CARBON, NITROGEN AND HUMIC SUBSTANCES IN BIOGENIC AND PHYSICOGENIC AGGREGATES OF A SOIL WITH A 10-YEAR HISTORY OF SUCCESSIVE APPLICATIONS OF SWINE WASTE†

[CARBONO, NITROGENO Y SUSTANCIAS HÚMICAS EN AGREGADOS BIOGENICOS Y FISIOGÉNICOS DE UN SUELO CON HISTORIA DE 10 AÑOS DE APLICACIONES SUCESIVAS DE DESECHOS PORCINOS]

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SUMMARY

Applications of swine waste on the soil promote changes in soil aggregation pathways and, consequently, in the chemical attributes of these aggregates. This study aimed to evaluated the effects of different sources and amounts of swine waste on the levels of total organic carbon (TOC), total nitrogen (TN), and C and N levels of humic substances (HSs) of the biogenic and physicogenic aggregates in an Ultisol with a history of applications of swine waste for 10 years. Undisturbed soil samples were collected at the depths of 0-5 cm and 5-10 cm, in the treatments without application of waste (CONTROL), with the application of pig slurry (PS) and deep litter (DL) at doses equivalent to one and two times the nitrogen recommendation for maize and oats (PS1X, PS2X, DL1X and DL2X, respectively). The aggregates were separated according to the biogenic and physicogenic formation pathways. Subsequently, TOC, TN, as well as C and N of HSs were quantified: humin (C-HUM/N-HUM), humic acids (C-HAF/N-HAF) and fulvic acids (C-FAF/N-FAF). The application of DL increased the total C and N levels of the aggregates and of the HSs. The continuous application of swine waste have increased TOC, TN, C-HUM, C-HAF, and N-HUM in the biogenic aggregates when compared to physicogenic ones.
Keywords: pig slurry; deep litter; no-tillage system; humin; humic acid; fulvic acid.

RESUMEN

Las aplicaciones de desechos de cerdos en el suelo promueven cambios en las vías de agregación del suelo y, consecuentemente, en los atributos químicos de estos agregados. Se evaluó los efectos de diferentes fuentes y cantidades de desechos de cerdos sobre los contenidos de carbono orgánico total (COT), nitrógeno total (NT) y los niveles C y N de las sustancias humicas (SHs) de los agregados biogénicos y fisiogénicos de Argissolo con histórico de aplicaciones de desechos de cerdos por 10 años. Se recolectar muestras indeformadas de suelo en las profundidades 0-5 cm y 5-10 cm, en los tratamientos sin aplicación de desechos (TESTEMUNHA), con aplicación de desecho líquido de cerdos (DLS) y cama superpuesta de cerdos (CSS) en dosis equivalente a una y dos veces la recomendación de nitrógeno para el maíz y la avena (DLS1X, DLS2X, CSS1X y CSS2X, respectivamente). Los agregados se separaron según las vías de formación em biogénicos y fisiogénicos. Posteriormente, se cuantificaron

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COT, NT, así como C y N de SHs: humina (C-HUM/N-HUM), ácidos húmicos (C-HAF/N-HAF) y ácidos fúlvicos (C-FAF/N-FAF). La aplicación de CSS incrementó los niveles totales de C y N de los agregados y de lãs SHs. La aplicación continua de desechos de cerdos ha incrementado el COT, NT, C-HUM, C-HAF y N-HUM en los agregados biogénicos en comparación con los fisiogénicos.
Palabras clave: desecho líquido de cerdos; cama superpuesta de cerdos; sistema de siembra directa; humina; ácido húmico, ácido fúlvico.

INTRODUCTION

The state of Santa Catarina, Brazil, stands out nationally because it is one of the main agricultural producers, especially in the animal production sector, with emphasis on swine breeding activities. The current production system in the state is predominantly intensive and in small properties, generating, in addition to high productivity, large volumes of waste (ABCS, 2014). This waste, when successively applied to the soil, may modify the chemical, physical and biological attributes of the soil (Correia et al., 2011, Lourenzi et al., 2013, Comin et al., 2013, Giacomini et al., 2013, Mafra et al., 2014).
Several soil attributes are used as soil quality indicators (SQI). Soil organic matter (SOM) is considered one of the best indicators of SQ. Furthermore, the determination of C and N levels of the different SOM compartments are used to show differences in soil use systems under diversified conditions Vezzani and Mielniczuk (2009). Organic fractions of the soil are divided into different stages of decomposition: organic residues, light fraction, living biomass of the soil, non-humic substances and humic substances. Within humic substances, the presence of three main fractions is possible: humin, humic acids and fulvic acids. Humic acids are fractions that have little solubility in the acidic conditions normally found in soils. These compounds are responsible for the greater part of the cation exchange capacity (CEC) of organic origin and in superficial layers of sandy soils, differently from fulvic acids, which are responsible for the greater generation of loads of organic origin in clay soils Mendonça and Rowell (1996). Humin is the fraction bounded to the minerals of the soil and although it presents low reactivity, it is responsible for aggregation mechanisms and in the majority of tropical soils it represents the greater part of humified carbon (Stevenson, 1994, Benites et al., 2003, Loss et al., 2010).

Humic substances have the ability to interact with the clay fraction and they play an important role in soil fertility and structure, as well as in the immobilization of heavy metals and pesticides (Stevenson, 1994, Dick et al., 2009). The humic fractions of SOM reflect the changes that have occurred through anthropic alterations and at the same time they are stable in the face of short-term spatial and temporal variations when compared to some biological and biochemical indicators normally evaluated, suggesting that the characterization of these fractions presents a great potential in the evaluation of changes in SQ Benites et al (2010).

Depending on the soil management system, for example, the no-tillage system (NTS) or the conventional tillage system (CTS), with or without the use of organic or mineral fertilizers, changes in the edaphic attributes may occur, with emphasis on changes in the soil aggregation, for it is an attribute strongly influenced by soil management. These changes may also culminate in changes in the aggregate formation pathways, which are classified as biogenic and physicogenic, and these patterns are established according to their morphology (Pulleman et al., 2005, Velasquez et al., 2007, Jouquet et al., 2009, Loss et al., 2014).

When studying the impact of earthworm activity on the aggregation and incorporation of SOM in different long-term cropping systems, being permanent pastures (PP), CTS and a CTS, however, organic (CTSo), Pulleman et al (2005) have observed that the biogenic aggregates found in PP and CTSo contained higher TOC levels than the physicogenic aggregates, and the inverse trend was observed for the CTS aggregates. The comparison of the different types of aggregates has revealed that the soil macroaggregates in PP were considerably enriched in carbon contained in the particulate organic matter and microaggregates. In general, the results have demonstrated that earthworms may directly initiate the formation of microaggregates, which, in turn, contribute to the physical protection of SOM against microbial decomposition.

