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## C-CO<sub>2</sub> Emissions, Carbon Pools and Crop Productivity Increased upon Slaughterhouse Organic Residue Fertilization in a No-Till System

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Jucimare Romaniw, João Carlos de Moraes Sá,  
Ademir de Oliveira Ferreira and  
Thiago Massao Inagaki

Additional information is available at the end of the chapter

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### Abstract

The use of slaughterhouse organic residues (SORs) as a form of fertilization in no-till systems could be an alternative to promote their appropriate disposal. This chapter reports a study on a Haplic Cambisol (Inceptisol) regarding the influence of different rates of SORs applied isolated or together with synthetic mineral fertilizers (SMFs) for 5.5 years in a no-till system with diverse crop rotation. We evaluated crop productivity and several soil organic matter pools affected by the SOR and SMF combinations in a field experiment. In addition, a laboratory incubation experiment was performed with different rates of SORs to evaluate C-CO<sub>2</sub> emissions and C dynamics. The SOR applications provided significant increases in crop productivity, soil organic matter pools and C-CO<sub>2</sub> emissions. The SOR applications provided significant increases in crop productivity, soil organic matter pools and C-CO<sub>2</sub> emissions. The treatment with 50% SOR + 50% SMF was the best alternative to provide higher crop productivity, while the higher use of SOR promoted more increments in soil organic matter levels. Despite the increase in C-CO<sub>2</sub> emissions due to the use of SORs, higher C levels were observed as a function of SOR rates. We conclude that the application of SORs combined with SMFs represents an efficient strategy to reduce costs and increase C levels, providing agronomic and environmental benefits.

**Keywords:** Carbon sequestration, soil organic matter, conservation agriculture, global warming, greenhouse gases

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## 1. Introduction

The world population is expected to reach more than 9 billion people by mid-century, creating enormous pressure over the global food supply. Concurrently in the food chain, meat production is an activity that causes the greatest environmental impact due to the inefficiency of the transformation of nonusable parts for direct consumption in reusable by-products [1]. Thus, the amount of waste to be recycled and reused for various purposes will increase significantly, negatively contributing to environmental sustainability due to its disposal in the environment in an inappropriate manner and thereby generating increased emissions of greenhouse gases (GHGs) [2-4]. This change may trigger an increase in the planet's average temperature by up to 5.8°C over the next 100 years [5]. It is estimated that alterations in soil management provide about 20% of the total emissions of greenhouse gases (GHGs) [6].

Global meat consumption is estimated to increase by 72% between 2000 and 2030, and much of this increase will be due to the consumption of poultry and pork [7]. Around 96 and 113 million tons of poultry and pork, respectively, are produced in the world. The Americas are responsible for 43.7% of the world's chicken production and 17.4% of pork production [8].

The United States accounts for 17% of the world's poultry meat production, China accounts for 13% and Brazil, becoming the third largest producer, accounts for 12%. China accounts for 60% of pork production, followed by the United States (10.5%), Russia (5.5%), Spain (3.9%) and Brazil (3.71%) [8]. The waste from slaughterhouses of poultry, pork and cattle has caused serious environmental consequences due to its improper disposal in the environment [2-4].

Poultry and pork production will generate 121 and 509 million tons of carbon dioxide, respectively, via carbon (C-CO<sub>2</sub>) equivalent until 2020, with a prospective increase of 47% in 2030 [7]. The use of organic waste from the meat processing industry could increase the potential for soil carbon (C) drain and promote reduction in GHG emissions compared with industrial fertilizers derived from fossil fuels, thereby minimizing its environmental impact [9]. In addition to reducing its environmental impact, the organic waste produced in the slaughtering system is an organic fertilizer option for soil due to the presence of essential nutrients for plant growth and mainly due to its high content of organic matter, which acts positively on physical, chemical and biological soil properties, thus promoting plant development [10-12].

The use of industrial organic waste in combination with crop residues that return to the soil increases the C accumulation rate in the long term [13]. The C accumulation potential in the soil is governed by many factors, such as climate and soil type [14, 15], crop systems [16], soil management, including conservational systems [17, 18], and soil fertilization [19].

Thus, industrial organic waste presents several benefits regarding soil quality improvement and agronomic production increase [20]. However, the potential that these residues have to promote C compensation to the soil-plant-atmosphere system has been scarcely explored compared with the use of industrial mineral fertilizers.

## 2. Problem statement

The organic residues used in agriculture as fertilizers often originate from three main activities: agricultural, urban and industrial. Among agricultural residues, manure (cattle, porcine and poultry) is the most commonly used. With regard to the organic residues from urban activities, the products generated from composting of urban garbage and sewage sludge are the most used ones [21, 22]. Lastly, the waste generated in the food processing industry is the most used in the production of organic fertilizers.

The residues generated from chicken and poultry slaughterhouses have been causing serious pollution problems to the soil, surface water and groundwater. According to COWI Consulting Engineers and Planners AS [23] and Matos [24], 20% and 30% of chicken and swine weight, respectively, are considered inedible (blood, feathers, hairs, nails, fat, etc.). Part of the residues generated is destined for industrial purposes (e.g., animal food production), and approximately 20-22% are discarded in the environment.

Several reports have demonstrated the benefits of using organic fertilizers to the chemical properties of soil. According to Rasmussen and Collins [25], the use of organic fertilizers in agriculture aims to increase the soil organic matter content. The soil organic matter plus the clay soil content form an absorption complex that increases soil chemical properties. The complex, in this way, is capable of retaining the nutrients as nitrogen that would be eventually leached. Organic fertilizers also add micronutrients and macronutrients to the soil. Therefore, organic fertilizers increase the soil cation exchange capacity (CEC), provide better water retention, create complex toxic elements [26-29] and determine the biological and physical qualities of soil.

Marchesini et al. [30] reported crop yield increases provided by the use of organic fertilizers, which are more persistent despite presenting lower and slower effects compared with synthetic mineral fertilizers (SMFs). This could be due to their lower and progressive nutrient release and plant root system development.

However, it is important to emphasize that since agricultural soils can be considered a destination for residue waste, we must respect the limits imposed by legislation, avoiding overpowering the soil's capacity [29, 31, 32]. Although the practice can work in ameliorating soil conditions, it can also cause contamination, consequently affecting crop yield and quality [33].

