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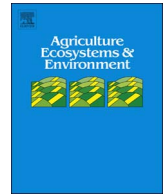
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## Research Paper

# Effects of organic and conventional crop management on vineyard biodiversity



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## ABSTRACT

Although organic farming is rapidly expanding in the vineyards of southern Europe, conventional crop management, using treatments that require a number of chemical inputs to guarantee yields is still the most common approach to crop management. To gauge the effects of these management systems on biodiversity, communities of vascular plants, butterflies, moths and birds were studied in vineyards in the Priorat Appellation of Origin (Catalonia, NE Spain). Measurements inside plots (all four taxonomic groups) and in grass strips between crop lines (only butterflies and vascular plants) were taken in organically and non-organically treated vineyards. Crop treatment was found to have an important effect, stronger on the most sessile organisms. Organic farms hosted consistently richer communities of both vascular plants and butterflies, a trend that was also observed – albeit less significantly – in moths. The weaker response in this group was probably due to insufficient sampling. Birds, the most vagile of the surveyed taxa, showed no significant response to treatments. Grass strips acted in all cases as reservoirs of biodiversity and hosted richer assemblages. The current trend of placing vineyards on slopes without terracing should ensure the existence of uncultivated strips within the vineyards to enhance the biodiversity of these agroecosystems. As well, parameters such as altitude and urban surface area are important drivers of biodiversity in this region. Our results suggest that organic farming may contribute to halting the widespread decrease that is occurring in communities of butterflies and other insects in this region.

## 1. Introduction

Globally, both social pressure and legislation are encouraging the implementation of crop production systems that are more environmentally sustainable and respectful. The focus is no longer only on yields but also on the quality, health and environmental security of products and procedures. Implementation of the European Union's agri-environment programs has been compulsory for member states since 1992, although they are still voluntary for individual farmers (CEU, 1992). The agri-environmental measures these programs imply are aimed at enhancing environmental biodiversity in and around farmlands, and reward the farmers who put them into practice for the ecological services their lands provide (Bradley et al., 2002). Organic farming (OF) is based mainly on the premise of enhancing biodiversity and using only natural products, that is, no synthetic fertilizers, herbicides or pesticides, or genetically modified varieties are employed during the

cultivation process. It also advocates that agroecosystems should have the potential to regulate basic services such as pollination and pest control if they are properly managed and biodiversity is preserved (Altieri and Farrell, 1995). The use of pesticides and herbicides, along with certain aggressive mechanical practices, can seriously harm the biodiversity of agroecosystems, a key component of their capacities to self-regulate and be self-sustainable. Moreover, the ecosystem services provided by healthy agroecosystems transcend the boundaries of farms and stimulate a number of off-farm benefits ranging from protection from erosion and water flow regulation and purification, to pest control and carbon sequestration (Garbach et al., 2014). Nevertheless, to our knowledge no estimates of their global impact exist.

Over the last two decades, OF practices have greatly expanded in Catalonia (NE Spain) and in the period 1995–2015 the amount of OF cultivation rose from just 4936 to 142,024 ha (CCPAE, 2016). Of the crops responsible for this dramatic increase, vineyards are by far the

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most significant: in 2015, organic vineyards occupied 11,706 ha, 36% of all the organic crop surface area in Catalonia (excluding organic pastures, which are widespread in the Pyrenees). Despite the obvious economic importance of organic vineyards (their production in 2014 was estimated in Catalonia at €43.1 million – [CCPAE, 2016](#) –), debate still continues in the farming local community as to whether or not it is worthwhile following the strict criteria imposed by OF. One of the arguments put forward by the Catalan government to promote this type of farming is that it benefits biodiversity; yet to date no studies have ever been carried out to test this assumption in this region.

It could be argued that the lack of consensus about the benefits of OF in vineyards is also reflected in the contrasting conclusions reached by a limited number of studies. [Bruggisser et al. \(2010\)](#), working on Swiss vineyards and focussing on three trophic levels, did not find an increase of diversity in any of the groups studied (vascular plants, grasshoppers and spiders). In fact, grasshopper diversity was even lower in organic compared to conventional vineyards. This result was explained in the context of the intermediate disturbance hypothesis ([Huston, 1979](#)) considering that disturbance in OF vineyards was too low to be beneficial for biodiversity (i.e. only a few highly competitive species prevailed under this kind of management). On the other hand, research carried out in N Italy has demonstrated a positive effect of OF in vineyards both in vascular plants ([Nascimbene et al., 2012](#)) and in some guilds of arthropod predators ([Caprio et al., 2015](#)). Moreover, [Kehinde and Samways \(2012\)](#) found an increase of monkey beetles (an important pollinator guild) but not of bees in OF compared to conventional South African vineyards. Likewise, [Thomson and Hoffmann \(2009\)](#) reported an increase in the abundance of natural enemies (including egg parasitoids) of an important local pest in Australian vineyards that included adjacent natural vegetation.

Therefore, although responses have proven to be idiosyncratic among taxonomic groups, results mostly suggest that OF in vineyards indeed contributes to promoting ecosystem services such as pollination and pest control.

Within this context, we undertook a study aimed at testing for the first time the biodiversity effects of OF in vineyards in N Spain. We designed a multi-taxon approach, carried out in 2014–2015, for one of the main areas devoted to viticulture in Catalonia as a way of critically exploring and understanding how crop management (OF vs. conventional farming – CF-) affects biodiversity across a range of taxa.

Our study focused on four different taxonomic groups (namely, plants, butterflies, moths and birds) with contrasting ecological attributes (e.g. mobility) that would enable us to gain some understanding of the effects of different types of farming systems at ecosystem level. We hypothesized that, firstly, differences in management practices would result in richer assemblages wherever no synthetic pesticides or herbicides are applied (OF) and secondly, that the magnitude of the response of a set of taxa would be linked to their mobility. We expected sessile organisms (i.e. plants) to be more affected than vagile ones (i.e. butterflies, moths and birds). However, birds and butterflies differ in terms of the spatial scale at which their biological processes occur ([Seto et al., 2004](#)). Therefore, given that (i) most butterflies and moths have life cycles that are closely linked to local conditions and to specific host plants, and that (ii) birds, with greater mobility, are more generally affected by vegetation structure at larger spatial scales, we predicted a stronger response to different management treatments in Lepidoptera than in birds.