In order to increase soil aggregation in agroecosystems, the management should aim to increase the entries of SOM, as well as the reduction of disturbances and the loss rates of this SOM by processes such as decomposition and erosion. Improvement of soil structure depends on management practices, which include methods of preparation, nutrient cycling and management of plant and animal waste. Thus, within this context, the NTS is highlighted with the use of organic fertilization via swine waste. This combination (NTS fertilized with swine waste) for a long time may influence the formation pathways of soil aggregates, as well as it may increase carbon and nitrogen levels in these aggregates.

Therefore, more studies are required which address the separation and characterization of soil aggregates due to changes resulting from soil management in the formation of aggregates, in relation to qualitative and quantitative aspects, since these are incipient in the face of the complexity and difficulty to establish patterns for the different forming pathways of the aggregates (Velasquez et al., 2007, Jouquet et al., 2009, Cecillon et al., 2010, Batista et al., 2013, Loss et al., 2014). According to Loss et al (2014) and Silva Neto et al (2016), information on the soil aggregation pattern results in a simple SQ indicator, since the aggregates are sensitive to the impacts caused by natural and anthropogenic processes, which affect the aggregate formation pathways.

This study aimed to evaluate the effects of different sources and amounts of swine waste on the levels of total carbon and nitrogen and of the humic substances of the biogenic and physicogenic aggregates in an Ultisol with a history of continuous applications of swine waste for 10 years.

MATERIAL AND METHODS

Location and characterization of the study area

The experiment was carried out in 2002 in a Typic Hapludult (Soil Survey Staff, 2010), cultivated under a NTS with the succession of black oat/maize, without the use of agrochemicals, in a rural property located in the municipality of Braço do Norte, SC (28º 14’20.7’’ S, 49º 13’55.5’’ W and altitude of 300 m). The climate of the municipality is type Cfa (humid subtropical climate) according to the classification of Köppen, with average annual temperatures of 18.7ºC, being the maximum of 35ºC and the minimum of 0ºC. There is not a defined dry season, and the rainfall concentration tends to occur in Summer months, with an average annual rainfall of 1,471 mm EPAGRI (2000). Before the installation of the experiment, in the 0-10 cm layer, the soil presented the following parameters, according to Tedesco et al (1995): pH_{H2O} 5.1; clay 330 g kg^{-1}; exchangeable Ca, Mg and Al, 3.0, 0.8 and 0.8 cmol$_c$ dm^{-3}, respectively (extracted by KCl 1 mol L^{-1}); available P and K 19 and 130 mg dm^{-3}, respectively (extracted by Mehlich-1), organic matter 33.0 g kg^{-1} (Embrapa.,1997).

Prior to the installation of the experiment, the area was covered by a pasture naturalized predominantly of *Paspalum notatum*, *Paspalum plicatulum*, *Eryngium ciliatum* and *Stylosanthes montevidensis*, being swine waste sporadically applied. In December 2002, 6 Mg ha^{-1} of limestone were applied to the soil surface to raise the pH in water to 6.0 CFS-RS/SC (1995). Then, five treatments were installed: CONTROL (without fertilization); pig slurry - PS1X (fertilization with PS, equivalent to the recommendation of 90 kg N ha^{-1} year^{-1} for maize and oats crops); PS2X (fertilization with PS, equivalent to the double (180 kg ha^{-1}) of the recommendation of N ha^{-1} year^{-1} for maize and oats crops); deep litter – DL1X (fertilization with DL, equivalent to the recommendation of 90 kg N ha^{-1} year^{-1} for maize crop) and DL2X (fertilization with DL, equivalent to the double (180 kg ha^{-1}) of the recommendation of N ha^{-1} year^{-1} for maize crop).

The experimental design was a randomized block with five treatments and three replicates. Each one of the blocks had dimensions of 4.5 x 30 m, separated by a corridor one meter wide. Each treatment consisted of experimental units (plots) with 4.5 x 6.0 m (27 m^2). The PS was collected in a full-cycle breeding system deep pit located on the same property in which the experiment was installed. DL was obtained at the Federal Agrotechnical School of Concórdia, SC, where the swine breeding system was made with a shavings substrate.

The required quantity of waste to meet the demand for N in the maize/oats succession in each treatment used over the period from 2002 to 2012 was established according to the recommendation proposed by the Soil Fertility Commission CFS RS/SC (1995) and CQFS-RS/SC (2004), both from the states of Rio Grande do Sul and Santa Catarina. Hence, the amount of PS1X and PS2X to be applied was defined by the estimate of dry matter (DM) and of the nutrient concentration in the waste. On the other hand, the applied amount of DL1X and DL2X was calculated based on the mineralization of 50% of the ammoniacal N contained in the residue.

The amounts of PS and DL applied during the experimental period, as well as the macronutrients contained in the waste, are shown in Table 1. PS and DL were the only sources of nutrients added in NTS for the oats/maize succession over the experimental period, which was from 2002 to 2012 (Table 1). The doses of PS in each agricultural year were applied to the soil surface and parceled out four times, totaling 40 PS applications (ten years of experimentation and four splits), namely: the first application was carried out on the week of maize sowing; the second one, at 51 days after sowing (DAS) the maize; the third one, at 95 DAS the maize and the fourth one, at 15 DAS the oats. For DL, ten applications were performed during the experimental period, with each application being carried out on the soil surface, on average 15 to 30 days before the implantation of each maize crop (Summer season). In the black oat cycle, no application of DL was performed.

Table 1. Amount of pig slurry and deep litter applied to the soil and chemical characterization of the waste during the experimental period.

Agricultural year	AV	DM	C/N	Ph	EC	Ca	Mg	N	P	K
	----	%	----	----	ds m^{-1}	---------------------kg ha^{-1}----------------------				
2002/12	**Pig slurry (PS)**									
	539.0	2.3	4.4	8.1	9.3	68.75	26.38	126.25	37.88	62.75
2002/12	**Deep litter (DL)**									
	153.10	51.0	13.2	8.8	5.9	322.38	97.88	171.06	103.13	169.50

AV=applied volume from 2002 to 2012, being for PS in m^3 ha^{-1} and for DL in Mg ha^{-1}; DM=dry matter; EC=electric conductivity. For waste, there is the sum of the total quantities applied over the period of 2002-2012. For the other parameters, we have the mean of the values obtained over the course of 2002-2012.

