

# Plants Biodiversity in Olive Orchards and Surrounding Landscapes from a Conservation Biological Control Approach <sup>†</sup>

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**Abstract:** Many natural control agents of olive pests need pollen and nectar from non-crop plants in order to complete their life cycles. However, a deep knowledge about the occurring plant species in the agroecosystem is necessary to select the plant species to be maintained or enhanced from a conservation biological control approach. Thus, in this study the goal was to increase the understanding about the plants biodiversity in an important olive producing region in the northeast of Portugal. For that, in a weekly basis during spring and every other week in summer and autumn blooming plant inventories were accomplished in three olive orchards with spontaneous vegetation and its surroundings (woody and herbaceous vegetation areas) from April to December of 2012 and 2013. The percentage ground cover for each flowering plant species was recorded following the Daubenmire cover scale modified by Bailey. A total of 258 plant species belonging to 47 families were identified. The most abundant family was Asteraceae followed by Poaceae, Fabaceae and Brassicaceae. Several species were specific of each land use and presented different flowering periods, representing a potential variety of food sources across the seasons. Additionally, some of the identified species are known for their implications as providers of key requisites for natural control agents. These results provided with valuable information for the implementation of conservation biological control measures.

**Keywords:** food sources; pollen; nectar; natural enemies; pest control; mediterranean areas

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## 1. Introduction

Conservation of beneficial arthropods can involve an important economic value through the provision of multiple ecosystem services (e.g., pollination, pest control, decomposition) [1,2]. This involves a deep knowledge of the occurring natural resources required by natural enemies and pests as well as the occurring resources in the agricultural landscape. Flowering plants are in many cases among the most important natural resources for arthropods because many of them rely on flowering resources for survival and reproduction in some stages of the life cycle (e.g., [3]).

The olive orchard agroecosystem is one of the main crops in Mediterranean areas around the world. In the northeast of Portugal, one of the most important olive producer regions in the country, the crop is attacked by several pests, such as the olive fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae)

or the olive moth *Prays oleae* (Bern.) (Lepidoptera: Praydidae). These pests coexist with multiple beneficial arthropods such as the predator *Chrysoperla carnea* s.l. (Neuroptera: Chrysopidae), several species of syrphids (Diptera) (predators and pollinators) or parasitoids. These arthropods may benefit from flower resources occurring in the agricultural landscape [4–10]. Vegetation within and/or around the olive orchard have been associated to an increase of natural enemies [7,11–13], specific plants were associated to the increase of predator abundance in olive ground covers [13] and in some cases a pest reduction was associated to an increase of natural enemies [7,14].

Additionally, a variation in the plant communities in different olive producer regions is expected according to multiple factors such as environmental conditions or management practices at the landscape or at the local scale. In this context, the knowledge of the plant community in different agricultural areas is necessary for determining which plant resources should be maintain, enhance or studied for conservation biological control. Thus, the goal of this work was to describe the flowering plant community in spring, summer and autumn in the ground cover of olive orchards and adjacent herbaceous and scrubland seminatural areas from the northeast of Portugal in order to analyze its potential for enhancing conservation biological control in olive orchards.

## 2. Experiments

### 2.1. Study areas

The study areas were three olive orchards of approximately 2 ha in area (Cedães: 41°29'16" N–7°07'34" W, Paradela: 41°32'8" N–7°07'29" W, and Guribanos: 41°34'12" N–7°09'59" W) and two surrounding non-crop areas next to each olive grove, one herbaceous vegetation plot and one scrubland, both of approximately 1 ha. During the experimental years, the olive groves were not tilled and were not sprayed with pesticides.

### 2.2. Flowering Plant Inventories

Five flowering plant inventories were carried out every week from April through June and every other week from July through December 2012 and 2013. Inventories were carried out in circular plots of 25 m<sup>2</sup> (olive groves and herbaceous patches) and three plots of 100 m<sup>2</sup> (scrubland patches). The plots were larger in the scrublands because the larger size of the plant species (trees and shrubs) required inventories to be conducted in larger plots to record the occurring plants. This resulted in a total of 39 samples of plant inventories per sampling date for characterizing the plant community of the olive grove agroecosystem. The percentage ground cover for each flowering plant species was recorded following the Daubenmire cover scale modified by Bailey [15].

