

Artigos

Role of soil fauna to litter decomposition in pine stands under Atlantic Forest biome

Papel da fauna do solo na decomposição da serapilheira em povoamentos de pinus sob bioma Mata Atlântica

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ABSTRACT

Litter production and its decomposition play an important role in the terrestrial carbon cycle and soil quality, being a crucial process involved in ecosystem productivity and functioning. Understanding this process under pine commercial stands or pine invasions is crucial to design forest management or ecosystems restoration. We conducted a study in a commercial pine plantation (*Pinus taeda* L.) under Atlantic Forest biome to investigate (1) the contribution of soil fauna to pine litter mass loss; (2) the litter fauna feeding activity within seasons. Litter bags with pine needles (5 g dry weight) were prepared as exclusion treatments for different sized soil fauna by varying mesh size (2 mm and 0.06 mm). Litter bags were removed after 60, 120, 180, 240, 300, and 365 days to estimate the decomposition rates. *Bait lamina* sticks were exposed seasonally and horizontally in soil surface to determine the fauna feeding activity on litter. We found: (1) no differences in litter mass loss and decomposition rate when soil macrofauna and most of the mesofauna were excluded; (2) feeding activity of litter organisms was higher in the summer, probably stimulated by a combination of high temperature and rainfall. In commercial pine stands, we can conclude that the nutrient cycling is slower than in natural areas, related to litter traits, as described in the scientific literature. Our results indicated that soil macro and mesofauna play a low role in the pine litter decomposition, where the organic material decomposition is more related to microorganism's activity.

Keywords: *Bait lamina*; Forest ecology; Litter bags; Soil invertebrates

RESUMO

A produção de serapilheira e sua decomposição desempenham um papel importante no ciclo do carbono terrestre e na qualidade do solo, sendo um processo crucial envolvido na produtividade e funcionamento do ecossistema. Compreender esse processo em áreas comerciais de pinus ou invasões de pinus é crucial para projetar o manejo florestal ou a restauração de ecossistemas. Conduzimos um estudo em uma plantação comercial de pinus (*Pinus taeda* L.) no bioma da Mata Atlântica para investigar (1) a contribuição da fauna do solo para a perda de massa da serapilheira; (2) a atividade alimentar da fauna edáfica na serapilheira dentro das estações do ano. As bolsas de decomposição com acículas de pinus (5 g, peso seco) foram preparadas como tratamentos de exclusão para a fauna do solo de diferentes tamanhos, variando o tamanho da malha (2 mm e 0,06 mm). As bolsas de decomposição foram removidas após 60, 120, 180, 240, 300 e 365 dias para estimar as taxas de decomposição. *Bait laminas* foram expostos sazonalmente e horizontalmente na superfície do solo para determinar a atividade de alimentação da fauna na serapilheira. Encontramos: (1) nenhuma diferença na perda de massa da serapilheira e na taxa de decomposição quando a macrofauna do solo e a maior parte da mesofauna foram excluídas; (2) a atividade alimentar dos organismos da serapilheira foi maior no verão, provavelmente estimulada por uma combinação de alta temperatura e umidade. Em plantios comerciais de pinus, podemos concluir que a ciclagem de nutrientes é mais lenta do que em áreas naturais, o que está relacionado às características das acículas, descritas na literatura. Nossos resultados indicaram que a macro e a mesofauna do solo desempenham um papel menor na decomposição da serapilheira em plantios comerciais de pinus, indicando que o processo de decomposição em tais povoamentos está mais relacionada à atividade dos microrganismos.

Palavras-chave: *Bait lamina*; Ecologia florestal; Bolsa de decomposição; Invertebrados do solo

1 INTRODUCTION

Species of *Pinus* are commonly planted for forestry purposes and have strong economic impacts on the local and national economies of many countries worldwide (CUBBAGE *et al.*, 2020). However, their impact on global biodiversity is also under concern, as wood production has increased and exotic species are currently considered as a choice to achieve high production levels (DODET; COLLET, 2012). Understanding ecosystem functioning in such systems is needed to improve economic and environmental aspects, as well as to establish recovering strategies for invasive areas.