Synthetic fertilizers that come from nonrenewable sources are commonly used in agriculture. In 2012, Brazil consumed more than 29 million tons of industrial fertilizers [34]. Therefore, lowering their use by replacement with organic residues in this way can positively contribute to environmental conservation.

In the search for more sustainable practices, correct management of organic residues in agriculture is an important process to promote their environmental, social and economic benefits. Exploring the potential of organic residues can promote their proper destination, increase soil quality and promote economic benefits.

In this light, the specific objectives of this research were (a) to assess the contribution of slaughterhouse organic residues (SORs) from poultry and porcine activities to carbon (C) alterations as well as (b) to study crop performance under a no-till system with organic residue applications with and without synthetic mineral fertilizers.

### 3. Materials and methods

The experiment was established in April 2009 at the State University of Ponta Grossa Farm (Fazenda Escola Capão da Onça) in the city of Ponta Grossa, Paraná, in southern Brazil (25° 05' S and 50° 05' W). The climate is classified as Cfb according to the Köppen system [19], with cold and humid winters and occasional frosts between May and July. The annual mean precipitation during the experimental period of 44 years is 1545 mm, with higher precipitation levels in the summer and no dry period defined. The mean maximum temperature is 24°C, and the minimum is 13.3°C. The soil is classified as Haplic Cambisol with medium texture, and it represents 27% of the region [35]. The results of the soil fertility analysis performed before the experiment were pH (CaCl<sub>2</sub>, 1M), cation exchange capacity = 11.2 cmolc dm<sup>-3</sup>, soil density = 1.35 Mg m<sup>-3</sup>, total organic C = 11.9 g kg<sup>-1</sup>, total organic nitrogen = 16.12 g kg<sup>-1</sup>, available P = 38.1 mg kg<sup>-1</sup> and available K = 0.24 cmolc dm<sup>-3</sup>.

The experimental design was of completely randomized blocks with six treatments and three replications. The following treatments were performed: T<sub>1</sub> = control, with no slaughterhouse organic residue (SOR) or synthetic mineral fertilizer (SMF) applications; T<sub>2</sub> = 100% SMF, with all plant nutrient supply applied via synthetic mineral fertilizer; T<sub>3</sub> = 100% SOR, with all plant nutrient supply applied via slaughterhouse organic residue; T<sub>4</sub> = 75% SOR + 25% SMF; T<sub>5</sub> = 50% SOR + 50% SMF; and T<sub>6</sub> = 25% SOR + 75% SMF. For T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub>, the rates of SORs applied were equivalent to 75%, 50% and 25% of the residues used in T<sub>2</sub>, respectively. The rates of SMFs applied were based on the soil fertility analysis and the recommendation for the crops used in the region.

In T<sub>3</sub> (100% SOR), 94, 21 and 19 kg ha<sup>-1</sup> of N, P and K, respectively, were applied. These were equivalent to 2 Mg ha<sup>-1</sup> bio-fertilizer. The crop sequence from 2009 to 2012 was the alternation of crops used in the summer and winter seasons in the region.

Crop yield was determined by harvesting 5 m of the three central rows in the summer and winter season crops. The grains were submitted to a cleaning process to remove impurities and then dried for humidity correction. Grain weight was corrected to 14% humidity for beans and 13% for the other crops. The unit was converted to kg ha<sup>-1</sup> and then to Mg ha<sup>-1</sup>. For black oat, we determined the dry mass production collecting two points with 0.17 m<sup>2</sup> at each plot. The soil total organic carbon (TOC) content was determined through the dry combustion method using an elementary C/N analyzer (TruSpec CN LECO® 2006, St. Joseph, EUA).

The results for content and the TOC stock in whole samples, the particle-size fractions and the SOM labile compartments were subjected to analysis of variance (ANOVA). The means that were significantly different from the *F* test were compared using the LSD test at 5% probability (*I* = 0.05) using SISVAR 5.1 [36].

## 4. Results and discussion

### 4.1. Crop response upon the use of slaughterhouse organic residues (SORs)

Combinations of 25% + 75%, 50% + 50% and 75 + 25% mineral fertilizer with organic fertilizer demonstrated significantly higher yields in several crop seasons compared with fertilization with 100% mineral fertilizer (**Table 1**). Sutton et al. [37] studied the effects of waste residue rates from ruminant animals and did not find differences in corn productivity among the rates or between mineral and organic fertilizers in 5 years of use. Despite the lack of significant difference, crop yields were always higher in organically fertilized plots.

		Treatments					
Crops	Crop season	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
		-----Mg ha <sup>-1</sup> -----					
Beans	2009/2010	2.76 <sup>ns</sup>	2.85 <sup>ns</sup>	3.25 <sup>ns</sup>	3.21 <sup>ns</sup>	3.39 <sup>ns</sup>	3.16 <sup>ns</sup>
Wheat	2010	0.67 <sup>b</sup>	1.18 <sup>a</sup>	1.11 <sup>a</sup>	1.35 <sup>a</sup>	1.06 <sup>a</sup>	1.20 <sup>a</sup>
Soybean	2010/2011	2.11 <sup>ns</sup>	2.51 <sup>ns</sup>	2.34 <sup>ns</sup>	2.38 <sup>ns</sup>	2.50 <sup>ns</sup>	2.28 <sup>ns</sup>
Oats	2011	1.71 <sup>b</sup>	4.49 <sup>a</sup>	3.88 <sup>a</sup>	3.50 <sup>ab</sup>	4.43 <sup>a</sup>	3.20 <sup>ab</sup>
Corn	2011/2012	7.00 <sup>c</sup>	12.22 <sup>a</sup>	9.93 <sup>b</sup>	11.56 <sup>ab</sup>	10.17 <sup>ab</sup>	9.59 <sup>b</sup>
Wheat	2012	3.36 <sup>b</sup>	3.57 <sup>ab</sup>	3.61 <sup>ab</sup>	3.47 <sup>ab</sup>	4.06 <sup>a</sup>	4.02 <sup>ab</sup>
Soybean	2012/2013	2.42 <sup>c</sup>	2.74 <sup>ab</sup>	2.94 <sup>a</sup>	2.93 <sup>a</sup>	2.81 <sup>ab</sup>	2.99 <sup>a</sup>
Oats	2013	1.35 <sup>c</sup>	2.18 <sup>ab</sup>	2.48 <sup>a</sup>	2.06 <sup>ab</sup>	2.06 <sup>ab</sup>	2.12 <sup>ab</sup>
Corn	2013/2014	7.21 <sup>c</sup>	11.18 <sup>a</sup>	10.67 <sup>ab</sup>	11.64 <sup>ab</sup>	12.03 <sup>a</sup>	11.79 <sup>a</sup>
Wheat	2014	1.03 <sup>c</sup>	2.11 <sup>a</sup>	1.59 <sup>b</sup>	2.40 <sup>a</sup>	2.12 <sup>a</sup>	1.28 <sup>bc</sup>
Beans	2014/2015	1.77 <sup>c</sup>	2.72 <sup>a</sup>	2.10 <sup>b</sup>	2.28 <sup>ab</sup>	2.68 <sup>a</sup>	2.63 <sup>a</sup>
Accumulated		31.40 <sup>c</sup>	47.75 <sup>a</sup>	43.90 <sup>ab</sup>	46.79 <sup>a</sup>	49.82 <sup>a</sup>	44.26 <sup>ab</sup>

