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Diversity Loss of Epigeic Collembola after Grassland Conversion into *Eucalyptus* Forestry in Brazilian Pampa Domain

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Abstract: The Brazilian Pampa is a rich domain mainly represented by grasslands. Conversion of native vegetation into *Eucalyptus* plantation leads to soil degradation and losses on local fauna and flora. The objectives of this study were to compare the taxonomic structure and abundance of epigeic springtails (Collembola) in two different types of land-use in the Brazilian Pampa, native grassland and *Eucalyptus* plantation, as well as to understand the processes that may cause species loss after grassland afforestation. Specimens were sampled in 10 paired plots of grasslands and *Eucalyptus* in southern Brazil. After sampling, all specimens were sorted, counted and identified. We evaluated the taxonomic composition, alpha and beta diversity, and used Random Forest Analysis to understand the influence of environmental factors on the structure and composition of Collembola communities. We sampled 1249 specimens in 26 morphospecies, and our data support there are significant losses in native Collembola communities after the conversion of grasslands into *Eucalyptus* plantations regarding abundance, species composition, richness, and alpha diversity. Species turnover better explained the beta diversity, and plant richness and dominance were the main environmental factors driving the Collembola diversity. These results deepen the knowledge of the impacts of native grassland conversion on soil fauna.

Keywords: land-use; native grasslands; soil ecology; soil mesofauna; springtails

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1. Introduction

The Brazilian Pampa consists of remnants of very old natural ecosystems which witnessed cold and dry climates recorded even before the expansion of the Atlantic Forest in the extreme south of the country, currently the Rio Grande do Sul state (RS) [1,2]. In Brazil, this biome covers an area of 178,243 km^2, corresponding to 63% of RS and 2.07% of the national territory [3,4], structured in three main types of phytoecological units: native forest, with 9591.05 km^2 of coverage (currently 5.38% of the biome in Brazil), native grassland, with 41,054.61 km^2 (23.03%), and native transitional vegetation, with 23,004.08 km^2 (12.91%) [5].

Behind the apparently uniformity of the Pampa, studies found that this domain is highly diverse. For instance, Menezes et al. [6] recorded 56 plant species per square meter within their native vegetation. Likewise, the soil organism richness is quite high in native grasslands, such as for springtails, beetles and spiders [7–13]. Unsurprisingly, the land-use intensification, more precisely the conversion of native vegetation, is one of the main causes of local biodiversity loss [14–16]. In Brazilian Pampa the native vegetation has lost more than 50% (2.5 million hectares) of its natural extent during the past 35 years, and in 2018, converted areas (7,893,373 ha) surpassed the native areas (6,519,015 ha) in terms of their extension, while it seems the conversion rate is still rising [17].

Eucalyptus spp. represent the most planted trees in the world, and Brazil is their largest producer, holding about 7.5 million hectares of cultivation. In the Pampa, the extension of *Eucalyptus* plantation strongly increased since 2006, now occupying about 480,000 hectares [17]. However, most of the converted areas occurred in shallow soils, which in turn are also areas more susceptible to degradation [18]. In addition, there is little understanding of how the *Eucalyptus* plantation affects the local biodiversity in Pampa, and consequently, the biodiversity-related ecological functions.

Native vegetation loss is thought to affect secondary trophic levels (i.e., soil fauna) by changing the quantity and quality of food resources, as well as physicochemical properties of the soil [19,20]. Such disturbances strongly affect soil fauna, which occupies a key role in the maintenance of terrestrial ecosystems. As they are very diverse and belong to several functional groups, soil fauna can directly influence ecological functions, such as: biological control (predators, herbivores, parasites), soil decomposition and fertility (decomposers, fungivores), water regulation and soil formation (ecosystem engineers) [21–24]. Moreover, the diversity within each functional group strongly affects important ecosystem processes such as nutrient cycling, pest control, and primary productivity [25–29]. Springtails (Collembola) are among the most common, widespread and abundant forms of soil decomposers [30]. Besides being the base of food chains involving small predators, i.e., mites, beetles, pseudoscorpions, spiders, they directly feed on organic debris and fungi, impacting the rate of organic matter decomposition in the soil [30–32]. Therefore, as Collembola influence nutrient cycling and soil fertility, it is crucial to deepen our understanding about their response to the environmental changes caused by grassland afforestation.

A recent study demonstrated that Collembola diversity is lower in *Eucalyptus* plantation compared to native grassland [7]. However, considering this study was carried out in a single site of the Brazilian Pampa, our work had as main objective to investigate the impact of *Eucalyptus* plantation on epigeic Collembola communities at a regional scale and identify whether those patterns observed by Winck et al. [7] holds true at a broader scale. Precisely, we addressed the following questions:

(i) How is the epigeic Collembola diversity affected after changes in land-use/land-cover (LULC) from native grassland to *Eucalyptus* plantation?
(ii) Is the species composition of epigeic Collembola communities in the *Eucalyptus* plantation a subgroup of the community present in the original native vegetation? Or is it originated from species replacement?
(iii) What were the most important environmental factors affecting the structure and composition of the epigeic Collembola community?

Our basic hypothesis is that the conversion to *Eucalyptus*, by significantly altering the diversity of plants, the vegetation structure and soil properties, led to the reduction of diversity within epigeic Collembola communities and altered its composition mainly via species replacement as seen in previous studies [7,21].

2. Materials and Methods

2.1. Sites Description and Sampling Design

Our experimental sites were located in the Brazilian Pampa, RS, southern Brazil. The region has a subtropical humid climate with regular monthly rains, being classified as “CFa” climate according to the Köppen-Geiger system [33]. Average temperatures range between 16 and 22 °C, being the coldest month of the year July (with lows down to 10 °C) and the hottest February (with highs of 38 °C). The predominant soils are Acrisols and Leptsols and the dominant vegetation consists of a mosaic of grasses, shrubs, and forbs [34,35].

