



How seasonality, semi-natural habitat cover and compositional landscape heterogeneity affect pollen collection and development of *Apis mellifera* colonies in Mediterranean agro-sylvo-pastoral systems

Alberto Satta · Matteo Lezzeri · Giuseppe Brundu · Ignazio Floris · Nicola Palmieri · Roberto Antonio Pantaleoni · Panagiotis Theodorou · Michelina Pusceddu

Received: 18 April 2023 / Accepted: 16 November 2023 / Published online: 13 February 2024
© The Author(s) 2024

Abstract

Context The reduction in the abundance and diversity of flowering plant species, mainly due to landscape alteration and consequent habitat loss, negatively affects honey bee populations and is the main driver of their decline. To safeguard pollination service and food security, we need to know how landscape characteristics affect the availability of pollen sources and consequently colony performance across seasons.

Objectives We therefore here explore how seasonality, percentage of semi-natural habitat cover and compositional landscape heterogeneity affect the abundance, richness, diversity and composition of pollen collected by honey bees and the development of their colonies in a Mediterranean area where agro-sylvo-pastoral systems are widely spread.

Methods For the purpose of our study, pollen samples were collected over a year from colonies of *Apis mellifera* placed in four sampling sites selected across a gradient of landscape heterogeneity and where semi-natural habitats were predominant at various levels within an agro-sylvo-pastoral zone. Simultaneously, the extension of the sealed brood of the same colonies was periodically evaluated.

Results We found that the amount of pollen collected by honey bees was positively correlated with the percentage of semi-natural habitat cover and that increasing habitat diversity at the landscape level had a significant positive effect on the richness and diversity of pollen collected by honey bees. In addition, season significantly affected the amount, diversity and composition of pollen collected by honey bees. Furthermore, bee colony growth was positively correlated with richness, diversity and amount of pollen collected by honey bees.

Conclusion Our study highlights the importance of an abundant and diverse pollen diet for honey bee colony development, which in agro-sylvo-pastoral systems seems to be ensured by a heterogeneous

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10980-024-01826-y>.

A. Satta (✉) · M. Lezzeri · G. Brundu · I. Floris · N. Palmieri · R. A. Pantaleoni · M. Pusceddu
Department of Agricultural Sciences, University of Sassari, Viale Italia 39A, 07100 Sassari, Italy
e-mail: albsatta@uniss.it

A. Satta · G. Brundu · I. Floris · M. Pusceddu
National Biodiversity Future Centre (NBFC), Piazza Marina 61, 90133 Palermo, Italy

P. Theodorou (✉)
General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Hoher Weg 8, 06120 Halle (Saale), Germany
e-mail: panatheod@gmail.com

P. Theodorou
German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Puschstraße 4, 04103 Leipzig, Germany

landscape dominated by different types of semi-natural habitats.

Keywords Agroforestry · Brood cell number · Honey bees · Land-use · Pollen diversity

Introduction

Pollen provides honey bees (*Apis mellifera*) with proteins, lipids (including essential fatty acids and sterols), vitamins, phenolic compounds, flavonoids and other essential micronutrients (Haydak 1970; Campos et al. 2008). Pollen protein concentration is positively correlated with ovarian development and egg laying (Human et al. 2007), whereas lipids are used for energy and as a structural component of cell membranes (Brodschneider and Crailsheim 2010; Avni et al. 2014). Sterols, obtained exclusively from pollen, are precursors of moulting hormones and, therefore, essential for larval development (Vaudo et al. 2015). Pollen also promotes the production of wax, which is used to make honeycombs, and royal jelly, which is the food of worker and drone larvae until the third day of life and of queens for their entire life (Hepburn 1986; Brodschneider and Crailsheim 2010). Pollen feeding also modifies both individual and social immunocompetence, based on haemocyte concentration, fat body content, and phenoloxidase and glucose oxidase activity (Alaux et al. 2010). In addition, by activating nutrient-sensing and metabolic pathways, pollen positively influences the expression of genes involved in the production of some antimicrobial peptides and affects longevity (Alaux et al. 2011). Furthermore, the access of bees to a polyfloral pollen diet can mitigate the negative impact of pesticides (Schmehl et al. 2014) and the fitness consequences of parasites and pathogens, such as the mite *Varroa destructor* (Annoscia et al. 2017) and the microsporidian *Nosema ceranae* (Di Pasquale et al. 2013). Therefore, the development, productivity, and health of honey bee colonies are strictly dependent on the availability of different pollen sources over time.

The reduction in the abundance and diversity of flowering plant species, mainly due to landscape alteration and consequent habitat loss, negatively affects bee populations due to nutrient deficiencies and is the main driver of their decline (Naug 2009; Pott et al. 2010; Ollerton et al. 2014; Goulson et al.

2015; Millard et al. 2021). In general, intensive agricultural practices do not ensure good health conditions for honey bees, as they can offer limited pollen sources concentrated in short periods of time (Naug 2009; Pott et al. 2010; Ollerton et al. 2014; Goulson et al. 2015; Millard et al. 2021). Therefore, understanding how the abundance and diversity of pollen sources affect honey bee physiology, health and development can help identify stressful or bee-friendly landscapes and point to appropriate management practices to improve pollinator conservation (Alaux et al. 2017). Yet little is known about the effects of landscape heterogeneity on the composition of pollen collected by honey bees. Requier et al. (2015) found that habitat composition in intensive agricultural landscapes influences the pollen diet of honey bees. In their study, honey bees collected pollen mainly from tree species and weeds and not from crops. On the contrary, in an agricultural area near Würzburg (Germany), Danner et al. (2017) found that the amount and diversity of pollen collected by honey bees were not influenced by landscape diversity, because honey bees compensated for the low landscape diversity by increasing foraging distance. In another agricultural area in Germany, characterised by strawberries and oilseed rape crops surrounded by a patchy landscape of arable land, semi-natural habitats, forests and urban areas, Bänisch et al. (2020) documented that honey bees collected pollen mainly from oilseed rape and semi-natural habitats. In a mountainous cultivated area of northern Italy, Malagnini et al. (2022) found that season drove the diversity of pollen collected by honey bees, and landscape composition affected pollen diversity in late summer, when pollen collection is essential for the overwintering of honey bee colonies. The contrasting findings obtained in these studies were probably due to not only the specific landscape contexts (Catford et al. 2022) in which the observations were made, but also the application of different experimental protocols, especially the duration of the monitoring period, the frequency of pollen sample collection, and the size of the study area. Given the importance of such studies to understand the effects of land use management on pollinator insect decline, further observations are needed in habitats different from those investigated so far.

Our study aimed to explore how seasonality, the percentage of semi-natural habitat and compositional

landscape heterogeneity (i.e., diversity of habitat types) affect pollen collection and the development of honey bee colonies in an area in northwest Sardinia (Italy), where land-use systems are characterised mainly by a heterogeneous mosaic of semi-natural habitats, such as forests, shrublands, and rangelands with sparse trees (*dehesas*) intermixed with small tree plantations, olive grooves and vineyards, and large extensive forage crops. These composite and patchy landscapes, known as agro-sylvo-pastoral systems (or agroforestry), are common in the Mediterranean area and their features can differ significantly, depending on the degree of diversification and integration of agricultural practices (Pardini and Nori 2011). In general, these land-use systems are more stable and show a higher species richness compared to intensive agricultural areas, due to higher resource diversity and connectivity that likely promote the survival of honey bees and, more generally, pollinating insects (Sjödin et al. 2008; Bagella et al. 2013; Floris et al. 2016). Within this agro-sylvo-pastoral context, we selected four 2-km radius sampling areas along a gradient of landscape heterogeneity and semi-natural habitat cover, which is reported to offer a greater diversity of floral resources (Odoux et al. 2012; Wratten et al. 2012; Requier et al. 2015). At each sample site, we placed a honey bee apiary to address the following questions: (1) Does seasonality, percentage of semi-natural habitat cover and landscape diversity affect the amount, richness, diversity, and composition of the pollen collected by honey bees? and (2) Because pollen collection can affect the development of bee colonies (Keller 2005a), do landscape diversity and percentage of semi-natural habitat cover impact colony development? We hypothesized that seasonality, landscape diversity and semi-natural habitat cover affect pollen diversity and, therefore, also honey bee colony development.