Litter decomposition plays an important role in the terrestrial carbon cycle and soil quality. In forest ecosystems, litter production and its breakdown are part of the nutrient cycling, being a crucial process for the ecosystem productivity (HUANG; ZHAO;

LI, 2017). The environmental conditions and vegetation type are important factors in determining the quality and the amount of material that reaches the soil (MCCAY; CARDELÚS; NEATROUR, 2013). Studies have been performed to investigate the litter decomposition of pine species (HE *et al.*, 2020), discussing climatic conditions and litter characteristics that influence this process. However, there is no sufficient information about the role of macro and mesofauna groups in pine litter breakdown. Previous studies have shown the effects of *Pinus* species to understory plant species (FOCKINK *et al.*, 2020) and soil invertebrates' community (ORTIZ *et al.*, 2021) in pine plantation and invasions, which point impacts for ecosystems where *Pinus* spp. are non-native.

In productive systems, litter decomposition is the main route for the transfer of the vegetation nutrients to the soil, besides protecting the soil from erosion, supplying organic material and nutrient to soil fauna and plants, maintaining and improving the physical, chemical and biological properties of the soil (LIU *et al.*, 2017). The soil-litter macro and mesofauna have a crucial role in ecosystem functioning and soil quality, by their feeding and dig, acting on the fragmentation of plant residues, improving soil physical structure and chemical quality, and establishing relationships at different levels with microorganisms (improving and controlling microorganism's populations) (COYLE *et al.*, 2017). Although several studies embraced pine litter decomposition, further research is needed to establish more quantitative relationships among soil fauna and its role in nutrient cycling (NEHER; WEICHT; BARBERCHECK, 2012).

In this context, this study was designed to answer the following questions: (1) What is the contribution of soil macro and mesofauna to pine litter decomposition at commercial stands inside the Atlantic Forest biome? (2) What is the *P. taeda* L. litter decomposition rate in a plateau region in southern Brazil? (3) Does the soil fauna feeding activity differ among seasons in these systems and region? We hypothesized that, as pine litter has both physical and chemical properties that impair several soil invertebrates groups, and such material presents slow decomposition rates in all biomes, the exclusion of soil macro and mesofauna would be of low importance to

litter decomposition, which could indicate that this process is specially carried out by microorganisms. To test this hypothesis, we conducted a field experiment to investigate the comparative pine litter decomposition rates in litter bags with a mesh size that allowed and excluded the entrance of macro and mesofauna.

2 MATERIAL AND METHODS

2.1 Site description

The experiment was located in the vicinity of the Federal University of Santa Catarina (UFSC) at the municipality of Curitibanos, in the plateau region (987 m altitude) of Santa Catarina State, Brazil. The climate according to Köppen-Geiger is temperate (Cfb), humid mesothermal, and mild summer, showing an average annual temperature between 16°C and 17°C. The biome of the region is Atlantic Forest, composed of Mixed Ombrophilous Forest (Araucaria Forest). According to Barbosa *et al.* (2017), the predominant soil type is characterized as very clayed Aluminic Humic Cambisol. The study was conducted in a commercial pinus plantation of the species *Pinus taeda* L. (loblolly pine), owned by Agropastoril Gaboardi Ltda company. This plantation was established in 2005 (13 years old when our experiment was carried out) with an initial spacing of 2.5 x 2.5 m among trees, for timber production to the sawmill. Selective pruning was carried out in 2013 and 2017, but in this last period, the plots with the experiments were excluded. There was no use of chemicals in this area. The air temperature and rainfall data during the experiment were provided by the Meteorological Station of CIRAM/EPAGRI.

2.2 Evaluation of the litter decomposition by the litter bags method

Three plots of 10 m X 30 m were delineated (300 m² per plot), 10 m distant from each other, and 20 m from the edge. Recently pine needles which had fallen in collectors (1.0 x 1.0 m) were transported to the laboratory, where they were dried in

air circulation and renewal oven at 60°C, until reaching a constant weight (~7 days). Dry needles (5 g) were weighed in nylon bags (*litter bags*), with meshes of 0.06 mm and 2 mm, here named as fine and coarse mesh bags, respectively, with dimensions of 15 cm × 15 cm. The objective was to have bags that excluded the entrance of the edaphic macro and mesofauna (fine mesh) and bags that allowed the entrance of these organisms (coarse mesh), which allowed to monitor the decomposition with and without the presence of fauna. The bags in each plot were arranged in rows 0.50 m distant among them, and 0.30 m distant among bags.

