Changes in edaphic mesofauna by successive application of pig slurry and poultry litter in Tifton 85 pasture

Rodrigo Ferreira da Silva¹, Alex Negrini¹, Douglas Leandro Scheid¹, Claudir José Basso², Juliano Cesar da Silva¹, Gilvan Moisés Bertollo³

¹ Universidade Federal de Santa Maria, Campus Frederico Westphalen, Frederico Westphalen, RS, Brasil. E-mail: rodrigosilva@smail.ufsm.br (ORCID: 0000-0002-1747-2149); alex.negrini@iffarroupilha.edu.br (ORCID: 0000-0002-1640-2720); douglasscheid@gmail.com (ORCID: 0000-0002-2056-6488); julianocesaragro@hotmail.com (ORCID: 0000-0003-1666-0540)
² Universidade Federal de Santa Maria, Campus Santa Maria, Santa Maria, RS, Brasil. E-mail: claudirbasso@gmail.com (ORCID: 0000-0002-3013-5702)
³ Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Departamento de Estudos Agrários, RS, Brasil. E-mail: gilvanbertollo@yahoo.com.br (ORCID: 0000-0002-8443-6711)

ABSTRACT: The application of organic or animal waste can alter the edaphic fauna. This study aimed to determine the influence from successive application of pig slurry (PS) and of poultry litter (PL) in the edaphic mesofauna in a Tifton 85 pasture. The experimental design was randomized blocks in a factorial arrangement (3 x 2), composed by 3 fertilization treatments with poultry litter (zero, 1.5 and 3.0 mg ha⁻¹ of poultry litter), and two of PS (zero and 40 m³ ha⁻¹), with 4 replicates. After collecting the edaphic fauna with PROVID type traps, we evaluated the relative frequency of the organisms and per functional groups, springtails, mites, abundance, Margalef richness, Simpson dominance index, Shannon diversity and Pielou index. The microphages relative frequency rises after four applications of pig slurry and poultry litter. The isolated use of pig slurry and poultry litter reduces Shannon diversity due to the increase in springtail population, while a joint application of 40 m³ ha⁻¹ of PS with 1.5 t ha⁻¹ PL induces diversity in the edaphic fauna. In periods of water deficit, Tifton 85 fertilization with pig slurry stimulates the fauna density and the Collembola order, in relation to the poultry litter.

Key words: diversity; organic waste; relative frequency; soil fauna

Alteração na mesofauna edáfica pela aplicação sucessiva de dejeto líquido de suínos e cama de aves em pastagem de Tifton 85

RESUMO: A aplicação de resíduos orgânicos oriundos de dejetos de animais pode alterar a fauna edáfica. O trabalho objetivou determinar a influência da aplicação sucessiva de dejeto líquido de suínos (DLS) e cama de aves (CA) na mesofauna edáfica em pastagem de Tifton 85. O delineamento experimental foi blocos casualizados em arranjo fatorial (3 x 2), sendo 3 tratamentos de adubação com cama de aves (zero, 1,5 e 3,0 t ha⁻¹ de cama de aves), dois de DLS ( zero e 40 m³ ha⁻¹), com 4 repetições. Após a coleta da fauna edáfica com armadilhas do tipo PROVID, avaliou-se a frequência relativa dos organismos e por grupo funcional, colêmbolos, ácaros, abundância, riqueza de Margalef, índice dominância de Simpson, diversidade de Shannon e o índice de Pielou. A frequência relativa de micrógafos aumenta após quatro aplicações de dejeto líquido de suínos e cama de aves. A utilização isolada de dejeto líquido de suínos e cama de aves reduz a diversidade de Shannon em decorrência do aumento na população de colêmbolos, enquanto a aplicação conjunta de 40 m³ ha⁻¹ de DLS com 1,5 t ha⁻¹ de CA induz diversidade da fauna edáfica. Em períodos de déficit hídrico, a adubação da Tifton 85 com dejeto líquido de suínos estimula a densidade da fauna e população da ordem Collembola em relação à cama de aves.