Methods

Study area, sampling sites, semi-natural habitat cover and landscape diversity

The study was carried out in Logudoro, a region in northwest Sardinia (Italy) that covers approximately 708 km² where agro-sylvo-pastoral land-use systems are very common. Within this area, dominated by

hills of volcanic origin, limestone areas, wide flat land and oak forests that interrupt large rangelands, we selected four sampling sites located in four municipalities (Codrongianos, Florinas, Ittiri and Villanova Monteleone, hereinafter referred to as Villanova) characterised by an area with similar type of management (extensive sheep or cattle breeding) but by a different overall landscape heterogeneity. The longest distance between the sampling sites was approximately 25 km (Codrongianos–Villanova), and, to ensure spatial independence, the shortest distance was 5 km (Codrongianos–Florinas).

In each sampling site, we placed in a private land a small apiary consisting of four honey bee colonies that represented our sampling unit for the pollen analysis. For each sampling site, we estimated the total and percent cover of habitat types within a 2-km radius around each apiary. This distance was considered large enough because in complex ecosystems, with habitat types composed of annual and/or perennial plant species, honey bee activity is typically concentrated within 1–2 km from the apiary (Steffan-Dewenter and Kuhn 2003). The coordinates of the apiaries and the average altitude of the surveyed areas are reported in the supplementary Table S1. For the evaluation of landscape heterogeneity, we used photointerpretation (a polygon vector layer was created by digitizing every type of habitat and land-use) on Google Satellite imagery using QGIS (2023) by applying the same legend as in *Carta della Natura della Sardegna* (Map of Nature of Sardinia) (Camarda et al. 2015). The Google Satellite imagery was cross-checked with the imagery available on the Sardinian Regional cartographic portal (<https://www.sardegnaoportale.it/>), as the availability of multitemporal imagery from different platforms and sensors can provide a more reliable photointerpretation.

Overall, a total number of 26 habitats and land-use types were identified and mapped at the four sampling sites, with a total of 16 semi-natural habitat types and 10 habitat types with a relevant level of anthropisation and often rich in introduced and cultivated plant species (Fig. S1 and Table S2). For the purposes of our study, semi-natural habitats were defined as any habitat within or outside crops containing a community of non-crop plant species (Holland et al. 2017). Codrongianos was dominated by arable land and herbaceous crops (Mediterranean subnitrophilous grass communities

and arable land with unmixed crops grown by low-intensity agricultural methods), with semi-natural habitats covering only 24% of the total area of the sampling site, whereas Villanova was dominated by semi-natural habitats (such as maquis, Tyrrhenian *Quercus suber* forests and Dehesa), covering approximately 95% of the total area. Florinas and Ittiri presented a more intermediate percentage, with 79% and 81% of semi-natural habitats (such as Oleo-lentisc brush and matorral, Mediterranean xeric grasslands and Dehesa), respectively (Table S2).

To quantify compositional landscape heterogeneity, the Shannon habitat diversity index (SHDI) was calculated for each location using the 26 habitat types detected (Nagendra 2002). SHDI was calculated according to the following formula:

$$\text{SHDI} = - \sum_{i=1}^m p_i \log p_i$$

where m is the number of habitat types at each sampling site and p_i is the proportion of each habitat type of all habitat types within each sampling site. The SHDI assesses the heterogeneity of the territory by considering the richness and evenness of each habitat type. A higher value of SHDI indicates greater compositional landscape heterogeneity. The Shannon index was calculated using the R package ‘vegan’ (Oksanen et al. 2022).

Apiary management

At each sampling site, a small apiary, composed of four colonies prepared with *Apis mellifera* queens with a homogeneous genetic profile (sisters), was established 5 months before the beginning of sampling (September 2020). The colonies were maintained in new Dadan-Blatt hives of different colours to reduce drifting. During the experiment, each colony was monitored every 10 days to assess the presence of the queen, brood, and food provisions. In addition, the total sealed brood extension was estimated by using one-sixth of a Dadant-Blatt frame (1880 mm²) as a unit of measure, and the number of capped cells was estimated by multiplying the number of sixth of sealed brood by 780 (Marchetti 1985).

Pollen collection

Each honey bee colony was equipped with a pollen trap placed at the entrance of the hive (Keller 2005b). The pollen traps were activated 48 h before the sampling date to collect enough pollen and a more representative pollen sample (from a variety of floral sources). Pollen was collected passively when foragers passed through the 5 mm diameter holes of the trap, causing the pollen load to fall from their corbicula into a storage drawer below. Samples were collected at each site from February 2021 to October 2021. In February, July, August, September and October, two samples were taken in each month (first and last decade of the month), whereas in March, April, May and June, three samples were taken per month (first, third and last decade of the month). If the check carried out on the honey bee colony revealed that the queen was missing or that a swarming was in progress or had just occurred, that pollen sample was not considered in our analyses. In total, we collected 85 samples in Codrongianos and Villanova, 84 samples in Florinas and 81 samples in Ittiri. All collected samples were weighted and stored at -20 °C until the pollen analysis was performed.

Pollen analysis

From each pollen sample, we weighted 5 g of pollen which were then dissolved in 15 ml of distilled water and vortexed for 5 min. An aliquot of 0.5 ml of the obtained suspension was resuspended in 7.5 ml of distilled water and vortexed again. Two drops of this final suspension were evenly distributed on a 2-cm² surface of a microscope slide using a Pasteur pipette. Once the suspension of water and pollen had dried, we placed a drop of glycerine jelly on the coverslip that covered the sediment. For each pollen sample, we made three microscope slides which were observed under an optical microscope (Kyowa, Biolux 12). A minimum of 700 pollen grains were counted for each microscope slide, but, when necessary, many more pollen grains were counted (up to 1200 grains). Pollen grains were identified at the lowest possible taxonomic level following the methodological standard proposed by the International Commission of Bee Botany (Louveaux et al. 1978; Ricciardelli D'albore 1998; Von der Ohe et al. 2004), the palynological database (PalDat

1997; PollenAtlas 2022; Pollen-Wiki 2022), and the reference collection available at the Palynology Laboratory in the Department of Agricultural Sciences of the University of Sassari (Italy). Because identification of pollen is not always possible at the species level, terms like ‘pollen type’, ‘pollen group’ or ‘pollen form’ are used instead. These terms refer to the type or types of pollen characteristic of a species (e.g., *Trifolium repens*) which, in turn, has pollen grains similar to those of other species of the same genus or a group of similar genera (Persano Oddo and Ricciardelli D’Albore 1989). Similarly, to what we did for landscape diversity, the Shannon pollen diversity index (SPDI) was estimated for each pollen sample as follows:

$$\text{SPDI} = - \sum_{i=1}^m p_i \log p_i$$

where m is the number of pollen types in each sample and p_i is the proportion of each pollen type out of all pollen types within each sample (Lau et al. 2019; Malagnini et al. 2022). Higher values of SPDI reflect higher taxonomic diversity in the pollen sample. We used this index, in addition to pollen richness (number of pollen taxa), to compare taxonomic diversity at temporal and site levels. Shannon index was calculated using the R package ‘vegan’ (Oksanen et al. 2022).

Statistical analysis

To investigate the effects of seasonality, percentage of semi-natural habitat cover and habitat diversity on the amount of pollen collected by bees, we used a linear mixed model (LMM). The monthly average of pollen collected per hive was used as a response variable. Month, percentage of semi-natural habitat cover, and habitat diversity were used as fixed factors and colony nested within locality was used as a random factor. In a separate LMM, we replaced semi-natural habitat cover and habitat diversity with sampling site. Further Tukey’s HSD post hoc tests were performed using the R package ‘multcomp’ (Hothorn et al. 2008) adjusted for multiple comparisons by using the Tukey post-hoc test with false discovery rate (FDR) correction to compare differences in the amount of pollen collected between sampling months and localities.

To investigate the effects of seasonality, percentage of semi-natural habitat cover and habitat diversity (SHDI) on pollen richness and pollen diversity (SPDI), we used a generalized linear mixed model (GLMM) with a negative binomial error structure and a LMM, respectively. Month of sampling, percentage of semi-natural habitat cover, and habitat diversity were used as fixed factors and colony nested within locality was used as a random factor. For the model of pollen richness, the amount of pollen collected by bees was included as an additional predictor to control for sampling effects. Further Tukey’s HSD post hoc tests were performed using the R package ‘multcomp’ (Hothorn et al. 2008) adjusted for multiple comparisons using the false discovery rate (FDR) to compare differences in pollen diversity between the sampling months.

