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### How weed management influence plant community composition, taxonomic diversity and crop yield: A long-term study in a Mediterranean vineyard

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### ABSTRACT

Agricultural expansion and intensification is a major driver of biodiversity loss. Conventional weed management (e.g. tillage, herbicide) has encouraged the promotion of a few more competitive species over a wide range of arable plants that are now in decline. A reduction in plant diversity would negatively affect the provision of key ecosystem services in agroecosystems. In vineyards, the use of plant covers has emerged as the principal alternative to conventional weed management, providing multiple ecosystem services such as enhance biodiversity, but usually with a decrease in yields in Mediterranean vineyards. This long-term study assessed how weed management (herbicide, mowing, tillage) influenced plant community composition, taxonomic diversity and vineyard yield. Plant surveys and grape yield measurements were carried out from 2015 to 2018 in an experiment established in 2008. Effects on plant community composition were analysed, focusing on two groups: noxious grapevine weeds and terophytic grassland species. In addition, three fundamental components of taxonomic diversity were measured: β-diversity, evenness and species richness. Linear mixed models and generalised linear mixed model were used to examine the response of different variables to weed management. Position (row, inter-row) as well as the indirect effect of adjacent management on the subplots were also included as fixed factors. Furthermore, the percentage of bare soil, related to disturbance degree associated with management, was explored as a potential predictor of taxonomic diversity and vineyard yield. Results indicated that plant community composition was affected by weed management and to a lesser extent by position. Noxious grapevine weeds were more abundant in herbicide-sprayed rows, while terophytic grasslands species showed a higher presence in mown subplots. Weed management had a strong effect on bare soil, which proved to be a good predictor of the variables studied. Indeed, the highest species richness were associated with the lowest percentages of bare soil (mown subplots), while the lowest values were found at high percentages of bare soil (tilled subplots). Herbicide showed intermediate values, with a response dependent on adjacent management. On the other hand, vineyard yields were higher with increased bare soil, although with the consequent loss of species richness. Conversely, mowing in spontaneous plant covers caused a decrease in yield, but still production was within the maximum limit established by the Regulatory Council, while increasing the species richness and a significant presence of therophytic grasslands species. These findings could foster the development of more sustainable management in Mediterranean vineyards.

### 1. Introduction

In recent years, worldwide experts have warned of a massive global loss of biodiversity, which is considered to be the sixth mass extinction of Earth's history (Barnosky et al., 2011; Dirzo et al., 2014; Ceballos et al., 2015). Agricultural expansion and intensification is a major driver of this biodiversity loss (Tilman et al., 2001; Dudley and Alexander, 2017), although has also alleviated poverty and increased global food security (FAO et al., 2021). Therefore, finding a balance between agricultural production and biodiversity is one of the main challenges

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Received 12 August 2021; Received in revised form 13 November 2021; Accepted 30 November 2021 Available online 11 December 2021 0167-8809/© 2021 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). humanity will have to face in the future (Norris, 2008; Foley, 2011; Garnett et al., 2013). Actually, it is part of the objectives of the current European policy included in the Farm to Fork Strategy.<sup>1</sup>

In the Mediterranean basin, there has been no expansion of agricultural area, but an intensification of existing farming systems, which had happened at two spatial scales: field and landscape (Emmerson et al., 2016). Intensification at field level (which involves the increasing use of agrochemicals, mechanisation and irrigation, among others) has particularly affected plant communities present in agroecosystems, causing a decline in their populations in recent decades (Storkey et al., 2012). These arable plant communities, usually referred to as weeds, are generally considered pest organisms that significantly reduce crop yields (Oerke, 2006). Therefore, in a context of agricultural intensification aimed at maximising yields, weed management has been focused at eradicating the presence of weeds through chemical and mechanic control. This has led to the disappearance of characteristic arable plants or rare plant species and the selection of a few more competitive species adapted to intensive management (Storkey et al., 2012). In this regard, it is important to emphasise that certain arable plant species serve as shelter and food for insects, birds and mammals (Marshall et al., 2003; Hyvönen and Huusela-Veistola, 2008; Evans et al., 2011), thus being essential to provide ecosystem services such as pest control (Crowder and Jabbour, 2014; Furtado et al., 2016), crop pollination (Nicholls and Altieri, 2013; Bretagnolle and Gaba, 2015) or nutrient cycling (Steinbeiss et al., 2008). Hence, a reduction in plant diversity would negatively affect the provision of these ecosystem services.

In Mediterranean vineyards, one of the three historical crops of the Mediterranean basin, weed management in recent decades mainly consisted of tillage, both in row and vineyard inter-row, and the application of herbicides (mainly glyphosate) usually restricted to the vineyard row. These management practices, which involve the maintenance of bare soil throughout the season, have an adverse effect on biodiversity (Kazakou et al., 2016; Hall et al., 2020), causing also other environmental problems that are of particular concern in the Mediterranean basin such as soil erosion, with higher rates in vineyards compared to other crops, and loss of soil fertility (Novara et al., 2011; Prosdocimi et al., 2016; Rodrigo-Comino, 2018). In line with the European policies,<sup>2</sup> new and more sustainable weed management methods have been proposed in recent years to deal with these problems. The use of plant covers (i.e., both sown cover crops and spontaneous plant covers) in vineyards, usually managed by mowing, has emerged as the principal alternative to conventional weed management (Baumgartner et al., 2008), by providing multiple ecosystem services such as improved erosion control and water retention, increased soil organic carbon, contribution to pest control and increased biodiversity (Ruiz-Colmenero et al., 2013; Simoes et al., 2014; Muscas et al., 2017; Daane et al., 2018; Guzmán et al., 2019; Novara et al., 2019; Hall et al., 2020). In terms of biodiversity, in fact, vineyards are singular agroecosystems with potential for biodiversity conservation when managed with plant covers (Shields et al., 2016; Paiola et al., 2020). For example, vineyards offer the possibility of preserving dry grassland plants (Nascimbene et al., 2016) which could provide a beneficial effect on butterfly abundance as a consequence of restoring native plants in vineyards (James et al., 2015). However, use of plant covers can also have detrimental effects as declining yields (Celette and Gary, 2013; Muscas et al., 2017); but in vineyards there is a paradox: more is not always better. Indeed, plant covers contribute to the control grapevine vigour and may have a positive effect on wine quality (Guerra and Steenwerth, 2012). Furthermore, in many wine-growing regions, production is generally regulated by Regulatory Councils to preserve the quality of the wine, by establishing a maximum permitted yield limit (Meloni et al., 2019).