Evaluation of the formation pathways of the aggregates

In 2012, in each treatment, undisturbed samples of soil in the layers of 0-5 cm and 5-10 cm depths were collected between the lines of maize with the aid of a spade, a hoe and a spatula. After the collection, the samples were bagged, labeled and transported to the laboratory where they were dried in the shade and sieved in a set of mesh sieves of 9.5; 8.0 and 4.0 mm to obtain the aggregates, according to EMBRAPA (1997). After the separation of the aggregates, three samples composed of evaluated treatment were obtained, being each composed sample formed by two simple undisturbed samples collected in each experimental unit.

For the separation of the aggregates, according to the formation pathway, the aggregates contained in the range of 9.5 to 4.0 mm were used. These aggregates were observed under a binocular microscope and manually separated, according to Velasquez et al (2007) and Loss et al (2014). The separation of the aggregates was done through morphological patterns, being the physicogenic defined by presenting angular and subangular forms (Figure 1a), and the biogenic are those in which it is possible to visualize rounded forms, provided by the intestinal tract of the macrofauna individuals of the soil and, or, those associated with root activity (Figure 1b).
Subsequently, Mergem-Junior (2017) has determined the relative contribution of the aggregates in terms of mass, weighing all the biogenic and physicogenic aggregates that were identified and, thus, quantified the fraction of physicogenic and biogenic aggregates in relation to the initial mass, as shown in Table 2.

Table 2. Percentage (%) of the biogenic and physicogenic aggregates in different soil use systems in Braço do Norte, Santa Catarina, Brazil.

Treatments	Depth (cm)	%BA	%PA
CONTR	0-5	33	67
	5-10	19	81
PS1X	0-5	95	5
	5-10	59	41
PS2X	0-5	93	7
	5-10	89	11
DL1X	0-5	93	7
	5-10	55	43
DL2X	0-5	91	9
	5-10	66	34

Physico-chemical analysis of the aggregates

After the separation and quantification of the aggregates, for the chemical and physical evaluation, the aggregates were crumbled and passed through a 2.00 mm mesh sieve to obtain the air-dry fine earth (ADFE). The granulometric composition of the biogenic and physicogenic aggregates was also performed by Mergem-Junior (2017), as shown in Table 3.

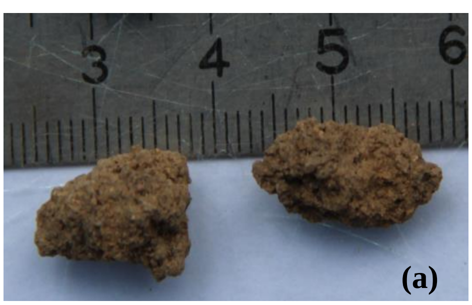

Figure 1. Physicogenic (a) and biogenic (b) aggregates of an Ultisol after applications of pig slurry and deep litter in Braço do Norte, Santa Catarina, Brazil.

Table 3. Granulometric composition (g kg^{-1}) of the biogenic and physicogenic aggregates in different soil use systems in Braço do Norte, SC.

Treatments	Clay		Silt		Sand	
	Bio	Phys	Bio	Phys	Bio	Phys
			0-5 cm			
Control	144	225	300	236	556	540
PS1X	116	169	329	278	555	552
PS2X	142	163	295	288	562	550
DL1X	80	163	306	270	613	567
DL2X	72	120	353	311	575	570
			5-10 cm			
Control	137	213	295	245	568	542
PS1X	129	221	282	239	589	540
PS2X	138	148	323	309	539	543
DL1X	172	200	251	240	578	561
DL2X	164	191	294	249	542	560

Control (without waste fertilization); PS1X = pig slurry, 1 time the recommendation of N; PS2X = pig slurry, 2 times the recommendation of N; DL1X = deep litter, 1 time the recommendation of N; DL2X = deep litter, 2 times the recommendation of N. Bio = biogenic, Phys = physicogeni

The TOC and TN levels of the biogenic and physicogenic aggregates were determined by the dry combustion method, in a C and N autoanalyzer at 900ºC (CHN-1000 from Leco) at the Isotopic Ecology Lab of the Center of Nuclear Energy in Agriculture (CENA) - Piracicaba.

The humic substances were separated into three fractions: fulvic acids fraction (FAF), humic acid fraction (HAF) and humin (HUM), using the differential solubility technique established by the International Humic Substances Society Swift (1996). After the separation of the fractions, the quantification of C in the fractions (C-HUM, C-FAF and C-HAF) was done according to a methodology recommended by Yeomans and Bremner (1988). The quantification of N (N-HUM, N-FAF and N-HAF) was done according to Tedesco et al (1995).

The data were analyzed for normality of residuals and homoscedasticity of variances using the Lilliefors and Barttlet tests, respectively. Afterwards, they were submitted to analysis of variance and mean values, when significant, were compared by the Scott-Knott test at 5%.

RESULTS AND DISCUSSION

Total carbon and nitrogen contents in the aggregates

TOC levels varied from 27 to 63.2 g kg^{-1} in the biogenic aggregates and from 21.2 to 51.9 g kg^{-1} in the physicogenic aggregates. Regarding TN levels, the variations were from 2.4 to 5.5 g kg^{-1} and from 1.7 to 4.7 g kg^{-1}, respectively, for biogenic and physicogenic aggregates. In the biogenic and physicogenic aggregates, both TOC and TN were higher in treatments with DL, at both depths. The treatments with PS and control had the lowest TOC and TN levels in both depths and did not differ between them. When comparing the TOC and TN levels between the aggregate types, the biogenic ones presented higher levels than the physicogenic ones, in all treatments and depths, except for the control and DL2X, for TN levels, which did not differ ($p<0.05$) in the 5-10 cm layer (Figure 2).

The dynamics of TOC and TN levels is controlled by the natural contribution of vegetal residues in the soil, as well as by the addition of organic residues of various natures and by the continuous transformation of these residues under the action of biological, chemical and physical factors. Thus, the highest TOC and TN levels in the DL areas resulted from the higher dry mass values of this residue (51% for DL and 2.3% for PS, Table 1) and from the C/N ratio (13.2 for DL and 4.4 for PS, Table 1) when compared to the values observed in PS. This higher C/N ratio leads to a lower rate of decomposition of SOM by microorganisms (Prescott., 2005, Giacomini and Aita., 2008) and, consequently, to an increase in TOC and TN levels in the soil in areas with DL when compared to the areas with PS and control.