The mechanical loss of needles until the arrival in the study area was estimated in ten bags, which returned to the lab for a new weighting on the same day. These values were used as a correction in the decomposition rate calculation, deducting this mechanical loss.

Decomposition bags were exposed for 365 days and removed after 60, 120, 180, 240, 300, and 365 days of exposure. In each removal time, five bags of each mesh were collected (randomly) per plot to estimate the decomposition rates. The reminiscent litter mass (%) was calculated by the expression $MR\% = (M_f / M_i) \times 100$. Where: MR% = dry weight percentage of remaining litter mass; M_f = dry weight of final litter mass; M_i = initial dry weight of litter mass.

After the remaining mass calculation throughout the period, the k decomposition constant was calculated, according to Thomas and Asakawa (1993), with the exponential model: $X_t = X_0 \cdot e^{-kt}$. Where: X_t = remaining dry material weight after t days; X_0 = weight of the dry material placed in bags at zero time ($t = 0$). To calculate the k decomposition constant, the total period of exposure (365 days) was taken into account, therefore, data from the last sampling were used for this purpose.

The half-life time ($t_{1/2}$) was calculated according to Rezende *et al.* (1999), which expresses the time required for half of the residues decomposing or for half of the

nutrients contained in these residues being released, the time equivalent to the decomposition of 95% of the residue was also estimated, considering the Equation (1) and (2):

$$T_{1/2} = \ln(2) / K \quad (1)$$

$$T_{95\%} = 3 / K \quad (2)$$

In where: $\ln(2)$ and 3 is a constant value and K = constant of decomposition calculated by the previous formula ($X_t = X_o \cdot e^{-kt}$).

2.3 Feeding activity of soil fauna by the *bait lamina* method

The *bait lamina* method standardized by ISO 18311 (ISO, 2016) was used to evaluate the feeding activity of the litter organisms. This method consists of plastic laminas with 120 mm in length and 1 mm in thickness, containing 16 holes, each with 2 mm of diameter and with a distance between them of 5 mm. The lamina holes were manually filled with an organic mixture containing 70% of cellulose, 27% of wheat flour, and 3% of active carbon. The litter fauna feeding activity was evaluated with the *bait lamina* method in the four seasons of the year; however, we chose to use the laminas horizontally (Figure 1), i.e. on the litter and not as commonly used, buried in the ground. The bait material does not necessarily mimic the complexity of the natural litter material, but they provide a standard method of comparison across seasons.

A total of 96 *bait laminas* was used for each season of the year, which were distributed among the plots. Each plot received 32 *bait laminas*, distributed in three groups of 8 *bait laminas* each. Horizontal *bait laminas* were attached to the surface with a wire in the shape of a reversed "U". The laminas were exposed in the field for 30 days. The laminas were carefully removed and visually examined against the light, counting and recording the holes with more than 30% of consumption.

Figure 1 – *Bait lamina* sticks arranged horizontally on the pine litter layer in pine stand

Source: Authors (2018)

2.4 Data analysis

Normality and homoscedasticity of litter bags and *bait lamina* data were checked by Shapiro-Wilk e Bartlett tests. The litter decomposition data (remaining % for each mesh size and exposure period) were subjected to Variance Analysis (ANOVA) two-way, following a randomized block design in a factorial scheme (treatment x time), by the statistical software R (R CORE TEAM, 2018). The Tukey test ($p < 0.05$) was adopted for comparing the averages, and the Pearson Correlation Coefficient to determine the linear correlation coefficient between the litter mass loss and two qualitative variables (temperature and rainfall). The consumption percentage of the *bait lamina* was compared among the seasons using the non-parametric Kruskal-Wallis test ($p < 0.05$).