Recent studies in vineyards have demonstrated the effects of weed management practices on the composition and diversity of plant communities. For example, it has been shown that tillage favours small ruderal species (Fried et al., 2019; Guerra et al., 2021), while herbicide may favour perennial (Fried et al., 2019; Hall et al., 2020) or more competitive species (Guerra et al., 2021). Regarding plant diversity, most studies in vineyards agree that plant covers favoured species richness compared to other management practices as tillage or herbicide application (Kazakou et al., 2016; Fried et al., 2019; MacLaren et al., 2019; Hall et al., 2020). However, other aspects of taxonomic diversity such as  $\beta$ -diversity (Anderson et al., 2011) or evenness have not been considered in these studies, and may be important for understanding how weed management affects plant diversity. Neither, with the exception of the work by Fried et al. (2019), has position (vineyard row or inter-row) been assessed as a factor that could explain differences in plant community composition or diversity, especially considering that irrigation is applied in the rows, thus creating a micro-environment. Finally, another factor that usually has not been explored, with the exception of the study by Kazakou et al. (2016), was the relationship between diversity and vineyard yield.

In order to answer these questions, a long-term study was initiated in 2008 to analyse the effects of weed management in vineyards on plant community composition, taxonomic diversity and vineyard yield, also considering position (row, inter-row) as a factor.

Firstly, this approach proposed an analysis of the plant community composition based on two main groups: noxious grapevine weeds and therophytic grassland species, the latter considered characteristic of the Natura 2000 habitats "6220 Pseudo-steppe with grasses and annuals (*Thero-Brachypodietea*)" (San Miguel, 2008). This is a priority habitat under the Habitats Directive (i.e., Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora) and, like other European grasslands ecosystems, is declining primarily as a result of agricultural intensification and conversion to arable land (Wall-isDeVries et al., 2002; San Miguel, 2008; Habel et al., 2013). The working hypothesis was that more intensive management (e.g., tillage, herbicide) could favour weed species considered more harmful to the vineyard, while spontaneous plant covers would promote grassland species that are not common in agroecosystems, since grasslands patches are present in the vicinity of the experimental field (Fig. 1).

Previous works have studied the response of plant communities to different disturbance gradients in agroecosystems (Swanton et al., 1993; Zanin et al., 1997; Gaba et al., 2014). In this regard, weed management can be defined as a disturbance (White and Pickett, 1985), the effect of which on plant communities mainly depends on its intensity (proportion of biomass removed) and frequency (time elapsed between disturbance events) (White and Jentsch, 2001). The present study proposes a disturbance-based approach for assessing the effects of different weed management (herbicide, mowing, tillage) on taxonomic diversity. For this purpose, the percentage of bare soil (hereinafter referred to as bare soil) was used as an indicator of the intensity and frequency of disturbance. In this regard, previous research explored the relationship between plant cover (i.e., the inverse of bare soil) and plant diversity in grasslands (Casado et al., 2004; Sanaei et al., 2018), but not in agroecosystems. This disturbance-based approach is intimately linked to ecological succession theory (Connell and, Slatyer, 1977; Huston and Smith, 1987), formerly used in weed ecology to study weed flora dynamics (Swanton et al., 1993; Zanin et al., 1997).

Finally, this study explored the relationship between biodiversity and vineyard yield. Earlier studies have examined the relationship between soil coverage and vineyard yield, but only in plant covers and with discrete data (Delpuech and Metay, 2018). Since the present work proposed the usefulness of bare soil as a predictor of biodiversity and vineyard yield, we hypothesised that the more intensive the weed management (i.e., higher bare soil), the lower the plant diversity and the higher yield, while the less intensive the weed management (i.e., lower bare soil), the higher the plant diversity and the lower yield.

<sup>&</sup>lt;sup>1</sup> https://ec.europa.eu/food/horizontal-topics/farm-fork-strategy\_ena

<sup>&</sup>lt;sup>2</sup> Regulation of the European Parliament and of the Council: https://eur-lex. europa.eu/legal-content/EN/TXT/?uri=COM%3A2018%3A392%3AFIN



Fig. 1. Land-use map of the study zone. Surface area occupied by Natura 2000 habitats "6220 Pseudo-steppe with grasses and annuals (*Thero-Brachypodietea*)" has been represented from *Spanish Inventory of Land Species* (MAGRAMA, 2014).

Unravelling these questions could provide a deeper understanding of the effect of weed management on plant communities, allowing the development of a more balanced and sustainable weed management in vineyards, one of the most important and widespread crops in the Mediterranean basin.

### 2. Materials and methods

### 2.1. Study site

The study was conducted in an vineyard of the IMIDRA experimental farm El Socorro (40°07'58"N, 3°22'33"O; altitude 755 m.a.s.l.), located in a wine region in the centre of Iberian Peninsula (Colmenar de Oreja, Madrid, Spain), within the area regulated by "Vinos de Madrid" DOC Regulatory Council. The experimental farm is located within an agricultural matrix of rainfed Mediterranean crops, with the presence of forest patches of Pinus spp. and Quercus spp. and remnants of grasslands corresponding to Natura 2000 Habitat 6220 (Fig. 1). The study site is characterised by a semiarid continental Mediterranean climate with an average annual rainfall of 436.2 mm and an average annual temperature of 13.5 °C (data from El Socorro weather station for the period 1999–2017). The soil, formed by Quaternary eluvial deposits, is a Calcic Haploxeralf (pH 8.4) with a clay-loam texture. The experimental field is a 6700 m<sup>2</sup> vineyard of Tempranillo variety (clone 771/pattern 110 R), grown in cordon Royat formation, with a planting frame of  $2.0\ m\times 1.1\ m$  and deficit drip irrigation in the vineyard row.

### 2.2. Experimental design

A long-term experiment was originally established in 2008 to evaluate different management systems in vineyards: MS1 (intensive), interrows tilled with cultivator (up to three passes throughout the grapevine growth cycle) and rows sprayed with herbicide (up to two applications of glyphosate); MS2 (eco-till) inter-rows tilled with cultivator and rows tilled with inter-vine cultivator (up to three passes in both cases); MS4 (low-input), spontaneous plant cover on the inter-rows (spontaneous vegetation managed by two mowing passes) and rows sprayed with herbicide (same as MS1); MS5 (eco-cover), inter-rows with spontaneous plant cover (same as MS4) and rows mown with mower inter-vine (up to two passes).