These results corroborate the ones found by Brunetto et al (2012) and Comin et al (2013), which when evaluating TOC levels in an NTS area of cultivation, with a oats/maize succession after eight years of application of different amounts and sources of waste (PS and DL), have observed that the application of PS in general did not alter TOC levels when compared to the control, while the application of DL increased TOC levels up to a depth of 20 cm.

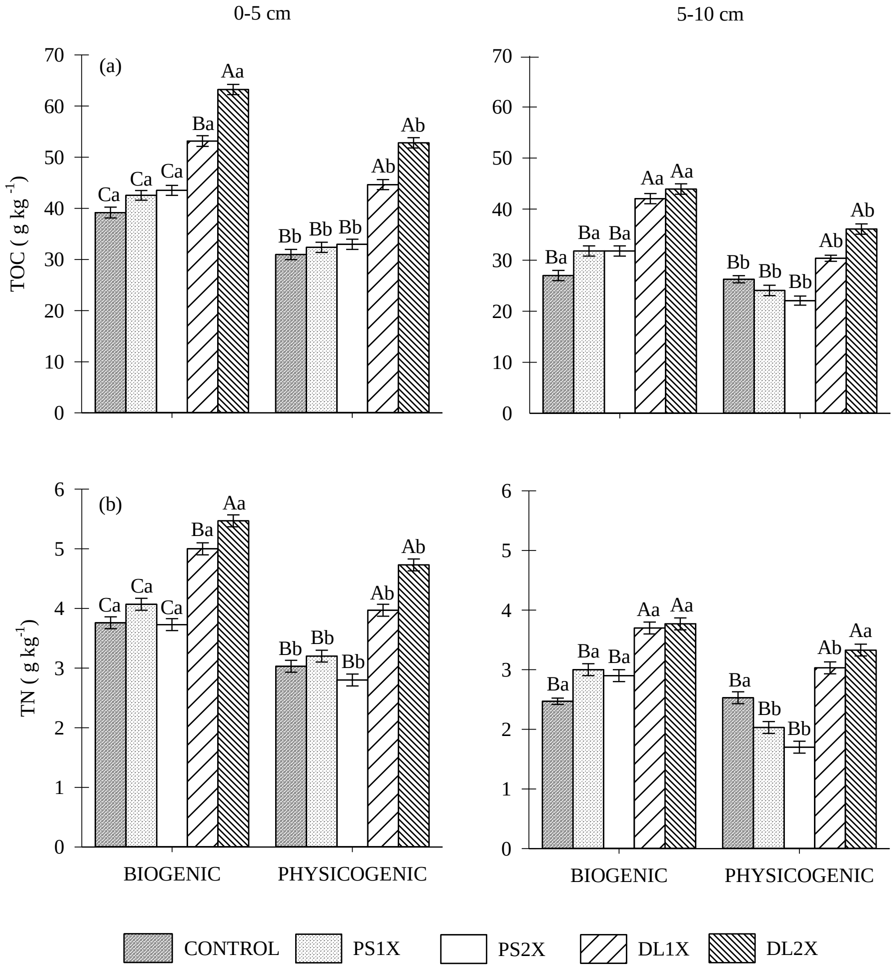

Figure 2. Total organic carbon (TOC, a) and total nitrogen (TN, b) levels in the biogenic and physicogenic aggregates of an Ultisol after applications of pig slurry (PS) and deep litter (DL), Braço do Norte, Santa Catarina, Brazil. Means followed by the same upper-case letter do not differ among treatments for each type of aggregate, and means followed by the same lower-case letter do not differ between types of aggregates for each treatment (Scott-Knott test, $p<0.05$). Control = without waste fertilization; PS1X = pig slurry, 1 time the recommendation of N; PS2X = pig slurry, 2 times the recommendation of N; DL1X = deep litter, 1 time to recommendation of N; DL2X = deep litter, 2 times the recommendation of N.

In another study, it has been suggested that the long-term use of PS is more efficient in increasing TOC levels in the soil than mineral fertilizer Mafra et al (2014). These authors have conducted a long-term experiment (11 years) in Campos Novos-SC in a Oxisol cultivated with a black oat/maize succession in NTS using the application of PS at doses of 50, 100 and 200 m^3 ha^{-1} in comparison to mineral fertilizer (NPK) and have concluded that the used doses increased TOC stocks in the soil in the order of 3.8; 6.2 and 9.1 Mg ha^{-1}, respectively, for the doses 50, 100 and 200 m^3 ha^{-1}, comparatively to the mineral fertilization that had a total stock of 7.1 Mg ha^{-1} in the 0-20 cm layer. In addition, they have found that the use of PS also increased the addition of C by the crop phytomass (maize and black oats), as well as the humification of SOM in comparison to mineral fertilization.

The use of PS as fertilizer does not always increase C and N levels (Comin et al., 2013, Dortzbach et al., 2013). However, in this study it has provided the increase of the yield of oats dry matter by up to 34% and of maize grains by up to 90% when compared to the control (Table 4). The dry mass of oats in the treatments with the application of manure had an increase, which varied from 25% to 37%, in relation to the control. The same result was found for corn grain production, which increased from 72% to 107%. It is suggested that a greater area of root system exploration and a greater yield of root exudates that stimulate microbial biomass may have influenced the higher TOC and TN levels in the biogenic aggregates when compared to the physicogenic ones. The higher proportion of biogenic aggregates compared to physicogenic ones (Table 3) also corroborate these results.

Table 4. Average yield per harvest of dry matter of oats and maize grains, in Mg ha^{-1}, during 10 years of application of swine waste.

Treatments	DM of oats	DM of maize grains
CONTROL	6.5	2.9
PS1X	8.1 (25%)	5.0 (72%)
PS2X	8.7 (34%)	5.5 (90%)
DL1X	8.2 (26%)	5.6 (93%)
DL2X	8.9 (37%)	6.0 (107%)

CONTROL= without waste fertilization; PS1X = pig slurry, 1 time the recommendation of N; PS2X = pig slurry, 2 times the recommendation of N; DL1X = deep litter, 1 time the recommendation of N; DL2X = deep litter, 2 times the recommendation of N. The number in parentheses represents the increase in % in relation to the control.