To test for the effect of seasonality, percentage of semi-natural habitat cover, and habitat diversity on pollen composition, we performed a permutational multivariate analysis of variance using the adonis function, with 1000 permutations, as implemented in the R package ‘vegan’ (Oksanen et al. 2022). In the adonis permutational multivariate analysis, the Bray-Curtis distance matrix of pollen species composition was the response variable, with habitat diversity and month as fixed factors. The strata (block) argument was set to locality so that randomizations were constrained to occur within locality and not across all sample sites. In a separate adonis model, we replaced percentage of semi-natural habitat cover and habitat diversity with sampling site without the use of a strata (block) argument. We used non-metric multidimensional scaling (NMDS) within the package ‘vegan’ to visualize the variation in pollen composition.

To investigate the effects of seasonality, percentage of semi-natural habitat cover, habitat diversity, pollen diversity, richness and abundance and pollen species composition on the number of sealed brood cells, we used a GLMM with a negative binomial error structure. Month, percentage of semi-natural habitat cover, habitat diversity, pollen diversity, pollen richness, pollen abundance, and pollen species composition were used as fixed factors and colony was used as a random effect factor nested within locality. We used non-metric multidimensional scaling (NMDS) and the first axis scores (Axis 1:0.281) to represent the pollen species community

composition. We used the Sobel method to test for significant indirect effects of landscape heterogeneity on the number of sealed brood cells (Sobel 1982).

All statistical analyses were performed using the statistical software R 4.2.2 (R Core Team 2021). All mixed model analyses were performed using the package ‘lme4’ (Bates et al. 2015). We checked each model for multicollinearity using variance inflation factors with a cut-off value of 3 (Zuur et al. 2009). All model (GLMM and LMM) assumptions were checked visually using the R package ‘DHARMA’ (Hartig 2022). The model assumptions were satisfied (residuals normally distributed, homogeneity of variance, and no outliers).

Results

Semi-natural habitat cover and habitat diversity

The highest value of semi-natural habitat cover (95%) was observed at the Villanova sampling site followed by Ittiri (81.6%) and Florinas (79.8%). The lowest value of semi-natural cover (24%) was observed in Codrongianos. Regarding the Shannon habitat diversity index, the highest values, i.e., 1.936 and 1.906, were observed at the sampling sites of Florinas

and Ittiri, respectively. The lowest value (1.523) was observed in Codrongianos, whereas an intermediate value (1.690) was found in Villanova (Table S1).

Amount of pollen samples

The weight of the pollen samples ranged from 0.9 to 493.2 g per day throughout the study period. The amount of pollen collected by bees was positively related to the percentage of semi-natural habitat cover (LMM; $\chi^2=7.234$, $df=1$, $P=0.007$; Fig. 1a). Consequently, we observed statistically significant differences in the amount of pollen collected across study areas (LMM; $\chi^2=7.584$, $df=3$, $P=0.05$). We found significantly higher amounts of pollen collected by honey bees in Villanova compared to the other three localities in February (LMM; Tukey post-hoc test with false discovery rate (FDR) correction, $P\leq 0.001$; Fig. S2 and March (LMM; FDR correction, $P\leq 0.001$; Fig. S2). The amount of pollen collected in Florinas was significantly higher than the amount of pollen collected in Codrongianos in February (LMM; Tukey post-hoc test with FDR correction, $z=3.119$, $P=0.010$), and higher than that observed in Ittiri and Codrongianos in April (LMM; Tukey post-hoc test with FDR correction, $P\leq 0.05$; Fig. S2). Finally, the amount of pollen collected in Ittiri was higher than

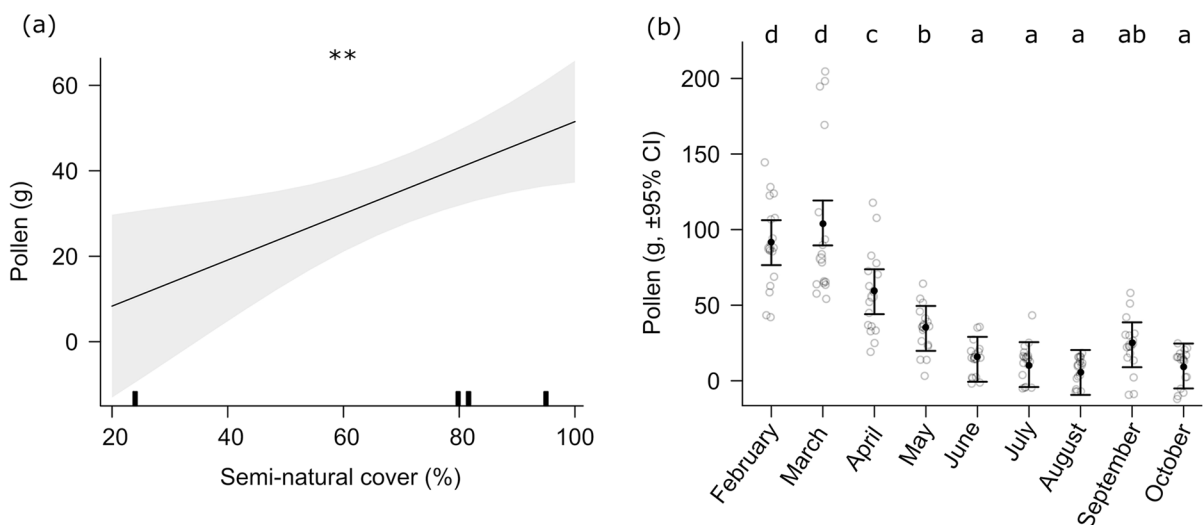


Fig. 1 **a** Relationship between semi-natural habitat cover (%) and amount of pollen (g) collected by a honey bee colony. The plotted line shows the predicted relationship and the shaded area indicates the 95% confidence intervals: ** $P\leq 0.01$.

b Amount of pollen collected per month by a honey bee colony (g; mean \pm 95% CI). Different lowercase letters indicate significant differences between months (LMM; Tukey post-hoc test with false discovery rate (FDR) correction; $P\leq 0.05$)

in Codrongianos in February (LMM; Tukey post-hoc test with FDR correction, $z=2.809$, $P=0.026$; Fig. S2). In contrast, habitat diversity did not affect the amount of pollen collected by honey bees (LMM; $\chi^2=2.507$, $df=1$, $P=0.113$; Fig. S3). Furthermore, we observed a significant seasonal variation in the amount of pollen collected by honey bees (LMM; $\chi^2=251.836$, $df=8$, $P<0.001$; Fig. 1b), with the highest values observed in March (100.49 ± 65.25 SD; Fig. 1b) and the lowest in August (1.625 ± 4.140 SD; Fig. 1b).

Richness and diversity of pollen samples

We analyzed a total of 335 pollen samples in which 168 different pollen taxa (pollen richness) were identified. In the Table S3, all pollen taxa with their monthly relative abundance (%) for each study area are listed. The number of pollen taxa ranged from 113 (Codrongianos) to 131 (Florinas) and was strongly and positively correlated with habitat diversity (GLMM; $\chi^2=3.636$, $df=1$, $P=0.05$; Fig. 2a). Percentage of semi-natural habitat cover did not affect pollen richness (GLMM; $\chi^2=0.379$, $df=1$, $P=0.537$; Fig. S4). Season had a strong effect on pollen species richness (GLMM; $\chi^2=376.066$,

$df=8$, $P<0.001$; Fig. 2b), with significantly higher pollen species richness in February, March, April, and May compared to June, July, August, September and October (GLMM; Tukey post-hoc test with false discovery rate (FDR) correction; $P \leq 0.05$; Fig. 2b). No relationship was found between the amount of pollen collected and pollen species richness (GLMM; $\chi^2=1.007$, $df=1$, $P=0.315$; Fig. S5).

