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Aggregation Index and Carbon and Nitrogen Contents in Aggregates of Pasture Soils under Successive Applications of Pig Slurry in Southern Brazil

Cristiano Santos [1], Arcângelo Loss [1,*], Marisa de Cássia Piccolo [2], Eduardo Girotto [3], Marcos Paulo Ludwig [4], Julia Decarli [4], José Luiz Rodrigues Torres [5] and Gustavo Brunetto [6]

[1] Center of Agricultural Sciences, Federal University of Santa Catarina, Itacorubi, Florianópolis 88034-000, SC, Brazil; docris@gmail.com
[2] Center of Nuclear Energy in Agriculture, University of São Paulo, Piracicaba 13416-970, SP, Brazil; mpiccolo@cena.usp.br
[3] Federal Institute of Education, Science and Technology of Rio Grande do Sul, Campus Bento Gonçalves, Bento Gonçalves 95700-206, RS, Brazil; eduardo.girotto@bento.ifrs.edu.br
[4] Federal Institute of Education, Sciences, and Technology of Rio Grande do Sul, Ibirubá Campus, Ibirubá 98200-000, RS, Brazil; marcos.ludwig@ibiruba.ifrs.edu.br (M.P.L.); julia.decarli90@gmail.com (J.D.)
[5] Federal Institute of Triângulo Mineiro, Uberaba 38064-790, MG, Brazil; jlrtorres@iftm.edu.br
[6] Center of Rural Sciences, Federal University of Santa Maria, Camobi, Santa Maria 97105-900, RS, Brazil; brunetto.gustavo@gmail.com
* Correspondence: arcangelo.loss@ufsc.br

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Abstract: Pig slurry (PS) applications affect soil aggregation and carbon and nitrogen contents in aggregates. The objective of this study was to evaluate changes caused by successive applications of PS and mineral fertilizer on soil aggregation and carbon (C) and nitrogen (N) contents in aggregates of a clayey Typic Hapludox cultivated with *Cynodon dactylon* cv. Tifton-85 in southern Brazil. The treatments consisted of six annual applications of PS (100, 200, 300, and 400 kg N ha^{-1}) and urea (200 kg N ha^{-1}), and a control with no fertilizer application. Soil samples were collected in March 2019 and evaluated for aggregate stability, through the geometric mean diameter of aggregates (GMD), and GMD sensitivity index (SI_{GMD}), and mass of macro-, meso-, and microaggregates. Total organic carbon and nitrogen contents were determined in macroaggregates and microaggregates. Applications of PS to pasture soils increase dry matter production of Tifton-85 and can increase soil aggregation by increasing the mass of macroaggregates. The highest PS rates decreased aggregate stability, resulting in lower macroaggregate mass, GMD, and SI_{GMD}, and higher microaggregate mass. PS applications to pasture soils can increase C and N contents in macro and microaggregates, and improve soil aggregation when using the rates of 100 or 200 kg N ha^{-1}, mainly in subsurface layers.

Keywords: *Cynodon* sp.; soil aggregation; swine slurry; mineral fertilizer

1. Introduction

Livestock is an important socioeconomic sector in Brazil due to its employment and income generation; the livestock market reached more than BRL200 billion in 2019, which includes bovine (210 million) and swine (40 million) animals [1,2]. Most bovines in Brazil are dependent on pastures, which reached an area of 162 million hectares in 2019, with 1.32 animals per hectare [3]. However, fertilizer applications and production management systems for pasture soil are often inefficient regarding sustainability.

Increases in technology and yield with intensive animal productions have generated high amounts of wastes, reaching 17.2 m^3 year^{-1} animal^{-1} [4], which need to be properly disposed or reused. Wastes from pig production are usually managed in liquid form, called pig slurry (PS) or liquid swine manure, which are stored in anaerobic ponds and mainly used as soil fertilizer for agricultural crops, including pastures [5–10] due to the high organic matter and macro and micronutrient contents (N, P, K, Ca, Mg, Mg, Fe, Zn, Cu, and B); however, the concentration of these nutrients presents high variation [11]. PS is an alternative to commercial fertilizers to increase biomass production and quality, and reduce environmental impacts and production costs [12,13] with similar results [14–16].

PS applications can improve soil attributes [17,18], such as aggregation, aeration, water infiltration, and soil bulk density [19,20]. PS is also a source of N, presenting similar results to urea [21], with the advantage of a reduction in costs [8], providing a residual effect due to the gradual release of N [22], and a significant increase in biomass production [7,8,22,23].

Changes in soil chemical [24,25], physical [16,26,27], and biological [28–30] attributes have been found with application of PS, with positive effects on soil quality, and crop yields [10,31,32]. These benefits are mainly due to increases in organic matter contents, which favor soil aggregation and availability of nutrients to plants. A meta-analysis study showed that manure applications have increased agricultural yields of 7.6%, on average, compared to mineral fertilizers; in addition, these fertilizers have also shown increases in soil pH (3.3%), water-stable aggregation (28.8%), soil organic carbon (17.7%), and total (15.5%) and available nitrogen (16.0%), and decreases in soil bulk density (−3.9%), compared to mineral fertilizers [33].