To explore the effects of grassland conversion to *Eucalyptus* plantation (timber production), we established a paired experimental design in four sites in Brazilian Pampa, with a high coverage of both land-use types: Pinheiro Machado (PIM), Jaguarão (JAG), Lavras do Sul (LAV) and São Gabriel (SAG) (Figure 1). All grassland sites were managed under extensive grazing with high animal pressure. For *Eucalyptus* plantations, plant density was about 0.11 kg/m^2 and the plantations were installed between 2007 and 2011. Within these sites we defined 10 paired plots: 10 of Grasslands and 10 of *Eucalyptus* (GE) (further details are presented in Table A1, Appendix A). All of the samplings were carried out once between November 2018 (late spring) and March 2019 (summer) in two land-use types in Brazilian Pampa, however, the pairs of GE were always sampled at the same time, in the same day.

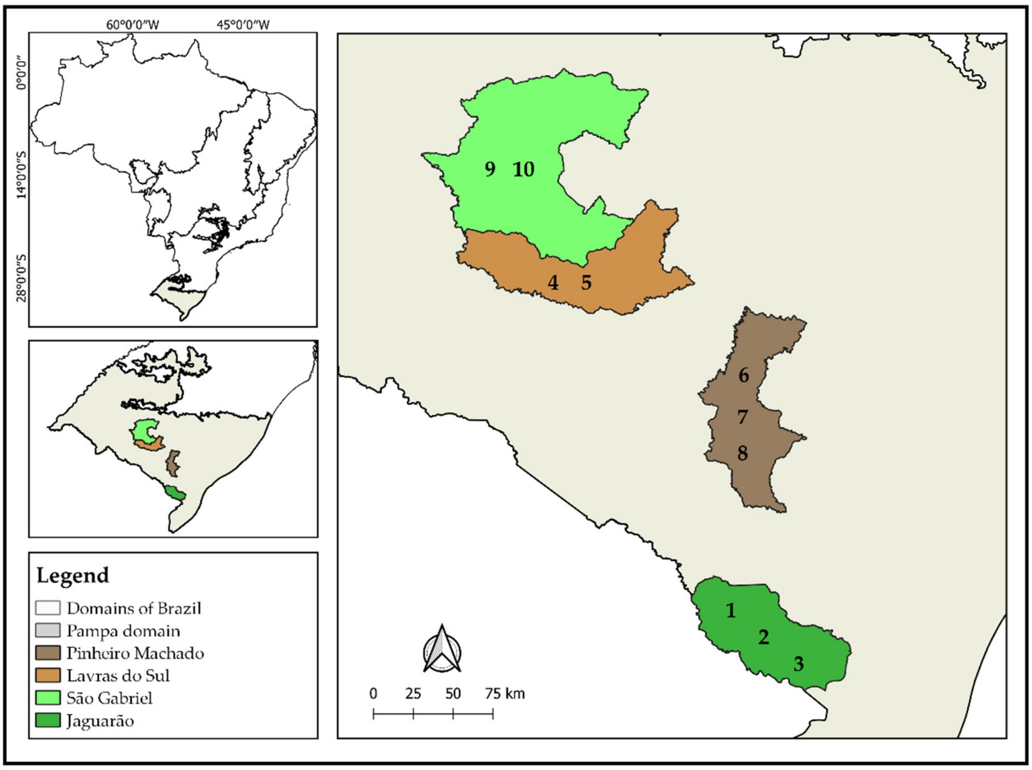

Figure 1. Sampled sites (municipalities of RS, Brazil) distribution within the Brazilian Pampa. The numbers within each site represent the IDs and locations of the paired plots of Grasslands and *Eucalyptus* (GE).

At each plot and along a 250 m transect, soil properties were measured in three subplots spaced 83 m apart. Sites from the same pair (GE) were sampled always in the same day, controlling possible variation of climatic conditions. Soil texture, expressed as the percentages of sand, silt and clay, were measure at three standard depths (10, 20, 30 cm) using the Bouycous method, as described by [36]. For soil bulk density, three core soil samples of 8.5 cm diameter and 5.0 cm depth were taken along the 250 m transect. Each sample was dried at 105 °C and then weighted to obtain its density in g/cm^2. Vegetation was sampled in ten 1 m^2 subplots, five adjacent to the Collembola sampling subplots and extra five equally spaced along the 250 m transect for a better characterization of the vegetation. In each subplot we measured: plant species composition, plant functional groups [37], and habitat structure (plant height, measured with a ruler, one measure on each corner of the 1 m^2 square subplot and one at the center). From plant functional classification, we obtained richness of functional groups, as non-grass-like species and rosettes (*Eryngium* spp. like species). Finally, from raster layer datasets, we described for each plot: the annual temperature and precipitation (resolution of 1000 m, WorldClim), annual evapotranspiration (resolution of 250 m, MODIS Web), land-surface temperature (resolution of 250 m, MODIS), and elevation (resolution of 90 m, SRTM DEM) [38–40].

2.2. *Collembola Sampling and Taxonomic Identification*

Collembola was surveyed in five subplots spaced 50 m apart. The specimens were sampled using pitfall traps which consisted of 50 mL Falcon tubes with 30 mm of diameter filled with 70% ethanol plus a drop of detergent. At each subplot we placed three pitfalls, adding up to 15 traps per transect (land-use) and 30 per sampled pair. Traps remained installed for 24 h. We choose such sampling methodology since we were aiming to investigate the epigeic springtails. However, it is important to state that pitfall traps show limitations in sampling all springtail communities, as many taxa live belowground (as the euedaphic Isotomidae) and would be better sampled by the use other methodologies such as Berlese-Tullgren funnels.