### 2.3. Data Analysis

#### 2.3.1. Alpha Diversity

The richness, the Pielou's evenness index, the Shannon-Wiener diversity index and the Simpson diversity index were calculated to describe the plant communities in spring, summer and autumn in the olive orchards, herbaceous and scrubland plots using the "vegan" package [16] in R software [17].

#### 2.3.2. Beta Diversity

Venn graphics were drawn to visualize the number of shared and unshared species among land uses by season. Plant communities by land use and location were visualized using nonmetric multidimensional scaling (NMDS) (999 permutations) plots (bray distance and k = 2) after grouping data by the mean of the percentage observed in the samples each date (metaMDS function from "vegan" package).

A Permutational Multivariate Analysis of Variance (PERMANOVA) for each season was used to analyze the differences among plants communities from the different land uses (olive orchards, herbaceous and scrubland patches) and locations (Cedães, Paradela, Guribanos) using the function

*adonis2* from the same package. Square root of the abundance matrix was used for minimizing the influence of most abundant groups. Then, the Bray-Curtis dissimilarity matrix was used as response and land use and location as explanatory variables. The main effects and the interaction were analyzed. Permutations were constrained by sample. 999 permutations were used. In order to analyze which levels of the significant explanatory variables and/or interactions were significantly different a pairwise comparison for each group level with Bonferroni correction for multiple testing was performed using pairwise permutational MANOVA with the function *pairwise.perm.manova* from the “RVAideMemoire” package (Hervé, 2020). Following the ‘marginality principle’ when non-null-interactions stood out, main effects were not analyzed (Nelder, 1977). The plant species driving the differences were analyzed with a similarity percentage analysis (SIMPER) of the square rooted abundance matrix using the *simper* function in the “vegan” package. The variance among communities grouped by land use-location were tested using *betadisper* function in the same package followed by a permutation test for homogeneity of multivariate dispersions using the *permutest* function in the same package. When differences were found, pairwise differences between groups were checked using a Tuckey’s HSD test using the *TukeyHSD* function.

### 3. Results

#### 3.1. Alpha Diversity: Diversity Incides

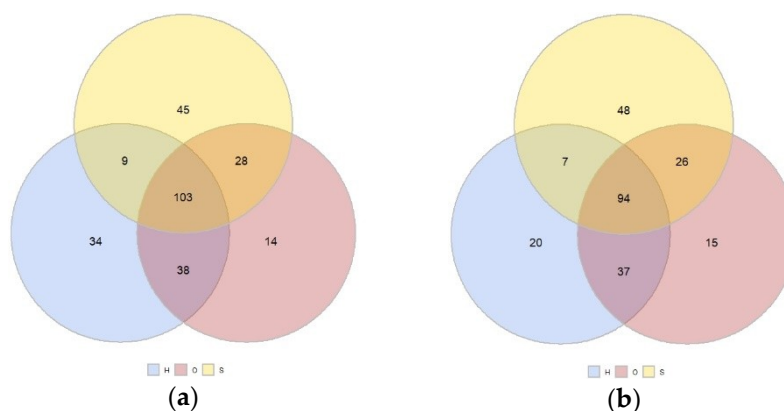
The Table 1 shows the values of the richness, the Pielou’s evenness index, the Shannon-Wiener diversity index and the Simpson diversity index of the plant communities blooming in spring, summer and autumn in the olive orchards, herbaceous and scrubland plots.

