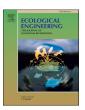
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# Abundance and diversity of mesofauna in relation to minesoil properties after 14.6 years of revegetation with grasses

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#### ABSTRACT

Global annual coal consumption may reach 8038 Mt. in 2025, resulting in continuous post-coal minesoils creation. In Brazil, the Candiota Mine represents the largest country's coal reserves and originates minesoils with high acidity, compaction, and depletion of organic carbon content and soil biological attributes, hindering the reestablishment of vegetation, mesofauna and microbial activity. This study aimed to assess mesofauna attributes and microbial activity in a minesoil restored for 14.6 years with perennial grasses in Candiota, and to explore their relationships with soil physical, chemical, and humus properties. Our previous research showed that Urochloa brizantha improved soil physical and chemical attributes more effectively than other grasses, suggesting stronger links between soil biology and these properties in this treatment. After 14.6 years of revegetation with Hemarthria altissima, Cynodon dactylon, Panicum maximum, Urochloa humidicola, and U. brizantha a series of minesoil biological, chemical and physical attributes (0.00-0.10 m layer) were assessed. Control site consisted of minesoil with spontaneous vegetation. U. brizantha treatment exhibited significantly lower bulk density (1.2 Mg m<sup>-3</sup>) and higher macroporosity (0.16 m<sup>3</sup> m<sup>-3</sup>) and total porosity (0.48 m<sup>3</sup> m<sup>-3</sup>) compared to C. dactylon (1.4 Mg m<sup>-3</sup>, 0.07 and 0.40 m<sup>3</sup> m<sup>-3</sup>, respectively). Treatments did not differ regarding total carbon and carbon content in humic fractions, but mostly increased it compared to control. The total number of fauna individuals across treatments ranged from 596 (P. maximum) to 1146 (U. brizantha) at the litter-soil interface and from 1305 (H. altissima) to 2397 (U. brizantha) in the minesoil. In the control these numbers were lower (539 and 483, respectively). Mites and springtails predominated in all treatments at both litter-soil interface (34-50 % and 36-44 %, respectively) and minesoil (76-85 % and 9-17 %, respectively). Treatments did not differ regarding fauna diversity. Axis 1 and 2 of canonical correspondence analysis (CCA) explained 51 and 45 % of data set variability, respectively. Moreover, CCA revealed that interrelations of fauna and microbial biomass carbon with other soil attributes were unspecific for grass species, contradicting our hypothesis. Despite the outstanding performance of *U. brizantha* to ameliorate physical soil attributes, further research is required before one grass species is prioritized for restoration of minesoil ecological functions.

#### 1. Introduction

Coal is a major global energy source, accounting for approximately 37 % of the world's total energy demand (WCA, 2021). By 2025, global coal consumption is projected to reach 8038 Mt., with China leading at 4337 Mt., followed by the USA at 383 Mt., Europe at 552 Mt., Russia at

227 Mt., and Africa at 190 Mt. (IEA, 2023).

In Brazil, coal remains a significant energy resource, particularly in the southern region, which hosts the country's largest coal reserves and the Candiota Mine—the largest coal mine in Brazil. This mine contains approximately 1.2 billion tons of coal, which is extracted through opencast mining methods (Pinto et al., 2020). Consequently, large areas

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in this region are susceptible to degradation due to coal mining, as has been observed in other parts of the world (Guzman et al., 2019; CEW, 2023). The Candiota Mine is located within the Pampa, a biome characterized by natural grasslands. The ongoing mining activities threaten to exacerbate the degradation of this ecosystem, which is already under significant anthropogenic pressure (Oliveira et al., 2017).

After coal deposits are extracted, the mining pit is refilled with rocks and unused coal remnants, collectively referred to as sterile material. The topographical reconstruction of the mined area is completed by placing a layer of soil, known as topsoil, over the sterile material (Pinto et al., 2020). The resulting reconstructed profiles are termed minesoils (Sencindiver and Ammons, 2000) or Technosols (WRB, 2015).

Recently formed minesoils often exhibit characteristics such as excessive compaction, acidity, poor structure, and low fertility and soil organic matter (SOM) content (Zhen et al., 2015; Da Silva et al., 2007). Additionally, they typically show little to no pedogenetic development in relation to the underlying materials (Filcheva et al., 2021). As a result, minesoils are highly fragile, which is why the minesoils at Candiota are unsuitable for agricultural use, even after completing the restoration protocols required by the regional environmental agency (CRM, 2023).

Successful revegetation of minesoils is a critical first step in the postmining reclamation process, as plant species must be adapted to the challenging soil conditions. Grass species, in particular, are commonly used for initial revegetation due to their ability to adapt to these conditions and effectively improve the physical, chemical, and biological properties of minesoils (Feng et al., 2021; Kumari and Maiti, 2022).

Revegetation is essential for promoting the return of fauna and microbial activity to minesoils by providing habitats that serve as food sources and protection for soil organisms against climate stress and predators (Lu et al., 2021). The re-establishment of mesofauna is especially important for the re-carbonization of minesoils, as these organisms can decompose biomass, thereby enhancing soil organic carbon (SOC) accumulation and humification (FAO, 2020). This process is fundamental for initiating the restoration of minesoil ecosystem functions (Castellanos-Barliza and Léon-Peláez, 2023).

Litter is a crucial source of soil SOC accumulation, along with roots, and forms the foundation of the soil food web. It plays a key role in regulating the rates of SOM decomposition, humification, and aggregate formation (Frouz et al., 2013). In this context, Pen et al. (2022) found that litter with higher nitrogen concentrations and a narrower C:N ratio may promote the abundance and diversity of soil fauna by providing more niche differentiation opportunities due to increased nutrient availability. Furthermore, the food preferences and ingestion behavior of soil fauna can influence the distribution and biomass of microbial communities, thereby affecting humus formation (Bonfanti et al., 2024).

To monitor the restoration of minesoils in the Candiota region, we established a long-term field experiment in which minesoils are revegetated with different perennial grasses. To the best of our knowledge, this is the only long-term field experiment in the Pampa Biome designed with experimental and statistical rigor to track minesoil restoration. Over the first 10 years of the study, we observed successful adaptation of the perennial grasses to both the minesoil and the climate (Pauletto et al., 2016). This led to several improvements, including the alleviation of soil compaction, enhanced soil aggregation and porosity, and increased SOC content, particularly in the top 10 cm of the minesoil (Leal et al., 2016; Stumpf et al., 2016, 2018; Miguel et al., 2023). Notably, these studies consistently highlighted the superior performance of *Urochloa brizantha* compared to the other grasses, as indicated by higher SOC stocks and root density throughout the minesoil profile in this treatment.

The vigorous growth and active root systems of grasses are known to stimulate microbial activity and functioning, which is key to SOM accrual (Bilgo et al., 2012; Tian et al., 2023). For instance, SOM humification and stabilization into aggregates and organo-mineral complexes have been observed in the minesoil of Candiota (Leal et al., 2015a, 2015b, 2016; Stumpf et al., 2018). This rationale has motivated detailed

studies about the role of grasses on the recovery of faunal attributes and minesoil ecosystem functions in our experimental site. Fernandez et al. (2023a, 2023b) confirmed greater mesofauna abundance (especially mites and springtails) and diversity as well as microbial activity after 18 years of revegetation of the Candiota minesoil with grasses compared to a control (spontaneous vegetation, mainly grasses). Nevertheless, the seasonal and spatial distribution of faunal organisms living at the litter–soil interface and soil interior as well as its relations with physical and chemical attributes of the minesoil as affected by grass species remain unstudied.

