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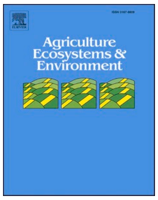

Vineyard footprint on pollinators is mediated by flower vegetation, organic farming, seasonal and weather factors, a case study from North Italy

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ABSTRACT

Intensive, industrialized agriculture is considered a major driver of pollinator decline and viticulture may play a relevant role in this context. A global priority is to find ways to decrease the agricultural impact on biodiversity and to undertake an ecological intensification of farms, especially for maintaining pollinator biodiversity. To recommend practical ways to support pollinators, we explored if they react to the intensive vineyard production in a valley in Northern Italy: we tested if environmental, weather and management parameters could be responsible for shaping pollinator abundance, diversity and functional trait distribution across different wine farms, sampled with observation plots and transect walks. Results demonstrated both some effects shared across pollinator groups and some idiosyncratic responses. Generally, management factors including the herbaceous vegetation cover, weed height and its flower diversity showed strong and positive linear relationships with the abundance (+13 % by unit) and diversity of pollinators (+15 % by unit), while organic farming was associated with a slight decline in the abundance of the overall pollinators (-10 % by unit) and of hoverflies and butterflies. Regarding the temporal and weather factors, pollinators decreased with wind intensity and seasonal progression, while a positive effect was found for intermediate values of air temperature and sampling hour, thus affecting insect activity. The community composition analysis showed that environmental and management factors translated in specific distributions of bee and hoverfly functional traits across sites. Farming practices allowing herbaceous cover, weed height and flower diversity are overwhelmingly important for pollinators to assure shelter and nutritional resources and should be systematically incorporated to mitigate vineyard impact. Furthermore, measures that support pollinators should also consider pollinator phenological dynamics associated with temporal and environmental parameters to accordingly modulate the time of agricultural treatment application. Overall, our study provides a knowledge basis for the development of pollinator-friendly vineyard practices to foster the ecological value of farms.

1. Introduction

Agricultural practices are one of the main global drivers of biodiversity decline, including the insect collapse (Ollerton et al., 2014; Sánchez-Bayo and Wyckhuys, 2019). The intensity of agricultural impact on biodiversity is mainly linked to two key factors: firstly, landscape homogenisation due to the increasing sizes of cultivated fields substituting natural areas (Neira et al., 2024; Rey Benayas and Bullock, 2012); secondly, the implementation of intensive agricultural regimes and the heavy use of agrochemicals (Ellis et al., 2020; Knapp et al., 2022). Conversely, certain agricultural landscapes have the potential to host rich and diverse biological communities and hence understanding which parameters could support biodiversity in agricultural ecosystems is now crucial (Bommarco et al., 2021; Granata et al., 2023; Raderschall et al., 2021; Tommasi et al., 2021). Only by reconciling and aligning farming practices with ecosystem functionality will it be possible to restore biodiversity in crop areas, while promoting the long-term resilience of both agricultural production and the broad ecosystem.

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Table 1
Abundance models: estimate, standard error and marginal and conditional R^2 of the final models, empty cells are for variables that did not pass the selection procedure based on delta AICc.

	Total pollinator abundance	Butterfly abundance	Honeybee abundance	Syrphid abundance	Wild bee abundance
	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE	Estimate ± SE
Intercept	2.187 ± 0.043	0.505 ± 0.09	0.259 ± 0.109	0.026 ± 0.145	0.843 ± 0.087
Weed cover (management)	0.129 ± 0.029	0.086 ± 0.071	0.254 ± 0.082		0.173 ± 0.05
Weed Shannon diversity (management)		0.046 ± 0.06	−0.166 ± 0.071	0.113 ± 0.098	
Weed height (management)			−0.222 ± 0.076		
Organic farming (management)	−0.101 ± 0.091	−0.108 ± 0.199		−0.209 ± 0.264	
Sampling date (environment)		0.053 ± 0.078	0.34 ± 0.106	−0.253 ± 0.098	−0.346 ± 0.066
Time (1ˆorder) (environment)	0.087 ± 0.037	0.256 ± 0.074	0.303 ± 0.108	0.473 ± 0.104	−0.248 ± 0.066
Time (2ˆorder) (environment)	−0.145 ± 0.032	−0.191 ± 0.076	−0.309 ± 0.112	−0.064 ± 0.095	−0.047 ± 0.053
Temperature (1ˆorder) (environment)	0.12 ± 0.033	0.07 ± 0.089	0.082 ± 0.118	−0.66 ± 0.128	0.781 ± 0.101
Temperature (2ˆorder) (environment)	−0.095 ± 0.035	−0.22 ± 0.075	−0.285 ± 0.104	−0.249 ± 0.096	−0.233 ± 0.091
Wind intensity (=1) (environment)	−0.039 ± 0.059		0.008 ± 0.111	−0.083 ± 0.143	−0.064 ± 0.108
Wind intensity (=2) (environment)	−0.267 ± 0.09		−0.285 ± 0.3	−0.266 ± 0.312	−0.464 ± 0.18
Marginal / Conditional R2	0.298 / 0.298	0.193 / 0.321	0.325 / 0.357	0.213 / 0.241	0.633 / 0.644

Pollinator biodiversity provide crucial ecosystem services by contributing to the processes of pollination and hence directly and indirectly assuring many other processes involving plants (Bartholomée and Lavorel, 2019), including many Sustainable Development Goals (Patel et al., 2020) and even human health (Smith et al., 2022, 2015). Pollinators are therefore pivotal characters of ecosystem functioning, and pollinator biodiversity is a crucial aspect especially in agricultural lands, where they are key to ecosystem functioning both for direct and indirect reasons.

Pollinator occurrence largely depends on the management strategies of agricultural lands, which can be strongly impactful for biodiversity and pollinators at the field level (Brambilla and Gatti, 2022; Nicholls and Altieri, 2013). Many studies highlighted that decreasing the intensive management of ground vegetation can benefit pollinators, for instance by keeping high flower diversity (Lowe et al., 2021; Tommasi et al., 2021) or by sowing flowers (Griffiths-Lee et al., 2022; Nichols et al., 2019). Low-intensity farming could also host heterogeneous microhabitats within and around fields, by leaving uncultivated patches, wild flowers, shrubs and trees that might even constitute ideal shelter and foraging areas for pollinators (Langlois et al., 2020). Within agricultural fields in general, a more biodiversity-friendly farming practice and the maintenance of elements belonging to semi-natural habitats are crucial aspects for maintaining biodiversity (Tscharntke et al., 2022), which is especially plausible in the context of vineyards (Paiola et al., 2020). Managing fields in this way provides positive feedbacks to the ecosystem services both at the field and landscape scale.

In this study, we investigated how pollinators respond to abiotic parameters describing seasonal and weather aspects and if they indicate the management footprint of vineyards. Previously, animals such as birds or butterflies were adopted as indicators in vineyards for their notorious sensitivity and flagship roles (Brambilla and Gatti, 2022; Cabodevilla et al., 2021). Similarly, also predatory mites are often recorded as bioindicators in vineyards because they cover essential ecological roles as predators of phytophagous mites (Tixier, 2018): for instance, phytoseiid and tydeoid mites increase in Austrian vineyards managed with integrated practices and having more spontaneous vegetation (Möth et al., 2021). Parasitoid wasps are also acknowledged in vineyard for their role in controlling pest dynamics (Schindler et al., 2022) and, for example, promoting inter-row ground management also improves hymenopteran parasitoids of leafhoppers (Cargnus et al., 2024). Therefore, it is likely that also pollinator groups including species with very different biological traits might serve as good candidates for indicating disturbance and management levels in agroecosystems (Granata et al., 2023; Tommasi et al., 2021).

It is appropriate to study pollinators in vineyards, because they have been previously employed as indicators of vineyard agricultural practices in a number of countries and case studies (e.g., Griffiths-Lee et al., 2022; Kehinde et al., 2018; Puig-Montserrat et al., 2017), as they promptly react to harmful vineyard practices (Kratschmer et al., 2021; Rocher et al., 2024). Moreover, peaks of pollinators at local scales could also point out positive situations if their biodiversity is particularly thriving (Kratschmer et al., 2019). They are especially sensitive to vineyard management, given their sensitivity to vegetation parameters including ground cover and flowering diversity (Granata et al., 2023; Kratschmer et al., 2021). Patterns of pollination in differently managed crops also have the potential to resume other taxa because those elements promoting pollinators benefit other beneficial arthropods like predators and parasitoids (Möth et al., 2021; Rocher et al., 2024; Schindler et al., 2022). Thus, providing habitats for pollinators in vineyards also enhances other ecosystem services like biological pest control, soil quality or landscape aesthetics (Wratten et al., 2012). Moreover, pollinators are indicators at the landscape scale, reacting to land consumption and landscape diversity surrounding vineyards (Granata et al., 2023; Kratschmer et al., 2019). This makes them good candidate sentinels of sustainable practices. Lastly, although most of the wine production results from self-pollination, it has been shown that cross pollination by insects can significantly contribute to vine production (Dobrei et al., 2021; Vorwohl, 1977): for instance, vine berry per bunch increases with native vegetation cover in relation to more pollination activity in the farms (Baronio et al., 2021) and up to 2 % more mass is recorded in vineyards supported by pollinators (Martignago et al., 2017). For all these reasons, it is appropriate to support pollinators in agricultural areas like the vineyards, especially in regions that are dominated by crops or are heavily affected by other human practices in the surroundings (*e.g.*, industries, urbanization).

