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ECOLOGY

Diversity of Collembola and occurrence of *Talitroides sylvaticus* in a *Pinus elliottii* Engelm afforestation

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ABSTRACT. A 52-year-old pine forest is characterized as an environment with low plant diversity and large accumulation of allelopathic litter with poor nutrient content. Collembola is sensitive to environmental conditions and may have difficulties living in these forests. This study aimed (1) to evaluate the abundance and richness of Collembola in the soil of a 52-year-old pine afforestation with different moisture contents; and (2) to identify the landhopper sampled unintentionally. For sampling, pitfall traps were set up for four days, in areas of a *Pinus elliottii* afforestation with high and low soil moisture located at the *Universidade Federal de Santa Maria*. Collembola specimens were counted and identified to family and genus level. The total abundance of springtails was greater in the low soil moisture area. The genera *Desoria* and *Lepidocyrtus*, and specimens of the family Onychiuridae were more abundant in low soil moisture, meanwhile, the genus *Ceratophysella* was more abundant in the high soil moisture. The landhopper *Talitroides sylvaticus* was sampled unintentionally by traps set up in the soil with higher moisture and calcium content. Six genera of Collembola and the landhopper *T. sylvaticus* were identified in soil of a *P. elliottii* afforestation and their distribution is influenced by soil moisture.

Keywords: Crustacea; pine; soil moisture; springtails.

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Introduction

Pinus species are cultivated in roughly 2 million hectares in Brazil (Sistema Nacional de Informações Florestais [SNIF], 2019), 288 thousand in the state of Rio Grande do Sul (Associação Gaúcha De Empresas Florestais [AGEFLOR], 2020). Pine forests are often mentioned as 'green deserts', because they form microclimates, modify soil properties, inhibit the development of other plant species, homogenize the litter with needles and produce allelopathic substances (Pompeo et al., 2016; Spiazzi et al., 2017). Needles have poor nutritional quality, high content of tannins, terpenes, and other secondary metabolites that make difficult consumption by many soil organisms, favoring groups that can digest them (Kleinpaul, Schumacher, Brun, Brun, & Kleinpaul, 2005; Zhang, Wang, & Liu, 2018). In addition, substances are released from needles during decomposition, such as monoterpenes, which is harmful to many soil organisms and plants (Schlyter & Birgersson, 1999). Environmental homogenization in pine forests results in low diversity of food and reduces species richness of soil fauna (Mboukou-Kimbatsa & Bernhard-Reversat, 2001; Silva, Lima, Andrade, & Brown, 2019).

Springtails (Collembola) are organisms of the soil mesofauna responsible for ecosystem services, such as biological control, dispersion of microorganisms, nutrient mineralization, production of humic substances, and others (Čuchta, Kaňa, & Pouska, 2019; Potapov et al., 2020). In forest soils, springtail density can reach up to 40,000 organisms per square meter (Orgiazzi et al., 2016). Furthermore, they are excellent bioindicators of soil quality due to their sensitivity to environmental factors. Marx (2008) demonstrated that sites with intermediate soil moisture harbor higher abundances, followed by wet and dry areas. In native forests from southern Brazil, the abundance of springtails of the order Entomobryomorpha are directly related to rainfall during the soil sampling period (Rieff, Luz, Sousa, & Sá, 2014).

Soil moisture is also a decisive factor for the life cycle and distribution of Crustacea. These organisms habitually live in marine environments, although some species are adapted to terrestrial environments, especially forest soils (Sousa & Dangremond, 2011). Members of the family Gammaridae, superfamily Talitridae, live on the soil/litter interface (Friend & Richardson, 1986), associated with humid environments where they find shelter from dehydration. Some species of the family Talitridae were co-introduced with species of the exotic flora, and were associated with disturbed areas and areas of *Eucalyptus* spp. afforestation (Kotze & Lawes, 2008).