These soil characteristics have been used as indicators of soil quality, but the effect of PS applications still requires further studies regarding their effects on them. Bertagnoli et al. [34] evaluated the application of different PS rates and found increases in soil macroaggregate mass, and Ferreira et al. [35] found increases in total organic carbon (TOC) (67%) and total nitrogen (TN) (126%) contents in soil aggregates using PS combined with mineral fertilizer, but no improvement in soil physical attributes, with decreasing soil aggregation indexes and geometric mean diameter of aggregates (GMD), and increases in mass of microaggregates in all treatments. Moreover, according to Barbosa et al. [36], despite the use of animal manure as fertilizer improving soil structure, positive and negative effects of this practice remain inconclusive, since manure application can increase soil dispersible clay contents, disaggregation, susceptibility to erosion, and contamination of surface waters. The authors in [37] evaluated PS applications to supply N for eight years and found increases in TOC contents up to 30 cm depth. In addition, Loss et al. [16] evaluated the use of animal manure for 11 years under a no-tillage system and found that applications of PS increase soil TOC and TN contents and the GMD, but reduce mesoaggregates ($2.0 > Ø \geq 0.25$ mm) and microaggregates ($Ø < 0.25$ mm) in the soil surface layer when compared to the NPK and control treatments, and that animal manure promoted the dispersion of clays in the 5–10 and 10–20 cm layers, resulting in lower soil aggregation in depth.

According to [38], pasture soils showed a higher aggregate stability (GMD) than cultivated soils due to the extensive rooting of grasses, higher TOC content, permanent plant coverage, and higher soil conservation. The authors in [39] reported that the greater stable macroaggregates in the pasture soils may be due to the higher amount of microbial biomass, plants residue, plants root, polysaccharides, and humic materials in the macroaggregates of these soils. Gol [40], Masciandaro [41], and Loss et al. [42] reported significantly larger water stable aggregates, TOC, and TN contents in pasture soils than in cultivated soils.

Long-term studies evaluating C and N contents in aggregates of pasture soils with application of PS and mineral fertilizer are incipient. Thus, considering the wide use and effects of mineral and organic soil fertilizers on the maintenance of soil quality, the hypothesis raised is that long-term PS applications to pasture soils, using proper rates, increase soil aggregation and C and N contents in aggregates, when compared to mineral fertilizers. Therefore, the objective of this study was to evaluate changes caused by successive applications of PS and mineral fertilizer on soil aggregation and carbon (C) and nitrogen (N) contents in aggregates of a clayey Typic Hapludox cultivated with Tifton-85 in southern Brazil.

2. Materials and Methods

2.1. Study Area

The experiment was conducted at the Federal Institute of Education, Science, and Technology of Rio Grande do Sul (IFRS), in Ibirubá, RS, Brazil (28°39′09″ S, 53°06′20″ W, and altitude of 421 m), in an area cultivated with Bermuda grass (*Cynodon dactylon* (L.) Pers., cv. Tifton-85) intended for hay production for approximately 10 years. The region presents a Cfa2, subtropical humid climate, according to the Köppen classification [43]. The historical monthly mean minimum and maximum temperature and rainfall depths of the region, according to the Brazilian National Institute of Meteorology [44] is shown in Table 1.

Table 1. Historical monthly mean minimum and maximum temperature and rainfall depths (mean of 1981–2010) in Ibirubá, RS, Brazil.

Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean minimum temperature (°C)											
18.0	17.4	16.1	13.3	10.4	9.0	8.2	9.6	10.5	13.0	15.1	17.0
Mean maximum temperature (°C)											
30.1	28.9	28.4	25.8	21.5	19.5	19.0	21.2	22.1	25.3	28.1	30.0
Mean rainfall depth (mm)											
153	162	112	156	117	128	166	145	167	219	154	132

The soil of the area was classified as Typic Hapludox according to Soil Survey Staff [45], Dystric Rhodic Ferralsol (Typical) according to WRB [46], and as *Latossolo Vermelho Distroférrico típico*, according to Santos et al. [47]. Prior to the establishment of the experiment, soil samples had been collected in the area in May 2012 and subjected to chemical and physical analysis, according to the methods described by Tedesco et al. [48]; the results are shown in Table 2.

Table 2. Chemical and physical attributes of the 0–10 and 10–20 cm layers of the topsoil (Typic Hapludox) of the experiment area. Ibirubá, RS, Brazil.

Layer (cm)	pH (H_2O)	pH (SMP)	Ca	Mg	Al	H + Al	P	K	SOC(%)	Clay (%)	Silt (%)	Sand (%)
			(cmol$_c$ dm^{-3})				(mg dm^{-3})					
0–10	5.8	6.0	9.8	5.9	0.0	4.4	50	288	4.3	46	20	34
10–20	5.2	5.7	4.9	3.1	0.5	6.2	23	232	2.6	59	15	26

pH H_2O determined in a soil to water ratio of 1:1; pH SMP was determined in Ca acetate buffer pH 7.5; H + Al determined based on the SMP index; SOC (soil organic carbon) determined by the dry combustion method, using an autoanalyzer (LECO TruSpec CHNS); P available and K exchangeable was extracted with Mehlich-1.

