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The Temporal and Spatial Variation of Arthropod Associations Inhabiting Non-Crop Vegetation in a Sisal Crop, *Agave sisalana* in the Caatinga Biome

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Abstract: Sisal, *Agave sisalana* Perrine, is cultivated for fiber production, with Brazil being its leading producer. Nowadays, given the increasing interest in organic products, the market for sisal could become an economical alternative for rural areas with low economic inputs. However, sisal is threatened by different pests and diseases. Conservation biological control could contribute to the limitation of these plant enemies, but this agroecosystem is poorly known. In this context, we aimed: (i) to identify the diversity of plants and arthropods and their potential relations, (ii) to study the spatial patterns of arthropods and plants in function of the proximity to the margin of the field, and (iii) to determine the minimum sampling effort needed to record the occurring biodiversity in a sisal crop. Arthropods were sampled using pit-fall traps located close to the border and in the inner plant of the sisal crop from June to September. Simultaneously, plant species and their abundance in quadrats next to each pitfall were recorded. Diversity indexes were calculated to describe the biodiversity, a redundancy analysis was performed to analyze relations among arthropods and plants and the spatial distribution was evaluated using the non-parametric Wilcoxon rank-sum test. The redundancy analysis and the Wilcoxon test revealed a temporal and spatial distribution of arthropods and plants during the period of study. Results indicated (i) similar temporal diversity patterns from June to July for both plants and arthropods, with a maximum in July, whereas in September the biodiversity increased for arthropods and decreased for plants; (ii) the importance of particular plant species for Collembola; and (iii) that arthropods seem to colonize the sisal crop from the fields beyond the crop during the rainy season. These results provide new information about arthropods and plant biodiversity from an agroecosystem in a semi-arid region and raise further queries about the management of sisal crops.

Keywords: biodiversity; semi-arid; arthropod–plant interactions; spatial pattern; sampling protocol

1. Introduction

Agave sisalana Perrine (Sisal), with origins in Mexico, is used for fiber production. This plant is a xerophyte, tolerating prolonged droughts and high temperatures that can survive in poor soils from drought-prone tropical regions and can establish itself in a broad range of environments from sub-humid to arid and semi-arid areas [1]. In Brazil, the plant's largest producer, the production is concentrated in Bahia, followed by Paraíba, Rio Grande do Norte and Ceará [2]. However, in the last decades, its production decreased. In the 1970 decade, the production in the country exceeded 700,000 tons, whereas during 2015, it was reduced to 91,100 tons, relative to a worldwide production of 257,800 tons [3]. This reduction has been mainly attributed to competition with synthetic fibers, low market prices, and unfavorable weather conditions in the main producer countries. However, with the increasing interest in organic products, the interest in natural fibers is rising. Despite the low representation of the Sisal crop in the Brazilian economy, it represents the potential of improving the productivity of semi-arid regions, which have few economic alternatives, and of benefiting the rural population that has low financial incomes. However, fiber extraction or crop production techniques have not been modernized, and there are no mechanization or chemical fertilizers [1,4].

Agave species are attacked by pests and diseases. The bole rot, *Aspergillus niger* van Tieghem, which was found for the first time in Brazil in 2006 [5], is a significant disease that attacks sisal. The Coleoptera *Scyphophorus acupunctatus* Gyllenhal, native to Mexico and well-established in Central and South America, the Caribbean, Africa, and Asia, is considered the major *Agave* pest worldwide [6]. Currently, *S. acupunctatus* is mainly controlled with synthetic insecticides, but their effectiveness is reduced because larvae and adults frequently feed in the interior of the *Agave* plants [7]. Crop diseases and pests can be regulated through Conservation Biological Control, which enhances the environment for their natural enemies. However, enhancing the performance of natural enemies without increasing the crop diseases or pests requires a deep knowledge of the natural enemies' needs in each agroecosystem [8]. In general, the *Agave* agroecosystem, and particularly sisal, is poorly known. Valdés-Estrada et al. [9] studied the effect of plant extracts against *S. acupunctatus* larvae, and several works evaluated different baits for trapping adults, but very few studies addressed the diversity of arthropods or plants in *Agave* crops in a conservation biological control frame. González-Castillo et al. [10] studied the arthropods diversity in *Agave durangensis* Gentry crops in Mexico, but to the best of our knowledge, no study addressed this topic in *A. sisalana*. Moreover, in general, biodiversity is less studied in seasons of resource scarcity [11].

In this context of a general lack of knowledge regarding the sisal agroecosystem, we aimed: (i) to identify the diversity and potential relations between arthropods and the occurring plants in a sisal crop; (ii) to study the spatial patterns of arthropods and plants in function of the proximity to the margins of the field; and (iii) to determine the minimum sampling effort to record the occurring biodiversity in a sisal crop.

