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# Bioassay

# Do restoration techniques and types of weed control influence the composition of edaphic entomofauna?

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**Abstract.** Agroforestry systems (AFS) are generally referred to as systems where perennial plants are associated with annual or perennial crops, spanning from relatively simple agroforestry systems characterized by only two associated species to very complex ones, close to natural systems. However, there are few studies on edaphic entomofauna in newly implanted restoration systems. We investigated the composition of edaphic entomofauna in areas managed under different restoration techniques (agroforestry system or mixed-planting) and types of weed control (chemical or mowing). In each treatment, we performed collections with pitfall traps in July and December 2017. A total of 11,727 specimens distributed in 11 orders and 45 families were collected. Most of the individuals collected were Collembola (53.86%) and Hymenoptera: Formicidae (31.50%). No significant interaction was observed between restoration techniques and types of weed control combined. However, for abundance, both restoration techniques and types of weed control were individually significant, with chemical control showing a higher abundance in relation to mowing, and agroforestry concerning mixed-planting. There was no significant difference in richness in any of the variables studied. We conclude that the agroforestry system and the chemical control can be viable for edaphic entomofauna, although future research is necessary to evaluate the dynamic of edaphic entomofauna during the development of agroforestry systems.

**Keywords**: insects, forest restoration, pitfall traps, shade tree, diversity.

The world's population growth and the change in diet composition related to welfare levels are putting pressure on agriculture to produce food that meets human needs (Odegard & van der Voet 2013). As a result, human activities to convert forests into agricultural lands and pastures have increased, significantly changing their composition and structure, as well as available resources (Armenteras et al. 2013).

To mitigate this impact, it is necessary to integrate the development of societies with the maintenance and restoration of degraded ecosystems (Perring et al. 2015). Ecological restoration is the process that objective to assist the recovery of an ecosystem that has been degraded, leading the ecosystem to a recovery trajectory, thus allowing the adaptation and maintenance of the component species to local and global changes (Gann et al. 2019). Among the ecological restoration techniques, the Agroforestry Systems (AFS) are multifunctional systems that can provide a wide range of economic, sociocultural, and environmental benefits (FAO 2017), allowing the conservation of areas, maintenance of agricultural production, and food security of communities (Moreno-Calles & Casas 2010; FAO 2017).

In addition to vegetation, ecological restoration implies the recovery of the entire set of attributes of the original ecosystem (Amazonas et al. 2018), such as the entomofauna community. However, little is known about insect dynamics in early areas of AFS, which has remained relatively understudied (Bos et al. 2007; Dantas et al. 2012; Mazón et al. 2018; Aquino et al. 2020; Paiva et al. 2020). We hypothesized that different restoration techniques and types of weed control change the composition of edaphic entomofauna.

The study was carried out in an early forest restoration area (about 1.38 ha) of the Federal University of São Carlos in Araras, São Paulo, Brazil (22°31'15"S; 47°38'38"W; 696 m altitude). The regional climate is classified as mesothermic Cwa, with an average annual temperature of 20.3 °C, with hot and humid summers, cold and dry winters, and annual precipitation of 1,300 mm (Alvares et al. 2013). The vegetation type is the Seasonal Semideciduous Forest (Amazonas et al. 2018). Sugarcane was cultivated until 2017.

Eight plots with six rows of 1,080 m² each were designed. Four plots correspond to an agroforestry system containing seedlings of 10 pioneer and secondary native tree species (Tab. S1, Supplementary online material), and Catuai 144 coffee cultivar. Crops were rotated with soybean cultivar Syngenta Intacta, sorghum cultivar BRS716 BM-757, and carioca beans. The spacing between the trees is 2 m, meaning that each plot has 120 plants, with interspersed coffee planting and 6 m between rows. The other four plots correspond to a mixed-planting (MP) containing seedlings of 20 native tree species (each plot has 240 plants), with 3 x 2 m spacing. The plots were implanted in February 2017.

In each of the plots, in addition to the restoration technique (agroforestry or mixed-planting), a different type of weed control (chemical or mowing) was applied, using a completely randomized design in a 2 x 2 factorial scheme, with the treatments (two replications for each): agroforestry system - chemical weed control (AFc), agroforestry system - weed control by mowing (AFm), mixed-planting - chemical weed control (MPc) and mixed-planting - weed control by mowing (MPm). The chemical control was performed at 90 days (Glyphosate, Roundup™), at 180 days (Paraquat, Gramoxone 200™), and with a single application of glyphosate + metsulfuron-methyl (Zartan™) in September 2017. The weed control by mowing was performed every 90 days.