T<sub>1</sub> = Absolute control (without SORs and SMFs); T<sub>2</sub> = 100% SMF; T<sub>3</sub> = 100% SOR; T<sub>4</sub> = 75% SMF + 25% SOR; T<sub>5</sub> = 50% SMF + 50% SOR; T<sub>6</sub> = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at *P* < 0.05. “ns” indicates not significant by the *F* test at *P* < 0.05. Source: Romaniw [60].

**Table 1.** Crop yields affected by slaughterhouse organic and mineral residues in no-till system.

Considering the six crop seasons accumulated (**Table 1**), the treatments with the highest productivities were T<sub>4</sub> (75% SMF + 25% SOR) and T<sub>5</sub> (50% SMF + 50% SOR), representing increases of 49.0% and 58.7% in relation to the control treatment (without SMFs and SORs), respectively.

Many authors [31, 38-40] have reported increases in crop yields due to the use of organic sources in fertilization. However, some of them can only be observed from medium-term to



long-term courses due to the slow and gradual soil property change, as observed in Table 1, with changes among the fertilizer treatments observed only after the third crop.

Through cost-benefit analysis (**Table 2**), we could identify that the increase in crop yield and the mineral fertilizer cost reduction, in response to the increase in SOR rates, reflected in increases in net earnings in comparison with SMF fertilization. With the application of the lowest SOR rate (25%), there were savings of 292.57 USD per hectare compared with the mineral fertilizer, but the maximum savings of 1170.27 USD per hectare was achieved with the application of 22 Mg ha<sup>-1</sup> SOR along the 11 crop seasons.

Crops	Treatments				
	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
	-----U\$ ha <sup>-1</sup> -----				
Beans	280.40	125.63	241.71	203.02	164.32
Soybean	240.86	125.63	212.06	183.25	154.44
Corn	529.65	125.63	428.64	327.64	226.63
Oats	301.51	125.63	257.54	213.57	169.60
Wheat	508.79	188.44	428.71	348.62	268.53
Accumulated	1861.22	690.95	1568.65	1276.09	983.52

\*Base values were obtained from SEAB [41]. T<sub>1</sub> = Absolute control (without SORs and SMFs); T<sub>2</sub> = 100% SMF; T<sub>3</sub> = 100% SOR; T<sub>4</sub> = 75% SMF + 25% SOR; T<sub>5</sub> = 50% SMF + 50% SOR; T<sub>6</sub> = 25% SMF + 75% SOR. \*SMF rates were recommended according to cultivated crop and soil analysis, and the SOR rate was fixed at 2 Mg ha<sup>-1</sup>.

**Table 2.** Fertilization costs (SORs and SMFs) along the crop seasons.

**4.2. Soil organic matter (SOM) pools in crop systems affected by SOR application**

As soil organic matter (SOM) is closely linked to C, it is essential to note that it is found in highly variable situations in terms of level of decomposition, chemical composition, size, level of recalcitrance as well as chemical and physical protection. For this reason, fractionation methods were used (chemical or physical) to classify and quantify the effects of SOR application on the SOM pools. In addition, the use of SORs as fertilizers in the medium and long terms can increase TOC content and microbial activity, which results in the recovery of soil quality and increases crops’ productive potential.

Analyzing the use of slaughterhouse waste over SOM pools, we could observe an increase in total organic carbon (TOC) stocks at the 0–20 cm layer through the use of a combination of 50% SOR + 50% SMF (**Table 3**). This increase was 33.8% higher than that in the control (without SORs and SMFs) and 28.8% higher than that in T<sub>2</sub> (100% SMF). Previous studies developed by Filho et al. [42] and Zhang et al. [43] concluded that fertilization with organic waste in long-term experiments elevated soil carbon (C) and nitrogen (N) levels.

Soil layer (cm)	Treatments					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
	TOC content, g kg <sup>-1</sup>					
0–5	18.33 <sup>ns</sup>	18.03	20.80	19.67	21.60	18.70
5–10	13.97 <sup>ns</sup>	13.50	13.43	13.97	15.87	13.07
10–20	13.00 <sup>ns</sup>	13.37	12.53	12.70	14.27	13.17
	TOC stock, Mg ha <sup>-1</sup>					
0–5	11.45 <sup>ns</sup>	9.15	12.52	17.13	18.59	16.78
5–10	8.70 <sup>ns</sup>	10.17	9.45	9.24	10.13	10.85
10–20	15.94 <sup>ns</sup>	18.18	17.17	17.31	19.59	18.04
0–20	36.09 <sup>b</sup>	37.50 <sup>b</sup>	39.14 <sup>b</sup>	43.67 <sup>b</sup>	48.31 <sup>a</sup>	45.66 <sup>b</sup>

T<sub>1</sub> = Absolute control (without SORs and SMFs); T<sub>2</sub> = 100% SMF; T<sub>3</sub> = 100% SOR; T<sub>4</sub> = 75% SMF + 25% SOR; T<sub>5</sub> = 50% SMF + 50% SOR; T<sub>6</sub> = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at  $P < 0.05$ . “ns” indicates not significant by the  $F$  test at  $P < 0.05$ . Source: Romaniw et al. [54].