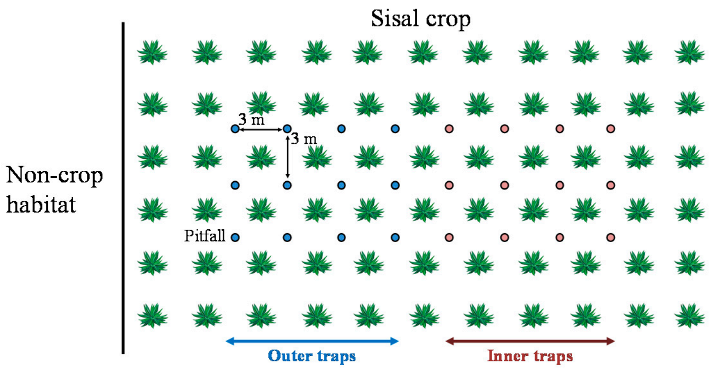
2. Materials and Methods

2.1. Study Area

The study area was a three year old sisal crop (1 ha) located in Antônio Gonçalves municipality—Bahia (10°34′22″ S, 40°16′26″ O), Brazil. No pesticides were applied during the period of study. Bovine manure was used to fertilize, rows were hoeing, sprouts were removed every four months, and biomass was incorporated into the rows. The crop was located in the Caatinga biome, which is composed of semideciduous forest and deciduous forest in the driest areas. This forest composition varies from spiny trees, deciduous or semideciduous—often with a ground layer of small deciduous shrubs and annual herbs, with a predominance of *Fabaceae*—to deciduous woodland of lower stature, with a high proportion of shrubs and subshrubs, and characterized by the presence of many cacti, bromeliads, and *Euphorbiaceae*. In the rainy season, green foliage covers the trees, and annual herbs cover the ground.

2.2. Arthropods and Plants Sampling

From June to September 2015 (cold and dry season), a total of 24 pit-fall traps (7.5 cm in diameter and 6 cm high) were located in the ground and in three inter-rows (eight traps per inter-row) and separated by at least 3 m. Traps were sampled on a monthly basis from June to September. They were placed within the crop according to the distance to the border; thus, 12 traps were located closer to the border of the plantation (outer traps) and the other 12 were closer to the inner part of the plantation (Figure 1).



(a) (b)

Figure 1. (**a**) Diagram of the experimental design; (**b**) planting area of sisal culture in Antônio Gonçalves, Bahia, Brazil.

The traps were provided with water (200 mL) and four drops of liquid detergent. After 24 h, the arthropods were collected and kept in 70% alcohol in the laboratory until identification to order and/or morphospecies. In the same period, the plant species and their abundance within 24 quadrats (50 × 50 cm)—each one corresponding to one pit-fall trap—were recorded.

2.3. Data Analysis

2.3.1. Arthropods and Plants Diversity

The Shannon diversity index (ShDI), the Simpson diversity index (SiDI), and the equitativity (E) were calculated using Past software, version 2.16 (2012), for both arthropods and plants [12]. Additionally, for each plant, the importance value index (IVI) was calculated.

To explore the potential relations of arthropods with plant species, a redundancy analysis (RDA) was performed using the function *rda* from the "*vegan*" package [13] in R [14]. Arthropod morphospecies from each pit-fall were used as response data and IVIs of the identified plant species next to each pit-fall were used as explanatory variables. Hellinger transformation, recommended for the ordination of species abundance data [15], was applied to the arthropods data matrix. The forward selection was used to reduce explanatory variables using the *ordistep* function from the "*vegan*" package. The significance of the RDA was tested using the *anova* function from the "*stats*" package from base R [14].

2.3.2. Analysis of Differences between Outer and Inner Samples

Statistical differences between outer and inner samples were assessed for total arthropod abundance (N) and richness (S) using the non-parametric Wilcoxon rank-sum test that estimates the probability that a randomly chosen subject from the first group (inner) has a higher weight than a randomly selected subject from the second group (outer). For that, the *wilcox.test* function from the "*stats*" package was used [14].

2.3.3. Sampling Protocol

The proportion of the recorded taxa and the inventory quality [16] was followed to determine the minimum sampling effort. Accumulation curves for morphospecies of arthropods and species of weeds were calculated using the software Estimates 8.2 [17] separately for each month (from June to September). This method shows the rate at which new taxa are added to the inventory within a defined area. An increasing number of samples leads to an increased number of taxa until a plateau is reached. The resulting diagram indicates the cumulative number of taxa recovered according to the increase in the number of samples [16]. Then, the curves were modeled using Statistica 7.0 [18] and adjusted by the Clench model [19] according to the equation:

$$S_n = a \times n/(1 + b_n), \quad (1)$$

where S_n is species richness, a represents the increase rate at the beginning of the collection, b is a parameter related to the curve shape, and n is the sampling effort.