Similarly, pollen diversity was positively related to habitat diversity (LMM; $\chi^2=3.509$, $df=1$, $P=0.061$; Fig. 3a), although it was marginally statistically significant. Percentage of semi-natural habitat cover did not affect pollen diversity (GLMM; $\chi^2=0.190$, $df=1$, $P=0.662$; Fig. S6). Pollen diversity varied significantly across the season (LMM; $\chi^2=205.810$, $df=8$, $P<0.001$; Fig. 3b) with significantly higher pollen diversity in February, March, April, May and August compared to June, July, September and October (LMM; Tukey post-hoc test with false discovery rate (FDR) correction; $P \leq 0.05$; Fig. 3b).

Species composition of pollen samples

Pollen species composition differed significantly between months (adonis; $F=35.52$, $P<0.01$, $R^2=0.503$; Fig. 4a) but was not affected by the

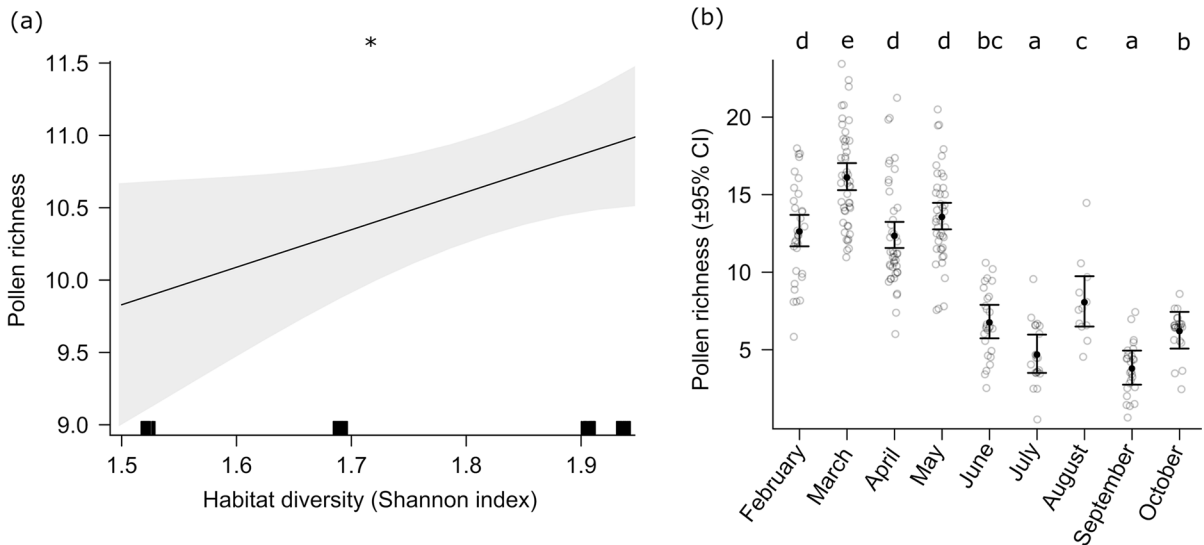


Fig. 2 a Relationship between habitat diversity (Shannon habitat diversity index) and pollen species richness. The plotted line shows the predicted relationship and the shaded area indicates the 95% confidence intervals: * $P \leq 0.05$.

b Pollen species richness (mean \pm 95% CI) per month. Different lowercase letters indicate significant differences between months (LMM; Tukey post-hoc test with false discovery rate (FDR) correction; $P \leq 0.05$)

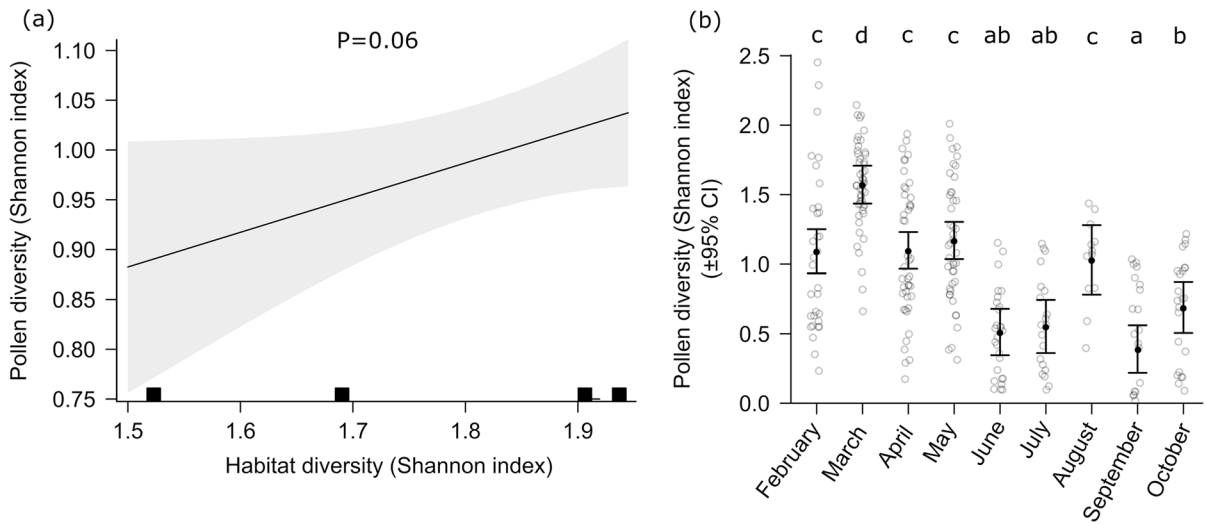


Fig. 3 **a** Relationship between Shannon habitat diversity index and Shannon pollen diversity index. The plotted line shows the predicted relationship and the shaded area indicates the 95% confidence interval. **b** Pollen Shannon diversity (mean \pm 95% CI) per month. Different lowercase letters indicate significant differences between months (LMM; Tukey post-hoc test with false discovery rate (FDR) correction; $P \leq 0.05$)

CI) per month. Different lowercase letters indicate significant differences between months (LMM; Tukey post-hoc test with false discovery rate (FDR) correction; $P \leq 0.05$)

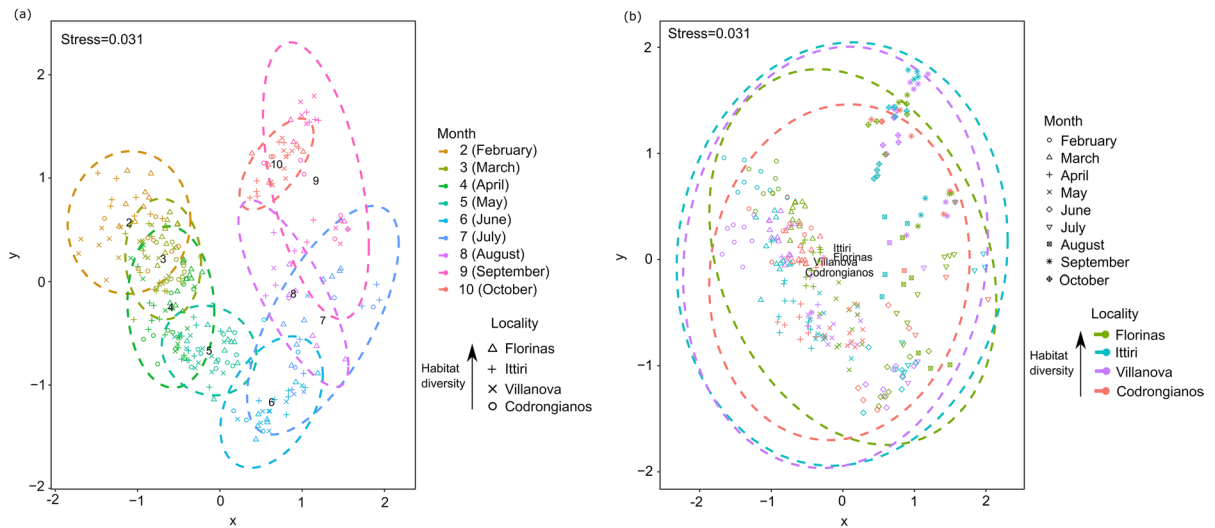


Fig. 4 **a** Non-metric multidimensional scaling (NMDS) ordination of pollen taxa composition across the season. **b** Non-metric multidimensional scaling (NMDS) ordination

of pollen taxa composition across the four sampling sites (habitat diversity). Stress levels are reported in the top left of the figures

percentage of semi-natural habitat cover, habitat diversity or sampling site (adonis; $F=2.246$, $P>0.05$, $R^2=0.008$; $F=1.934$, $P>0.05$, $R^2=0.007$; $F=3.387$, $P>0.05$, $R^2=0.036$, respectively; Fig. 4b). Indeed, considering only the most abundant pollen taxa in each location (Fig. 5), it can be observed that

the main pollen sources were very similar in all 4 localities. Overall, 47 pollen taxa dominated our pollen samples (Fig. 5). In February, pollen from *Rhamnus alaternus* (a dioecious Mediterranean shrub or treelet, which is commonly present in habitat types such as Oleo-lentisc brush and Maquis, was