## 2. Material and methods

### 2.1. Study area

The study area consisted of the Priorat Appellation of Origin (DOQP in its original acronym), a wine-producing area located in the county of the same name in Catalonia (41° 8' N, 0° 49' E, see [Fig. 1](#)). It has a Mediterranean climate influenced by the proximity of the sea (mean

annual precipitation around 600 mm). Topographically complex, the Priorat lies between two mountainous ranges and has an average altitude of  $472 \pm 250$  m a.s.l. It has a surface area of roughly 18,000 ha, of which 1887 ha are covered by vineyards, the main economic activity in the area. Most of the vineyards are managed following CF procedures, although the presence of OF is increasing.

### 2.2. Plot selection and characterization

A total of 10 OF and 10 CF crop plots were selected for the study. Given that the region's vineyards vary greatly in size and are subject to a wide range of environmental conditions (e.g. exposure and altitude), a pre-selection of plots was made to minimize the effect of confounding factors.

To exclude the possible effect of agricultural parcel size, an up-to-date cadastral map of holdings was simplified by merging all neighbouring parcels (i.e. plots) into a number of production blocks. Of these, only blocks with surface areas within the 25th–75th percentile were selected (ranging between 0.6 and 2 ha). A digital elevation map of the region ([ICGC, 2013](#)) was used to estimate the altitude and exposure of each block. Since both insolation and altitude are factors that heavily influence community assemblages and are therefore prone to bias the results of surveys, only blocks between 150 and 550 m a.s.l. oriented predominantly southwards (between 135° and 230°) were selected. To prevent the influence of riparian habitats and neighbouring forests on biodiversity, buffers of 300 and 50 m were drawn around streams and forest patches, respectively, on the 0.25 m resolution land cover map ([DMAH, 2005](#)) and blocks falling within these buffers were excluded. From the remaining blocks, we also removed those containing a mixture of OF and other management practices. Finally, we selected 10 plots to represent OF management systems and 10 to represent CF management systems out of 20 blocks that shared uniform environmental and physical conditions. Information on the management practices of both OF and CF plots was provided by the DOQP bureau ([Table 1](#)). All geographic computations were conducted using QGIS ([QGIS Development Team, 2013](#)).

To characterize each plot, a set of nine geographical variables was generated by taking into account the influence of physical conditions (altitude, roughness calculated as the standard deviation of elevation and slope) and habitat features (land covered by vineyards, shrub, forest, herbaceous vegetation and other habitat types, and the Shannon index of landscape diversity) around the vineyards. These variables were computed for three incremental buffer distances around the plots (500, 2000 and 4000 m). Physical variables were estimated from a digital elevation model with a resolution of  $15 \times 15$  m ([ICGC, 2013](#)), while habitat features were taken from a land cover map of Catalonia ([DMAH, 2005](#)).

To check whether there were differences between treatments (OF and CF) in the nine variables in each buffer, Monte-Carlo tests were run using 1000 permutations of the t-student test. This test is more robust than conventional non-parametric tests and no particular distribution of the data needs to be assumed ([Gotelli and Ellison, 2004](#)). No significant differences were found between groups (OF vs. CF) when running the Monte-Carlo test for each independent variable ( $t/\leq 0.03$ ;  $p > 0.21$ ; 27 tests in total, corresponding to the nine geographical variables calculated at each of the three buffer distances; see results in Appendix A in Supplementary material). All variables were therefore found to be suitable for use in the model building process (see 'Species richness modelling').

All geo-processing and computation was performed with R ([R Core Team, 2015](#)). The Shannon index of landscape diversity was estimated with the package "vegan" for R ([Oksanen et al., 2015](#)).