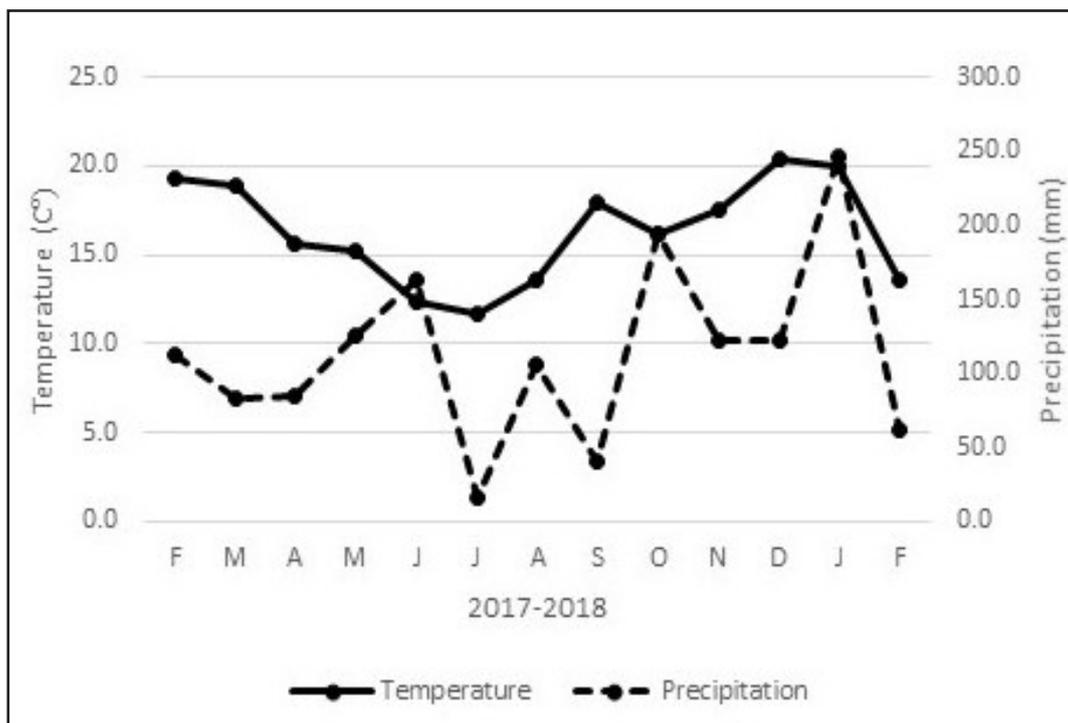
3 RESULTS AND DISCUSSION

3.1 Climatic conditions

The climatic conditions during the litter bags and *bait lamina* exposure were according to the expected for the period. The total rainfall was 1378.2 mm, with

maximum values in October 2017 (194 mm) and January 2018 (245 mm), and the lowest in July (15.2 mm) and September 2017 (40.8 mm). The highest average temperature occurred in December 2017 (20.3°C) and the lowest in July 2017 (11.7°C) (Figure 2).

Figure 2 – Average monthly values of temperature and precipitation in the study area. Data from Meteorological Station of CIRAM, EPAGRI, SC, Brazil



Source: Authors (2018)

3.2 Litter decomposition

The litter mass loss is shown in Figure 3. No statistically significant difference was found by the Tukey test ($p < 0.05$) between mesh sizes; however, statistical differences were observed in the sampled times, within each mesh size, i.e. including or excluding the edaphic macro and mesofauna (Table 1). After 365 days, the total litter mass loss corresponded to 43.2% in mesh 2 mm and 45.4% in mesh 0.06 mm, not differing significantly among them.

Table 1 – Reminiscent litter mass in litter bags excluding (fine mesh, 0.06 mm) or not (coarse mesh, 2.00 mm) soil macro and mesofauna, exposed in a pine plantation, compared by two-way ANOVA (mesh x time), followed by Tukey test ($p < 0.05$)