After the collections, the biological material was investigated using a stereomicroscope and the springtails were sorted, quantified, and morphotyped. We considered as rare species the taxa with 10 or less specimens per land-use. Posteriorly, glass slides were mounted following Arlé and Mendonça [41] and Jordana et al. [42] methods combined, in which the specimens were cleared in Nesbitt's and Arlé's fluids and were mounted in glass slides in Hoyer's medium. Glass slides were dried at 50 °C for about three days and were studied using a Leica DM750 optical microscope with phase contrast. The taxonomic identification was made following specially the keys and diagnoses provided by Jordana et al. [42], Massoud [43], Betsch [44], Christiansen and Bellinger [45], Bretfeld [46], Potapov [47], Bellini and Godeiro [48], Cipola et al. [49], and Bellinger et al. [50]. The specimens were deposited at the Collembola Collection of the Biosciences Center, Federal University of Rio Grande do Norte, Brazil (CC/UFRN).

2.3. *Ecological Data and Statistical Analysis*

All statistical analyses were performed using the R software (version 4.1.2). Our data were organized into two matrices: **matrix W**, with the abundance and species richness of springtails of each community in different land-uses (native grasslands and *Eucalyptus* plantation), and **matrix E**, with the environmental data.

2.3.1. Species Composition

Species composition was investigated using Principal Coordinate Analysis (PCoA) based on Euclidian distance after Hellinger transformation. We used "*vegan::vegdist*" to calculate the distance matrix, and "*stats::cmdscale*" function to run PCoA [51]. Then, a Permutation Multivariate Analysis of Variance (PERMANOVA) with 999 permutations ("*vegan::adonis*" function) was calculated to explore differences in species composition between both land-uses.

2.3.2. Beta Diversity

For each pair of LULC, we identify whether the species composition of Collembola in *Eucalyptus* plantation resulted from species replacement after the conversion or represented a subset of species which previously inhabited the original grasslands. For that, we used the function "*betapart::beta.temp*" [52] to compute the temporal dissimilarity using Sørensen dissimilarity index (βSOR). Here we are considering that the native grassland sites were a state prior to the *Eucalyptus* plantation. We also computed both turnover (Simpson index of dissimilarity, βSIM) and nestedness (nestedness resultant index of dissimilarity, βSNE) components [53–55].

2.3.3. Alpha Diversity

We calculated the total abundance of Collembola and alpha diversity indices of each community. Species richness (S) was estimated by rarefaction method using the "*iNEXT::iNEXT*" function [56]. We also calculated the Shannon index (H'), which considers the species richness and each species relative abundances, and Pielou evenness index (J') which indicates if the different species have similar or divergent abundances in the investigated communities [57,58]. Both H' and J' were calculated using the "*vegan::diversity*" function [51]. The total abundance and alpha diversity were tested for their normality and homoscedasticity using Shapiro-Wilk and Levene tests, respectively. For that, we used both "*stats::shapiro.test*" and "*DescTools::LeveneTest*" functions. Posteriorly, the effects of land-use types on alpha diversity were determined by Linear Mixed Models (LMM), which were calculated using the "*nlme::lme*" function [59]. Land-use types (grasslands and *Eucalyptus* plantation) were the predictor variables (fixed effect), and each pair nested within the sites were inserted as random effects in the models to account for paired experimental design.

2.3.4. Effects of the Environmental Factors on the Epigeic Collembola Community

To explore the responsiveness of the epigeic Collembola community to environmental factors, we run the Principal Component Analysis (PCA) and the Random Forest Analysis (RF). The PCA was applied to identify the collinearity among variables and select those which could be used in RF (Figure A1, Appendix A), using "*FactorMineR::PCA*" function, and correlated variables were removed from the dataset. To estimate the importance of different environmental factors on Collembola diversity we used the increases in MSE (mean squared error) of variables, in which higher MSE% values imply on more important variables. The RF analyses were run using "*randomForest*" and "*rfpermute*" packages [60,61].

3. Results

3.1. Epigeic Collembola Community in the Two Land-Use Types

From a 24-h sampling, we collected a total of 1249 specimens of springtails belonging to 26 morphospecies, 19 genera and 11 families in both native grasslands and *Eucalyptus* plantation. The taxonomic composition, total and relative abundances of each taxon in both land-use types are detailed in Table 1. We could not clearly assign any species to taxa already recorded in Brazil or other countries of the Neotropical Region, suggesting at least part of these morphospecies may be actually new to the science.

The LMM revealed significant differences in epigeic Collembola abundances between both land-use types ($p = 0.0059$, Table 1). Furthermore, the abundance within each taxonomic level varies between land uses. For example, we recorded a total of 979 individuals belonging to 17 genera and 23 morphospecies in native grasslands. The family with the highest abundance was Entomobryidae (with 57.69%), followed by Isotomidae (21.52%), Hypogastruridae (8.58%), Sminthurididae (6.43%), Bourletiellidae (1.83%), Katiannidae (1.92%), Neanuridae (1.63%), and Brachystomellidae (0.40%). In *Eucalyptus* plantation, however, we only found a total of 270 individuals belonging to 8 genera and 10 morphospecies. As observed for native grasslands, the family with the highest abundance was Entomobryidae (with 59.26%), but it was followed by Paronellidae (13.70%), Orchesellidae (11.49%), Hypogastruridae (7.04%), Isotomidae (4.44%), Neanuridae (2.59%), and Dicyrtomidae (1.48%).

