



# Article Effectiveness of Multifunctional Margins in Insect Biodiversity Enhancement and RTE Species Conservation in Intensive Agricultural Landscapes

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Abstract: Starting in the 1950s, agricultural production has been remarkably intensified, resulting in modern management systems where a severe increase in field size led to an elimination of edges and other ecologically valuable structural elements. The resulting habitat loss caused dramatic changes in natural communities. The aim of this work is to test whether there are statistically significant differences in insect abundance over time by using multifunctional margins that are seed mixtures of autochthonous species planted in combined strips, which are the fastest way to provide significant biodiversity benefits within farmed landscapes, enhancing the diversity and abundance of insects, birds, and small mammals, offering resources and reservoirs. This study was carried out in three intensive fruit farms in Spain over a three-year period (2013–2015). Each field was divided into two zones: the margin where a multifunctional margin was planted, and another that remained unchanged in the field. A clear trend to increase RTE species throughout the years in all farms was observed. Moreover, the margin showed a significant difference with respect to the field in the average number of insect species and individuals. The use of margins improves the appearance of RTE species in mean percentages ranging between 12.06 and 25.26% according to the sampling area. Margins also favour the increase in species (148.83-232.84%) and individuals (207.24-586.70%) in agricultural landscapes. These results clearly show that margins are an essential tool to fight insect decline in intensive farming areas.

**Keywords:** agro-ecosystems; sustainability; habitat management; cover plants; natural enemies; RTE species; stone fruits

# 1. Introduction

The substitution of heterogeneous agricultural landscapes by homogeneous ones, together with the increased adoption of intensive agricultural practices, has resulted in accelerated environmental deterioration and the associated loss of whole taxonomic groups [1–5]. Various studies show that the influence of changing agricultural practices since the second half of the 20th century negatively impacted species richness, abundance, and biomass, and led to shifts in species composition [6–14]. Reviews prove that six key factors are responsible for the biodiversity decline: (1) habitat loss, fragmentation, and degradation; (2) invasive species; (3) parasites and diseases; (4) non-sustainable use of pesticides; (5) extinction cascades; and (6) climate change [15–17].



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Biodiversity decline has been mainly reported in wild bees, honeybees, hoverflies, butterflies, wasps, birds, and mammals [18,19] through comprehensive studies carried out in Europe and North America. Given the fact that these regions boast a long history of agricultural activity, it is likely that farming impacted the most sensitive species even before studies started to investigate such effects. It is therefore not possible to claim that these studies are representative of what is happening globally [20,21], as intensive farming activities set in quite late in many tropical areas [19].

Pollinating insects have been severely affected by land-use change and are suffering the highest decline [10,15,22–27]. A recent review on land-use intensity showed a divergent effect on cropland pollinator biodiversity between non-tropical and tropical areas. In non-tropical areas, species and abundance did not differ significantly among minimal, light, and intense land-use, while in tropical areas species richness decreased by 44–49% in an intense land-use scenario compared to one with minimal farming activities [19].

Global crop production heavily depends on ecosystem services from pollinators. The value of such services is estimated at USD 235–577 billion per year the world over [28]. For this reason, over the past 40 years, several studies have tried to investigate the causes behind declining insect populations and proposed measures to protect and enhance biodiversity in agricultural landscapes [29–37]. Most commonly, these studies focused on implementing hedgerows, field margins, floral margins, or flower and herb strips, which provide significant biodiversity benefits within farmed landscapes. As a matter of fact, these measures are suitable to enhance the diversity and abundance of insects, birds, and small mammals, offering them habitats, nesting places, and food resources [34,35,37–51].

Studies focusing on biodiversity in rural areas are critically important to gain a deeper knowledge of how ecosystems in such areas function and to understand what type of measures are suitable to protect and conserve biodiversity and to enhance biodiversity in agricultural landscapes [52–59].

In the present work, we focus on RTE (Rare, Threatened, and Endangered) species and we test three hypotheses. First, we check whether the implementation of multifunctional margins effectively increases the probability of finding RTE species. Our second hypothesis is that the use of field margins correlates with an increase in the number of species. Finally, we test the assumption that the use of field margins also boosts the number of individuals. The second and the third hypotheses are tested irrespective of whether a species or an individual falls into the RTE category. These hypotheses were studied in three intensive fruit farms in Spain.