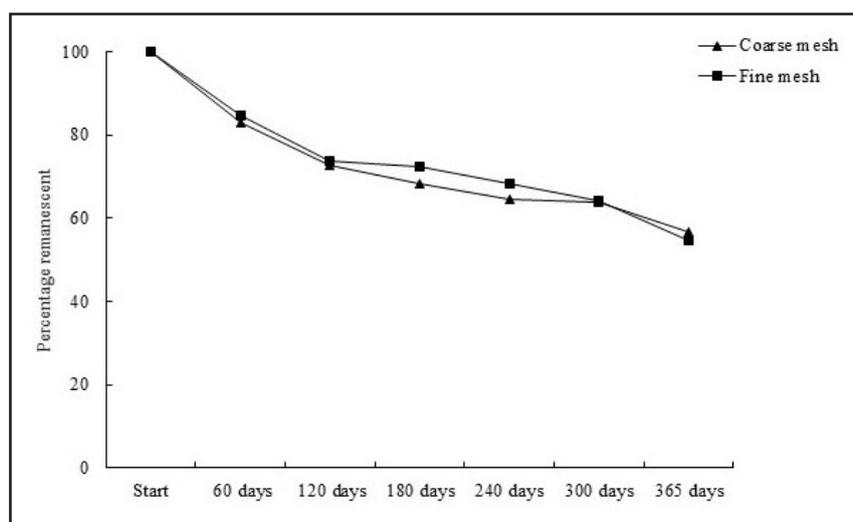
Litter bags mesh size	Time (days)					
	60†	120	180	240	300	365
0.06 mm ‡	83.0 Aa	72.6 Ba	68.2 Ba	64.5 Aba	63.9 Aba	54.6 Ca
2.00 mm	84.6 Aa	73.7 Ba	72.2 Ba	68.4 Aba	64.2 Aba	56.8 Ca

Source: Authors (2018)

In where: Averages followed by the same lowercase letters in the column not differ, by the Tukey Test ($p < 0.05$); Averages followed by the same uppercase letters in the lines do not differ by the Tukey Test ($p < 0.05$).

Regarding the decomposition dynamics, the mass loss was higher in the first 120 days, for both mesh sizes (Figure 3). When studying the correlation between rainfall and temperature with litter mass loss, no significant correlations were observed by Pearson Correlation Coefficient analysis: for rainfall and the coarse mesh bags, $p=0.78$ and $R=0.14$; rainfall and fine mesh bag, $p=0.59$ and $R=0.27$; temperature and coarse mesh, $p=0.92$ and $R=0.05$; temperature and fine mesh, $p=0.69$ and $R=0.20$.

Figure 3 – Reminiscent litter mass (%) in litter bags excluding (fine mesh, 0.06 mm) or not (coarse mesh, 2 mm) soil fauna, exposed on the litter layer of a *Pinus taeda* stand



Source: Authors (2018)

The estimated time to decompose 50% and 95% of the total litter mass is presented in Table 2. According to this calculation, the average time of litter renewal would be higher than 1 year for both treatments.

Table 2 – Decomposition constant (K) and the time (days) required for decomposition of 50% ($T_{1/2}$) and 95% (T 95%) of *P. taeda* litter mass, excluding (mesh 0.06 mm) or not (mesh 2.00 mm) the main soil macro and mesofauna groups

Litter bags mesh	kg g ⁻¹ day ⁻¹	T _{1/2} (days)	T 95% (days)
0.06 mm	0.0017	418	1810
2.00 mm	0.0015	448	1937

Source: Authors (2018)

The presence of large fauna has a recognized impact on decomposition in terrestrial ecosystems around the world (a review in HANDA *et al.*, 2014). While soil fauna has an important role to organic material decomposition in the rainforest, influencing N and C cycles (e.g., YANG; CHEN, 2009), and it is the main driver on *Pinus massoniana* litter decomposition and breakdown of its refractory compounds (ZHANG *et al.*, 2019), our field experiment using litter bags showed no significant differences in litter mass loss when macrofauna and most of the mesofauna were excluded. Our results showed that soil macro and mesofauna play a low role in the pine litter decomposition, indicating that the organic material decomposition in pine stands is more related to microorganism's activity. Besides microflora (e.g., bacteria, fungi), the role of microfauna cannot be excluded, as these small organisms could enter in the fine mesh bags. Neher, Weicht and Barbercheck (2012) showed that litter decomposition and N availability in pine forest soils were correlated with microarthropods and nematodes communities. In the present study, we assumed that decomposition in fine mesh litter bags could involve both microflora and microfauna groups.

The role of litter fauna on litter mass loss is dependent on litter composition (AUGUSTO *et al.*, 2014). Low-quality litter can show significantly lower fauna effects

in the decomposition process (LI *et al.*, 2015a). Pine species including *P. taeda* are recognized as low quality showing slow decomposition rates, which depends on climatic conditions and its effects on microbial growth rate (OLSSON *et al.*, 2019). Higher decay in litter reminiscent material at the beginning of the process can be related to the decomposition of water-soluble compounds and some labile organic components, while the lower decay in late stages is related to recalcitrant components (ZHANG *et al.*, 2019). Needles of coniferous species contain more recalcitrant compounds such as cellulose, hemicelluloses, lignin, and tannins when compared with broad-leaved tree species (GUO *et al.*, 2009), besides needles being a hard material to be breakdown by soil faunal groups, which justify the main role of microorganisms, especially fungi (PURAHONG *et al.*, 2014), in this process.