The original experimental consisted of a randomised blocks design with four replications, thus involving 16 plots (4 management system $s \times 4$  blocks) each including five 50 m inter-rows: three treated interrows and a buffer inter-row on each side to limit the edge effect. Using this original design, we divided each of the 16 original plots into rows and inter-rows, thus distinguishing 32 subplots (4 management systems  $\times$  4 blocks  $\times$  2 positions). Thus, for example, we divide "plot 2" into "subplot 2R" (row) and "subplot 2IR" (inter-row). Inter-row and row were assessed separately, since the deficit irrigation applied on the vineyard row and, to a lesser extent, the shade produced by the grapevines themselves, generate different conditions for plant growth, especially in the summer months. Consequently, hereafter we will use the terms rows (irrigated subplots) and inter-rows (non-irrigated subplots). Then, from this new design, we focused on how the combination of weed management practices (herbicide, tillage, mowing) and position (row, inter-row) could shape vineyard plant communities. Therefore, five combinations were identified (Fig. 2): herbicide-sprayed rows, mown inter-rows, mown rows, tilled inter-rows and tilled rows. It should be noted that experimental design did not include the herbicide treatment inter-rows since this is not a common practice used in Mediterranean vinevards.

In addition, whether the management of adjacent subplots had an effect on the plant community in study subplots was also considered. For example, it was assumed that community composition would not be the same in an herbicide-sprayed row adjacent to a tilled inter-row as compared to one adjacent to a mown inter-row. Based on these assumptions, the design included eight environmental sites (Fig. 2): 1) tilled inter-row next to herbicide; 2) herbicide-sprayed row next to tillage; 3) tilled inter-row next to tillage; 4) tilled row next to tillage; 5) mown inter-row next to herbicide; 6) herbicide-sprayed row next to mowing; 7) mown inter-row next to mowing; and 8) mown row next to mowing.

### 2.3. Field data collection

Plant surveys were conducted in 2015, 2016 and 2018 during the



Fig. 2. Diagram of the management systems and sites defined according to the combination of weed management (herbicide, mowing, tillage) and position (R, row; IR, inter-row). Management systems: MS1, intensive management; MS2, eco-tillage management; MS4, lowinput management; MS5, eco-cover management. Sites: 1, tilled inter-row next to herbicide; 2, herbicide-sprayed row next to tillage; 3, tilled inter-row next to tillage; 4, tilled row next to tillage; 5, mown inter-row next to herbicide; 6, herbicide-sprayed row next to mowing; 7, mown inter-row next to mowing; 8, mown row next to mowing.

first half of May, just before weeding operations, coinciding with the time of year when the vast majority of the plant species sampled were in bloom. In each of the 16 plots (e.g., plot 2), sampling points were located in pairs, six in the row (e.g., subplot 2R) and six in the inter-row (e.g., subplot 2IR). Sampling points were separated by 1 m and a distance of 5.5 m between pairs within the same subplot (Fig. 2).

Each sampling point consisted of a 33 cm  $\times$  66 cm quadrat, in which all plant species were identified and the percentage cover of each species was estimated. In addition, the percentage of bare soil within each quadrat was also assessed. Data from the sampling points were pooled per subplot to calculate average cover and to build a "subplot by species" matrix. Species were identified according to Castroviejo (1986–2012) and the nomenclature was subsequently updated following the International Plant Names Index (IPNI, 2020). Those species considered, according to Ríos and Salvador (2009), characteristic of the Pseudo-steppes with grasses and annuals of the *Thero-Brachypodietea* in Natura 2000 habitats were classified as therophytic grassland species (hereinafter referred to as TGS). The remaining species were classified where appropriate, in accordance with the independent criteria of five experts of the Spanish Weed Science Society (SEMh), as noxious grapevine weeds (hereinafter referred to as NGW).

Grape yields were also measured from 2016 to 2018, to analyse possible competence effects between plant communities and the vineyard. For this purpose, a set of 10 grapevines were randomly marked in the central rows of each of the 16 plots (from the 2008 original design), harvested by hand and subsequently weighed to obtain the weight per grapevine. Yield per hectare was estimated as: Yield (kg/ha) = (grapevines per hectare \* (1 – (gaps per plot/planting grapevines per plot)) \* weight per grapevine), where the percentage of gaps refers to the empty spaces (i. e., dead grapevines) calculated beforehand.

### 2.4. Data analysis

### 2.4.1. Plant community composition

Vegetation cover data for each species were first optimised using the Hellinger transformation in order to avoid problems associated with Euclidean distances (Legendre and Gallagher, 2001). Then, the Bray-Curtis dissimilarity was used to compute the distance between subplots according to their community composition. Subsequently, a non-metric multidimensional scaling (Kenkel and Orloci, 1986; Clarke, 1993) was performed using Bray-Curtis distances to display the position of the subplots in an ordination space. Then, a permutational multivariate analysis of variance (Anderson, 2001) was conducted to assess the statistical significance of differences in plant community composition, both according to "weed management" and "position" as nested fixed factors (hereinafter referred to as WM:P), and according to site (as explained in the experimental design, Section 2.2). These analyses were carried out with the *vegan* package (Oksanen et al., 2020) for R (R Core Team, 2020).

### 2.4.2. Plant taxonomic diversity

In order to understand how different weed management practices

affect plant taxonomic diversity, three fundamental components of taxonomic diversity were measured at different levels using *vegan* package:  $\beta$ -diversity, evenness and species richness.

Firstly, to disentangle how species composition varies spatially depending on weed management,  $\beta$ -diversity was calculated from multivariate dispersion according to Anderson et al. (2006), with  $\beta$ -diversity defined as variation in community structure among sample units (Anderson et al., 2011). For this purpose,  $\beta$ -diversity was measured at the sampling point level to capture fine-scale variation. Sampling points were grouped according to the nested fixed factors WM:P, as well as to site. Then, based on Bray-Curtis distance matrix, the distance between each sampling point and the centroid of each group was calculated using the function *betadisper* of *vegan* package. This was computed separately for each year, so that the  $\beta$ -diversity would only take into account spatial variation and not temporal variation.