Given the above considerations, the objective of the present work was to assess biological attributes of the Candiota minesoil after 14.6 years of revegetation, and moreover to comprehensively understand its interrelations with soil physical and chemical attributes. Specifically, we aimed to 1) evaluate for the first time in our experiment the seasonal abundance and diversity of mesofauna organisms at the litter–soil interface and soil interior as function of the grasses used for minesoil revegetation, and 2) deepen our understanding of the most suitable grass species for minesoil revegetation from the perspective of restoring minesoil ecological functions. In view of the outstanding amelioration of the minesoil structure exerted by *Urochloa brizantha* and considering that this favors biological activity in the minesoil, we hypothesized that this treatment would show clearer associations between mesofauna attributes, microbial activity, and soil chemical and physical properties, as well as with SOM humification.

#### 2. Materials and methods

#### 2.1. Study site characterization

The study site (31° 33′ 56" S, 53° 43′ 30" W) is located in the Candiota Mine domain in Rio Grande do Sul State, Southern Brazil (Fig. 1). The climate according to Köppen's classification is subtropical humid (Cfa) (Alvares et al., 2013), the average annual air temperature is 17 °C and average annual precipitation is 1400 mm, including cold winter and hot summer.

The mining area is under the concession of "Companhia Riograndense de Mineração", which conducted the topographic reconstitution of the studied mined area in early 2003. This process included the deposition of nearly 40 cm of topsoil on the overburden layer. The topsoil consisted mainly of the B horizon of the original soil of the premining area, a typical Rhodic Lixisol (IUSS, 2014) and exhibited 46 % clay, 21 % silt, 33 % sand, SOM content <1.2 %, and pH ( $\rm H_2O$ ) of 5.6 (Stumpf et al., 2016). Further information about the basic properties of this minesoil can be found in Leal et al. (2016).

The experiment was established right after the topographic reconstitution of the land in a randomized block design with four replicates (plots of 4  $\times$  5 m) (Fig. 1). The treatments were installed in November 2007 and consisted of perennial summer grasses used for revegetation of the minesoil: *Hemarthria altissima, Cynodon dactylon* cv Tifton, *Panicum maximum* cv Tanzania, *Urochloa humidicola* and *Urochloa brizantha*. Adjacent to the experimental site, an area of the same minesoil with spontaneous growth of eucalyptus (*Eucalyptus* spp.) and annual grasses was used as control site.

Prior to the implementation of the experiment, the minesoil was ploughed to a depth of approximately 15 cm using a bulldozer, followed by liming (10.4 Mg ha $^{-1}$  with 100 % neutralizing power) and fertilization (900 kg ha $^{-1}$  of NPK 5–20–20 fertilizer). Annual fertilizations (250 kg ha $^{-1}$  of NPK 5–30–15 and 250 kg ha $^{-1}$  of ammonium sulphate) were carried out in all experimental plots until 2020. Thereafter, no further fertilization was performed due to consistent accumulation of established green biomass.

# 2.2. Litter and soil moisture analyses

The thickness of the litter deposited on the minesoil surface was

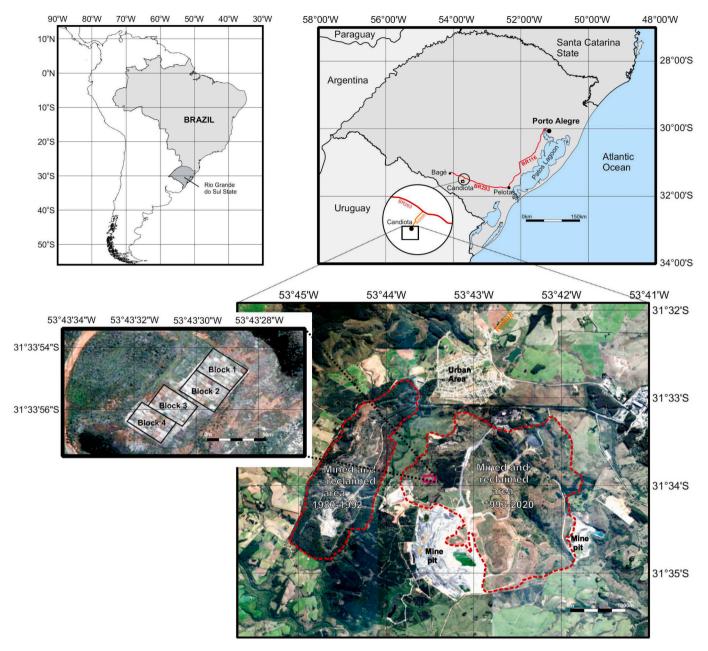


Fig. 1. Location of the experimental site in Candiota, Rio Grande do Sul State, Southern Brazil. Source: Miguel et al. (2023).

measured using a ruler. The total carbon and nitrogen content of the litter was determined by dry combustion on a Thermo Finnigan Flash EA 1112 Elemental Analyzer. For soil gravimetric moisture measurements, 15 g of soil were placed in an oven at 105 °C until constant weight (Teixeira et al., 2017). The litter thickness and soil gravimetric moisture were measured in summer, autumn and winter. The carbon and nitrogen content of litter was determined in litter samples collected in summer. These analyses were performed on composite samples and served mostly to support the interpretation of soil biological attributes, rather than to its isolated interpretation.

# 2.3. Soil biological attributes

# 2.3.1. Fauna abundance, diversity and evenness indexes

On 3rd of March (summer), 3rd of June (autumn) and 24th of August (winter) 2022, faunal organisms inhabiting the minesoil interior and the litter–soil interface were sampled at the experimental plots and control site.

Rainfall and average air temperature throughout the fauna sampling period was recorded by an automatic station located within the Candiota Mine area. In summary, between January and March 2022 (respective to summer sampling), the average air temperature ranged from 23 to 26  $^{\circ}$ C, and precipitation in these months was 98, 79, and 49 mm, respectively. Between April and May 2022 (respective to autumn sampling) the average air temperature varied from 14.3 to 18.6  $^{\circ}$ C, and precipitation in these months was 142 and 100 mm, respectively. From June to August 2022 (respective to winter sampling), the average air temperature ranged between 11.8 and 14.6  $^{\circ}$ C, and precipitation in these months was 207, 175, and 53 mm, respectively. Detailed information on precipitation, maximum and minimum temperature throughout the experimental period is provided in Supplementary Material.

The sampling of the fauna inhabiting the interior of the minesoil was conducted in two steps. In each plot, two steel cylinders of  $84.7~{\rm cm}^3$  and  $10~{\rm cm}$  length each were used to collect soil samples at the 0.00– $0.10~{\rm m}$  layer. In the laboratory, the Tullgren Extraction Funnel method (Bachelier, 1978) was applied to extract fauna from the soil cores. For

that, the cylinders were carefully placed on sieves with 2 mm mesh at the top of the funnels. Then, 40-watt lamps were switched on above the soil samples for a period of 48 h to induce the organisms to move downwards. The organisms were captured in a collection cup attached to the base of the funnel and preserved in 70 % alcohol until identification. This procedure was the same for the samples collected in summer, autumn and winter.