Knowledge regarding the fauna of Collembola and crustacean in Brazilian *P. elliottii* forests is very limited (Troian, Baldissera, & Hartz, 2009; Bugs, Araujo, Mendonça Júnior, & Ott, 2014; Buckup & Bond-Buckup, 1999), therefore, the environmental conditions in a 52-year-old pine forest are very conducive to studying groups of organisms adapted to these adverse conditions. Thus, the goal of this study was (1) to evaluate the abundance and richness of springtails in soil of a 52-year-old pine afforestation with different soil moisture contents; and (2) to identify the landhopper sampled unintentionally.

Material and methods

Site description

Soil fauna was sampled in an afforestation of *P. elliottii* of 6.2 hectares and 2 x 2 meters spacing at the *Universidade Federal de Santa Maria*, Santa Maria, state of Rio Grande do Sul, Brazil (29°43′20″ S, 53°42′48″ W, 95 m). The area was planted in 1966, and since then the pine trees were never pruned or cut. The soil is classified as Gray-Brown Argisol, and the climate is Cfa Subtropical humid, according to the Köppen classification (Moreno, 1961). The average temperature reaches 18.5°C, and the rainfall is well distributed reaching 1,580 mm per year. Values of temperature and rainfall in the period close to and during the sampling are illustrated in Figure 1. Weather data was obtained from an official station, located 530 m from the study area (Instituto Nacional de Metereologia [INMET], 2017).

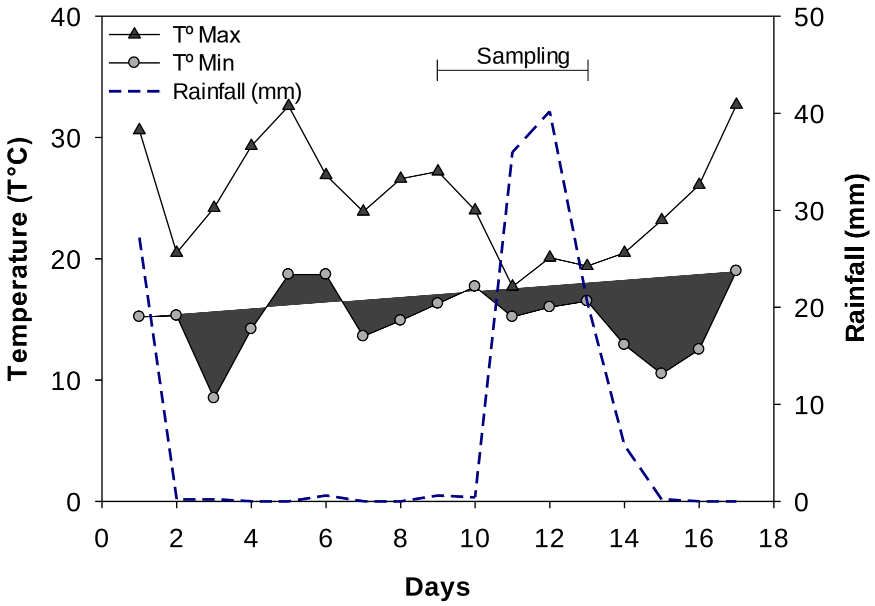

Figure 1. Temperature (maximum and minimum) and rainfall (mm) during the fauna sampling period in the pine afforestation. Santa Maria, state of Rio Grande do Sul.

Soil fauna sampling

Collembola specimens were sampled in the spring, during four days (09 – 13 Oct. 2017). Pitfall traps (diameter 10 cm and depth 20 cm) protected from rainfall were added with 500 mL ethanol 70% (v v^{-1}), used as a preservative solution. Traps were set up at six points distant seven meters from each other. Three points were located in the high moisture area (Ug 0.35 g g^{-1}), at an approximate distance of 3 m from a stream. The other three points were located in the low moisture area (Ug 0.25 g g^{-1}), far 12 m from the same stream, and in a slightly more elevated area of the forest. At each point, three pitfall traps were set up, totaling 18 traps. After four days, traps were taken from the field and samples were processed at the laboratory. Specimens were carefully cleaned up with water, and kept in ethanol 70% ($v\ v^{-1}$). Springtails were classified into families and genera according to specialized keys (Cipola, Silva & Bellini, 2018; Bellinger, Christiansen, & Janssens, 1996-2022), once the identification into species was not possible due to the scarce number of records for the region (Bellinger et al., 1996-2022). The unique landhopper species sampled was identified according to Buckup and Bond-Buckup (1999).