The edaphic entomofauna was collected in July 2017 (dry season) and December 2017 (rainy season). Five pitfall traps were distributed along a 20 m transect within each plot. The traps remained in the field for 120 hours before being collected (Machado et al. 2015). They were made with 10 × 12 cm plastic cups (diameter × depth and volume of 500 mL) containing 250 mL of a 4% formaldehyde solution with a few drops of a neutral detergent (Brown & Matthews 2016). All collected material was placed in individual plastic containers containing 70% alcohol for further screening and identification.

The specimens were identified using Rafael et al. (2012) and Fujihara et al. (2016), at the taxonomic level of order or family. According to Vanin (2012), the order Collembola belongs to class Ellipura, however, it was considered in our study due to its fast response to environmental characteristics (Ortiz et al. 2019). The specimens were named according to Rafael et al. (2012).

The data were analyzed using the faunal indexes proposed by Silveira Neto et al. (1995), based on the calculations of constancy, abundance, dominance, and frequency indexes. Besides, the Shanon Wiener diversity index and Pielou equitability were calculated. For the analyses, the ANAFAU software was used (Moraes et al. 2003).

Abundance and richness were used to determine the effect of factors: restoration techniques (AFS versus MP) and types of weed control (chemical versus mowing) within their respective areas. For each of the variables considered, the assumptions of normality and homogeneity of the variances were verified, using the Shapiro-Wilk and Levene tests, respectively, at the 5% significance level (Levene 1960; Shapiro & Wilk 1965). For the abundance data, the absence of the normality assumption and homogeneity of the variances ($p < 0.01$) were observed. Thus, a Box-Cox transformation (Box & Cox 1964) was performed on the data set. For the analysis of variance (ANOVA) and subsequent Tukey test, the statistical software RStudio version 3.5.3 (R Core Team 2019) was used.

A total of 11,727 specimens from 11 orders and 45 families were collected. Most of the individuals collected were Collembola (53.86%), Hymenoptera: Formicidae (31.50%), Coleoptera: Carabidae (3.53%), Diptera: Cecidomyiidae (1.62%), and Dermaptera: Labiduridae (1.13%). The Shanon Wiener diversity index and Pielou equitability showed the highest values in AFS compared to mixed-planting (Tab. 1). According to faunal indexes proposed by Silveira Neto et al. (1995), Collembola and Formicidae were superabundant, super dominant, super frequent, and constant in all treatments (Tab. S2, Supplementary online material).

**Table 1.** Total abundance and relative frequency (%) of edaphic entomofauna in an early forest restoration area in Araras, state of São Paulo, Brazil. AFc: agroforestry system - chemical weed control. AFm: agroforestry system - weed control by mowing. MPc: mixed-planting - chemical weed control. MPm: mixed-planting - weed control by mowing. RF: relative frequency. *Others: larva of Hymenoptera: Chalcidoidea; nymphs of Hemiptera: Cercopidae, Cicadellidae, Delphacidae, and Heteroptera.