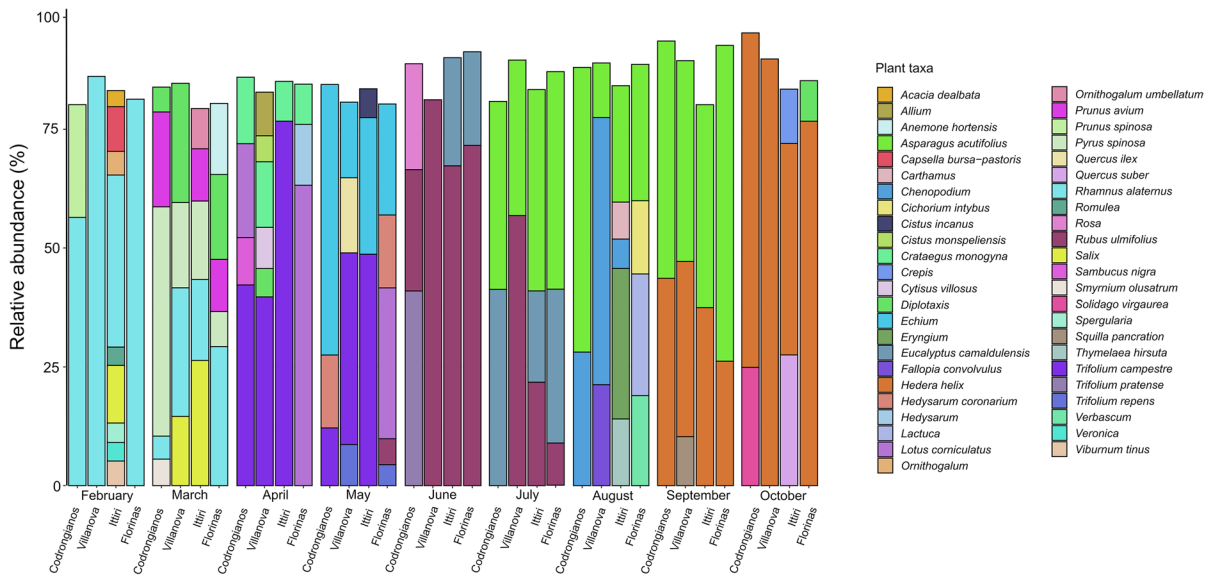


Fig. 5 Summary of the relative abundance of pollen taxa identified in pollen samples. For plotting purposes, we set the relative abundance threshold to 80%. Sites are sorted from low to high habitat diversity (left to right)

the most abundant in all four localities. In March, the composition of the pollen sample was highly variable in all four localities, with the most abundant pollen being from *Rhamnus alaternus*, *Pyrus spinosa*, *Prunus avium*, *Salix* and *Diplotaxis*. In April and May, the most represented pollen were from *Echium* and some spontaneous fodder legumes, such as *Trifolium campestre* and *Lotus corniculatus*. In June, the most important sources of pollen were *Rubus ulmifolius* and *Trifolium pratense*, the latter only in Codrongianos, followed by *Eucalyptus camaldulensis* in Florinas and Ittiri and *Castanea sativa* in Villanova. In July, the most important pollen sources were *Eucalyptus camaldulensis*, *Rubus ulmifolius* and *Asparagus acutifolius*. The records of *Asparagus acutifolius* pollen lasted until September. In August, in addition to pollen from *Asparagus acutifolius*, other dominant pollen were from *Chenopodium*, *Lactuca*, *Fallopia convolvulus*, *Verbascum* and *Thymelaea hirsuta*. In September, besides *Asparagus acutifolius* pollen, we recorded a relevant presence of *Hedera helix* pollen, which became the dominant pollen in all four sampling sites in October, when a secondary presence of *Solidago virgaurea* (an ornamental non-native species), *Diplotaxis* and *Quercus suber* was found also in Codrongianos, Florinas and Ittiri, respectively.

Colony development

The mean number of sealed brood cells in the four sampling sites during the experiment is reported in Fig. 6. The number of sealed brood cells was positively associated with pollen diversity (GLMM; $\chi^2=33.659$, $df=1$, $P<0.001$; Fig. 7a) with the amount of pollen collected by honey bees (GLMM; $\chi^2=18.309$, $df=1$, $P<0.001$; Fig. 7b) and with the percentage of semi-natural habitat cover (GLMM; $\chi^2=3.985$, $df=1$, $P=0.045$; Fig. 8). Accordingly, we found that sampling site (GLMM; $\chi^2=18.467$, $df=3$, $P<0.001$; Fig. 6) and season (GLMM; $\chi^2=106.194$, $df=8$, $P<0.001$; Fig. 6) significantly affected the number of sealed worker brood cells. Considering sampling sites, we found significantly higher amounts of sealed brood in Villanova and Florinas compared to the other two localities in April (GLMM; Tukey post-hoc test with FDR correction; $P\leq 0.01$; Fig. 6) and May (GLMM; Tukey post-hoc test with FDR correction; $P\leq 0.01$; Fig. 6). Significantly higher amounts of sealed brood were found in Villanova compared to Codrongianos in June (GLMM; Tukey post-hoc test with FDR correction; $P\leq 0.01$; Fig. 6).

Due to multicollinearity with pollen Shannon diversity, pollen species richness was not included in the model used above. A separate model with

Fig. 6 Number of sealed brood cells (mean \pm SE) in the four study areas during the experiment

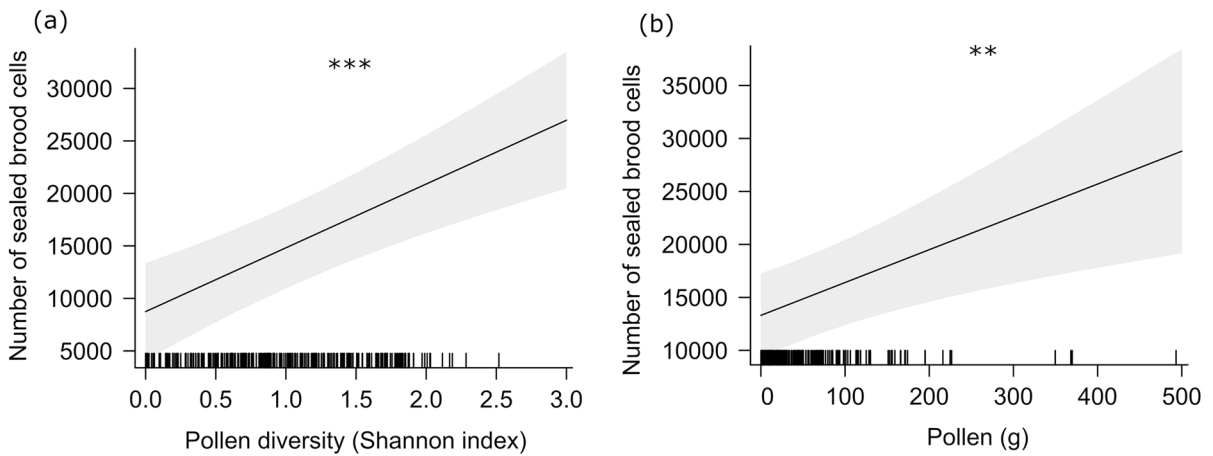
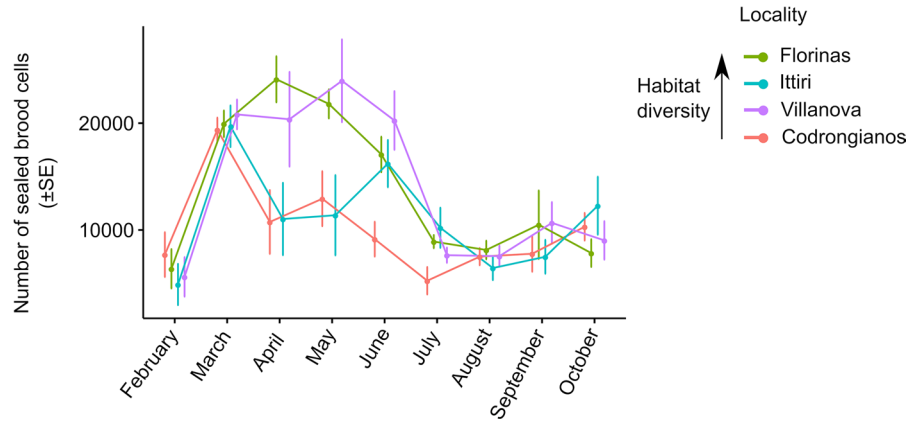


