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Collembolans and mites communities as a tool for assessing soil quality: effect of eucalyptus plantations on soil mesofauna biodiversity

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This study aimed to assess the population dynamics of collembolans and mites in an area under eucalyptus cultivation and native grassland, and their use as bioindicators to quantify changes in soil quality. Soil samples were collected monthly and the micro-arthropods were extracted by the Berlese-Tullgren modified funnel method. There were differences in the abundance of mites and collembolans in the area, showing that eucalyptus alter the diversity of the micro-arthropods edaphic. The abundance of mites and collembolans was found to be an excellent tool for studying the impact of farming on edaphic biodiversity.

Keywords: Acari, biological indicators, environmental change, springtails.

SOIL is a critical part of the terrestrial ecosystem, and supports several forms of life[1]. Monitoring soil quality is an increasingly more relevant topic within research units. Most concepts related to soil quality have been based on the premise that the various soil components are integrated and depend on each other to fulfill their specific function. Soil quality is the result of continuous conservation and degradation processes, and it represents the capacity of soil to function as a healthy living ecosystem[2]. Soil biological quality is the ability to support and shelter a wide diversity of edaphic organisms. This is essential to maintain the integrity of terrestrial ecosystems and help them combat issues such as climate change, pest infestation, pollution and agriculture[1].

The abundance and diversity of collembolans have been widely used to assess the environmental impact of pollutants in the soil[3] or land-use effects[4]. Mites and collembolans are micro-arthropods belonging to the

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mesofauna and are key components of soil biota. They are very abundant, acting in the formation and transformation of the soil along its life cycle, allowing for insights into soil ecological conditions. Several species have already been recognized as useful biological indicators of soil quality[5]. Furthermore, soil micro-arthropods are considered to play an important role in nutrient turnover[6]. Changes in plant diversity and vegetation patterns can create gradients which also shape the composition and distribution of micro-arthropods[7], influencing litter decomposition and nutrient recycling[8]. Because of the variety of roles played by invertebrates in the soil system, organic matter degradation and nutrient cycling, they become important indicators of changes in soil quality[9].

The *Eucalyptus* spp. monoculture has long been questioned about its effects on the biodiversity of edaphic organisms. These monocultures can affect both the species diversity and the distribution of the organisms[10]. Other factors like the diversity of organic matter, composition and type of residues (plant type or plant part), and decomposition stages, can also influence arthropod communities[11]. Monocultures decrease the variation in residue characteristics, which exert a strong influence in the biota composition. From a conservational perspective, the endemic biota is particularly vulnerable to ecosystem disturbance[12].

Our hypothesis was that eucalyptus monoculture alters the edaphoclimatic structure and can create microhabitats, affecting the mites and collembolans communities, shifting dominance. Families with potential use as bioindicators of soil quality in areas under eucalyptus monoculture were identified. The objective of this study was to analyse patterns of seasonal distribution of micro-arthropods among different vegetation cover (native grassland and eucalyptus monoculture) in South Brazil and micro-arthropods communities as a tool for assessing soil quality.

The two study areas are located at the UFRGS Agronomy Experimental Station (EEA), in Eldorado do Sul, RS, Brazil. The climate in the region, according to Köppen's classification, is humid subtropical with hot summer type *Cfa* (humid temperate climate with hot summer[13], with an annual rainfall of 1.445 mm on an average[14]. Soil samplings were done in two adjacent areas: one under eucalyptus cultivation – EC (30°05′20.28″S and 51°41′16.37″W) and the other in a native grassland – NG (30°05′25.87″S and 51°41′09.48″W). Additional soil samples from top to 20-cm depth were taken from each of the evaluated sites for chemical characterization. Parameters evaluated were pH, P and K, organic matter content, Al and Al + H, Ca, Mg and cation-exchange capacity, as described in ref. 15 (Table 1).