## 2. Materials and Methods

#### 2.1. Areas of Study

The study was carried out in three highly productive Spanish stone fruit farms located in Águilas (Murcia; 37°37′04.7″ N, 0°47′08.1″ W), Alcarràs (Lérida; 41°34′39.2″ N, 0°30′24.4″ E), and Fuliola (Lérida; 41°43′13.62″ N, 0°59′53.56″ E) (Figure 1). Lérida farms have a slightly continental Mediterranean climate with hot summers and cold winters where the annual rainfall is approx. 340 mm, while Águilas presents a semi-arid Mediterranean climate with hot summers and mild winters with an annual rainfall of 201 mm [60].

The crops covered in our study are peaches (on the Águilas farm) and nectarines (on the Alcarràs and Fuliola farms), which are planted in a standard-conventional design, with tree lines separated by 5 m and with trees of a same row planted approximately every 2 m. All fields are of equal size (6 ha each).

During the study, all farms stuck to their preferred agricultural practices such as tillage, sowing, and fertilisation, and phytosanitary treatments remained unchanged. Any management measures were was confined to the crop to avoid trying not to interfere with the multifunctional margin.



Figure 1. (A) Farm location in Spain. (B) Multifunctional margin in Fuliola (Lérida) farm.

#### 2.2. MFM Mixture Plant Selection

Multifunctional margins (MFM) are seed mixtures of autochthonous species planted in combined strips, which are the fastest way to provide significant biodiversity benefits within farmed landscapes, enhancing the diversity and abundance of insects, birds, and small mammals, offering resources and reservoirs. The selection of plant species was based on several fundamental criteria, such as the strict use of native species, ensuring a smooth climatic adaptation; being non-weed for the crop; featuring easy maintenance and capacity for self-sowing, as well as staggered flowering phenologies; and finally, being attractive for pollinators and natural enemies.

MFM were established using an herbaceous mixture consisting of *Borago officinalis* L. (10%), *Calendula officinalis* L. (22.5%), *Coriandrum sativum* L. (10%), *Diplotaxis catholica* (L.) DC. (5%), *Echium vulgare* L. (5%), *Melilotus officinalis* (L.) Pall. (12.5%), *Nigella damascena* (L.) (5%), *Salvia verbenaca* L. (10%), *Silene vulgaris* (Moench) Garcke (10%), and *Vicia sativa* L. (10%). This mixture was sown in January 2013 in an area of 200 m length  $\times$  3 m width separated about 5 m from the field edge to favour management by an electric drill with air distribution after the soil preparation by flail mower and subsequent covering of the seed with a drag. The sowing dose used was 15 kg/Ha. The margins were mowed in autumn and then left to regrow.

#### 2.3. Experimental Design and Sampling

The experiment was conducted for 3 years (2013–2015) to investigate the dynamics of effects of multifunctional margins on RTE species. On each farm a plot was selected, and this field was divided into two zones: field and margin. The field was kept clean of cover plants, weeds, or spontaneous vegetation through the application of a residual herbicide at the beginning of the season and through the mechanical removal of weeds during the season. In the field margin, a line of herbaceous mixture was sown.

The insect abundance was assessed visually and using a sweeping net. All observations were made by moving in a zigzag along fixed transects of 50 m  $\times$  2 m during 15 min per line and four times per day to avoid the light and temperature gradient and obtain a more representative sample. Observed and captured specimens were merged to perform the corresponding analyses. Sampling took place five times a year following the stone fruit tree growth stages (shooting, blossoming, fruit setting, harvest, and senescence of leaves) coinciding with the vegetative period of the multifunctional margin plants.

Collected specimens were preserved in cyanide to keep them intact and to avoid discoloration. All specimens were identified to species level using appropriate entomological literature (see [61–75]). After their identification, the species were catalogued according to the IUCN Red List [76]. For the data analysis, we focused only on RTE species. Specimens are deposited in the entomological collection of the National Museum of Natural Sciences (Madrid, Spain; MNCN).

