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Response of the Coccinellidae Community within Sustainable Vineyards to the Surrounding Landscape

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Abstract: The family Coccinellidae (Coleoptera) includes important predatory natural enemies in agricultural crops. To survive, this group uses different occurring resources across the landscape; therefore, the landscape can influence the Coccinellidae community in agroecosystems. In this context, this work aims to evaluate the response of the Coccinellidae community to the landscape context within a gradient of distances from vineyards managed under sustainable production methods. For that, Coccinellidae were sampled in thirty-five vineyards distributed by six wine Protected Designation of Origin (PDO) regions of Portugal, and landscape metrics—composition and configuration—were calculated in a 2000 m, 1500 m, 1000 m, 750 m, and 500 m buffers around the vineyards. Then, Coccinellidae species were identified, and the response of the Coccinellidae to the landscape metrics was analyzed. In total, 326 Coccinellidae from 21 species were collected. The most abundant species were *Scymnus apetzi*, *Scymnus interruptus*, *Scymnus subvillosus*, *Coccinella septempunctata*, and *Stethorus pusillus*, which together represented 83.4% of the total collected individuals. Most specimens were concentrated in July and at the vegetation cover. Results from the statistical analysis (Generalized Linear Mixed Models) indicated that the presence of seminatural habitat surrounding the crop may favor Coccinellidae, while habitat fragmentation seems detrimental for them. The potential relevance of the identified Coccinellidae for pest control in vineyards is discussed.

Keywords: seminatural areas; landscape diversity; ladybirds; viticulture; *Scymnus* spp.

Citation: Taranto, L.; Rodrigues, I.; Santos, S.A.P.; Villa, M.; Pereira, J.A. Response of the Coccinellidae Community within Sustainable Vineyards to the Surrounding Landscape. *Agronomy* **2022**, *12*, 2140. https://doi.org/10.3390/agronomy12092140

Academic Editor: José Casanova Gascón

Received: 18 August 2022
Accepted: 5 September 2022
Published: 9 September 2022

1. Introduction

Vineyards occupy a large proportion of Portugal's territory, the fifth largest wine producer country in Europe and the eleventh worldwide [1]. One of the most important challenges for grapevine growers is arthropod pests due to their significant production and economic losses [2,3]. Pest populations in vineyards can be influenced by different factors such as climatic conditions [4,5], local management practices [6–8], or the structure of the agricultural landscape [9]. There is growing evidence that landscapes composed of complex habitat mosaics generally provide more crop protection against pests than a simplified landscape [10]; however, in some cases, complex landscapes may also favor colonization by crops' pests [11]. In vineyards, a complex mosaic of both managed and natural habitats may maximize various ecosystem services (e.g., crop pollination and yield, biological pest control) [12], while the landscape simplification would have negative effects on natural pest control in vineyards [7,13–16]. Moreover, complex trophic interaction networks integrated by co-existing arthropods in the vine ecosystem [4,6], can maintain the pest populations under economic threshold levels [17]. Several works indicated that beneficial arthropods in Mediterranean vineyards can be enhanced through habitat manipulation, namely, establishing or maintaining non-crop vegetation within and/or surrounding the vineyards [15,18–22]. Ground cover vegetation promotes habitat complexity in vineyards, creating a microclimate that benefits natural enemies, e.g., mitigating temperature and increasing humidity [23], or providing shelter and food resources for arthropods [24,25]. On a broader scale, this can be promoted by: encouraging land uses that sustain the presence of natural or seminatural edge habitats; or large areas of uncultivated lands such as grasslands and natural or planted forests [26].

The family Coccinellidae (Coleoptera), commonly referred to as ladybirds or lady beetles, is considered one of the most important groups of predatory natural enemies, presenting species with different degrees of prey specialization [27]. Coccinellidae species can feed on the hemipteran suborder Sternorrhyncha (aphids, adelgids, scales, mealybugs, whiteflies, psyllids), ants (Hymenoptera: Formicidae), mites (Acari: Tetranychidae), leaves of plants, pollen, nectar honeydews, and fungi [28–31]. In vineyards, Coccinellidae may be important predators of several pests belonging to latter insect groups, such as vine mealybug *Planococcus ficus* Signoret (Hemiptera: Pseudococcidae) [32], or the Acari European red mite *Panonychus ulmi* Koch and the two-spotted spider mite *Tetranychus urticae* Koch (Tetranychidae). These pests feed on the leaves of the vines, weakening them [33]. Coccinellidae have also been referred to as potential predators of lepidopteran pests in Australia [24], and therefore they may also be potential predators of the European grapevine moth, *Lobesia botrana* Denis and Schiffermüller (Lepidoptera: Tortricidae) [11,34].