**Table 3.** Total organic carbon (TOC) contents and stocks in response to the use of mineral fertilizers and slaughterhouse organic waste applied in isolated and combined forms in no-till system.

de Andrade et al. [44] observed higher increases in TOC in the second year after the application of sewage sludge biosolids in sugarcane, emphasizing that such effects could be further increased in subsequent years.

The contents and stocks of mineral-associated organic carbon (MAOC) and the contents of particulate organic carbon (POC) presented different responses upon the fertilization treatments (**Table 4**).

Layer (cm)	Treatments					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
	POC content, g kg <sup>-1</sup>					
0–5	5.00 <sup>ns</sup>	5.16	5.98	5.42	6.28	4.76
5–10	2.96 <sup>ns</sup>	2.66	2.79	2.67	2.83	2.71
10–20	2.80 <sup>ns</sup>	2.68	2.60	2.50	3.37	2.57
	MAOC content, g kg <sup>-1</sup>					
0–5	50.00 <sup>ns</sup>	44.73	49.70	49.27	52.53	44.73
5–10	38.53 <sup>ns</sup>	37.07	35.90	39.30	40.13	37.87
10–20	35.00 <sup>ns</sup>	24.20	33.83	36.97	41.93	35.73
	POC stock, Mg ha <sup>-1</sup>					
0–5	2.03 <sup>ab</sup>	2.18 <sup>a</sup>	2.04 <sup>ab</sup>	2.13 <sup>ab</sup>	1.75 <sup>ab</sup>	1.39 <sup>b</sup>



Layer (cm)	Treatments					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
5–10	1.17 <sup>bc</sup>	1.06 <sup>c</sup>	1.28 <sup>bc</sup>	1.19 <sup>bc</sup>	1.98 <sup>a</sup>	1.79 <sup>ab</sup>
10–20	2.15 <sup>ns</sup>	2.29	2.20	2.10	2.29	2.97
0–20	5.35 <sup>ns</sup>	5.53	5.52	5.42	6.02	6.15
MAOC stock, Mg ha <sup>-1</sup>						
0–5	9.41 <sup>bc</sup>	6.97 <sup>c</sup>	10.48 <sup>abc</sup>	15.00 <sup>ab</sup>	16.84 <sup>a</sup>	15.39 <sup>ab</sup>
5–10	7.54 <sup>ns</sup>	9.11	8.17	8.05	8.14	9.06
10–20	13.79 <sup>b</sup>	15.89 <sup>ab</sup>	14.97 <sup>ab</sup>	15.21 <sup>ab</sup>	17.31 <sup>a</sup>	15.07 <sup>ab</sup>
0–20	30.74 <sup>c</sup>	31.97 <sup>bc</sup>	33.62 <sup>bc</sup>	38.25 <sup>abc</sup>	42.29 <sup>a</sup>	39.51 <sup>ab</sup>

T<sub>1</sub> = Absolute control (without SORs and SMFs); T<sub>2</sub> = 100% SMF; T<sub>3</sub> = 100% SOR; T<sub>4</sub> = 75% SMF + 25% SOR; T<sub>5</sub> = 50% SMF + 50% SOR; T<sub>6</sub> = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at  $P < 0.05$ . "ns" indicates not significant by the  $F$  test at  $P < 0.05$ . Source: Romaniw et al. [54].

**Table 4.** Contents and stocks of particulate organic carbon (POC) and mineral-associated organic C (MAOC) in response to the application of mineral fertilizers and slaughterhouse waste isolated and combined in no-till system.

Layer (cm)	Treatments					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
C-OXP content, g kg <sup>-1</sup>						
0–5	1.29 <sup>a</sup>	2.34 <sup>b</sup>	2.74 <sup>b</sup>	2.49 <sup>b</sup>	2.72 <sup>b</sup>	2.86 <sup>b</sup>
5–10	0.89 <sup>a</sup>	1.71 <sup>bc</sup>	1.74 <sup>bc</sup>	1.54 <sup>b</sup>	1.89 <sup>c</sup>	1.80 <sup>bc</sup>
10–20	0.67 <sup>a</sup>	1.61 <sup>b</sup>	1.43 <sup>b</sup>	1.28 <sup>b</sup>	1.66 <sup>b</sup>	1.68 <sup>b</sup>
C-HW content, g kg <sup>-1</sup>						
0–5	0.42 <sup>a</sup>	0.67 <sup>a</sup>	1.29 <sup>b</sup>	2.07 <sup>c</sup>	2.	2.33 <sup>c</sup>
5–10	0.50 <sup>a</sup>	0.69 <sup>a</sup>	0.69 <sup>a</sup>	1.93 <sup>b</sup>	2.02 <sup>b</sup>	1.95 <sup>b</sup>
10–20	0.51 <sup>a</sup>	0.67 <sup>a</sup>	0.57 <sup>a</sup>	1.54 <sup>b</sup>	1.95 <sup>b</sup>	2.03 <sup>b</sup>
C-OXP stock, Mg ha <sup>-1</sup>						
0–5	0.81 <sup>a</sup>	1.48 <sup>b</sup>	1.70 <sup>b</sup>	1.57 <sup>b</sup>	1.71 <sup>b</sup>	1.80 <sup>b</sup>
5–10	0.62 <sup>a</sup>	1.20 <sup>b</sup>	1.22 <sup>b</sup>	1.08 <sup>b</sup>	1.33 <sup>b</sup>	1.26 <sup>b</sup>
10–20	0.91 <sup>a</sup>	2.21 <sup>b</sup>	1.95 <sup>b</sup>	1.75 <sup>b</sup>	2.28 <sup>b</sup>	2.30 <sup>b</sup>
0–20	2.35 <sup>a</sup>	4.88 <sup>b</sup>	4.90 <sup>b</sup>	4.40 <sup>b</sup>	5.32 <sup>b</sup>	5.36 <sup>b</sup>
C-HW stock, Mg ha <sup>-1</sup>						
0–5	0.26 <sup>a</sup>	0.42 <sup>a</sup>	0.81 <sup>b</sup>	1.31 <sup>c</sup>	1.30 <sup>c</sup>	1.47 <sup>c</sup>
5–10	0.35 <sup>a</sup>	0.48 <sup>a</sup>	0.48 <sup>a</sup>	1.35 <sup>b</sup>	1.41 <sup>b</sup>	1.37 <sup>b</sup>
10–20	0.70 <sup>a</sup>	0.91 <sup>a</sup>	0.79 <sup>a</sup>	2.10 <sup>b</sup>	2.67 <sup>b</sup>	2.78 <sup>b</sup>