Regarding morphospecies abundance, we recorded 10 abundant species in native grasslands, with *Entomobrya* sp.1 and *Entomobrya* sp.3 as the most abundant ones, respectively. We also found 13 rare species, and *Rastriopes* sp.1 and *Sminthurinus* sp.1 were the rarest taxa. On the other hand, in the *Eucalyptus* plantation we observed four abundant species, with *Lepidocyrtus* sp.1 and *Entomobrya* sp.4 as the most abundant taxa, and six rare species, being *Entomobrya* sp.1 and *Ptenothrix* sp.1 the rarest ones (Table 1).

3.2. Effects after Grassland Conversion into Forestry

The PERMANOVA analysis revealed that native grassland conversion to *Eucalyptus* strongly affects epigeic springtails morphospecies composition ($F = 6.304$; $R^2 = 0.25957$; $p < 0.001$). This result is evidenced in PCoA (Figure 2), which represent 51% of the data variation, and we found a clear separation between both native grassland and *Eucalyptus* plantation. Furthermore, we found an association of *Entomobrya* sp.1 and *Entomobrya* sp.2 to the native grasslands, while *Dicranocentrus* sp.1 and *Entomobrya* sp.4 were associated with *Eucalyptus*. In addition, *Eucalyptus* crops PCoA values were more dispersed than the native grasslands ones, indicating high variability of morphospecies composition in the former land-use.

Table 1. Morphospecies and abundance of epigeic springtails (Collembola) in different land-use types in Brazilian Pampa grasslands.

Family/Morphospecies		**Native Grassland**		***Eucalyptus***	
		Total Abundance	**Relative Abundance (%)**	**Total Abundance**	**Relative Abundance (%)**
Bourletiellidae	*Rastriopes* sp.1	01	0.10	00	0.00
	Prorastriopes sp.1	14	1.43	00	0.00
	Prorastriopes sp.2	03	0.30	00	0.00
Brachystomellidae	*Brachystomella* sp.1	04	0.40	00	0.00
Dicyrtomidae	*Ptenothrix* sp.1	00	0.00	04	1.48
Entomobryidae	*Entomobrya* sp.1	192	19.61	02	0.74
	Entomobrya sp.2	62	6.33	00	0.00
	Entomobrya sp.3	174	17.77	06	2.22
	Entomobrya sp.4	10	1.02	53	19.63
	Entomobrya sp.5	10	1.02	00	0.00
	Lepidocyrtus sp.1	111	11.33	99	36.67
	Seira sp.1	06	0.61	00	0.00
Hypogastruridae	*Hypogastrura* sp.1	14	1.43	00	0.00
	Xenylla sp.1	70	7.15	19	7.04
Isotomidae	*Desoria* sp.1	80	8.17	12	4.44
	Desoria sp.2	65	6.63	00	0.00
	Folsomia sp.1	22	2.24	00	0.00
	Isotomurus sp.1	18	1.83	00	0.00
	Proisotoma sp.1	26	2.65	00	0.00
Katiannidae	*Katianna* sp.1	13	1.32	00	0.00
	Katianna sp.2	03	0.30	00	0.00
	Sminthurinus sp.1	02	0.20	00	0.00
Neanuridae	*Pseudachorutes* sp.1	16	1.63	07	2.59
Orchesellidae	*Dicranocentrus* sp.1	00	0.00	31	11.49
Paronellidae	*Trogolaphysa* sp.1	00	0.00	37	13.70
Sminthurididae	*Sphaeridia* sp.1	63	6.43	00	0.00

Linear Mixed Model

Land-Use Types	**Total Abundance**	**F Value**	***p* Value**
Grassland	979	12.86	0.0059
Eucalyptus	270		

Even though seven morphospecies were shared by the two land-uses, we found 16 morphospecies restricted to grasslands and only three to *Eucalyptus* plantations. The restricted species found in grassland sites were *Prorastriopes* sp.1, *Prorastriopes* sp.2, *Rastriopes* sp.1, *Brachystomella* sp.1, *Entomobrya* sp.2, *Entomobrya* sp.5, *Seira* sp.1, *Hypogastrura* sp.1, *Desoria* sp.2, *Folsomia* sp.1, *Isotomurus* sp.1, *Proisotoma* sp.1, *Katianna* sp.1, *Katianna* sp.2, *Sminthurinus* sp.1 and *Sphaeridia* sp.1, while those ones restricted to *Eucalyptus* plantation were *Ptenothrix* sp.1, *Dicranocentrus* sp.1, and *Trogolaphysa* sp.1. (Table 1, Figure 2). Furthermore, after exploring β-diversity components in each pair of LULC (Table A2, Appendix A), we observed that the differences in species composition between both LULC resulted more from species turnover (βSIM ranging from 0.5 to 1) rather than species nestedness (βSNE ranging from 0 to 0.2).

We found clear evidence that grassland conversion into *Eucalyptus* lead to a decrease of species richness (Figure 3A), resulting in a significant difference between both LULC ($p = 0.0038$). Specifically, epigeic Collembola richness was 2.3-fold higher in native grasslands (S′ = 23) compared to *Eucalyptus* plantations (S′ = 10). The alpha diversity analysis also remarked that grassland areas had a higher species diversity (H′ = 2.317, $p = 0.0032$) compared to *Eucalyptus* (H′ = 1.545) (Figure 3B). However, no statistical difference was observed in the evenness index (Figure 3C, $p = 0.31$).

