments. Eur J Soil Sci. 2010;61:231-42. https://doi.org/10.1111/j.1365-2389.2009.01223.x