In order to sample the fauna inhabiting the litter–soil interface, pitfall traps (two per plot) were installed using the holes (8.47 cm² surface area each) left after removal of the steel cylinders used to collect fauna from minesoil interior. The traps remained in the field for seven consecutive days. This procedure was the same for the summer, autumn and winter sampling. The same procedures were adopted to sample fauna in the control site.

Organisms collected from pitfall traps and soil cores were photographed with a CARL ZEISS STEMI 305 trinocular stereomicroscope, quantified, and classified according to Gallo et al. (1988). To calculate the abundance and relative frequency of the fauna inhabiting the litter–soil interface and the minesoil, the mesofauna individuals observed in the three sampling seasons were summed up. The number of individuals of the main fauna groups (mites and springtails) is also presented per sampling season. The abundance of the fauna is expressed as the total number of individuals collected in each treatment and location (litter–soil interface and minesoil).

For each sampling season (summer, autumn and winter) and location (litter–soil interface and minesoil) the Shannon-Wierner diversity index (H') and Pielou's evenness index (J) were calculated using the DivEs - Diversity of Species® software (2018–2021, v. 4.17). The H' index indicates the diversity of the soil fauna organisms, and it is calculated according to Eq. (1):

$$\dot{H} = -\sum_{i=1}^{s} pi.ln(pi)$$
 (1)

where: s = number of fauna groups; pi = proportion of individuals of each group in relation to the total: ni/N (ni = number of individuals in group i, N = number of individuals in all groups).

The J index indicates the uniformity of fauna individuals, i.e., how individuals are distributed among the different taxa in the sample. The J index is calculated according to Eq. (2):

$$J = \frac{H'}{ln(s)}$$
 (2)

# 2.3.2. Soil microbial biomass carbon and microbial respiration

In March 2022, soil samples were collected from the 0.00–0.10 m layer of the minesoil in the different treatments and in the control site using a cutting shovel for the determination of microbial biomass carbon (MBC), microbial respiration (MR), and soil chemical properties (see Section 2.4). The soil samples collected for MBC and MR analysis were preserved at 4 °C until analysis. The MBC was quantified by the microwave-radiation-extraction method (Ferreira et al., 1999). For that, 32 g of fresh soil were weighed in duplicate and amended with 50 mL 0.5 M  $\rm K_2SO_4$  as extractant. One replicate was subjected to irradiation in a microwave oven while the other was not. The samples were titrated with 0.25 M  $\rm FeSO_4$ , and the MBC content (mg C kg soil $^{-1}$ ) was calculated as described in Eq. (3):

$$MBC \left( mg \ C \ kg \ soil^{-1} \right) = \frac{Ct - Cnt}{KC} \tag{3}$$

where:  $C_t = \text{carbon content}$  in the treated sample;  $C_{nt} = \text{carbon content}$  in the untreated sample; and  $k_C = \text{carbon mineralization rate (0.33)}$  according to Sparling and West (1988).

Soil microbial activity was assessed by measuring soil MR according to Anderson and Domsch (1993). For this,  $100 \, g$  of fresh soil with known moisture content were placed together with cups containing  $20 \, \text{mL}$  of  $1 \, g$ 

M NaOH into a hermetically sealed bottle. After 21 days, the solution was removed for titration with 1 M HCl. The MBC and MR data were used to calculate the metabolic coefficient for  $CO_2$  (q $CO_2$ ) according to Anderson and Domsch (1993), as shown in Eq. (4):

$$\label{eq:qco2} \text{qCO}_2 \; \left( \text{mg CO}_2 - \text{C g}^{-1} \; \text{MBC h}^{-1} \right) = \frac{\text{MR} \; \left( \text{mg CO}_2 - \text{C kg soil}^{-1} \; h^{-1} \right)}{\text{MBC (mg C kg soil}^{-1})} \quad (4)$$

#### 2.4. Soil chemical and physical attributes

Soil samples (0.00–0.10 m layer) collected in March 2022 were subjected to pH determination in water (w:v, 1:1) and total organic carbon (TOC) content determination by the Walkley-Black combustion method (Teixeira et al., 2017). This method was chosen because it is the official method referred to in the manual of recommendation of fertilization and liming for the region where the experiment is located (CQFS, 2016).

The chemical fractionation of SOM was conducted according to Benites et al. (2003). For that, 1 g of soil was used and the separation of fulvic acid, humic acid and humin was performed according to the differential solubility of these fractions in alkaline and acid solutions followed by centrifugation. The carbon content in fulvic acids (C-FA), humic acids (C-HA) and humin (C-HU) extracts was determined via wet oxidation. The extracts were oxidized with potassium dichromate ( $K_2Cr_2O_7$  0.042 M for fulvic and humic acid, and 0.1667 M for humin) at acidic pH (2.0) and the excess of  $K_2Cr_2O_7$  was determined by titration with ferrous ammonium sulfate ( $Fe(NH_4)_2(SO_4)_2$ . 6  $H_2O$ ), 0.0125 M for fulvic and humic acid, and 0.25 M for humin. The degree of humification of the SOM was expressed as C-HA/C-FA ratio (Filcheva et al., 2021).

Undisturbed soil samples were collected with steel cylinders (86.70 cm $^3$ , 10 cm length) in the 0.00–0.10 m layer for determination of soil bulk density (Bd) and porosity according to Teixeira et al. (2017). In the laboratory, the samples were saturated with water by capillarity for 24 h, and then placed on a tension table, where they were equilibrated to a tension of 6 kPa for determination of macroporosity (Ma). After equilibration, the samples were oven-dried at 105  $^{\circ}$ C until constant weight for determination of microporosity (Mi) and Bd. Total porosity (Tp) was calculated by the sum of Ma and Mi.

# 2.5. Statistical analysis

The normal distribution of the data was verified using the Shapiro-Wilk test and the homogeneity of variances was verified with Levene's test. After confirmation of normal distribution and homogeneity of variances the data were subjected to one-way analysis of variance (ANOVA). When ANOVA indicated significant effect of treatments (grass species), the means of variables were compared by Tukey test (p < 0.05) with Bonferroni correction. The C-FA and C-HA data failed the normal distribution test. Therefore, the Kruskal-Wallis' test was used to verify the effect of the treatments on C-FA and C-HA, which was not significant. Therefore, the means of C-FA and C-HA in the different treatments were not compared statistically. These analyses were conducted using Sigmaplot 12.3.

Canonical Correspondence Analysis (CCA) was performed using the software Past v. 4.03 (Hammer et al., 2001) to assess the relationship between the environmental attributes (soil Bd, Ma, Tp, TOC, C-FA, C-HA, C-HU and pH) and the response variables (abundance of mites, springtails, and other organisms, and MBC). Environmental variables were normalized (0 to 1) to ensure their comparable weight on CCA ordination despite their different units. Response variables were transformed by  $\log(x+1)$  to reduce the influence of high mites and springtails abundance and improve data distribution. For the CCA biplot, the scaling type 2 method was applied, which maximizes the correlation between treatments and variables, providing a more accurate representation of the data structure. The biplot quadrants were identified as Q1, Q2, Q3 and Q4 for guidance of results and discussion.