Here, we expected that pollinators could simultaneously be influenced by temporal, environmental and management variables. The environmental parameters include abiotic conditions, such as the air

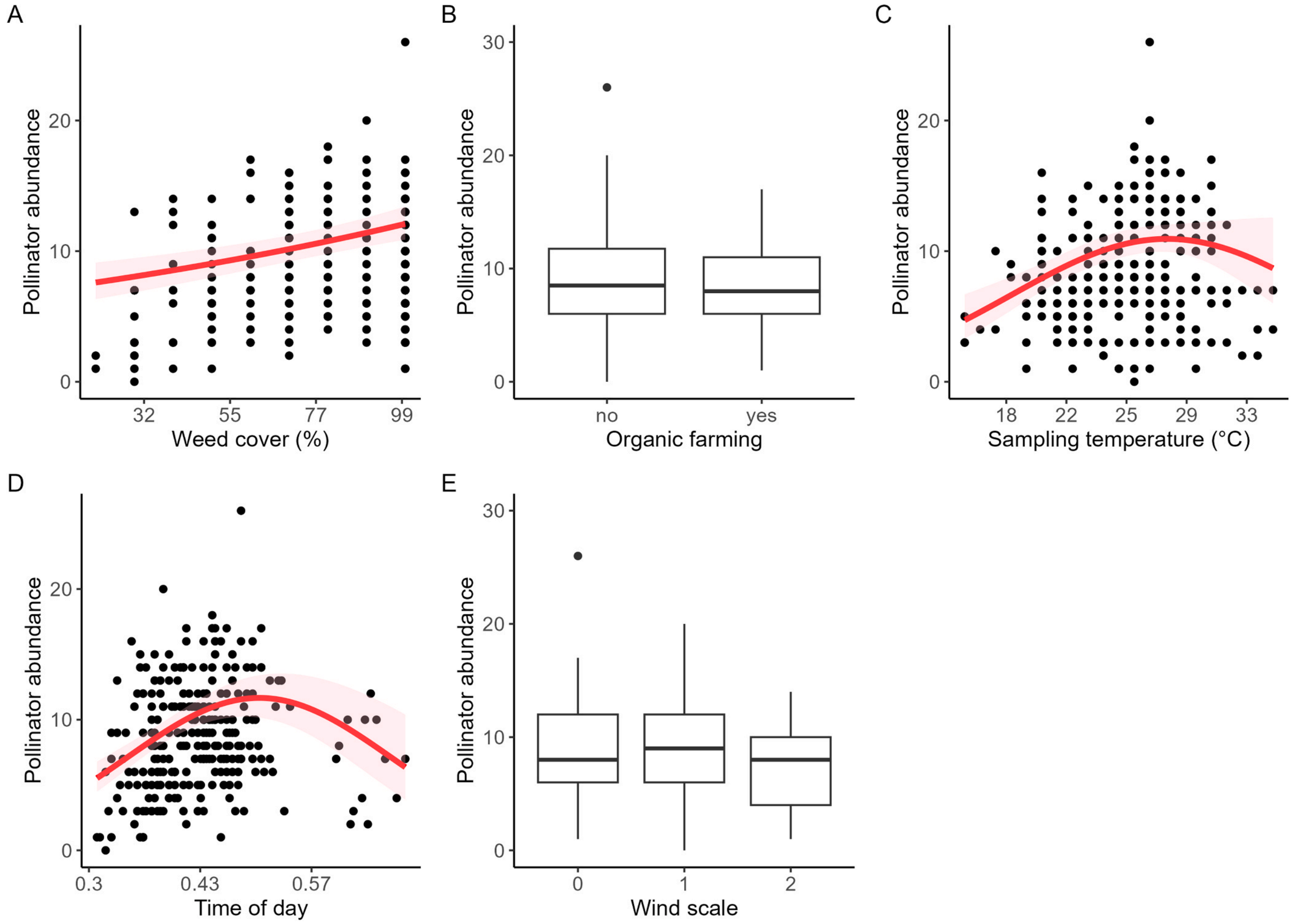

Fig. 1. Pollinator abundances (all groups together) on flowers in relation to management and environment variables describing (a) the ground vegetation cover, (b) organic/non-organic farming regimes, (c) air temperature at the moment of sampling, (d) the time of sampling and (e) the wind speed scale (0,1, 2, from absent to moderate wind, respectively). Raw data are obtained from 5-minute observations on plots. Predicted lines and confidence intervals are from regression models with variable selection procedures (the unselected variables are not plotted).

temperature or wind intensity affecting insect mobility (Vicens and Bosch, 2000), and time or date of sampling shaping seasonal or daily phenologies of pollinators. The vineyard management may also exert an important effect: farming intensity is inversely proportional to the amount, diversity and height of the herbaceous cover between rows of vines, hence affecting the availability of trophic resources, shelters and/or nesting sites. Moreover, organic and conventional farming regimes may have different effects on different groups (Winter et al., 2018). We hypothesize that pollinators will respond to all these factors in terms of varying diversity, abundances and distribution of functional traits across different vineyard parcels.

In particular, we aimed to test the pollinator response to the different environmental conditions and inter-row management practices in vineyards. We investigated this topic in an area that is intensively dedicated to wine production but where the link between wild pollinators and vineyard farming has not been explored so far. In addition, studies dedicated to pollinators in vineyards has rarely focused on integrating both biotic and abiotic factors influencing pollinators, usually preferring to focus on farming variables only. Instead, here we assess the role of factors describing management (organic/conventional treatments, vegetation cover, height and diversity) and the temporal-physical environment (weather conditions, hour and day of sampling) on the abundance and diversity of pollinators as a whole and as single pollinator groups, in order to gain knowledge integrating both biotic and abiotic factors necessary for the development of pollinator-friendly viticultural practices or the timing of treatment applications to promote ecosystem services in the area and increase pollinator conservation in agriculturally dominated landscapes.

2. Materials and methods

2.1. Study area and pollinator sapling

In this study, we focused on intensive vineyards in Northern Italy (Valtellina), a long valley in the Alps with a longitudinal direction, hosting approximately 1000 ha of vineyards (Lorusso, 2014). In this area, the peculiarity of wine production is that vineyards are located along steep mountainsides of the Alps on the south-facing slopes of the mountains and are subjected to a harsh climate especially during the summer. There, farming strategies range from systems with rows oriented north to south where the slope is steeper to east-west rows where the topography allows. Viticulture is among the most profitable sectors of Italian agriculture and shapes the economy, landscape and culture of winemaking regions, where it is often practised in intensive ways. During the study, 30 vineyards were monitored once a month between June and September 2022 (Fig. A1 in Appendix). The investigated vineyards were chosen to include a wide variety of environmental and management conditions, while maintaining a distance of at least 200 m between monitored fields.