## 2.4. Statistical Analysis

First, an exploratory data analysis was initially performed to describe the behaviour of the percentage of RTE species, the abundance of species, and the abundance of individuals under the evaluated factors. Second, to test our hypotheses, we used an approach based on fitting regression models to explain the presence of RTE species, the number of species, and the number of individuals. Then, we estimated three generalised linear models (GLM), one for a binary variable and two for count data, respectively.

#### 2.4.1. Model for RTE Species

For evaluating the impact of MFM and their consistency through time in decreasing the pressure over RTE species, a logistic regression model is fitted. To perform this, we define the binary random variable:

$$Z = \begin{cases} 1 & \text{if there is at least one identified insect specimen considered at risk} \\ 0 & \text{in any other case} \end{cases}$$
(1)

Based on Equation (1), we establish the logistic regression model as follows:

$$\log\left(\frac{\pi_{ijkl_{o_k}}}{1 - \pi_{ijkl_{o_k}}}\right) = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijkl_{o_k}} \begin{cases} i = 1, 2 \\ j = 1, 2, 3 \\ k = 1, 2, 3 \\ l = 1, 2, \dots, o_k \end{cases}$$
(2)

where  $\pi_{ijkl_{o_k}}$  represents the probability of finding at least one individual of a species at RTE in the *i*th zone, *j*th year, and *k*th farm.  $o_k$  is the number of identified species in the *k*th farm. The left part in Equation (2) is known as the logit function and it is interpreted as the logarithm of odds [77]. In Equation (2),  $\alpha_i$  is the *zone*,  $\beta_j$  is the year, and  $\gamma_k$  is the farm.

## 2.4.2. Models for Abundance of Species and Individuals

Similarly, as the previous case, to test the effect of the implemented measures and their evolution in the abundance of *species* and *individuals*, we define two GLM for count data, respectively, as follows:

$$g(n_{ijkl}) = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \varepsilon_{ijkl} \begin{cases} i = 1, 2 \\ j = 1, 2, 3 \\ k = 1, 2, 3 \\ l = 1, 2 \end{cases}$$
(3a)

where  $n_{ijkl}$  represents the number of species in the *i*th zone, *j*th year, *k*th farm, and *l*th type of species (see Equation (1)).

$$g(n_{ijklm_{o_k}}) = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \varepsilon_{ijklm_{o_k}} \begin{cases} i = 1, 2 \\ j = 1, 2, 3 \\ k = 1, 2, 3 \\ l = 1, 2 \\ m = 1, 2, \dots, o_k \end{cases}$$
(3b)

where  $n_{ijklm_{o_k}}$  represents the number of individuals in the *i*th zone, *j*th year, *k*th farm, and *l*th type of species.  $o_k$  is the number of identified species in the *k*th farm.

In Equations (3a) and (3b), g is a monotonous function that linearises the relationship between the response variable and the systematic component of the model, such as loga-

rithm or root square for a Poisson model. It is usually assumed that the response variable, i.e.,  $n_{ijkl}$  or  $n_{ijklm_{o_k}}$ , follows a Poisson distribution when its mean and variance are equal or a negative binomial when its variance is greater than its mean (overdispersion) [78]. Here,  $\alpha_i$  is the *zone*,  $\beta_i$  is the *year*,  $\gamma_k$  is the *farm*, and  $\delta_l$  is the *type of species*.

The parameters of three models in Equations (2), (3a) and (3b) were estimated via maximum likelihood. For the models in Equations (3a) and (3b), we consider and evaluate a Poisson and a negative binomial distribution for the response variable. The fitted count data models are compared to choose the best probability distribution of the response variable by using a likelihood ratio (LR) contrast [79]. We address all statistical data analysis in R statistical software [80]. Particularly, for fitting the models, we use the glm and glm.nb function from packages stats and MASS, respectively. For statistical inference over the fitted GLM, we use the deviance, AIC, and BIC functions from stats package, Anova function from car package, and Irtest from Imtest package.