Physical and chemical properties of soil

Soil samples were collected (0–20 depth) to analyze the physical and chemical properties of each of the six points. Samples were mixed into a composite sample for the areas of high and low soil moisture content. The properties analyzed were: clay content (densimeter), organic matter (Walkley-Black), pH (water 1:1), phosphorous (Mehlich-1), calcium (EDTA), magnesium (EDTA), sulfur ($Ca_3(PO_4)_2$), cation exchange capacity (CEC), potential acidity (H+Al) and base saturation (V%). The chemical properties from both high and low moisture soil areas in the afforestation are listed in Table 1.

Table 1. Chemical, physical, and litter properties of the soil in low and high soil moisture areas in a 52-year-old *Pinus elliottii* afforestation.

Chemical properties	High soil moisture 0 – 20 cm		Low soil moisture 0 - 20cm	
Clay ($g\ kg^{-1}$)	290.0		240.0	
Gravimetric moisture ($g\ g^{-1}$)	0.35		0.25	
pH in H_2O (1:1)	4.8		4.1	
Organic matter ($g\ kg^{-1}$)	31.0		35.0	
P ($mg\ dm^{-3}$)	6.9		7.5	
K ($mg\ dm^{-3}$)	56.0		48.0	
Ca ($cmol_c\ dm^{-3}$)	7.1		1.1	
Mg ($cmol_c\ dm^{-3}$)	2.7		0.5	
S ($mg\ dm^{-3}$)	17.6		40.4	
CEC ($cmol_c\ dm^{-3}$)	18.6		36.2	
V (%)	53.1		4.8	
H+Al ($cmol_c\ dm^{-3}$)	8.7		34.5	
Litter ($Mg\ ha^{-1}$)	69.26		103.66	
Physical properties	0 - 5 cm	5 – 10 cm	0 - 5 cm	5 - 10 cm
Density ($g\ cm^{-3}$)	1.380	1.440	1.470	1.520
Total porosity ($cm^3\ cm^{-3}$)	0.454	0.424	0.420	0.399
Macroporosity ($cm^3\ cm^{-3}$)	0.050	0.065	0.122	0.106
Microporosity ($cm^3\ cm^{-3}$)	0.404	0.359	0.298	0.293

For physical property analysis, two undeformed samples (0-5 cm depth and 5 -10 cm depth) from three points of each area were collected using steel cylinders, totaling 12 samples. Soil density (graduated cylinder method), total porosity (saturation by water), microporosity, and macroporosity (tension table) were determined, according to Teixeira, Donagemma, Fontana, and Teixeira (2017). The gravimetric moisture was determined after drying soil at 105°C. For each sampling point, three samples of litter were collected using a metallic frame (25 x 15 cm). Samples were dried in a forced air oven at 65°C to constant weight.

Data analysis

For the Collembola community, we calculated the diversity profile by the Renyi index (function renyiresult, 'BiodiversityR' package); the dissimilarity between areas was calculated by the Jaccard index. A permutational multivariate analysis of variance, using distance matrices (function 'adonis') based on the Jaccard index (function 'vegdist'), was applied to evaluate the difference in springtail community between areas with low and high soil moisture. Medians of abundance of Collembola and Crustacea in the areas of low and high soil moisture were compared by the Mann-Whitney test. All statistical analyses were run using R version 3.6.0 (R Core Team, 2019).

Results

A total of 2,528 specimens of Collembola were sampled and identified in the *P. elliottii* afforestation, belonging to *Desoria*, *Ceratophysella*, *Lepidocyrtus*, *Calvatomina*, and *Entomobrya* (Table 2 and Figure 2). Specimens of Onychiuridae were not identified at the genus/species levels due to the lack of literature and records for the region. The low soil moisture area showed an abundance of springtails eight times fold higher than the high soil moisture area. Specimens of Onychiuridae largely dominated the low soil moisture area, representing 89% individuals sampled and being 45 times more abundant in this environment compared to the high moisture soil. In this sense, *Desoria* and *Lepidocyrtus* were more abundant in the low soil moisture. Nevertheless, *Desoria* was superior in the high soil moisture area, with 48% relative frequency of individuals. *Cerathophysella* was found in a greater number in the high moisture area. The abundance of *Entomobrya* and *Calvatomina* was very low in the pine afforestation soil. Unintentionally, 16 specimens of *Talitroides sylvaticus* Haswell belonging to the family Talitridae (Crustacea) were collected. Their abundance was superior in the high moisture area (Table 2).