Pollinator abundances were recorded with observation plots, an

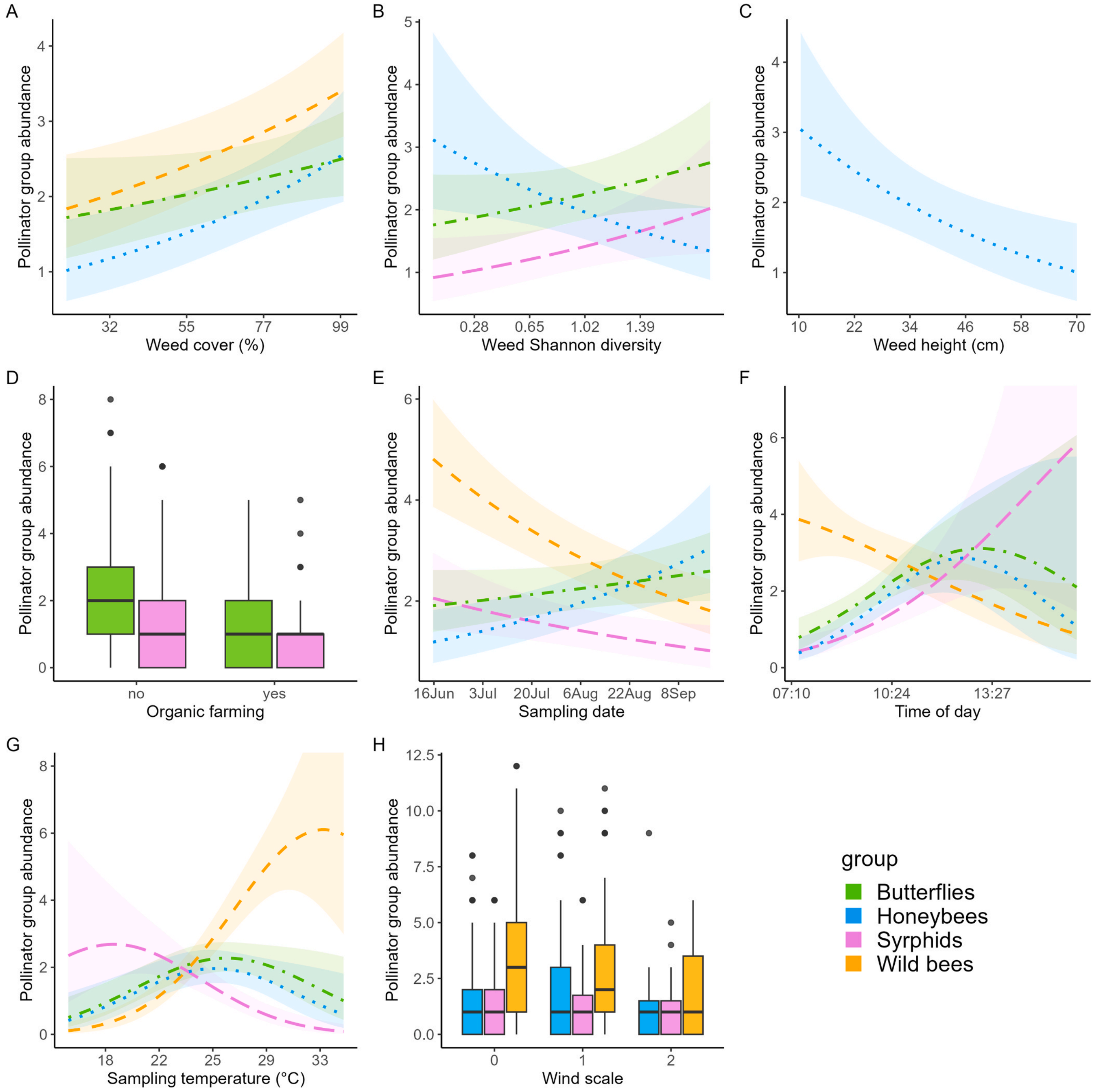

Fig. 2. Single pollinator-group abundances on flowers in relation to management and environment variables describing (a) the ground vegetation cover, (b) the Shannon diversity index of ground vegetation, (c) the height of the ground vegetation, (d) organic/non-organic farming regime, (e) the sampling date, (f) the time of sampling, (g) the air temperature at the moment of sampling, (h) the wind speed scale (0,1, 2, from no to moderate wind, respectively). Raw data are obtained from 5-minutes observations on plots. Predicted lines and confidence intervals are from regression models with variable selection procedures (the unselected variables are not plotted).

appropriate method for recording broad taxonomic categories and assuring that what is being counted is visiting flowers, instead of traps that record flying insect and do not guarantee their role as pollinators (Hutchinson et al., 2022). For each sampling date, data on pollinator abundance visiting flowers were collected from 3 plots (2 m diameter) for each vineyard parcel, chosen randomly in the rows and changed after each sampling day, but always within the same parcel. Given the configuration of vineyard parcels in Valtellina, all plots within a parcel are subject to similar ranges of conditions in terms of aspect, slope, and vineyard arrangement and management. Pollinator abundance was evaluated counting the pollinators found on the flowers during a 5-minute observation period for each plot (Fig. A2 in Appendix), as in Granata et al. (2023). Only insects belonging to macro-groups as honeybees, wild bees and bumblebees, hoverflies and butterflies were counted. In addition, we carried out net samplings during walks across the vineyard for 20 minutes (free transects), targeted on wild bees (including bumblebees) and hoverflies found on flowers, as these are the main pollinator groups used in agricultural studies (Sommaggio and Burgio, 2014; Tommasi et al., 2021) and they are groups used as indicators of human pressures also in many landscape contexts (Biella et al., 2022b). We preferred active transect walks instead of traps because the latter often exclude medium and large pollinators (*i.e.*, size

Table 2 –
Diversity models: estimate, standard error and marginal and conditional R^2 of the final models, empty cells are for variables that did not pass the selection procedure based on delta AICc.

	Wild bee and syrphid diversity	Wild bee diversity	Syrphid diversity
	Estimate ± SE	Estimate ± SE	Estimate ± SE
Intercept	1.275 ± 0.046	1.067 ± 0.06	0.463 ± 0.083
Weed cover (management)	0.151 ± 0.052	0.076 ± 0.052	
Weed Shannon diversity (management)	0.137 ± 0.052	0.108 ± 0.057	
Weed height (management)		0.121 ± 0.056	0.103 ± 0.068
Organic farming (management)			−0.222 ± 0.193
Sampling date (environment)	−0.215 ± 0.064	−0.25 ± 0.094	0.181 ± 0.057
Time (1ˆorder) (environment)		−0.205 ± 0.069	
Time (2ˆorder) (environment)		−0.118 ± 0.057	
Temperature (1ˆorder) (environment)	0.163 ± 0.069	0.358 ± 0.08	−0.222 ± 0.06
Temperature (2ˆorder) (environment)	−0.082 ± 0.046	−0.086 ± 0.08	−0.058 ± 0.06
Wind intensity (=1) (environment)			
Wind intensity (=2) (environment)			
Marginal / Conditional R2	0.351 / 0.369	0.648 ± 0.648	0.198 ± 0.398

bias, Prendergast et al., 2020), the former allow recording insects visiting flowers and exclude non-pollinating ones. The captured insects were placed in a solution of ethanol 80 % v/v and stored in a freezer at −21°C until subsequent analyses. Pollinator sampling in plots and along transects was not particularly standardized in terms of time of day and weather conditions because the aims of the study specifically included a test on those factors, although the sampling occurred during times of high insect activities and in sunny days (no rainy days or times after rains) in order to avoid limitations from unfavourable weather conditions.

2.2. *Management and environmental data collection*

Within each plot, environmental and management parameters were recorded. Environmental variables measured during the pollinator sampling were: sampling date, sampling time of day, air temperature in °C (measured with an environmental thermometer at 1.5 m above ground) and wind intensity estimated by the operator on a scale from 0 to 2 (*i.e.*, 0 = no wind; 1 = weak; 2 = moderate wind).

The management was described through a number of parameters: (i) the mean height of the ground vegetation (where low vegetation is associated with frequent mowing) after measuring 5 different spots per plot; (ii) the cover of the ground vegetation (ground percentage occupied by vegetation, estimated visually, where high bare ground signals the use of frequent mechanical mowing); (iii) the composition of the ground vegetation, measured as the abundance of each plant species in terms of number of stalks in the observation plots to derive the Shannon index (using the "Diversity Indices" command of Past v4.13; Hammer et al., 2001); (iv) organic or conventional farming regime (by asking farmers whether their vineyards were under certified organic farming during the sampling time).

2.3. *DNA barcoding, pollinator diversity and community composition*

Collected wild bees and hoverflies were identified by means of a morphological examination and DNA barcoding. Firstly, specimens were sorted in morphospecies (*i.e.*, after careful scrutiny, the samples with very similar morphological features are grouped together) and then these were taxonomically identified with DNA barcoding, by analysing a middle leg from 1–3 specimens for each morphospecies, processed following established protocols (Biella et al., 2022a; Cornalba et al., 2024). Starting from gDNA extracted from a leg, the standard 5'-end COI mitochondrial barcode region was amplified (658 bp) using primers LCO1490/HCO2198, then sequenced and validated (more details are provided in Text A1 in Appendix). The sequence match parameters and the Neighbour Joining trees of similar sequences obtained from the Identification Engine Tool of BOLD Systems (https://www.boldsystems.org/index.php/IDS_OpenIdEngine) were carefully evaluated and the species identity assigned based on the best match scores and the congruence of matching sequences belonging to a coherent clade in the Neighbour Joining tree (Table A1 in Appendix); in case of ambiguous outputs, a careful morphological examination was conducted to reach a final identification.