2.2. Treatments and Sampling

A randomized block experimental design was used, with four replications. The experimental area was prepared in October 2012, and the application of the treatments started in November 2012, using 4 × 5 m plots (20 m^2). The treatments consisted of six annual applications of fertilizer containing N, using an organic source (pig slurry—PS) at the rates of 0 (Control, no N fertilizer application), 100 (PS100), 200 (PS200), 300 (PS300), and 400 (PS400) kg N ha^{-1}, and a mineral source (urea) at the rate of 200 kg N ha^{-1} (Min200). The mineral treatment included phosphorus (P) and potassium (K) applications, using potassium chloride and triple superphosphate as sources; the rates used were based on soil analysis and the Tifton-85 biomass accumulation. P and K sources were manually applied to the soil surface. The amounts of P and K applied annually were, respectively, 90 kg of P_2O_5 ha^{-1} and 120 kg of K_2O ha^{-1}. The PS rates used were based on its N contents, which were estimated according to the methodology proposed by the Soil Chemistry and Fertility Committee [15]. To estimate the amounts of PS to be applied based on N contents, five subsamples of manure were evaluated. This procedure was conducted every year. The PS applied presented the following characteristics (average contents) (Table 3).

Table 3. Characteristics of the pig slurry (PS) used (mean of the 2012–2019 applications; data expressed on a wet basis). Ibirubá, RS, Brazil.

Dry Matter (%)	pH	Total C ($g\ kg^{-1}$)	Total N ($g\ kg^{-1}$)	TAN ($g\ kg^{-1}$)	C to N Ratio	Total P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)
2.71	7.30	30.10	3.23	2.38	9.32	0.010	0.049

TAN = total ammonia N (NH_3^+, NH_4^+).

The total annual amounts of PS and urea were divided into three equal applications, carried out after each one of the three cuts of the grass, carried out for dry matter evaluation, and in the six agricultural years (2012/2013, 2013/2014, 2014/2015, 2015/2016, 2016/2017, and 2017/2018). The grass cuttings were removed from the area before the applications.

Samples of the 0–5, 5–10, and 10–30 cm layers of the soil of each plot were collected in March 2019; undisturbed samples were used to determine the soil bulk density, using a ring with known volume (Kopecky rings) of 50 cm^3, and soil aggregation, according to the methods described by the Embrapa [49] and Tedesco et al. [48]; disturbed samples were used for the chemical analysis. In each plot of each treatment, three undisturbed and disturbed subsamples were collected to compose an undisturbed/disturbed sample, making up four undisturbed/disturbed samples per treatment and for each depth.

2.3. Soil Aggregation Indexes

Soil aggregates stability analyses were carried out using undisturbed soil samples, which were air dried, manually crushed, and passed through 8.0 and 4.0 mm mesh sieves. A 25 g subsample of the soil aggregates retained in the 4.0 mm mesh sieve was placed on a 2.0 mm mesh sieve, which was the first of a set of sieves with decreasing mesh diameters (2.0, 1.0, 0.5, 0.25, 0.106, and 0.053 mm, according to the method described by the Embrapa [49]. The aggregates on the 2.0 mm mesh sieve were wetted and, after 5 min, the set of sieves was submerged in water for a vertical wet sieving for 15 min, using a Yoder device [50]. The soil retained in each sieve was removed with water jets and placed in previously weighed aluminum containers, identified, and taken to a forced air-circulation oven at 60 °C until constant weight.

The dry soil retained in each sieve was used to calculate the dry weight of the aggregate according to each diameter class, as described by Costa Junior et al. [51]: 8.0 > Ø ≥ 2.0 mm (macroaggregates), 2.0 > Ø ≥ 0.25 mm (mesoaggregates), and Ø < 0.25 mm (microaggregates). These data were used to calculate the geometric mean diameter (GMD), aggregate stability index (ASI), index of percentage of aggregates with diameter larger than 2 mm (AGRI), and GMD sensitivity index (SI_{GMD}), as used by Torres et al. [52]:

$$\text{GMD} = \exp\left\{\sum [(\ln [xi] * [pi])] / \sum [pi]\right\} \quad (1)$$

where ln [xi] is the natural logarithm of the mean diameter of aggregate classes; and pi is the weight (g) of aggregates retained in each sieve.

$$\text{ASI} = [(\text{PA} - \text{wp} < 0.25)/\text{PA})] \times 100 \quad (2)$$

where PA is the weight of the sample; wp < 0.25 is the weight of aggregates <0.25 mm (g)

$$\text{AGRI} = \text{wi} > 2 \times 100 \quad (3)$$

where wi > 2 is the proportion of aggregates >2 mm.

$$\text{SI}_{\text{GMD}} = \text{GMDt}/\text{GMDc} \quad (4)$$

where GMDt is the GMD of each treatment, and GMDc is the GMD of the control in the respective soil layer.

2.4. C and N in Soil Aggregates

The total organic carbon (TOC) and total nitrogen (TN) contents were determined in undisturbed soil samples of macroaggregates (8.0 > Ø ≥ 0.25 mm) and microaggregates (Ø < 0.25 mm). The samples were ground in a mortar and a subsample of two grams was placed in Eppendorf containers and sent for analysis by the dry combustion method, using an autoanalyzer (TruSpec CHNS; LECO Corporation, St. Joseph, MI, USA), at 1000 °C, in the Nutrient Cycling Laboratory (LCN) of the of Center of Nuclear Energy in Agriculture (CENA) of the University of São Paulo (USP), in Piracicaba, SP, Brazil.

2.5. Statistical Analysis

The results were subjected to normality (Lilliefors) and homogeneity (Cochran) tests. The results of the treatments were subjected to analysis of variance (ANOVA) by the F test and significant means were compared by the Scott–Knott test at 5% probability using the Sisvar 5.6 program [53].