The litter moisture, provided by microclimate, can be an important factor in the decomposition process. Litter from the fine mesh litter bags presented visually higher moisture (with small drops of water) than that from the coarse mesh. Furthermore, the presence of fungal mycelia was pronounced inside the fine mesh bags. Similar characteristics like fungi mycelia were also observed by Gama-Rodrigues, Barros and Santos (2003) when studying the decomposition of species of the *Eucalyptus* genus. As the fine bags probably decrease the moisture loss, it could favor microorganism's activity and therefore affect the decomposition rates, according to local climatic conditions. On the other hand, the coarse mesh could favor the leaching and loss of mass. The authors concluded that different mesh sizes can be used to reliably quantify the role of soil macro and mesofauna in litter mass loss from litter bags and that the loss of material in the coarse mesh is related to these organisms by promoting litter decomposition as well as due to greater litter fragmentation, losing undecomposed fragments from the bags.

Decomposition constant and the time to decompose 50 and 95% of *P. taeda* litter indicated a slow process for this species at the study site. Litter mass loss and its chemical composition are dependent on both plant species and climate conditions

(LI *et al.*, 2015b). Several authors discussed that increasing temperature can improve the pine litter decomposition (SALAH; SCHOLLES, 2011), enhancing biological activity and degradation of chemical compounds such as hemicellulose (COÛTEAUX; ALOUI; BESSON, 2002).

Management of the forest stands can also influence the colonization of the litter layer in stands (MORRISON, 2003). When the pruning and trimming are not performed, the forest canopy protects the litter from solar incidence, enabling a more humid microclimate, stimulating the decomposition. However, in stands where a management system is conducted or even where there are natural deaths of some trees, there is a higher incidence of sunstroke on the litter and soil, thereby changing the abiotic conditions, which influence on the decomposition process.