After species identification, the pollinator diversity data were obtained using the *Diversity Indices* function of the Past software v4.13 to calculate the Shannon diversity index at the site level for each sampling date (Hammer et al., 2001) by aggregating bees and hoverflies species that could describe their overall diversity, and also separately of each of these two groups in other to gain a more specific insight. Moreover, a species by site matrix was built by keeping sampling dates independent, to facilitate relating species abundances and their functional traits to environmental variables recorded at the same time. The functional traits of bees and hoverflies were collated describing body size (small < 8 mm, 7 mm < medium < 12 mm and large > 11 mm), larval feeding habit (pollinivorous, detritivorous, carnivorous) and egg-laying substrate (in the soil, on plants, in debris, in cavities within materials); trait data were taken from the published literature (Scheuchl and Willner, 2016; Speight et al., 2015; Westrich, 2019).

2.4. *Statistical analysis*

Pollinator data were analysed with regression models evaluating the effect of the environmental and management parameters by means of variable selection methods. The procedure was performed on (i) pollinator total abundance, where the total sums of pollinators are considered, (ii) separately, the abundances of each pollinator group (i.e., honey bees, wild bees including bumblebees, butterflies, hoverflies), (iii) the diversity index of both wild bees and hoverflies together, (iv) separately, the diversity indices of wild bees and of hoverflies. The models were fitted with generalized linear mixed regressions models. The abundance data from individual plots per date were fit with a negative binomial error distribution and the site identity as grouping (random) factor; the diversity data for each parcel at each sampling day were fit with a Gaussian error distribution and site identity was kept as grouping (random) factor. For the analyses on bee and hoverfly diversity indices, a zero-inflation parameter on the intercept was necessarily added to the models. The function *glmmTMB* was used for these analyses (Brooks et al., 2017). Predictors of the environment influencing pollinator activity were: the sampling date to consider seasonal environmental changes, the sampling time of day to consider variations within the day, the sampling air temperature and wind intensity category to describe physical properties of the environment at the moment of sampling; The variables describing the management were: mean height of the ground vegetation, the cover of the ground vegetation, the flower diversity of the ground vegetation, organic or conventional farming regime. Collinearity was tested with the VIF index, using the function *vif* in the *CAR* package in R (Fox and Weisber, 2019), with a threshold collinearity coefficient of 5 (if VIF was greater than 5, only one of the

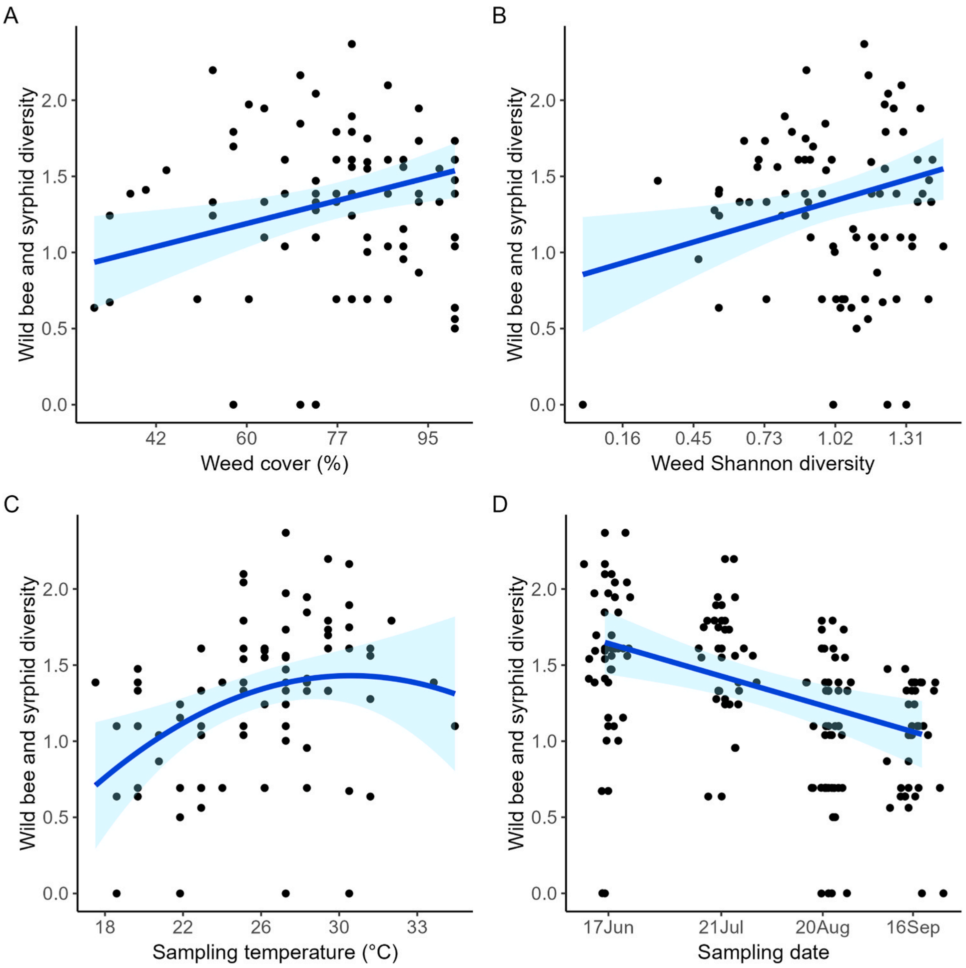

Fig. 3. Pollinator diversity on flowers based on wild bee and hoverfly data together in relation to selected management and environment variables describing (a) the ground vegetation cover, (b) the Shannon diversity index of ground vegetation, (c) the air temperature at the moment of sampling and (d) the sampling date. Predicted lines and confidence intervals from regression models with variable selection procedures are shown (the unselected variables are not plotted).

collinear parameters would be kept). Model and variable selection were performed following the strategy of Granata et al. (2023), using the function dredge in the *MuMIn* package (Barton, 2023), which evaluates all possible models based on the Akaike Information Criterion corrected for small sample size (AICc). After excluding the models containing non-informative parameters that does not explain enough variation to allow their inclusion (Arnold, 2010), it select models within a delta AICc less than 2 compared to the best supported model. If more than one model fulfilled such criteria, an average model was fitted using the function *model.avg.*

Bee and hoverfly community data were analysed by means of a fourth-corner analysis in order to relate the species abundances in each site at each date with the environmental and management variables measured at the same times and the taxa traits. The fourth-corner analysis was conducted by fitting a sequence of generalized linear models with negative binomial error distribution and a model selection procedure based on adding a LASSO penalty algorithm (Least Absolute Shrinkage and Selection Operator) to find the model with the set of variables that minimized the BIC. The analyses were run with the *traitglm* function (with) in the *mvabund* package in R.

3. Results

During the plot observations 489 butterflies, 427 honeybees, 295 hoverflies and 809 wild bees were counted, for a total of 2020 pollinators belonging to any of these groups; transect walks retrieved 76 species, belonging to 58 wild bee and 18 hoverfly species.

3.1. Abundance models

In the analyses considering the total abundance of pollinators, the informative management variables were the ground vegetation cover and the organic farming, with a rather strong positive influence and a weak negative effect, respectively (Table 1, Fig. 1). The most important environmental variables were the sampling temperature, the sampling time of day, both with positive (hump-shaped) quadratic relationships (*i.e.*, a positive effect of intermediate values), and the wind speed, the latter showing a weak negative effect.