Layer (cm)	Treatments					
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
0–20	1.31 <sup>a</sup>	1.82 <sup>a</sup>	2.08 <sup>a</sup>	4.76 <sup>b</sup>	5.38 <sup>b</sup>	5.61 <sup>b</sup>

T<sub>1</sub> = Absolute control (without SORs and SMFs); T<sub>2</sub> = 100% SMF; T<sub>3</sub> = 100% SOR; T<sub>4</sub> = 75% SMF + 25% SOR; T<sub>5</sub> = 50% SMF + 50% SOR; T<sub>6</sub> = 25% SMF + 75% SOR. Means followed by the same letters per row do not differ among themselves by the LSD test at  $P < 0.05$ . “ns” indicates not significant by the  $F$  test at  $P < 0.05$ . Source: Romaniw et al. [54].

**Table 5.** Contents and stocks of C oxidizable by potassium permanganate (C-OXP) and hot water (C-HW) in response to the use of mineral fertilizers and organic residues from slaughterhouses applied alone or in combination under a no-till system.

For POC at the 0–20 cm layer, the treatments that provided the highest increases were T<sub>5</sub> (50% SMF + 50% SOR) and T<sub>6</sub> (25% SMF + 75% SOR), with increments of 12.5% and 14.9%, respectively, in comparison with the control (**Table 4**). For MAOC at the same depth, the highest increases were also provided by T<sub>5</sub> and T<sub>6</sub>, with increments of 37.6% and 28.5%, respectively, in comparison with the control. The increase and maintenance of labile SOM pool stocks are essential for the amelioration of soil quality and for the sustainability of crop systems, since they are essential for soil microbial activity [45].

The C-HW content decreased with soil depth, suggesting a stratification profile in the soil (**Table 5**). This fact is already well reported in no-till systems [46–48], and due to the addition of SORs, this response was even more pronounced, leading to higher biomass-C input from crop residues over the soil surface.

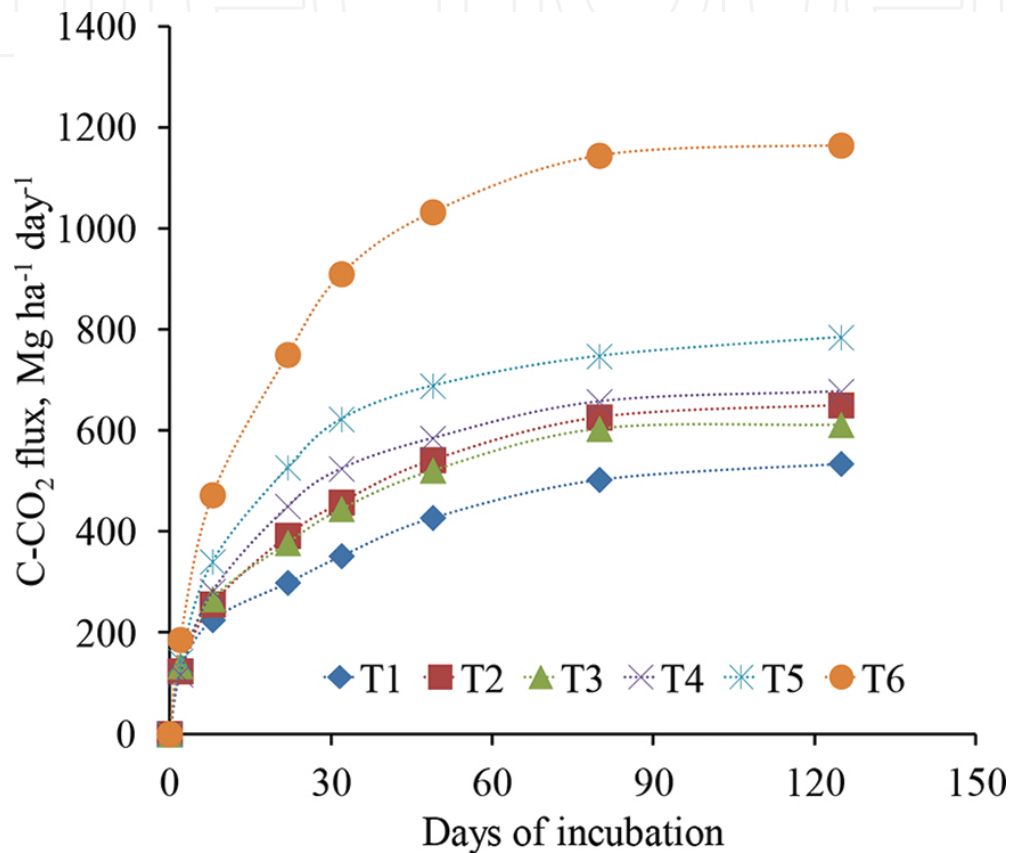
In addition, the SOR application in combination with SMFs increased the C-OXP and C-HW stocks regardless of the SMF combination. Considering the C-OXP pool at the 0–20 cm layer, the treatments that provided the highest increases were T<sub>5</sub> (50% SOR + 50% SMF) and T<sub>6</sub> (75% SOR + 25% SMF), representing increases of 126.4% and 128.0%, respectively, in comparison with the control. For C-HW at the same depth, the T<sub>5</sub> and T<sub>6</sub> treatments were also the ones that provided the highest increases, with increments of 310.7% and 328.2%, respectively, in comparison with the control.

This impact of the combinations of SORs and SMFs, in the short term, could be attributed to the increase of labile SOM pools, promoting higher soil biological activity [48]. The input of organic residues also plays an important role in soil aggregation [49] and higher C protection [50]. Similar results were also found by Kanchikerimath and Singh [51] and Rudrappa et al. [52] in the medium term (more than 5 years) in India.

Thus, fertilization with SORs favors soil microbial activity and stimulates soil organic matter mineralization [53]. Therefore, combinations of SORs and SMFs can lead to higher C inputs, and depending on the soil layer, they can even surpass the increments provided by isolated SMFs.