The control site was not part of the experimental design, and thus it was not included in the statistical analyses. The means of the response variables of the experimental treatments were compared to the control using percentage differences (positive or negative) to monitor the recovery of soil attributes by the perennial grasses.

#### 3. Results

#### 3.1. Soil moisture, litter and fauna attributes

On average, soil gravimetric moisture in the treatments was 0.7–9.1 % higher compared to the control across the different seasons, and the litter thickness in the treatments was on average 2.4- (summer), 1.4-(autumn) and 2.5-fold (winter) greater compared to the control (Table 1). The C:N ratio of the litter in the treatments ranged from 32.5 (*Urochloa brizantha*) to 66.0 (*Hemartria altissima*) and in the control it was 34.7 (Table 1).

Across the seasons and treatments, a total of 4299 and 8904 organisms were found inhabiting the litter-soil interface and the minesoil, respectively (Table 2), totaling 13,203 organisms distributed in 9 taxonomic groups. At the litter-soil interface, the number of organisms observed in Hemarthria altissima, Cynodon dactylon, Panicum maximum, Urochloa humidicola and Urochloa brizantha treatments was 832, 868, 596, 857 and 1146 respectively, while the number of organisms inhabiting the minesoil in these respective treatments was greater: 1305, 1945, 1605, 1652 and 2397 (Table 2). The total number of organisms summing that inhabiting the litter-soil interface or the minesoil in the Hemarthria altissima, Cynodon dactylon, Panicum maximum, Urochloa humidicola and Urochloa brizantha treatment was 2137, 2813, 2201, 2509 and 3543, respectively (Table 2). These numbers were respectively greater by 109, 175, 115, 145 and 247 % compared to the control site. These differences were mainly influenced by the proportionally greater number of organisms in the minesoil interior in the treatments compared to the control, which ranged from 170 (Hemarthria altissima) to 396 % (Urochloa brizantha).

In most treatments, Collembola, Arachnida, Coleoptora and Hymenoptera were more abundant at the litter–soil interface, while the groups

Table 1
Soil gravimetric moisture and litter thickness measured in summer, autumn and winter, and carbon (C) and nitrogen (N) content and C:N ratio of litter measured in summer in the experimental area (perennial grasses treatments) and in the control site.

Treatments	Summer	Autumn	Winter	
	Soil gravimetric moisture (%)			
Hemartria altissima	23.8	24.5	22.4	
Cynodon dactylon	23.1	26.9	23.7	
Panicum maximum	19.8	27.5	26.1	
Urochloa humidicola	22.4	28.2	24.0	
Urochloa brizantha	25.6	25.4	25.6	
Control site	16.5	23.8	20.1	
	Litter thickness (cm)			
Hemartria altissima	15.4	13.2	14.3	
Cynodon dactylon	8.2	7.6	10.4	
Panicum maximum	4.1	5.6	6.2	
Urochloa humidicola	10.9	9.2	8.7	
Urochloa brizantha	8.8	7.8	7.3	
Control site	4.2	6.1	3.8	
	Summer			
	$C (g kg^{-1})$	$N (g kg^{-1})$	C:N ratio	
Hemartria altissima	429.0	6.5	66.0	
Cynodon dactylon	410.5	7.2	57.0	
Panicum maximum	374.3	9.3	40.2	
Urochloa humidicola	436.0	9.8	44.5	
Urochloa brizantha	393.6	12.1	32.5	
Control site	391.8	11.3	34.7	

Acari, Diptera, Diplura and Enchytraeidae were more abundant into the minesoil. In contrast, the number of individuals in the groups Collembola, Arachnida, Coleoptera, Diptera, Hymenoptera and Enchytraeidae in the control was higher at the litter-soil interface than in the minesoil (Table 2).

At the litter–soil interface, springtails were more abundant in winter and less in summer (Fig. 2a), whereas into the minesoil the opposite occurred (Fig. 2b), regardless of grass species. Emphasis is given to *Urochloa brizantha* and *Urochloa humidicola*, which contained the highest number of springtails (364 and 332, respectively) at the litter–soil interface in winter (Fig. 2a) and also 116 and 146 springtails into the minesoil in summer, respectively, which are higher than that observed in *Hemarthria altissima* (109) and *Panicum maximum* (98) (Fig. 2b). The lowest number of springtails was observed in autumn (1 to 6), regardless of treatment and spatial distribution of individuals (litter–soil, soil) (Fig. 2a-b).

Unlike springtails, the greatest abundance of mites was recorded in summer, regardless of the spatial localization of individuals (litter–soil interface, soil interior), except for *Panicum maximum* treatment, where abundance of mites at the litter–soil interface was higher in winter (130) than in summer (104) (Fig. 2c-d). Furthermore, mites were more abundant in the minesoil than at the litter–soil interface, regardless of season and grass species. For instance, across treatments, the total number of mites at the litter-soil interface ranged from 246 to 557 (Fig. 2c), while in the soil interior it ranged from 1018 to 2030 (Fig. 2d). Comparing the treatments, the number of mites stood out in *Urochloa brizantha* both at the litter–soil interface (557) and into the minesoil (2030) (Fig. 2c-d).

Like springtails, the lowest abundance of mites occurred in autumn, regardless of treatment, with 1 to 12 individuals at the litter–soil interface (Fig. 2c) and 19 to 42 individuals into the minesoil (Fig. 2d). In winter, the abundance of mites at the litter-soil interface varied less remarkably between 103 (*Hemarthria altissima*) and 163 (*Urochloa brizantha*) (Fig. 2c), whereas into the minesoil this variation was larger, between 365 (*Hemarthria altissima*) and 613 (*Urochloa brizantha*) (Fig. 2d).

Compared to the control, the total number (accounting for the three seasons) of mites and springtails in the treatments was about  $195-612\,\%$  and  $64-238\,\%$  greater, respectively, at the litter-soil interface, and about  $164-427\,\%$  (mites) and  $164-420\,\%$  (springtails) greater into the minesoil (Table 2).

In the treatments, Hymenoptera was the third most abundant group both at the litter-soil interface (6.8–14.1 %) and into the minesoil (2.0–6.1 %) (Table 2), mainly due to the contribution of ant counts (data not shown). Notably, Hymenoptera individuals were absent at the litter-soil interface in the control (Table 2), probably due to the lower abundance of litter (Table 1).

The H' index found in the treatments at the litter–soil interface varied between 1.3 and 2.1 and it was higher in summer, except for *Urochloa brizantha*, where H' was higher in autumn (Fig. 3a). Into the minesoil, the H' index in the treatments varied from 0.7 to 2.0 and it was higher in autumn, regardless of the treatment (Fig. 3b). Compared to the treatments, the H' in control was always lower across seasons, regardless of spatial distribution of organisms (litter-soil, into minesoil), except in winter at litter-soil interface, where H' in control (1.9) was higher compared to all treatments (1.3–1.8) (Fig. 3a).