The EC area was under eucalyptus cultivation for more than 15 years, and the NG area, used as reference, was used for beef cattle, and kept under moderate grazing intensities. Soil samples were taken monthly from January to September 2009, with 4 replications per site, a total of 72 samples. Samples were collected using metal cylinders, with 7 cm in diameter and 7.5 cm high (total volume of 288.5 cm^3). Once removed from the soil, cylinders were covered with plastic wrap and identified. Samples were placed in coolers for transportation, in order to prevent moisture loss and temperature variations.

Mites and collembolans from the samples were extracted immediately after collection, using the modified Berlese–Tullgren funnel method. Soil samples were placed in the collecting funnels under 40 W bulbs, controlled with a dimmer that modulates light intensity and temperature (38 ± 4°C). The bulbs produce light and heat on the soil sample surface, generating a drying layer, causing the displacement of the organisms downwards, forcing them to fall into the funnel and are collected in a bottle containing 20 ml of preserving solution (70% alcohol and 1% glycerin). To facilitate efficiency of the extraction, soil cylinders were inverted, as explained by Edwards and Fletcher[16], and the extraction period was seven days.

All specimens of mites and collembolans were separated and put in slides to facilitate identification and preservation of the collection. For mesofauna identification a stereoscope (40 ×) and a phase contrast microscope were used. The identification and classification of collembolans were based on information from the literature[17–20]. Mites were identified through an identification key from the literature[21,22]. After the species identification, slides were labeled and stored in the soil mites and collembolans collection of the soil microbiology laboratory, at the Soil Department, College of Agronomy, UFRGS. The ecological parameters were evaluated based on the number and ratio of captured families and their classification, using the Shannon–Wiener index (H′), Simpson's dominance (C) and Pielou equality (J') indices[23]. The parameters were calculated using the PAST software[24].

The abundance of mites and collembolans recorded over nine months was analysed using two-way analysis of variance (ANOVA), considering the study area and the organisms group (mites or collembolan) as independent variables. Means were separated by Tukey's HSD test, considering $\alpha = 0.05$ for significant differences. Normality and homogeneity of data were checked using the Kolmogorov–Smirnof and Bartlett tests respectively. When normality and/or homogeneity criteria were not met, data was transformed by $(x + 1)^{1/2}$ prior to analysis. Statistical analysis was done using STATISTICA 5.0 (Statsoft, Inc., Tulsa, USA).

For multivariate analysis, the average number of individuals, for each sampling area and for each month, and mean values of environmental variables were used to build data matrices. Prior to analysis, taxa data were transformed using the equation by $(x + 1)^{1/2}$. A principal component analysis (PCA), calculated using CANOCO[25], was used to summarize environmental variables into

Table 1. Chemical characterization of the soil surficial layer (top 20 cm), for both studied areas of the *Eucalyptus* cultivations (EC) and native grassland (NG)

		mg/dm^3			$cmol_c/dm^3$				
Areas	pH	P	K	OM (%)	Al + H	Al	Ca	Mg	CEC (%)
EC	4.8	5.8	79	1.3	12.3	1.4	0.7	0.6	13.8
NG	5.4	4.5	59	2.3	5.5	0.2	2.8	1.5	9.9

P, Phosphorus; K, Potassium; OM, Organic matter; Al + H, Potential acidity; Al, Aluminum; Ca, Calcium; Mg, Magnesium; CEC, Cation-exchange capacity.

Table 2. Family composition of the mites and collembolans communities (total, average per month ± SD; $n = 4$)

