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Invertebrate Community of Scots' pine Coarse Woody Debris in the Southwestern Pyrenees under Different Thinning Intensities and Tree Species

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1. Introduction

Coarse woody debris (CWD) is very important for forest ecosystems due to its multiple ecological functions, such as regulating carbon and nutrient cycles and providing different structural properties to forest soils, such as affecting sediment transport storage and water retention. Previous works have also emphasized the importance of CWD for species diversity conservation.CWD is usually divided into five different decay classes from fresh (class 1) to fully decomposed (class 5) (Waddell, 2002). Chronologically, freshly woody debris is colonized by fungi and xylophagous insects that disperse these fungi (Hanula, 1996), followed by Diptera larvae, mites, and centipedes and coleopteran predators (Savely, 1939). Consequently, greater abundances of xylophages and their predators are found in the initial decomposition classes. As decomposition proceeds, social insects such as ants or termites excavate their nests (Harmon *et al.*, 1986) feeding on fungi and broken sapwood. Hence, saprophages and parasitoids are more abundant in intermediate stages (Harmon *et al.*, 2020; Magnússon *et al.*, 2016). In the most advanced stages, fungi, organisms that feed on rotten wood and their predators become dominant (Savely, 1939; Fager, 1968; Vanderwel, 2002).

CWD production rates can be influenced by forest management practices (Magnússon *et al.*, 2016; Radtke et al., 2009). Forest thinning is commonly used to control stand density and productivity (Fujimori, 2001), and to manage competition and nutrient cycling in forests, which is particularly important in mixed forests (Primicia *et al,* 2013; González de Andrés, 2019). Previous research, conducted by our team in mixed forests of Scots pine (*Pinus sylvestris* L.) and European beech (*Fagus sylvatica* L.) in the Southwestern Pyrenees, has found that forest thinning reduces litter biomass and nutrient reserves, alters litter production and decomposition rates (Blanco *et al.*, 2008; Martinez, 2015) decreases the volume and biomass of fallen woody debris (Martinez, 2015; Herrera-Alvarez, 2015), and changes the microclimate (Primicia *et al.*, 2013).

Based on this background, it is reasonable to think that, if in the forests object of this study (temperate mixed Scots pine and European beech forests) litter invertebrates have been affected by forest management and canopy type (Jabat, 2006), woody debris colonization by invertebrates may have been altered. The main objective of this research was to study the colonization process by mesofauna in CWD after applying different thinning intensities, as well as to determine whether the type of canopy and the decomposition class of CWD could influence the abundance, richness, and diversity of invertebrate present. In this context, it is expected that certain abiotic variables such as water content of woody debris and type of canopy cover under different intensities of thinning can explain the results found.

2. Materials and Methods

2.1. Study Area

This research was carried out in a mixed natural forest in the Southwestern Pyrenees (Navarre Province, Northern Spain), being the dominant tree species Scots pine and as codominant species European beech. These two species are the most important for European silviculture, and they share their distribution zones in large regions.

2.2. Experimental Design

The experimental design consisted of nine 40 × 30 m plots. Three treatments were replicated three times: control (0% of basal area removed); intermediate thinning (20% of the Scots pine basal area eliminated in 1999 and again in 2009), and intense thinning (30% of the Scots pine basal area was removed in 1999 and 40% in 2009). In 1999, the stumps were left on the ground and most of the trunks and cut branches were removed (Blanco *et al.,* 2005). In 2009, the stumps were left on site but the trunks and large branches were removed for commercial use, leaving the rest of the harvested material in the plots. Each plot was mapped into two sub-plots depending on the types of arboreal canopy: composed of pure pine crowns or composed of a mixture of pine and beech crowns (and other broadleaved species if present).