Coccinellidae are widely known for their predatory feeding habits [35], but can supplement their most common diet with non-prey supplementary foods, such as pollen, nectar, or insect honeydew [36], and even rely on them when food is scarce [29,37]. Moreover, both preys and supplementary foods (e.g., flowers) occur seasonally and patchily across the landscape where predators disperse, searching for food [38,39]. The effect of the landscape on Coccinellidae within vineyards was demonstrated in some cases. For example, in vineyards from Australia, several species of Coccinellidae from the vine canopy were aggregated within the crop, in rows near woody vegetation [24], and in vineyards from Spain, the richness and diversity of Coccinellidae were positively related with a more complex shape of the landscape within a 200 m buffer [40]. However, there is not sufficient investigation about how the landscape structure at different distances from the vineyards affects Coccinellidae populations within the crop. In Portugal, the coccinellidofauna was described by Raimundo and Alves [41], but habitats and the basic ecology knowledge about this group in Portugal are still insufficient. In the northeastern of the country, several species of Coccinellidae were previously found as important potential predators of aphids, coccids, mites, and scale pests in olive, chestnut, and almond groves [31,42,43]. In olive groves, Coccinellidae prey on the black scale, *Saissetia oleae* (Olivier) [44], and they were a well-represented group of natural predators in citrus [45]. However, there is a gap in knowledge of coccinellidofauna in Portuguese vineyards. In this context, this research aims to analyze the response of the Coccinellidae community to the surrounding landscape at different scales in the most important wine producer regions of Portugal, considering a period with suitable conditions for Coccinellidae (end of spring/ beginning of summer) and a period with unsuitable conditions (autumn).

2. Materials and Methods

2.1. Study Sites

A total of 35 vineyards in six wine Protected Designation of Origin (PDO) regions of Portugal were selected to include a vast extension of territory and maximize the recorded information about the Coccinellidae community in sustainable Portuguese vineyards. The surveyed Wine regions were Bairrada, Beira Interior, Douro, Trás-os-Montes, Península de Setúbal, and Vinhos Verdes (Figure 1). Among them, the climate varies across two climate regions, according to Koppen-Geiger's classification: one with a temperate climate with rainy winter and dry and hot summer (Csa), including the interior regions of Douro Valley, Trás-os-Montes, and Península de Setúbal, and the other with temperate climate with rainy winter and dry and mild summer (Csb), including Vinhos Verdes, Bairrada and Beira Interior [46], commonly observed in Mediterranean areas [47,48].

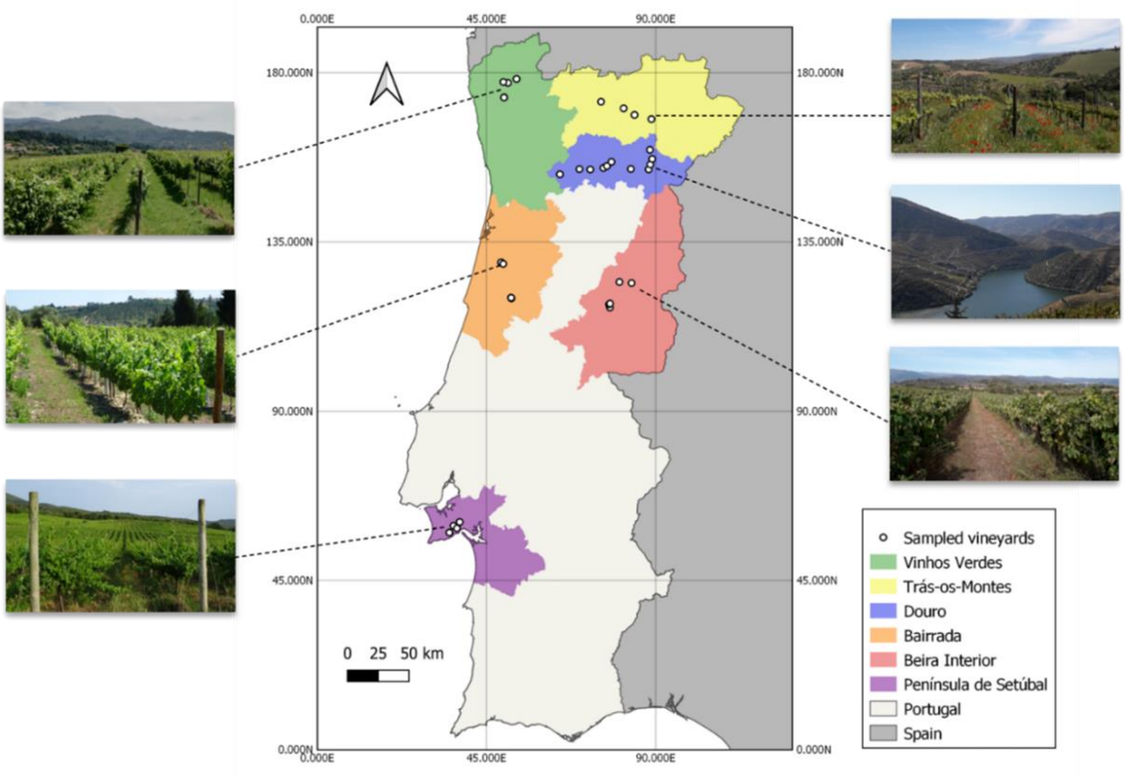


Figure 1. Map of Portugal with indicated sampled vineyards and, respectively PDO Wine Region. The map was projected in ETRS89/PT-TM06.