	EC		NG		Tukey (0.05)	
	Total	$\bar{x} \pm SD$	Total	$\bar{x} \pm SD$	*F*	*P* value
Mites						
Actinedida						
Eupodidae	43	4.8 ± 6.1	47	5.2 ± 2.7		
Cunaxidae	12	1.3 ± 2.1	9	1 ± 2.2		
Penthalodidae			2	0.2 ± 0.6		
Tarsonemidae	3	0.3 ± 0.9[a]				
Astigmata						
Acaridae	7	0.8 ± 1.1[b]	43	4.8 ± 5.2[a]	74,259	<0.05
Endeostigmata						
Pachygnathidae	47	5.2 ± 4.2[a]	29	3.2 ± 2.4[b]	22,88	<0.05
Mesostigmata						
Ascidae	3	0.3 ± 0.7[b]	27	3 ± 2.5[a]	40,431	<0.05
Phytoseiidae	3	0.3 ± 0.7				
Mesostigmata sp.	15	1.7 ± 3.0				
Uropodidae	3	0.3 ± 0.5	1	0.1 ± 0.3		
Oribatida						
Galumnidae	6	0.7 ± 1.1	2	0.2 ± 0.6		
Oribatida sp.	86	9.6 ± 2.8[a]	52	5.8 ± 5.5[b]	23,793	<0.05
Not identified						
NI 1			1	0.1 ± 0.3[a]		
NI 2	3	0.3 ± 0.9				
NI 3	6	0.7 ± 0.7	1	0.1 ± 0.3		
Collembola						
Entomobryidae	27	3.0 ± 3.4[a]	4	0.4 ± 0.5[b]	64,92	<0.05
Hypogastruridae	8	0.9 ± 1.3[b]	14	1.6 ± 1.5[a]	8,359	<0.05
Onychiuridae	10	1.1 ± 1.4	9	1.1 ± 0.6		
Poduridae	10	1.1 ± 1.6	12	1.3 ± 1.9		
Symphypleonidae	23	2.6 ± 2.4[a]	5	0.8 ± 0.8[b]	32,887	<0.05
Total	315	35 ± 7.1	258	28.7 ± 8.7		

Obs., Averages followed by the same letter did not differ statistically by the Tukey's HSD test ($P < 0.05$).

fewer components that can be used to analyse relationships between the different sampling areas (EC e NG).

A total of 17 morphospecies were identified from the 681 micro-arthropods specimens captured. Morphospecies were classified into 5 orders, 12 families and 3 unidentified. Most individuals were mites (Acarina), representing 68.7% of the total collection (Table 2). Oribatida was the most representative group of mites, with 86 (EC site) and 52 (NG site) individuals, and differed significantly between treatments ($P < 0.05$) (Table 2). The number of morphospecies belonging the Ascidae and Acaridae families was higher in soil samples collected in the NG site. However, the opposite was observed for Pachygnathidae (ENDEOSTIGMATA). Average number of species in the collembolan families was significantly higher in the EC site (Entomobryidae: 3.0 ± 3.4; Symphypleonidae: 2.6 ± 2.4) than in the NG site; though the NG site contributed higher number of species in the Hypogastruridae family (Table 2).

In this case, the average abundance of micro-arthropods was overestimated in numbers of mites and collembolans per square meter and compared with the

Table 3. Number of mites and collembolans m^{-2} of soil (monthly average; $n = 4$) from areas under EC and NG

	Mites				Collembolans			
	$\bar{x}$		Tukey (HSD)		$\bar{x}$		Tukey (HSD)	
Month	EC	NG	*F*	*P*	EC	NG	*F*	*P*
January	1,465[b]	2,548[a]	8,31	0.027	446[a]	127[b]	10,23	0.018
February	2,102	2,675			446[a]	64[b]	9,37	0.022
March	1,783	1,529			1,019[a]	318[b]	11,45	0.012
April	1,019	1,274			382	382		
May	1,083	955			701	318		
June	1,338	955			701[a]	127[b]	8,82	0.024
July	1,847[a]	955[b]	13,53	0.010	382	446		
August	2,293[a]	1,274[b]	15,76	0.007	446	318		
September	2,166[a]	1,465[b]	13,54	0.010	446	701		
Total	15,095	13,630			4,968	2802		

[a]Averages followed by the same letter did not differ statistically by the Tukey's HSD test ($P < 0.05$) between areas for mites and collembolans.