Subsequently, evenness (Pielou, 1966) and species richness were calculated at subplot level from "subplot by species" matrix, this latter estimated for the total number of species found in each subplot (S) as well as for the set of therophytic grassland species ( $S_{TGS}$ ) and noxious grapevine weeds ( $S_{NGW}$ ) found in each subplot.

In addition, to explore the relationship between diversity and vineyard yield, species richness and TGS richness were also calculated by management system (i.e., MS1, MS2, MS4 and MS5).

### 2.4.3. Statistical analyses

In this paper, the response of different variables to weed management (WM) and position (P) were analysed using mixed models, considering the nested fixed factors WM:P. Furthermore, alternative models were constructed using site (i.e., the eight environmental sites) as fixed factor. As noted above, when referring to "site", the indirect effect that adjacent management may have on each subplot was taken into consideration.

Firstly, the effect of WM:P and site on the distribution of the ten most abundant species, bare soil and the percentage of NGW and TGS was examined. For this purpose, generalised linear mixed models were fitted with a beta regression using "loglog" as link function. Beta regression is suitable for fitting models with continuous response variables that are constrained between zero and one (Ferrari and Cribari-Neto, 2004). Subsequently, the effect of WM:P and site on taxonomic diversity indices was analysed using linear mixed models, except for  $\beta$ -diversity which were fitted by a generalised linear mixed model with beta regressions and "loglog" as link function. Finally, the effect of management systems (i.e., MS1, MS2, MS4 and MS5) on bare soil, species richness, TGS richness and vineyard yield was also studied using linear mixed models, considering "management system" as fixed factor. Block and year were considered as random effects in all studied models.

To select the best models, corrected Akaike Information Criterion (AICc) was used, which allows direct comparison of models with different parameter combinations, including a correction for small sample sizes (Burnham et al., 2011). For each variable, the model with the lowest AICc score was selected. For all selected models, anova tests (type III) were used to assess the statistical significance of the fixed

effects using the car package (Fox and Weisberg, 2019). Linear mixed models were fitted with the lme4 package (Bates et al., 2015) and beta regression models with the betareg package (Cribari-Neto and Zeileis, 2010). To estimate the model fit, pseudo  $R^2$ , marginal and conditional  $R^2$  were calculated using the *performance* package in R (Lüdecke et al., 2021). Pseudo  $R^2$  was used as a measure of fit beta regression models. For all other models, marginal (estimates only the variance of the fixed effects) and conditional  $R^2$  (both fixed and random effects) were calculated. When a significant effect of WM:P on any of the response variables was found, the Student's t-test was used for pairwise comparison between the different levels, adjusting the P-values with the Bonferroni correction. In order to study the usefulness of bare soil as a predictor of biodiversity and vineyard yield, the relationship among bare soil, plant taxonomic diversity and vineyard yield was first explored using linear and polynomial regression models. Once the relationship between the variables studied was confirmed to be monotonic, the associations between them were analysed using Spearman's correlation.

All statistical analyses were performed with R software (R Core Team, 2020).

### 3. Results

During plant surveys, a total of 59 herbaceous species were identified. The complete list of all species is shown in the Appendix A (Table A1), with their mean relative abundance values and classification as NGW or TGS, if applicable. The most abundant species were, in this order: *Medicago minima* (relative abundance of 19.7%), *Bromus madritensis* (14.5%), *Lamium amplexicaule* (10.6%), *Convolvulus arvensis* (6.6%) and *Sonchus asper* (6.6%). The most present families were Asteraceae (with 15 taxa and a relative abundance of 15.3%), Poaceae (10 taxa, 20.3%) and Fabaceae (8 taxa, 22.6%).

# 3.1. Effect of weed management practices and position (inter-row, row) on plant community composition

The results of the permutational multivariate analysis of variance (Appendix A, Table A3) showed significant differences in the composition of plant communities according to weed management ( $R^2 = 0.48$ ; P < 0.001), position ( $R^2 = 0.08$ ; P < 0.001) and WM:P ( $R^2 = 0.52$ ; P < 0.001), although with a slightly better fit when composition was analysed by site ( $R^2 = 0.56$ ; P < 0.001). This is consistent with the non-



**Fig. 3.** Non-metric multidimensional scaling ordination of experimental subplots according to site. Ellipses of standard deviation and centroids are shown for each site, having been coloured based in nested effect of weed management and position (WM:P). Stress value generated by the algorithm is indicated in the lower left corner. Stress values equal to or below 0.05 indicate good fit. Codes for WM:P are: HR, herbicide-sprayed rows; MIR, mown inter-rows; MR, mown rows; TIR, tilled inter-rows; TR, tilled rows.

metric multidimensional scaling analysis, which clearly showed the subplots grouped by sites in the ordination space (Fig. 3), with the x-axis discriminating between weed management and the y-axis between positions. Results from this analysis also showed that site 6 (herbicide-sprayed row next to mowing) was closer to mown sites than site 2 (herbicide-sprayed row next to tillage), the latter being closer to tilled sites.

Regarding the distribution of the ten most abundant species, the models using WM:P as a fixed factor resulted in the best fits for those species showing highest coverage (see Appendix B). Table 1 show the relative covers of the 10 most abundant species according to WM:P. *Crepis vesicaria* subsp. *taraxacifolia, Festuca myuros, Galium parisiense,* and *M. minima* showed the highest percentages of cover in mown subplots, although when comparing between mown and herbicide-sprayed rows, only *F. myuros* and *M. minima* showed significantly higher cover. *Bromus madritensis* and *S. asper* reached the highest covers in herbicide-sprayed rows (27.44% and 21.39%, respectively). In tilled subplots, *L. amplexicaule* was the species with the highest cover on both rows and inter-rows, while *C. arvensis* and *Diplotaxis erucoides* showed significantly higher covers in tilled rows compared to mown and herbicide-sprayed rows.

Nested fixed factors WM:P had a strong influence on the percentage of NGW and TGS (Fig. 4), being especially significant in the case of TGS (pseudo  $R^2 = 0.76$ ). Significantly higher percentage of NGW was observed in herbicide-sprayed rows compared to that observed in tilled and mown rows. Differences were also observed according to position (inter-row, row), with higher percentages of NGW in rows, although significant differences were only found when comparing mown subplots. Regarding the percentage of TGS, significantly higher values were observed in mown subplots, with the highest values reached in mown inter-rows.