3. Results

3.1. Aggregate Classes and Aggregation Indexes

3.1.1. Mass of Aggregates

The treatments with PS affected the soil macro, meso, and microaggregates in all soil layers (0–5, 5–10, and 10–30 cm) (Figure 1). Significantly higher macroaggregate mass was found for the treatments PS100 and PS200 in the 0–5 cm layer when compared to the other treatments. In the 5–10 cm layer, only the Control treatment differed from the others, presenting the lowest macroaggregate mass. In the 10–30 cm layer, PS200, PS300, and PS400 presented higher macroaggregate mass than the treatments MIN200 and PS100; however, the Control treatment presented higher macroaggregate mass when compared to all treatments.

Mesoaggregate mass was lower than 0.5 g in the samples of all treatments in the 0–5 cm layer; the highest contents were found for the PS100 and Control treatments. The Control presented significantly higher contents in the 5–10 cm layer when compared to the other treatments. In the 10–30 cm layer, the mesoaggregate mass decreased as the PS contents were increased, with PS400 presenting similar contents to the Control and lower than the MIN200.

The microaggregate mass was significantly higher for PS300 and PS400 in the 0–5 cm layer and for PS400 in the 5–10 cm layer. PS300 and PS400 presented higher contents than the other treatments in the 10–30 cm layer, except for the MIN200, which showed the highest contents in this layer.

3.1.2. Geometric Mean Diameter (GMD)

The GMD of the aggregates showed significant differences between treatments, with higher GMD for the surface layer in all treatments (Figure 2). The treatments PS100 and PS200 presented the highest GMD in the 0–5 and 5–10 layers. P200 presented the highest GMD in the 10–30 layer, but was similar to the Control.

GMD represents the estimated size of the most frequent aggregate class. Higher GMD (Figure 2) was found for treatments with PS when compared to mineral fertilizer due to the higher amount of macroaggregates (Figure 1) in these treatments. The treatment PS200 showed higher GMD than the other treatments in all evaluated soil layers, except for the treatment PS100, which did not differ from treatment PS200 in the 0–5 and 5–10 cm.

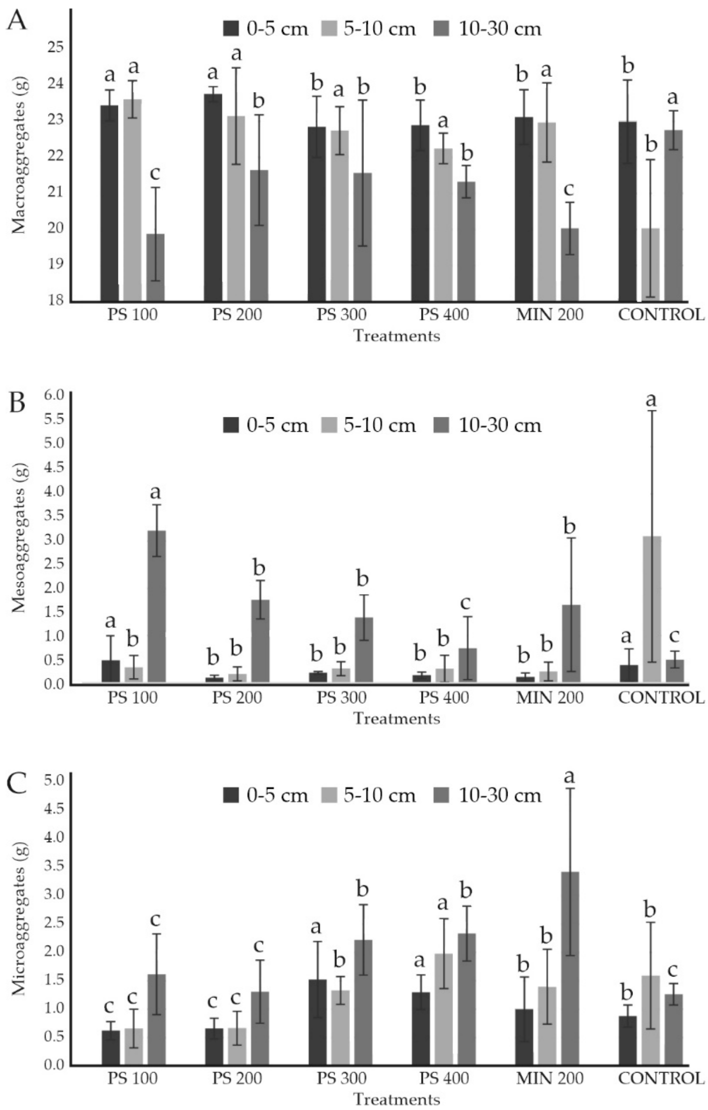

Figure 1. Macro (**A**), meso (**B**), and microaggregate (**C**) contents in the 0–5, 5–10, and 10–30 cm layers of a Typic Hapludox treated with different rates of pig slurry (PS) and mineral fertilizer. Ibirubá, RS, Brazil. PS100 = 100 kg N ha^{-1} using pig slurry (PS); PS200 = 200 kg N ha^{-1} N using PS; PS300 = 300 kg N ha^{-1} using PS; PS400 = 400 kg N ha^{-1} using PS; MIN200 = 200 kg N ha^{-1} using urea; Control = no fertilizer application. Bars with the same letter within the same soil layer are not different from each other by the Scott–Knott test ($p < 0.05$).