#### 3. Results

## 3.1. Diversity of Insects

A total of 3305 insects were captured during the three-year research programme. Of these insects, 1828 individuals were sampled from Águilas and 894 and 583 individuals from Alcarràs and Fuliola, respectively (Table 1). In Águilas, 154 species (74 from the field and 139 from the margin) were identified, of which 57 species (24 from the field and 54 from the margin) are listed in the Red List. On the other hand, 46 species were captured (31 from the field and 45 from the margin) from Alcarràs, of which 19 species are registered in the Red List (12 in the field and 19 in the margin). Finally, in Fuliola, 57 species (39 from the field and 26 from the margin) had been catalogued in the Red List.

	Leasting	20	013	2014		2015	
	Location -	Field	Margin	Field	Margin	Field	Margin
	Águilas	26	54	51	79	57	131
Species	Alcarràs	11	14	23	36	25	44
-	Fuliola	12	12	22	42	36	53
	Águilas	52	389	122	377	141	747
Individuals	Alcarràs	35	32	91	233	104	399
	Fuliola	38	33	73	107	127	205

Table 1. Abundance of species and individuals by farm and zone through the years.

Analysing the species by category, we observe that all species were catalogued as LC (Least Concern) except *Epeolus cruciger* (Panzer, 1799) (Hymenoptera: Apidae) and *Halictus quadricinctus* (Fabricius, 1776) (Hymenoptera: Halictidae), which belong to the NT (Near Threatened) category. Of these NT species, one individual of *Epeolus* was captured from Águilas in the margin zone (in 2015), while three *Halictus* individuals were found in Alcarràs and Fuliola also in the multifunctional margin in 2015. However, these two NT species were never captured from the field zone.

## 3.2. GLM Modelling

Figure 2 and Table A1 show the changes in the average of percentage of presence of RTE species, the total number of identified species, and the total number of insects between zones, farms, and years, respectively. All three measures of biodiversity and abundance show a trend of increasing their average through the years in all farms. However, these trends differ between farms, varying the rate of change. In all the cases, the zones in the margins of the farms have higher averages in comparison with the zones in the fields. In most of the cases, the variability of the percentage of RTE species, the number of species, and the number of individuals in the margins is bigger than in the fields, showing the



complexity of insect population dynamics among the contrasting farming environments. Finally, there is not an observed interaction effect among the zones and the years.

**Figure 2.** Plot of means and standard error bars of the percentage of RTE species, the total number of species, and the total number of individuals between zones across the farms through the years. (a) Percentage of RTE species. (b) Abundance of species. (c) Abundance of individuals.

## 3.2.1. Model for RTE Species

We estimated a logistic regression model based on Equation (2). The reference categories were Location: Aguilas, Year: 2013, and Zone: Field. Table 2 presents the statistics for the goodness of fit and the analysis of deviance to the adjusted model. The LR test shows that the model has a better fit than the null model (model without explanatory variables). We then concluded that the model is acceptable to explain the percentage of RTE species as a function of the examined systematic component, i.e., zones, years, and farms, since the deviance statistic is also statistically significant. The analysis of deviance also shows that the related parameters are all statistically significant, which means that there are differences due to main effects, zones, and years, and the blocking effect the farm.

**Table 2.** Statistics of goodness of fit and the analysis of deviance table (Type II Wald chi-square tests) in the fitted logistic regression model for the percentage of RTE species.

	Statis	tics of Goodnes	s of Fit	
Likelihoo	d ratio (LR)		104.8 ***	
Devia	nce (D)		1420.5 *	
А	JC		1432.5	
В	BIC		1464.5	
	Analy	sis of Devianc	e Table	
Source	LR Chisq	Df	<i>p</i> –value	
Farm	11.42	2	0.003	**
Year	61.62	2	$4.170 \times 10^{-14}$	***
Zone	33.80	1	$6.125  imes 10^{-9}$	***

\*\*\* [0, 0.001]; \*\* [0.001, 0.01]; \* [0.01, 0.05]; [0.05, 0.1]; [0.1, 1].