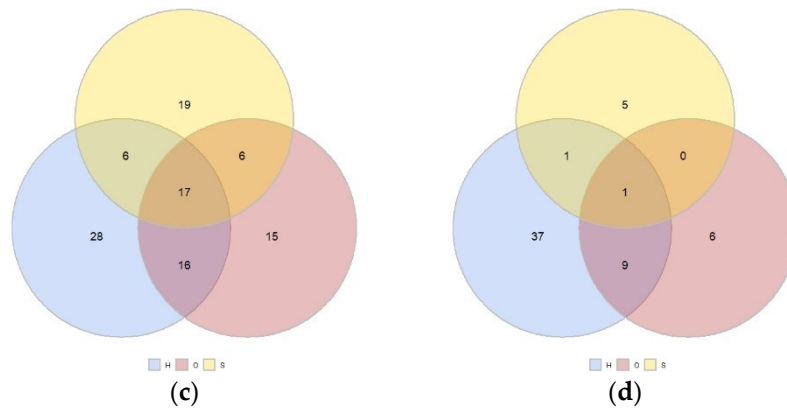
**Table 1.** Biodiversity indexes calculated the plant communities blooming by season and land used (Olive—olive orchards; Herb—herbaceous plots; Scrub—scrubland plots).

Biodiversity Indexes	Spring			Summer			Autumn		
	Olive	Herb	Scrub	Olive	Herb	Scrub	Olive	Herb	Scrub
Richness	109	97	105	40	53	38	14	35	6
Pielou	0.749	0.644	0.664	0.593	0.642	0.718	0.605	0.624	0.150
Shannon	3.514	2.946	3.089	2.188	2.548	2.612	1.596	2.217	0.269
Simpson	0.957	0.917	0.915	0.790	0.879	0.879	0.666	0.830	0.109

#### 3.2. Plants Communities in Spring, Summer and Autumn by Land Use and Location

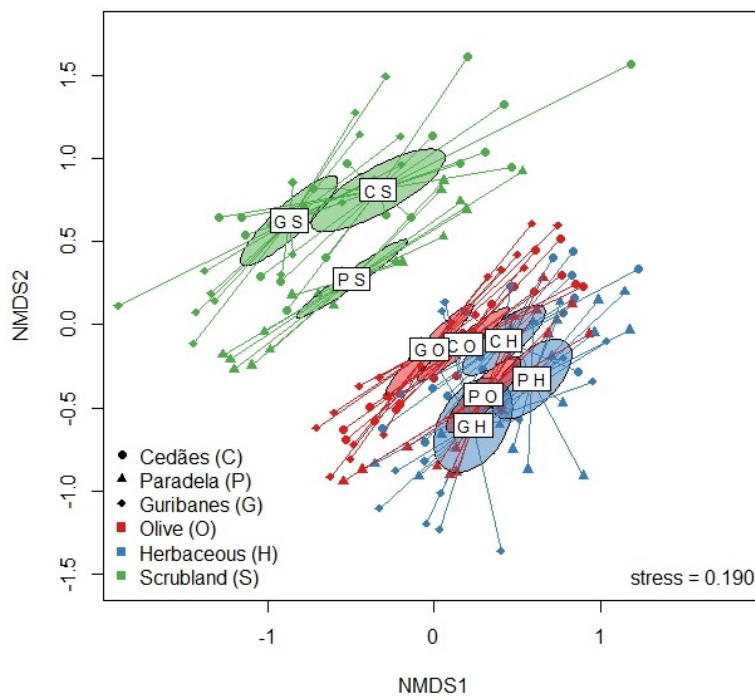
The Venn graphics showed that in all seasons several species were exclusive from each land use (Figure 1).





**Figure 1.** Venn graphics indicating the number of shared and non-shared species of the plant community blooming in the entire sampling period (a), spring (b), summer (c) and autumn (d). H—herbaceous plots, O—olive orchards, S—Scrubland plots.

The low number of shared species in summer and autumn did not allow to draw the NMDS plot for those seasons. The NMDS plot for visualizing the plant community blooming in spring by season and location is showed in the Figure 2.



**Figure 2.** Non-metric multidimensional scaling (NMDS) analysis for flowering plant species (percentage of ground cover) in spring by the interaction of land use [olive (O), herbaceous patches (H), Scrubland (S)], and location [Cedães (C), Guribanés (G) and Paradelá (P)]. Sites are connected to the centroid in each group, and the 95% confident interval of each centroid is shown by shaded ellipses.