The J index at the litter–soil interface of treatments across seasons varied between 0.4 and 0.9 and it was always lower in winter (Fig. 3c). The J index of treatments into the minesoil varied from 0.2 and 0.8 across seasons, and it was consistently higher in autumn (Fig. 3d). In the control, the J index across seasons varied from 0.4 to 0.8 at litter-soil interface (Fig. 3c), and from 0.2 to 0.5 into minesoil (Fig. 3d). Compared to the treatments, the J in the control was consistently higher at litter-soil interface in winter (Fig. 3c), but consistently lower into minesoil in autumn (Fig. 3d).

Table 2
Abundance (total number of individuals) and relative frequency (%, within parentheses) of faunal taxonomic groups inhabiting the litter–soil interface and the minesoil (0.00–0.10 m layer) revegetated with perennial grasses and in the control.

Taxonomic groups	Hemarthria altissima	Cynodon dactylon	Panicum maximum	Urochloa humidicola	Urochloa brizantha	Control	
	Number of individuals inhabiting the litter-soil interface						
Acari	285 (34.2 %)	349 (40.2 %)	239 (40.1 %)	325 (37.9 %)	577 (50 %)	81 (15 %)	
Collembola	352 (42.3 %)	345 (39.8 %)	217 (36.4 %)	375 (43.8 %)	446 (38.2 %)	132 (24.5 %)	
Arachnida	25 (3 %)	15 (1.8 %)	20 (3.4 %)	14 (1.7 %)	3 (2 %)	21 (3.9 %)	
Coleoptera	36 (4.3 %)	14 (1.6 %)	13 (2.2 %)	14 (1.6 %)	20 (1.7 %)	188 (34.9 %)	
Diptera	4 (0.5 %)	6 (0.7 %)	4 (0.7 %)	9 (1 %)	12 (1 %)	9 (1.7 %)	
Diplura	8 (1 %)	4 (0.5 %)	4 (0.7 %)	4 (0.5 %)	4 (0.3 %)	1 (0.2 %)	
Hymenoptera	117 (14.1 %)	126 (14.5 %)	95 (16 %)	108 (12.5 %)	80 (6.8 %)	0 (0.0 %)	
Enchytraeidae	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	1 (0.1 %)	1 (0.9 %)	95 (17.6 %)	
Outros	5 (0.6 %)	9 (1 %)	4 (0.7 %)	7 (0.8 %)	3 (0.3 %)	12 (2.2 %)	
Total	832	868	596	857	1146	539	
	Number of individuals inhabiting the soil interior						
Acari	1018 (78 %)	1471 (76 %)	1322 (82 %)	1305 (79 %)	2030 (85 %)	385 (80 %)	
Collembola	169 (13 %)	333 (17 %)	176 (11 %)	252 (15 %)	210 (9 %)	64 (13.3 %)	
Arachnida	1 (0.1 %)	9 (0.5 %)	2 (0.1 %)	4 (0.3 %)	6 (0.3 %)	0 (0.0 %)	
Coleoptera	1 (0.1 %)	5 (0.3 %)	0 (0.0 %)	1 (0.6 %)	15 (0.6 %)	3 (0.6 %)	
Diptera	5 (0.4 %)	5 (0.3 %)	13 (0.8 %)	8 (0.5 %)	37 (1.5 %)	0 (0.0 %)	
Diplura	7 (0.5 %)	40 (2 %)	8 (0.5 %)	15 (0.9 %)	21 (0.9 %)	2 (0.4 %)	
Hymenoptera	79 (6.1 %)	65 (3.3 %)	53 (3.2 %)	54 (3.2 %)	49 (2 %)	18 (3.7 %)	
Enchytraeidae	6 (0.5 %)	2 (0.1 %)	11 (0.7 %)	2 (0.1 %)	13 (0.5 %)	2 (0.4 %)	
Others	19 (1.5 %)	15 (0.8 %)	20 (1.2 %)	11 (0.7 %)	16 (0.7 %)	9 (1.9 %)	
Total	1305	1945	1605	1652	2397	483	

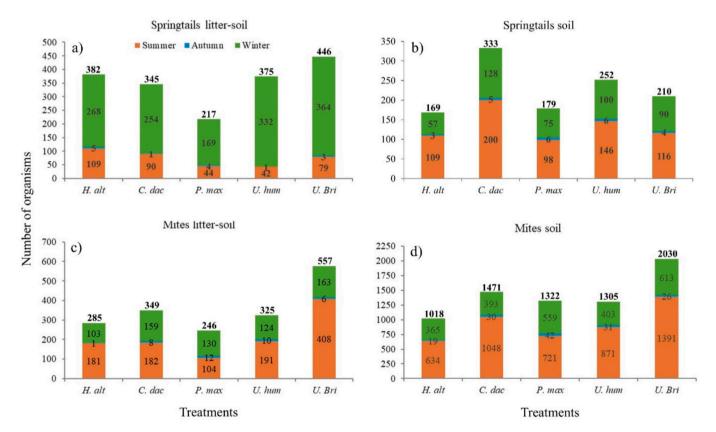


Fig. 2. Abundance of springtails at the litter–soil interface (a) and soil (b), and abundance of mites at the litter–soil interface (c) and soil (d) in the different treatments in three sequential seasons of the year (summer, autumn and winter). Numbers at the top of the bars indicate the total number of organisms summing the three seasons.

# 3.2. Soil microbial biomass carbon and microbial respiration

The treatments exhibited average MBC values between 542 (*Cynodon dactylon*) and 904 mg C kg $^{-1}$  (*Urochloa brizantha*), and MR values between 0.50 (*Urochloa brizantha*) and 0.73 mg CO2-C kg $^{-1}$  h $^{-1}$  (*Hemarthria altissima*) (Table 3). The treatments did not differ

statistically with respect to MBC and MR (Table 3). Compared to the control, the MBC was about 67–178 % higher and MR was about 28–50 % lower in the treatments (Table 3).

The qCO<sub>2</sub> values in the treatments were consistently lower (65–78 %) compared to the control ( $3.7 \times 10^{-3}$  mg CO<sub>2</sub>-C g<sup>-1</sup> MBC h<sup>-1</sup>), except for *Urochloa humidicola*, which was only 3 % lower compared to the

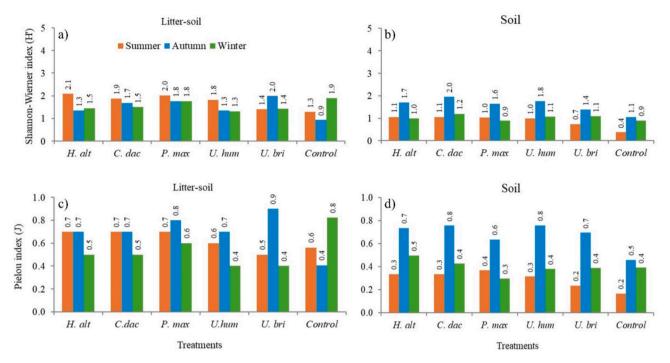


Fig. 3. Shannon-Wierner diversity index (H') at the litter–soil interface (a) and soil (b), and Pielou's evenness index (J) at the litter–soil interface (c) and soil (d) in the different treatments in three sequential seasons of the year (summer, autumn and winter).