Table 3 shows the estimated parameters of the logistic regression model and the odds ratio with their 95% confidence intervals. This fitted model shows that, holding farm and year at a fixed value, the odds of getting at least one individual of an RTE species in the margin (Zone: Margin = 1) over the odds of getting at least one individual of an RTE species in the field (Zone: Margin = 0) are  $\exp(0.78) = 2.18$ . In terms of percent change, we can say that the odds for the margin are 118% higher than the odds for the field. Similarly, the associated coefficients with year show that, holding farm and zone at a fixed value, we will see a 135% and 277% increase in the odds of obtaining at least one individual of an RTE species in the years 2014 and 2015 with respect to the year 2013 since  $\exp(0.85) = 2.35$  and  $\exp(1.33) = 3.77$ .

**Table 3.** Estimated regression coefficients, odds ratios, and 95% confidence intervals in the fitted logistic regression model for the percentage of RTE species.

Estimate	OR	2.5%	97.5%
-2.85	0.06	0.04	0.08
0.40	1.49	1.06	2.09
0.49	1.63	1.19	2.22
0.78	2.18	1.67	2.85
0.85	2.35	1.64	3.41
1.33	3.77	2.67	5.4
	-2.85 0.40 0.49 0.78 0.85	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

## 3.2.2. Models for Abundance of Species and Individuals

We fitted four count GLM based on Equations (3a) and (3b) by considering a Poisson and a negative binomial response. Table A2 presents the statistics for the goodness of fit to the estimated models. For the case of the number of identified species, based on the LR test and deviance statistic, both models have approximately the same fit. However, AIC and BIC statistics are slightly lower for the model that assumes the Poisson distribution for the response variable, which means that the Poisson distribution seems to be an adequate probabilistic schema for the number of species. For the case of the number of identified individuals, the LR test shows a better fit in the model that uses a negative binomial distribution for the response variable, which means that the variance of the count of individuals increases more rapidly than their mean and the negative binomial distribution is more accurate as a probabilistic schema for the number of individuals. Moreover, the other statistics of goodness of fit such as AIC and BIC are considerably lower for the model that assumes the negative binomial distribution for the response variable.

Based on the previous results, we selected the *Poisson model* for the *number of species* and the *negative binomial* for the *number of individuals* as preferred models. Tables 4 and 5 show the analysis of deviance and the estimated parameters with their associated confidence interval for the preferred GLM, respectively. In both cases, the statistical inference in the models shows that the effects, zone, year, and farm, are statistically significant. The related parameters are also significant and reveal an increase in the number of species and individuals with time and in the margins. However, there is a difference between the model for the abundance where the parameter associated with the RTE species is significant in the case of the number of species but not in the number of individuals.

**Table 4.** Analysis of deviance table (Type II Wald chi-square tests) in the fitted count regression model for the number of identified species and individuals.

Model fo	Model for the Number of Identified Species				Model for th	l for the Number of Identified Individuals				
Source	LR Chisq	Df	<i>p</i> -Value		Source	LR Chisq	Df	<i>p</i> -Value		
Farm	141.0	2	$<2.2 \times 10^{-16}$	***	Farm	15.1	2	0.0005293	***	
Zone	56.8	1	$4.85  imes 10^{-14}$	***	Zone	128.7	1	$<2.2 \times 10^{-16}$	***	
Year	103.6	2	$<2.2 \times 10^{-16}$	***	Year	66.3	2	$4.11  imes 10^{-15}$	***	
Type of species	21.2	1	$4.09  imes 10^{-6}$	***	Type of species	1.6	1	0.2106602		

<sup>\*\*\* [0, 0.001].</sup> 

Table 5. Estimated regression coefficients in the fitted count regression model for the number of identified species and individuals.