2.3. Samples Collection and Laboratory Work

Thirty-six samples of pine woody debris were collected in late April 2015. The decay classes collected were class 3 and 4 according to the classification by Waddell (2002). These samples were divided according to the definition by Harmon (1986), and were randomly collected under thinning treatments such as control, intermediate (20%) and intense (40%) thinning. Eighteen samples were collected under pure pine canopy, and 18 under mixed canopy of pine, beech and other deciduous, collecting one sample of each decay class in each sub-plot. Collected samples were approximately wood cylinders 10 cm long and 5 in diameter. In the laboratory, these samples were weighed (to ± 0.1 g) as fresh weight and then placed in a Berlese–Tullgren funnel (a 40 W lightbulb on top of a sample container 15 cm length × 8 cm diameter with 2 mm nylon mesh at the bottom) for mesofauna extraction for six days, collecting the mesofauna in glass containers with 70% ethanol. After extracting the mesofauna, woody samples were dried for 72 h in an oven at 70 °C and weighed. Water content percentage of coarse woody debris was determined by the difference of fresh and dry weight.
The contents of each container were analyzed with a stereoscope, the invertebrates being identified with a key guidebook Barrientos (1988) and following a list of taxonomic groups of invertebrates previously found in leaf litter at the same plots by Jabat (2006). In this way, the invertebrates found were classified according to their order and some even suborder. Total abundance of each taxonomic group was divided by sample weight.

2.4. Data Analysis

We estimated total abundance, richness and the Shannon–Wiener diversity index per sample. The effects of thinning, canopy type and CWD decay class on total abundance of macro and mesofauna, species richness, Shannon–Wiener index, and CWD water content were analyzed using the nonparametric Kruskal–Wallis test, as data did not follow a normal distribution. Generalized mixed models (GLM) with Poisson distribution were used to analyze the interactions between thinning intensity, canopy type, and CWD decay class for the variables of water content of CWD and total abundance of invertebrates per gram of decayed wood. Principal component analyses (PCA) were performed considering the variables that represented the variance in a greater percentage, being such variables: CWD water content, total abundance per gram of oribatid mites, other mites, Collembola, immature stages of macrofauna (larvae), immature mites, Coleoptera, and total abundance. The software used in this research was R Studio version 3.1.3. (RStudio, 2020) and IBM SPSS Statistics version 25 (IBM Corporation, Armonk NY, USA, 2017).

3. Results

3.1. Invertebrate Community Composition

We registered a total of 8348 individuals belonging to 19 taxonomic groups in all different treatments combined (Table 1). The largest number of individuals (accounting for 96.75%) belonged to the mesofauna, being dominated by the Acariform order with 80.07%, subdivided into oribatid mites (19.11%), other mites (14.52%), and immature mites (46.44%) (Table 1). The Collembola order was also one of the most abundant taxonomic groups with a percentage of 16.67%. Nematodes had the lowest total abundance (0.01%) within the mesofauna. The macrofauna only represented 3.28% of the total number of individuals, subdivided into the classes Insecta (1.09%), Arachnida (0.19%), Chilopoda (0.19%), Pauropoda (0.07%), Oligochaeta (0.04%), Symphyla (0.24%), and larvae (immature stages of macrofauna) (1.43%) (Table 1).

Table 1. Total number of individuals of the taxonomic groups identified in decomposing woody debris, the taxa are classified by size (mesofauna and macrofauna). Cl.: class, Sb. Cl.: sub-class; O.: order; Fil.: Filum. In bold the taxonomic groups divided in Fil and Class.

Taxonomic Group	No. of Individuals	Fraction (%)
All samples	8348	100
Mesofauna	**8077**	**96.75**
Cl. Arachnida	**6684**	**80.07**
Sb. cl. Acarina		
Super O. Acariform	6684	80.07
O. Oribatid	1595	19.11
Other mites	1212	14.52
Immature mites	3877	46.44
Cl. Entognatha	**1392**	**16.67**
Sb. cl. Collembola		
O. Collembola	1392	16.67
Fil. Nematoda	1	0.01
Macrofauna	**271**	**3.25**
Cl. Insecta	**91**	**1.09**
Sb. cl. Pterygota		
O. Diptera	17	0.17
O. Thysanoptera	4	0.05
O. Coleoptera	44	0.53
O. Hymenoptera	25	0.3
O. Hemiptera	1	0.01
Cl. Arachnida	**16**	**0.19**
O. Pseudoscorpionida	1	0.01
O. Araneae	15	0.18
Cl. Chilopoda	**16**	**0.19**
O. Geophilomorpha	9	0.11
O. Lithobiomorpha	9	0.11
Unidentified Chilopoda	3	0.04
Cl. Pauropoda	**6**	**0.07**
Cl. Clitellata	**3**	**0.04**
Sb. cl. Oligochaeta	3	0.04