4.3. SOR rates affecting C-CO<sub>2</sub> emission and soil organic matter pools in incubated soil

The mean C-CO<sub>2</sub> flux rate of incubated soils with SOR applications varied from 0.30 to 2.79 Mg ha<sup>-1</sup> at the lowest rate (0 Mg ha<sup>-1</sup>) at the beginning of the incubation and at the highest SOR rate (16 Mg ha<sup>-1</sup>) at the end of the incubation process (**Figure 1**). At the highest SOR rate, there was an increase in the C-CO<sub>2</sub> flux equivalent to seven times compared with the beginning of the process (0.45 Mg ha<sup>-1</sup> C-CO<sub>2</sub>).



**Figure 1.** C-CO<sub>2</sub> flux in incubated soils with different rates of SOR. T<sub>1</sub> = Control (0 Mg ha<sup>-1</sup> SOR); T<sub>2</sub> = 1 Mg ha<sup>-1</sup> SOR; T<sub>3</sub> = 2 Mg ha<sup>-1</sup>; T<sub>4</sub> = 4 Mg ha<sup>-1</sup>; T<sub>5</sub> = 8 Mg ha<sup>-1</sup>; T<sub>6</sub> = 16 Mg ha<sup>-1</sup>. Source: Romaniw [60].

The mean C-CO<sub>2</sub> fluxes observed due to the increasing SOR applications of 0, 1, 2, 4, 8 and 16 Mg ha<sup>-1</sup> were 10.3, 12.5, 12.6, 13.0, 15.0 and 22.4 kg ha<sup>-1</sup>, respectively. Therefore, only the highest SOR rate of 16 Mg ha<sup>-1</sup> is out of the ideal range of 9.8–19.5 kg ha<sup>-1</sup> as evaluated by the Soil Quality Kit test in long-term experiments [55].

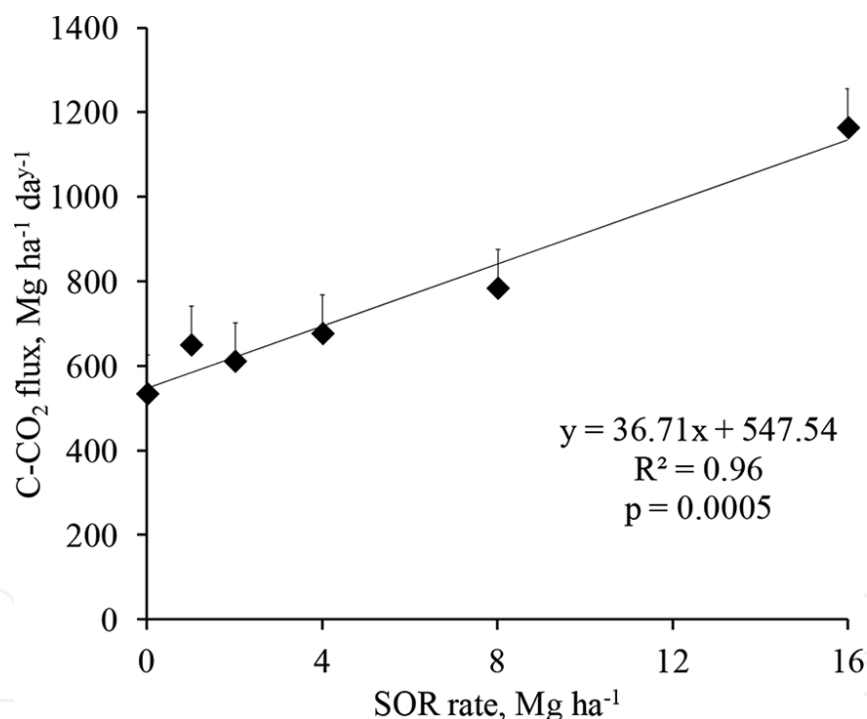
With the increase in SOR rates in incubated soils, we could observe a more pronounced effect 60 days after applications (**Figure 1**), which indicates that the CO<sub>2</sub> emission rates of microbial biomass decrease as C starts being fixed in the soil.

After the initial increase on day 45, in general, CO<sub>2</sub> emissions among the treatments tended to be similar to the control soils. After 60 days of incubation, all treatments started emitting a

similar amount of CO<sub>2</sub>. Such evolution was also observed by Sánchez-Monedero et al. [56] in an incubation experiment with composted sewage sludge at different stabilization degrees.

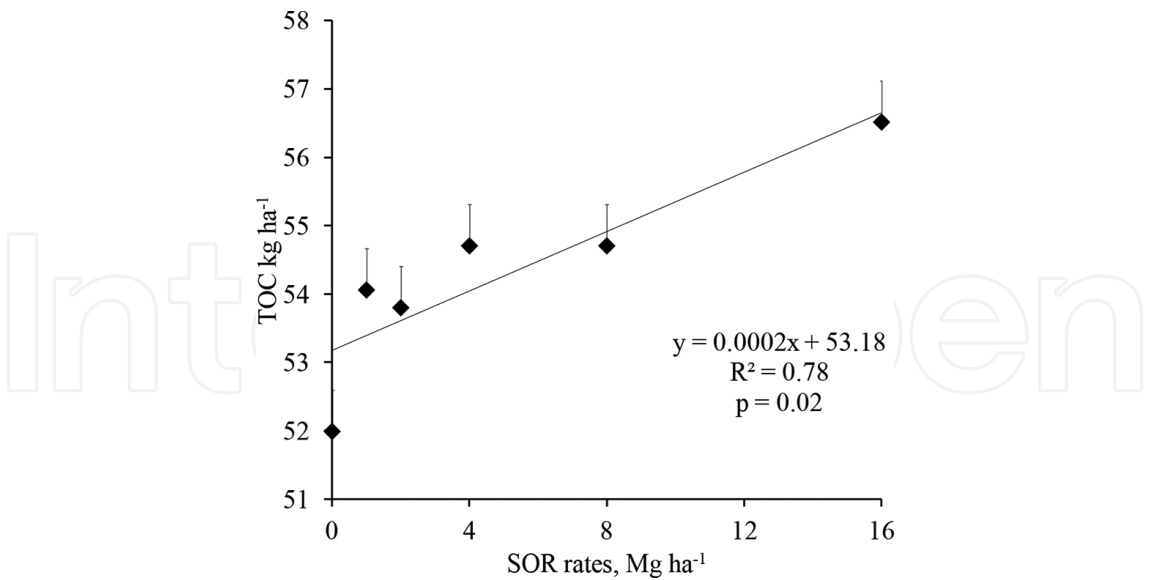
According to **Figure 1**, there is a tendency for stabilization of the C-CO<sub>2</sub> emissions after 80 days of incubation. This fact may be related to the availability of substrate for microbial activity as reported by Campbell et al. [57]. The balanced fertilization with SORs as a source of labile carbon supports the microbial activity, resulting in increases in C-CO<sub>2</sub> emissions.