Model for the N	Number of Ide	ntified Spe	Species Model for the Number of Identified Individuals				
Parameter	Estimate	2.5%	97.5%	Parameter	Estimate	2.5%	97.5%
Intercept	2.70	2.48	2.92	Intercept	-0.76	-1.00	-0.52
Location: Alcarras	-0.96	-1.15	-0.77	Location: Alcarras	0.49	0.23	0.76
Location: Fuliola	-0.81	-0.99	-0.64	Location: Fuliola	0.01	-0.24	0.27
Zone: Margin	0.57	0.42	0.72	Zone: Margin	1.20	0.99	1.40
Year: 2014	0.67	0.46	0.89	Year: 2014	0.56	0.31	0.81
Year: 2015	0.99	0.79	1.19	Year: 2015	1.03	0.78	1.27
Type of species: RTE	-0.34	-0.49	-0.20	Type of species: RTE	0.16	-0.09	0.42

## 4. Discussion

Even if biotopes in the Mediterranean area are often not suitable for human intervention, urbanisation, infrastructures, and agricultural activities have led to isolation and fragmentation and the emergence of isolated biodiverse patches within the landscape [81]. The creation of multifunctional margins (MFM), banker plants, headlands, or hedges sown with a species-rich seed mixture has resulted in the fast recovery of insects, with biodiverse and abundant populations [37,51,82–89]. According to our first working hypothesis, the integration of multifunctional margins increases the probability of finding RTE species. There are clear differences between field margin and field. While there is a clear trend in both zones for the likelihood of identifying more RTE species, this trend is much stronger in field margins than in the fields. As a matter of fact, the probability of finding RTE species in Águilas increased from 13.63% to 33.76% in the margin and from 6.49% to 14.28% in the field. In Alcarràs, the probability significantly rose from 13.04% to 39.13% in the margin and from 10.89% to 21.73% in the field. In Fuliola, the values went up from 7.01% to 45.61%

in the margin and from 8.77% to 33.33% in the field. Looking at the mean value for all three farms taken together, the probability increased from 12.06% to 37.35% in the margin and from 7.78% to 19.84% in the field. We must be aware that an increased probability of finding RTE species can also be triggered by the occurrence of only one or two species in the area examined. Therefore, it is necessary to closely look at the trend in the number of species and individuals in order to obtain a more approximate interpretation of the data. Indeed, the use of plant mixtures can play a very important role in the speed of biodiversity enhancement. Similar observations were also made by authors such as Hannon and Sisk [90], who considered that flowering shrubs are important in attracting bees (that were otherwise uncommon in the landscape) and further pollinators that may play a valuable role in pollinating agricultural crop plants.

According to our second working hypothesis, selecting the right plants can improve the number of insect species. The results from the present study show that there is a clear increase in the number of species both in the field and in the margin. Within three years, the number of species identified in Águilas grew from 26 to 57 (by 119.23%) and from 54 to 131 (by 142.59%) in the margin zone. A similar development could be registered for Alcarràs and Fuliola. There, the increase in the field was 127.27% and 200.00%, respectively, and the increase in the field margin was 214.28% and 341.66%. All three sites taken together, the number of species identified in the field rose by 148.83% and in the field margin by 232.84%. This confirms the findings of several other studies that MFM play an important role in the presence of abundant insect species [50,76–80,83,84]. However, only two such studies examined insect species over three consecutive growing seasons. Miranda-Barroso et al. [91] registered an increase of 102.47% in an alfalfa field, while Peris-Felipo et al. [92] found that the number of species identified on five intensively managed wine farms rose by 12.10% after three years of using cover crops. On the other hand, the analysis of RTE species of the three studied stone fruit farms showed that 42.77% of species (77 of 180 spp.) are extinction RTE according to the IUCN Red List (2021), while Miranda et al. [91] noted a value of 33.74% (55 of 163 spp.). These results underline the importance of MFM and plant mixtures as strategies to support the conservation of species in intensively farmed areas. With regard to this, various authors have suggested that future conservation strategies need to assess whether it is better to minimise further habitat losses or whether it makes more sense to enhance agricultural landscapes with measures such as smartly composed multifunctional margins [17,35,37,44–51,91–94].