All the sampled vineyards are under sustainable production management (certified organic or integrated production). Spontaneous vegetation cover was maintained in the inter-rows during the collection periods. Additional data are given in Table S1 and shows the vineyard's location, phenological stage, area, spacing, vine variety, orientation, training system, chemical treatments (when applied), and soil management.

2.2. Experimental Design and Species Identification

Sampling was performed on three dates: July (beginning of summer—with suitable conditions for Coccinellidae), September, and October (autumn—with unsuitable conditions for Coccinellidae) of 2019. In each vineyard, 10 samples of vegetation cover and 10 samples of the canopy were collected using an entomological sweep net of 38 cm diameter. Each sweep was performed by moving the entomological sweep net 180 degrees, 10 times at the vegetation cover and 50 times at the canopy. The net was emptied into plastic bags, and 0.3 mL of diethyl ether was added to kill the arthropods immediately and avoid intraguild predation within the bags. In the laboratory, they were stored in a freezer at −20 °C until identification.

Identifications at the species level were carried out using Raimundo and Alves [41]. Genitalia was extracted and observed when needed. For the extraction of the genitalia, a modified Majerus and Kearns [49], method was followed. Thus, the abdomen was detached from the body with a scalpel blade and boiled into 10% potassium hydroxide solution for 2 to 5 min to soften the hard body structures and partially clear the tissues. The processed abdomen was moved to a petri dish with a drop of glycerin, and the genitalia was operated on with the help of two needles under the binocular stereoscopic. Male and female genitalia were put into glass microscope slides with a drop of glycerin, fixed with coverslips, sealed on the edges, and identified. All specimens were stored in the Mountain Research Center (CIMO) collection at the Polytechnic Institute of Bragança, Bragança, Portugal.

2.3. Landscape Variables

Landscape configuration and composition metrics were calculated within 2000, 1500, 1000, 750, and 500 m diameter buffers constructed around each vineyard (overlapping vineyards were excluded to avoid spatial autocorrelation. Thus, 25, 27, 28, 29, and 30 vineyards were selected respectively for 2000, 1500, 1000, 750, and 500 m buffers. The map "Carta de Uso e Ocupação do Solo de Portugal Continental para 2018" [50], was used to obtain the land uses and respective areas within each circle. The map buffers were constructed using the *buffer*, *intersect*, and *aggregate* functions from the "raster" package [51], and the *msexplode* function from "rmapshaper" package [52], in R software [53]. To obtain more accurate landscape variables, the small polygons (<25 m^2 because the resolution of orthophotos for COS2018 is 25 m^2) generated in the buffer edges during the intersection process were merged to a larger adjacent polygon using ArcGIS, version 10.3.1 [54]. Then, landscape variables were calculated using the extension Patch Analyst of the ArcGIS.

The land-use classes considered to calculate the landscape metrics were vineyards, seminatural areas, olive orchards, other crops, other orchards, pasture, bared areas, artificial territory (i.e., urban territory, buildings), and water/humid areas.

At the landscape level (i.e., considering all land uses), the calculated metrics for further analysis were the Simpson's diversity index (SEI) and the mean patch fractal dimension (MPFD). SEI was calculated to quantify the landscape composition and represents the probability that any land types randomly selected would be different from one another. MPFD was calculated to quantify the degree of complexity of the landscape by measuring the complexity of a polygon by relating perimeter and area [55]. At the class level, the considered landscape metrics were the areas of artificial territories (AT), olive orchards (Oli), vineyards (Vin), other crops (Oth), and seminatural areas (SNA) because they were the most variable across regions or because of its potential importance for Coccinellidae.

2.4. Coccinellidae Response to Landscape Variables

The response of Coccinellidae (richness and abundance) and of the tribe Scymnini (the most abundant tribe) abundance to the calculated landscape variables at the different buffers along the sampling period were analyzed using a series of separated generalized mixed models (GLMMs) (one model for each buffer: 500, 750, 1000, 1500, and 2000 m). Thus, the following explanatory variables were considered for the models construction: the coordinates of the sampling sites (longitude -X and latitude -Y), the landscape diversity index (SEI), the landscape complexity (MPFD), areas of artificial territories (AT), olive orchards (Oli), vineyards (Vin), other crops (Oth) and seminatural vegetation (SNA), the date (July, September, and October), and the habitats (two levels: ground cover vegetation and canopy). Before running the models, collinearity among the standardized continuous explanatory variables was evaluated, and non-collinear variables were selected for each buffer. For that, a Variance Inflation Factor (VIF) value higher than 3.5 was not allowed, and the Pearson correlations (calculated using the function *cor* from base R [53]) were lower than 0.7 in all cases, minimizing potential model misspecifications [56]. Thus, the selected explanatory variables for the full models were:

- At 2000, 1500, and 500 m buffers the full models included Y, SEI, MPFD, AT, Oli, Vin, SNA, Date, and Habitat. X and Oth were excluded because X was negatively correlated with AT, and Vin with Oth. The maximum VIF among the continuous variables was 3.48, 2.75, and 2.89, respectively.
- At the 1000 and 750 m buffers the full model included X, Y, SEI, MPFD, AT, Oli, Vin, SNA, Date, and Habitat. Oth was excluded from the model because it was negatively

correlated with Vin. The maximum VIF among the continuous variables was 2.82, 2.89, respectively.

The Poisson (for count data) and negative binomial—quadratic parameterization (nbinom2)—to account with overdispersion distributions [57], were used for the models. The distribution used for each model is indicated in Table S2. Backward selection was performed until all explanatory variables were significant or the model validation failed. The most explanatory model (keeping the highest number of explanatory variables) within <2 ΔAIC was selected [58]. The function *glmmTMB* from the glmmTMB package was used for the models fit [59]. Models were validated using the *simulateResiduals* function from DHARMa package [60].

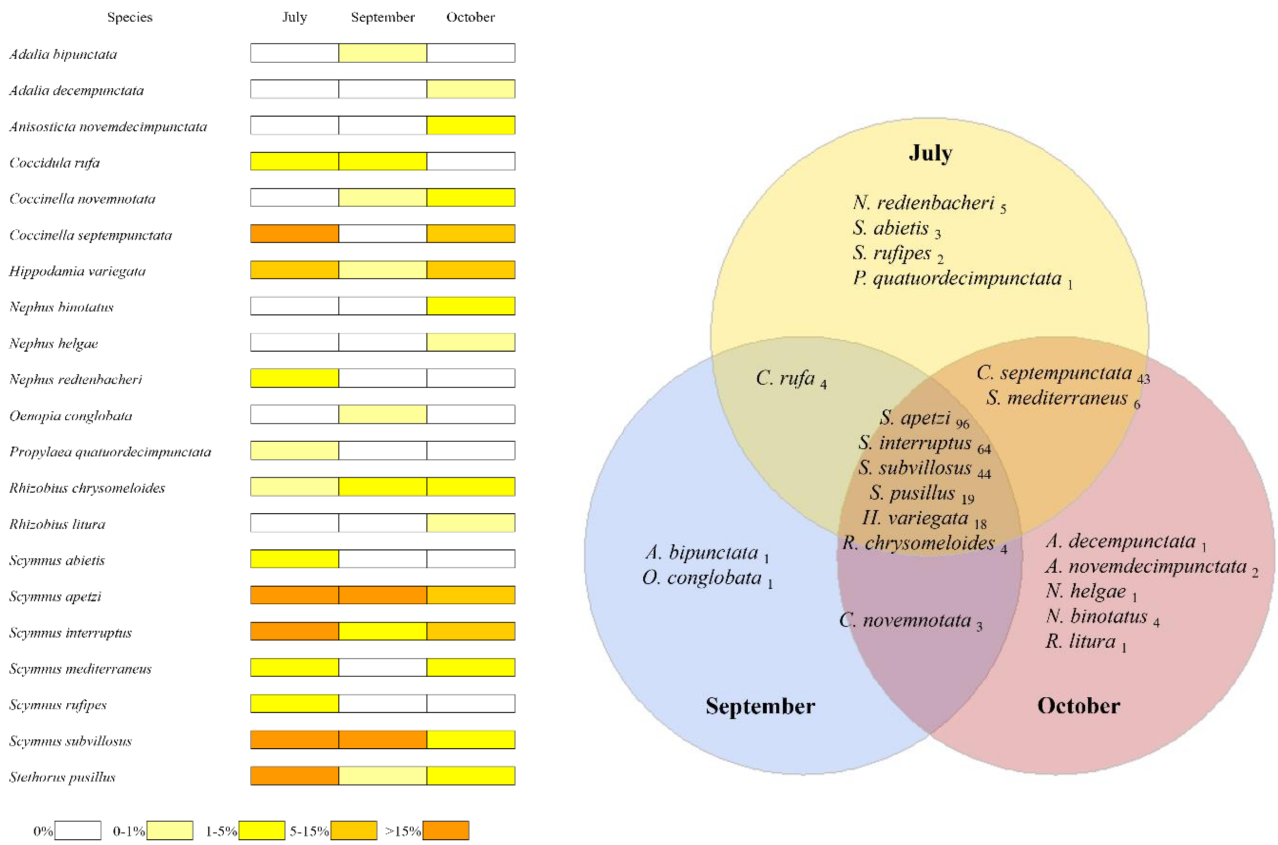
3. Results

3.1. Community Description

In total, 326 Coccinellidae individuals belonging to 21 species were identified. Six of the most abundant Coccinellidae species represented 87.1% of the total collected individuals and belonged to three tribes: Scymnini [*Scymnus apetzi* (Mulsant) totaling 96 individuals, *Scymnus interruptus* (Goeze) 64 individuals, *Scymnus subvillosus* (Goeze) 44 individuals], Coccinellini [*Coccinella septempunctata* (Linnaeus) 43 individuals, *Hippodamia variegata* (Goeze) 18 individuals] and Stethorini [*Stethorus pusillus* (Herbst) 19 individuals] (Table 1). The highest abundance was recorded in July, with 210 individuals and 13 species, followed by October, with 63 individuals and 14 species. September had the lowest abundance and richness, with nine species and 52 individuals, and 10 species (Table 1 and Figure 2). The GLMM results showed that Coccinellidae and Scymnini were more abundant in July than in September and October and in the vegetation ground cover than in the canopy (Table S2).