Cl. Symphyla	**20**	**0.24**
Immature macrofauna stages (larvae)	**119**	**1.43**

3.2. Influence of Treatments in CWD Invertebrate Community

Total abundance and species richness decreased with thinning intensity although no significant differences were found between treatments. No clear patterns for the Shannon–Wiener index were found (Table 2). Interestingly, in spite of not detecting direct significant effects of thinning, GLM results indicated a significant interaction between thinning intensity and decay class (D_{df2}= 3.557, p = 0.050), for which CWD in heavily thinned plots had the lowest invertebrate abundance.

Species richness was significantly higher in mixed patches than in pure pine patches (X^2_{df1}= 4.016, p= 0.031). However, no significant differences between canopy types were found for total abundance and the Shannon–Wiener index (Table 2). In addition, GLM results indicated that the interaction between pure pine canopy and decay class significantly affected total abundance, with the highest decomposed CWD (class 4) under pine canopy having the lowest abundance (Z = 2.148, p= 0.032).

Although the Shannon–Wiener index was not significantly different between decay classes, total abundance (X^2_{df1}= 16.400, p < 0.001) and richness were significantly influenced by them (X^2_{df1} = 7.123, p = 0.008). Regarding water content, it was significantly higher in class 4 than in class 3 (X^2_{df1} = 8845, p = 0.003) (Table 2).

Table 2. Mean and SE number of captured individuals per gram of CWD in different treatments of thinning intensity, canopy type, and CWD decay class (significant differences at *p* < 0.05 in bold, *n* = 36).

Variable		**Water content (%)**	**Total abundance (individuals g^{-1})**	**Richness (number of taxa)**	**Shannon–Wiener Index**
Thinning Intensity	**0%**	56.10 ± 11.63	5.46 ± 6.52	6.83 ± 2.37	1.20 ± 0.28
	20%	57.84 ± 20.20	4.14 ± 5.44	6.33 ± 2.35	1.11 ± 0.29
	40%	45.54 ± 11.51	3.04 ± 3.93	5.83 ± 1.99	1.17 ± 0.39
	p	0.695	0.671	0.571	0.742
Canopy Type	**Mixed**	60.45 ± 8.75	3.97 ± 4.91	7.08 ± 1.62	1.07 ± 0.35
	Pure Pine	60.20 ± 17.04	3.20 ± 4.61	5.08 ± 2.19	1.18 ± 0.40
	p	0.462	0.155	**0.031**	0.837
CWD Decay Class	**Class 3**	31.27 ± 8.33	1.32 ± 1.67	5.42 ± 1.98	1.13 ± 0.34
	Class 4	89.47 ± 13.30	5.86 ± 5.63	6.75 ± 2.78	1.14 ± 0.35
	p	**0.002**	**<0.001**	**0.008**	0.899

4. Conclusions

Our results provide some of the first evidence of the interactive effects that overstory composition and thinning can have an impact on CWD invertebrate taxonomic groups and richness in mixed forests of the two European tree species more widely distributed (*Pinus sylvetris* and *Fagus sylvatica* L.). As thinning and type of canopy cover modifies the moisture–radiation–wind balance in the forest soil, CWD moisture content seems to be reduced when tree density reduction crosses a threshold around 20% of initial basal area. Such change can affect differently invertebrate taxa, with some being more sensitive to moisture reduction than others are.

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