Finally, following our third hypothesis, we observed that the number of individuals increased significantly in the field and margin over time. Numerous researchers have studied the effect of the impact of plant mixtures on the abundance of insects [49,50,82–88,91,92]. Our results demonstrate that, in Águilas, the number of individuals in the field grew from 52 to 151 (190.38%), while the number went up from 389 to 747 in the field margin (plus 92.03%). In Alcarràs and Fuliola, the number of individuals collected in the field rose by 197.14% and 234.21%, respectively. In the field margins of these farms, the increase registered was 521.21% and 1146.87%, respectively. With all three farms taken together, the number of individuals identified increased by 207.24% in the field and by 586.70% in the field margin. These values are bigger than those obtained by Miranda-Barroso et al. [91] in one alfalfa field and Peris-Felipo et al. [92] in five intensively managed wine farms, where they observed an increase of 97.64% and 40.01%, respectively.

The results obtained show that the implementation of MFM and the appropriate selection of plant species based on basic criteria (see Materials and Methods) substantially increase the number of pollinating insect species and individuals over the years. This phenomenon emerges even more clearly in areas where the abundance of individuals was low at the beginning of the study. An increase in the number of pollinating insect species and individuals is of particular relevance in crops such as stone fruits, where they improve fruit curdling. Apart from these agronomic benefits, there are also ecological ones. Indeed, MFM play an important role as an insect reservoir and help to mitigate the impact from intensive agriculture on insect populations.

We could not obtain significant results when analysing the number of individuals and distinguishing whether these were RTE or not. There is a very high variability in the number of individuals registered. This is less so the case when there is at least one RTE as in hypothesis 1 and Equation (1).

## 5. Conclusions

Implementing multifunctional margins in intensive farmed landscapes clearly promotes biodiversity conservation. Field margins sown with plant mixtures indeed play an important role for the conservation of RTE species and for enhancing the abundance of species and individuals in the short term and keeping it up in the longer term. In addition, we conclude that the implementation of these measures should be considered an essential and permanent strategy for biological conservation.

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## Appendix A

**Table A1.** Descriptive statistics measures of the percentage of RTE species, the total number of species, and the total number of individuals between zones across the farms through the years.

Farm	Year	Zone	% R	% RTE Species Number of Species Number of Individua	er of Individuals			
ГаШ	Tear	Zone	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
	0010	Field	0.06	0.25	13	4.2	0.3	1.2
	2013	Margin	0.14	0.34	27	8.5	2.5	21.5
Aquilas	0014	Field	0.12	0.32	26	10.6	0.8	2.2
Aguilas	2014	Margin	0.21	0.41	40	9.2	2.4	17.8
	2015	Field	0.14	0.35	29	9.2	0.9	2.9
	2015	Margin	0.34	0.47	66	19.1	4.9	37.8
	2012	Field	0.11	0.31	6	0.7	0.8	2.4
	2013	Margin	0.13	0.34	7	1.4	0.7	1.5
. 1	0014	Field	0.20	0.40	12	3.5	2.0	3.6
Alcarras	2014	Margin	0.33	0.47	18	4.2	5.1	15.2
	0015	Field	0.22	0.42	13	3.5	2.3	4.3
	2015	Margin	0.39	0.49	22	5.7	8.7	26.5
	2012	Field	0.09	0.29	6	1.4	0.7	1.4
	2013	Margin	0.07	0.26	6	2.8	0.6	1.5
<b>T</b> 1. 1	0014	Field	0.16	0.37	11	2.8	1.3	2.0
Fuliola	2014	Margin	0.35	0.48	21	1.4	1.9	2.4
	0015	Field	0.33	0.48	18	1.4	2.2	3.4
	2015	Margin	0.46	0.50	27	0.7	3.6	3.6

Test	Poisso	n	Negative binomial	
Likelihood ratio (LR)	322.6	***	94.6	***
Deviance (D)	18.1		18.1	
AIC	198.2		200.2	
BIC	209.3		212.9	
Mode	l for the Number o	of Identified In	dividuals	
Test	Poisso	n	Negative bi	nomial
Likelihood ratio (LR)	1824.2	***	203.8	***
Deviance (D)	13,247.7		1316.6	***
AIC	15,205.9		5015.2	
BIC	15,243.3		5057.9	

Table A2. Statistics of goodness of fit for the fitted count regression models.

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