3.1.3. Aggregation Indexes

The treatments PS100 and PS200 resulted in the highest ASI values in 0–5 and 5–10 cm layers evaluated, however, without differing from the control (0–5 cm) and Min200 treatments (5–10 cm) (Table 4). In the 10–30 cm layer, treatments PS400 and Min200 showed the lowest ASI values.

PS200 presented the highest AGRI values in the 0–5 cm layer, and the PS100, PS200, and Min200 presented the highest AGRI in the 5–10 cm layer. The control treatment presented the highest AGRI in the 10–30 cm layer (Table 4); PS200, PS300, and PS400 showed higher ASI values compared to Min200, and PS100 did not differ from the Min200 treatment in the 10–30 cm layer.

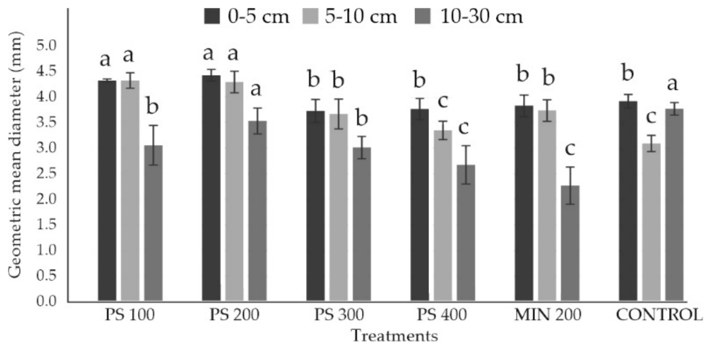

Figure 2. Geometric mean diameter in the 0–5, 5–10, and 10–30 cm layers of a Typic Hapludox treated with different rates of pig slurry (PS) and mineral fertilizer. Ibirubá, RS, Brazil. PS100 = 100 kg N ha^{-1} using pig slurry (PS); PS200 = 200 kg N ha^{-1} N using PS; PS300 = 300 kg N ha^{-1} using PS; PS400 = 400 kg N ha^{-1} using PS; MIN200 = 200 kg N ha^{-1} using urea; Control = no fertilizer application. Bars with the same letter within the same soil layer are not different from each other by the Scott–Knott test ($p < 0.05$).

Table 4. Aggregate stability index (ASI), index of percentage of aggregates with diameter larger than 2 mm (AGRI), and GMD sensitivity index (SI_{GMD}) in the 0–5, 5–10, and 10–30 cm layers of a Typic Hapludox treated with different rates of pig slurry (PS) and mineral fertilizer. Ibirubá, RS, Brazil.

Treatments	ASI (%)			AGRI (%)			SI_{GMD}		
	0–5	5–10	10–30	0–5	5–10	10–30	0–5	5–10	10–30
PS100	96.16 a	97.33 a	94.03 a	90.02 c	95.75 a	79.28 d	1.10 a	1.40 a	0.81 b
PS200	97.58 a	96.12 a	94.66 a	96.96 a	94.61 a	88.48 b	1.13 a	1.39 a	0.94 a
PS300	93.79 b	93.78 b	92.57 a	88.59 c	92.73 b	87.64 b	0.95 a	1.19 b	0.80 b
PS400	94.28 b	91.92 b	89.25 b	93.43 b	90.96 b	84.50 c	0.96 a	1.08 b	0.71 b
Min200	95.20 b	95.16 a	87.75 b	94.69 b	93.90 a	78.97 d	0.98 a	1.21 b	0.60 b
Control	96.04 a	92.10 b	94.59 a	93.58 b	74.05 c	92.60 a	1.00 a	1.00 b	1.00 a
F-value	5.73	14.36	26.24	28.38	198.98	87.48	1.74	7.79	6.43
CV (%)	2.05	2.06	2.09	2.08	2.11	2.17	9.80	8.25	12.35

Means followed by the same lowercase letter in the columns are not different from each other by the Scott–Knot test ($p < 0.05$). PS100 = 100 kg N ha^{-1} using pig slurry (PS); PS200 = 200 kg N ha^{-1} N using PS; PS300 = 300 kg N ha^{-1} using PS; PS400 = 400 kg N ha^{-1} using PS; MIN200 = 200 kg N ha^{-1} using urea; Control = no fertilizer application.

In the superficial layer (0–5 cm), the treatments showed no differences between them (Table 4). However, it is evident that treatments PS100 and PS200 presented, respectively, SI_{GMD} of 10 and 13% higher than the reference treatment (control). PS100 and PS200 presented the highest SI_{GMD} in the 5–10 cm layer. These values were, respectively, 40% and 39% higher than the reference treatment (Control). In the 10–30 cm layer, PS200 presented the highest SI_{GMD} in relation to the other treatments, and did not differ from the control treatment.

3.2. C and N in Aggregates

Considering the differences between treatments, in the 0–5 cm and 10–30 cm layers, the treatments presented similar N contents in macro and microaggregates. In the 5–10 cm layer, the Control presented significant lower N contents in macro and microaggregates than the other treatments, and PS400 presented significantly higher N contents in macroaggregates (Table 5).

In the 0–5 cm layer, the highest C contents were found in the treatments PS100, PS200, and PS400 for micro and PS100, PS300, and PS400 for macroaggregates, with the Control presenting the lowest contents for both aggregate classes. In the 5–10 cm layer, the highest C contents were found in the PS100 for micro and PS400 for macroaggregates, with the Control presenting the lowest contents for both aggregate classes. In the 10–30 cm layer, the highest C contents were found in the P100 and Control for micro and in the PS200 and

Control for macroaggregates (Table 5). The C and N contents were, in general, lower in the 10–30 cm layer, which was probably because the PS was applied to the soil surface without incorporation.

