



FERTILIZER AND LIME IMPACTS ON EDAPHIC MESOFAUNA IN A SOUTHERN BRAZIL LOBLOLLY PINE SYSTEM

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Abstract: Mesofauna play an important role in nutrient biocycling, but little attention has been given to fertilizer and lime impacts on forest floor and soil populations. The aim of this study was to evaluate the effect of fertilization and liming on edaphic mesofauna in a *Pinus taeda* L. system (Paraná State, Brazil). Lime and fertilizer were applied over litter in a six-year-old *P. taeda* system using three treatments: complete fertilization (N, P, K, Zn, Cu, B, Mo, and limestone), complete fertilization without lime, and a control. Four years later, winter and summer samples were collected from soil (0-5 cm) and litter for mesofauna and chemical properties. Results confirmed the importance of litter as a niche for mesofauna (94 % of total mesofauna; 286,627 individuals·m⁻²) compared to control soil (6 % of total mesofauna; 17,833 individuals·m⁻²). Seasonality was an important factor, with summer populations (135,000 individuals·m⁻²) being 3-fold higher than winter (42,000 individuals·m⁻²). Complete fertilization and fertilization without lime increased Oribatid mites in summer litter but decreased Collembola Arthropoda. In the summer, the mesofauna population in soil was lower under complete fertilization. Thus, fertilization and lime applications to *P. taeda* systems can change edaphic mesofauna in soil and litter.

Keywords: *Pinus taeda* L., Collembola, litter, mites, Acari, soil fertility, soil food web.

1. Introduction

Pinus plantations in Brazil began in 1960 and currently (2022) occupy around 1.93 million hectares of the total reforested area (9.93 million hectares) in Brazil (IBÁ 2022). More than 88.9 % of *Pinus* forests in Brazil are located in the southern region where climate is considered subtropical (IBÁ 2022).

Pinus taeda L. is the most commonly planted species since it is well adapted to soils and climatic conditions of southern Brazil and generally has very high yields (Motta et al, 2014). However, there are indications that very low soil fertility could limit pine growth at some sites (Moro et al, 2014; Consalter et al, 2021 a; Pereira et al, 2022). leading to litter accumulation (Reissmann and Wisniewski, 2000) and changes in soil fertility that may influence decomposition (Yu et al, 2015; Bonanomi et al, 2017; Krishna and Mohan, 2017). However, soil fertility management can increase (Consalter et al, 2021 a; Pereira et al, 2022), maintain (Adam et al, 2021), or decrease (Jandl et al, 2003) forest floor mass. This differential response to nutrient additions could be related to the balance between changes in litterfall input, rate of litter decay, and amount of carbon that remains in the humic fraction.

Mesofauna are well known to play important roles in litter decomposition, which could alter litter amounts on forest floors (Gomes et al, 2007; Wang et al, 2015; Nascimento et al, 2021). In general, the orders Acari (Arthropoda: Arachnida) and Collembola (Arthropoda: Hexapoda) represent between 75 and 97% of mesofauna individuals (Higvar and Amundsen, 1981; Jandl et al, 2003; Illig et al, 2010). However, other groups such as Diptera (Souto et al, 2008) or Isopoda (Ribeiro et al, 2014) may be more abundant under some conditions.

Changes in soil fertility by use of fertilizers, acidity correctives, and organic residues can alter abundance and diversity of litter and/or soil mesofauna (Ribeiro et al, 2014; Wang et al, 2016). Large increases in mesofauna populations were observed with N and P application as reflected by 2-3 fold increases in Acari abundance (in litter + soil) with little impact on Collembola populations (Bird et al, 2004). Cole et al. (2006) reported that liming increased Collembola populations, while Giracca et al. (2008) found a small effect of isolated liming on populations of Acari and Collembola.

Over the long term, fertilizer and lime use in *Pinus* plantations could be required for environmental sustainability. However, little is known on how these inputs affect pine plantation mesofauna under the subtropical climatic conditions of southern Brazil (Batista et al, 2015; Liebsch and Mikich, 2017). Thus, our objective was to evaluate the influence of fertilizer and lime application on mesofauna in a *P. taeda* system located in southern Brazil. Our hypothesis was that high seasonal climate variability directly influences the quantity of mesofauna individuals. Thus, number of individuals found in winter periods would be smaller and consequent treatment effects would be less during this seasonal period. Furthermore, since most mesofauna individuals inhabit litter, it is presumed that fertilization and liming could alter litter quality leading to changes in population behavior and community structure.