The higher TOC and TN levels in the biogenic aggregates compared to the physicogenic ones occur because they are formed mainly by the factors fauna of the soil and root system activity. According to Batista et al. (2013), in a study whose objective was to analyze the biological influence on the formation pathways of aggregates, the authors have evaluated TOC levels, aggregate stability and the edaphic macrofauna in different production systems and at two times of the year. The biogenic aggregates showed a positive correlation with TOC levels and with the edaphic macrofauna, mainly earthworms.
In another study conducted in the southwest of Paraná, Brazil, Loss et al. (2014) have evaluated the formation pathways of 15-year old aggregates in NTS, 56-year old CTS, secondary forest (30 years old) and pasture (30 years old). The authors have separated and quantified the proportion of soil aggregates into biogenic and physicogenic and characterized them regarding soil fertility, levels of TOC, TN, granulometric and chemical fractionation of SOM. Between the aggregates, the authors have observed that the biogenic ones were more efficient in the increase of soil fertility, TN, TOC and C levels of the fractions of SOM in comparison to the physicogenic aggregates.

The use of swine waste may also contribute to the factors that influence the formation of biogenic aggregates, with emphasis on the biological factor. In a study conducted by Morales et al. (2016), in the same area of this study, the levels of carbon microbial biomass (CMB) and basal respiration (BR) were measured after nine years of successive applications of swine waste to evaluate the response of soil microbiota. The CMB and BR levels were higher in the treatment with DL, which shows that the applications of swine waste in the long term increased soil microbial activity and organic matter level, especially when applied in the form of DL.

In this study, no differences were verified for TN levels between the control and PS treatments. These results differ from those found by Grohskopf et al. (2015). By evaluating TN levels in the soil after 10 years of annual application of PS and mineral fertilizer (NPK) in a Oxisol cultivated under an NTS with the oats/maize succession, Grohskopf et al. (2015) have observed that the application of PS at doses 0, 25, 50, 100 and 200 m^3 ha^{-1} per year increased TN at the depths of 0-2.5, 2.5-5.0 and 5-10 cm in relation to the control (zero dose) and in comparison to the mineral fertilization with the doses of 100 and 200 m^3 ha^{-1}. TN levels for the depths of 0-2.5 and 5-10 cm vary from 3.2 to 2.2 g kg^{-1} (0 m^3 ha^{-1}), 4.1 to 2.4 (50 m^3 ha^{-1}), 4.6 to 2.6 (100 m^3 ha^{-1}) and 3.9 to 2.5 g kg-1 (NPK).

Carbon levels of the humic substances

The levels of C-HUM varied from 14.5 to 46.6 g kg^{-1} in the biogenic aggregates and from 11.1 to 36.1 g kg^{-1} in the physicogenic ones. In both types of aggregates, the highest levels were found in the DL treatments and the lowest ones in the other treatments for both depths, except for the DL1X treatment of the biogenic aggregates, which at the depth of 5-10 cm did not differ from the control and from the treatments with PS. When compared between the aggregates, in the biogenic ones the highest levels of C-HUM were observed in all treatments at the depth of 0-5 cm. On the other hand, at the depth of 5-10 cm, the C-HUM of the biogenic aggregates was higher than the physicogenic ones in the treatments with PS and DL2X, however, it did not differ from the levels found in the control area and it was lower in the DL1X treatment (Figure 3a).

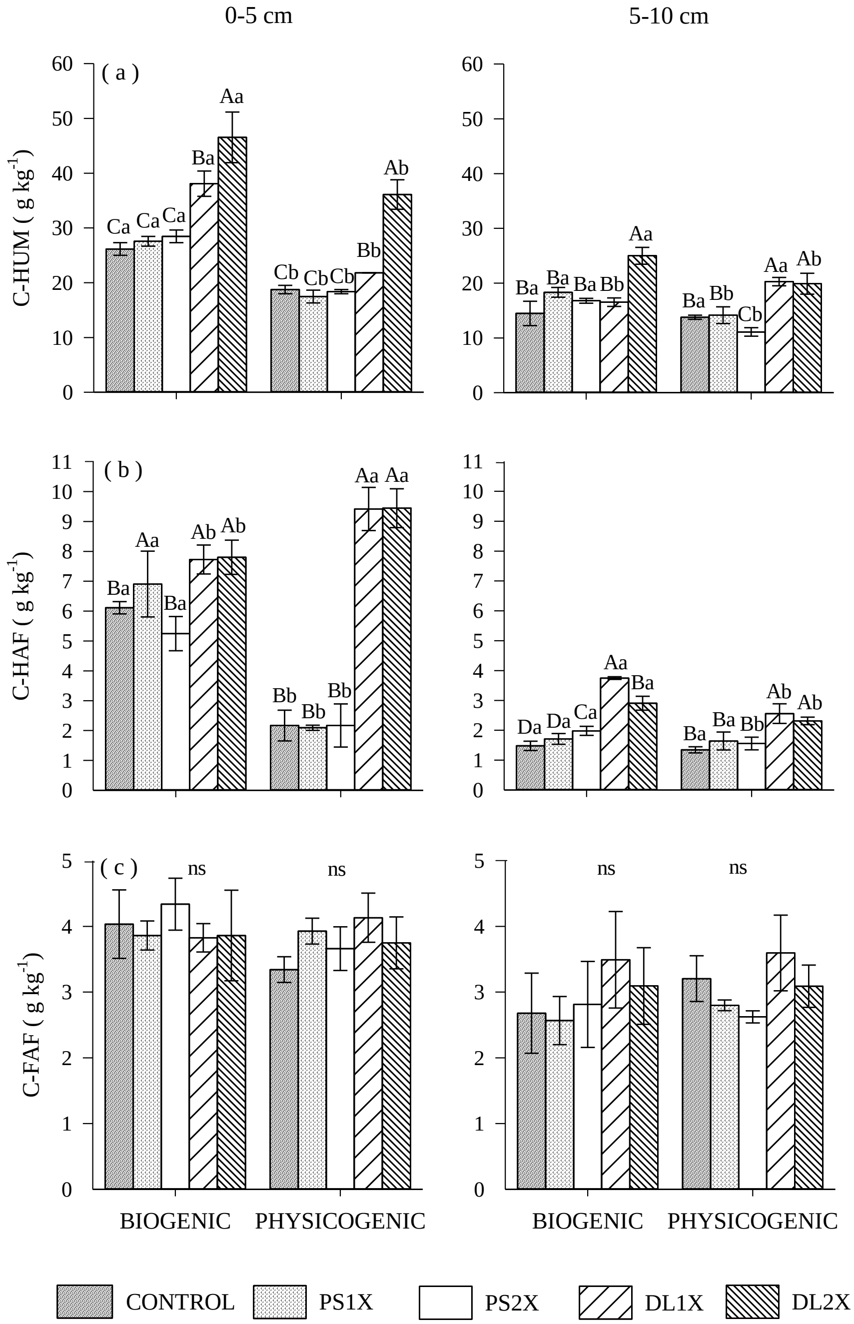

Figure 3. Carbon levels of the fractions humin (a), humic acids (b) and fulvic acids (c) in the biogenic and physicogenic aggregates at the depths of 0-5 cm and 5-10 cm of an Ultisol after applications of PS and DL, Braço do Norte, SC, Brazil. Means followed by the same upper-case letter do not differ among treatments for each type of aggregate, and means followed by the same lower-case letter do not differ between types of aggregates for each treatment (Scott-Knott test, $p<0.05$). Control = without waste fertilization; PS1X = pig slurry, 1 time the recommendation of N; PS2X = pig slurry, 2 times the recommendation of N; DL1X = deep litter, 1 time to recommendation of N; DL2X = deep litter, 2 times the recommendation of N.