The analyses focused on each pollinator-group abundance demonstrated that while some factors were important to most of the pollinator groups, other variables influenced only single groups (Table 1, Fig. 2). Butterflies were positively influenced by the ground vegetation cover and flower diversity, sampling date, time of day (quadratically) and sampling temperature (quadratically), with a negative effect of organic farming. Honeybees were positively related to the ground vegetation cover and sampling date, quadratically to the time of day and sampling temperature, and negatively to the moderate winds, ground vegetation flower diversity and height. Syrphids were positively influenced by the ground vegetation flower diversity, quadratically by the time of day, and, with negative effects, by organic farming, wind speed and sampling date. Wild bees were positively influenced by the ground vegetation cover and quadratically by the sampling temperature, and negatively by

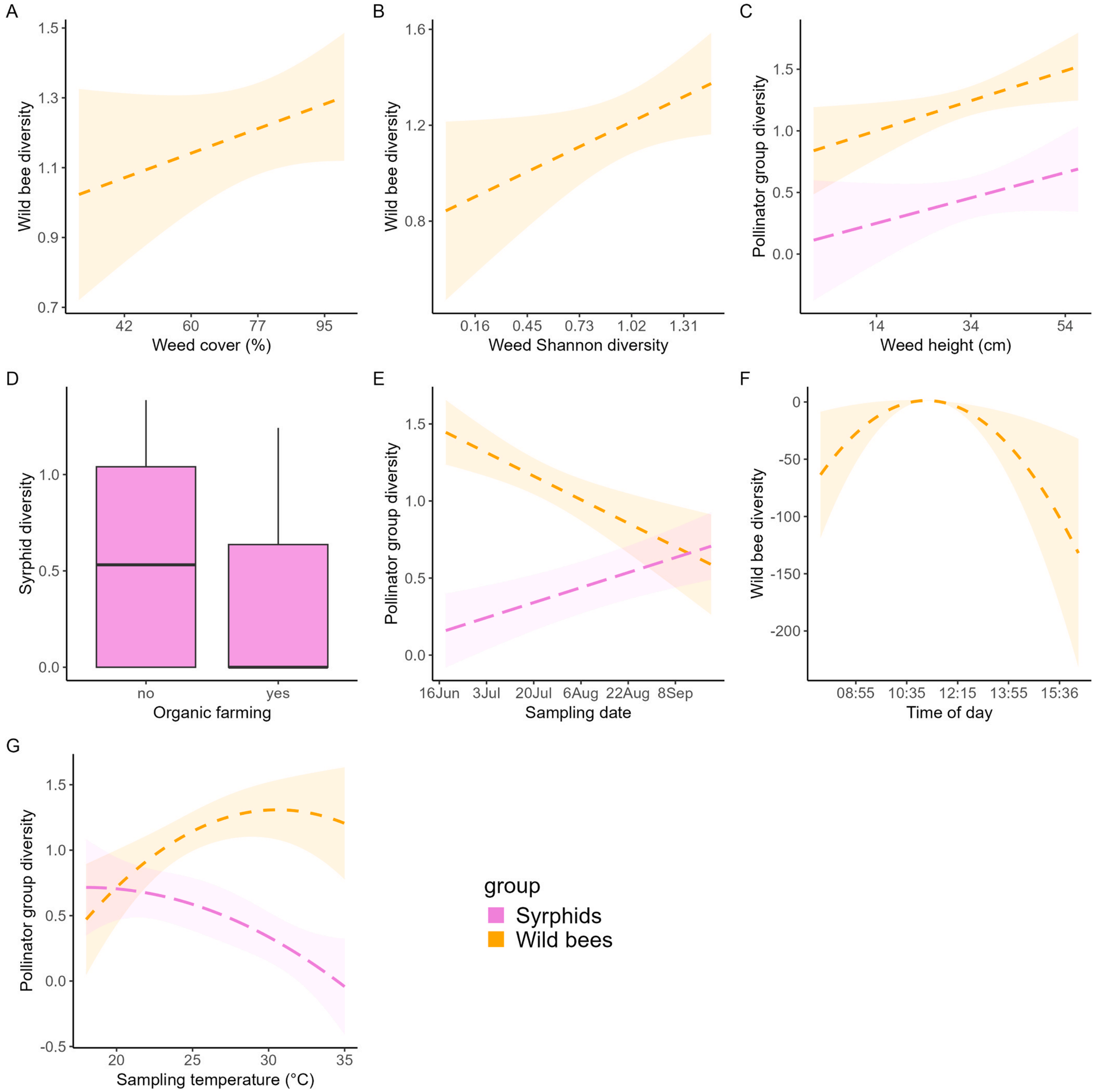

Fig. 4. Diversity indices of wild bees and of hoverflies modelled separately in relation to selected management and environment variables describing (a) the ground vegetation cover, (b) the Shannon diversity index of ground vegetation, (c) the height of the ground vegetation, (d) organic/non-organic farming regime, (e) the sampling date, (f) the time of sampling, (g) the air temperature at the moment of sampling. Predicted lines and confidence intervals from regression models with variable selection procedures are shown (the unselected variables are not plotted).

the wind speed, sampling date and time of day.

3.2. Bee and hoverflies diversity

The analysis of pollinator diversity based on both wild bee and syrphid data together demonstrated a positive influence of the management variables promoting the ground vegetation cover and flower diversity, which were related to a linear increase of pollinator diversity (Table 2, Fig. 3). In parallel, air temperature resulted to be quadratically related to pollinator diversity, indicating a slight decrease only at high temperatures, while sampling date caused a linear decline.

When analysed separately, wild bee diversity confirmed most of trends found with the pollinator diversity, showing a positive linear relationship with the weed cover, height and diversity; A slight quadratic relationship was detected with the air temperature and a linear decrease with the time of day and sampling date (Fig. 4). Syrphid diversity depended on the management described by the weed height in a positive way, while it decreased with the organic farming; A linear decline is found with the air temperature and a linear increase is detected with the sampling date (Fig. 4).

3.3. Bee and hoverfly community composition

The best model selected several variables influencing the community

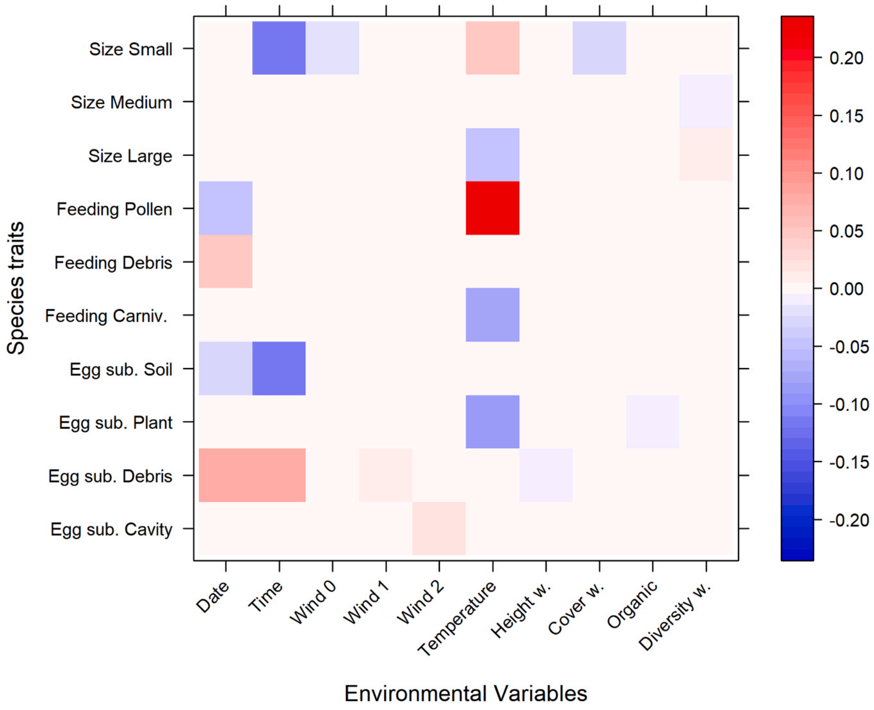

Fig. 5. Pollinator community composition and species trait variation explained by environmental or management variables from a fourth-corner model. Colors indicate the intensity and sign of the fourth-corner coefficients. "sub." stands for "substrate", "Wind 0", "Wind 1", "Wind 2" refers to the wind speed scale (from no to moderate wind, respectively). "w." stands for "weed" and "Carniv." for "carnivorous".

composition. The environmental factors of the sampling date, time and temperature were related to many traits (*i.e.*, 3–5 traits), while management variables were associated to just a few traits and usually with lower fourth-corner coefficients (Fig. 5).

4. Discussion

In this study, we investigated the effects of management and environment on pollinators occurring in vineyards, in a study area of Northern Italy. We focused on pollinator abundance, diversity and community composition. We detected effects shared across pollinator groups from both the environmental and management parameters, but also different responses and idiosyncrasies between pollinator groups and species traits. These differential responses must be considered when planning and applying agricultural and species conservation practices in order to minimize the impacts of vineyard cultivation on pollinators.