Table 2. Taxonomic information of Collembola, total abundance, and richness of Crustacea and Collembola in areas with high and low soil moisture of a 52-year-old pine afforestation.

Family	Genus	Low soil moisture	High soil moisture
Onychiuridae	-	2011	44
Isotomidae	*Desoria*	202	136
Hypogastruridae	*Ceratophysella*	14	81
Entomobryidae	*Lepidocyrtus*	19	7
Dicyrtomidae	*Calvatomina*	0	2
Entomobryidae	*Entomobrya*	1	0
Total Abundance (1)	Collembola ns	2,247	281
	Crustacea *	2	14
Richness	Collembola	5	5
	Crustacea	1	1

(1)Medians compare by Mann-Whitney test. Significance codes: * p = 0.0229; ns = non-significant.

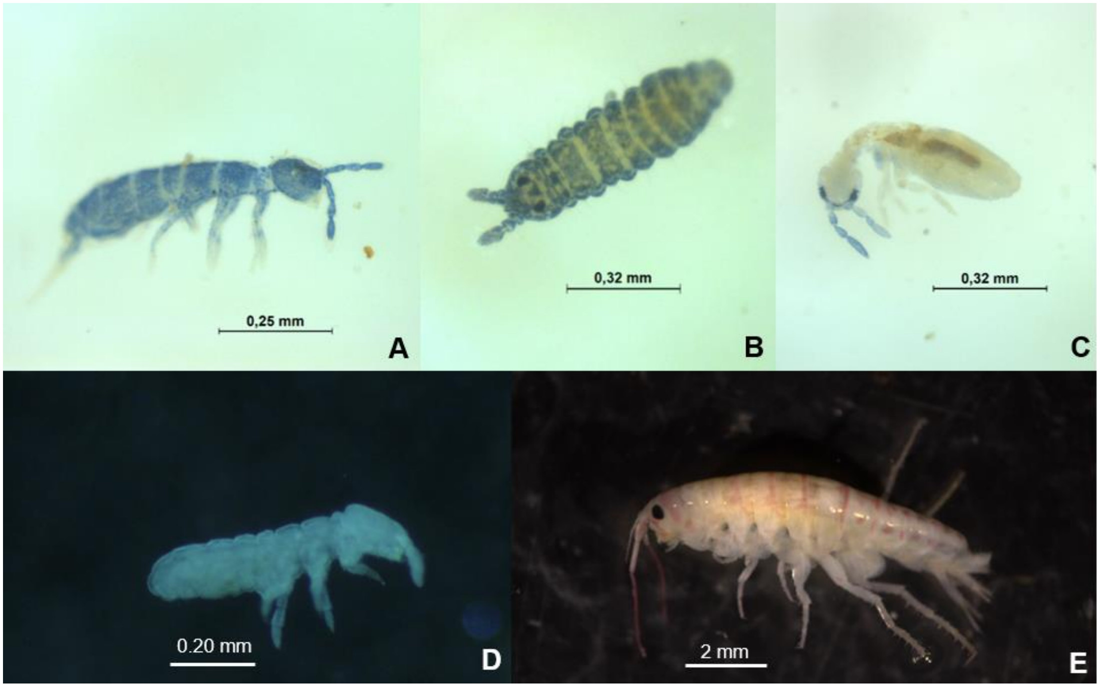

Figure 2. Specimens of Collembola (A, B, C e D) and Crustacea (E) from a 52-year-old pine afforestation. A: *Desoria*, Isotomidae, B: *Ceratophysella,* Hypogastruridae. C: *Lepidocyrtus,* Entomobryidae, D: Onychiuridae sp. E: *Talitroides sylvaticus.*

The calculated Jaccard Index was 66.7%, indicating similarity in the composition of genera and families of Collembola between high and low soil moisture areas (p = 0.23928). The Renyi diversity profiles showed that the species richness of springtails was five (alpha = 0) in both areas (Figure 3). However, the Shannon index (alpha = 1) and the Simpson index (alpha = 2) were higher in the area with high soil moisture. Moreover, this area was more even (alpha =0) and the lower proportion of dominant groups contributed to higher diversity.