Table 3 Microbial biomass carbon (MBC), microbial respiration (MR), and metabolic coefficient ( $qCO_2$ ) in the minesoil revegetated with perennial grasses and in the control site.

Treatments	MBC	MR	$\rm qCO_2$
	mg C kg <sup>-1</sup>	$\overline{\mathrm{mg~CO_2\text{-}C~kg^{-1}~h^{-1}}}$	$\overline{\mathrm{mg~CO_2\text{-}C~g^{-1}~MBC~h^{-1}}}$
Hemarthria altissima	681 ± 326 <sup>ns</sup> (+109 %)	$0.73 \pm 0.03$ ns (-28 %)	$1.3 \times 10^{-3}  (-65  \%)$
Cynodon dactylon	$542 \pm 347 \ (+67 \ \%)$	$0.55 \pm 0.41 \ (-46 \ \%)$	$1.3 \times 10^{-3} \ (-65 \ \%)$
Panicum maximum	$770 \pm 284 \ (+137 \ \%)$	$0.64 \pm 0.33 \ (-37 \ \%)$	$0.8 \times 10^{-3}  (-78  \%)$
Urochloa humidicola	$643 \pm 411 \ (+98 \%)$	$0.55\pm0.45~(-46~\%)$	$3.6 \times 10^{-3} (-3 \%)$
Urochloa brizantha	$904 \pm 806 \ (+178 \ \%)$	$0.50\pm0.16~(-50~\%)$	$1.1 \times 10^{-3}  (-70  \%)$
Control	$325 \pm 55.97$	$1.01\pm0.43$	$3.7\times 10^{-3}\pm 0.001$

 $<sup>\</sup>pm$  Standard deviation. ns: not significant. Increase (+) or decrease (-) compared to the control.

control (Table 3). The greater  $qCO_2$  in control and *Urochloa humidicola* treatment unaccompanied by greater MBC likely indicates higher stress of microorganisms in these sites.

# 3.3. Soil chemical and physical attributes

The soil pH in the treatments ranged from 5.2 to 5.9, without statistical differences, and it was comparable to the pH in the control (5.4) (Table 4).

The TOC content in the treatments ranged from 13.8 (*Urochloa humidicola*) to 16.4 g kg<sup>-1</sup> (*Panicum maximum*), without statistical differences between treatments (Table 4). These values were about 39–55 % higher than the control (Table 4). These TOC increments in treatments compared to the control are mostly attributed to an increase in C-FA and C-HU content, which were 36–82 % and 0–62 % greater than in the control, respectively (Table 4). For C-HA, only *Panicum maximum* increased C-HA (57 %) compared to control, whereas in the other treatments C-HA was identical or up to 71 % lower (*H. altissima*)

Table 4
Soil pH, total organic carbon content (TOC), carbon content as fulvic acid (C-FA), humic acid (C-HA) and humin (C-HU), and C-HA/C-FA ratio in the minesoil revegetated with perennial grasses and in the control site.

Treatments	pH	TOC	C-FA	C-HA	C-HU	C-HA/C-FA
		g kg <sup>-1</sup>			<u>.</u>	
H. altissima	$5.3\pm0.2^{\text{ns}}~(-2~\%)$	14.7 $\pm$ 1.7 $^{\mathrm{ns}}$ (+39 %)	1.7 $\pm$ 0.1 $^{\mathrm{ns}}$ (+55 %)	0.2 $\pm$ 0.1 $^{\rm ns}$ (–71 %)	$3.3\pm0.6$ ns (+57 %)	0.1
C. dactylon	$5.9 \pm 0.4 \ (+9 \ \%)$	$15.9 \pm 1.2 \ (+50 \ \%)$	$1.5 \pm 0.1 \ (+36 \ \%)$	$0.4 \pm 0.5 \; (-43 \; \%)$	$2.1\pm0.8$ (+0 %)	0.3
P. maximum	$5.2 \pm 0.3$ (-4 %)	$16.4 \pm 3.3 \ (+55 \ \%)$	$1.7 \pm 0.5 \ (+55 \ \%)$	$1.1\pm0.2$ (+57 %)	$3.4 \pm x0.5 \ (+62 \%)$	0.6
U. humidicola	$5.6\pm0.3$ (+4 %)	$13.8 \pm 2.5 \ (+30 \ \%)$	$1.8 \pm 0.2 \ (+64 \ \%)$	$0.7 \pm 0.6 \ (0 \ \%)$	$3.0 \pm x0.7 \ (+43 \%)$	0.4
U. brizantha	$5.2 \pm 0.5~(-4~\%)$	$14.8 \pm 3.3 \ (+40 \ \%)$	$2.0 \pm 0.2 \ (+82 \ \%)$	$0.6 \pm 0.3~(-14~\%)$	$3.2 \pm 0.6 \ (+52 \ \%)$	0.3
Control	$\textbf{5.4} \pm \textbf{0.6}$	$10.6 \pm 0.2$	$1.1\pm0.2$	$0.7\pm0.2$	$2.1\pm0.7$	0.6

 $<sup>\</sup>pm$  Standard deviation. ns: not significant. Increase (+) or decrease (-) compared to the control.

Table 5
Bulk density (Bd), macroporosity (Ma), microporosity (Mi) and total porosity (Tp) in the minesoil revegetated with perennial grasses and in the control site.

Treatments	Bd	Ма	Mi	Тр	
	Mg m <sup>-3</sup>	$m^3 m^{-3}$			
Hemartria altissima	1.45 ± 0.06 ab (+7 %)	0.06 ± 0.02 b (-57 %)	$0.35 \pm 0.02$ <sup>ns</sup> (+25 %)	$0.41 \pm 0.04$ ab ( $-2$ %)	
Cynodon dactylon	$1.37\pm0.11$ b (+1 %)	$0.07 \pm 0.02 \text{ b } (-50 \text{ \%})$	$0.33 \pm 0.01 \ (+18 \ \%)$	$0.40 \pm 0.02 \text{ b } (-5 \%)$	
Panicum maximum	$1.32\pm0.08$ ab ( $-3$ %)	$0.11\pm0.05$ ab ( $-21$ %)	$0.33 \pm 0.03 \ (+18 \ \%)$	$0.43 \pm 0.05 \text{ ab (+3 \%)}$	
Urochloa humidicola	$1.32\pm0.08$ ab ( $-3$ %)	$0.11\pm0.05$ ab ( $-21$ %)	$0.34 \pm 0.01 \ (+21 \ \%)$	$0.45 \pm 0.05 \text{ ab (+7 \%)}$	
Urochloa brizantha	$1.19\pm0.16$ a ( $-13$ %)	$0.16\pm0.03$ a (+14 %)	$0.32 \pm 0.02 \ (+14 \ \%)$	$0.48 \pm 0.04$ a (+14 %)	
Control	$1.36\pm0.07$	$0.14\pm0.4$	$0.28\pm0.02$	$0.42\pm0.04$	

 $<sup>\</sup>pm$  Standard deviation. Equal lower-case letters in the column indicate no significant difference by Tukey's test (p < 0.05). ns: not significant. Increase (+) or decrease (-) compared to the Control.

compared to control (Table 4).