### 2.3. Sampling

The study was conducted in 2013 and 2014. Plants, butterflies and

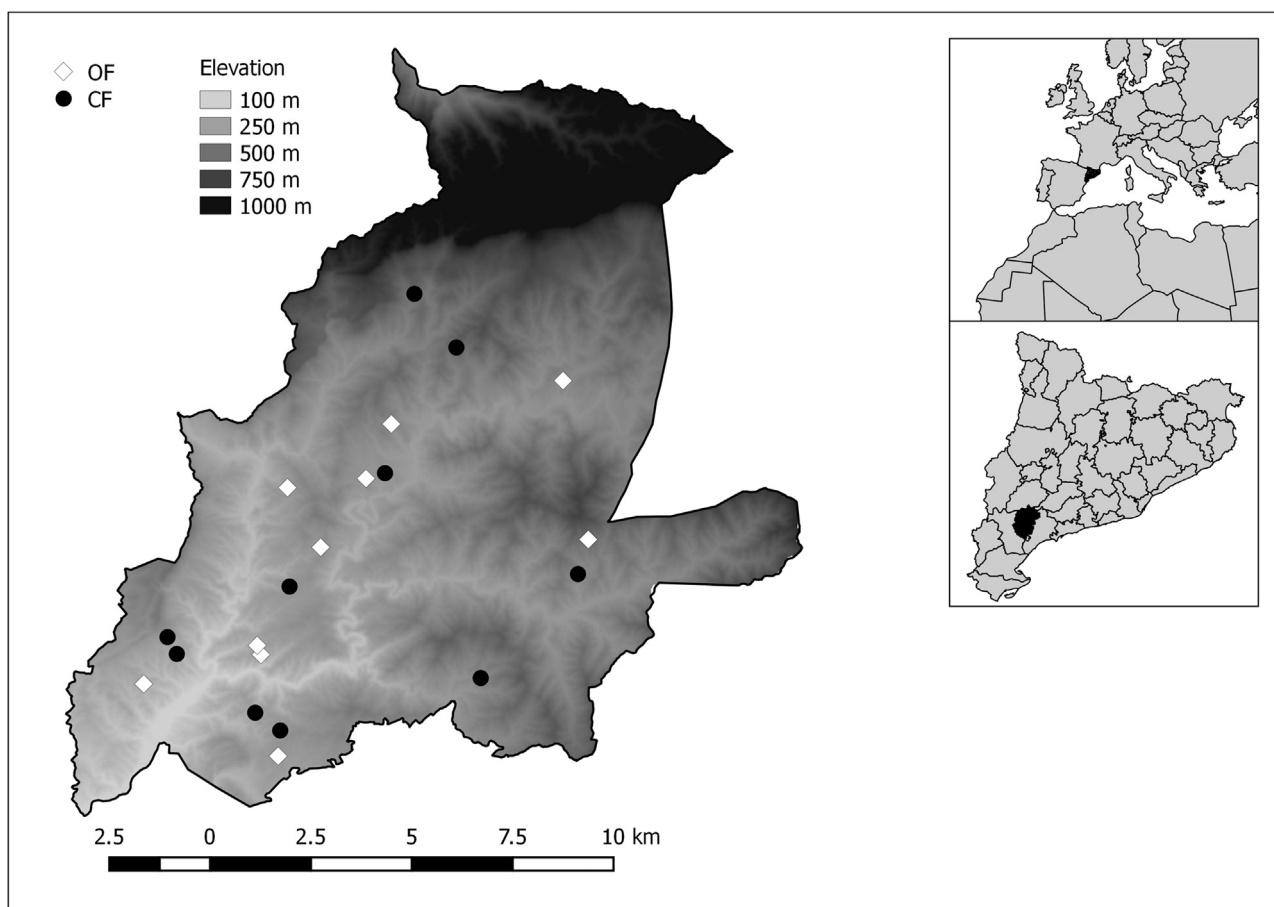


Fig. 1. Study area. Location of the sampling locations in the Priorat PDO (Protected Designation of Origin). Organic farming (OF) and conventional farming (CF) plots are shown.

moths were sampled in both years but birds only in 2013. For vascular plants and butterflies two subsamples per plot were obtained: one in the crop lines and the other in the grass strips that exist within the vineyards (or in the boundaries between vineyards sharing the same management). For both moths and birds only one sample at plot level was obtained. Locations of survey plots, central line of vegetation survey quadrats, and butterfly transects are presented in the Supplementary material (see Appendix B in Supplementary material).

### 2.3.1. Vegetation

Vascular plants were sampled within randomly assigned quadrats of 16 m<sup>2</sup> (8 × 2 m) in April 2013 and May 2014 (i.e. the flowering period of most species). Each year, plots were surveyed twice on the same day (crop lines and grass strips): all vascular plants were identified and assigned a cover value ranging from 0 to 5 following Braun-Blanquet (1932). One of the plots lacked naturalized grass strips and hence two simultaneous surveys were conducted in the crop lines, resulting in a total of 21 surveys carried out in quadrats in the crop lines and 19 in

Table 1

Summary of the main management treatments applied in the conventional and organic farming vineyards of the Priorat Appellation of Origin. Minimum and maximum number of annual treatments is shown.

| Target  | Treatment   | Conventional farming                                    | Organic farming   |
|---|---|---|---|
|   |   | Applications per year                                   | Applications per year                                   |
| Powdery mildew ( <i>Uncinula necator</i> )                                  | Sulfur based fungicides   | 4   | 3–4   |
| Mildew  | Copper based fungicides   | 0–2   | 0–2   |
| European grapevine moth ( <i>Lobesia botrana</i> )<br>2nd & 3rd generations | <ul style="list-style-type: none"> <li>• Bt</li> <li>• Spinosad (products approved in organic agriculture)</li> </ul> | 0–2 (treatment prevented when sexual confusion is used) | 0–2 (treatment prevented when sexual confusion is used) |
| European grapevine moth ( <i>Lobesia botrana</i> )<br>2nd generation        | <ul style="list-style-type: none"> <li>• Chlorpyrifos</li> <li>• Methyl Chlorpyrifos</li> </ul>                       | 0–1 (treatment prevented when sexual confusion is used) | 0   |
| European grapevine moth ( <i>Lobesia botrana</i> )<br>3rd generation        | <ul style="list-style-type: none"> <li>• Fenoxicarb</li> <li>• Tebufenocide</li> </ul>                                | 0–1 (treatment prevented when sexual confusion is used) | 0   |
| European grapevine moth (applied from may)                                  | • Sexual confusion  | 0–1   | 0–1   |
| Weeds   | • Glyphosate  | 0–2   | 0   |
| Weeds   | • Mowing  | 2–3   | 2–3   |
| Weeds   | • Tillage   | 0–2   | 0–2   |

grass strips.

### 2.3.2. Butterflies

Counts of adult butterflies were made along fixed 100-m transects following the standard technique described in Van Swaay et al. (2008). Each year, plots were surveyed four times in April–August, a period that encompasses the flight periods of all species present in the area. Counts were undertaken only in sunny weather with no strong wind, between 11a.m. and 4p.m. In all, 9 plots of each treatment were sampled each year, and 288 transect counts were performed, i.e. eight counts (four within crop lines, four within grass strips) per plot per year.

### 2.3.3. Moths

Moths were surveyed from the end of April to early September using standard 6W actinic light Heath traps. Sampling nights were selected to coincide with the new moon to avoid moonlight interference and optimal weather conditions (i.e. absence of rain and of strong wind; Yela and Holyoak, 1997). On every sampling night two traps were operated simultaneously in the middle of one OF plot and one CF plot during the first 3–4 h after sunset. In total, 18 plots were sampled in 2013 and 20 in 2014. Collected moths were anaesthetized with ethyl acetate and, after preliminary trials and counts, most of the macro moths were released *in situ*. The rest of the samples were carefully preserved by freezing for further study in the laboratory. Some of the sampled specimens were mounted and genitalia structures were studied for correct identification (especially for the microlepidoptera).