Fig. 7 Relationships between **a** pollen diversity and **b** amount of pollen and number of sealed brood cells. The plotted lines show the predicted relationships and the shaded areas indicate the 95% confidence intervals: *** $P \leq 0.001$; ** $P \leq 0.01$

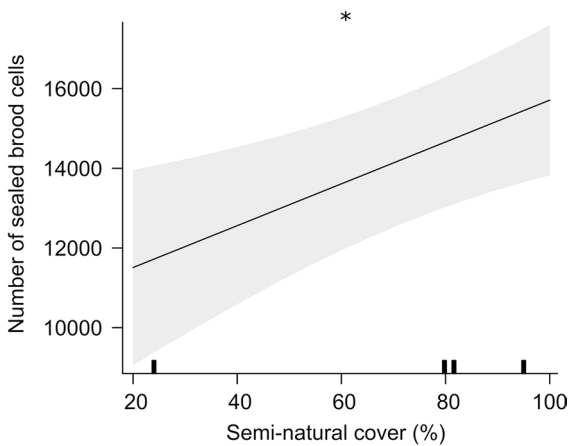


Fig. 8 Relationship between semi-natural habitat cover (%) and number of sealed brood cells. The plotted line shows the predicted relationship and the shaded areas indicate the 95% confidence intervals: * $P \leq 0.05$

pollen species richness revealed a positive influence of pollen richness on the number of sealed worker brood cells (GLMM; $\chi^2 = 8.001$, $df = 1$, $P = 0.004$; Fig. S7). Habitat diversity and pollen composition were not associated with the amount of worker sealed brood cells (GLMM; $P > 0.05$). We found an indirect positive effect of habitat diversity on the number of sealed brood cells mediated by its impact on pollen Shannon diversity (Sobel’s test; 2.109, $P = 0.034$) and pollen species richness (Sobel’s test; 2.049, $P = 0.040$).

Discussion

In this study, we conducted a one-year survey (from February to October) of pollen collected by honey

bees in a Mediterranean agro-sylvo-pastoral area of north-western Sardinia (Italy), to assess the effects of seasonality, percentage of semi-natural habitat cover and compositional landscape heterogeneity (habitat diversity) on the amount, richness and diversity of pollen collected by honey bee colonies and on honey bee colony development.

For the analysis of pollen samples, we used standard melissopalynological techniques instead of molecular approaches based on DNA metabarcoding due to the difficulties of the latter in pollen quantification (Lamb et al. 2019; Piñol et al. 2019; Baksay et al. 2020). As expected, we found strong seasonal patterns in the amount of pollen collected by honey bees, with the highest amount observed during late winter and early spring (especially March). This seasonal difference reflects the development needs of colonies, with an increased protein demand for brood rearing beginning in late winter (Mattila and Otis 2006) and to a lesser extent in late summer according to the typical bistagial pattern of bee colony development in the Mediterranean environment (Ruttner 1986; Floris et al. 2016). Furthermore, it also reflects the increased availability of pollen due to the concomitant flowering of many plant species. Indeed, as in the case of *Rhamnus alaternus* (Gutián 1995), most Mediterranean plant species have a single-peaked flowering period in late winter and early spring (Bosch et al. 1997).

The observed differences in the amount of pollen collected among the four sampling sites were positively correlated with the percentage of semi-natural habitat cover but not with habitat diversity. These results appear to be consistent with the foraging strategy of honey bees, which involves the recognition of food sources in a large area around the nest, followed by the selection of a few flower species to forage on, according to criteria such as their abundance and nutritional value (Visscher and Seeley 1982; Winston 1991; Hendriksma and Shafir 2016). In fact, our pollen analysis revealed that pollen harvests were derived mainly from only one or few floral sources, such *Rhamnus alaternus* in February, *Lotus corniculatus* or *Trifolium campestre* in April and *Echium* sp. (very likely *Echium plantagineum*) in May. All these plant species are very common in semi-natural habitats such as Mediterranean evergreen shrubland, wild-olive-lentisc evergreen shrubland, western garrigues and Dehesa (rangelands

with sparse oak trees). Importantly, Codrongianus had the lowest percentage of semi-natural habitats (24%) among the 4 sites, and this could explain the lower pollen yield detected there compared to Villanova (95% of semi-natural habitats) and Florinas (79% of semi-natural habitats). In contrast to our results, Danner et al. (2017) reported no quantitative differences in the amount of pollen collected by honey bees among 16 different sites located in an agricultural area of Germany. However, in that context, the maximum cover of semi-natural habitats was only 14% and honey bees had the opportunity to compensate for resource scarcity by increasing foraging distances to obtain sufficient amount of pollen for their colonies (Danner et al. 2017).

Considering that the pollen traps used in our study can retain about 30% of all the collected pollen (Keller et al. 2005b), the amount of pollen collected by the bees over the entire observation period (February-October) was approximately 46 kg in Villanova, 30 kg in Florinas, 23 kg in Ittiri and 19 kg in Codrongianos. Similarly, Winston (1991) reported that honey bee colonies require between 15 and 30 kg of pollen a year but could collect up to 55 kg. Moreover, it should be noted that in all sites most of the pollen was collected in spring (80% in Florinas, Ittiri and Codrongianos and up to 90% in Villanova), whereas very poor harvests were recorded during autumn and even less in summer. Therefore, it is likely that, under such conditions, honey bees and all other pollinating insects would benefit from conservation efforts aimed at increasing floral resources that could fill the temporal and spatial gaps in the availability and diversity of food resources. This would provide greater continuity of bee activity and probably extend their honey production season.

The total number of 168 pollen taxa that we found in the pollen samples is very similar to the values reported by Köppler et al. (2007), who compared the spectra of pollen collected from 4 different subspecies of *Apis mellifera* in Germany. Considering that a pollen taxon does not refer to a single plant species, but more frequently to a genus or a botanical family (Louveaux et al. 1978), it can be expected that the number of plant species in our pollen samples was much higher. In our sampling sites, species that are non-native in Sardinia and have been introduced for cultivation, such as *Eucalyptus* and *Castanea*, and that are commonly absent in the habitat types classified as

semi-natural in the present study, gave a contribution to pollen collection. However, these non-native species occupied a limited area in all four sites used in our study. Access to a large number of flowering plant species is certainly beneficial for honey bee colonies, because it increases immunocompetence (Alaux et al. 2010) and tolerance to pathogens (Di Pasquale et al. 2016). As observed for the amount of pollen, pollen richness and diversity varied significantly throughout the season, with the highest values observed in March and the lowest in September. The observed trend is consistent with the wild plant flowering seasonal pattern normally observed in a region characterized by a Mediterranean climate. Different trends have been reported in other climatic regions (Malagnini et al. 2022) or in the presence of wide areas with mass flowering crops, such as oilseed rape (Danner et al. 2017).

Our results also highlighted that pollen richness and pollen diversity were positively correlated with habitat heterogeneity (Shannon's habitat diversity index), although we acknowledge the small size of our experimental design. These findings suggest that honey bees effectively exploit the increased foraging opportunities offered by a more diverse landscape, with a resultant gain in pollen richness and diversity. Our results are in line with Montoya-Pfeiffer et al. (2021), who also reported a higher diversity of pollen collected by honey bees in more structurally diverse landscapes. Nevertheless, contrasting results have been reported in other agricultural landscapes (Piroux et al. 2014; Danner et al. 2017; Malagnini et al. 2022), probably due to differences in the type of management of those areas and landscape heterogeneity, considering that intensive human activities can reduce flowering plant species and their availability. For example, the use of herbicides can reduce or eliminate flowering plants to forage on (Bohnenblust et al. 2016; Cullen et al. 2019). Another negative impact on flower richness and availability could result from intensive grazing, which can lead to a rescission of flowers before the flowering period (Rakosy et al. 2022), to an increase in late-flowering spiny species, or to a floristic homogenisation.