Palavras-chave: diversidade; resíduos orgânicos; frequência relativa; fauna do solo
Introduction

Animal waste is receiving recurring attention from governments and researches, mainly due to the chemical fertilizers cost and the social pressure for a sustainable agriculture, whereby recycling of nutrients can contribute not only to cost reduction but also to the reduction of environmental pollution (Gualberto et al., 2016). Pasture-based animal production systems have sought production models based on improved soil quality, in which pasture areas can benefit by the rational use of animal manures for fertilization, ensuring the production, economy and conditions for environmental protection (Alonso & Costa, 2017). For that matter, the continuous use of pig slurry (PS) in areas with annual crops provides a higher concentration of P, K, Cu and Zn in the topsoil compared to mineral fertilization (Scherer et al., 2010), while the poultry litter presents N, Ca, P and Mg in adequate concentrations to meet the plants development demand (Silveira Junior et al., 2015). The high production of animal waste due to the expansion of the poultry industry and the pig activity allow the availability of these wastes and their uses in organic fertilizer form.

In order for these wastes employment in agriculture make the system more sustainable, profitable and with no damage to the environment, it is necessary that the amount of nutrients applied to the soil does not cause significant alterations in its environment (Alves et al., 2018). The mesofauna organisms play a fundamental role in the decomposition of plant material, nutrient cycling and regulation of the soil biological processes (Berude et al., 2015), since they are responsible for the fragmentation of residues, the mixing of mineral and organic particles, redistribution of organic matter, among other services (Parron & García, 2015). However, as consequence to the environmental changes sensitivity, edaphic fauna has been used as an indicator of soil quality (Rovedder et al., 2004). Thus, the assessment of the soil community, as bioindicators, has been efficient in the comparisons of different environmental conditions (Santos et al., 2016), since the diversity monitoring allows the understanding of these organisms functionality (Moço et al., 2005). Due to the interaction of mites and springtails with soil, these organisms are being used as indicators of the soil quality, since any alterations in the environment affect its population structure (Huber & Morselli, 2011).

Among the summer grasses, Tifton 85 (Cynodon spp.) stands out due to the combination of its genotypic and phenotypic characteristics, which generate a high production of dry mass (DM), well-developed rhizomes, rapid sprout and high digestibility, making its supply to be possible both in the pasture and hay forms (Farinatti et al., 2009). Tifton 85 has high responsiveness to nitrogen fertilization and the usage of residues from animal production increased Tifton 85 dry matter yield, with increased yield and crude protein levels (Scheffer-Basso et al., 2008). In this context, cultivated areas with this fodder are an alternative to the disposal of residues from animal production.

Research results indicate that the addition of organic waste combined with NPK substantially increases the abundance of individuals and families from the edaphic fauna (Lin et al., 2013), while the application of 200 m² of PS increased the springtail population (Tessaro et al. al., 2011). However, there are reports of a reduction in the abundance of mites and enchytraeids with the increasing amount of PS applied in soil with perennial pasture (Domek-Chruściłka & Seniczak, 2005), a reduction in the springtail population, possibly due to the increase in Cu and Zn concentrations from the PS applications (Antonioli et al., 2013), and lower individuals density in treatments with poultry litter in relation to the mineral fertilizer (Matos-Moreira et al., 2012). Thus, the influence of successive applications of pig slurry and poultry litter on the soil fauna community has not yet been clarified. This study aimed to determine the influence of pig slurry and poultry litter applications on the soil mesofauna in Tifton-85 pastures.

Materials and Methods

We carried this study out in the experimental area from Federal University of Santa Maria, Frederico Westphalen campus, RS. The region climate is of the Cfa type according to Köppen, with average annual temperature of 18ºC and annual precipitation between 1,800 and 2,100 mm. The soil was characterized as Red Latosol (Embrapa, 2013), whose chemical and physical analysis of the 0-0.20 m layer presented: pH(H₂O): 4.6; SMP index: 5.5; 630 g kg⁻¹ clay; 29 g kg⁻¹ organic matter; P-mehlich-1 13 mg kg⁻¹; 252 mg kg⁻¹ potassium; 3.1 cmol dm⁻³ calcium; 1.7 cmol dm⁻³ magnesium; H + Al 5.6 cmol dm⁻³; Al 1.0 cmol dm⁻³. Six months prior to the experiment, 4.9 t ha⁻¹ of dolomitic limestone was applied to raise the pH of the soil to 6, following the recommendations of the fertilization and liming manual (SBCS, 2004).