The PERMANOVA in spring, summer and autumn showed that the plant communities varied with environment and location (Table 2). The Tuckey’s HSD test indicated that: (i) in spring all plots presented different plant communities ( $p < 0.05$  in all cases); (ii) in summer herbaceous plot in Cedães and the herbaceous plot in Paradelá did not present differences in their community composition ( $p =$

0.108) and all the other plots presented different community compositions ( $p < 0.05$  in all cases), and (iii) in autumn did not find differences among the plant community in the scrubland from Cedães and any of the plots, among the plant community in the herbaceous plot from Cedães and the herbaceous plot in Paradela, the olive orchards of Cedães and Guribanés and the scrubland from Cedães and Guribanés, and among the herbaceous plot in Paradela and the olive orchards of Cedães and Guribanés and the scrubland in Cedães ( $p > 0.108$  in all cases). The Tuckey’s HSD test indicated that the communities among all the other plots in autumn differed ( $p < 0.05$  in all cases).

**Table 2.** PERMANOVA results for the variations of the plant communities in spring, summer and autumn by location and land use.

		df	SS	R <sup>2</sup>	Pseudo-F	p-Value
Spring	Location	2	12.525	0.044	20.479	0.001
	Land use	2	34.441	0.122	56.313	0.001
	Location: Land use	4	21.601	0.076	17.660	0.001
	Residual	702	214.672	0.758		
	Total	710	283.239	1.000		
Summer	Location	2	13.188	0.072	22.556	0.001
	Land use	2	16.827	0.092	28.781	0.001
	Location: Land use	4	20.925	0.114	17.895	0.001
	Residual	452	132.134	0.722		
	Total	460	183.074	1.000		
Autumn	Location	2	9.045	0.136	17.299	0.001
	Land use	2	8.594	0.129	16.435	0.001
	Location: Land use	4	10.058	0.151	9.618	0.001
	Residual	149	38.955	0.584		
	Total	157	66.651	1.000		

According to the permutation test for homogeneity, the variance of plant communities by land use and location in spring, summer and autumn were heterogeneous (spring:  $F = 3.236$ ,  $df = 8$ ,  $p = 0.002$ ; summer:  $F = 2.357$ ,  $df = 8$ ,  $p = 0.026$ ; autumn:  $F = 3.626$ ,  $df = 8$ ,  $p = 0.003$ ). The Tuckey HSD test showed that: (i) in spring the differences were due to the lower data dispersion around the centroid in the scrubland from Guribanés than in the herbaceous patches from Guribanés and Paradela (Figure A1a and A2a); (ii) in summer the differences were due to the higher data dispersion around the centroid in the scrubland from Guribanés than in the herbaceous patches of Guribanés ( $p = 0.033$ ) and the olive orchard of Paradela ( $p = 0.009$ ) (Figure A1b and A2b); (iii) and in autumn the Tuckey’s HSD test found differences in the data dispersion of herbaceous plot in Guribanés with the olive orchard ( $p = 0.030$ ) and herbaceous plot in Cedães ( $p = 0.011$ ) and the scrubland plot in Paradela ( $p = 0.035$ ) (Figures A1c and A2c). This was probably due to the fact that the herbaceous plot in Guribanés coped with most of the plant diversity in autumn. In the cases in which the variance of plant communities was different, the differences found by the PERMANOVA could be due to the heterogeneity of the data and not to a difference in the community composition.

### 3.3. Simper Analysis: Species Diving the Differences among Land Uses and Sites and Most Abundant Species

The simper analysis performed a total of 36 comparisons between plots in each season. Only the significant pairs in the Tuckey’s HSD test after the permanova were analyzed. During spring a between 17 and 48 species (of a total of between 117 and 193 species) were responsible for the 70% of the differences among the community composition of flowering plants in the plots. The species that most contributed to the difference in each comparison was responsible for between 2% and 10% of the difference and coincided with the most abundant plants in the plots (Table 3). Also in some cases *Coleostephus myconis* (L.) Cass., *Cistus ladanifer* L. and *Cytisus multiflorus* (L’Her.) Sweet, were the species that most contributed to the difference among plots. During the summer between 5 and 10

species (of a total of between 25 and 76 species) were responsible for the 70% of the differences among the community composition of flowering plants in the plots. The species that most contributed to the difference in each comparison was responsible for between 2.1% and 24.01% of the difference and, excepting *Andryala integrifolia* L., they coincided with the most abundant plants (Table 3). During the autumn between 2 and 11 species (of a total of 4 and 16 species, up to 50 in the comparisons with the herbaceous plot in Guribanés). were responsible for the 70% of the differences among the community composition of flowering plants in the plots. The species that most contributed to the difference in each comparison was responsible for between 2.53% and 67.03% of the difference and they coincided with the most abundant plants (Table 3). Also in some cases *Foeniculum vulgare* Mill. Was the species that most contributed to the difference among plots.