# 3.2. Effect of weed management practices and position (inter-row, row) on bare soil

The best fits resulted from models using WM:P as nested fixed factors (see Appendix B), thus showing a strong effect of WM:P on bare soil (pseudo  $R^2 = 0.86$ ). This significant effect was also evident depending on weed management, with higher bare soil in tilled rows (75.69%) compared to herbicide-sprayed (52.69%) and mown (18.19%) rows (Fig. 5). However, there was no consistent response of the bare soil depending on the position, with no significant differences between rows and inter-rows in tilled subplots, while in mown subplots, rows showed significantly higher bare soil than inter-rows.

# 3.3. Effect of weed management practices and position (inter-row, row) on taxonomic diversity

The results of this study have shown significant effects of WM:P on all taxonomic diversity indices (Table 2). Thus, WM:P had a strong influence on  $\beta$ -diversity (pseudo R<sup>2</sup> = 0.53), with the lowest values reached in mown subplots. Evenness was moderately affected by WM:P (marginal  $R^2 = 0.19$ ), with the highest values in tilled subplots, although the differences were only statistically significant when comparing tilled subplots versus mown inter-rows. Also, WM:P had a moderate effect on NGS richness (marginal  $R^2 = 0.15$ ), with significant differences between inter-rows and rows in mown subplots. Nevertheless, for species richness and TGS richness, a better fit was found in models using "site" as an explanatory variable (Fig. 6; Appendix B), which explained a large proportion of the observed variation (marginal  $R^2\,=0.52$  and 0.68respectively). Thus, mown sites (i.e., sites 5, 7 and 8) showed a significantly higher species richness and TGS richness than tilled sites (i.e., sites 1, 3 and 4), while a non-homogeneous response was found in herbicide-sprayed sites (i.e., sites 2 and 6). Indeed, for species richness no significant differences were found between site 2 and the tilled sites, and no significant differences were found between site 6 and the mown

### Table 1

Relative covers (expressed in percentages) of the 10 most abundant species according to nested fixed factors "weed management" and "position": HR, herbicide-sprayed rows; MIR, mown inter-rows; MR, mown rows; TIR, tilled inter-rows; TR, tilled rows. Fixed terms effect significance level was tested using Anova test (type III, \*\*\* P < 0.001). For pairwise comparison, the Student's *t*-test was used, adjusting the *P*-values with the Bonferroni correction. Different letters in the same column indicate significant differences between nested factors.

	Bro	Con	Cre	Dip * **	Gal	Lam	Med	Son	Ver	Vul * **
HR	27.44 a	9.44 b	3.50 b	5.00 bc	3.44 bc	1.78 b	13.78 b	21.39 a	0.83 b	1.28 b
MIR	10.17 b	0.62 c	9.71 a	0.79 c	8.63 a	0.00 b	39.21 a	1.25 b	1.20 b	7.42 a
MR	13.08 b	4.67 bc	6.17 b	2.25 bc	7.83 ab	0.00 b	33.25 a	7.42 b	0.92 b	5.92 a
TIR	11.88 b	5.92 bc	0.25 c	5.54 b	1.12 c	23.75 a	9.88 b	1.29 b	12.88 a	0.29 b
TR	13.75 b	19.08 a	0.00 c	12.00 a	0.33 c	23.42 a	4.75 b	3.75 b	7.50 ab	0.00 b

Species codes: Bro, Bromus madritensis; Con, Convolvulus arvensis; Cre, Crepis vesicaria subsp. taraxacifolia; Dip, Diplotaxis erucoides; Gal, Galium parisiense; Lam, Lamium amplexicaule; Med, Medicago minima; Son, Sonchus asper; Ver, Veronica hederifolia; Vul, Festuca myuros.



**Fig. 4.** Percentage of noxious grapevine weeds (NGW) and terophytic grassland species (TGS) according to nested effect of weed management and position (WM:P). *P*-values were estimated from an Anova test (type III). Pseudo  $R^2$  indicates the variance explained by fixed factors. For pairwise comparison, the Student's *t*-test was used, adjusting the P-values with the Bonferroni correction. Boxes with different letters are significantly different. Codes for weed management (lower row) and position (upper line) in the x-axis are: H-R, herbicide-row; M-IR, mowing-inter-row; M-R, mowing-row; T-IR, tillage-inter-row, T-R, tillage-row.



**Fig. 5.** Percentage of bare soil according to nested effect of weed management and position (WM:P). *P*-value was estimated from an Anova test (type III). Pseudo R<sup>2</sup> indicates the variance explained by fixed factors. For pairwise comparison, the Student's *t*-test was used, adjusting the *P*-values with the Bonferroni correction. Boxes with different letters are significantly different. Codes for weed management (lower row) and position (upper line) in the x-axis are: H-R, herbicide-row; M-IR, mowing-inter-row; M-R, mowing-row; T-IR, tillage-inter-row, T-R, tillage-row.

sites as well. Similarly, no significant differences were observed for TGS richness between site 6 and mown rows, and between site 2 and tilled inter-rows.

## 3.4. Relationship between bare soil, taxonomic diversity and vineyard yield

As observed at subplot level, a strong positive correlation (r = 0.73) between bare soil and  $\beta$ -diversity was found (Fig. 7a). Likewise, a positive but weak correlation (r = 0.39) was observed between bare soil

and evenness (Fig. 7b). In contrast, a strong negative correlation (r = -0.74) was found between bare soil and species richness (Fig. 7c).

Furthermore, a strong negative correlation (r = -0.85) was found between bare soil and species richness according to management system (Fig. 8a). In contrast, a strong positive correlation (r = 0.73) between bare soil and vineyard yield was observed (Fig. 8b). Consequently, a moderate negative correlation between species richness and vineyard yield was found (Fig. 8c). Indeed, management systems MS1 and MS2, with bare soil ranging from 51% to 89%, resulted in higher yields than management systems MS4 and MS5, particularly with MS5 that attained significantly less yield (Fig. 9, Table 3). Conversely, the highest values of species richness and TGS richness were found in MS4 and MS5, with an average of more than six TGS in each management (Table 3).

### 4. Discussion

Our results have shown a significant effect of weed management on plant community composition and taxonomic diversity indices, while position affected composition, but no significant effect on taxonomic diversity was observed. Supporting one of the work hypotheses, bare soil has been shown to be a good predictor for plant taxonomic diversity and vineyard yield depending of weed management.