### 2.3.4. Birds

Acoustic and visual censuses of birds were conducted in each plot during the 2013 breeding season. Following the census periods established by the Catalan Common Bird Survey (Herrando et al., 2008), the local version of the Pan-European Common Bird Monitoring Scheme, one visit was made between April 15 and May 15 and a second between May 15 and June 15. Data from both visits were pooled to estimate species richness. Plots were not resampled until at least 15 days after the first visit. Four sampling points were defined in each plot (at vertices if plots were rectangular or, alternatively, as far apart from each other as possible). Censuses consisted of 15-min counts from each point, giving a one-hour effort per plot and sample. All birds seen or heard within the plots were identified and counted. All censuses were conducted between 08.00 and noon.

## 2.4. Species accumulation curves

Four data matrices with the number of individuals sampled (butterflies, moths and birds) or the presence/absence (vegetation) of the species in each focal group were created; species accumulation curves were used to estimate species richness (Gotelli and Colwell, 2001). The expected richness functions were calculated with EstimateSv. 9.1.0. (Colwell, 2013) after 100 randomizations (default option) of the observed number of species as accumulated samples. To ascertain the completeness of the inventory for each focal group, we used the Clench equation to adjust the species accumulation curves (Díaz-Francés and Soberon, 2005). To fit the Clench equation to the functions provided by EstimateS, we used the non-linear estimation module of Statistica v7.0 (Stat Soft Inc.) following the procedure outlined by Jiménez-Valverde and Hortal (2003). Asymptotic species density was calculated by dividing the intercept by the slope of the function fitted when a good fit between both curves was obtained ( $r^2 > 0.99$ ). The completeness of the inventories was recorded as the ratio between the number of observed species and the number of expected species. Vegetation inventories were treated at qualitative level (presence/absence) and we used species density (i.e. the number of species detected per sampled plot) as a measure of diversity rather than species richness (i.e. the number of species related to the number of individuals sampled; Gotelli and Colwell, 2001).

## 2.5. Species richness modelling

We were interested in understanding how the species richness of different taxonomic groups in vineyards (dependent variable) was influenced by the chosen geographical factors and the two management treatments (OF and CF). Thus, we constructed generalized linear models with the nine geographical variables, the two types of management and for plants and butterflies their position in vineyards (crop lines or grass strips) as fixed factors.

Following the procedure recommended by Zuur et al. (2007), all independent variables were examined prior to model building (nine geographical variables for each of the three buffers, thereby 27 in total). Firstly, we tested for the existence of outliers and non-linear relationships between species richness and the independent variables (plotting and visual exploration). We explored the collinearity between variables using pair-wise correlation tests and rejected those with correlations of  $\pm 0.7$  or greater, following Dormann et al. (2013), who suggest the  $|r| > 0.7$  threshold on descriptive ecological studies. In addition, variance inflation tests (VIF) were made starting from a saturated model with all independent variables, followed by a step-wise exclusion of those variables with  $VIF > 5$  (Zuur et al., 2007).

No apparent outliers were found and no consistent non-linear relationships emerged after visually examining the richness of the different taxa against the geographical variables. Therefore, no data transformation was performed. However, strong correlations (i.e.  $r/|r| \geq 0.7$ ) between some variables occurred at all buffer distances. The Shannon index of landscape diversity showed a strong correlation with herbaceous vegetation cover and altitude at all buffer distances, and was thus excluded from the models. Similarly, roughness and slope were correlated with altitude over distance and were also discarded. VIF values for all these variables were accordingly high, a further reason for their exclusion. The remaining six variables (altitude and vineyard, shrub, forest, herbaceous vegetation and other habitat type cover) had differential relationships at the various buffer distances, and a constant agreement between correlations and VIF values. The only exception was altitude in the 2000-m buffer, which had a VIF that was greater than the threshold of five but correlations at most of  $0.7/$ with the other non-collinear variables, and was eventually included in the models to preserve at least one physical variable. The final set of geographical variables and factor interactions used in the model building at the various buffer distances is given in Table 2. All these variables were standardized to improve the comparability of the coefficients and the convergence of the models. Standardization was done by subtracting the mean value, as implemented in the “scale” function in base R package (R Core Team, 2015).

Birds were the only taxonomic group with a single year of sampling and so, unlike the other groups, had no replications. Accordingly, general linear models (GLM) were constructed for birds and generalized linear mixed models (GLMM), which have the ability to deal with random factors and are thus better suited to replicated designs, for the rest of taxonomic groups. Separate models were built for each group and for each buffer distance, resulting in 12 sets of models.

GLMMs were built using sampling location nested with year of sampling as a random factor. The Laplace approximation was used to estimate parameter likelihood (Bolker et al., 2009). Starting with the 12 initial saturated models, all possible models were run using the packages MuMIn (Barton, 2015) and bestglm (McLeod and Xu, 2014) for the GLMMs and the GLMs, respectively. We based model selection on the Akaike Information Criterion (AIC) and selected our best model as the one with the lowest AIC value. The models with their AIC differing by less than 2 from the AIC of the best model were considered as top-ranked models (statistically equivalent to the best model of the set). Goodness-of-fit was assessed using pseudo-R-squared. However, this interpretation needs to be taken with caution since it is not a straightforward measure of the explained variance as  $R^2$  is in linear models, but rather a measure of the improvement of the selected model

**Table 2**

Sets of variables, factors, interactions and random factors included in the saturated model for each considered buffer and all taxonomic groups (Veg: vegetation and But: butterflies).