2. Material and Methods

The study area was located in the municipality of Jaguariaíva located in the east-central region of Paraná State, Brazil. This experimental area had an altitude of 928 m, latitude 24°12'51.25", and longitude 49°36'33.62". The area was classified as subtropical according to Köppen with an average summer temperature of 22 °C and average precipitation of 1,460 mm (Alvares et al, 2013).

The soil was classified as a Yellow Red Latosol; chemical and granulometric attributes are shown in Table 1. Furnas Sandstones and Itararé formations were the two parent materials for soils formed in this region. The region was located in a transition area among three ecosystems: Cerrado, native subtropical fields, and subtropical forests.

Table 1 – Soil chemical and physical analysis of a Yellow Red Latosol cultivated with *Pinus taeda* L. in the region of Jaguariaíva, Paraná, Brazil.

Depth (cm)	pH CaCl ₂	pH SMP	C g·dm ⁻³	Al ³⁺	H + Al	Ca ²⁺ cmolc·dm ⁻³	Mg ²⁺	K ⁺	CEC
0-5	3.24	4.94	32.55	2.58	12.40	0.15	0.10	0.04	12.69
05/out	3.81	5.79	13.65	1.48	5.83	0.15	0.10	0.03	6.10
out/20	3.94	5.98	11.20	1.28	5.05	0.15	0.10	0.02	5.32
20-40	3.98	6.00	9.40	1.23	5.08	0.13	0.10	0.01	5.31
40-60	4.07	6.26	7.43	1.00	4.38	0.13	0.10	0.02	4.62

Depth (cm)	P	Cu	Mn mg·dm ⁻³	Fe	Zn	V	m %	Clay	Sand
0-5	3.85	0.89	0.74	44	0.23	2.5	90	5.4	83.7
05/out	2.00	0.97	0.09	35	0.24	4.5	84	4.2	81.3
out/20	1.10	0.95	0.08	38	0.15	5.1	83	3.9	82.0
20-40	0.83	0.94	0.08	40	0.14	4.7	84	3.4	79.7
40-60	0.30	1.11	0.15	28	0.15	5.5	80	3.1	77.6

CEC: cation exchange capacity; V: base saturation; m: Al³⁺ saturation.

The study area originally had natural vegetation (Cerrado and native subtropical fields) that had been used for extensive long-term cattle and equine grazing. In 1992, the area was planted with *Eucalyptus* spp. and harvested in 2002. In 2003, the area was reforested with *P. taeda* (2 x 3 m spacings). There were no indications of previous lime and fertilizer use and pine plantings also occurred without the use of correctives and fertilizers.

The current experiment was implemented six years after the *P. taeda* planting. The experimental design consisted of randomized blocks containing seven treatments and four replicates. The technique used was diagnosis by subtraction (or omission of nutrients) for a total of seven treatments as follows: complete (N, K₂O, P₂O₅, Zn, B, Cu, and lime), complete less macronutrients, complete less micronutrients, complete less K, complete less Zn, complete less lime, and a control. Three of these treatments were selected for mesofauna evaluation: complete (Complete), complete less lime (- Lime), and control (Control).

Each plot was 104 m² with a useful area consisting of 16 central trees that corresponded to an area of 90 m². Rates equivalent to 40, 60, 80, 3.0, 2.0 and 1.5 kg·ha⁻¹ of N, P₂O₅, K₂O, Zn, B, and Cu, respectively, were applied in two years (November 2008 and February 2010). Respective nutrient sources were urea, triple superphosphate, potassium chloride, zinc sulfate, ulexite, and copper sulfate. Molybdenum was added at 20 g·ha⁻¹ as sodium molybdate. Dolomitic limestone was applied at a rate of 1,300 kg·ha⁻¹ as a source of Ca and Mg.