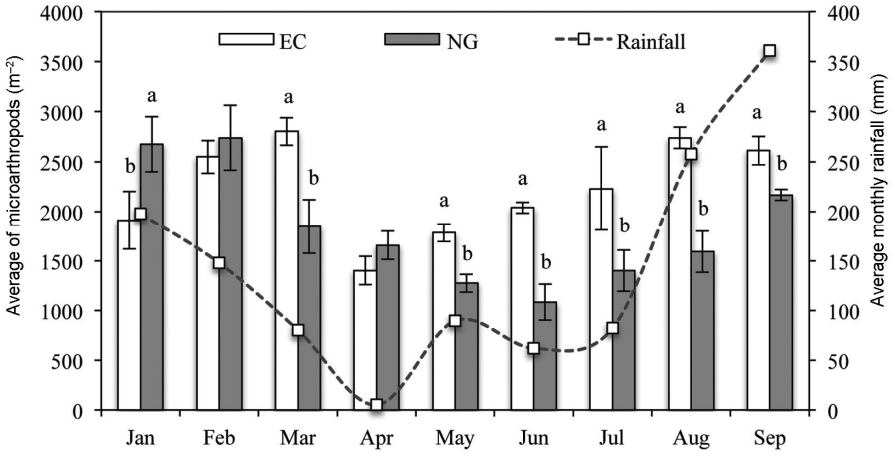

Figure 1. Average number of micro-arthropods/m^{-2} on the soil samples in *Eucalyptus* cultivation (EC) and native grassland (NG), and average monthly rainfall (mm). $n = 4$ replicates per treatment and error bars represent ± SD with the same letter are not significantly different (Tukey's honestly significant difference, $P < 0.05$).

average precipitation (mm) of the areas located. Micro-arthropods were influenced by the type of vegetation (EC or NG) and by average monthly rainfall (mm) (Figure 1). On the other hand, the lowest precipitation (April 2009) did not coincide with the lowest number of micro-arthropods in the soil samples, and so there was no statistical difference between both sites for April 2009 (one-way ANOVA, Tukey $P > 0.05$). As was observed for micro-arthropods, abundance was higher in EC site than in NG site for most of the period evaluated.

The differences observed between average number of micro-arthropods in the soil samples from March and June (Figure 1) were influenced by the lower amount of collembolans (Table 3). Mites were more abundant in July, August and September in the EC area, but the opposite was observed for the springtail communities. In January, NG showed a higher abundance of mites, with 2548 individuals m^{-2} soil, and the EM a higher number of collembolans (446 individuals m^{-2} soil) (Table 3).

For the NG site, April 2009 was observed as having the highest diversity of micro-arthropods (Shannon–Wiener Index), lowest dominance (Simpson's dominance index; Table 4) and the greatest heterogeneity (Simpson – 1 D-index). The highest Margalef index was in January, April and May for NG, and in June, July and August for EC (Table 4). In contrast, biodiversity as determined by the Equitability_J index was not significantly affected by either EM or NG (Table 4).

The result of the PCA is demonstrated through the relationship between the principal component 1 – PC1 (x-axis) and principal component 2 – PC2 (y-axis). The variability in the data was explained in 85.97% and 4.03% by CP1 and CP2 respectively, a total of 90% of variability of mite abundance (Figure 2). The PCA was

Table 4. Diversity indices estimated from mites and collembolans extracted from soil samples from EC and NG sites