Table 1. Number of individuals (n) and percentage per sampling date (%) of Coccinellidae by tribes.

Taxa	July		September		October	
	n	%	n	%	n	%
Coccinellini						
Adalia bipunctata (Linnaeus, 1758)			1	1.88		
Adalia decempunctata (Linnaeus, 1758)					1	1.58
Anisosticta novemdecimpunctata (Linnaeus, 1758)					2	3.17
Coccinella novemnotata (Herbst, 1793)			1	1.88	2	3.17
Coccinella septempunctata (Linnaeus, 1758)	29	13.81			14	22.22
Oenopia conglobata (Linnaeus, 1758)			1	1.88		
Propylaea quatuordecimpunctata (Linnaeus, 1758)	1	0.47				
Hippodamia variegata (Goeze, 1777)	10	4.76	1	1.88	7	11.11
Coccidullini						
Rhizobius chrysomeloides (Herbst, 1792)	1	0.47	2	3.77	4	6.34
Rhizobius litura (Fabricius, 1787)					1	1.58
Coccidula rufa (Herbst, 1783)	2	0.95	2	3.77		
Scymnini						
Nephus binotatus (Brisout de Barneville, 1863)					4	6.34
Nephus helgae (Fürsch, 1965)					1	1.58
Nephus redtenbacheri (Mulsant, 1846)	5	2.38				
Scymnus abietis (Paykull, 1798)	3	1.42				
Scymnus apetzi (Mulsant, 1846)	68	32.38	18	33.96	10	15.87
Scymnus interruptus (Goeze, 1777)	51	24.29	4	7.54	9	14.29
Scymnus mediterraneus (Iablokoff-Khnzorian, 1972)	4	1.91			2	3.17
Scymnus rufipes (Fabricius, 1798)	2	0.95				
Scymnus subvillosus (Goeze, 1777)	18	8.57	22	41.51	4	6.34
Stethorini						
Stethorus pusillus (Herbst, 1797)	16	7.61	1	1.88	2	3.17
Total abundance	210		53		63	



(**a**) (**b**)

Figure 2. Relative abundance of Coccinellidae species found in the vineyards in July, September, and October (**a**); Venn diagram showing Coccinellidae species in vineyards in July, September, and October. The overlapping areas indicate species shared by two or three dates. The number of specimens (subscripts) is indicated (**b**).

3.2. Coccinellidae Response to Landscape Structure

Results showed that some of the landscape metrics affected Coccinellidae and that the effects depended on the buffer size where the metrics were calculated. At 500 m, 750 m, and 1000 m, seminatural vegetation (SNA) areas significantly increased the Coccinellidae abundance. Scymnini abundance also had a positive influence by seminatural areas at 500 m, 750 m, 1500 m, and 2000 m buffers (Figure 3). Landscape diversity (SEI) significantly decreased the Coccinellidae abundance (at 1500 m) and Scymnini abundance at 500 m and 1000 m buffers (Figure 4).