Soil and litter collections were conducted in August 2012 (winter) and March 2013 (summer). Litter was collected at three random points in the middle of tree lines using a square template (0.2 x 0.2 m). After litter collection, soil was collected up to a 5 cm depth at the same point. At each collection point, temperature was checked and composite soil samples (three 0-5 cm subsamples) were collected and stored in sealed plastic bags. These samples were used for chemical analysis and determination of gravimetric moisture. Chemical analysis followed the methodology described by Silva (2009) where the following attributes were determined: pH in solution of CaCl₂ 0.01 mol·L⁻¹, Al³⁺, Ca²⁺, Mg²⁺ and K⁺ exchangeable, potential non-exchangeable acidity (H + Al), available P (Mehlich I), and organic carbon (Marques and Motta, 2003). Litter was collected from the three selected treatments (Complete, - Lime, and Control).

Assessments of mesofauna organisms in soil and litter samples were conducted using a modified Tullgren-Berlese funnel (8 cm diameter) system according to procedures described elsewhere (Wiggins and Curl, 1979; Runion et al, 1994; Karyanto et al, 2008). Funnels were placed in plastic cartridges to minimize organism and moisture losses. Soil samples (0-5 cm depth) were collected with an auger (3.8 cm diameter) and kept in plastic bags to avoid loss of mesofauna organisms. In the laboratory, soil samples were transferred to Tullgren-Berlese funnels. Funnels containing either litter or soil were placed in holding racks arranged in series under



25 W lamps for a period of seven days. Organisms migrating in advance of the slowly drying litter or soil were collected in plastic bottles containing a preservation solution (70 % alcohol, 28 % water, 1 % glycerin, 1 % formalin). Subsequently, the different taxonomic groups were screened and counted using a stereoscopic microscope. Organisms evaluated were the Acari subclass belonging to the class Arachnida and Collembolas belonging to the class Insecta. All soil abundance data were extrapolated to number of individuals per square meter based on the Tullgren-Berlese funnel area for litter and auger area for soil. Each litter sample was oven-dried at 60 °C, ground for digestion (500 °C), and solubilized in 3 mol·L⁻¹ HCl solution (Martins and Reissmann, 2007). The determination of elements was by: flame emission spectrometer (Digimed, MD-62) for K; UV/VIS spectrometer (Bel Photonics, SP2000) colorimetry via ammonium molybdate-vanadate for P; and atomic absorption spectrometry (Varian, AA240FS) for Ca, Mg, Fe, Mn, Zn, and Cu.

For statistical analysis, we use the Kolmogorov-Smirnov test to observe for data normality. Mesofauna data were submitted to analysis of variance (ANOVA) using Quasi-Poisson distribution. Tests were performed with the R-Studio® program. To explore main variation trends of soil mesofauna and the relationship between physical and chemical variables of soil and litter, principal component analysis (PCA) was applied using Canoco 4.5 for Windows.

3. Discussion and Results

Results in Table 2 indicated that lime decreased soil acidity. The effect of lime was increased pH, decreased Al³⁺ and potential acidity (H + Al), and increased soil bases (Ca²⁺ and Mg²⁺). However, pH was not high in the complete treatment since lime rate was intended to increase soil Ca²⁺ and Mg²⁺ rather than adjust pH. Fertilization also increased soil P (Table 2), which can negatively impact mycorrhizal development (Higo et al, 2020). Increased P was due to soil surface application and low mobility within the soil profile (Barcellos et al, 2015). Although K⁺ was applied in higher quantities than P, increase in K⁺ was less expressive (Consalter et al, 2021 b). This was due to high mobility of K⁺ within soil and high plant uptake (Marschner 2012; Barcellos et al, 2015; Consalter et al, 2021 a).

Table 2 – Soil chemical analysis, temperature, and moisture (0-5 cm layer) for two seasons in a *Pinus taeda* L. system subjected to fertilization and liming in Jaguariaíva, Paraná, Brazil.