The C-HA/C-FA ratio in *Hemarthria altissima* (0.1), *Cynodon dactylon* (0.3), *Urochloa humidicola* (0.4) and *Urochloa brizantha* (0.30) were lower than that in *Panicum maximum* (0.6) and control (0.6), indicating greater humification degree of SOM in the last two (Table 4).

The minesoil Bd was statistically lower in *Urochloa brizantha* (1.19 Mg m $^{-3}$ ) than in *Hemarthria altissima* (1.45 Mg m $^{-3}$ ), and these values were 13 % lower and 7 % higher than the control (1.36 Mg m $^{-3}$ ), respectively (Table 5). The Bd in the other treatments did not differ statistically from these treatments (Table 5). The Ma was significantly higher in *Urochloa brizantha* (0.19 m $^3$  m $^{-3}$ ) than in *Hemarthria altissima* (0.06 m $^3$  m $^{-3}$ ) and *Cynodon dactylon* (0.07 m $^3$  m $^{-3}$ ). The Ma in the *Panicum maximum* and *Urochloa humidicola* treatment did not differ from these treatments (Table 5). Compared to control, the Ma in the treatments was about 21–57 % lower, except for *Urochloa brizantha* which exhibited Ma 14 % higher compared to control (Table 5).

The Mi ranged from 0.32 to 0.35 m $^3$  m $^{-3}$ , without statistical differences between treatments. Noteworthily, treatments increased Mi by about 14 % (*Urochloa brizantha*) to 25 % (*Hemarthria altissima*) compared to the control (Table 5). The Tp differed statistically only between *Urochloa brizantha* (0.48 m $^3$  m $^{-3}$ ) and *Cynodon dactylon* (0.41 m $^3$  m $^{-3}$ ), which were 14 % higher and 2 % lower, respectively compared to the control (Table 5).

Overall, after 14.6 years of revegetation, the performance of *Urochloa brizantha* stood out in terms of the recovery of the minesoil structure (lower Bd, higher Ma and Tp), particularly if compared to *Hemarthria altissima* and *Cynodon dactylon*.

# 3.4. Canonical correspondence analysis

The axis 1 (CCA1) and 2 (CCA2) of the CCA biplot explained 51.44 and 44.98 % of the variation in the data set (Fig. 4). *Urochloa brizantha* was clearly ordinated in Q3 together with TOC, Ma, and Tp in the CCA biplot, whereas *Hemarthria altissima* and *Urochloa humidicola* were ordinated in the opposite quadrant (Q2) together with Bd, C-FA and pH (Fig. 4). *Panicum maximum* was ordinated in Q1 together with C-HA, whereas *Cynodon dactylon* was ordinated in Q4 along with C-HU (Fig. 4). Notably, mesofauna attributes (abundance of mites, springtails, and other organisms) and MBC were plotted mostly close to the intersection of CCA1 and CCA2 (Fig. 4), revealing their unspecific association with other soil attributes, regardless of treatment.

#### 4. Discussion

The greater nitrogen content and lower C:N ratio of *Urochloa brizantha* litter (Table 1) may have favored the abundance of mites and springtails at the litter–soil interface as well as the abundance of mites in the soil (Fig. 2, Table 2). Whereas the lower nitrogen content and greater C:N ratio of *Hemarthria altissima* litter (Table 1) may have led to the lower abundance of mites and springtails at the litter–soil interface and soil interior compared to most of the other treatments (Table 2). The greater thickness of litter and soil moisture (especially in summer) in the treatments compared to the control (Table 1) likely explain the greater abundance of springtails in the treatments (Table 2). Zagatto et al. (2019) observed that the abundance of springtails in soil was strongly related to soil moisture and season-dependent, similarly to our findings. This is in line with Potapov et al. (2023), who observed relatively low abundance of springtails in tropical ecosystems where the litter layer is

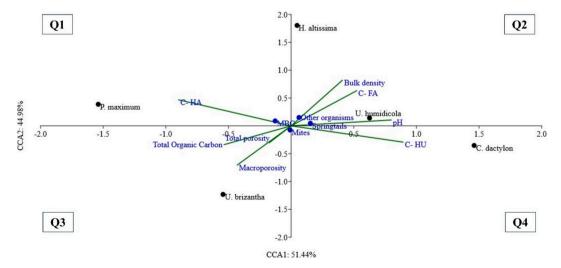


Fig. 4. Canonical correspondence analysis of abundance of mites, springtails, and other organisms and microbial biomass carbon (MBC) in the minesoil under *H. altissima, C. dactylon, P. maximum, U. humidicola* and *U. brizantha*, using soil bulk density, macroporosity, total porosity, pH, total organic carbon, carbon as fulvic acid (C-FA), humic acid (C-HA) and humin (C-HU) as environmental variables. Q = quadrant.

usually shallow due to the fast decomposition of plant residues.

The H' values  $\leq$ 2.1 observed in all treatments (Fig. 3a-b), regardless of the season and the habitat of the organisms, indicate low diversity of fauna (Góes et al., 2021; Fernandez et al., 2023a). Likely, the low diversity of plant species within treatments (monoculture of grasses) and across treatments (all perennial grasses) may have contributed to these results (Urbanowski et al., 2021; Xie et al., 2022). Additionally, in degraded areas the revegetation time is a major factor allowing niche diversification and associated increase in mesofauna diversity (Menta et al., 2018; Correia et al., 2020). These data suggest that mesofauna succession in our experimental area is incipient and possibly limited by plant diversity. The J index values found in our study can be attributed to the large predominance of mites over other groups, evidencing the great adaptability of these organisms to the adverse minesoil and local weather conditions, as verified elsewhere (Manu et al., 2019).

The acidity of the minesoil after 14.6 years of revegetation was expected because minesoil liming occurred solely in 2003, when soil pH increased to 7.4 (Santos, 2006). Soil acidification over years of recultivation is attributed to multiple factors: rainfall-associated leaching of basic cations (minimum average monthly rainfall in the mined area is 60 mm (Alvares et al., 2013), nitrogen fertilization of grasses, roots exudation, SOM decomposition, and acidity generated by the parental material used for topographic reconstitution of the area (Li et al., 2020). Soil pH <5, which may be restrictive for soil colonization by mesofauna (Lavelle et al., 1995; Geissen et al., 2007), was not observed in our experiment. On the other hand, all treatments exhibited similar soil pH (5.2-5.9), hampering mesofauna diversification across treatments driven by soil pH. However, benefits of active (with perennial grasses) compared to passive revegetation (control) are clearly indicated by the greater abundance of mesofauna across the taxonomic groups in the experimental plots, both at the litter-soil interface and soil interior (Table 2). Even though litter in the control exhibited a C:N ratio (34.7) narrower than in all treatments (except Urochloa brizantha), its thickness was considerably lower compared to most of the treatments across the seasons. Similarly, soil moisture in the control was lower in all seasons compared to the treatments (Table 1), likely forcing mesofauna organisms to search for other habits with greater offer of food and protection against summer conditions (Lu et al., 2021).