The proportions of the recorded taxa, $F(\%)$, were calculated for arthropods and weeds using the equation:

$$F(\%) = S_{obs}/(a/b) \times 100, \quad (2)$$

where S_{obs} represents the observed richness, and a/b is the asymptote of the curve.

The quality of the inventory was calculated as:

$$C_i = a/(1 + bn)^2, \quad (3)$$

where C_i is the slope of the curve at each sampling point. When $C_i < 0.1$, the inventory can be considered complete and reliable. The number of samples needed for $C_i = 0.1$ was calculated using Equation (3).

3. Results and Discussion

3.1. Arthropods and Plants Diversity

In this work, the diversity of arthropods from the soil and non-crop plants of a sisal agroecosystem was identified during the winter season, which is characterized by cold and and dry conditions, in Bahia, Brazil. Regarding the arthropods, a total of 3259 specimens were captured in the pit-fall traps. They belonged to Araneae, Acari, Diptera, Hymenoptera, Collembola, Coleoptera, Orthoptera, Hemiptera, Blattodea, and Lepidoptera (Figure 2) and were separated into 153 morphospecies.

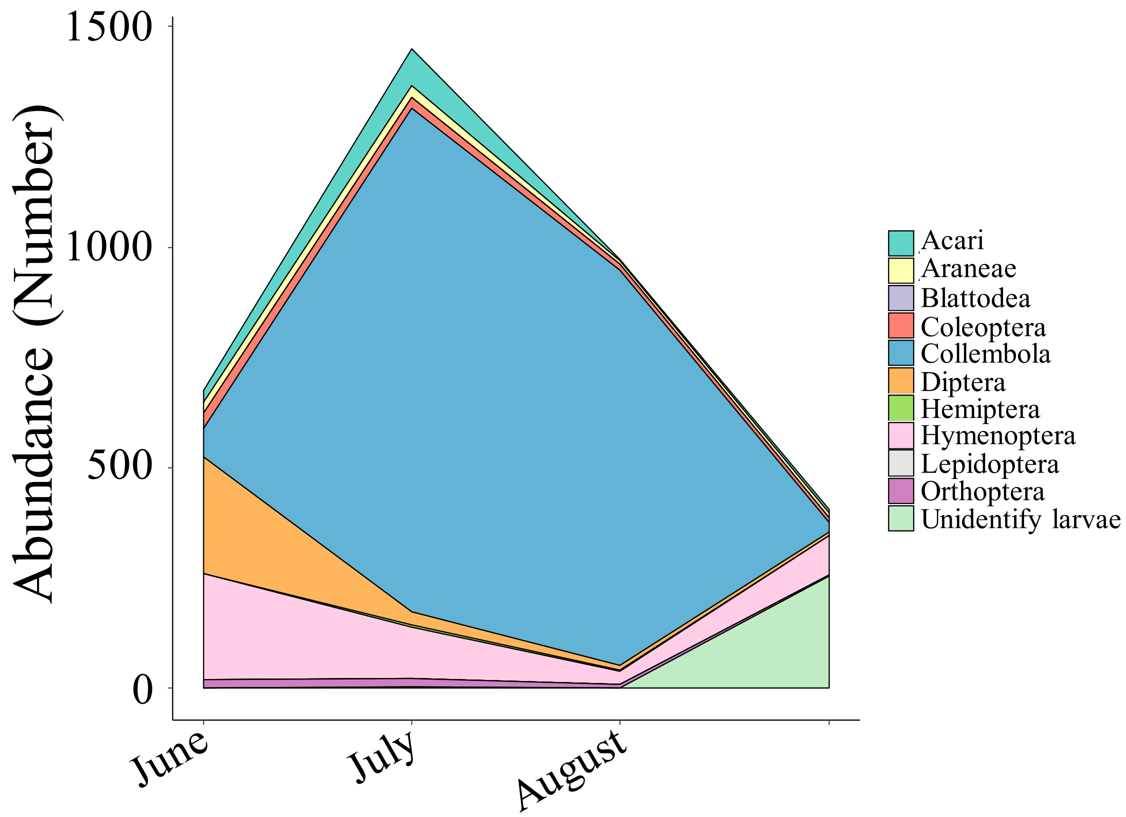


Figure 2. Abundance (number) of the identified taxa in a sisal crop from June to September 2015.