Treatments	pH CaCl ₂	pH SMP	H+Al	Al ³⁺	Ca ²⁺ Mg ²⁺ K ⁺	Mg ²⁺	K ⁺	SB
	cmol _c ·dm ⁻³							
Summer								
Complete	4.72	5.7	6.6	0.3	3.2	2.6	0.09	5.9
- Lime	3.46	4.9	11.4	2.8	0.5	0.1	0.09	0.7
Control	3.39	4.7	13.2	3.1	0.2	0.1	0.07	0.3
Winter								
Complete	4.70	6.0	5.1	0.2	2.96	2.4	0.10	5.4
- Lime	3.25	5.0	10.8	2.6	0.20	0.1	0.13	0.4
Control	3.23	4.9	12.5	2.9	0.15	0.1	0.06	0.3
Treatments	CEC effective	CEC pH 7.0	P	C	V	m	SM	T
	cmol _c ·dm ⁻³							
Summer								
Complete	6.3	12.5	52.8	21.4	47	6	7.5	20.0
- Lime	3.6	12.1	46.7	21.1	6	79	7.7	20.0
Control	3.4	13.6	3.0	23.5	3	89	8.6	20.0
Winter								
Complete	5.6	10.6	68.5	28.3	51	3	9.8	16.0
- Lime	3.0	11.2	39.6	33.2	4	85	9.7	16.0
Control	3.2	12.8	5.0	28.4	3	90	9.2	16.0

SB: sum of bases; CEC: cation exchange capacity; V: base saturation; m: Al³⁺ saturation; SM: soil moisture; T: temperature.

Chemical analysis of litter (Table 3) indicated fertilization and liming affects total Cu and Zn concentrations, which was associated with the high capacity of organic matter to adsorb Cu and Zn, as was observed by Consalter et al. (2021 b) under similar conditions. In contrast, Mg²⁺ concentration was high in the second litter sample, due to the continued presence of lime in the fine litter fraction four years after complete fertilization.



Table 3 – Nutrient concentrations in litter for two seasons in a *Pinus taeda* L. system subjected to fertilization and liming in Jaguariaíva, Paraná, Brazil.

Treatment	P	K	Na	Mg	Cu	Mn	Fe	Zn
Summer								
Complete	0.20	0.58	0.08	2.23	81.1	223	978	95
- Lime	0.19	0.53	0.05	2.50	116.7	254	811	129
Control	0.14	0.40	0.05	2.38	8.3	227	700	14
Winter								
Complete	0.19	0.65	0.09	2.61	137.1	48	476	35.7
- Lime	0.06	0.42	0.11	1.20	78.3	49	575	43.9
Control	0.05	0.32	0.08	1.00	15.5	53	875	21.0

Regarding edaphic mesofauna in litter, Figure 1 shows summer collections of Oribatid mites (84,618 individuals·m⁻²), Collembola Arthropleona (47,134 individuals·m⁻²), and Collembola Symphypleona (2,918 individuals·m⁻²). This was significantly higher than the winter ($p < 0.001$) collection of Oribatid mites (18,452 individuals·m⁻²), Collembola Arthropleona (23,555 individuals·m⁻²), and Collembola Symphypleona (418 individuals·m⁻²). This difference was likely due to lower winter temperatures, which limits Acari food resources and overall reproduction of edaphic mesofauna (Langan et al, 2006; Souto et al, 2008; Giracca et al, 2008; Battigelli 2011). Seasonality explains the absence of treatment effects on winter litter samples. Silvan et al. (2000) noted that the frequency of these organisms in litter makes them more or less susceptible to seasonal effects.