The levels of C-HAF varied from 1.9 to 7.8 g kg^{-1} in the biogenic aggregates and from 1.4 to 9.4 g kg^{-1} in the physicogenic ones. The results showed a similar behavior to the one of C-HUM, in which, in the two types of aggregates, the highest levels of C-HAF were found in the treatments with DL at both depths, and the lowest levels in the control and PS, with the exception of the PS1X treatment at the depth of 0-5 cm, which did not differ from DL. Between the aggregates, at the depth of 0-5 cm, the levels of C-HAF were higher in the biogenic aggregates for the control and PS treatments, whereas in the treatments with DL the levels were higher in the physicogenic aggregates. At the depth of 5-10 cm, in the PS2X and

DL treatments, the levels were higher in the biogenic aggregates and did not differ among the other treatments (Figure 3b). The levels of C-FAF varied from 2.6 to 4.3 g kg^{-1} in the biogenic aggregates and from 2.6 to 4.1 g kg^{-1} in the physicogenic ones, and did not present differences ($p<0.05$) among treatments and nor between the types of aggregates in the two evaluated depths (Figure 3c).

Among the studied fractions and corroborating other studies (Passos et al., 2007, Fontana et al., 2006 and 2010, Loss et al., 2010, Borges et al., 2015), the HUM fraction that has higher mineral interaction (Stevenson., 1994, Fontana et al., 2006, Dick et al., 2009), contains most of the C levels (40 to 73%), followed by HAF (5 to 21%) and FAF (6 to 13%). These higher levels of carbon in the form of HUM suggest higher cation retention and higher aggregation of the soil Souza and Melo (2003).

The highest levels of C-HUM and C-HAF, which occurred in general in the treatments with DL, are associated with the highest TOC levels found in the treatments with DL (Figure 2), i.e., due to the higher C/N ratio of DL when compared to PS (Table 1), which favors a slower mineralization and leads to the formation of more stable humic substances.

Between the types of aggregates, the presented results indicate that in the biogenic aggregates there is a more favorable environment for the formation of humic substances. It should be noted that the use of PS and DL in general favors the formation of C-HUM and C-HAF in the biogenic aggregates, because for the control, at the depth of 5-10 cm, no differences were observed between the types of aggregates (Figure 3). At depth of 0-5 cm, the levels of C-HUM and C-HAF were also higher in the biogenic aggregates of the control treatment to compared the physicogenic ones. This is due to the influence of plant residues (maize and oats) in the 0-5 cm layer, because NTS is used, which favors the accumulation of carbon in the soil surface layer, especially the SOM stable fractions, such as HUM and HAF (Loss et al., 2013). The fact of having more carbon in depth, where it has less effect of the vegetal residues, can be an indicative of effect of the use of pig swine for 10 years. These higher levels of C-HUM and C-HAF in the biogenic aggregates are directly related to the action of soil fauna factors (macro and microfauna) and to the root system, which are mainly related to the formation of biogenic aggregates, in addition of occurring with more intensity on the soil surface due to the contribution of plant material and the absence of soil revolving. These results are similar to those found by Loss et al (2014), which have also quantified the carbon levels of humic fractions in biogenic and physicogenic aggregates in NTS, pasture and forest, and have also found higher levels of C in the biogenic aggregates.

Only at the depth of 0-5 cm the levels of C-HAF were higher in the physicogenic aggregates for the treatments with DL (Figure 3b). This may be related to the clay levels, which were higher in the physicogenic aggregates only for DL1X and DL2X compared to the biogenic ones (Table 4). Studies conducted by Santana et al (2011) and Mujuru et al (2013) suggest that in soils with a higher level of clay there is a predominance of carbon in the more stable fractions through a strong interaction between the organic and inorganic portions of the soil, forming complexes with strong bonds between clays and humic acids. In many soils, between 50 and 100% of the carbon is complexed with the clay fraction Manahan (2012).

The C-HAF/C-FAF ratio is widely used when the chemical fractionation of SOM is performed, as it indicates the level of humification, or carbon mobility in the soil Benites et al (2003). If the result of this ratio is higher than 1.0, the predominance of C-HAF, i.e., a higher level of humification of the SOM with more stable organic material, is observed. In this case, the formation of biogenic aggregates is favoring the humification of SOM, since values of this ratio higher than 1.0 were found in all treatments at the depth of 0-5 cm and only for DL1X at the depth of 5-10 cm (Table 5).

In the physicogenic aggregates, only the treatments with use of DL at the depth of 0-5 cm presented values higher than 1.0, which indicates that the treatment with the use of DL favors the humification of SOM regardless of the formation pathway of the aggregate. These results are corroborated by the higher values of the C-HAF/C-FAF ratio in the treatments with DL, both for the biogenic aggregates and for the physicogenic aggregates at both depths (Table 5).

The higher values of the C-HAF/C-FAF ratio in the treatments with DL compared to the ones with PS indicate that the DL presents better quality and favors the formation of HAF. This higher quality may be related to the higher values of dry mass, C/N ratio and nutrient levels N, P, K, Ca and Mg in comparison to PS (Table 1). With a higher index of the C-HAF/C-FAF ratio, better conditions for the establishment of physical and chemical properties beneficial to the development of plants are obtained, as reported by Guareschi et al (2013), and corroborated by other authors (Fontana et al., 2006, Loss et al., 2010, Borges et al., 2015).

Table 2. C-HAF/C-FAF and (C-HAF+C-FAF)/C-HUM ratio in the biogenic and physicogenic aggregates of anUltisol with application of different sources and amounts of swine waste, Braço do Norte, Santa Catarina, Brazil.