These taxa include several functional groups, such as predators, parasitoids, pollinators, or decomposers, that could play important roles in the sisal agroecosystem. Their abundance varied during the period of study. In June, the most abundant taxa were Diptera and Hymenoptera, with 38.4% and 35.5%, respectively, and the SiDI, ShDI, and E were 0.85, 2.43, and 0.66, respectively. The highest abundance of specimens occurred in July (1452) and August (975). During these months the SiDI, ShDI, and E decreased (July—SiDI = 0.53/ShDI = 1.46/E = 0.38; August—SiDI = 0.26/ShD = 0.77/E = 0.22), with Collembola being the dominant taxa and representing 78.6% and 91%, respectively. In September, the most abundant taxon was Hymenoptera, representing 58% of the total, and the SiDI, ShDI, and E increased (0.59, 1.65, and 0.50, respectively). To the best of our knowledge, there are no other studies that addressed arthropods diversity in a sisal crop. However, in *Agave tequilana* Weber var. Azul crops from Mexico, Gonçalez Castillo et al. [10] found ShDIs between 1.5 and 3.5, with higher richness and special relevance of Hemiptera, Coleoptera, Diptera, and Hymenoptera orders. Other studies evaluated the arthropods colonizing plants of *Agave palmeri* Engelmann [20].

Regarding the plants, in this study, a total of 21 species belonging to 8 families and 5 species in seedling stage were identified (Table 1). The richest families were *Asteraceae*, *Malvaceae*, and *Fabaceae*, and the diversity varied during the period of study. In June, only the *Commelinaceae Commelina erecta* L. was identified. In July, 13 species were identified. The highest IVI was presented by *Amaranthaceae Alternanthera tenella* Colla (IVI = 40.04), followed by species belonging to *Asteraceae*, *Malvaceae*, and *Fabaceae*. In August, 15 species were identified. The most represented species belonged to *Asteraceae* and were followed by *A. tenella* (IVI = 22.54). Finally, in September, 10 species were identified, and the highest IVI was displayed by Fabaceae *Chamaecrista rotundifolia* Moench (IVI = 20.15), followed by Asteraceae *Lepidaploa cotoneaster* (Willd. ex Spreng.) (IVI = 18.09) and *Malvaceae Pavonia cancellata* (L.) Cav. (IVI = 11.67) (Table 1). The plant diversity indexes decreased from July to September (July—SiDI = 0.50, ShDI = 0.78, E = 0.31; August—SiDI = 0.44, ShDI = 0.74, E = 0.29; September—SiDI = 0.29, ShDI = 0.47, E = 0.20). Thus, both plants and arthropods showed similar temporal patterns from June to July, whereas in September the biodiversity increased for arthropods and decreased for plants. Generally, the results agree with the seasonal patterns of tropical ecosystems such as the Caatinga, where soil arthropod abundance and richness occur following the dry and rainy seasons, being directly influenced by the weather conditions and food resources, such as non-crop plants [21].

The identified plants comprise multiple potential uses and may provide additional ecosystem services to the sisal crop. Among the agronomic potential uses, pest/diseases control, biostimulant properties, or the improvement of soil characteristics are included. In a study carried out in urban ecosystems, the increasing patch size and decreasing patch isolation of *C. erecta* resulted in higher overall parasitism rates over the leaf miner, *Liriomyza commelina* (Frost) (Diptera: Agromyzidae) [22]. An extract from *Artemisia absinthium* L. showed biostimulant properties in soybean cultivation [23] and long-term *C. rotundifolia* mulching significantly influenced the soil chemical properties and bacterial communities of persimmon orchards [24]. Extracts from this plant have also shown promising biopesticide activity; however, it may affect the survival and metabolic activity of key soil organism [25]. *Centratherum punctatum* Cass. showed pesticide activity against the mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) [26] and extracts from *Solanum agrarium* Sendtn. showed molluscicidal activity against the snail *Biomphalaria glabrata* (Say), the intermediated host of *Schistosoma mansoni* Sambon (Diplostomida: Schistosomatidae) [27]. Moreover, several of the identified plants, such as *C. erecta*, *Lourteigia ballotaefolia* (Kunth) R.M.King and H.Rob., *A. absinthium*, *Centratherum punctatun* Cass., *Sida* spp., and *Solanum lycocarpum* A.St.-Hil., contain compounds with medical properties or have been traditionally used as medicinal plants [28–34].

Concerning the relations among arthropods and plant species, to date, the knowledge about weed–arthropod interactions in agricultural landscapes is insufficient [35].

Table 1. Plant importance value index (IVI) collected from June to September 2015 in a sisal crop.