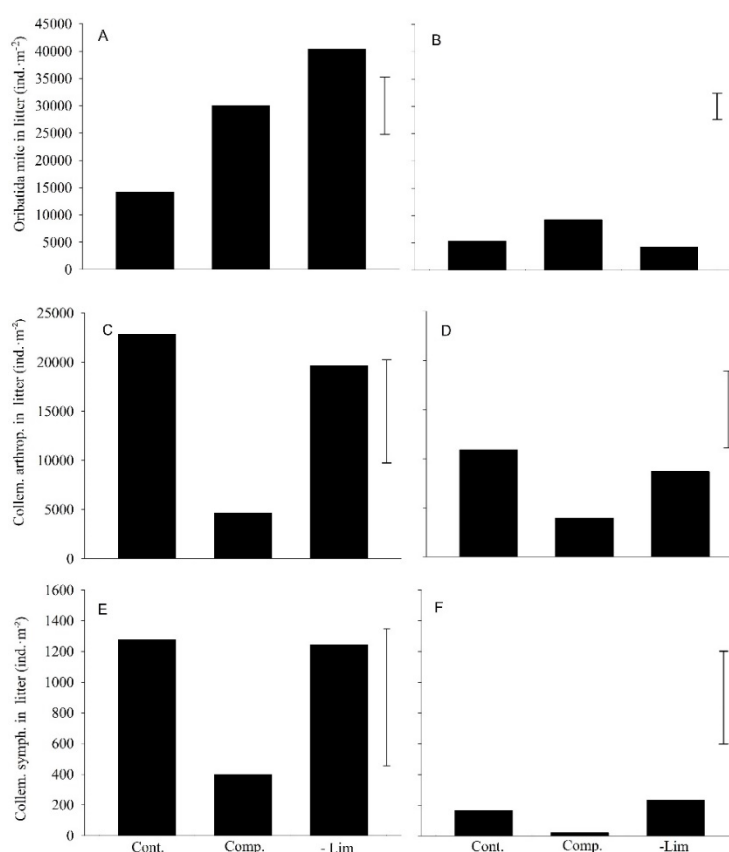


Figure 1 – Oribatid mites collected in summer (A) and winter (B), Collembola Arthropleona (Collemb. Artrop.) collected in summer (C) and winter (D), and Collembola Simphypleona (Collemb. Symph.) collected in summer (E) and winter (F) in the Control (Cont.), complete with lime treatment (Comp.), and complete without lime (- Lim.). Horizontal bars represent the high significant difference according to the Tukey test with 5 % of probability.

In summer, Oribatid mites and Collembola Arthropleona in litter responded to fertilization and liming in different ways. While Oribatid mites increased with complete fertilization and lime (compared to control; $p \leq 0.05$; Figure 1A), liming did not benefit



Collembola Arthropleona (Figure 1C) since individual numbers were higher in the control and complete fertilization without lime. These results reflect the difference in trophic activities of each organism. Since Oribatid mites use higher diversity food resources, inclusive of litter (Maraun et al, 2003), they are considered the main decomposers of soil organic matter (Schneider et al, 2005; Erdmann et al, 2012). Thus, nutritional quality of litter directly benefited Oribatid mites (Gergócs et al, 2015), since fertilization increased P, K, Cu, Fe, and Zn levels in organic matter deposited in soil (Table 3).

For the treatment without lime, the higher number of Collembola Arthropleona in litter can be related to food preference by fungi (Higvar and Amundsen, 1981; Sadaka-laulan et al, 1998; Maraun et al, 2003). Fungi are the main microorganism of the soil food web with litter low quality (Wardle et al, 2004) and soil with high acidity (Souto et al, 2008; Rousk et al, 2009), as was true for the soil in this study (Table 1). In addition, use of lime decreased ectomycorrhiza fungi in *P. taeda* litter in the same area. Therefore, we infer that liming indirectly decreased Collembola Arthropleona by decreasing fungi development in litter. All study results indicated that adding lime to *P. taeda* L. systems can shift nutrient cycling in soil and litter, since organic matter decomposition by edaphic mesofauna is directly related to chemical composition of litter (Reeleder et al, 2006; Yang and Chen, 2009; Silva et al, 2011).

Principal component analysis (PCA) also demonstrated that differences in trophic structure were influenced by treatment (Figure 2). In the summer (Figure 2A), principal component 1 (PC1) explained 42 % of data variability, and PC1 was associated with separation among Collembola and litter Mg, Ca, Fe, and K. Principal component 2 (PC2) grouped Oribatid mites with Mn, Ca and Mg content and explained 25.6 % of mesofauna data variability. This result reinforces the importance of litter nutritional quality for Oribatid mites. Conversely, Collembola Arthropleona and Symphypleona correlated with P but not with soil bases. The winter season PCA (Figure 2B) exhibited the same tendency as summer, where Oribatid mites benefited from the high nutritional quality of litter, while Collembola benefited from nutrient poor niches.