## 4.1. Weed management and position (row, inter-row) affect plant community composition

Tillage favoured a small group of fast-growing ruderal terophytes (Kazakou et al., 2016; Guerra et al., 2021) such as *L. amplexicaule*, which adapt to a recurrent disturbance. In contrast, many species in the local species pool seemed to be well adapted to mowing, with significant presence of TGS. Thus, a highly significant presence of TGS was observed in mown subplots relative to tilled or herbicide-sprayed subplots. At these sites, plant cover was dominated by *M. minima*, a characteristic species of the Natura 2000 habitats 6220 (San Miguel, 2008). Mowing not only promoted a higher cover percentage of TGS, but a significantly higher number as well (see Table 2, S<sub>TGS</sub>). Mediterranean

#### Table 2

Mean values of the different diversity indices according to the nested fixed factors "weed management" and "position": HR, herbicide-sprayed rows; MIR, mown interrows; MR, mown rows; TIR, tilled inter-rows; TR, tilled rows. Fixed terms effect significance level was estimated from an Anova test (type III; \*\* P < 0.01, \*\*\* P < 0.001). Conditional (R<sup>2</sup>c) and marginal (R<sup>2</sup>m) r-squared values indicate the variance explained by the full model and by fixed factors, respectively. For pairwise comparison, the Student's *t*-test was used, adjusting the P-values with the Bonferroni correction. Different letters in the same column indicate significant differences between nested factors.

	β	J,	S	S <sub>NGW</sub>	S <sub>TGS</sub>
	* **	* **	* **	* *	* **
HR	0.24 a	0.73 ab	11.72 b	3.78 ab	2.39 b
MIR	0.17 b	0.70 b	16.46 a	2.62 b	5.58 a
MR	0.18 b	0.73 ab	16.00 a	4.42 a	4.83 a
TIR	0.26 a	0.78 a	10.21 c	3.25 ab	1.87 bc
TR	0.25 a	0.79 a	8.58 c	3.17 ab	0.83 c
R <sup>2</sup> c	_	0.34	0.57	0.19	0.65
R <sup>2</sup> m	_	0.19	0.48	0.15	0.63
pseudo R <sup>2</sup>	0.53	-	-	-	-
distribution	beta	gaussian	gaussian	gaussian	gaussian

 $\beta$ ,  $\beta$ -diversity according to Anderson et al. (2006); J', evenness; S, species richness; S<sub>NGW</sub>, noxious grapevine weeds richness; S<sub>TGS</sub>, therophytic grassland species richness.



**Fig. 6.** Species richness (a) and TGS richness (b) according to site. *P*-values were estimated from an Anova test (type III). Marginal R-squared ( $R^2m$ ) indicate the variance explained by fixed factors. For pairwise comparison, the Student's *t*-test was used, adjusting the *P*-values with the Bonferroni correction. Boxes with different letters are significantly different. Sites: 1, tilled inter-row next to herbicide; 2, herbicide-sprayed row next to tillage; 3, tilled inter-row next to tillage; 5, mown inter-row next to mowing; 7, mown inter-row next to mowing; 8, mown row next to mowing.

= -0.74



grasslands have historically been subject to periodic grazing, that is, a disturbance similar to mowing, hence fostering species adapted to above-ground biomass removal (Perevolotsky and Seligman, 1998; (Peco et al., 2012). In addition, the presence of these therophytic grasslands habits in the vicinity of the study area (Fig. 1), could lead to seed dispersal from these spaces to the vineyard. Finally, since mowing was carried out when most of these species already dispersed seeds, such results could be expected.

On the other hand, the percentage of NGW was significantly higher in herbicide-sprayed rows, mainly due to a greater presence of *S. asper* (the most abundant NGW species). Along with *S. asper*, *B. madritensis* was also promoted by herbicide application on vineyard rows. Recent work has shown that herbicide application in vineyards can favour perennial species which are able to resprout after herbicide application (Fried et al., 2019; Hall et al., 2020). Nevertheless, both *S. asper* and *B. madritensis* are annual species lacking below-ground organs. Guerra et al. (2021) indicated that *S. asper* could escape herbicide pressure due to its delayed germination and emergence pattern, but this would not apply to *B. madritensis*. Moreover, a negative effect of tillage has been reported on nearby species such as *Sonchus oleraceous* (Widderick et al., 2002; Chauhan et al., 2006) and *Bromus diandrus* Roth. (Recasens et al., 2016), since tillage promotes seed burial and these species find it difficult to emerge from deeper soil layers. In addition, establishment of these species may be limited in mown subplots due to interception of seed rain by grass cover, as has been documented for *S. asper* (Doisy et al., 2014).

The percentage of NGW was higher in the vineyard rows than in vineyard inter-rows. Therefore, competitive species such as *C. arvensis* 



**Fig. 8.** Scatterplots showing the relationship of percentage of bare soil with species richness at management system level (a), with vineyard yield (b), and the relationship between species richness and vineyard yield (c). Dots have been filled according to management system (MS). Spearman correlation coefficient (*r*) and *P*-value (\*\*\* P < 0.000) are indicated on scatterplots. Regression equation: (a) S = 30.33 - 0.20 BareSoil (R<sup>2</sup> = 0.71); (b) Yield = 1.83 + 0.02 BareSoil (R<sup>2</sup> = 0.52); (c) S = 36.60 - 5.86 Yield (R<sup>2</sup> = 0.38). Confidence intervals of 0.95 are indicated by light-grey shaded. Codes for MS are: MS1, intensive management; MS2, eco-tillage management; MS4, low-input management; MS5, eco-cover management.



**Fig. 9.** Vineyard yields by management system, expressed in kg ha<sup>-1</sup>. *P*-value was estimated from an Anova test (type III). Pseudo R<sup>2</sup> indicates the variance explained by fixed factors. For pairwise comparison, the Student's *t*-test was used, adjusting the *P*-values with the Bonferroni correction. Boxes with different letters are significantly different. Dotted red line indicates the production limit of 7000 kg ha<sup>-1</sup> defined by the "Vinos de Madrid" DOC Regulatory Council. Codes for MS are: MS1, intensive management; MS2, eco-tillage management; MS4, low-input management; MS5, eco-cover management.