The increase in SOR rates resulted in a linear tendency with the C-CO<sub>2</sub> flux (**Figure 2**). This tendency is probably related to the SOR C:N ratio and structure, which provides higher surface contact with soil particles. These factors allied to ideal conditions of humidity and temperature increase the microbial activity, leading to higher C-CO<sub>2</sub> emission rates [58]. The increase in C-CO<sub>2</sub> emission with higher SOR rates at the end of the incubation period is probably related to the fast soil microbiota growth and the decomposition of higher organic material amounts. This fact indicates that such higher SOR rates could cause a higher liberation of organic materials in the soil, which easily decompose due to temperature and humidity conditions.



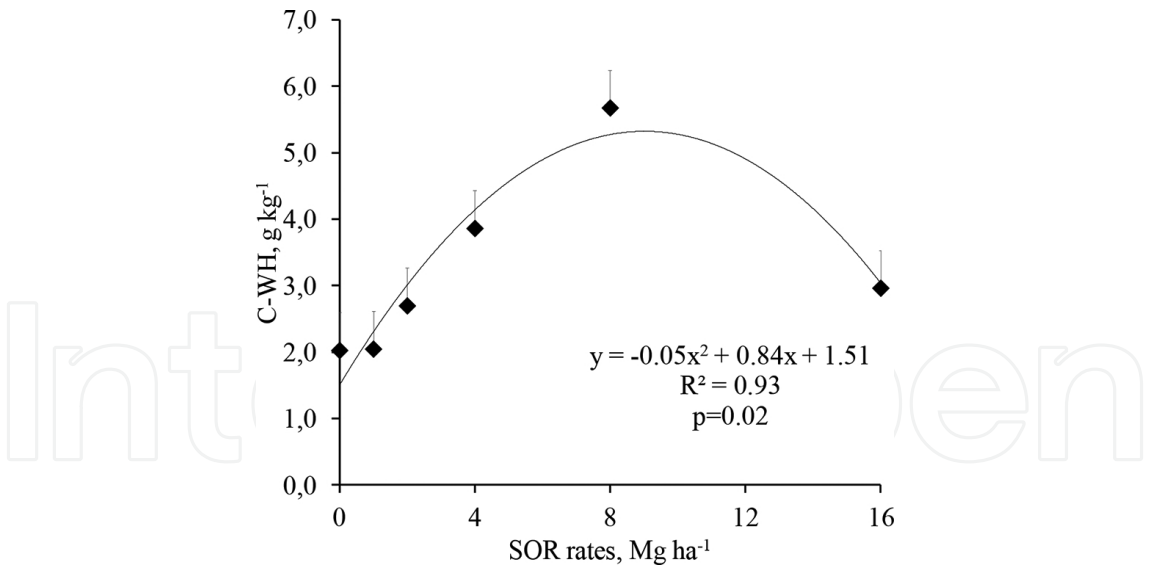
**Figure 2.** Accumulated C-CO<sub>2</sub> emissions affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha<sup>-1</sup> after 125 days of incubation. Source: Romaniw [60].

Although the increase in SOR rates resulted in higher C-CO<sub>2</sub> emissions, linear increases in the TOC were observed ( $P < 0.05$ ) (**Figure 3**), indicating its influence over soil carbon mineralization. The fast mineralization at the beginning of the incubation process is mainly related to the amount of labile carbon available. As the decomposition process begins, the influence of labile fraction lessens due to its easy degradation [58, 59]. In general, all samples with SOR application presented higher TOC contents compared with the control.



**Figure 3.** TOC content affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha<sup>-1</sup> after 125 days of incubation. Source: Romaniw [60].

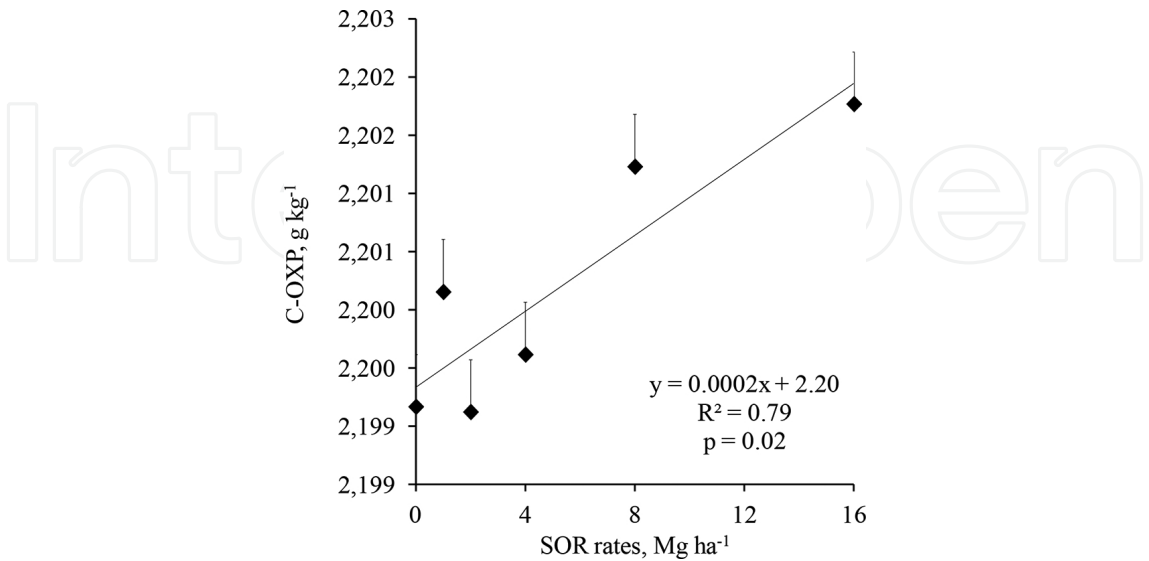
The C-HW content decreased at the SOR rate of 16 Mg ha<sup>-1</sup>. The high SOR rate possibly caused a reduction in soil aeration, leading to lower microbial activity and carbon mineralization (C-HW) (**Figure 4**).