Family/Species	IVI			
	June	July	August	September
Amaranthaceae				
Alternanthera tenella Colla	0	40.04	22.54	9.19
Asteraceae				
Artemisia absinthium L.	0	0	0.68	0
Centratherum punctatum Cass.	0	6.73	11.91	0
Lepidaploa cotoneaster (Willd. ex Spreng.)	0	13.14	8.5	18.09
Lourteigia ballotaefolia (Kunth) R.M.King and H.Rob.	0	36.23	26.19	9.85
Tridax procumbens L.	0	6.23	0	0
Commelinaceae				
Commelina erecta L.	100	7.16	7.45	0
Fabaceae				
Calopogonium sp.	0	9.67	6.97	6.56
Chamaecrista rotundifolia Moench	0	0	0	20.15
Mimosa sp.	0	3.32	5.75	2.71
Malvaceae				
Sida cordifolia L.	0	9.98	5.72	5.62
Pavonia cancellata (L.) Cav.	0	2.38	0	11.67
Sidastrum paniculatum (L.) Fryxell	0	0	3.89	0
Sida sp.	0	0	1.07	0
Poaceae				
Specie 1	0	1.43	0	0
Rubiaceae				
Borreria verticilata (L.) G.Mey.	0	0	1.95	0
Solanaceae				
Solanum lycocarpum A.St.-Hil.	0	3.74	1.07	0
Solanum agrarium Sendtn.	0	2.56	0	0
Unidentified				
Specie 2	0	0	0	0.96
Specie 3	0	0	0	1.05
Specie 4	0	0	1.07	0
Specie 5	0	0	3.04	0

In this work, the RDA revealed some relations among arthropods and plants. After the forward selection, the plant species were reduced to *A. tenella* (P1), *C. punctatum* (P6), *L. ballotifolia* (P2), *S. lycocarpum* (P7), unidentified species 2 (P20), *P. cancellata* (P9), and *C. rotundifolia* (P12). The RDA was significant (F = 4.969, df = 7, p-value = 0.001) and resulted in seven canonical axes with a cumulative contribution to the variance (explained by the explanatory variable IVIs of plant species) of 28.33%. The adjusted R2 reduced the variance explained to 22.63%. The percentages of accumulated constrained eigenvalues show that the first axis explained 22.63 $\times$ 66.17 = 14.97% and the second 22.629 $\times$ 27.81 = 6.29% of the variance. The first residual eigenvalue explained 20.21% of the variance, more than all RDA eigenvalues. This means that the first residual structure of the data has more variance than the structures that can be explained by the explanatory variables [36]. This could be due to a potential influence of other variables not included in the analysis. Further studies should address the ecological mechanisms that trigger these correlations and other possible factors influencing them.

The triplot (Figure 3) shows that plants played an essential role in the dispersion of the sites along the first axis. The plants *A. tenella* (P1), *C. punctatum* (P6), *L. ballotifolia* (P2), and unidentified species (P20) characterized sites sampled in July and August, and the arthropod 61, and to a lesser degree the arthropod 50 (both Collembola) were correlated with those plants. *S. lycocarpum* (P7), *P. cancellata* (P9), and *C. rotundifolia* (P12) characterized sites sampled in September and were associated with the arthropod 112 (larvae). The other arthropods showed shorter projections, indicating that they occurred during most of the sample months or were related to several plants. The vegetation can influence arthropods through different processes. For example, foliar herbivory may indirectly affect the soil biota and affect below-ground processes through the effects on plants. These effects may manifest either as changes in plant C allocation and root exudation or as in root biomass and morphology, altering plant litter quality [37]. The decomposers of the plant litter, such as Collembola, could be affected by changes produced in the litter by P1, P6, P2, and P20 and the higher precipitation during those months. Moreover, the presence of Collembola can modify the chemical composition of the soil affecting differently different species of plants [38]. Therefore, the high amount of Collembola during July and August could affect the soil's chemical composition and, consequently, the results found in September. Salamon et al. [39] investigated the relation among Collembola species and abundance to manipulation of the plant species richness and the plant functional groups number. Their results suggested that the presence of certain plant species and functional groups may be more important for the collembolan community structure than the diversity of plant species and functional groups per se, which is in line with our results. Contrastingly, the other arthropods might be more related to the diversity of plants. Further studies are needed to clarify this hypothesis.