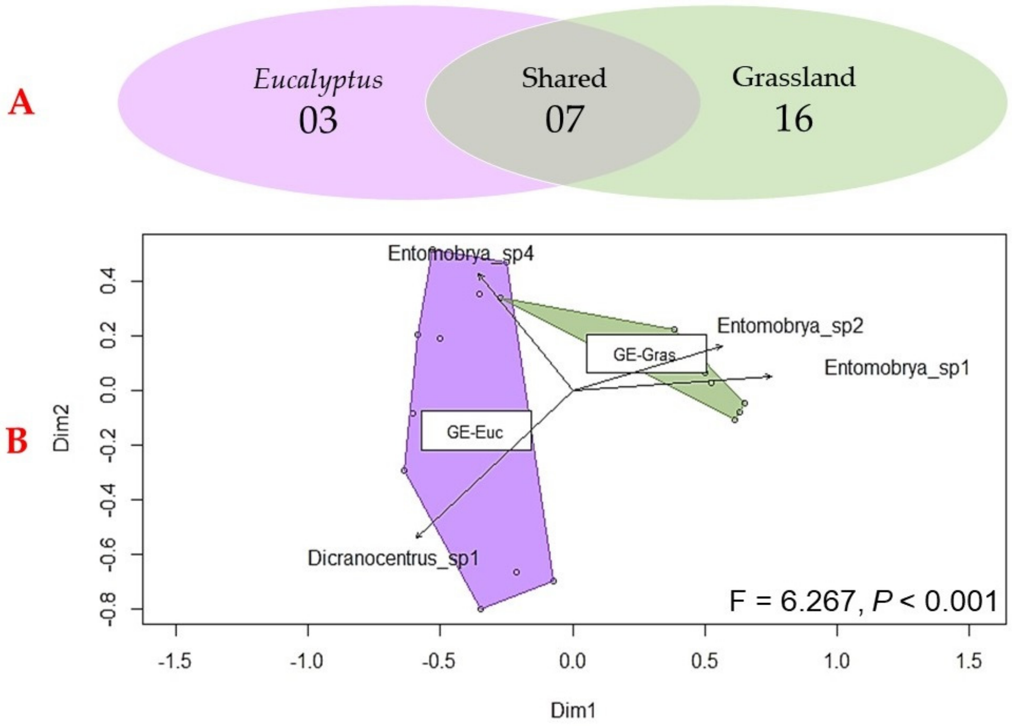

Figure 2. Comparison between epigeic Collembola communities in different LULC in Brazilian Pampa grasslands: (**A**) Venn diagrams showing shared morphospecies between Grassland and *Eucalyptus*; (**B**) Species composition (green polygon—grasslands, and purple polygon—*Eucalyptus* plantation).

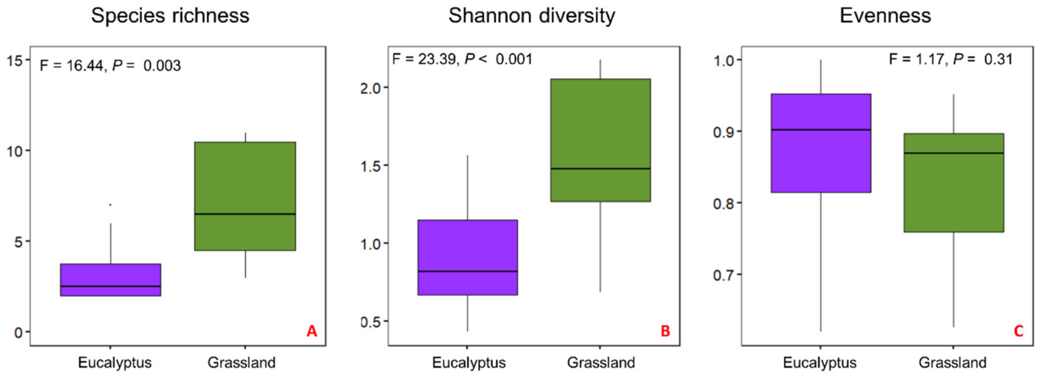

Figure 3. Comparison between epigeic Collembola communities in different LULC in Brazilian Pampa grasslands: (**A**) species richness; (**B**) Shannon index and; (**C**) evenness index.

Lastly, Random Forest analyses showed that plant richness and plant dominance were the major driving factors influencing epigeic Collembola abundance and composition in the sampled sites, while plant richness was the main predictor favoring epigeic Collembola diversity (Figure 4).