The two sampled areas showed differences regarding the soil chemical and physical properties (Table 1). The high soil moisture area stood out by the content of calcium and magnesium, respectively, 545% and 440% superior to the low soil moisture area. On the other hand, values of OM, P, S, CEC, H+Al were higher in the low moisture area. The litter, composed of needles of *P. elliottii*, showed a high amount of dry mass, especially in the low soil moisture area. The analysis of physical properties demonstrated that the high moisture area soil is less dense due to a higher total porosity, with a predominance of micropores.

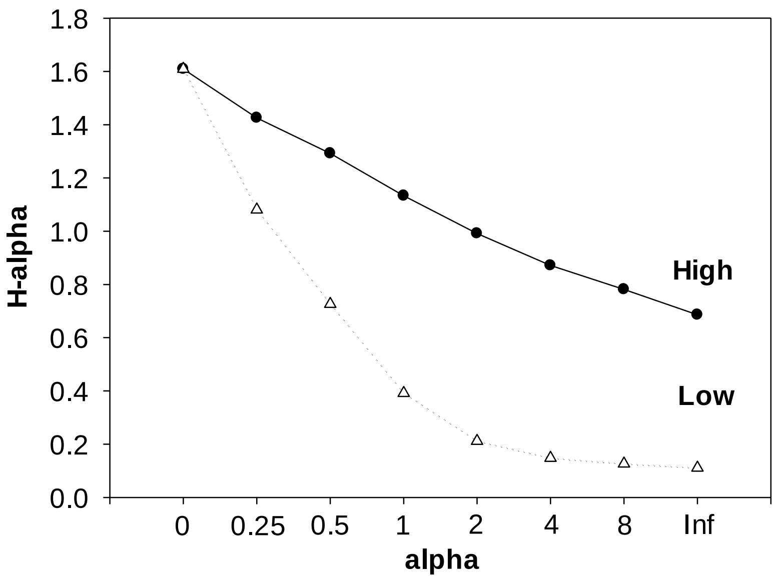

Figure 3. Renyi diversity profiles of the Collembola community in areas of high and low soil moisture.

Discussion

The *P. elliottii* afforestation showed a low abundance of Collembola, with an average of 31 and 249 springtails per pitfall trap, in the high and low soil moisture areas. In the same pine afforestation, in a soil fauna study, Pessotto et al. (2020) obtained a greater abundance of springtails per trap, although in a longer sampling period (seven days). Silva, Saidelles, Vasconcellos, Webber, and Manassero (2011) also reported a high average abundance (1736 per pitfall trap) of Collembola in a pine forest close to the study region. In addition to the sampling time and environmental conditions in the study local (moisture, structure of the understory, soil surface, litter availability, etc.), seasonality may also influence the springtail abundance. In native and *Eucalyptus* forests, Ortiz et al. (2019) found a superior number of springtails per pitfall trap in the summer compared to the winter.

The soil moisture in the pine afforestation changed the population dynamics of Collembola, and the response relied on the genera and families, despite having the greater total abundance of individuals in the low moisture soil. Water excess in the soil may have reduced the availability of oxygen and impaired the population growth of some genera and families. Xu, Kuster, Goerg, Dobbertin, and Li (2012) indicate that springtails might be sensitive to excess of moisture in the environment and that specimens of some genera, such as *Parisotoma*, may be more abundant in low moisture areas. In our study, it should be considered that the low soil moisture area showed a higher amount of litter, higher organic matter content, phosphorous, and sulfur in the soil, which may have favored the grown of springtails (Eaton, 2006; Russell & Gercócs, 2019).