This study consisted of the edaphic fauna evaluation in experiment with Tifton pasture (Cynodon sp.), with four applications of organic residues being carried out at 3 months intervals. The Tifton 85 seedlings were planted in September 2011, when the experimental area was uniformly covered by the forage (Figure S1 A), which was then cut, having the removal of the vegetal residue of the area in order to simulate animal grazing, followed by application of the treatments in 20 m² plots. The collection of the edaphic fauna organisms occurred 15 days after the application, since according to Baretta et al. (2011), this time is considered sufficient for a response from the fauna to the imposed differences on the environment, with the first collection on February 15 and the second after the fourth application on November 15, all identified during the experiment conduction. We evaluated the fertilization treatments within each different time, considering that, in the literature, the influence of the collection periods on the fauna density, species richness, Shannon and Pielou indexes is well known (Young et al., 2005).

The experimental design was randomized blocks in a factorial arrangement (3 x 2), having three fertilization treatments with poultry litter (zero, 1.5 and 3.0 t ha⁻¹ of...
poultry litter), and two PS (zero and 40 m³ ha⁻¹), with 4 replicates. The mean applied PS composition was 3.58 kg m⁻³ of N; pH 7.63; Dry Mass of 46.81 g kg⁻¹ and the PL (four batches) was 29.81% of C-org; 2.94% N; 0.40% P₂O₅; 2.47% K₂O; pH 7.7. The edaphic fauna was collected by PROVID method means (Figure S1 B) (Antoniolli et al., 2006), remaining in the field for five days, containing 350 mL of 70% alcohol. The edaphic organisms collected in the traps were identified according to the class or order (Figure S1 C, D, E, F, G and H), aided by a binocular stereoscopic microscope with a 60-fold increase and then classified into functional groups, according to Lavelle (1996).

Then, we calculated the abundance and relative frequency of the organisms and per functional group, as well as the biodiversity indexes:

- Margalef Richness (Equation 1):
  \[ I = \frac{(n-1)}{\ln N} \]  
  \[ (1) \]

in which:
- n - number of species;
- N - total number of individuals in the sample.

- Simpson Index, dominance form (Equation 2):
  \[ S = \sum \left( \frac{n_i}{N} \right)^2 \]  
  \[ (2) \]

in which:
- \( n_i \) - number of individuals from group “i”.

- Shannon Diversity (Equation 3):
  \[ H = -\sum \Pi \log \Pi \]  
  \[ (3) \]

in which:
- \( \Pi \) - group i proportion in the sample total.

- And Pielou Evenness (Equation 4):
  \[ e = \frac{H}{\log S} \]  
  \[ (4) \]

in which:
- \( H \) - Shannon index;
- \( S \) - total number of groups in the community, (Eq. 1), (Eq. 2), (Eq. 3) e (Eq. 4) according to Odum (1986).

The data were submitted to the \((X+0.5)^{0.5}\) transformation and the analysis of variance, and when significant, the means were compared by the Tukey test at the 5%, significance level, employing the SISVAR program (Ferreira, 2011) and the Principal Component Analysis (PCA) by the InfoStat program (Di Rienzo et al., 2013).

**Results and Discussion**

The analysis of the main components indicated an alteration in the edaphic fauna community, in the first collection the first two main components explained 77.7% of the data variance, and the treatments without and with PS were in opposite positions, with the 40 m³ of PS treatment grouping with the density and number of springtails (Figure 1A). Soil moisture shortage can restrict metabolic processes and increase the mortality rate of springtails, as they are sensitive to moisture restrictions (Assad, 1997). When analyzing the rainfall data, we observed a water deficit in the period before the first collection (Figure 1). Thus, the PS applied, which has by characteristic 95.32% humidity, seems to have influenced for a larger springtails individuals population. Consequently, the results of this study infer that PS, when applied in periods of water deficit, contributes to the springtail population due to the moisture provided in this residue type.

In the second collection, the two main components explain 89% of the data variance, and the treatments without PS and with application of 1.5 and 3.0 t ha⁻¹ of PL were grouped with

Figure 1. Main Compounds analysis for the first (A) and the second (B) collections of edaphic fauna in a Tifton cultivated area submitted to the application of 0 and 40 m³ ha⁻¹ of PS, 1.5 and 3.0 t ha⁻¹ of PL, 40 m³ of PS + 1.5 t of PL and 40 m³ of PS + 3.0 t ha⁻¹ of PL. Being: PS = pig slurry; PL = poultry litter.
density, number of springtails and dominance (Figure 1B). This treatments influence on the main components contrasts with what was discussed in the first collection, indicating an alteration in the edaphic fauna response with respect to the application of organic or solid organic fertilizer. In the analysis of Figure 2, it can been seen that the period prior to the second collection (November 15) was characterized by high rainfall and, thereby, humidity was not the limiting factor for the springtail population. However, research results infer that poultry litter application has repercussions on a microphage group domain (Almeida et al., 2017), since the organic material supplied in greater quantity by PL benefits the springtail population (Cutz-Pool et al., 2007). Thus, in periods without water restriction, the poultry litter allows a higher springtail population in relation to PS.