| Variable                                      | Type               | 500-m buffer |      |      |      | 2000-m buffer |      |      |      | 4000-m buffer |      |      |      |
|---|--------------------|--------------|------|------|------|---------------|------|------|------|---------------|------|------|------|
|   |                    | Veg          | But  | Moth | Bird | Veg           | But  | Moth | Bird | Veg           | But  | Moth | Bird |
|   |                    | GLMM         | GLMM | GLMM | GLM  | GLMM          | GLMM | GLMM | GLM  | GLMM          | GLMM | GLMM | GLM  |
| Block nested within year                      | Random factor      | ●            | ●    | ●    |      | ●             | ●    | ●    |      | ●             | ●    | ●    |      |
| Treatment (OF or ICM)                         | Factor             | ●            | ●    | ●    | ●    | ●             | ●    | ●    | ●    | ●             | ●    | ●    | ●    |
| Location (crop line or grass strip)           | Factor             | ●            | ●    |      |      | ●             | ●    |      |      | ●             | ●    |      | ●    |
| Treatment*Location                            | Factor interaction | ●            | ●    |      |      | ●             | ●    |      |      | ●             | ●    |      |      |
| Altitude (average)                            | Variable           | ●            | ●    | ●    | ●    | ●             | ●    | ●    | ●    | ●             | ●    | ●    | ●    |
| Forest (cover)                                | Variable           | ●            | ●    | ●    | ●    | ●             | ●    | ●    | ●    | ●             | ●    | ●    | ●    |
| Vineyard (cover)                              | Variable           | ●            | ●    | ●    | ●    |               |      |      |      |               |      |      |      |
| Shrub (cover)                                 | Variable           |              |      |      | ●    | ●             | ●    | ●    | ●    | ●             | ●    | ●    | ●    |
| Herbaceous (cover)                            | Variable           | ●            | ●    | ●    | ●    |               |      |      |      |               |      |      |      |
| Other, mainly urban & infrastructures (cover) | Variable           | ●            | ●    | ●    | ●    | ●             | ●    | ●    | ●    | ●             | ●    | ●    | ●    |

over the null model. Thus, values close to 1 indicate an excellent fit, while values close to 0 indicate a low or poor fit; no comparison is possible between models built from different datasets (UCLA, 2011).

**3. Results**

A large amount of convergent models were obtained (N = 704), out of which a number of top-ranked models were selected for every taxa at each considered buffer distance (7.8 ± 4.0 top rank models per taxa and distance). Fig. 2 summarizes these results showing the size of all significant standardized effects of the models. Hence, the relative weight of every considered factor and variable can be visually interpreted. To provide a more detailed outcome of the models, Table 3 shows the results of a subset of the four best models for the three considered buffer distances. A comprehensive detailed list of models is given in the Appendix C in Supplementary material.

**3.1. Vegetation**

The vegetation inventories identified 168 species of vascular plants, 132 of which were found in OF plots and 125 in CF plots. The grass strips hosted a total of 143 species, whereas 123 species were found in the crop lines of the vineyards (see Appendix D.1 in Supplementary material).

Species accumulation curves revealed that vegetation species density was higher under OF than CF treatments (see Appendix E.a in Supplementary material); greater differences were detected in vegetation communities in grass strips, where there was higher species density

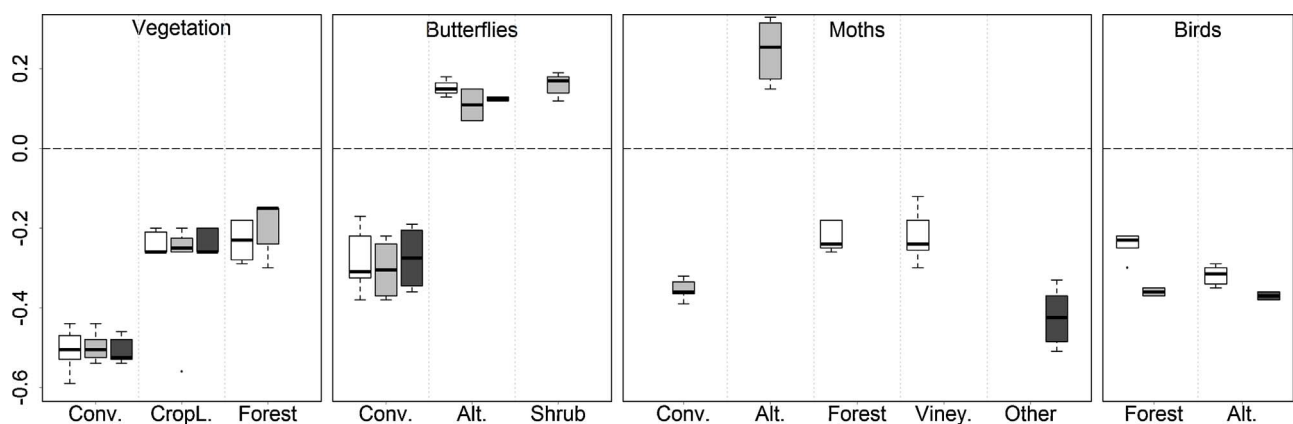
than within the crop lines (see Appendix E.b in Supplementary material). Vegetation inventories were still far from the asymptote (Table 4).

A total of 14, eight and six top ranked models were obtained for the 500, 2000 and 4000 m buffers respectively. The estimate for the CF treatment had the highest absolute value (range from -0.54 to -0.44 ± 0.13–0.17) and was significant in all selected models (P ≤ 0.01), with a negative relationship between vegetation species density and CF. The second strongest and most prevailing estimate was for the ‘crop line’ sampling location (range from -0.26 to -0.20 ± 0.05–0.07), which was also significant in all models (P ≤ 0.01) and was associated with a decrease in plant species density. However, no selected model showed any significant interaction between management treatment and location in the plot. Finally, at distances of 500 and 2000 m, forest coverage also had a negative effect on vegetation species density (range from -0.15 to -0.28 ± 0.07–0.11), an effect that was stronger at 500 m (present in all models) but weaker and less prevailing at 2000 m.