4.1. The role of farm management

Farm management influences the vegetation in terms of cover, height and plant diversity of the herbaceous layer (Winter et al., 2018). In our study, the cover of the ground vegetation and its flower diversity played important roles on the abundance and diversity of pollinators, in most cases with strong positive linear relationships. Results of a similar type were observed in vineyards and also in other agricultural areas (Granata et al., 2023; Tommasi et al., 2021). This relationship is due to the fact that both the cover and the flower diversity of ground vegetation are proxies of flower resource availability, a crucial parameter for pollinators (Ollerton, 2017), even at the level of their nutritional needs. Another important parameter is weed height, that was linearly related to bee and hoverfly diversities but uninfluential for their abundances. The height of herbaceous vegetation is an emerging aspect that is often recorded as a meaningful parameter for pollinator diversity as well as for other beneficial arthropods, even in other land use types like urban green areas (Proske et al., 2022). This clearly means that field practices devoted to containing and reducing height, cover and flower diversity of ground vegetation have direct negative effects on pollinators (Griffiths-Lee et al., 2022).

Reasons why pollinators increase where the management type improves the conditions of the ground vegetation could involve the functional traits and life cycles of the single pollinator groups. In fact, a previous study shows that grassland strips in agricultural context increases the functional diversity of the pollinator guild (Maas et al., 2021). In line with that, we found positive relationships with some specific pollinator traits. The small body sizes were promoted by the weed cover, maybe because it assures more shelter and hiding places (Dennis et al., 1998); the abundance of medium sizes increased by the flower diversity, likely because medium size bees can access many flower sizes (Dafni and Kevan, 1997); the weed height favouring hoverflies laying in watery debris, probably because weed height promotes moisture and litter (Deutsch et al., 2010). Thus, the ensemble of vegetation features between rows should be managed to promote wilderness and ecological intensification of vineyards, sustaining more abundant and functionally diverse pollinators.

Organic farming is usually considered and promoted as a less impacting farming regime compared to conventional ones (Hole et al., 2005). However, our results show that it causes declines in the overall pollinator abundances, and specifically also for hoverflies and butterflies, while no effect is found in honeybees and wild bees. Likewise to our study, negative responses on pollinator abundance to organic farming was previously detected in Southwestern France (Ostandie et al., 2021), but vineyard organic farming was not significantly related to pollinator presence in some other cases (Brittain et al., 2010); several studies show that bees are not responsive to organic farming in vineyards (Kehinde et al., 2018), which is consistent with the absence of responses in our study for this insect group. For hoverflies and butterflies, the literature is less concordant with our results. On hoverflies, no effect of organic farming was found in a study in Italy (Sommaggio and Burgio, 2014), while we found a negative abundance and diversity trend for this taxon. Conversely, while we detected less butterflies, a study found a positive effect of organic farming on butterflies, but this was actually mediated by a higher diversity of flowers in those study sites (Puig-Montserrat et al., 2017). Similarly, another study found a role of inter-row alternate management rather than organic farming alone on butterflies (Brambilla and Gatti, 2022). Reasons underlying the differential responses to organic regime across studies and pollinator groups are still to be fully understood, but it is probable that organic regimes correlate with other management practices that in turn may favour some pollinators or limit other ones in crops under this regime.

4.2. *The role of environmental parameters*

Abiotic and temporal parameters, such as the air temperature, wind intensity, date and time of sampling, were the key drivers of pollinator abundances and diversity. While bee and hoverfly abundance and diversity declined along the summer season, butterfly and honeybees abundance increased. This pattern is generally concordant with the known seasonal trends and the life cycles of insects studied here. For instance, it is known that many solitary insects avoid the summer heat while eusocial ones like the honeybees increase with increasing colony sizes over time, and this trend is also confirmed by the literature in other Italian vineyards (Brambilla and Gatti, 2022; Granata et al., 2023). Community composition analysis highlights that seasonal progression also influences a number of functional traits. For instance, it correlates with a decline in hoverflies feeding and laying in debris and increasing pollinators nesting in soils and with small sizes. This analysis clarifies that seasonal variation in the total or group abundance and diversity could be related to species abundance trends over time, positively or negatively affected by the seasonal patterns of the environmental conditions.

Pollinator activity depended also on wind and temperatures. Pollinators declined with wind intensity, which is known to affect pollinator movements (Vicens and Bosch, 2000). A positive, yet quadratic, relationship was detected with the air temperature in most pollinator groups in terms of their abundances and diversities. This trend is consistent with the location of the study areas and the time of sampling: the sites are on south-facing slopes that during sunny days can become very warm, hence decreasing pollinator presence for physiological reasons. Interestingly, this is consistent with declining trends of bee and hoverfly abundances recorded in areas subjected to particularly warm climates at a regional scale (Biella et al., 2022b). Therefore, even at the daily and seasonal level, pollinators tend to avoid hours and days where the environmental conditions are thermally harsher, which could be informative for farm management practices for example for the timing of agricultural treatments.

In spite of the general trends with temperature and time, when comparing single groups, the predicted regression peaks are not overlapping, showing that the responses of the single groups to the same parameters (*i.e.*, temperature, time) resulted in slightly idiosyncratic responses. Once again, the community composition analysis indicates that this could be mediated by the relative variations in species abundances with different traits: temperature correlates with increasing plant or soil egg-laying species and large ones, while decreasing pollen feeders and small sizes. These specific responses clarify that promoting pollinators in agricultural lands cannot ignore the requirements of single pollinator groups and that general cross-taxonomic guidelines should be integrated with actions tailored on specific insect groups.

4.3. *Good practices to support pollinator diversity*

To support pollinators in agricultural areas and in vineyards in particular, adequate strategies should be regularly applied in single farms, and upscaled to the regional level (e.g., across consortia of producers) in order to reach a significant positive impact. The strategies should strongly acknowledge the ecology of pollinating insects, at least in terms of ensuring the availability of nutritional sources and shelters to sustain their populations in the face of the potentially impacting farming practices (Kevan et al., 1990).

From our results, some management practices emerged as very relevant to pollinators in the vineyards and a larger adoption of those positive aspects would further support pollinators at a broader scale. Farm management should promote the wilderness of the ground vegetation and more systematically maintain the herbaceous ground cover, flower diversity and tall herbs. This can be easily applied between vine rows and even further boosted by dedicating some areas to sown wildflowers (Griffiths-Lee et al., 2022) or by alternating those rows subject to mowing (Brambilla and Gatti, 2022).

Apart from the management alone, the responses of pollinators to weather conditions that we detected in our study highlight that biodiversity strategies cannot ignore the abiotic parameters. For instance, understanding the environmental conditions when pollinators are less active could provide precious additional information on the timing of application of actions possibly harming pollinators (e.g. pesticides, tillage, mowing). In other words, from the patterns observed in our abundance and diversity data, it could make sense to apply harmful treatments later during the day and later during the season so that to likely minimize the impact on pollinators and other nontarget animals, compared to when they are applied earlier in the morning and in the season: it is well known that timing of application could be crucial to avoid side effects on beneficial organisms (Zhang et al., 2023). This is very relevant for the ecological vineyard management.

5. Conclusions

In this study, we found evidence that the management strategies, the temporal and abiotic conditions determine the impact of vineyard farming on pollinators. Here, we show that simple elements promoting ground vegetation positively correlate to pollinator presence, leading to actions that farmers could easily apply in their vineyards. Furthermore, the differential responses found in different pollinator groups indicate that such a biological complexity should be considered to find effective ways to support pollinators and decrease vineyard impact, moving towards more sustainable agricultural practices. Therefore, our study provides a basis of knowledge for the application of the principles of ecological intensification in the context of agricultural production (MacLaren et al., 2022; Rossing et al., 2021).

CRediT authorship contribution statement

Paolo Biella: Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Fausto Ramazzotti:** Writing – review & editing, Investigation, Data curation. **Giulia Parolo:** Writing – review & editing, Investigation, Data curation. **Andrea Galimberti:** Writing – review & editing, Data curation. **Massimo Labra:** Writing – review & editing, Conceptualization. **Mattia Brambilla:** Writing – review & editing, Methodology, Conceptualization.

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.

Data availability

Data used for this study are available in a public repository (Fighsare) at this link: https://doi.org/10.6084/m9.figshare.26954548.v1.