Table 5. Nitrogen (N) and carbon (C) contents in macro and microaggregates of the 0–5, 5–10, and 10–30 cm layers of a Typic Hapludox treated with different rates of pig slurry (PS) and mineral fertilizer. Ibirubá, RS, Brazil.

Treat	Micro	Macro	CV (%)	F-Value	Micro	Macro	CV (%)	F-Value
	Nitrogen (g kg^{-1})				Carbon (g kg^{-1})			
				0–5 cm				
PS100	1.85 Ab	2.28 Aa	10.17	1.21	21.52 Ab	27.93 Aa	9.34	15.52
PS200	1.89 Ab	2.55 Aa	21.10	3.99	22.78 Ab	24.12 Ba	7.13	2.32
PS300	1.66 Ab	2.28 Aa	11.65	2.01	19.09 Bb	27.17 Aa	5.57	78.44
PS400	2.09 Ab	2.88 Aa	19.98	5.04	23.61 Ab	29.23 Aa	13.34	5.07
Min200	2.02 Ab	2.64 Aa	17.68	4.60	19.02 Bb	25.45 Ba	13.73	8.83
Control	1.45 Ab	2.35 Aa	29.77	5.06	13.30 Cb	22.88 Ba	17.87	17.54
F-value	0.84	0.68			9.06	2.92		
CV (%)	28.39	22.45			12.45	10.79		
				5–10 cm				
PS100	1.67 Aa	1.72 Ba	10.86	0.05	17.57 Aa	18.97 Ba	8.59	1.53
PS200	1.27 Ab	1.75 Ba	11.60	14.17	14.82 Bb	19.85 Ba	6.47	40.39
PS300	1.27 Ab	1.75 Ba	11.18	14.60	13.53 Bb	18.82 Ba	6.53	49.68
PS400	1.52 Ab	2.32 Aa	31.71	3.54	14.02 Bb	21.32 Aa	8.89	42.27
Min200	1.39 Ab	1.74 Ba	13.73	5.34	14.26 Bb	18.33 Ba	12.37	8.15
Control	0.90 Bb	1.10 Ca	10.00	8.00	9.50 Cb	13.87 Ca	7.15	54.85
F-value	5.45	4.78			8.70	28.95		
CV (%)	16.94	20.50			12.66	5.04		
				10–30 cm				
PS100	1.05 Aa	1.17 Aa	9.33	3.71	11.22 Ab	14.78 Ba	7.39	27.52
PS200	0.87 Ab	1.37 Aa	5.76	115.55	9.54 Bb	16.68 Aa	6.77	128.51
PS300	1.12 Ab	1.55 Aa	14.40	1.75	9.82 Bb	15.90 Ba	3.63	339.37
PS400	0.93 Ab	1.25 Aa	12.99	10.17	9.52 Bb	15.39 Ba	9.40	4977
Min200	0.98 Ab	1.25 Aa	13.75	6.76	9.45 Bb	15.22 Ba	6.35	108.50
Control	1.07 Ab	1.65 Aa	14.60	16.71	11.07 Ab	18.12 Aa	6.41	114.25
F-value	1.06	2.33			5.23	5.41		
CV (%)	19.67	17.94			7.08	6.62		

Means followed by the same uppercase letter in the columns comparing treatments are not different from each other by the Scott–Knot test ($p < 0.05$). Means followed by the same lowercase letter in the rows comparing macro and microaggregates are not different from each other by the t Student teste ($p < 0.05$). PS100 = 100 kg N ha^{-1} using pig slurry (PS); PS200 = 200 kg N ha^{-1} N using PS; PS300 = 300 kg N ha^{-1} using PS; PS400 = 400 kg N ha^{-1} using PS; MIN200 = 200 kg N ha^{-1} using urea; Control = no fertilizer application. Treat = treatment; CV rows refer to the coefficient of variation in the treatments and the CV columns refer to the aggregate classes.

Considering the differences in C and N between macro and microaggregates, significant differences were also found in all treatments and soil layers, except for N in the 5–10 cm layer. Higher C and N contents were found in macroaggregates. The C and N contents found for the treatments with PS were, in general, higher when compared to the Control and similar to those in the Min200 treatment.

4. Discussion

4.1. Aggregate Classes and Aggregation Indexes

The results (Figure 1) showed improvements for treatments with PS regarding soil aggregation by increasing the macroaggregate mass. PS100 and PS200 presented, in general, higher macroaggregate and lower microaggregate mass than the MIN200 and Control treatments, denoting that these are the most efficient rates to improve aggregation of clayey soils. According to Loss et al. [42], soil aggregation is a good indicator of soil physical quality, mainly the macroaggregate mass (>2 mm). The macroaggregate mass in the surface layer in all treatments were probably improved by the high biomass contents in this layer and the Tifton-85 root system activity.

Soil aggregation is highly affected by the root system quality, mainly of grasses, whose fasciculate root system promote aggregation by the release of exudates and entanglements within soil particles, increasing macroaggregate formation and stability [54]. In addition, PS applications stimulate soil microbial activity and mycelium and glomalin production by mycorrhizal arbuscular fungi, which contribute to soil aggregation [55,56].