The identified organisms of the edaphic fauna were distributed in the functional groups: Microphages, Social, Predator, Herbivorous, Saprophyte and Others, with the Microphage group being the one with the highest relative frequency in relation to the others, increasing from 77.4 to 97.3% from the first to the second collection (Table 1). Tessaro et al. (2011) verified that the PS addition and other animal waste in the soil favor the springtails. In this study, PS and PL, applied on Tifton pasture, contributed to increase the relative frequency of the Microphages, represented by the Collembola group. The Hymenoptera group increased the relative frequency in the first collection and reduced in the second, with the application of the organic fertilizers (Table 1). The first collection result followed the proposed by Alves et al. (2008), in which the organic fertilization with pig waste contributed to a higher frequency of this order. However, climate factors such as temperature and mainly humidity regulate this order, in a way that humidity excess reduces the frequency of Hymenoptera (Silva et al., 2017). The high humidity from rainfall during the second collection period (Figure 1), added to the PS humidity reduced the relative frequency of Hymenoptera, while in the first collection, with water deficit (Figure 1), the PS application added moisture into the soil, increasing this group frequency.

In the fertilization treatments, there was a significant interaction for Collembola, Acarina, density, Shannon diversity, Simpson dominance and Pielou evenness in both the first and second collections (Table 2). In the first collection, the springtails number was higher with the application of 40 m³ ha⁻¹ of PS and in the second collection only with application of 1.5 and 3 t of PL (Table 2). PS allows an increase in the springtail population due to their food habit mainly based on fungi or plant residues (Lavelle, 1996). The Tifton-85 dry matter production responds positively to the pig slurry application.

![Figure 2](image-url) Extract of the fortnight precipitation of 2012 seasons summer (A) and spring (B) in Frederico Westphalen. Meteorological data: INMET.

| Functional group | Organisms | 1st collect | 2nd collect |
|------------------|-----------|-------------|-------------|
| Microphages      |           |             |             |
| Collembola       | 0.4       | 0.2         | 0.6         |
| Socials          | 3.7       | 3.6         | 3.9         |
| Hymenoptera      | 3.7       | 3.6         | 9.3         |
| Predator         | 2.5       | 1.3         | 2.9         |
| Coleoptera       | 1.4       | 0.8         | 1.4         |
| Araneae          | 1.1       | 0.5         | 1.5         |
| Herbivore        | 1.2       | 6.5         | 4.5         |
| Hemiptera        | 1         | 1           | 0.6         |
| Orthoptera       | 1.0       | 5.5         | 3.9         |
| Others           | 1.9       | 3.4         | 17.5        |
| Diptera          | 0.4       | 0.7         | 3.6         |
| Acarina          | 1.5       | 1.7         | 13.9        |

Table 1. Relative frequency (RF: %) per functional groups (FG) and of edaphic fauna organisms in the first and second collections, in Tifton cultivated area submitted to the application of 0 (control group unfertilized), 40 m³ ha⁻¹ of PS, 1.5 e 3 t ha⁻¹ of PL, 40 m³ de PS + 1.5 t de PL e 40 m³ de PS + 3 t ha⁻¹ de PL ha⁻¹.

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application, presenting linear increments in the first two cuts (Camargo et al., 2011), this leads to potential additions of vegetable residues in the area submitted to PS application, favoring the fungi and bacteria populations, and can be, along with the provided humidity from PS in the drought period, the cause of the increase in the springtail population, mainly due the opportunistic characteristic of these organisms, since they increase their frequency when there is an improvement in the environment conditions (Santos et al., 2007).