### Table 3

Mean values of bare soil cover, species richness (S), therophytic grassland species richness (S<sub>TGS</sub>) and vineyard yield according to management system (MS). Fixed terms effect significance level was estimated from an Anova test (type III; \*\*\* P < 0.001). Conditional (R<sup>2</sup>c) and marginal (R<sup>2</sup>m) r-squared values indicate the variance explained by the full model and by fixed factors, respectively. For pairwise comparison, the Student's *t*-test was used, adjusting the *P*-values with the Bonferroni correction. Different letters in the same column indicate significant differences between MS.

	Bare soil (%)	S	STGS	Yield (kg grapevine <sup>-1</sup> )		
	* **	* **	* **	* **		
MS1	67.25 b	17.00 b	2.60 b	2.72 a		
MS2	77.19 a	13.42 b	1.58 b	2.79 a		
MS4	25.94 c	22.75 a	6.25 a	2.02 ab		
MS5	11.67 d	24.25 a	6.67 a	1.59 b		
R <sup>2</sup> c	0.94	0.69	0.74	0.82		
R <sup>2</sup> m	0.93	0.64	0.74	0.32		

Management system: MS1, intensive management; MS2, eco-tillage management; MS4, low-input management; MS5, eco-cover management.

were more abundant in rows, particularly in tilled rows (Table 1), in agreement with the findings of Fried et al. (2019). Similar results were reported by Guerra et al. (2021), who found that more competitive species were located in the vineyard rows where irrigation was applied.

# 4.2. Effects of weed management on bare soil from a disturbance-based approach

Based on the results, a gradient from greater to lesser degree of disturbance as a function of weed management could be established, with bare soil values in the following order: tillage > herbicide > mowing.

Tillage and herbicide application involve mechanical and chemical removal of plant biomass both above and below-ground, thus resulting in significantly higher bare soil than mowing, the latter primarily affecting only above-ground plant biomass.

After tillage or herbicide application, following a process of secondary succession (Connell and Slayter, 1977), plant species can recolonise gaps generated by disturbance (Bullock et al., 1995; Pakeman and Small, 2005). This gap was evident in tilled subplots, but also occurred after the application of the herbicide used in this study, since glyphosate is an herbicide with relatively short persistence in soil (Simonsen et al., 2008), which generated gaps available to plants shortly after its application. Consequently, a reduction in bare soil was expected as the succession process progressed (e.g., Coiffait-Gombault et al., 2012). The differences observed in bare soil between these subplots and the tilled subplots (Fig. 5) could be due to the time elapsed between the last disturbance event and the sampling time (8–9 months for herbicide, while only 2–3 months for tillage).

### 4.3. How weed management influence plant taxonomic diversity

As mentioned above, weed management had a strong effect on bare soil, which could be assumed as an indicator of the disturbance degree associated with management. From the findings of this long-term study, three basic assumptions could be drawn: (a) The higher disturbance degree, the higher  $\beta$ -diversity (Fig. 7a). This is consistent with the observations of Catano et al., (2017), who pointed out in a meta-analysis that β-diversity was generally higher in disturbed relative to undisturbed plant communities, as well as with Derrouch et al. (2021), who have recently noted in agroecosystems how β-diversity decreased over time as the degree of disturbance decreased, leading to a homogenisation of weed community assemblages. (b) The higher disturbance degree, the higher evenness (Fig. 7b). This is in agreement with previous work (Biswas and Mallik, 2010; Svensson et al., 2012; Sanaei et al., 2018) which observed that evenness increases with increasing levels of disturbance. In this study, mowing communities were more stable but at the same time less evenly distributed (lower J'), with clearly dominant species such as M. minima. (c) The higher disturbance degree, the lower

species richness (Fig. 7c). This is consistent with recent work in vineyards (e.g., Kazakou et al., 2016; Hall et al., 2020), as well as in other agroecosystems that have shown a decrease in species richness as the disturbance gradient increased (Feldman et al., 1998; Derrouch et al., 2021), or the agriculture intensification increased (Carmona et al., 2020). However, some works in grasslands have observed a unimodal relationship between species richness and cover, with a decrease at high herbaceous cover values (Grytnes, 2000; Casado et al., 2004). In fact, a vast literature has shown that the highest values of species richness were observed in plant communities subjected to intermediate disturbances (intermediate disturbance hypothesis; Grime, 1973; Connell, 1978). This is probably valid for low-disturbance plots of high productivity, where a species exclusion process occurs due to competition for light (Grime, 1973; Hautier et al., 2009; Boch et al., 2021). But these conditions can hardly be found in agroecosystems (with the possible exception of field edges), since these are by definition subject to recurrent disturbances. Therefore, mowing could be considered as a weed management with an intermediate degree of disturbance. In this study, bare soil values close to 0% occur in disturbed-by-mowing subplots, strongly associated with increased stress-tolerant dimension, but not competitiveness (Guerra et al., 2021).

These assumptions would be modelled in this study by two ecological processes: the environmental filtering on the species pool and the impact of spatial mass effects on species richness. Firstly, weed management worked as a filter in the species pool, which could condition the taxonomic diversity observed in each management. Thus, since tillage involves recurrent cultivator passes, bare soil was very high and only a few species were able to reoccupy that space. In contrast, although the frequency of disturbance at mowing was similar, the intensity was not, since biomass was only removed from 2 to 4 cm above the ground, thus allowing small terophytes and hemicryptophytes with rosette leaves to remain. As a result, species exclusively grown at mowing, such as *Leontodon longirrostris* or *Rostraria cristata*, with a more stress-tolerant strategy and a greater investment of resources in vegetative or generative tissues, had limited survival capacity in intensively disturbed environments such as tillage.

Secondly, our findings about the effect of herbicide application on species richness have revealed a response dependent on the adjacent management (Fig. 6a). Thus, no significant differences were observed between herbicide-sprayed rows and adjacent subplots (e.g. between site 6 [herbicide] and site 5 [mowing]). In the similar vein, Fig. 3 shows how site 6 was closer to the mown sites than site 2, which was closer to the tilled sites. These results could be explained according to two basic aspects: (a) seed dispersal fit generally a negative exponential curve, with dispersed seed density decreasing with distance (Willson and Traveset, 2000), so that for most weed species the probability of seed dispersal is higher as the distance to the parent plant is shorter (Wallinga et al., 2002); and (b) related to dispersion mechanisms stated in point a, herbicide-sprayed rows could be subject to spatial mass effects (Shmida and Wilson, 1985) from adjacent subplots, in a manner analogous to what has been observed in field edges (Metcalfe et al., 2019). According to Shmida and Wilson, (1985), a flow of individuals from areas of high success (e.g., mown inter-rows) to unfavourable areas (e.g., herbicide-sprayed rows) would be likely. Therefore, after the gaps created by the herbicide application in site 6, these spaces would be colonised mostly by species present at site 5. For example, among the ten most abundant species at site 6, albeit only to a small extent, appeared common mowing species such as Astragalus hamosus, which however had a marginal presence at site 2 (see Appendix A, Table A2). Similarly, among the top ten species at site 2, there was typical tillage species such as L. amplexicaule, which nevertheless had a residual presence at site 6.