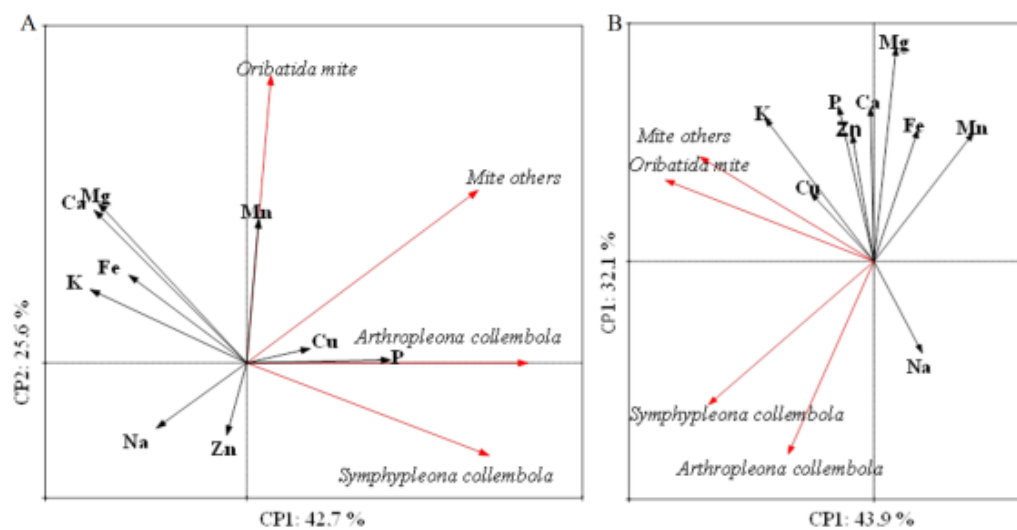


Figure 2 – Principal component analysis using edaphic mesofauna collected in the summer (A) and winter (B) as dependent variable (red arrow), and litter nutrient content as the environment explicative variable (black arrow).

In soil, Acari numbers were lower than found in litter (Figure 3). Collembola Symphypleona was only found in the complete fertilization and lime treatment, where population densities were 294 and 321 individuals·m⁻² in summer and winter, respectively. The lower population density in soil compared to litter was due to lower organic matter content in soil mineral horizons. For this reason, litter deposition in forest environments is essential for the survival of these edaphic arthropods (Rieff et al, 2016).

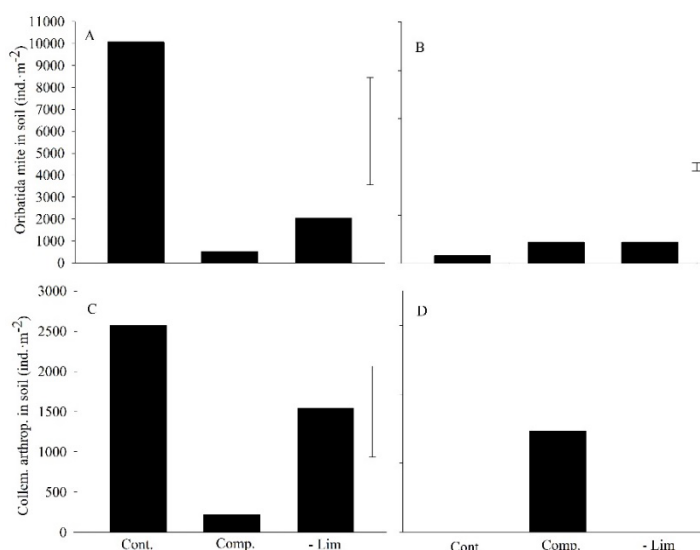


Figure 3 – Oribatid mites collected in summer (A) and winter (B) and Collembola Arthropleona (Collemb. arthrop.) collected in summer (C) and winter (D) in the Control (Cont.), complete with lime treatment (Comp.), and complete without lime (- Lim.). Horizontal bars represent the high significant difference according to the Tukey test with 5 % of probability.