	January	February	March	April	May	June	July	August	September
Eucalyptus cultivations									
Dominance_D	0.29	0.26	0.18	0.28[a]	0.19	0.19[b]	0.25	0.19	0.16
Shannon_H	1.39	1.53	1.95	1.44[b]	1.78	1.82[a]	1.73	1.87	1.95
Simpson_1-D	0.71	0.74	0.82	0.72	0.81	0.80[a]	0.75	0.81	0.83
Margalef	1.18[b]	1.35	2.38	1.29[b]	1.80b	2.02[a]	2.25[a]	2.13[a]	2.15
Equitability_J	0.87	0.85	0.85	0.89	0.92	0.87	0.79	0.85	0.89
Native grassland									
Dominance_D	0.27	0.29	0.15	0.14[b]	0.17	0.33[a]	0.25	0.18	0.16
Shannon_H	1.68	1.51	2.06	2.07[a]	1.96	1.31[b]	1.61	1.81	1.92
Simpson_1-D	0.74	0.71	0.85	0.86	0.83	0.67[b]	0.75	0.81	0.83
Margalef	2.14[a]	1.59	2.67	2.45[a]	2.67[a]	1.41[b]	1.94[b]	1.86[b]	1.98
Equitability_J	0.76	0.78	0.89	0.94	0.89	0.81	0.83	0.93	0.93

[a]The indices differences between treatments were compared within the column every month by Student's *t*-test ($P < 0.05$).

used to provide an ordering of the combinations and distributions of the micro-arthropod families in the soil. The principal response curve showed that mite communities had a disperse distribution and there was no specificity to certain area (Figure 2). There was no correlation between studied sites, and Acaridae, Eupodidae, Oribatida and Ascidae mite families were the most related to the NG area. Although individuals from the Oribatida family were found in both areas, this family was particularly related to the EM site (Figure 2).

According to our hypothesis, we found evidence that the vegetation can influence the distribution and abundance of edaphic micro-arthropods. The fact that a greater number of mites and collembolans was captured in soil samples from the area under eucalyptus can be related to other forest areas. Regarding the total number of mites captured in this study, those belonging to the suborder Oribatida were captured in greater proportions; 38.8% in the eucalyptus area and 25.3% in the native grassland area. Factors such as the higher deposition of litter on the soil surface of eucalyptus monoculture and the forest environment created by it may have influenced these results. The Oribatid influences the decomposition of organic matter and normally are components of the soil biota in environments with higher amount of organic material[26]. In forests, the deposition of surficial layers of organic material and the presence of fungi in the litter make the place an excellent environment for Oribatid[27]. Another factor that may be related is the vegetation type, because dense tree areas have a great amount of litter on the surface that can serve as protection for soil biota[28]. This also may be related to the resistance of the mites for changes in temperature and moisture[29]. The characteristics of the singular areas under the study may cause the differences in the presence of mite families such as Acaridae and Ascidae in the soil samples from the eucalyptus. The importance of these families has been associated with biological control because they are predators of various mite species[21].

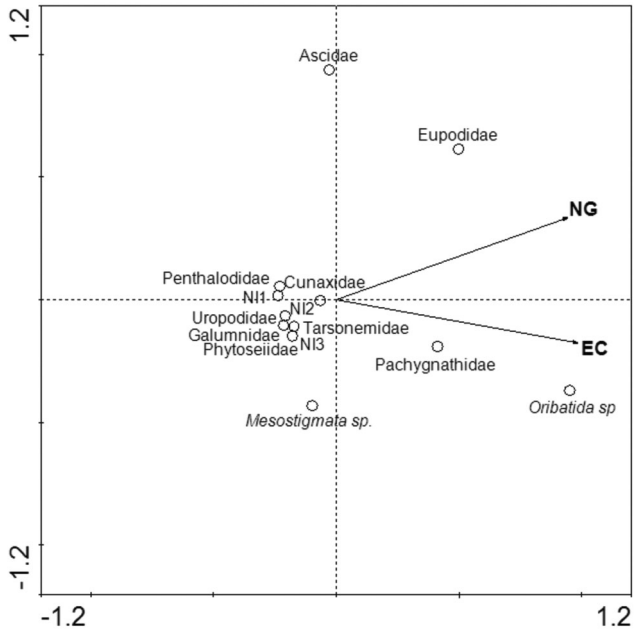

Figure 2. Principal component analysis biplot ordination of micro-arthropods (mites and collembolans) communities observed in the areas of soil sampling under EC and NG.