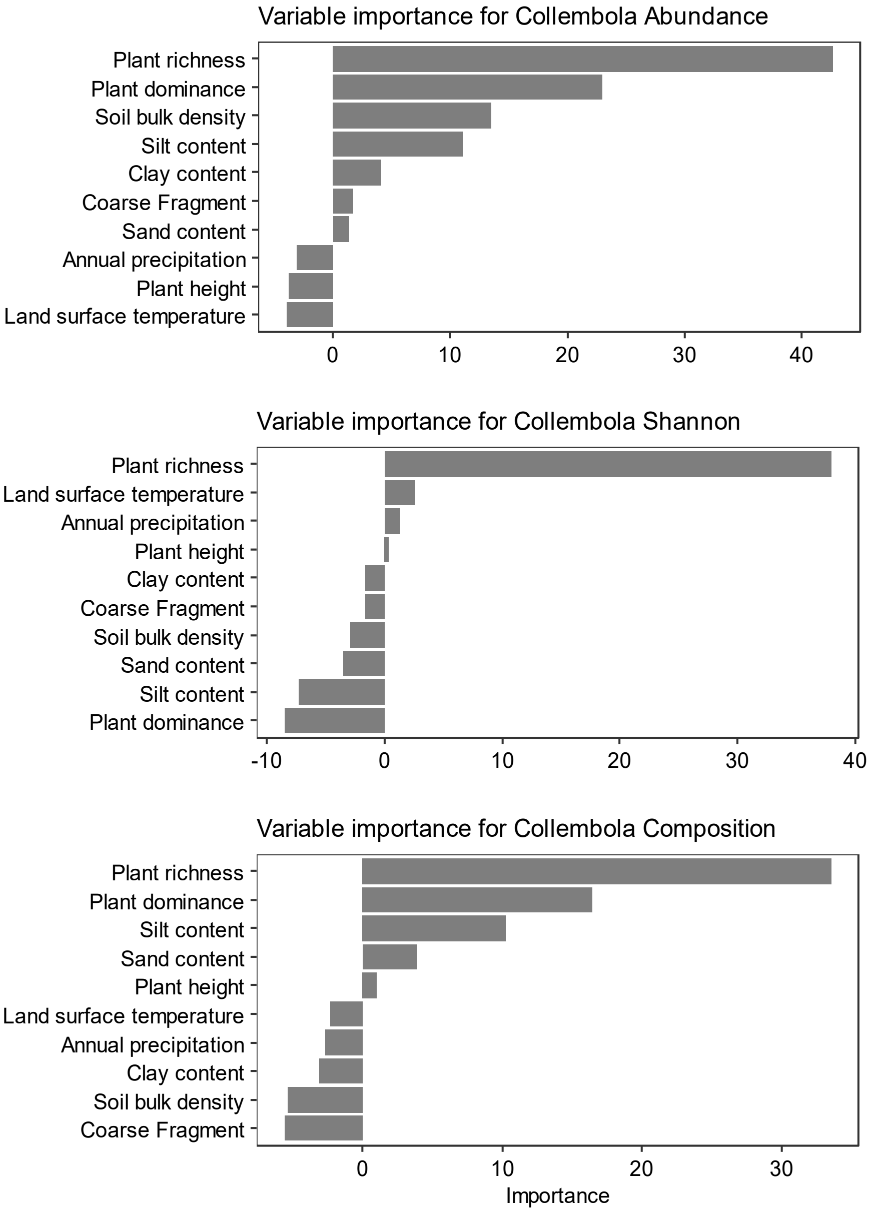

Figure 4. Effect of environmental variables on epigeic Collembola composition and structure using Random Forest Analysis. The accuracy importance measure was calculated for each tree and averaged over the forest (5000 trees). High percentage increase in the MSE (mean squared error) values implies in more important predictors.

4. Discussion

4.1. Taxa Occurrence in the Brazilian Pampa

Here we have compiled so far the most comprehensive taxonomically verified checklist of Collembola from the Pampa biome, considering different LULC types. Most of the studies that used Collembola as a model group to describe soil fauna communities were performed in few sites of the Pampa [7,8,62], while we carried out our study on a broad scale comprising sites with high potential of grassland afforestation. Also, although our identification has been up to the genus level for all collected families, as the species could not be clearly assigned to any nominal taxa, this is one of the few studies that detailed the communities of Collembola until such taxonomic resolution. Such results improved our understanding about the Collembola biodiversity in Brazilian Pampa, as well as allowed us to better identify the real impact of grassland afforestation. Currently, only three nominal (described) species of springtails are known for the RS state, and none of them were recorded from the Pampa domain [63], while we were able to sample 26 morphospecies (Table 1). These data highlight the limited taxonomic efforts concerning the Collembola in Pampa, a fauna which has an elevated potential of endemism but it is not known in its most basic aspects.

All families of springtails herein recorded were already registered in Brazil, while most genera gather nominal species previously recorded to the country, with the exception of *Rastriopes*, *Prorastriopes* (both Bourletiellidae) and *Katianna* (Katiannidae) [63]. Even so, the first two genera were previously recorded from the northeastern Brazil with morphospecies, while all of the three genera have nominal species found in the Neotropical Region [50,64].

We observed that Entomobryidae was the most abundant and rich family in both LULC types (Table 1). This pattern is expected, since this is the most diverse family of Collembola, with more than 1700 species and 40 genera described [50], and our methods tend to favor the sample of this group of organisms [65]. In Brazil, Entomobryidae is the most studied Collembola family, with 106 species described so far [63], and we identified that the dominant genera and species vary among the Brazilian regions. For instance, in Caatinga, a semiarid phytogeographic domain seen in northeastern Brazil, preliminary data point out *Seira* spp. as the major components of the Collembola communities [66,67], while *Entomobrya* spp. are notably the most representative in the Pampa, as found in previous works and in our study [7,8]. This variation is probably a consequence of the habitat characteristics (climatic, soil, and vegetation) of each region, which contribute significantly in structuring Collembola communities. However, detailed analysis of the influence of habitat characteristics on Collembola in a large geographic scale is still missing in Brazil for soil organisms.

4.2. Effects of LULC Changes on Epigeic Collembola Community

We tested the effects of grassland afforestation on the responses of Collembola communities in the Pampa biome. We found that (i) *Eucalyptus* plantation reduced the abundance and diversity of epigeic Collembola communities (Figure 3); and (ii) caused changes in species composition by the replacement of taxa possibly more adapted to closed canopy habitats. Also, as indicated by the Venn diagram (Figure 2A), (iii) native grassland areas present a higher number of unique morphospecies compared to *Eucalyptus* plantations, and most of them were classified as rare. Finally, we also found that (iv) the main environmental factors influencing the epigeic Collembola community were plant richness and dominance.

Our results agree with those from other studies previously carried out in the Brazilian Pampa which compared different land-use types and agricultural intensification [7,62]. In general, they found that *Eucalyptus* plantations cause reduction in functional and/or taxonomic diversity, and also alter species composition within the Collembola community. However, our study provides complementary information. For example, we identified that both LULC types shared only seven morphospecies out of 26, and the epigeic Collembola composition in *Eucalyptus* plantation resulted from species turnover (replacement) rather than nestedness. Such findings support the hypothesis that the conversion to *Eucalyptus* caused local extinctions, selecting species most adapted to the new conditions (i.e., shade habitat and niche simplification).