In the summer, the control presented higher populations ($p < 0.05$) of Oribatid mites (10,068 individuals·m⁻²) and Collembola Arthropleona (2,572 individuals·m⁻²). Improvement in soil chemical properties (Table 2) can increase the activity of edaphic macrofauna (Lima et al, 2010), which leads to decreases in Oribatid mite populations from macrofauna predation (Erdmann et al, 2012), considering the low mobility of this Acari in soil. The negative effect of liming on the Collembola Arthropleona community can be related to decreased fungi (food source) in high fertility soil (Wardle et al, 2004). These results were confirmed by PCA of edaphic mesofauna (Figure 4), which showed a grouping of Collembola Arthropleona, Collembola Symphypleona, and Oribatid mites with soil acidity in the summer.

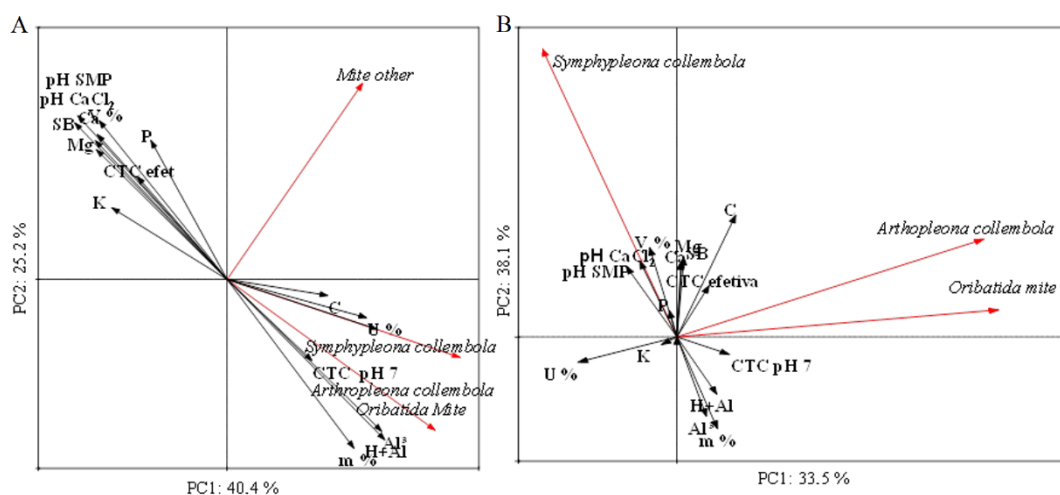


Figure 4 – Principal component analysis using edaphic mesofauna collected from soil in summer (A) and winter (B) as dependent variable (red arrow), and litter nutrient content as the environment explicative variable (black arrow).

Grouping of soil parameters for summer collection and edaphic mesofauna explained 40.4 % of all data variability, while PC2 explained only 25.2 % (Figure 4). Lower winter temperature decreased macrofauna predation (Lima et al, 2010), which lead to separation between Oribatid mites, Collembola Arthropleona, and the acidity component of soil. This was due to decreased predation activity of some macrofauna groups that eat Acari. PC1 explain 33.5 % of all data variability, while PC2 explain 38.1 %. The explanation for higher PC2 was due to the presence of Collembola Symphypleona only in the complete with liming treatment.

Thus, lime can change edaphic mesofauna populations. Change in the soil food web traits can lead to functional changes in soil organic matter decomposition that favor Oribatid mite development. While fungi development is favoured in low quality litter (Wardle et al, 2004) where the process of nutrient cycling slowed. This is due to the longer life cycle of fungi compared to bacteria



(Kooijman et al, 2016). Thus, changes in the soil food web must be considered for the development of a sustainable management plan for *P. taeda*.

4. Conclusion

Use of fertilization and lime on acidic soils cultivated with *Pinus taeda* L. changed litter quality, which consequently shifted the edaphic mesofauna community in both litter and soil. Seasonality was an important factor influencing edaphic mesofauna response. Winter decreased edaphic mesofauna populations, while fertilization did not affect mesofauna in this season. In summer, complete fertilization with lime favoured omnivores organisms in litter like Oribatid mites, while Collembola Arthropodea was impaired by fertilization and lime that limited the fungivory activity of Collembola. Although lime decreased Oribatid mites and Collembola Arthropodea in soil, findings indicated that litter was the primary habitat for edaphic mesofauna.

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