Acarine density was significantly higher with the application of mixtures from 40 m$^3$ of PS with 1.5 t of PL in the first collection, being reduced with the organic compounds applications in the second collection (Table 2). The initial results of this research are similar to that found by Geremia et al. (2015) that, when working with organic fertilizer, obtained higher occurrence values of mites in the treatments based on PL. However, other results highlighted a negative effect of the organic fertilizers application on the mite population, since according to Cao et al. (2011) the application of 15 t ha$^{-1}$ of the wheat straw mixture + chicken manure + cotton residues reduced the abundance of mites by the increase of predatory mites, which are more resistant to drought periods (Figure 2). Therefore, there is evidence of synergism between low rainfall and applications of manure rich in organic matter, as a cause for the decrease of the individuals mentioned above, while Tessaro et al. (2011) indicated a reduction in the mite population 42 days after PS application.

The highest density of the edaphic organisms in the first collection occurred with the application of PS + 3 t of PL, while in the second collection it was with application of PL (Table 2). Research results indicate that the addition of animal waste can influence the density of soil fauna individuals (Lin et al., 2013). Therefore, the PS and PL applications in soil cultivated with Tifton increases the density of soil fauna organisms, in this case, by the increase in the springtail population (Table 2), that was influenced by the soil humidity and by organic material addition, mainly via PL, whose C/N ratio is 10, being

### Table 2. Mean numbers from Collembola, Acarina, Density and Margalef Richness in the first and second collections, in a Tifton cultivated area submitted to the application zero and 40 m$^3$ ha$^{-1}$ de PS and zero, 1.5 e 3 t ha$^{-1}$ of PL.

| 1st collection | Collembola | Acarina |
|----------------|------------|---------|
| PL (t ha$^{-1}$) | Zero | 40 | Zero | 40 |
| Zero | 145Bb$^a$ | 238Aa | 38b | 5Ab |
| 1.5 | 154Ba | 214Ab | 4Bb | 12Aa |
| 3.0 | 142Bb | 233Aa | 68a | 12Aa |
| CV (%) | 1.92 | 8.71 |
| PL (t ha$^{-1}$) | Density | Margalef Richness |
| Zero | 181Bb | 275Ab | 2.67Aa | 2.18Ba |
| 1.5 | 196Ba | 274Ab | 2.52Aa | 2.55Aa |
| 3.0 | 192Ba | 290Aa | 2.76Aa | 2.45Aa |
| CV (%) | 2.10 | 12.75 |

| 2nd collection | Collembola | Acarina |
|----------------|------------|---------|
| PL (t ha$^{-1}$) | Zero | 40 | Zero | 40 |
| Zero | 330Bb | 999Ac | 1.00Ba | 1.75Aa |
| 1.5 | 5845Aa | 3480Bb | 1.00Ba | 2.25Aa |
| 3.0 | 4925Aa | 4625Bb | 0.00Ab | 0.00Ab |
| CV (%) | 1.84 | 27.9 |
| PL (t ha$^{-1}$) | Density | Margalef Richness |
| Zero | 3738Bb | 1041Ac | 2.57Aa | 2.03Ba |
| 1.5 | 5898Aa | 3529Bb | 1.80Ab | 2.16Aa |
| 3.0 | 4964Aa | 4658Bb | 1.90Ab | 1.64Ab |
| CV (%) | 1.9 | 8.92 |

*Means followed by the same uppercase letter in the line and lowercase in the column within each variable do not differ from each other by the Tukey test at the 5% error probability level

*In which: PS = pig slurry; PL = poultry litter
Table 3. Means from Pielou Evenness, Simpson Dominance and Shannon Diversity Index for the doses zero and 40 m³ ha⁻¹ of PS and zero, 1.5 and 3 (t ha⁻¹) in two collection periods for edaphic fauna over Tifton fertilization management.

| PS doses (m³ ha⁻¹) | PL (t ha⁻¹) | Zero | 40 | Zero | 40 | Zero | 40 |
|-------------------|-------------|------|----|------|----|------|----|
|                   |             |      |    |      |    |      |    |
| Pielou Evenness   |             | 0.48 | 0.55 | 0.95 | 0.97 | 0.67 | 0.42 |
|                   |             | 0.59 | 0.51 | 0.75 | 0.75 | 0.50 | 0.44 |
|                   |             | 0.15 | 0.33 | 0.87 | 0.84 | 0.13 | 0.26 |
| CV (%)            |             | 38.1 | 5.61 | 11.74 |  |
|                   |             |      |    |      |    |      |    |
| Simpson Dominance |             | 0.18 | 0.49 | 0.78 | 0.94 | 0.16 | 0.42 |
|                   |             | 0.01 | 0.52 | 0.98 | 0.96 | 0.01 | 0.44 |
|                   |             | 0.01 | 0.15 | 0.98 | 0.98 | 0.01 | 0.13 |
| CV (%)            |             | 5.23 | 0.34 | 12.51 |  |
*Means followed by the same uppercase letter in the line and lowercase in the column within each variable do not differ from each other by the Tukey test at 5% error probability level