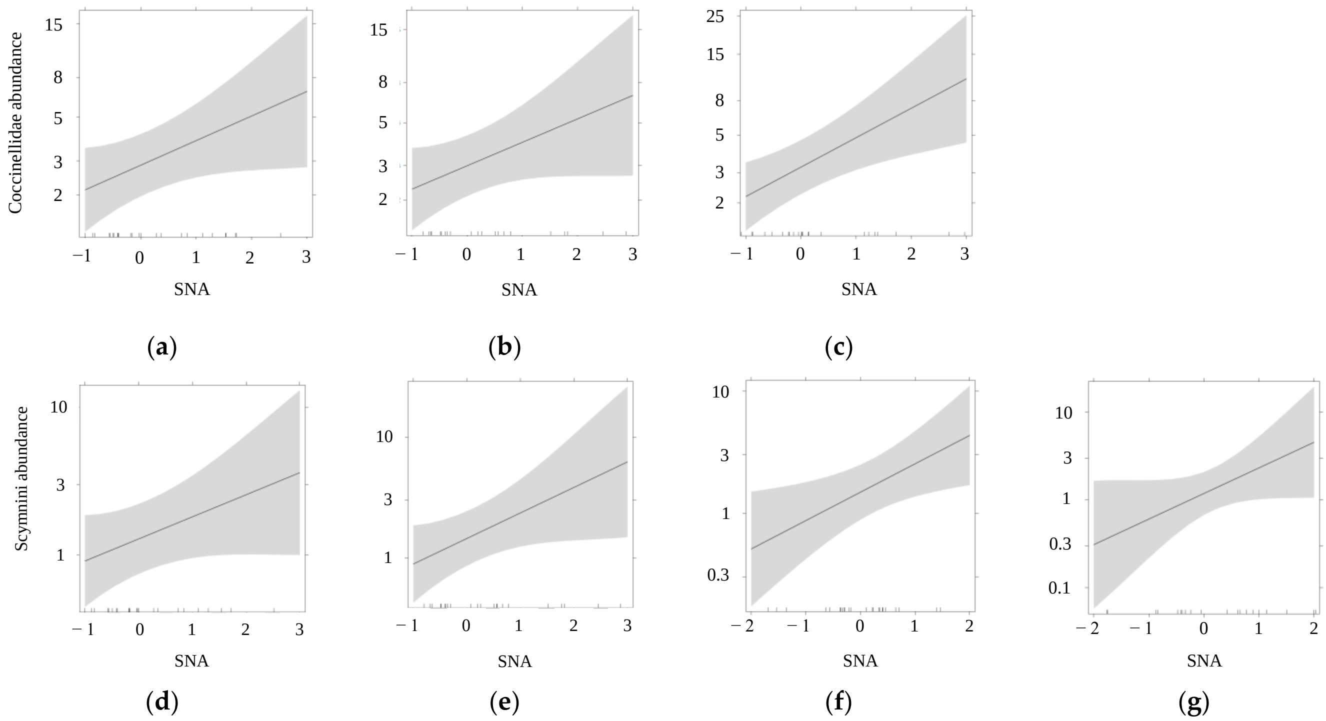


Figure 3. Response of the Coccinellidae and Scymnini abundance to Seminatural areas (SNA) (standardized) surrounding vineyards at different buffers (Coccinellidae: (**a**)—500 m; (**b**)—750 m; (**c**)—1000 m; Scymnini: (**d**)—500 m; (**e**)—750 m; (**f**)—1500 m; and (**g**)—2000 m).

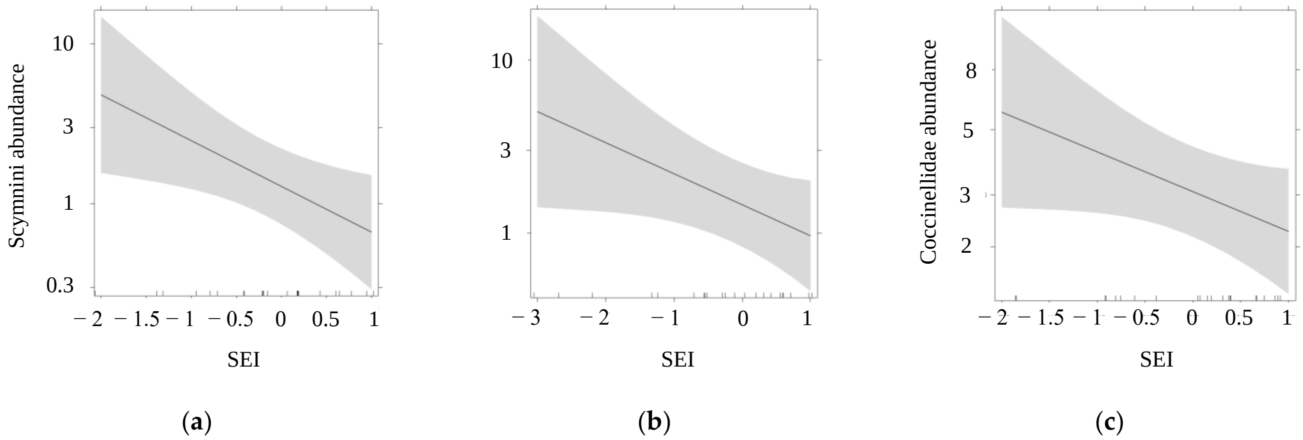


Figure 4. Response of the Scymnini and Coccinellidae abundance to landscape diversity (SEI) (standardized) surrounding vineyards at different buffers: (**a**) Scymnini—500 m; (**b**) Scymnini—1000 m; (**c**) Coccinellidae—1500 m.