Treatments	C-HAF/C-FAF		(C-HAF+C-FAF)/C-HUM	
	Biogenic	Physicogenic	Biogenic	Physicogenic
	0-5 cm			
CONTROL	1.53 B	0.65 C	0.39 A	0.29 B
PS1X	1.79 B	0.53 C	0.39 A	0.35 B
PS2X	1.22 B	0.59 C	0.34 A	0.32 B
DL1X	2.02 A	2.28 B	0.30 B	0.62 A
DL2X	2.06 A	2.53 A	0.25 B	0.37 B
CV%	14.64	7.93	8.62	11.73
	5-10 cm			
CONTROL	0.58 B	0.42 C	0.30 B	0.33 A
PS1X	0.68 B	0.58 B	0.23 B	0.32 A
PS2X	0.73 B	0.60 B	0.29 B	0.38 A
DL1X	1.10 A	0.73 A	0.44 A	0.30 A
DL2X	0.95 A	0.75 A	0.24 B	0.27 A
CV%	21.31	12.99	17.48	8.42

Means followed by the same letter in the column do not differ among them by the Scott-Knott test at 5%. CV = coefficient of variation. C-HAF: carbon of the humic acid fraction, C-FAF: carbon of the fulvic acid fraction, C-HUM: carbon of the humin fraction. CONTROL = without waste fertilization; PS1X =pig slurry, 1 time the recommendation of N; PS2X = pig slurry, 2 times the recommendation of N; DL1X = deep litter, 1 time to recommendation of N; DL2X = deep litter, 2 times the recommendation of N.

Another ratio studied is the (C-HAF/C-FAF)/C-HUM, which indicates the structural stability of SOM, in which the lower values indicate the predominance of C-HUM and a better chemical stability of SOM Benites et al (2003). In the present study, the values found were all lower than 1.0, indicating the predominance of C-HUM and a good chemical stability in all treatments, at both depths and in the two types of aggregates that were evaluated. These results may be due to the NTS with the oats/maize succession, which favors the accumulation of aerial and root biomass of oats and maize used in all treatments, especially the treatments with the use of DL in the biogenic aggregates at the depth of 0-5 cm, which presented the lowest values of this ratio (Table 5), indicating a better chemical stability than the use of PS and control.

Similar results to the ones of this study were also found by Fernandes et al (2017), which evaluated the levels of C-HUM, C-HAF, C-FAF, C-HAF/C-FAF and (C-HAF+C-FAF)/C-HUM in areas of forest and pasture in Pinheiral, RJ. The authors have found higher levels of C-HUM, C-HAF and C-HAF/C-FAF in the biogenic aggregates when compared to the physicogenic ones, as well as absence of differences for C-FAF and lower values of (C-HAF+C-FAF)/C-HUM in the biogenic aggregates.

Nitrogen levels of the humic substances

The highest proportion of TN of the soil is found in the humin fraction (N-HUM), the same pattern being verified by Souza and Melo (2000). The levels of N-HUM varied from 1.2 to 3.4 g kg^{-1} in the biogenic aggregates and from 1.0 to 3.0 g kg^{-1} in the physicogenic ones. In both types of aggregates, the highest levels were found in the areas with DL treatments and the lowest ones in the other treatments for both depths, with the exception of DL1X in the physicogenic aggregates at the depth of 0-5 cm, which did not differ from the control and PS areas. This behavior may be due to the fact that the N present in the swine manure is largely in the form of mineral N, that is, it is readily available to the plants and the rest is subject to losses by volatilization or surface runoff, not being accumulated in the soil (Ceretta et al., 2010).When compared between the aggregates, the highest levels of N-HUM were observed in the biogenic aggregates in all treatments at the depth of 0-5 cm, with the exception of PS2X, which did not differ between the types of aggregates. At the depth of 5-10 cm, higher levels of N-HUM for the biogenic aggregates were also observed, however, only for the PS1X and DL2X treatments. For the others, no statistical differences were found between the types of aggregates (Figure 4a).