Table 3. Most abundant flowering species by season, site and land use.

Season	Site	Olive Orchard	Herbaceous Plot	Scrubland Plot
Spring	Cedães	<i>Ornithopus compressus</i> L.	<i>Chamaemelum mixtum</i> (L.) All.	<i>Lavandula pedunculata</i> (Mill.) Cav.
	Guribanés	<i>Crepis capillaris</i> (L.) Wallr.	<i>Trifolium michelianum</i> Savi	<i>L. pedunculata</i>
	Paradeia	<i>Bunias erucago</i> L.	<i>Chrysanthemum segetum</i> L.	<i>L. pedunculata</i>
Summer	Cedães	<i>Chondrilla juncea</i> L.	<i>Chondrilla juncea</i> L.	<i>Helichrysum stoechas</i> (L.) Moench
	Guribanés	<i>Chondrilla juncea</i> L.	<i>Hypochaeris radicata</i> L.	<i>Centaurea aristata</i> subsp. <i>langeana</i> (Arènes) Dostál
	Paradeia	<i>Andryala integrifolia</i> L.	<i>Tolpis barbata</i> (L.) Gaertn.	<i>Daphne gnidium</i> L.
Autumn	Cedães	<i>Chondrilla juncea</i> L.	<i>Chondrilla juncea</i> L.	<i>Daphne gnidium</i> L.
	Guribanés	<i>Chondrilla juncea</i> L.	<i>Hypochaeris radicata</i> L.	<i>Arbutus unedo</i> L.
	Paradeia	<i>Chenopodium album</i> L.	<i>Chondrilla juncea</i> L.	<i>Daphne gnidium</i> L.

#### 4. Discussion

In this study community composition of flowering plants in an important olive producer region from the north of Portugal was described. The results indicate that the community composition vary across the year. The spring was the most biodiverse season followed by the summer and the autumn. The community composition of flowering plants was generally different by land use (olive orchards, herbaceous vegetation and scrubland), in the three studied sites and in the three seasons, indicating a high variability of plants in the studied region. However, the species which most contributed to the difference in each pair of comparisons varied among one or two species for each land use and site and corresponded with the most abundant species. The high variability may be linked to the high landscape complexity of the studied region, composed mainly for small and irregular patches of several land uses, including many semi-natural areas with different composition (see [10]).

The plants variability among seasons may contribute to a spillover of natural enemies among vegetation types and seasons. This type of spillover was described in olive orchards by [20] who identified movements of predators and parasitoids from ground cover to adjacent vegetation and to olive trees in different periods, in some cases corresponding to the development of important pests such as *P. oleae*. Additionally, Álvarez et al. [13] suggested that a spillover of natural enemies may occur between olive orchards and adjacent natural habitats when their resources differ in quality and quantity, as occurring in the present study.

The variability in the plant composition among land uses may also contribute to pest reductions. For example, the anthophagous generation of *P. oleae* (spring) was reduced in some years with the increase of its specific parasitoid *A. fuscicollis* in orchards with ground covers [7], the landscape composition negatively affected *P. oleae* abundance [10] and the predation of *B. oleae* in olive orchards was associated to the area of scrublands in autumn [14]. However, it is important consider also the potential risks of flowering plants through potential benefits for pests [8] or vectors of diseases [21,22].