# 4.4. Agronomic implications for a sustainable management in Mediterranean vineyards

As discussed above, herbicide-sprayed rows showed a higher

percentage of NGW, such as *S. asper* or *C. arvensis*, whose management could be problematic (Davis et al., 2018). In contrast, mowing emerged as the most effective control method to limit the presence of these species in vineyard inter-rows, but its effectiveness decreased in vineyard rows, where these NGW species with a more competitive strategy seem to be favoured by irrigation application (Guerra et al., 2021). According to the CSR strategy pointed out by these authors, it would be advisable to use tillage or mowing, as they emerge as more effective weed control methods to limit the presence of these noxious weeds.

Moreover, bare soil has been shown to be a good predictor of vineyard yields (Fig. 8b), revealing lower yields as the bare soil decreases, particularly in spontaneous plant cover management where lower yields were found, being significantly reduced in MS5 (i.e., mowing in rows and inter-rows). This is in line with Tesic et al., (2007) and Delpuech and Metay (2018), who observed that grapevine vigour and yield decreased when the percentage of soil cover increased with permanent cover crops. Likewise, other previous works have also found reductions in grapevine vigour and yield under plant covers in Mediterranean vineyards (Monteiro and Lopes, 2007; Lopes et al., 2011; Pou et al., 2011; Muscas et al., 2017). Decrease in vineyard yields could be due to resource competence, particularly for nitrogen and water (Monteiro and Lopes, 2007; Celette et al., 2009; Celette and Gary, 2013). Thus, in water competence with plant covers, grapevine roots seek water in ever-deepening soil layers (Morlat and Jacquet, 2003; Celette et al., 2008; Tomaz et al., 2017), developing the highest root density in a soil horizon where grapevines have less access to assimilable nitrogen (Celette et al., 2009). This would cause a nitrogen deficiency in grapevines which, combined with water stress, would lead to reduced vigour and lower yields (Celette and Gary, 2013; Guilpart et al., 2014; Verdenal et al., 2021), although the application of irrigation and an early removal of plant covers could reduce competition stress under Mediterranean conditions (Linares Torres et al., 2018). On this basis, our assumption is that under similar conditions, the higher the bare soil, the lower the competence for resources and the higher the vineyard yield. Nevertheless, yields of MS1 and MS2 are clearly above the limit set by "Vinos de Madrid" DOC Regulatory Council (7000 kg ha<sup>-1</sup>) (Fig. 9) or other emblematic DOCs such as Rioja (5000 kg ha<sup>-1</sup>) or Ribera del Duero (7000 kg ha<sup>-1</sup>). In this sense, as the literature points out, plant covers would help to control excessive vigour and favour more balanced production.

Since measuring bare soil is relatively easy and accurate by methods such as remote sensing (Ge et al., 2011; Lima-Cueto et al., 2019), the results of this study could lead to its widespread use in vineyards as a rapid method for rough estimation of crop yields and taxonomic diversity. However, it must be taken into account that this method would only be valid for spontaneous plant covers and does not make any sense in the case of sown cover crops, where the taxonomic diversity will be completely conditioned by the sown species. In any case, further studies in other settings will be needed to explore this issue in more depth.

# 4.5. Mowing management in Mediterranean vineyards could contribute to conservation of habitats of European interest

This study has revealed the dominance of TGS in soils managed with mowing, especially in inter-rows. These species favoured by mowing (e. g., *M. minima, Leontodon longirrostris*) are considered characteristic species of the Natura 2000 habitats 6220 (San Miguel, 2008), and the conservation of such a habitat is a priority within the European Union, in compliance with the Habitats Directive. In a time in which these grassland ecosystems are being threatened (WallisDeVries et al., 2002; San Miguel, 2008; Habel et al., 2013), an adequate management in Mediterranean vineyards could be an alternative for their conservation, since they would serve as a refuge for many of these species. This proposal is in accordance with what was outlined by Nascimbene et al. (2016) regarding grasslands species in Northern Italian vineyards. Preserving native plant covers could provide multiple ecosystem services (Daane et al., 2018) and it could also be an extra stimulus for

winegrowers, who could be recipients of subsidies from the new European policy of eco-schemes<sup>3</sup> related to protection of biodiversity, enhance ecosystem services and preserve habitats and landscapes, finding a balance between wine production and the conservation of natural habitats. In support of this idea, the results also showed that those management systems with mown plant cover, whether organic (MS5) or non-organic (MS4), presented high species richness and a yield that, as discussed above, was even above the production limits established by "Vinos de Madrid" DOC Regulatory Council.

### 5. Conclusion

Bare soil as an indicator of the disturbance degree associated with management, has proved to be a good predictor for taxonomic diversity and vineyard yield. Indeed, weed management increasing bare soil (e.g. tillage) resulted in a loss of species diversity and higher vineyard yields, while management enhancing spontaneous plant cover (e.g. mowing) led to richer diversity and lower yields. This long-term study also revealed how noxious species were more abundant in vineyard rows, especially in herbicide-sprayed rows. This should lead to a rethinking on how to manage weeds in vineyards. The increase in vineyard yields in management with higher bare soil far exceeded the limit allowed by the Regulatory Council. In contrast, management with spontaneous plant covers showed mean yields slightly surpassing this allowed limit, but with higher species richness and a very significant presence of therophytic grasslands species. This highlights the potential of vineyards as agroecosystems, which under proper management could contribute to the conservation of grasslands habitats of European interest, hence offering an opportunity for winegrowers interested in reconciling wine production and biodiversity conservation.

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### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2021.107816.

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<sup>&</sup>lt;sup>3</sup> See Article 28 of the proposed Regulation of the European Parliament and of the Council: COM/2018/392 final - 2018/0216 (COD)

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