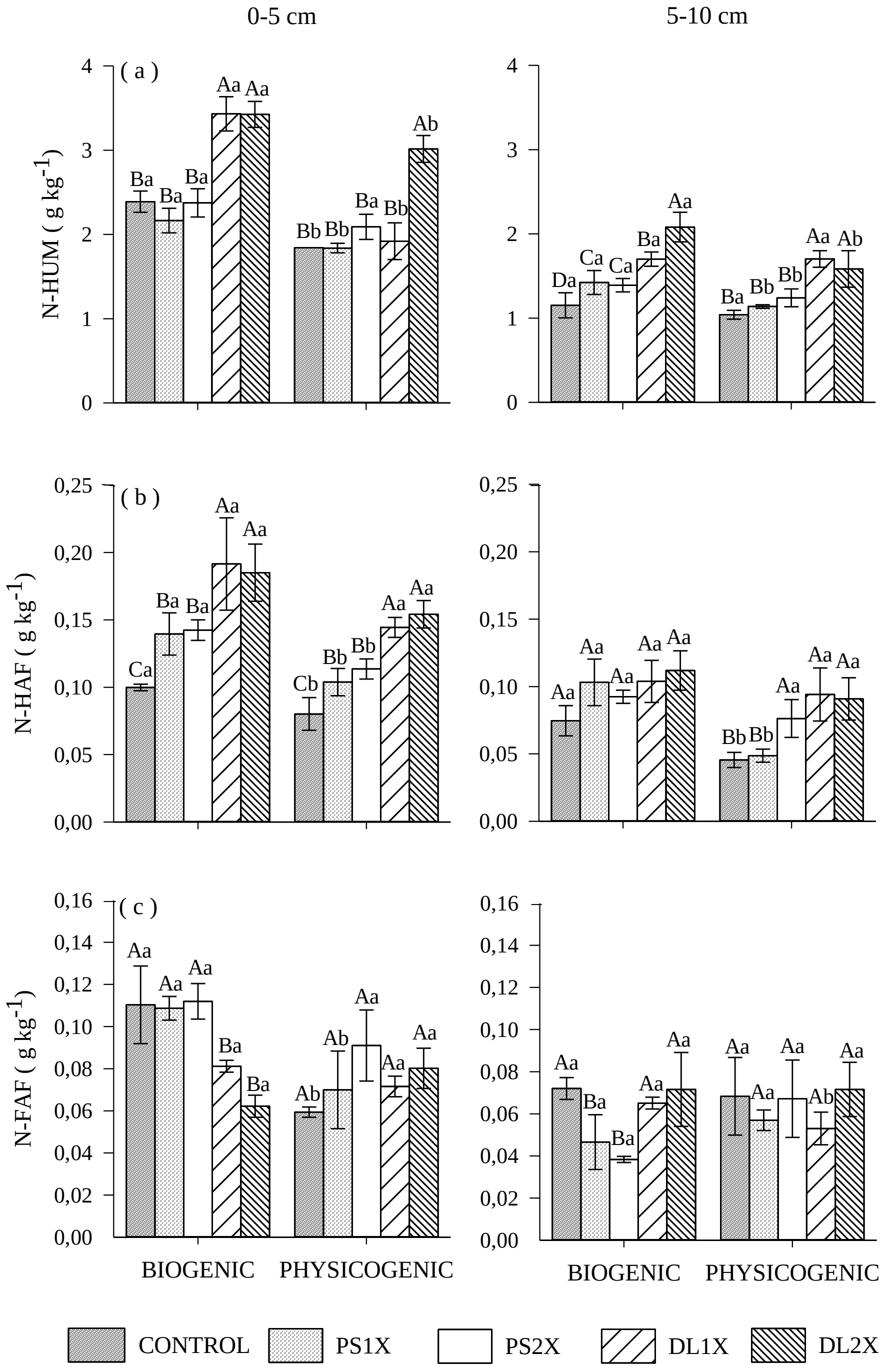

Figure 4. Nitrogen levels of the fractions humin (a), humic acids (b) and fulvic acids (c) in biogenic and physicogenic aggregates in an Ultisol after applications of PS and DL, Braço do Norte, SC, Brazil. Means followed by the same upper-case letter do not differ among treatments for each type of aggregate, and means followed by the same lower-case letter do not differ between types of aggregates for each treatment (Scott-Knott test, $p<0.05$). Control = without waste fertilization; PS1X = pig slurry, 1 time the recommendation of N; PS2X = pig slurry, 2 times the recommendation of N; DL1X = deep litter, 1 time to recommendation of N; DL2X = deep litter, 2 times the recommendation of N.

The levels of N-HAF varied from 0.08 to 0.19 g kg^{-1} in the biogenic aggregates and from 0.05 to 0.16 g kg^{-1} in the physicogenic ones. In the biogenic aggregates, for the N-HAF at the depth of 0-5 cm, the highest levels were found in the treatments with DL, intermediate ones in PS and the lowest levels in the control. At the depth of 5-10 cm no statistical differences among treatments were found. In the physicogenic aggregates, at the depth of 0-5 cm, the highest levels of N-HAF were also found in the treatments with DL, intermediate ones in PS and the lowest levels in the control. At the depth of 5-10 cm, the highest levels were found in the treatments with DL and PS2X. Between the aggregates, at the depths of 0-5 and 5-10 cm, the levels of N-HAF were higher in the biogenic aggregates compared to the physicogenic ones in the control and PS (0-5 cm) and only for the control and PS1X (5-10 cm) (Figure 4b).

The levels of N-FAF varied from 0.04 to 0.11 g kg^{-1} in the biogenic aggregates and from 0.05 to 0.09 g kg^{-1} in the physicogenic ones. In the biogenic aggregates, the highest levels were found in the PS and control treatments and the lowest ones in the DL treatment at the depth of 0-5 cm. At the depth of 5-10 cm, the highest levels were observed in the DL and control treatments and the lowest ones in the PS treatments. For the physicogenic aggregates, no differences were observed among the treatments at the two evaluated depths. When comparing the aggregates, higher levels of N-FAF were observed in the biogenic ones in the control and PS1X treatments at the depth of 0-5 cm. At the depth of 5-10 cm, only in the DL1X treatment higher values of N-FAF were observed for the biogenic aggregates (Figure 4c).

The highest levels of N-HUM and N-HAF for the treatments with DL when compared to the ones with PS in the biogenic and physicogenic aggregates at the two evaluated depths and only for the biogenic aggregates for C-FAF may be related to the quality of the organic material, i.e., in DL waste there are higher values of N when compared to PS waste (Table 1). As described by Assis et al. (2006), the presence of N in the humic fractions is an indicative that part of the N of the soil is stabilized in these fractions. In other words, in DL fertilized areas there is a greater chemical stability when compared to PS fertilized areas.

It should be noted that the levels of N-HUM in the biogenic aggregates at the depth of 5-10 cm and the levels of N-HAF in both types of aggregates at the depth of 0-5 cm were higher in the treatments with DL and PS in comparison to the control treatment (Figure 4). These results indicate that the use of swine waste favors the formation of these humic fractions in the soil, which was not observed for the total nitrogen (TN) of the soil, since the control and PS areas showed no differences between them (Figure 2).

Between the aggregate types, when differences were observed, the highest values occurred in the biogenic aggregates, as observed for the TOC, TN and carbon levels of the humic fractions, indicating that in the biogenic aggregates the edaphic conditions are better for the maintenance and formation of the N of the humic fractions, with emphasis on N-HUM that presented higher values in the biogenic aggregates for all treatments when compared to N-HAF and N-FAF. Similar to the carbon levels, and showing a linear relation, the nitrogen levels were higher in the N-HUM fraction (41.11% to 74.64%) compared to the N-HAF fraction (1.98% to 4.71%) and N-FAF (1.10% to 4.12%). These results follow the same pattern of the carbon, in which the entry and exit rates of C and N have favored the humification of SOM, thus presenting higher levels in the more stable fractions of the aggregates, with an emphasis on the biogenic ones, mainly due to the higher biological activity.

The N contents of HSs represent a passive fraction of SOM, because HSs are characterized as highly recalcitrant organic molecules in the soil, that is, they are more difficult to be altered by management practices (Stevenson, 1994). However, in this study, the use of swine manure (PS and DL) in oat/corn under NTS increased N-HUM levels (5-10 cm) compared to control treatment in biogenic aggregates. And at 0-5 cm depth, the use of DL and PS increased the N-HAF levels compared to the control in biogenic and physicogenic aggregates. This indicates that the use of swine manure (DL and PS) in NTS favors the humification of SOM, as the more stable fractions (HUM and HAF) increased in relation to the less stable fraction (FAF). This greater humification of SOM in areas with DL and PS areas is directly related to the higher dry mass production of black oats (Table 4). With greater mass of the root system there is also a greater production and distribution of root exudates. Thus, both soil aggregation and N protection within the aggregates are favored, increasing in the SOM stable fractions, such as N-HUM and N-HAF (Silva et al., 2008).

CONCLUSIONS

The applications of DL for 10 years have increased TOC, TN, C-HUM, N-HUM, C-HAF and N-HAF levels in relation to the control and PS treatments. The use of PS and DL has increased the levels of TOC, TN, C-HUM and N-HUM in the biogenic aggregates when compared to the physicogenic ones.

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