Our findings showed that pollen richness and diversity were not significantly affected by the percentage of semi-natural habitat cover, which was surprising. In addition, the multivariate permutational analysis of variance, performed considering all

pollen taxa, showed that pollen composition was significantly affected by season, but not by habitat diversity, proportion of semi-natural habitat cover or sampling site. These results were probably because both the potential vegetation (the semi-natural habitats identified at the 4 sites often represent different stages of the same potential vegetation) and the types of crops (olive groves, vineyards, cereals for fodder, *Eucalyptus* or *Pinus* small plantations or windbreaks) were similar among sites, although the four sampling sites were strongly shaped by a diverse degree of anthropisation, with varying extension of built up areas, agricultural areas and, conversely, semi-natural habitats. In addition, the landscape of the study areas is very patchy, so that the different habitats and land-use types co-exist in a relatively very small area.

Another important result of our study was the observed positive effect of the amount of pollen collected on honey bee colony development. This would be expected considering the importance of pollen for brood rearing and worker development (Haydak 1970; Brodschneider and Crailsheim 2010). Indeed, our result is consistent with other studies revised by Keller et al. (2005) based on estimates made over long periods of time. Considering that when two factors vary together, a causal relationship between them cannot be established, the positive relationship between the amount of pollen collected and brood development could also mean that it is the amount of brood that regulates the intensity of pollen collection. Alternatively, the opposite could be equally valid. However, in our comparative study, the amount of brood was also positively related to the percentage of cover of semi-natural habitats, which offer the spontaneous flora that is mainly visited by bees. This strongly suggests that the amount of pollen available in an area and thus collected by honey bees influences the amount of brood reared by the colonies.

Finally, our study also showed that, in addition to the amount of pollen, both the richness and diversity of the pollen collected also had a positive effect on the amount of brood reared, thus highlighting a positive indirect link between habitat heterogeneity and honey bee colony development. Although laboratory experiments have shown that diversity in pollen diet increases bee immunocompetence (Alaux et al. 2010, 2011), how pollen diversity may

affect colony development at the field level has not been elucidated yet. Despite that, it is known that the nutritional value of pollen from different plant species varies according to its absolute and relative content of essential nutrients such as amino acids, which are required in specific proportions for normal growth and development (de Groot 1953; Crailsheim 1990). Therefore, it is likely that an increase in habitat diversity, and thus in the availability of different pollen sources, optimizes the availability of specific nutrients needed to develop specific physiological aptitudes in bees. In fact, some studies showed that habitat composition can affect queen's fecundity (Clair et al. 2022) and the nutritional values of the bee bread (Donkersley et al. 2014). In particular, the nutritional values of the bee bread were found to be negatively correlated with increasing levels of arable and horticultural farmland surrounding the hives and positively correlated with the cover of natural grasslands and broadleaf woodlands (Donkersley et al. 2014).

In conclusion, although we acknowledge the small sample size and correlative design of our study, and the inherent methodological limitations of pollen morphological identification, our field experiment emphasizes that for honey bee colony development an abundant and diverse pollen diet is important, which in agro-sylvo-pastoral systems is ensured by a heterogeneous landscape dominated by different types of semi-natural habitats.

Acknowledgements The authors cordially thank Ana Helena Dias Francesconi for revising the manuscript.

Author contributions All authors contributed to the study conception and design. Collection of field data was performed by Matteo Lezzeri and Nicola Palmieri. Data preparation and statistical analysis were performed by Michelina Pusceddu, Alberto Satta and Panagiotis Theodorou. The first draft of the manuscript was written by Alberto Satta and Panagiotis Theodorou and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Open access funding provided by Università degli Studi di Sassari within the CRUI-CARE Agreement. This work was financially supported by Ministero dell'agricoltura, della sovranità alimentare e delle foreste, project For(m)aggi Api e Frutta: Integrazione apistico-frutticola di sistemi foraggero-zootecnici in ambiente mediterraneo asciutto (4APIFRUT).

Data availability Data will be made available on request.

Declarations

Competing interests The authors declare no competing interests with research described in this paper.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Alaux C, Ducloz F, Crauser D, Le Conte Y (2010) Diet effects on honeybee immunocompetence. *Biol Lett* 6(4):562–565
- Alaux C, Dantec C, Parrinello H, Le Conte Y (2011) Nutrigenomics in honey bees: Digital gene expression analysis of pollen's nutritive effects on healthy and *Varroa*-parasitized bees. *BMC Genom* 12:496
- Alaux C, Allier F, Decourtye A, Odoux JF, Tamic T, Chabirand M, Delestra E, Decugis F, Le Conte Y, Henry M (2017) A 'Landscape physiology' approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats. *Sci Rep* 7(1):1–10
- Annoscia D, Zanni V, Galbraith D, Quirici A, Grozinger C, Bortolomeazzi R, Nazzi F (2017) Elucidating the mechanisms underlying the beneficial health effects of dietary pollen on honey bees (*Apis mellifera*) infested by *Varroa* mite ectoparasites. *Sci Rep* 7(1):1–13
- Avni D, Hendriksma HP, Dag A, Uni Z, Shafir S (2014) Nutritional aspects of honey bee-collected pollen and constraints on colony development in the eastern Mediterranean. *J Insect Physiol* 69:65–73
- Bagella S, Satta A, Floris I, Caria MC, Rossetti I, Podani J (2013) Effects of plant community composition and flowering phenology on honeybee foraging in Mediterranean sylvo-pastoral systems. *Appl Veg Sci* 16(4):689–697
- Baksay S, Pornon A, Burrus M, Mariette J, Andalo C, Escaravage N (2020) Experimental quantification of pollen with DNA metabarcoding using ITS1 and trnL. *Sci Rep* 10(1):1–9
- Bänsch S, Tschartke T, Ratnieks FL, Härtel S, Westphal C (2020) Foraging of honey bees in agricultural landscapes with changing patterns of flower resources. *Agric Ecosyst Environ* 291:106792