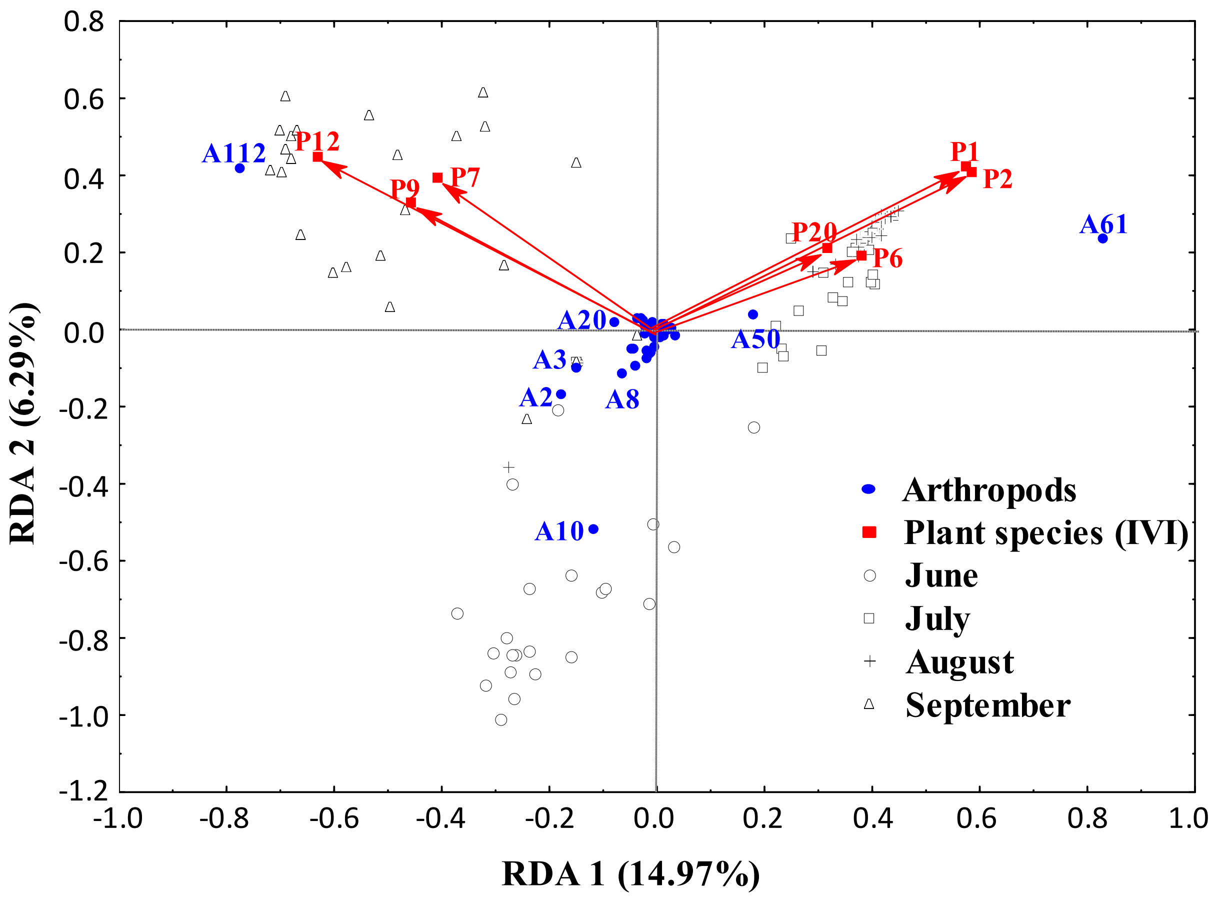


Figure 3. RDA triplot showing the relationships between the Hellinger-transformed abundance of arthropods (response variable) (•), the IVI of plant species (explanatory variables) (■), and sample points in June, July, August, and September (○, □, +, Δ, respectively). The percentage of variance explained by the two first canonical axes (RDA 1 and RDA 2) is indicated between brackets in the axes titles. P1-*Alternanthera tenella*, P6-*Centratherum punctatum*, P2-*Louteigia ballotifolia*, P7-*Solanum lycocarpum*, unidentified species 2 (P20), P9-*Pavonia cancellata*, P12-*Chamaecrista rotundifolia*. A2, A3, A8-Hymenoptera morphospecies; A10-Diptera morphospecies; A20-Unidentified; A50, A61-Collembola morphospecies; A112-Unidentified larvae.

In general, the Brazilian semi-arid biodiversity is poorly known [40], and particularly the biota of the Caatinga is poorly protected. For example, in 2004, several groups of animals in Bahia state were inventoried through Rapid Ecological Assessment, and 14 species of Asilidae (Diptera) were collected, constituting most of the new records for the state [41]. Designs of conservation strategies for this biome could reduce further habitat losses and desertification, maintain essential ecological services necessary for improving the rural population's living standards, and promote the sustainable use of the region's natural resources [42].