Specifically, specimens of *Desoria, Lepidocyrtus*, and the family Onychiuridae were more abundant in the low soil moisture area. *Desoria* acts in the decomposition of litter and their abundance is related to the amount of organic residues on the soil surface (Fujii, Saitoh, & Takeda, 2014). The organic matter content of the soil also influences the distribution of organisms of the family Isotomidae (including *Desoria*), and for this reason, they are considered indicators of changes in soil fertility (Salamon, Schaefer, Alphei, Schmid, & Scheu, 2004). Marx (2008) relates the presence of some organisms of this genus with low soil moisture. In contrast, in agriculture systems in southern Brazil, Sautter, Santos, and Ribeiro Júnior (1999) found individuals of the family Entomobryidae in no-till areas with high moisture and accumulation of organic matter.

In the area with low soil moisture, it was observed only one individual of *Entomobrya*. Chang, Wang, Liu, Callaham Jr. and Ge (2017) observed organisms of this genus in pine forests of different ages in China (from 26 to 260 years old), and its abundance did not vary significantly between forests. However, Fujii and Takeda (2012) report that the abundance of individuals of this genus is related to soil litter, which corroborates our results, in which *Entomobrya* was found in the area with the highest litter accumulation. Individuals of the family Onychiuridae largely dominated the area with low soil moisture. The high abundance of individuals of this family have been reported in a pine forest of around 80 years and justified by the high amount of litter in the soil (Chang et al., 2017). This condition also favors the development of fungi colonies, which are part of the diet of fungivorous springtails of this family (Potapov, Semenina, Korotkevich, Kuznetsova, & Tiunov, 2016; Samain, Baldy, Lecareux, Fernandez, & Santonja, 2019).

Some hygrobiont species of the *Ceratophysella* genus have been associated with high moisture and low pH of pine forest soils (Sławska, Bruckner, & Sławski, 2017), corroborating the observations of the present study. Organisms of the genus *Calvatomina*, belonging to the family Dicyrtomidae, might be sensible to drought environments and were also sampled in subtropical forests of high humid in Australia (Greenslade & Slatyer, 2017). In South Africa, Liu, Janion, and Chown (2012) report the presence of organisms of this family, and their invasive potential related to the high moisture of the litter of *Pinus radiate* forests. The litter moisture may also contribute to the greater abundance of springtails (Fujii et al., 2014) since the growth of fungi is favored in these conditions, which increases the availability of food for fungivorous springtails (Nakamori & Suzuki, 2010; Anslan, Bahram, & Tedersoo, 2018).

The moisture and the high content of calcium in the soil of the pine afforestation may have contributed to the occurrence of *Talitroides sylvaticus*, found in the area of high soil moisture. Landhoppers of the family Talitridae live in terrestrial environments and have nocturnal habits (Friend & Richardson, 1986). On the surface of the pine afforestation, *T. sylvaticus* finds conditions to avoid direct contact to solar radiation and consequently exoskeleton dehydration (Mendes & Ulian, 1987). Studies have also reported the presence of this landhopper in soils with high calcium content, an element necessary to exoskeleton formation, as observed in the present study (Ohta, Niwa, Agetsuma, & Hiura, 2014). *Talitroides sylvaticus* is an invasive species and its origin is unknown, although its presence was reported in the south of the state of Rio Grande do Sul state as well in urban areas, playgrounds, and botanic yards from Europe, where it was probably introduced by human activities (Buckup & Bond-Buckup, 1999).

Conclusion

Soil moisture changes the abundance, the structure of the community, and the diversity of Collembola in a 52-year-old *P. elliottii* afforestation. The low soil moisture area harbors a higher abundance of individuals of *Desoria*, *Lepidocyrtus*, *Entomobrya,* and the family Onychiuridae, meanwhile the high soil moisture area has a greater abundance of *Ceratophysella* and *Calvatomina,* as well as the larger diversity of springtails. The landhopper *Talitroides sylvaticus* sampled unintentionally was more abundant in the area with the high moisture and calcium in the soil.

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