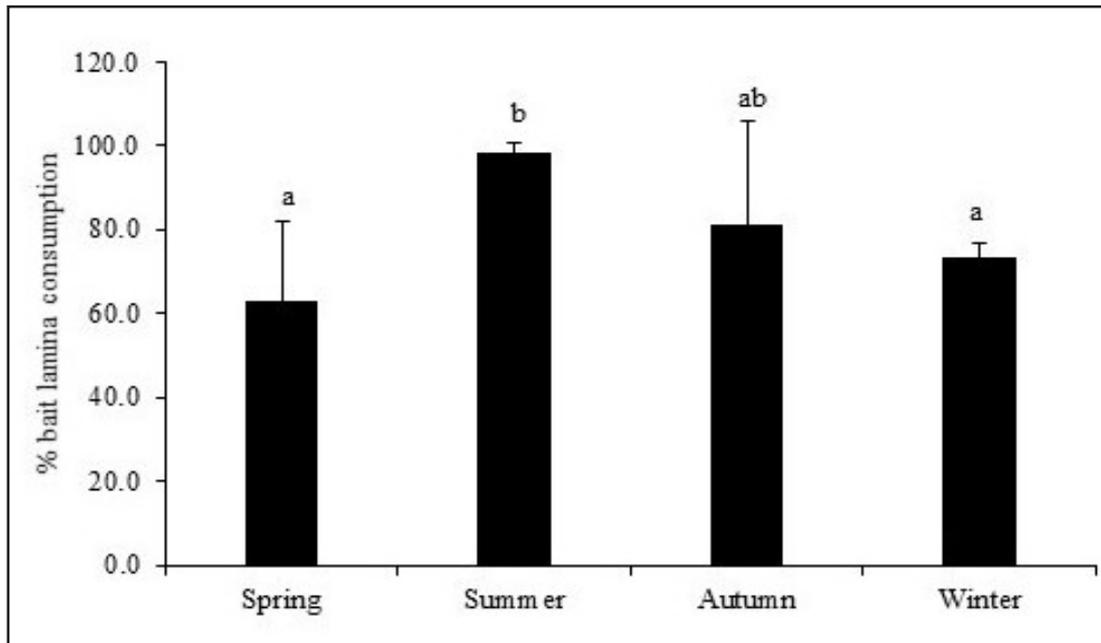
3.3 Feeding activity of soil fauna

The exposure period of 30 days proved to be efficient to get high decomposition rates of the *bait lamina* on the litter layer. The highest percentages of baits consumption were observed in summer (98.18%), followed by autumn (81.08%), while a reduced activity was observed in the spring (62.93%) and winter (73.09%). Statistical differences were observed between summer, spring, and winter. The results are shown in Figure 4.

Concerning *bait lamina* consumption, results indicated the higher feeding activity of litter organisms in summer, which corresponds to the warmer season combined with high humidity, as usual in this biome (Atlantic Forest/Mixed Ombrophilous Forest) and confirmed by the climatic data. In general, *bait lamina* consumption represents the feeding activity of both soil invertebrates and microorganisms (FILZEK *et al.*, 2004), being a recognized methodology for this purpose around the world, standardized by ISO and applied in forest areas (e.g., KLIMEK *et al.*, 2015; SANTANA *et al.*, 2018). The results showed the positive effect of high temperature and humidity in summer promoting a higher soil fauna activity (ROZEN *et al.*, 2010). Cold conditions or drought can reduce the fauna activity on litter surface, as a result of the migration of invertebrates to more

deep soil layers, searching by optimal conditions, or even causing the death of some faunal groups (SILVA *et al.*, 2013).

Figure 4 – Mean values (\pm SD) of *bait lamina* consumption (%) representing the litter organisms feeding activity within seasons, after 30 days of exposure on pine litter layer



Source: Authors (2018)

In where: Averages followed by the same lowercase letters did not differ (Kruskal-Wallis, $p < 0.05$).

The *bait lamina* test proved effectiveness when arranged on the surface (horizontally) to determine the litter organisms feeding activity on the litter surface, as seen in Podgaiski, Silveira and Mendonça Junior (2011). In pine stands, it was necessary to use an extended time of exposure (30 d) to achieve at least 40% consumption, when compared to studies carried out in other Brazilian biomes (e.g., ROMBKE *et al.*, 2006 in Central Amazonia). Podgaiski, Silveira and Mendonça Junior (2011) found similar results among vertically and horizontally exposed bait lamina in pasture areas in south Brazil, where the baits on soil surface showed slightly higher activity than the buried ones, probably related to high activity of surface organisms.

Changes in abiotic conditions caused by thinning, pruning, fire, or fragmentation can modify the litter organism's activity and consequently the decomposition dynamics, e.g., increasing light entrance and consequently increasing temperature and decreasing moisture. Therefore, climatic conditions and management practices should be considered when more accurate estimative is desired involving litter lifetime or nutrient cycling in pine stands or invasions.

Understanding forest ecosystem functioning is essential to wood production as well as conservation purposes (HANDA *et al.*, 2014). In general, the findings confirmed that forest stands of this exotic tree species do not serve as a food supply to maintain soil fauna biodiversity under Atlantic Forest biome, and, consequently, *P. taeda* stands do not promote soil fauna biodiversity and its associated functions in the soil ecosystem. Our finds bring some contribution to predicting the consequences of exotic trees stands for soil quality and biodiversity, and under climate global change.

4 CONCLUSION

Our results provided novel insights into pine litter decomposition dynamics and detritivore feeding activity in pine stands: (1) no significant differences in litter mass loss and decomposition rate when soil macrofauna and most of the mesofauna were excluded; (2) feeding activity of litter organisms was higher in the summer, probably stimulated by a combination of high temperature and rainfall. In general, we can conclude that soil macro and mesofauna play a low role in the pine litter decomposition, indicating that the organic material decomposition in pine stands is more related to microorganism's activity. In commercial pine stands, we can conclude that the nutrient cycling is slower than in natural areas, related to litter traits, as described in the scientific literature. Furthermore, these findings could be extrapolated to invasion areas, indicating that this exotic tree species could not serve as a food supply to maintain soil fauna biodiversity under Atlantic Forest biome, and, consequently, it could impair the soil ecosystem functioning in natural areas.

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