**3.2. Butterflies**

A total of 47 species of butterflies were found in the vineyards, of which 40 species were detected in OF plots and 38 in CF plots (see Appendix D.2 in Supplementary material). Inside the vineyards 37 species were reported, while 40 were found on grass strips.

Species accumulation curves showed that the butterfly species density was higher under OF than CF treatment (see Appendix E.c in Supplementary material); however, greater differences were detected in



**Fig. 2.** Significant effect sizes of variables included in the models. Standardized effects, when significant, are shown for the three considered distances. Boxplots include values of all top-ranked models when at least the effect was significant in one of them. White boxes represent 500 m buffers, light grey boxes 2000 m buffers and dark grey boxes 4000 m buffers. Where Conv.: effect of CF as compared to OF; CropL.: effect of sampling in the crop lines as compared to sampling in the grass strips; Alt.: effect of altitude; Forest: effect of forest coverage; Viney.: effect of vineyard coverage; Shrub: effect of shrub coverage; Other: effect of other land covers (see methods).

**Table 3**

Model results for richness considering the influence of land cover at a radius of 500 m around the vineyard plots. The four best models (increment of AIC value below 2) are given, with the total of best models indicated in the table headings. Standardised estimates are given for fixed effects included in the models and the SD of the estimates in parenthesis.  $P(z)$  indicates significant estimates (\*\*\*  $p < 0.001$ . \*\*  $p < 0.01$ . \*  $p < 0.05$ ). The grey shading indicates the variables excluded from the saturated models. *Treat-conv*: conventional farming treatment; *Altitude*, *Forest*, *Vineyard*, *Shrub*, *Herbaceous*: percentage cover at the specified radius; *Other*: percentage cover of all remaining land covers at the specified radius; *Loc-CropL*: locations in crop lines; *Loc-CropL:Treat-conv*: interaction between conventional treatment and sampling in the crop lines; *AIC*: Aikake Information Criterion; *Pseudo R-sq*: pseudo r-squared value.

|                             | Vegetation (14 models out of 160) |                     |                     |                    | Butterflies (11 models out of 80) |                    |                    |                    | Moths (12 models out of 64) |                    |                    |                    | Birds (6 models out of 64) |                    |                    |                    |
|-----------------------------|-----------------------------------|---------------------|---------------------|--------------------|-----------------------------------|--------------------|--------------------|--------------------|-----------------------------|--------------------|--------------------|--------------------|----------------------------|--------------------|--------------------|--------------------|
|                             | Model1                            | Model2              | Model3              | Model4             | Model1                            | Model2             | Model3             | Model4             | Model1                      | Model2             | Model3             | Model4             | Model1                     | Model2             | Model3             | Model4             |
| <b>Intercept</b>            | 3.15 ***<br>(0.09)                | 3.16 ***<br>(0.10)  | 3.17 ***<br>(0.09)  | 3.12 ***<br>(0.10) | 1.74 ***<br>(0.09)                | 1.68 ***<br>(0.11) | 1.64 ***<br>(0.08) | 1.76 ***<br>(0.10) | 3.35 ***<br>(0.12)          | 3.34 ***<br>(0.13) | 3.18 ***<br>(0.09) | 3.19 ***<br>(0.10) | 1.56 ***<br>(0.11)         | 1.55 ***<br>(0.11) | 1.58 ***<br>(0.10) | 1.51 ***<br>(0.15) |
| <b>Treat-conv</b>           | -0.50 ***<br>(0.13)               | -0.53 ***<br>(0.13) | -0.55 ***<br>(0.13) | -0.44 **<br>(0.14) | -0.31 *<br>(0.12)                 | -0.17<br>(0.15)    | -0.31 *<br>(0.12)  | -0.34 **<br>(0.13) | -0.32<br>(0.18)             | -0.29<br>(0.19)    |                    |                    |                            |                    |                    | 0.09<br>(0.20)     |
| <b>Altitude</b>             |                                   |                     |                     |                    |                                   |                    | 0.15 *<br>(0.06)   |                    |                             | 0.16<br>(0.09)     |                    |                    | -0.30 *<br>(0.12)          | -0.34 **<br>(0.12) | -0.35 **<br>(0.12) | -0.31 *<br>(0.12)  |
| <b>Forest</b>               | -0.18 *<br>(0.07)                 | -0.22 **<br>(0.07)  | -0.28 **<br>(0.09)  | -0.18 *<br>(0.07)  | -0.13<br>(0.07)                   | -0.13<br>(0.07)    | -0.13<br>(0.07)    |                    | -0.26 *<br>(0.12)           |                    | -0.25 *<br>(0.12)  |                    | -0.23<br>(0.13)            | -0.30 *<br>(0.15)  |                    | -0.22<br>(0.13)    |
| <b>Vineyard</b>             |                                   |                     | -0.11<br>(0.08)     |                    |                                   |                    |                    |                    | -0.27 *<br>(0.11)           |                    | -0.24 *<br>(0.11)  |                    |                            |                    |                    |                    |
| <b>Shrub</b>                |                                   |                     |                     |                    |                                   |                    |                    |                    |                             |                    |                    |                    |                            |                    |                    |                    |
| <b>Herbaceous</b>           | 0.11<br>(0.07)                    |                     |                     | 0.10<br>(0.07)     |                                   |                    |                    |                    | -0.18<br>(0.09)             |                    | -0.16<br>(0.10)    |                    |                            |                    |                    |                    |
| <b>Other</b>                |                                   |                     |                     |                    |                                   |                    |                    |                    |                             |                    |                    |                    |                            |                    |                    |                    |
| <b>Loc-CropL</b>            | -0.26 ***<br>(0.05)               | -0.26 ***<br>(0.05) | -0.26 ***<br>(0.05) | -0.21 **<br>(0.07) | -0.21<br>(0.11)                   | -0.07<br>(0.15)    |                    | -0.21<br>(0.11)    |                             |                    |                    |                    |                            |                    |                    |                    |
| <b>Loc-CropL:Treat-conv</b> |                                   |                     |                     | -0.13<br>(0.11)    |                                   | -0.31<br>(0.22)    |                    |                    |                             |                    |                    |                    |                            |                    |                    |                    |
| <b>AIC</b>                  | 598.15                            | 598.25              | 598.63              | 598.79             | 327.34                            | 327.44             | 328.83             | 328.86             | 319.66                      | 320.74             | 320.89             | 320.91             | 99.24                      | 100.17             | 100.35             | 101.02             |
| <b>PseudoR-sq</b>           | 0.945                             | 0.943               | 0.944               | 0.946              | 0.239                             | 0.259              | 0.202              | 0.201              | 0.992                       | 0.991              | 0.991              | 0.99               | 0.473                      | 0.501              | 0.385              | 0.479              |