| Class | Order | Family | AFc | AFm | MPc | MPm | Total | RF (%) |
|---|---|---|---|---|---|---|---|---|
| Ellipura | Collembola | | 2,037 | 2,882 | 811 | 586 | 6,316 | 53.86 |
| Insecta | Blattaria | Blattellidae | 0 | 0 | 0 | 0 | 1 | 0.0 |
| | Coleoptera | Carabidae | 118 | 58 | 91 | 147 | 414 | 3.5 |
| | | Chrysomelidae | 13 | 5 | 6 | 8 | 32 | 0.3 |
| | | Coccinellidae | 0 | 0 | 0 | 1 | 1 | 0.0 |
| | | Curculionidae | 0 | 0 | 1 | 1 | 2 | 0.0 |
| | | Elateridae | 2 | 0 | 0 | 0 | 2 | 0.0 |
| | | Lagriidae | 0 | 1 | 4 | 2 | 7 | 0.1 |
| | | Lycidae | 0 | 2 | 0 | 4 | 6 | 0.1 |
| | | Meloidae | 0 | 1 | 0 | 1 | 2 | 0.0 |
| | | Melyridae | 6 | 0 | 0 | 0 | 6 | 0.1 |
| | | Nitidulidae | 13 | 27 | 7 | 6 | 53 | 0.5 |
| | | Passalidae | 0 | 0 | 0 | 1 | 1 | 0.0 |
| | | Scarabaeidae | 8 | 6 | 0 | 2 | 16 | 0.1 |
| | | Scolytidae | 0 | 1 | 0 | 0 | 1 | 0.0 |
| | | Staphylinidae | 1 | 0 | 0 | 0 | 1 | 0.0 |
| | | Tenebrionidae | 17 | 16 | 4 | 11 | 48 | 0.4 |
| | Diptera | Agromyzidae | 0 | 6 | 1 | 2 | 9 | 0.1 |
| | | Cecidomyiidae | 69 | 39 | 32 | 50 | 190 | 1.6 |
| | | Chironomidae | 3 | 0 | 0 | 1 | 4 | 0.0 |
| | | Dolichopodidae | 2 | 2 | 1 | 1 | 6 | 0.1 |
| | | Lonchaeidae | 0 | 0 | 0 | 1 | 1 | 0.0 |
| | | Muscidae | 0 | 0 | 1 | 0 | 1 | 0.0 |
| | | Otitidae | 27 | 14 | 10 | 36 | 87 | 0.7 |
| | | Phoridae | 3 | 4 | 7 | 8 | 22 | 0.2 |
| | | Sciaridae | 35 | 12 | 10 | 16 | 73 | 0.6 |
| | | Tachinidae | 1 | 2 | 0 | 0 | 3 | 0.0 |
| | Dermaptera | Labiduridae | 56 | 58 | 13 | 6 | 133 | 1.1 |
| | Hemiptera | Alydidae | 0 | 2 | 4 | 9 | 15 | 0.1 |
| | | Aphididae | 4 | 20 | 6 | 8 | 38 | 0.3 |
| | | Cicadellidae | 1 | 4 | 5 | 15 | 25 | 0.2 |
| | | Coreidae | 0 | 0 | 0 | 11 | 11 | 0.1 |
| | | Largidae | 0 | 0 | 1 | 5 | 6 | 0.1 |
| | | Lygaeidae | 1 | 0 | 0 | 0 | 1 | 0.0 |
| | | Pentatomidae | 1 | 3 | 2 | 1 | 7 | 0.1 |
| | | Psyllidae | 0 | 0 | 0 | 1 | 1 | 0.0 |
| | | Reduviidae | 0 | 0 | 0 | 1 | 1 | 0.0 |
| | Hymenoptera | Formicidae | 917 | 1,188 | 523 | 1,066 | 3,694 | 31.50 |
| | | Ichneumonidae | 2 | 0 | 0 | 0 | 2 | 0.0 |
| | | Pompilidae | 0 | 1 | 0 | 0 | 1 | 0.0 |
| | Lepidoptera | Gelechiidae | 3 | 4 | 2 | 3 | 12 | 0.1 |
| | Orthoptera | Acrididae | 5 | 26 | 8 | 7 | 46 | 0.4 |
| | | Gryllidae | 21 | 28 | 7 | 25 | 81 | 0.0 |
| | Psocoptera | | 0 | 1 | 0 | 0 | 1 | 0.0 |
| | Thysanoptera | Phlaeothripidae | 0 | 1 | 0 | 1 | 2 | 0.0 |
| | | Others* | 12 | 13 | 40 | 24 | 345 | 2.9 |
| **Total** | | | **3,430** | **4,475** | **1,646** | **2,176** | **11,727** | **100** |
| **Observed richness** | | | **28** | **29** | **26** | **35** | | |
| **Diversity Index** | | | **2.40** | **2.64** | **2.20** | **2.35** | | |
| **Equitability** | | | **0.73** | **0.80** | **0.69** | **0.67** | | |

For the abundance data, after the Box-Cox transformation, normality ($p = 0.7564$) and homogeneity of variances ($p = 0.0637$) were observed, allowing the application of ANOVA and subsequent Tukey test to compare the means. For richness data, normality ($p = 0.0846$) and homogeneity of variances ($p = 0.2172$) were observed, and ANOVA and Tukey test were applied to the original data. In the ANOVA, no significant interaction was observed between restoration techniques and types of weed control, neither for the variable abundance nor for the variable richness (Tab. 2).