The lower macroaggregates and higher microaggregate values found for the MIN200 treatment when compared to PS200, PS300, and PS400 treatments in the 10–30 cm soil layer (Figure 1) may be due to the more pronounced soil organic matter (SOM) mineralization caused by the mineral nitrogen application (urea), which may lead to reductions in SOM contents, since this N becomes a raw material for decomposing microorganisms, accelerating SOM decomposition [35,57].

For the Control treatment, the highest macroaggregate mass in the 10–30 layer indicates that the treatments with PS and Min200 had a more pronounced effect in the superficial layers (0–10 cm). This is in accordance with the form of the application of fertilization, which is made on the surface of the soil and without incorporation into the soil. Thus, in depth (10–30 cm), it is highlighted that after five years of cultivation of Tifton 85, and without soil disturbance, the grass root system, together with the soil microbial community, increases the soil aggregation (higher mass of macroaggregates—Figure 1A, and lower mass of microaggregates—Figure 1C).

On the other hand, in the 5–10 cm layer, the higher mass of mesoaggregates in the control treatment indicates that the use of PS and Min200 favored the formation of more stable aggregates (larger mass of macroaggregates) compared to the control treatment, which showed a smaller mass of macroaggregates and a larger of mass of mesoaggregates. The addition of PS and Min200 favored the production of Tifton 85 biomass, as well as the soil microbial community, which favored the formation of more stable aggregates in the most superficial layer of the soil [26,31,34,39].

The higher GMD values found in treatments with PS is a result of the higher mass of macroaggregate and lower mass of microaggregate found in these treatments. In addition, it is also due to the C added by the PS, which assists in aggregate formation and stability, and the higher organic matter mineralization rate when applying high PS rates or mineral soluble fertilizers [35]. The C contents in micro and macroaggregates in treatments with PS were equal to, or higher than, those found in the Control and MIN200 treatments, and never lower in the 0–5 and 5–10 cm layers (Table 5).

Similarly, Loss et al. [16] evaluated a sandy loam soil subjected to 11 years of application of animal manure, including PS, and found lower meso and microaggregate mass, and higher macroaggregates contents and GMD values in the soil surface layer when compared to mineral fertilizer (N-P-K) and control treatments. Bertagnoli et al. [34] evaluated the effect of different PS rates on soil aggregation and found that soils that received the lowest PS rates (67% to 133%) improved the soil aggregation by reducing aggregates <0.25 mm up to 0.20 cm depth. However, Ferreira et al. [35] evaluated PS (at a rate equivalent to PS100) applied alone or combined with mineral fertilizer, and found increases in TOC and TN contents in a Typic Hapludult, but no improvements in soil physical attributes, with decreases in soil aggregation indexes, mainly in the 5–10 cm layer, due to decreases in GMD and increases in microaggregates contents in all treatments; they attributed these results to the negative pH values and increase in clay dispersion.

The treatments with the highest PS rates (PS300 and PS400) showed the highest microaggregate and lowest mesoaggregate mass. It reflected on the GMD, with PS300 and PS400 presenting lower GMD when compared to the lowest PS rates. This denotes that the use of high PS rates is hindering soil aggregation and may result in structural deficiencies over time. According to Barbosa et al. [36], PS applications (33 and 66 m^3 ha^{-1}) lead to rapid and dynamic changes in the dispersible clay content and aggregation processes, with increases in dispersible clay contents and mass of aggregates <0.250 mm, and decreases in soil flocculation and restructuring. In addition, Rauber et al. [58] found decreases in flocculation degree in soils with PS applications under different management systems. Clay dispersion is caused mainly by increases in soil pH and sodium contents and decreases in Ca and Mg contents and microbial activity [59], which are affected by PS applications.

The values found for ASI and AGRI are high in all treatments. This indicates that there is a higher mass of stable macroaggregates compared to meso and microaggregates, which can be seen in Figure 1. The high values of ASI and AGRI are due to the protection provided by the vegetation cover of the Tifton 85 grass, which protects the soil against the breakdown caused by the impact of raindrops and sudden variations in humidity. In addition, energy from organic matter from plant residues for microbial activity is provided, which produces substances responsible for the formation and stabilization of aggregates. These results are corroborated by Wendling et al. (2005) [60] and Torres et al. [52] who evaluated the soil aggregation indices (ASI and AGRI) in Red Latosol under different vegetation cover (grasses, legumes, and fallow); the authors found higher values of AGRI and ASI in soil with grass cover.

The ASI represents the total soil aggregation, disregarding the aggregate classes; thus, PS100 and PS200 presented the best overall aggregation in the 0–5 and 5–10 cm layers than compared to PS300 and PS400 (Table 4; Figure 2). The lowest indexes were in PS300 and PS400, denoting that the high PS rates are compromising soil aggregation, according to Barbosa et al. [36]. The higher ASI values corroborate the higher GMD values found in PS100 and PS200 treatments compared to PS300 and PS400 (Figure 2).

The AGRI represents the proportion of aggregates >2 mm; thus, it confirms the results found for macroaggregate mass for PS200 in the 0–5 cm layer. The lower values of AGRI for treatments PS300 and PS400, in the 5–10 cm layer, corroborate the lower values of GMD compared to treatments with lower doses of PS (PS100 and PS200) (Figure 2).