Although the natural communities were dominated by a few numbers of morphospecies (i.e., *Entomobrya* sp.1 and *Entomobrya* sp.2), the high richness and diversity found in the grassland could be related to the high number of habitat and feeding niches provided by a heterogeneous plant community [68–71]. Due to the selective grazing behavior of the animals (cattle and sheep) in native grasslands, the vegetation structure represents a mosaic of intensely grazed and ungrazed patches (i.e., hard leaves species such as *Eryngium* sp. and *Baccharis* sp. are less preferred by grazers) [72]. Such heterogeneous vegetation structure favors the emergence of different microhabitats and also contributes to a greater amount of available food resources for soil fauna communities [73]. More specifically for the Collembola community, since springtails are tiny organisms mostly ranging from 1 to 5 mm [50], it is expected that even microclimate variations within a given habitat strongly influence the composition and structure within the communities [74–76]. Plant diversity may favor the co-occurrence between different species with different food preferences, considering they feed on a wide range of food, such as living and dead plant tissues, pollen, grain, soil mesofauna, and fungi [31,77]. Finally, the presence of ungrazed patches can act as a refuge for diversity [73], as a shelter for rare species [78]. This statement may explain the high number of rare morphospecies found in grassland sites in our study.

In addition to the habitat simplification caused by grassland afforestation, some studies also supported that *Eucalyptus* strongly alters the physicochemical properties of the soil, which in turn act as ecological filters on the regional pool of species [7,79]. For instance, soils under *Eucalyptus* plantations tend to be drier, more acidic and nutrient-poor [80,81], and present low microbial biomass and activity [82]. Such constraints not only affect Collembola microhabitats, but also influence their food sources. Even further, these ecological filters induced by *Eucalyptus* plantation may select species with functional traits more adapted to lower pH, and closed habitats (with reduction of eyes and pigmentation, for example). In fact, such soil modifications may negatively affect the physiological functions of most Collembola, with consequences on their reproduction rates, life cycles, and overall survival [30,83–86]. So, the conversion of native grasslands into *Eucalyptus* results in changes in epigeic Collembola communities as we observed, which can include local extinctions [87].

4.3. Conservation Perspectives of Soil Fauna in the Brazilian Pampa

Our results support an alarming view of the strong negative effects of native grasslands conversion into *Eucalyptus* forestry on the epigeic Collembola diversity. If our data are representative of what happened in most converted areas, it is possible to expect an overall local loss of more than half of the species richness and more than two thirds of the total abundance of epigeic Collembola, with potentially devastating effects for the edaphic ecosystems and, consequently, for the ecosystem services provided by these animals [30–32]. In this scenario and considering the continuous losses of native grasslands in the Brazilian Pampa [17], it is expected that a significant amount of its Collembola diversity has been locally lost without ever been catalogued, described or studied. This is also probably valid for other representatives of the edaphic fauna as well, and conservation strategies must be implemented to preserve what remains of the native soil mesofauna diversity in the Brazilian Pampa. It is important to expand taxonomic inventories in the remaining fragments of native vegetation, in addition to studies that aim to understand the mesofauna community indexes and how the diversity is influenced by the conversion of natural landscapes. Furthermore, conservation strategies specifically designed for the soil invertebrates are rare or non-existent in most of Brazil, prevailing policies which aim to conserve vertebrates [88]. Although vertebrate management and conservation plans involve the protection of large areas, which end up protecting an entire biota associated with them including the edaphic fauna, it is not possible to affirm that such strategies cover the specific needs of the latter fauna [30,74].

5. Conclusions

The obtained results allow for better understanding on how native grassland conversion into *Eucalyptus* forestry impacts the epigeic Collembola community in Brazilian Pampa. We found the establishment and growth of such crops decrease the native abundance, species richness and alpha diversity of epigeic springtails, and the composition between the two different land-uses is modified due to species turnover. Moreover, our models support the loss of plant richness and dominance in artificial forests of *Eucalyptus* are key features to explain the decrease on abundance and composition of epigeic springtails in the sampled sites. All of these data combined with the known negative impacts of changes in land-use, especially in growing *Eucalyptus* monocultures, may have conservationist and applied ramifications, as they can point to more sustainable methods of land use.

Author Contributions: Conceptualization, C.D.D.d.S., B.C.B. and B.R.W.; methodology, C.D.D.d.S., B.C.B., L.d.S.M. and B.R.W.; software, C.D.D.d.S., V.M.R. and B.R.W.; validation, C.D.D.d.S., B.C.B. and B.R.W.; formal analysis, C.D.D.d.S. and B.R.W.; investigation, C.D.D.d.S.; resources, B.C.B. and B.R.W.; data curation, C.D.D.d.S., V.M.R. and B.R.W.; writing—original draft preparation, C.D.D.d.S., B.C.B., R.C.N. and B.R.W.; writing—review and editing, B.C.B., V.M.R., R.C.N., L.d.S.M. and B.R.W.; visualization, B.C.B., V.M.R., R.C.N., L.d.S.M. and B.R.W.; supervision, B.C.B. and B.R.W.; project administration, B.C.B. and B.R.W.; funding acquisition, B.C.B. and B.R.W. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, due to Brazilian laws which do not require permission from an institutional ethics committee on the use of animals for taxonomical/ecological studies with microarthropods.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are contained within the article. All biological material is deposited at CC/UFRN as previously stated.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