**Table 2**. Result of the analysis of variance for the abundance and richness variables of edaphic entomofauna in an early forest restoration area in Araras, state of São Paulo, Brazil. Restoration techniques: agroforestry system or mixed-planting. Types of weed control: chemical or mowing.

| Management | Abundance p-value | Richness p-value |
|---|---|---|
| Restoration techniques * Types of weed control | 0.6965 | 0.6465 |
| Restoration techniques | 0.0017* | 0.1712 |
| Types of weed control | 0.0091* | 0.0634 |

*Abundance data obtained after Box-Cox transformation (Box & Cox 1964). *Significant, at the 5% level.

However, for abundance, both the restoration techniques and the types of weed control were individually significant (Tab. 2). In this case, greater abundance was verified with the agroforestry restoration than in the mixed-planting, and in the chemical control than in mowing (Tab. 3). As for richness, neither the restoration techniques nor the types of weed control were significant (Tab. 2).

**Table 3.** Tukey test for the edaphic entomofauna abundance on each restoration technique (agroforestry system or mixed-planting) and type of weed control (chemical or mowing).

| | Restoration techniques | Mean | Types of weed control | Mean |
|---|---|---|---|---|
| **Abundance** | Agroforestry system | 3.87 **a** | Chemical | 3.84 **a** |
| | Mixed-planting | 3.54 **b** | Mowing | 3.57 **b** |

*Means followed by the same letter in the column do not differ at the 5% level.

Collembola is positively related to the increase in soil microbial biomass since it is a food source for these organisms. In addition, they benefit from low-intensity land use activities, such as forest restoration (Ortiz et al. 2019). A high occurrence of Collembola in early forest restoration areas was also observed by Machado et al. (2015) since these organisms are still adapting to the ecosystem balance. For example, at this early stage, their predators are absent.

Formicidae are considered ecosystem engineers affecting soil availability and resources, and their presence may facilitate the establishment of other functional groups in the ecosystem (Amazonas et al. 2018; Venuste et al. 2018). In the present study, a greater abundance of ants was observed in the AFS, as well as in Aquino et al. (2020), who also observed a greater abundance in the intercropping plots, demonstrating the importance of environmental complexity for these organisms. Their high abundance can be related to two factors, first, their high capacity for establishment in environments with different levels of disturbance, and second, the sampling method may have that could overestimate the abundance of these groups of aggregated distribution if sampling is done near nests (Machado et al. 2015; Amazonas et al. 2018; Aquino et al. 2020).

The fact that the AFS showed the highest abundance values over mixed-planting can be explained by the diversification of plant extracts and supply of resources in the AFS, with native tree species and crops growing together (Bos et al. 2007). According to Amazonas et al. (2018) and Aquino et al. (2020), the greater the complexity of the system, the more niches will be explored. We did not observe a significant difference in richness for restoration techniques and types of weed control and, unlike Aquino et al. (2020), the highest values of diversity and equitability were recorded in AFS.

One must also consider the intensity of management in an agroforest, which, when excessive, may cause damage to local biodiversity (Bos et al. 2007). However, the AFS differed from the MP only in terms of planting and harvesting sorghum and bean, with no negative effect observed on the abundance of entomofauna. As for the types of weed control, significant differences were observed in the abundance of entomofauna. The chemical control showed the highest abundance value compared to mowing, results also observed by Nakamura et al. (2008) and Watts et al. (2016), who found that the use of glyphosate had minimal impact on arthropods.

Our results demonstrate that the use of chemical control in restoration, in addition to being a technique that can help tree species in the establishment of seedlings and reduce environmental stress due to reduced competition with weeds (Campoe et al. 2014) did not cause a negative impact in the entomofauna. With the reduction of competition, there is the development of vegetation cover and improvement of soil quality, providing conditions such as humidity and shade, in addition to shelter, allowing the occurrence of different groups of invertebrates (Basset & Lamarre 2019).

We conclude that the agroforestry system and the chemical control can be viable for edaphic entomofauna. Nevertheless, future research is necessary to evaluate the dynamic of edaphic entomofauna during agroforestry systems restoration, as well as the changes in niches occupation and the role of some specific groups.

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## Authors' Contributions

AP, RTF, and ASP contributed to the study's conception and design. AP performed data collection. Identification and data organization were performed by AP and RTF. The draft of the manuscript was written by AP, RTF, and ASP. Statistical analysis was performed by AP and JR. All authors read and approved the final manuscript.

## Conflict of Interest Statement

The authors declare no conflict of interest.

## Supplementary Material

There is supplemental data for this manuscript.

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