The area under eucalyptus cultivation had higher number of collembolans than the native grassland area, especially those from the Entomobryidae family. The vegetation cover on the native grassland area may not favour the presence of this group[30]. Factors such as presence of trees, shading and litter availability have positive correlation with the number of individuals and richness of springtail species[31]. Still, the collembolans are a predominantly detritivorous group, and their density and diversity depend on the quality and quantity of decomposing plant litter as food source[32]. On the other hand, the presence of eucalyptus trees in the area may have provided a specific edaphoclimatic condition, shifting the native population of collembolans. The Collembola can

be considered generalist feeders that can utilize a large range of feed sources[33].

In contrast to our expectations, samples from the Eucalyptus area had larger amount of mites and collembolans than from the native grassland. Monitoring across times allowed evaluating the group distribution for each scenario. Also, Sabais *et al.*[34] showed that microorganisms only respond to long-term changes, of about four years after the change in plant diversity, suggesting that in the long run plant community structure has significant impacts on soil microorganisms.

Density and diversity of mites and collembolans were significantly affected by the botanical composition of each area. In April, there was a larger amount of mites in the eucalyptus area, characterized mainly by the increase in the Oribatida order. April also registered the lowest rainfall during the experimental period. This shows that, under native vegetation, abiotic effects in micro-arthropods can be less intense. Fluctuation in the number of specimens of soil mesofauna can also be influenced by several other factors, such as rainfall and temperature[35]. Comparing the population fluctuation of the groups in both areas, it appears that the native grasslands area had greater homogeneity, even in months where lower total amount of mites and collembolans was observed. The greatest diversity of mites and collembolans occurs in places, which present the highest variety of plant species, which will serve as feed for a large number of individuals.

The PCA analyses data suggest a substantial level of differentiation in mesofauna community structure between areas. Overall, the distinctiveness-captured organisms by area are greater than the differences between samples collected at the same sampling period in the two different vegetation types. Based on the results of the properties of the PCA factors in relation to the differences between the areas, it was possible to identify the influences of the vegetation communities on the soil mesofauna. Maintaining soil invertebrate biodiversity reflects the quality of soil as a habitat[36]. Therefore, it can be used as indicator in risk assessment studies, important to analyse the distribution of mesofauna groups by PCA.

In this study, the Oribatida group was more associated with the area under eucalyptus. Most of those species feed on fungi and decomposing plant materials with a relatively low energy content[37]. The Oribatida mites secrete oily substances that can provide protection from arthropod predators[38]. Another family that had a noted relationship with eucalyptus soil samples was Pachynathidae. These organisms are widely distributed in soil and may be found in various locations and under various kinds of vegetation[22].

The Acaridae has countless representative organisms in agricultural soils, where several are considered pests[39] and some others are important agents in nematode control[40]. Characteristics of the native grassland area may be influencing directly or indirectly to sustain a greater amount of individuals from this family on the soil. The Ascidae had a larger presence in the native grassland area, suggesting that the influence of eucalyptus may affect the presence of this family in the soil. Those are also considered mites, which are useful as biological indicators of soil conditions and changes in soil ecosystems[41]. The presence of mites and collembolans, especially for the Symphypleonidae and Entomobryidae families, can be influenced by the presence of litter in soils under Eucalyptus cultivation.

Integration of biological and ecological data on species assemblages, data on soil physical and chemical parameters, climate and vegetation, must provide assessments of habitat complexity and change under stress in larger spatial scales, and allow comparisons between habitats[26].

There was influence of vegetation types (native and eucalyptus) on the behaviour and distribution of mites and collembolans in the soil. This study demonstrated that long-term monitoring is an excellent tool for observing seasonal distribution of soil mesofauna groups. Overall, our results highlight the importance of the soil-dwelling mites and collembolans to observe changes in soil characteristics and influence on micro-arthropods. On the other hand, a significantly larger amount of mites and collembolans were found in the experimental plots under eucalyptus. However, the families of mites had a better distribution, as evaluated by PCA, which allowed the identification of potential bio-indicators of soil quality.

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