...an important food source for the springtails compared to PS, in a situation in which there is no water restriction in the soil, as discussed in Figure 1.

Margalef Richness showed a higher value for the zero treatment in the first collection, while in the second, with the application of 40 m³ ha⁻¹ of PS and 1.5 t of PL (Table 2). This index is characterized by the probability of sampled individuals in an area belonging to different species (Silva et al., 2013). Research results indicate that a balanced soil fertilization, with organic residues, increases Margalef Richness in relation to the control group or nitrogen-only application (Zhu & Zhu, 2015). However, Silva et al. (2014) found no difference for this index between PS doses and chemical fertilization in soil under minimum tillage and no-tillage. This study results indicate a decrease trend in the number of distinct species with the isolated addition of 40 m³ ha⁻¹ of PS, however, there is a positive effect of the PS addition with 1.5 t of BL.

There was no significant difference for fertilizer treatments in the first collection for Pielou uniformity, but in the second collection, it is evident that the treatment with zero and 40 m³ ha⁻¹ of PS plus 1.5 t ha⁻¹ of PL presented higher means than the others (Table 3). This result corroborates with those of Alves et al. (2008), who also found a significantly higher value of the Pielou index in treatments with 50, 100 and 200 m³ ha⁻¹ of PS in relation to mineral fertilization and the control group without fertilization. The results indicate that the joint application of 40 m³ ha⁻¹ of PS plus 1.5 t ha⁻¹ of PL increases the Pielou evenness of the soil fauna in Tifton 85 perennial pasture.

Simpson dominance was higher at the zero dose and 40 m³ ha⁻¹ of PS at the first collection, however, treatments with addition of organic wastes showed significantly higher dominance than the control group at the second collection (Table 3). Alves et al. (2008) results also demonstrated an increase in Simpson dominance values with organic fertilizer application. The occurrence of a particular group of soil fauna is due to the preparation type of the soil and, mainly, to the benefits of the vegetal residues kept on the soil surface, which provide a favorable environment for the survival of certain groups (Moço et al., 2005). Therefore, the increase in the springtail population, by the addition of animal waste (Table 2), increased the Simpson dominance in relation to the control group; however, if we consider the importance given to the individuals belonging to this edaphic fauna group as bioindicators of soil quality, it can be inferred that this dominance is beneficial to the studied soil (Rovedder et al., 2004).

Shannon diversity was significantly higher in both collection periods treatment with no application of organic residues and when associated with 40 m³ ha⁻¹ of PS with 1.5 t ha⁻¹ of PL (Table 3). Silvano (2011) found similar results in which the application of waste recurred into lower diversity values in relation to the control group. Alves et al. (2008) suggest that the quality and quantity of soil cover and/or stubble also influences the abundance and diversity of soil macrofauna. In this study, the lower diversity obtained with some treatments of organic residues is a consequence of the increase in the springtail population (Table 2) and indicates that the high application of residues doses from animal production can favor the development of certain organisms groups and, consequently, reduction of diversity. However, the joint application of 40 m³ ha⁻¹ of PS with 1.5 t ha⁻¹ or 3 t ha⁻¹ PL, in addition to allow the contribution of organic residue to the soil, also makes a greater diversity of the edaphic fauna possible.

**Conclusion**

The relative frequency of microphages increases with the joint application of 40 m³ ha⁻¹ of PS with 1.5 or 3 t ha⁻¹ of PL and after four applications.

The isolated use of pig slurry and poultry litter reduces Shannon diversity due to the increase in the springtail population, while the joint application of 40 m³ ha⁻¹ of PS with 1.5 t ha⁻¹ of PL induces edaphic fauna diversity.

In periods of water deficit, the fertilization of Tifton 85 with pig slurry stimulates the population of the Collembola order in relation to the poultry litter.

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