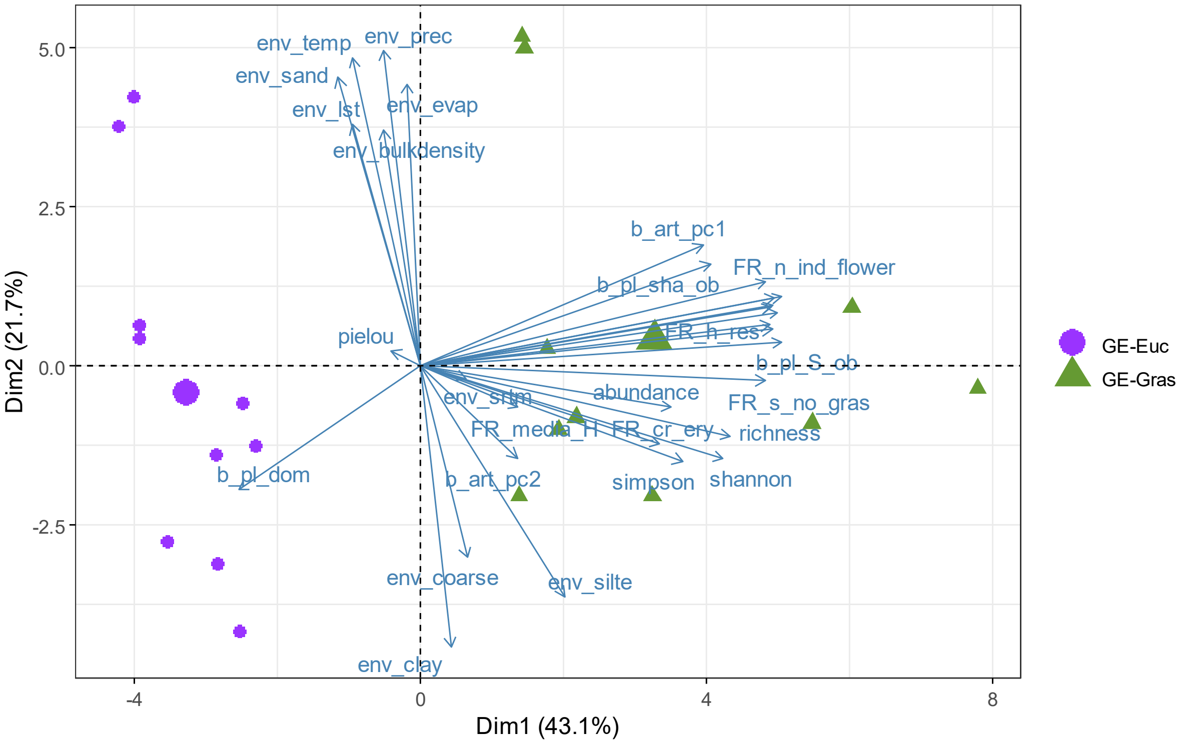

Figure A1. Principal component analysis of all ecological indices and environmental factors (plant community, climate, and soil) in different land-use types across Brazilian Pampa region.

Table A1. Sites, pairs and parcels identifications, different land-uses (Gras = native grassland, Euc = *Eucalyptus* plantation) and coordinates of the sampled sites of this study.

Site	Par	Lulc	Parcel	Geographic Coordinates	
				Longitude	Latitude
JAGUARÃO (JAG)	1	Gras	GE_JAG_Gras_1	−53,397,067	−32,239,946
		Euc	GE_JAG_Euc_1	−53,365,464	−32,239,994
	2	Gras	GE_JAG_Gras_2	−53,319,039	−32,210,709
		Euc	GE_JAG_Euc_2	−53,349,856	−32,240,435
	3	Gras	GE_JAG_Gras_3	−53,304,145	−32,227,443
		Euc	GE_JAG_Euc_3	−53,301,572	−32,245,974
LAVRAS DO SUL (LAV)	4	Gras	GE_LAV_Gras_2	−54,263,648	−30,885,178
		Euc	GE_LAV_Euc_2	−5,431,076	−30,860,155
	5	Gras	GE_LAV_Gras_3	−54,267,129	−30,962,529
		Euc	GE_LAV_Euc_3	−54,252,869	−309,676
PINHEIRO MACHADO (PIM)	6	Gras	GE_PIM_Gras_1	−53,602,698	−31,339,271
		Euc	GE_PIM_Euc_1	−5,359,796	−31,343,839
	7	Gras	GE_PIM_Gras_2	−53,577,106	−31,385,678
		Euc	GE_PIM_Euc_2	−53,571,586	−31,391,762
	8	Gras	GE_PIM_Gras_3	−53,499,849	−31,402,764
		Euc	GE_PIM_Euc_3	−53,509,913	−31,412,028
SÃO GABRIEL (SAG)	9	Gras	GE_SAG_Gras_1	−54,324,136	−30,059,119
		Euc	GE_SAG_Euc_1	−54,320,563	−30,049,282
	10	Gras	GE_SAG_Gras_2	−54,321,307	−30,083,604
		Euc	GE_SAG_Euc_2	−54,327,398	−30,069,851

Table A2. Beta diversity partitioning for each pair of Grassland and Eucalyptus (GE) plantation.

LULC Pairs	βSIM	βSNE	βSOR
GE_JAG_1	0.71	0.04	0.75
GE_JAG_2	1	0	1
GE_JAG_3	0.75	0	0.75
GE_LAV_2	0.5	0.1	0.6
GE_LAV_3	0.5	0.25	0.75
GE_PIM_1	0.67	0.2	0.87
GE_PIM_2	0.67	0.21	0.88
GE_PIM_3	1	0	1
GE_SAG_1	0	0.5	0.5
GE_SAG_2	1	0	1

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