3.2. Spatial Distribution from the Field Margins

Most of the arthropod species experience their habitats at spatial scales beyond the plot level, and there is a spillover of natural enemies across the crop–non-crop interface [20]. In this study, the abundance and richness of arthropods according to the distance from the field margins were evaluated during the sampling period to analyze potential distribution patterns related to the proximity to the crop borders. The results indicated a temporal and spatial distribution of the arthropods. In June, the total arthropod abundance (N) and the richness (S) were significantly higher in the outer than in the inner samples (N June: w = 110, *p*-value = 0.030; S June: 114.5, *p*-value = 0.014). In July, no differences were found in N between inner and outer samples, but S was significantly higher in outer samples (N July: w = 57, *p*-value = 0.402; S July: w = 114.5, *p*-value = 0.034). Both N and S were not significantly different between inner and outer samples in August and September (N August: w = 51.5, *p*-value = 0.248; S August: w = 88, *p*-value = 0.357; N September: w = 65.5, 0.729; S September w = 74.5, *p*-value = 0.906) (Figure 4).

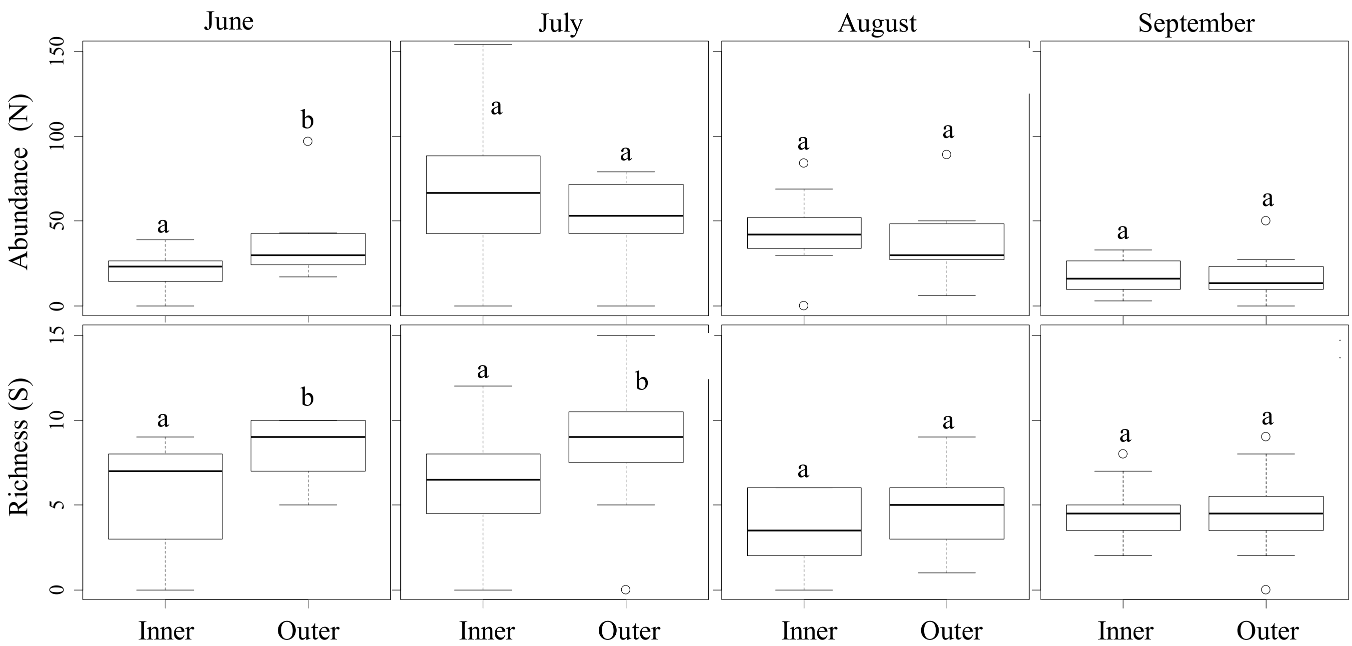


Figure 4. Boxplots of the abundance and richness of arthropods in the pit-fall traps closer (inner) and further (outer) from the limit of the crop during the months of study. Different letters indicate significant differences in the Wilcoxon rank-sum test.

This could indicate the colonization by arthropods along the sample period and agrees with several studies that found colonization of predators that overwinter in the field margins and disperse into the crop in the spring in latitudes where the spring is the rainy and favorable season for arthropods [8].

3.3. Sampling Protocol

The taxa accumulation curves are shown in Figure 5 for the studied months. The percentage of recorded richness varied from 67.25 to 75.51% in the case of the arthropods and from 76.29 to 90.17% in the case of the weeds. The quality of the inventories was always higher than 0.1 for arthropods, indicating that a more intensive sampling would be required for a representative inventory. The number of samples for C_i = 0.1 varied from 54 to 80. In the case of the weeds, the C_i was in all months lower than 0.1, indicating a good quality of the inventory, and the number of samples for Ci = 0.1 varied from 22 to 24. These parameters were not calculated for weeds in June, when only one species was found.