Springtails inhabiting the litter-soil interface tended to be more abundant in winter, while mites were more abundant in summer, as a natural behavior of these organisms (Xu et al., 2012). The greater abundance of mites into the minesoil in summer is likely due to a combined effect of soil coverage by the grasses leading to milder temperatures into the soil (Frouz, 2018; Manu et al., 2019). The general greater abundance of mites at the litter-soil interface in summer, especially in Urochloa brizantha, suggest that mites benefitted from lower litter C:N ratio and sufficient moisture to decompose litter. The incorporation of plant biomass into the soil would also explain the highest abundance of soil mites observed in this treatment compared to all other treatments. Nevertheless, the MBC, MR and qCO2 values in Urochloa brizantha did not differ from that of other treatments, except qCO2 in Urochloa humidicola (Table 3), challenging our assumption. Otherwise, greater microbial activity would be expected after the incorporation of fresh biomass into the soil in summer. Thus, it might be considered that litter may have been mostly decomposed above soil surface.

According to Frouz (2018), the main factor determining whether soil fauna induces an increase or decrease of microbial decomposition of biomass is the time elapsed since the litter was consumed, emphasizing that microbial activity increases during and shortly after fauna feeding. Nevertheless, despite litter, mesofauna and soil sampling (for MR analysis) in common occasion, abundance of measofauna and microbial activity were not clearly associated across treatments, not even in *Urochloa brizantha*, which assumably offered improved soil conditions for biological activity. Possibly, mites inhabiting the litter–soil interface in our experimental plots were rather generalists than specialized decomposers (Velasquez and Lavelle, 2019). According to Moradi et al.

(2017), generalist mite species predominate in early revegetation of minesoils, and are replaced by ecologically specialized species at later stages of revegetation. Overall, these observations highlight the initial stage of fauna succession in the minesoil of Candiota. In this way, our MR values were comparable to that reported by Fernandez et al. (2023b) after 18 years of Candiota minesoil revegetation with grasses (0.56–0.82 mg kg $^{-1}$ h $^{-1}$ ), revealing a steady state of microbial activity between 14.6 and 18 years of revegetation, likely in response to the unchanged revegetation system with permanent monoculture of grasses.

In our study, the greater MBC together with the lower qCO2 in the treatments compared to the control suggest less stress of microorganisms to process litter biomass (Bastida et al., 2008), probably due to the greater offer of litter in the actively revegetated plots. However, the similar content of TOC and humified C forms (C-FA, C-HA and C-HU) observed in all treatments (Table 4), reveals incipient and grassunspecific associations between mites, microbial activity and humus formation (Potapov et al., 2023; Frouz, 2018). Interestingly, Hemarthria altissima and Cynodon dactylon treatments exhibited the highest litter C: N ratios (66 and 57, respectively, Table 1) but not the highest abundance of mites and springtails at the litter-soil interface (Table 2), apparently disfavoring initial biomass degradation steps until SOM humification. This is supported by the lower C-HA/C-FA ratio found in Hemarthria altissima (0.1) and Cynodon dactylon (0.3) compared to the other treatments (Table 4). The Panicum maximum treatment deserves particular attention as it promoted the highest C-HA and C-HU content increments as well as substantial C-FA content increments in relation to the control and exhibited the highest C-HA/C-FA ratio among all treatments, evidencing the conversion of plant biomass into active and more stable (humified) C forms. On average, the C-FA, C-HA and C-HU content represented less than 14, 7 and 23 % of the TOC content, confirming that most of C in the minesoil remains in labile forms, likely due to depleted functions of microbial communities (Lavelle et al., 2006).

The outstanding performance of Urochloa brizantha to ameliorate minesoil physical attributes (Table 5) may be assigned to the greater development of the root system of this species in the minesoil. In fact, Pauletto et al. (2016) reported that the root density of Urochloa brizantha was in average 2.1-fold higher than that of the other grasses five years after start of minesoil revegetation. Accordingly, Stumpf et al. (2018) confirmed the effectiveness of this grass in breaking aggregates formed by compression and in forming well-structured aggregates. Nevertheless, the effectiveness of soil microorganisms to convert Urochloa brizantha biomass into SOC, inferred from the lowest qCO<sub>2</sub> found in this treatment compared to the others (Table 3) did not result in greater TOC content (Table 4). According to Xie et al. (2022), the conversion of plant biomass into SOM is positively correlated with soil fauna abundance, but also diversity. On one side, the similar H' and J indexes in the treatments (Fig. 3) may justify their similar TOC contents, and on the other side it supports the hypothesis that the low mesofauna diversity in the minesoil likely hinders the achievement of greater TOC contents.

The ordination of each treatment in a different quadrant of CCA biplot alongside the centralization of biological attributes around the intersection of CCA1 and CCA2, clearly shown unspecific interrelation of treatments with soil biological attributes. This occurred despite the improved physical structure of the minesoil promoted by Urochloa brizantha, as seen in Q3 of CCA (Fig. 4), contracting our hypothesis. Worth noticing, CCA Q2 clearly indicated persistent soil compaction in Hemarthria altissima treatment (Fig. 4), suggesting the limited potential of the root system of this grass in alleviating soil compaction, converging with findings of Pauletto et al. (2016) and Stumpf et al. (2016). Particularly, the C-HA vector points exclusively to Panicum maximum (Q1) and C-HU vector points exclusively to Cynodon dactylon (Q4) indicating the preferential form of C stabilization in these treatments (Fig. 4). However, these humic forms were not clearly associated with the investigated soil biological attributes, and it did not imply in soil structural enhancement.

#### 5. Conclusions

Our study confirmed the exceptional performance of *Urochloa brizantha* in improving the structure of minesoil and increasing the abundance of mites and springtails. However, we found that these attributes were not strongly interconnected and did not lead to humus accumulation in the minesoil, which contradicts our initial hypothesis. Therefore, it is plausible to conclude that, at the this reclamation stage, the observed populations of mites and springtails are largely unspecialized.

Although the vigor and resilience of grasses are prioritized to withstand the harsh conditions of both minesoil and climate at the study site, we hypothesize that the monoculture of grasses may limit niche diversification, thereby restricting gains in mesofauna diversity. This hypothesis warrants further investigation.

Although *Urochloa brizantha* has effectively improved minesoil structure, additional research is needed to determine whether other species might be more effective at promoting mesofauna diversity and supporting the recovery of soil ecological functions. This is particularly relevant for re-integration of these minesoils to the surrounding Pampa Biome. Our study provides a valuable baseline for monitoring mesofauna populations and assessing the progress of ecological minesoil reclamation in future research.

#### CRediT authorship contribution statement

Mauricio Silva Oliveira: Writing – original draft, Methodology, Investigation, Conceptualization. Otavio dos Anjos Leal: Writing – review & editing, Writing – original draft, Formal analysis. Maria Bertaso de Garcia Fernandez: Writing – review & editing, Writing – original draft. Nicolas Brüggemann: Writing – review & editing. Pablo Miguel: Methodology, Investigation, Data curation. Luiz Fernando Spinelli Pinto: Methodology, Data curation. Jakeline Rosa de Oliveira: Methodology. Gláucia Oliveira Islabão: Methodology. Lizete Stumpf: Writing – review & editing, Supervision, Formal analysis, Data curation.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2025.107578.

#### Data availability

Data will be made available on request.

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