- Bates D, Mächler M, Bolker B, Walker S (2015) Fitting Linear mixed-effects models using lme4. *J Stat Softw* 67(1):1–48
- Bohnenblust EW, Vaudo AD, Egan JF, Mortensen DA, Tooker JF (2016) Effects of the herbicide dicamba on nontarget plants and pollinator visitation. *Environ Toxicol Chem* 35(1):144–151
- Bosch J, Retana J, Cerdá X (1997) Flowering phenology, floral traits and pollinator composition in a herbaceous Mediterranean plant community. *Oecologia* 109:583–591
- Broschneider R, Crailsheim K (2010) Nutrition and health in honey bees. *Apidologie* 41(3):278–294
- Camarda I, Laureti L, Angelini P, Capogrossi R, Carta L, Brunu A (2015) Il Sistema Carta Della Natura Della Sardegna. ISPRA. Serie Rapporti 222:2015
- Campos MG, Bogdanov S, de Almeida-Muradian LB, Szczesna T, Mancebo Y, Frigerio C, Ferreira F (2008) Pollen composition and standardisation of analytical methods. *J Apicult Res* 47(2):156–163
- Catford JA, Wilson JR, Pyšek P, Hulme PE, Duncan RP (2022) Addressing context dependence in ecology. *Trends Ecol Evol* 37(2):158–170
- Clair ALS, Suresh S, Dolezal AG (2022) Access to prairie pollen affects honey bee queen fecundity in the field and lab. *Front Sustain Food Syst* 6:908667
- Crailsheim K (1990) The protein balance of the honey bee worker. *Apidologie* 21(5):417–429
- Cullen MG, Thompson LJ, Carolan JC, Stout JC, Stanley DA (2019) Fungicides, herbicides and bees: a systematic review of existing research and methods. *PLoS ONE* 14(12):e0225743
- Danner N, Keller A, Härtel S, Steffan-Dewenter I (2017) Honey bee foraging ecology: season but not landscape diversity shapes the amount and diversity of collected pollen. *PLoS ONE* 12(8):e0183716
- de Groot AP (1953) Protein and amino acid requirements of the honey bee (*Apis mellifica* L.). *Physiol Comp Oecol* 3:197–285
- Di Pasquale G, Salignon M, Le Conte Y, Belzunces LP, Decourtye A, Kretzschmar A, Suchail S, Brunet JL, Alaux C (2013) Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS ONE* 8(8):e72016
- Donkersley P, Rhodes G, Pickup RW, Jones KC, Wilson K (2014) Honeybee nutrition is linked to landscape composition. *Ecol Evol* 4(21):4195–4206
- Floris I, Bagella S, Caria MC, Ruii L, Buffa F, Satta A (2016) A Mediterranean silvo-pastoral system supporting beehive health and productivity. *Bull Insectology* 69(1):13–20
- Goulson D, Nicholls E, Botías C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347(6229):1255957
- Guitiñ J (1995) Sex ratio, reproductive investment and flowering phenology in dioecious *Rhamnus alaternus* (Rhamnaceae). *Nord J Bot* 15(2):139–143
- Hartig F (2022) DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.5, <https://CRAN.R-project.org/package=DHARMA>
- Haydak MH (1970) Honey bee nutrition. *Annu Rev Entomol* 15(1):143–156
- Hendriksma HP, Shafir S (2016) Honey bee foragers balance colony nutritional deficiencies. *Behav Ecol Sociobiol* 70(4):509–517
- Hepburn HR (1986) Composition and synthesis of beeswax. Honeybees and wax. Springer, Berlin, pp 44–56
- Holland JM, Douma JC, Crowley L, James L, Kor L, Stevenson DRW, Smith BM (2017) Semi-natural habitats support biological control, pollination and soil conservation in Europe. A review. *Agron Sustain Dev* 37:31
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in General Parametric models. *Biom J* 50(3):346–363
- Human H, Nicolson SW, Strauss K, Pirk CWW, Dietemann V (2007) Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*). *J Insect Physiol* 53(7):649–655
- Keller I, Fluri P, Imdorf A (2005a) Pollen nutrition and colony development in honey bees: part 1. *Bee World* 86(1):3–10
- Keller I, Fluri P, Imdorf A (2005b) Pollen nutrition and colony development in honey bees: part II. *Bee World* 86(2):27–34
- Köppler K, Vorwohl G, Koeniger N (2007) Comparison of pollen spectra collected by four different subspecies of the honey bee *Apis mellifera*. *Apidologie* 38(4):341–353
- Lamb PD, Hunter E, Pinnegar JK, Creer S, Davies RG, Taylor MI (2019) How quantitative is metabarcoding: a meta-analytical approach. *Mol Ecol* 28(2):420–430
- Lau P, Bryant V, Ellis JD, Huang ZY, Sullivan J, Schmehl DR, Cabrera AR, Rangel J (2019) Seasonal variation of pollen collected by honey bees (*Apis mellifera*) in developed areas across four regions in the United States. *PLoS ONE* 14(6):e0217294
- Louveaux J, Maurizio A, Vorwohl G (1978) Methods of melissopalynology. *Bee World* 59:139–157
- Malagnini V, Cappellari A, Marini L, Zanotelli L, Zorer R, Angeli G, Ioriatti C, Fontana P (2022) Seasonality and landscape composition drive the diversity of pollen collected by managed honey bees. *Front Sustain Food Syst* 6:865368
- Marchetti S (1985) Il Metodo dei sestri per la valutazione numerica degli adulti in famiglie di *Apis mellifera* L. *Apicoltura* 1:41–61
- Mattila HR, Otis GW (2006) Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. *J Econ Entomol* 99(3):604–613
- Millard J, Outhwaite CL, Kinnersley R, Freeman R, Gregory RD, Adedoja O, Gavini S, Kioko E, Kuhlmann M, Ollerton J, Ren Z-X, Newbold T (2021) Global effects of land-use intensity on local pollinator biodiversity. *Nat Commun* 12(1):2902
- Montoya-Pfeiffer PM, González-Chaves A, Nates-Parra G (2021) Effects of landscape structure and climate seasonality on pollen intake by honeybees in neotropical highland agroecosystems. *Agric Entomol* 23(4):452–462
- Nagendra H (2002) Opposite trends in response for the Shannon and Simpson indices of landscape diversity. *Appl Geogr* 22(2):175–186
- Naug D (2009) Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biol Conserv* 142(10):2369–2372
- Odoux JF, Feuillet D, Aupinel P, Loublier Y, Tasei JN, Mateescu C (2012) Territorial biodiversity and

- consequences on physico-chemical characteristics of pollen collected by honey bee colonies. *Apidologie* 43(5):561–575
- Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlenn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2022) *vegan: Community Ecology Package*. R package version 2.6-2, <https://CRAN.R-project.org/package=vegan>
- Ollerton J, Erenler H, Edwards M, Crockett R (2014) Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. *Science* 346(6215):1360–1362
- PalDat (1997) PalDat: A Palynological Database. Available online at: www.paldat.org (June 20, 2022)
- Pardini A, Nori M (2011) Agro-silvo-pastoral systems in Italy: integration and diversification. *Pastoralism: Res Policy Pract* 1(1):1–10
- Persano Oddo L, Ricciardelli D'Albore G (1989) Melissopalynological nomenclature. *Apicoltura* 5:63–72
- Piñol J, Senar MA, Symondson WO (2019) The choice of universal primers and the characteristics of the species mixture determine when DNA metabarcoding can be quantitative. *Mol Ecol* 28(2):407–419
- Piroux M, Lambert O, Puyo S, Farrera I, Thorin C, L'Hostis M, Vignes B, Bastian S (2014) Correlating the pollens gathered by *Apis mellifera* with the landscape features in western France. *Appl Ecol Environ Res* 12(2):423–439
- PollenAtlas (2022) June 20. Available online at <https://www.pollenatlas.net>
- Pollen-Wiki (2022) Available online at <https://www.pollen.tstebler.ch>
- Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. *Trends Ecol Evol* 25(6):345–353
- QGIS Development Team (2023) QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>. (January 10, 2023)
- Rakosy D, Motivans E, Ștefan V, Nowak A, Świerszcz S, Feldmann R, Kühn E, Geppert C, Venkataraman N, Sobieraj-Betlińska A, Grossmann A, Rojek W, Pochrzast K, Cielniak M, Gathof AK, Baumann K, Knight TM (2022) Intensive grazing alters the diversity, composition and structure of plant-pollinator interaction networks in central European grasslands. *PLoS ONE* 17(3):e0263576
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Requier F, Odoux JF, Tamic T, Moreau N, Henry M, Decourtye A, Bretagnolle V (2015) Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecol Appl* 25(4):881–890
- Ricciardelli D'Albore (1998) G Mediterranean melissopalynology. – Università degli Studi di Perugia, Facoltà di Agraria, Perugia. pp. 466
- Ruttner F (1986) Bee genetics and breeding. In: Rinderer TE (ed) *Geographical variability and classification*. Academic press, Cambridge, pp 23–56
- Schmehl DR, Teal PE, Frazier JL, Grozinger CM (2014) Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (*Apis mellifera*). *J Insect Physiol* 71:177–190
- Sjödin NE, Bengtsson J, Ekbom B (2008) The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *J Appl Ecol* 45:763–772
- Sobel ME (1982) Asymptotic confidence intervals for indirect effects in structural equation models. *Sociol Methodol* 13:290–312
- Steffan-Dewenter I, Kuhn A (2003) Honeybee foraging in differentially structured landscapes. *Proc. Royal Soc B: Biol Sci* 270:569–675
- Vaudo AD, Tooker JF, Grozinger CM, Patch HM (2015) Bee nutrition and floral resource restoration. *Curr Opin Insect Sci* 10:133–141
- Visscher PK, Seeley TD (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology* 63(6):1790–1801
- Von der Ohe W, Persano Oddo L, Piana LM, Morlot M, Martin P (2004) Harmonized methods of melissopalynology. *Apidologie* 35:18–25
- Winston ML (1991) *The biology of the honey bee*. Harvard University Press, London
- Wratten SD, Gillespie M, Decourtye A, Mader E, Desneux N (2012) Pollinator habitat enhancement: benefits to other ecosystem services. *Agr Ecosyst Environ* 159:112–122
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) *Mixed effects models and extensions in ecology with R*, vol 574. Springer, New York, p 574

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.