**Table 4**

Total species density, asymptotic species density and inventory completeness for the four taxonomic groups studied for sampled-based accumulation curves of the observed species ( $n = 20$  samples). All possible combinations of treatments (two farming levels x two plot locations) are presented when available. The asymptotic number of species was calculated by dividing the parameters of the Clench equation (a/b) adjusted to the observed species accumulation curve (see methods for details).

| Group       | Treatment | Location     | Total species density (mean ± SD) | Asymptotic species density | Inventory completeness (%) |
|-------------|-----------|--------------|-----------------------------------|----------------------------|----------------------------|
| Vegetation  | OF        | Grass strips | 106.58 ± 2.23                     | 134.69                     | 77.96                      |
|             |           | Crop lines   | 100.69 ± 2.91                     | 131.96                     | 78.06                      |
|             | CF        | Grass strips | 105 ± 3.22                        | 149.04                     | 70.45                      |
|             |           | Crop lines   | 77 ± 3.27                         | 111.96                     | 68.78                      |
| Butterflies | OF        | Grass strips | 33 ± 3.41                         | 42.99                      | 76.75                      |
|             |           | Crop lines   | 30 ± 2.68                         | 39.17                      | 76.59                      |
|             | CF        | Grass strips | 32 ± 2.35                         | 44.41                      | 72.05                      |
|             |           | Crop lines   | 22 ± 3.20                         | 34.57                      | 63.64                      |
| Moths       | OF        |              | 193.1 ± 7.83                      | 282.18                     | 68.40                      |
|             | CF        |              | 190 ± 7.76                        | 294.42                     | 64.53                      |
| Birds       | OF        |              | 33.51 ± 5.15                      | 45.15                      | 55.37                      |
|             | CF        |              | 29.71 ± 3.99                      | 40.67                      | 59.01                      |

butterfly communities in grass strips, where there was higher species density than in crop lines (see Appendix E.d in Supplementary material). The completeness of butterfly inventories was similar to that calculated for plants (Table 4).

A total of 11, 11 and 7 top ranked models were selected for the 500, 2000 and 4000 m buffers respectively (see Appendix C.2 in Supplementary material).

Treatment consistently appeared as the most important factor (range from  $-0.17$  to  $-0.38 \pm 0.12$ ), and was significant in 17 models ( $P \leq 0.05$ ), above all at the smallest distances. Altitude often had a significant positive effect and species density increased with elevation. Finally, for the 2000-m buffer, shrub cover had a moderate

positive effect in five of the 11 selected models.

### 3.3. Moths

In all, 2163 moths belonging to 242 species were captured (see Appendix D.3 in Supplementary material), of which 185 species were identified in OF plots and 189 in CF plots.

The species accumulation curves show that moth species density was slightly higher in OF than in CF treatments (see Appendix E.e in Supplementary material) but that differences were not significant. Moth inventories were somewhat less comprehensive than those of plants and butterflies. For this group, considerable further sampling would be

required to approach the asymptotic value in species density (Table 4).

A total of 12, 9 and 8 top ranked models were selected from the 500, 2000 and 4000 m buffers respectively (see Appendix C.3 in Supplementary material).

Results were not consistent over distances. Forest, herbaceous plant and vineyard cover had a significant ( $P < 0.05$ ) negative effect in three, one and four models, respectively. At the 2000-m buffer, altitude had a positive effect in five selected models; the CF treatment had a negative effect in one selected model ( $P < 0.05$ ). The strongest effects were found for the 4000-m buffer, where the cover of other habitat types (e.g. urban and infrastructures) negatively affected ( $P < 0.001$ ) all eight selected models, with no other variables or factors having a significant effect.

### 3.4. Birds

A total of 33 bird species were observed in the vineyards during the 2013 sampling season (see Appendix D.4 in Supplementary material), with 25 species being detected in the OF plots and 24 in the CF plots.

Species accumulation curves showed that bird species density was slightly higher under OF than CF treatments (see Appendix E.f in Supplementary material). The bird inventories were the least comprehensive of the four studied groups, being the species density accumulation curve far from its asymptote (Table 4).

Only six, one and one top ranked models were selected (see Appendix C.4 in Supplementary material).

Bird species density was essentially influenced negatively by altitude (range from  $-0.38$  to  $-0.29 \pm 0.12$ – $0.14$ ,  $P \leq 0.05$ ). Forest cover also had a negative impact on bird richness; this variable was included in six models and was significant at 500- and 2000-m distances (range  $-0.37$  to  $-0.30 \pm 0.11$ – $0.15$ ,  $P \leq 0.05$ ). Finally, management treatment had no effect on this taxa.

## 4. Discussion

Differences in species richness and biodiversity between OF and CF has been the object of a number of studies that have consistently revealed the beneficial effects of the former in a wide range of taxa (see e.g. Bengtsson et al., 2005; Hole et al., 2005 for thorough reviews). Several works have confirmed this general trend in vineyards, although some exceptions have also been found (e.g. Brittain et al., 2010; Bruggisser et al., 2010). Our study makes a novel contribution in an area (N Spain) where, despite the enormous economic importance of the wine industry, no previous attempts had been made to evaluate the impact that OF in vineyards has on biodiversity.