The SI estimates the intensity of changes in GMD caused by the different treatments. Thus, it showed that the effect on GMD caused by treatments PS100 and PS200 (0–10 cm layer) were, on average, 11.5% and 39.5%, respectively, higher than control treatment. The results found for these SI_{GMD} confirmed that PS100 and PS200 are the best treatments for improving aggregation. They presented, in the 5–10 cm layer, higher indexes than the other treatments, which was consistent with the GMD and aggregate size distribution found, denoting a better soil structuring and aggregate stabilization. The higher SI_{GMD} found for PS100 and PS200, when compared to the control treatment in the 0–10 cm layer (Table 4), is consistent with the higher macroaggregate mass (Figure 1) and GMD (Figure 2) found.

The results found for the ASI, AGRI, and SI indexes (Table 4) were lower in the soil 10–30 cm layer, which was probably due to the more favorable conditions for soil aggregation in surface layers, partly promoted by the grass biomass cover, which protects the soil against degradation by weather variables, adds organic matter, and promote microbial activity [52].

Wortmann and Shapiro [57] evaluated the effects of manure application (beef feedlot and pig slurry) and on soil aggregation and found that these manures increase the formation of macroaggregates and aggregate stability. They also found several benefits for the use of animal manure compared to mineral fertilizer, including the reduction in runoff and soil erosion; increases in water infiltration into the soil, possibly leading to a higher tolerance to drought; partial offset of higher soil P levels resulting from manure application; and decreases in P loss to local surface water. According Řezáčová et al. [56], the use of pig slurry as organic fertilizer improves soil aggregation through increases in abundance of eubacteria and products from arbuscular mycorrhizal fungi.

4.2. C and N in Aggregates

The highest C and N contents in PS treatments are connected to a better soil aggregation (macroaggregate mass and GMD), found mainly for PS200, which results in a physical protection against mineralization or occluded forms of the SOM containing C and N [42]. Moreover, according to McCarthy et al. [61], soils and treatments with increasing SOM in microaggregates are associated with encapsulation of colloidal SOM by minerals, thereby creating protected SOM-filled pores at the submicron scale within the microaggregate structure, maintaining most of the stable SOM (75%) in SOM-filled pores. Thus, considering the theory of aggregate hierarchy [62], which states that microaggregates are first formed freely, and then serve as building blocks for the formation of macroaggregates, and that these treatments tend to present higher aggregation due to the increases in SOM, C and N contents tend to be higher in macroaggregates. Francisco et al. [63] also found significantly higher C and N contents in soils treated with PS, when compared to the NPK and control treatments, and higher C and N in macroaggregates.

The results are also connected to the high biomass production by the grass in these treatments, which adds C and N to the soil via rhizodeposition. In addition, the low biomass production in the Control treatment may have affected the addition of C and N, mainly to the 0–10 cm layer. Adeli et al. [64] evaluated the effects of long-term PS applications on nutrient distribution in different soils and found increases in total soil C contents up to 60 cm depth, varying according to differences in the amount of plant residues and soil type.

The best results found for C and N contents are consistent with the best results found for soil aggregation; the lowest PS rates resulted in C and N higher than or equal to the contents found for the highest rates and, in general, better aggregation index. Increases in TOC and TN contents due to PS applications were also found by Mafra et al. [19], for a Typic Hapludox soils under a maize–oat rotation and a no-tillage system, using rates of 50 to 200 m^3 ha^{-1}; by Grohskopf et al. [65] for soils with maize and oat crops, with positive results in TN absorption by plants; and by Comin et al. [26] for the surface layer of soils cultivated with black oats and maize for 8 years. However, Loss et al. [27] evaluated PS applications in a no-tillage system for 10 years at rate equivalent to 100% of the recommended N (90 kg ha^{-1}) and found no changes in TOC and TN contents.

The similar C and N contents in the treatments with PS were probably due to the low C to N ratio (C:N) of the PS, which increases the SOM decomposition [66]. However, successive PS applications increase soil $N\text{-}NO_3^-$ and SOM contents, increasing N availability to crops; increase soil exchangeable K, Ca, and Mg contents, and pH; promote Al^{3+} complexation by adsorption to humic and fulvic acids of the SOM; and generate a slower SOM decomposition rate [37,67]. In addition, the lowest PS rates presented higher macroaggregate mass, which usually contain more organic carbon than microaggregates [68,69], compensating the lower addition of C by the PS. Moreover, the SOM decomposition is higher and the C:N is lower in microaggregates [56,70].

Some studies have shown divergent results for the effect of PS application to the soil surface on C and N contents and soil aggregation [19,26,27,29,37,66,71,72], which were probably due to differences in PS rates, initial soil characteristic, tillage system, and evaluation time. Moreover, according to Benedet et al. [25] long-term application of high PS rates can be a risk of contamination to soils and surface waters, mainly due to losses by runoff, and subsurface, by leaching. Basso et al. [73] reported that the use of low PS rates (20 and 40 m^3 ha^{-1}) minimizes N losses by volatilization; and $N\text{-}NO_3^-$ leachate, despite little being expressed when compared to the amounts added by PS applications, can surpass the concentration limits for groundwater quality [74].

5. Conclusions

Applications of pig slurry (PS) to soils cultivated with Tifton-85 grass increased the soil macroaggregate mass.

The highest PS rates decreased the geometric mean diameter, and resulted in higher microaggregate mass.

PS applications to pasture soils increased C and N contents in macro and microaggregates, and improved soil aggregation when using rates of 100 or 200 kg N ha^{-1}.

The use of successive applications of PS as a nitrogen fertilizer can substitute mineral fertilizer (urea) when using proper rates, with the advantage of improvements in soil aggregation and slower release of nitrogen.

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