### 5. Conclusions

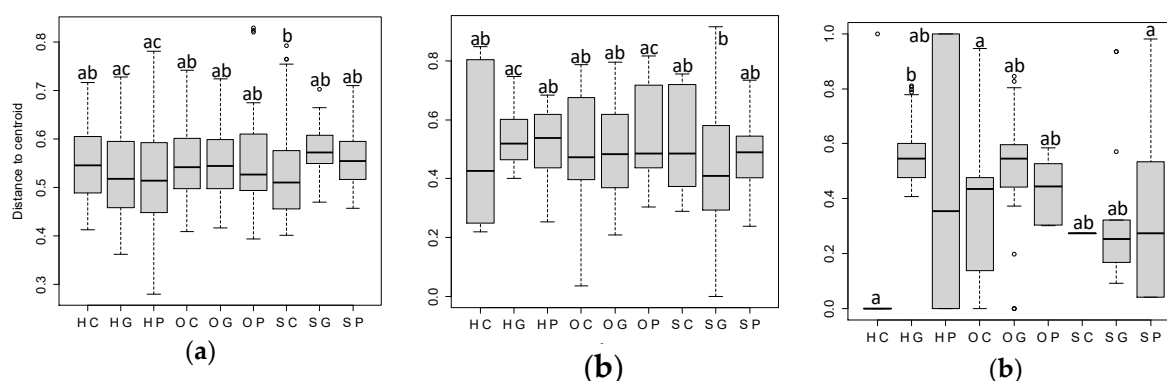
In this study the community composition of flowering plants in an olive agroecosystem was described. The results indicate a high variability among land uses, sites and season, which include exclusive flowering plant species and may potentially originate a spillover of arthropods. Further studies should study this potential spillover and effects on biological control.

**Author Contributions:** M.V. and J.P. conceived and designed the experiments; M.V. performed the experiments; M.V. and C.A. identified the plants; M.V. analyzed the data; M.V., S.S. and J.P. wrote the paper.

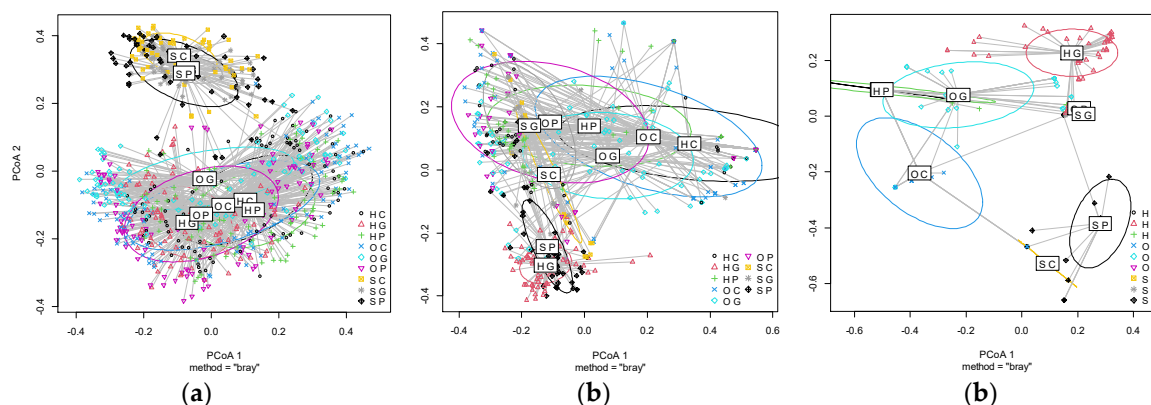
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### Appendix A



**Figure A1.** Variance of the plant communities in spring (a), summer (b) and autumn (c) by land use [olive (O), herbaceous patches (H), Scrubland (S)], and location [Cedães (C), Guribanés (G) and Paradela (P)] as the distance to centroid of the Bray-Curtis distance matrix. Different letters indicate significant differences using a Tukey HSD test. Upper and lower hinges in the boxplot represent the first and third quartiles, error bar indicate 95% confidence intervals, and bar represents the median.



**Figure A2.** Data points (points) and centroids (rectangles) across the two principal coordinates axes (PCoA1 and PCoA2) during spring (a), summer (b) and autumn (c). H—herbaceous patches, O—olive orchard, S—Scrubland, C—Cedães, G—Guribanês, P—Paradela.

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