Differences in species richness between these two types of managements emerged for both vascular plants and butterflies in our multi-taxon approach. In addition, although the treatment effects in the other two studied groups (moths and birds) were weak and non-significant (only in one model treatment had a significant effect in moths), the majority of the moth models showed a consistent positive relationship between species richness and OF. This result suggests that a similar interaction to that found for butterflies may also occur in moths.

Regarding local management, two major differences exist between OF and CF in our area: i) the use of herbicides (i.e. glyphosate), and ii) the use of synthetic insecticides (chlorpyrifos) and growth regulators (tebufenozide and fenoxycarb). Glyphosate is a broad-spectrum herbicide that affects both mono and dicotyledons, and hence can potentially have an important impact on vascular plant communities within the sprayed zone and within the reach of the spray drift (Marrs et al., 1993). Regarding the insecticides used in the region, tebufenozide is specifically targeted to lepidopterans, and fenoxycarb and chlorpyrifos are wide-spectrum insecticides. Hence all three can negatively affect insect communities of the vineyards, and more acutely the lepidopteran communities. Most likely these factors accounted for most of the loss of species richness of both plants and butterflies in CF. A similar

conclusion was reached by Nascimbene et al. (2012) in their study of Italian vineyards regarding the impact of herbicides.

Our results contrast with those found by Brittain et al. (2010) in vineyards of NE Italy, where OF did not have any effect on pollinator abundance and species richness, including butterflies. Brittain et al. (2010) concluded that this was because of the major impact of the surrounding landscape compared to the local management. However, their study area was located within an intensive agricultural landscape, dominated by large extensions of maize, soy and vine crops, that is, characterised by low heterogeneity and biodiversity (e.g. Benton et al., 2003). On the other hand, our study area is found in a region where the predominant landscape is constituted by a mosaic of a variety of land uses and an overall high spatial heterogeneity. In any case, our data supported the general statement that OF has positive effects on pollinators (Holzschuh et al., 2008).

An interesting but expected result was the greater effect that the type of farming treatment had on less mobile taxa (e.g. Fuller et al., 2005). Thus, plants, which rely strictly on the conditions of the soil in which they grow, were the most affected by OF farming, while the weakest effect was recorded in birds, which generally have home ranges that are larger than the considered plots. For instance, one of the more common bird species in the area with the smallest territories, the Sardinian Warbler (*Sylvia melanocephala*), inhabits patches of around 2 ha (Bas et al., 2005) and thus is very unlikely to occupy a single vineyard plot or to show clear responses at such a local scale. Although differences in avian richness and abundance between treatments in vineyards have been found by Jedlicka et al. (2011), in these authors' study plots were roughly six-times larger than those in the present study, which further suggests that plot size could partially explain our results. The weak effect in moths could also be related to a greater mobility in this group, given that the sampling method (i.e. light traps) attracts individuals from a certain distance (Muirhead-Thompson, 2012), therefore reducing possible local habitat effects. Alternatively, the incompleteness of the moth surveys (Moreno and Halffter, 2001) could be partly responsible for this weaker effect, above all because some of the species that predictably would benefit the most from OF are rare specialists living at low population densities and are hence more difficult to detect than generalist species.

Our data also highlight important differences in biodiversity in microhabitats within plots. For plants and butterflies, a consistent pattern emerged of richer assemblages in grass strips compared to crop lines. Because in our design grass strips were located among fields and not closer to the natural vegetation surrounding the vineyards, the positive effect of this microhabitat was genuine and could not be explained merely as a gradient in biodiversity rise with increasing distance from the vineyards. In our case, grass strips were clearly associated with richer plant communities which, in turn, favoured richer butterfly communities by providing both an increase of larval host plants and nectar sources for adults. Our results provide further evidence on the importance as reservoirs of biodiversity of the naturalized strips within or along the margins of the crops, that has already been highlighted by other authors (e.g. Le Coeur et al., 2002; Merckx et al., 2012).

Grass strips within and between vineyards are a particular relevant feature of the study system since, given the complex topography of the region, most vineyards are characterised by their ranks of farmed terraces. Currently, there is a trend towards establishing vineyards directly on slopes, which benefits grapes by improving the natural air-flow and avoids mechanization and the soil erosion it can cause. However, this practice prevents the natural occurrence of grass strips within and between plots. To increase biodiversity and enhance natural ecosystem services, our results suggest that plots should be small and surrounded by non-cultivated strips.

Finally, our data also highlight the importance of other geographical and landscape factors that must be taken into consideration when trying to explain richness patterns in the taxa found in vineyards. For



example, altitude had a strong influence on both birds and Lepidoptera — despite our attempt in our experimental design to restrict the altitudinal range of the plots. These two taxonomic groups had opposite responses to altitude, which agrees with previous knowledge of their ecology. The observed negative effect of both altitude and forest cover on bird richness resembles the effects detected by (Farina, 1997) in other Mediterranean agroecosystems and was expected given the regional scale of our sampling. On the other hand, altitude had a strong positive effect on butterflies, a result which is consistent with previous analyses showing a peak of diversity in this group at mid-mountain elevations in the study region (Stefanescu et al., 2011).

Perhaps the most unexpected finding was the negative influence of urban cover on moth diversity that was detected in the 4000-m buffer. We believe that this response could indirectly indicate how light pollution negatively affects this group. The detrimental effects of outdoor lighting on moths and other insects are well known (e.g. Frank et al., 2006), although to date no study has convincingly assessed its effects at community level. In our study area, small towns and villages probably act as light traps and negatively affect moth communities in vineyards over a considerable area (i.e. a few kilometres around settlements). Clearly, this possibility deserves further investigation and opens an interesting line for future research.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2017.04.005>.

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