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Appendix

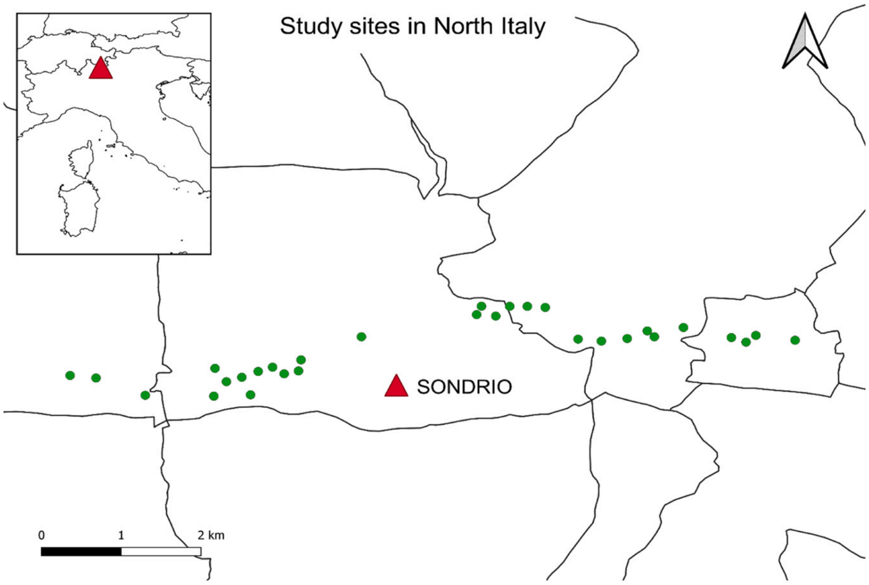

Figure A1 – Sampling sites in Valtellina (North Italy). Although the map is in two dimensions, the sites are actually located in south-facing slopes of steep mountains.

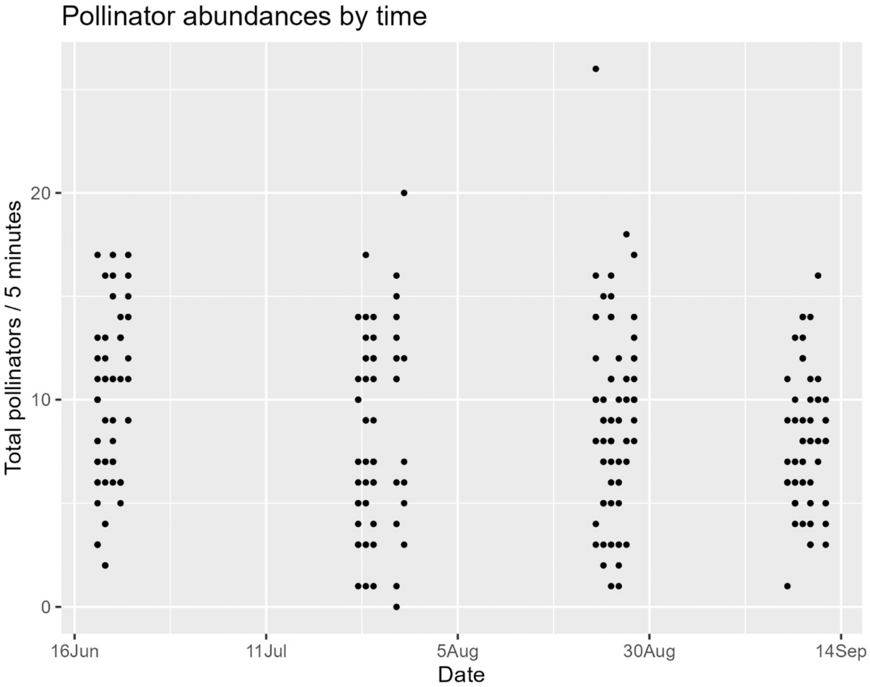

Figure A2 – Total pollinator abundance for each sampling plot in each sampling day. Raw data are obtained from 5-minutes observations plots.

Text A1 – Protocol of DNA barcoding

The extraction of gDNA was carried out following BIO-RAD® InstaGene extraction kit protocol, during which the leg fragments were incubated in a thermomixer with InstaGene® resin at 56°C – 1000 rpm for 50 minutes, followed by a resin inactivation phase of 10 minutes at 96°C – 300 rpm.

Amplification of the DNA barcode region (the 5’ terminal region of the COI mitochondrial gene) was carried out with the EuroClone® Wonder Taq Polymerase and Reaction buffer, using the universal primers LCO1490-HCO2198 (Folmer et al., 1994). Trials were conducted in 20 µL total volume reactions containing 4 µL of 10x Buffer solution (5x WonderTaq reaction Buffer EuroClone®), 1 µL of each primer (10 µM), 0.25 µL Taq polymerase (WonderTaq EuroClone®) and 2 µL of DNA template, under the following conditions: an initial step at 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 60 seconds, 50 °C for 45 seconds, elongation at 72 °C for 60 seconds, and a final extension at 72°C for 7 minutes. Gel electrophoresis run of resulting PCR products were performed on a 2 % agarose gel and visualised under UV light. After purification with the Qiagen® MinElute PCR Purification Kit, the sequencing phase was outsourced to Eurofins Genomics SRL®, which performed it using the Sanger method. After checking for the absence of pseudogenes by converted the sequence into an amino acids using EMBOSS Transeq (https://www.ebi.ac.uk/Tools/st/emboss_transeq/), the sequences was matched against to the ones already present in reference databases of BOLD Systems (https://www.boldsystems.org/).

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 3: 294–299

Table A1 – DNA barcoding results

Table A1 –
Samples identified with DNA barcoding and the taxonomic outputs, matching scores, project BOLD ZPLGP.

Sample code	Genetic identification	BOLD sequence ID	Matching value
G17_4AB	*Andrena fulvago*	ZPLGP025–24	100.00 %
G23_4A	*Andrena intermedia*	ZPLGP038–24	100.00 %
G17_4I	*Andrena thoracica*	ZPLGP026–24	100.00 %
A9_4AA	*Anthidiellum strigatum*	ZPLGP013–24	99.74 %
G24_4AI	*Anthidium manicatum*	ZPLGP040–24	100.00 %
G24_4F1	*Anthidium manicatum*	ZPLGP041–24	100.00 %
L29_4F	*Anthidium manicatum*	ZPLGP083–24	100.00 %
G25_4AA	*Anthidium oblongatum*	ZPLGP042–24	100.00 %
G16_3C	*Bombus argillaceus*	ZPLGP023–24	100.00 %
S15_3D	*Bombus hypnorum*	ZPLGP094–24	100.00 %
A19_3B1	*Bombus pascuorum*	ZPLGP004–24	100.00 %
G3_3A1	*Bombus terrestris*	ZPLGP047–24	100.00 %
L27_4AN	*Ceratina cucurbitina*	ZPLGP081–24	100.00 %
A19_4AJ	*Ceratina cyanea*	ZPLGP005–24	100.00 %
L23_4AJ	*Ceratina cyanea*	ZPLGP079–24	100.00 %
G16_4AE	*Coelioxys elongata*	ZPLGP024–24	100.00 %
S21_4AL	*Colletes halophilus*	ZPLGP095–24	100.00 %
G7_4P	*Colletes similis*	ZPLGP059–24	100.00 %
S3_2D3	*Dasysyrphus albostriatus*	ZPLGP133–24	99.84 %
S7_2D	*Dasysyrphus albostriatus*	ZPLGP135–24	99.85 %
L19_2M	*Episyrphus balteatus*	ZPLGP120–24	100.00 %
A3_2H	*Eristalis tenax*	ZPLGP106–24	100.00 %
G1_2B	*Eristalis tenax*	ZPLGP111–24	100.00 %
G8_4U	*Eucera interrupta*	ZPLGP062–24	100.00 %
G9_4Q	*Eucera interrupta*	ZPLGP063–24	100.00 %
S25_2P1	*Eumerus amoenus*	ZPLGP129–24	99.85 %
S25_2P2	*Eumerus amoenus*	ZPLGP130–24	99.67 %
A12_2J	*Eupeodes corollae*	ZPLGP098–24	100.00 %
G26_2J	*Eupeodes corollae*	ZPLGP115–24	100.00 %
L28_2J	*Eupeodes corollae*	ZPLGP121–24	100.00 %
S15_2J	*Eupeodes corollae*	ZPLGP123–24	100.00 %
S21_2J1	*Eupeodes luniger*	ZPLGP128–24	100.00 %
S27_2J1	*Eupeodes luniger*	ZPLGP132–24	100.00 %
S9_2J	*Eupeodes luniger*	ZPLGP138–24	100.00 %
G10_4V2	*Halictus maculatus*	ZPLGP019–24	99.37 %
G3_4C	*Halictus maculatus*	ZPLGP048–24	99.38 %
L23_4V	*Halictus maculatus*	ZPLGP080–24	99.39 %
G18_4D	*Halictus rubicundus*	ZPLGP029–24	99.64 %
G2_4E	*Halictus sexcintus*	ZPLGP037–24	100.00 %
G19_4D	*Halictus simplex*	ZPLGP033–24	100.00 %
G6_4D	*Halictus simplex*	ZPLGP055–24	100.00 %
G8_4R	*Halictus simplex*	ZPLGP060–24	100.00 %
L21_4V1	*Halictus simplex*	ZPLGP076–24	99.34 %
L6_4N1	*Halictus subauratus*	ZPLGP090–24	100.00 %
A22_4AK	*Heriades rubicola*	ZPLGP008–24	100.00 %
G6_4M	*Heriades rubicola*	ZPLGP056–24	100.00 %
L21_4AH	*Heriades rubicola*	ZPLGP074–24	100.00 %
L29_4H	*Heriades rubicola*	ZPLGP084–24	100.00 %
G12_4Z	*Hoplitis leucomelana*	ZPLGP022–24	99.84 %
G25_4AK	*Hoplitis leucumelana*	ZPLGP043–24	100.00 %
L11_4O	*Hylaeus angustatus*	ZPLGP065–24	100.00 %
A8_4O	*Hylaeus gibbus*	ZPLGP012–24	100.00 %
G25_4L2	*Hylaeus gibbus*	ZPLGP044–24	100.00 %
G7_4L	*Hylaeus gibbus*	ZPLGP057–24	100.00 %
G7_4O	*Hylaeus imparilis*	ZPLGP058–24	99.84 %
A19_4L1	*Hylaeus punctatus*	ZPLGP006–24	100.00 %
S7_2O1	*Lapposyrphus lapponicus*	ZPLGP136–24	100.00 %