The presence of artificial territory and vineyards increased Scymnini abundance at 750 m but not at the other buffers. Coccinellidae abundance was also positively influenced by the complexity of the landscape configuration (MPFD) at larger buffers (2000 m and 1500 m) (Figure 5), and vineyards showed a positive influence on Scymnini abundance at 2000 m, 1500 m, and 750 m (Table S2).

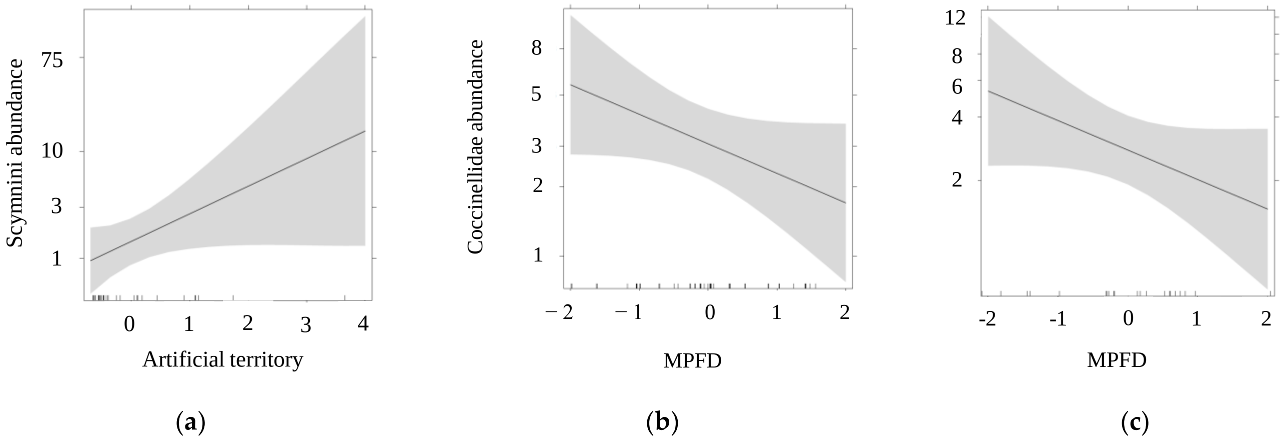


Figure 5. Response of the abundance of Scymnini to artificial territory and response of Coccinellidae to mean patch fractal dimension (MPFD) (standardized) surrounding vineyards at different buffers: (**a**) Scymnini—750 m; (**b**) Coccinellidae—2000 m; and (**c**) Coccinellidae—1500 m.

The richness of Coccinellidae was not affected by the landscape composition or configuration (Table S2).

4. Discussion

In this study, Coccinellidae, and particularly Scymnini abundances, responded to the composition and configuration of the surrounding landscape in Portuguese PDO regions. However, Coccinellidae richness did not vary with the landscape.

Commonly, a diverse landscape mosaic enhances beneficial arthropods [7,13–16]. However, in this study, the landscape diversity (SEI) decreased Scymnini abundance (at 500 and 1000 m buffers) and the abundance of Coccinellidae at 1500 m. A more diverse landscape mosaic may mean more resources for organisms but also implies a higher number of smaller patches and, therefore, a more fragmented area. Fragmented habitats reduce the possible habitat area and increase edge effects [61]. On the other hand, in a less diverse landscape (lower SEI) with larger patches, a larger center area not affected by external factors (such as the environmental and biotic changes associated with edges) will be available [62]. This suggests the need to find an equilibrium between the greater range of habitats provided by more diverse landscapes and the negative effects of habitat fragmentation. As mentioned, this effect was not detected at all buffers, and a partner on the scale effect was not revealed, indicating that more research is needed to confirm the results. However, the abundance of Coccinellidae and Scymnini were generally positively affected by the area of seminatural vegetation around the vineyards, indicating that the specific composition of the land use (SNA) and not the diversity of land uses (SEI) would favor the abundance of Coccinellidae and particularly of Scyminini. That agrees with Thomson and Hoffmann [63], who found a higher abundance of Coccinellidae in vineyards with adjacent woody vegetation, and with Ammann et al. [38], who found landscapes with higher proportions of forest edge to support higher abundance and densities of aphid predators (including Coccinellidae) as well as reduced aphid infestation on fava bean. Uncultivated habitats can provide refuge where Coccinellidae can survive for short periods when prey within the crop is insufficient [64]. Wood trees, like *Arbutus unedo* L., common in seminatural habitats from the studied areas [65], can host important natural enemies of vineyard pests, including Coccinellidae [21]. Also, increasing distances from adjacent vegetation were related to a predator reduction during spring [14], highlighting the positive impact of these habitats on Coccinellidae and other beneficial arthropods nearby vineyards [20,66]. These results indicate that land management focused on maintaining natural or seminatural habitats may support the Coccinellidae community preservation [26]. However, not all studies agree with this conclusion. For example, Judt et al. [22] did not find seminatural habitats to affect arthropods in vineyards, but in this case, although arthropods were collected in May and July, only two Coccinellidae individuals were recovered.