**Figure 4.** C-HW content affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha<sup>-1</sup> after 125 days of incubation. Source: Romaniw [60].

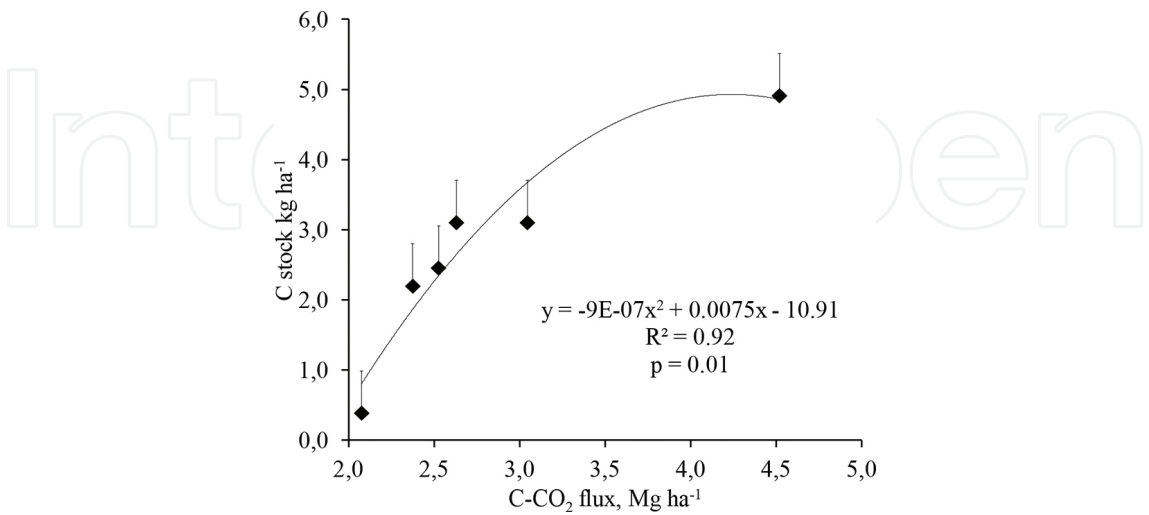
The SOR rate of 8 Mg ha<sup>-1</sup> provided increases in C-HW and C-XP (**Figures 4 and 5**), mainly because of the high microbial activity due to the availability of labile carbon. The proportions between labile and recalcitrant fractions differ in the fertilizer that presents a higher concentration of soluble fraction and that with lower fiber contents [61]. The differences in biochemical

composition can alter the structure of microbial biomass and affect its efficiency in C use, resulting in differences in C mineralization of different organic sources.



**Figure 5.** POXC content affected by SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha<sup>-1</sup> after 125 days of incubation. Source: Romaniw [60].

The C-CO<sub>2</sub> flux, when related to the TOC stock, expressed the amount of C-CO<sub>2</sub> lost by each Mg ha<sup>-1</sup> TOC produced according to the SOR rates applied (**Figure 6**). This parameter is a sensitive indicator of the environmental changes that can occur due the increasing SOR applications. It can be used to detect disturbances, reflecting the increase in C-CO<sub>2</sub> emission. The TOC stock was greater with the increase in SOR rates, which indicates higher potential for C-CO<sub>2</sub> sequestration.



**Figure 6.** Relationship between C-CO<sub>2</sub> flux and TOC stock in incubated soils for 125 days with SOR rates of 0, 1, 2, 4, 8 and 16 Mg ha<sup>-1</sup>. Source: Romaniw [60].



The increase in C-CO<sub>2</sub> emissions with SOR addition produced an initial increment and variability in TOC (**Figure 6**). This variability suggests disturbance in the microorganisms' activity through the SOR addition. The SOR applications provided accumulated emissions of 1.28, 1.56, 1.58, 1.63, 1.88 and 2.79 Mg ha<sup>-1</sup> C-CO<sub>2</sub> and fixations of 0.24, 1.52, 1.36, 1.92, 1.92 and 3.04 Mg ha<sup>-1</sup> TOC (reduced values from the initial TOC) in the soil after the 125-day incubation period. These results indicate that, although there is a pronounced flux of C-CO<sub>2</sub> with higher SOR applications, the TOC levels also increased. The TOC fixation was higher than the C-CO<sub>2</sub> flux for the 4, 8 and 16 Mg ha<sup>-1</sup> rates.

Therefore, SOR application can be considered a promising strategy in order to provide soil C sequestration, affecting directly the quality and productivity of the system.

## 5. Conclusions

The applications of poultry and pork slaughterhouse waste increased crop productivity, especially in T<sub>5</sub> (50% SOR + 50% SMF). The C labile pools (C-HW, C-OXP and POC) were higher in the treatments with elevated SOR applications (50% and 75%), thereby increasing soil quality and sustainability. In addition, fertilization with SORs demonstrated to be an alternative to minimize the costs and use of mineral fertilizers and increase C sequestration.

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## 7. Acronyms

SOR: Slaughterhouse organic residue

SMF: Synthetic mineral fertilizer

N: Nitrogen

C: Carbon

TOC: Total organic carbon

MAOC: Mineral-associated organic carbon

POC: Particulate organic carbon

C-OXP: Permanganate oxidizable organic carbon

C-HW: Hot water extractable organic carbon

GHG: Greenhouse gases

C-CO<sub>2</sub>: Carbon emitted as carbon dioxide.

## Author details

Jucimare Romaniw<sup>1\*</sup>, João Carlos de Moraes Sá<sup>2</sup>, Ademir de Oliveira Ferreira<sup>3</sup> and Thiago Massao Inagaki<sup>1</sup>

\*Address all correspondence to: [ju.romaniw@gmail.com](mailto:ju.romaniw@gmail.com)

1 State University of Ponta Grossa, Campus de Uvaranas Ponta Grossa, PR, Brazil

2 Department of Soil Science and Agricultural Engineering, State University of Ponta Grossa, Paraná, Brazil

3 Soil Organic Matter Laboratory (LABMOS), State University of Ponta Grossa, Paraná, Brazil

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