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Table A1 – (*continued*)

Sample code	Genetic identification	BOLD sequence ID	Matching value
G10_4J4	*Lasioglossum aeratum*	ZPLGP018–24	100.00 %
L12_4H	*Lasioglossum albipes*	ZPLGP066–24	99.50 %
A18_4A	*Lasioglossum brevicorne*	ZPLGP003–24	99.82 %
L11_4J1	*Lasioglossum brevicorne*	ZPLGP064–24	100.00 %
L9_4T	*Lasioglossum brevicorne*	ZPLGP093–24	99.84 %
S23_4R	*Lasioglossum brevicorne*	ZPLGP096–24	100.00 %
L14_4R	*Lasioglossum calceatum*	ZPLGP069–24	97.54 %
L4_4X	*Lasioglossum calceatum*	ZPLGP087–24	97.47 %
A29_4A	*Lasioglossum clypeare*	ZPLGP010–24	99.74 %
G19_4AF	*Lasioglossum costulatum*	ZPLGP031–24	100.00 %
L15_4P	*Lasioglossum costulatum*	ZPLGP070–24	99.84 %
A12_4AF	*Lasioglossum discum*	ZPLGP001–24	99.56 %
A9_4AF	*Lasioglossum leucozonium*	ZPLGP014–24	100.00 %
L16_4AF	*Lasioglossum leucozonium*	ZPLGP072–24	98.76 %
L4_4D	*Lasioglossum leucozonium*	ZPLGP086–24	98.72 %
L6_4Y	*Lasioglossum leucozonium*	ZPLGP091–24	98.67 %
L13_4T2	*Lasioglossum morio*	ZPLGP068–24	100.00 %
L15_4T1	*Lasioglossum morio*	ZPLGP071–24	100.00 %
A9_4H	*Lasioglossum nigripes*	ZPLGP015–24	98.58 %
S27_4E	*Lasioglossum nigripes*	ZPLGP097–24	98.72 %
G11_4A	*Lasioglossum nitidulum*	ZPLGP021–24	100.00 %
L12_4T1	*Lasioglossum nitidulum*	ZPLGP067–24	100.00 %
G19_4A	*Lasioglossum pauxillum*	ZPLGP030–24	100.00 %
G27_4M	*Lasioglossum pauxillum*	ZPLGP046–24	100.00 %
G30_4A1	*Lasioglossum pauxillum*	ZPLGP052–24	100.00 %
L21_4J	*Lasioglossum pauxillum*	ZPLGP075–24	100.00 %
G10_4J3	*Lasioglossum politum*	ZPLGP017–24	99.84 %
G17_4J	*Lasioglossum politum*	ZPLGP027–24	99.84 %
G30_4A2	*Lasioglossum politum*	ZPLGP053–24	99.79 %
L27_4T1	*Lasioglossum semilucens*	ZPLGP082–24	100.00 %
L29_4T	*Lasioglossum setolosum*	ZPLGP085–24	100.00 %
L22_4J1	*Lasioglossum villosulum*	ZPLGP077–24	100.00 %
L19_4AF	*Lasioglossum zonulum*	ZPLGP073–24	100.00 %
A2_4AH	*Megachile centuncularis*	ZPLGP007–24	98.40 %
A29_4AG	*Megachile melanopyga*	ZPLGP011–24	100.00 %
G24_4AH	*Megachile melanopyga*	ZPLGP039–24	100.00 %
L23_4AG	*Megachile melanopyga*	ZPLGP078–24	98.76 %
A28_4K	*Megachile pilidens*	ZPLGP009–24	100.00 %
G19_4AG	*Megachile rotundata*	ZPLGP032–24	99.84 %
G27_4K	*Megachile rotundata*	ZPLGP045–24	99.84 %
G5_4K	*Megachile rotundata*	ZPLGP054–24	100.00 %
A30_2Q	*Myathropa florea*	ZPLGP107–24	99.85 %
G8_4S1	*Nomia diversipes*	ZPLGP061–24	100.00 %
L6_4AC	*Nomia diversipes*	ZPLGP089–24	100.00 %
G10_4W	*Osmia aurulenta*	ZPLGP020–24	100.00 %
L9_4AM	*Osmia caerulescens*	ZPLGP092–24	100.00 %
G1_4C2	*Panurgus calcaratus*	ZPLGP016–24	99.53 %
G2_4B	*Panurgus calcaratus*	ZPLGP036–24	99.52 %
G19_2k	*Paragus bicolor*	ZPLGP103–24	99.48 %
A22_2L	*Paragus haemorrhous*	ZPLGP112–24	100.00 %
S12_2G	*Paragus haemorrhous*	ZPLGP116–24	100.00 %
S26_2G	*Paragus haemorrhous*	ZPLGP122–24	100.00 %
S3_2G	*Paragus haemorrhous*	ZPLGP131–24	100.00 %
G10_2G	*Paragus haemorrous*	ZPLGP134–24	99.69 %
G26_2L	*Paragus haemorrous*	ZPLGP114–24	100.00 %
G8_2F	*Paragus quadrifasciatus*	ZPLGP119–24	99.84 %
G3_4G	*Pasites maculatus*	ZPLGP050–24	99.69 %
G3_2C	*Physocephala vittata*	ZPLGP117–24	98.62 %
S18_2P	*Platycheirus sp.*	ZPLGP127–24	100.00 %
G18_4AA	*Pseudoanthidium scapulare*	ZPLGP028–24	100.00 %
L6_4AA	*Pseudoanthidium scapulare*	ZPLGP088–24	98.73 %
G19_4F	*Rhodanthidium septemdentatum*	ZPLGP034–24	99.01 %
G3_4F	*Rhodanthidium septemdentatum*	ZPLGP049–24	99.01 %
G17_2J	*Scaeva selenitica*	ZPLGP099–24	100.00 %
A16_2O	*Scaeva selenitica*	ZPLGP100–24	100.00 %
A16_2O1	*Scaeva selenitica*	ZPLGP101–24	100.00 %
A16_2O2	*Scaeva selenitica*	ZPLGP102–24	100.00 %
A17_2O1	*Scaeva selenitica*	ZPLGP104–24	100.00 %
A24_2O	*Scaeva selenitica*	ZPLGP105–24	100.00 %
A29_2O	*Scaeva selenitica*	ZPLGP109–24	100.00 %
A8_2J	*Scaeva selenitica*	ZPLGP113–24	100.00 %
S15_2O	*Scaeva selenitica*	ZPLGP124–24	100.00 %
S7_2O2	*Scaeva selenitica*	ZPLGP137–24	100.00 %
G1_2A1	*Sphaerophoria scripta*	ZPLGP110–24	100.00 %
G3_2E	*Sphaerophoria scripta*	ZPLGP118–24	100.00 %
G3_4H	*Sphecodes gibbus*	ZPLGP051–24	100.00 %

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Table A1 – (*continued*)

Sample code	Genetic identification	BOLD sequence ID	Matching value
A17_4AO	*Sphecodes niger*	ZPLGP002–24	99.84 %
G19_4H	*Sphecodes puncticeps*	ZPLGP035–24	99.34 %
A5_2N1	*Syritta pipiens*	ZPLGP108–24	99.84 %
S17_2D2	*Syrphus torvus*	ZPLGP126–24	100.00 %
S17_2D1	*Syrphus vitripennis*	ZPLGP125–24	100.00 %

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