Results also indicated that the complexity of the landscape configuration (MPDF) favored the Coccinellidae abundance at the largest buffers (2000 and 1500 m), being more abundant in vineyards when irregular patches constituted the landscape. The same pattern was observed in several studies when studying Coccinellidae assemblages [67–69], which found that higher configurational heterogeneity (normally related to higher intensification of agricultural landscapes) reduced Coccinellidae diversity and abundance.

In this study, the vineyard areas were related with an increase of Scymnini at 2000 m, 1500 m, and 750 m, which was expectable because they are common insects within vineyards [21,43].

Regarding the Coccinellidae community composition, the most abundant tribe was Scymnini. Scymnini species are voracious predators of aphids and coccids [29,70,71]. Among the five most abundant Coccinellidae, three species *(S. apetzi*, *S. interruptus,* and *S. subvillosus*) belong to Scymnini, and they occur in the canopy and the vegetation cover. These species are common in other Portuguese crops, such as chestnut, almond, or olive groves [31,43,72]. *Scymnus apetzi* and *S. interruptus* were also frequent species in Portuguese citrus groves [45], preying mainly on aphids [27,39,41]. Their high abundance in olive and citrus groves suggested that they probably feed on the black scale, *S. oleae*, a frequent coccid pest in both crops, as well as other coccids and pseudococcids [31,44]. *Scymnus subvillosus* is frequent in Mediterranean olive groves [72], and can feed on coccids, diaspidids, or aphids [45]. *Scymnus* are also considered abundant pseudococcid predators in vineyards [73]. The phenology of these species in other Portuguese crops is in accordance with our results [45,72]. The high abundance of these species in vineyards suggests their role as biological control agents.

Coccinellini was the second most abundant tribe found in the vineyards. This tribe is mainly composed by predators of aphids [74], psyllids, and Chrysomelidae [27]. It includes *C. septempunctata*, the third more abundant species in the study, which besides aphids [39], feed on pollen [27]. In Portugal, this species is frequent in chestnuts [43], citrus groves [45], and almond trees [42]. Its peak was in July, in September was not found, and reappeared with less abundance in October. The same pattern occurred in chestnuts [43], where *C. septempunctata* had its peak in August, was almost null in September, and recovered its activity in October. *Hippodamia variegate*, also Coccinellini, is an aphidophagous species [29,39], which was only found in the vegetation cover. *Hippodamia* genus is frequent in perennial crops [42,43,45,72], and in the spontaneous vegetation cover of legume crops [19]. In this agroecosystem, *H. variegata* was also found in the blooming herbaceous vegetation cover [9].

The tribe Stethorini was the third most abundant in this survey, and it was composed of only *S. pusillus*, the fifth more numerous collected species. This species is a well-known specialist of Tetranychidae [28], actively prey *P. ulmi*, and *T. urticae* [33], and can feed on extrafloral nectaries in the absence of prey [27]. *Stethorus pusillus* is extensively distributed throughout the most geographically diverse areas, and crops being successfully used in biological control in woody perennial systems, like vineyards [28].

5. Conclusions

The results of this study suggest that larger areas of seminatural habitats surrounding the vineyards would result in higher abundances of Coccinellidae, particularly of Scymnini, and that fragmentation may be detrimental for them, while the Coccinellidae richness would not be affected by the landscape. Moreover, Coccinellidae were concentrated in the ground cover vegetation and in July. This Coccinellidae relationship with the surrounding landscape and its community composition in Portuguese vineyards indicate that managing landscapes toward mosaics of less fragmented habitats, with prioritization of the presence of large natural areas, may benefit Coccinellidae species with important roles in biological control against vineyard pests.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy12092140/s1, Table S1: Wine Regions climatic information [46], vineyards' sampling dates, and management data of the plots. Tmin—Medium of the minimum temperature; Tmax—Medium of the maximum temperature; Total Prec.—Total Precipitation. Table S2: General Liner Mixed Models (GLMMs) for the Coccinellidae abundance and richness, and Scymnini abundance response to landscape variables, sampling period and habitat at different buffers (2000 m, 1500 m, 1000 m, 750 m, and 500 m). SEI—Simpson's diversity index; MPFD—mean patch fractal dimension; S- September; O—October; J—July; H—Herbaceous; C—Canopy.

Author Contributions: Conceptualization: J.A.P., I.R. and M.V.; Methodology: L.T., I.R., S.A.P.S. and J.A.P.; Statistical analysis: M.V.; Writing—Original draft preparation, review and editing, all authors contributed equally. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020 and UIDP/00690/2020) to the Associate Laboratory SusTEC (LA/P/0007/2020), and to the project OLIVESIM-Managing ecosystem services in olive groves using advanced landscape agent-based models PTDC/ASP-PLA/30003/2017.

Data Availability Statement: Data are available from the authors upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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