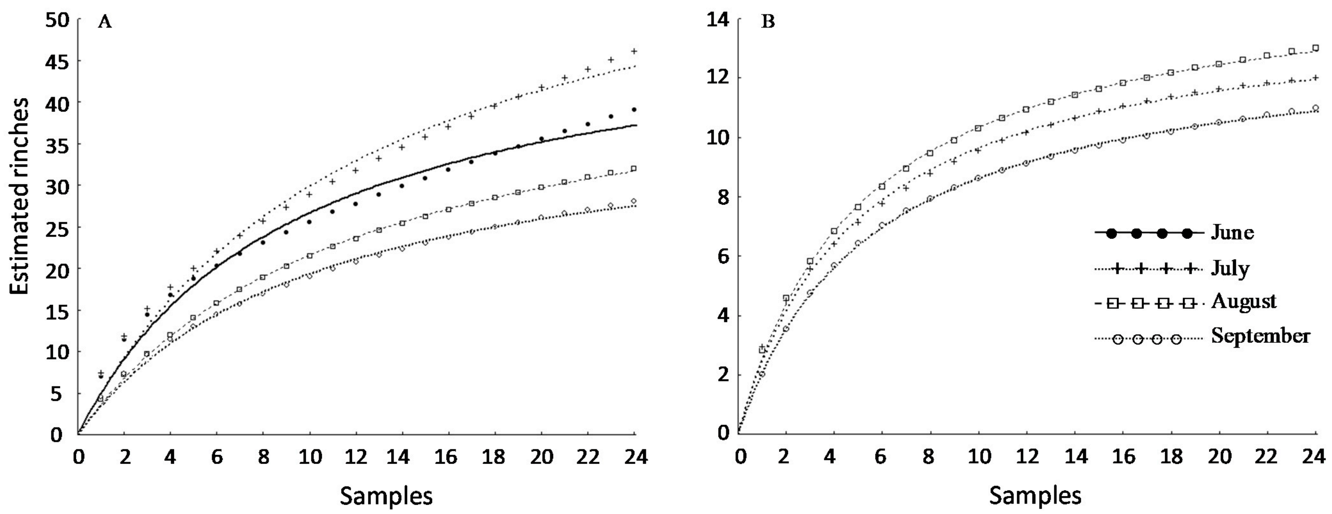


Figure 5. (**A**) Accumulation curves of estimated richness for morphospecies of arthropods and (**B**) plant species. Each mark (●, +, □, and ○) represents the mean of 500 randomizations in the months of study. Lines represent the estimated taxa accumulation.

Thus, in this study, the minimum number of samples needed to elaborate a comprehensive inventory of arthropods and plants in a sisal crop was determined. However, the biology and distribution of the species in the field can bring several constraints to optimizing a sampling protocol [16], and different sampling techniques, such as entomological net or chromotropic traps, could improve the quality of the inventory. For example, Prasifka et al. [43] (2007) compared pit-fall traps and litter bags and showed that litter bags most frequently succeeded in collecting certain groups of arthropods associated with moisture and sheltered areas and pit-fall traps most often captured taxa considered active at the ground level. Additionally, the minimum number of samples may be sufficiently satisfactory depending on the study's goals [16].

4. Conclusions

In conclusion, in this study the temporal and spatial patterns of biodiversity of arthropods and plants, as well as their relations, were identified in a sisal crop. Additionally, the most abundant arthropod groups were Diptera/Hymenoptera in June, Collembola in July and August, and Hymenoptera in September. Several functional groups –predators, parasitoids, pollinators and decomposer– were identified. Regarding the plants, *C. erecta* was the unique species in June and *A. tenella*, *L. ballotaefolia* and *C. rotundifolia* were the most representative in July, August and September respectively. The identified plants comprise multiple potential uses, such as pest/diseases control, biostimulant properties, the improvement of soil characteristics or medical purposes. Both plants and arthropods diversity showed similar temporal patterns from June to July, with a maximum in July, whereas in September the biodiversity increased for arthropods and decreased for plants.

Some relations among arthropods and plants were revealed. Particularly, Collembola were related with *A. tenella*, *C. punctatum*, and *L. ballotifolia* in July and August and *S. lycoparpum*, *P. cancelata*, and *C. rotundifolia* with larva in September. The other arthropods did not show particular associations, indicating the importance of certain plant species for Collembola while the rest of arthropods might be influenced by different variables, but further studies should investigate this aspect. Regarding the spatial distribution of arthropods, our results indicated that they colonized the crop throughout the sample period. Finally, a more intensive sampling would be required for a representative inventory of arthropods, while our results indicate a good quality of the inventory for plants.

To the best of our knowledge, this is the first time that the diversity of arthropods and plants, their associations, and their temporal and spatial distribution have been investigated in a sisal crop within the Caatinga bioma, revealing interesting arthropods and plant relations, as well as dynamic and potential uses.

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