

Simple and informative: applying a basic Anthophila monitoring scheme in a simplified insular ecosystem

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Abstract

The decline of pollinators and the consequent decay of pollination services call for the establishment of monitoring schemes for several groups of pollinators. For Anthophila (Hymenoptera), the design of monitoring schemes is still under development. The main difficulties lie in combining a reliable but field-feasible taxonomic identification with the collection of informative data about the consistency and functional role of pollinator populations. Here we report on the application of the Italian monitoring scheme for pollinators recently defined by ISPRA and the University of Turin in agreement with the European Pollinators Monitoring Scheme on the small island of Giannutri (Tuscany), a simplified insular ecosystem with a virtually unknown pollinator community. This island has recently experienced a drastic change in its bee community, as since 2018 honey bee (*Apis mellifera* L.) hives are regularly moved every year to the island for breeding purposes. In the spring 2021 we established six 250 m long fixed transects and performed a total of 48 surveys (8 for each transect), recording more than 2300 observations of 9 Anthophila bee taxa and the flowers they visited. By using generalised additive mixed models, we showed that the monitoring protocol has a good potential for monitoring Anthophila, as we could verify several expected relationships between Anthophila abundance and abiotic factors (season, hour of the day, distance from the apiary) and biotic factors (abundance of flower resources). More importantly, we verified that *A. mellifera* represents by far the most frequent Anthophila taxon. Our data do not show evidence for spatial partition between *A. mellifera* and the other most frequent taxa (*Bombus terrestris* L. and *Anthophora* spp.). The visit network based on transect observations also showed that these taxa largely overlapped in terms of visits to flower resources. Overall, our data showed that the monitoring protocol allows gathering informative data about Anthophila taxa abundance, interactions and flower-visits. Moreover, the spatial and flower-visit overlap suggest potential for competition between honey bees and wild pollinators, with a potential consequent resource depletion for the latter. While this hypothesis could only be assessed by a long-term monitoring and *ad hoc* honey bee removal experiments, our data show that this basic monitoring protocol produces rapid and valuable information about Anthophila community and dynamics.

Key words: Apoidea, Anthophila, pollination, pollinators, walking transects, honey bee, bumblebee, *Anthophora*.

Introduction

Animal-mediated pollination is crucial to ecosystem functioning and it is a key to global crop productivity and access to food (Potts *et al.*, 2016), with an estimate of around 90% of wild flowering plants and 75% of food crops which rely on animal pollination (Ollerton *et al.*, 2011; Halvorson *et al.*, 2021). Bees (Anthophila) play a major role in providing pollination services (Ollerton, 2017), with honey bees (*Apis mellifera* L. in particular), bumblebees and many wild bees having dominant positions in the plant-pollinator networks all over the world (Carre *et al.*, 2009; Neumann and Carreck, 2010; Klein *et al.*, 2017; Ollerton, 2017). As many other insect taxa, a significant fraction of Apoidea are experiencing a drastic decrease, at local, regional and global scales, in both diversity and abundance (Ollerton, 2017; Sánchez-Bayo and Wyckhuys, 2019), which often lead to local or global extinctions (Kosior *et al.*, 2007; Martins and Melo, 2010).

The understanding of what we are losing, where it is happening, and the causes of these declines is instrumental to mitigate this widespread and fast-paced loss. This is especially true at local scales and in small, fragile environments, where limited resources sustain reduced pollinator populations, which are thus particularly prone to

resource depletion and local extinction (Cox and Elmqvist, 2000; Liu *et al.*, 2021). Establishing a long-term monitoring system is the first step to provide information on trends of species diversity, abundance and biotic interactions over large temporal and spatial scales, retaining enough resolution on both scales in order to be useful to catch the local dynamics (Westphal *et al.*, 2008; Lebuhn *et al.*, 2013; Ollerton, 2017; O'Connor *et al.*, 2019). Based on these data, *ad hoc* experimental studies can then be implemented to identify and mitigate the drivers of the observed declines in bee communities.

There are many existing monitoring methods so far developed, such as pan traps, observation plots and walking transects (for methodological reviews and comparisons see for example Garratt *et al.*, 2019; O'Connor *et al.*, 2019; Breeze *et al.*, 2021). These methods have different efficacy for monitoring the many aspects of pollinator biodiversity. For example, pan traps provide species resolution data independently from the taxonomic expertise of the operator deploying the traps, but they might be biased in attractiveness to different species and might miss information about the relationships with flowering vegetation. Observational methods, such as observation plots or transects, strongly depend on observer expertise (both for bee detection and species recognition) but have the advantage of allowing the characterisation of plant-

pollinator interactions and the identification of which insect species (or taxa, more widely) are delivering pollination service to crops and/or wildflowers (Kleijn *et al.*, 2015; Gibbs *et al.*, 2017; Giovanetti *et al.*, 2021). Walking transects can be seen as a good compromise, as they provide reliable information on species community (Westphal *et al.*, 2008; Dennis *et al.*, 2009; Nielsen *et al.*, 2011), despite less than pan traps. They also provide information on plant-pollinator interactions (O'Connor *et al.*, 2019; Giovanetti *et al.*, 2021), even if are less reliable than observation plots. Notwithstanding the wealth of research into monitoring methods for bee pollinators, the adoption of national scale monitoring schemes using repeatable and standardised survey methods (Dicks *et al.*, 2016) is still lacking in most countries (see Powney *et al.*, 2019; Potts *et al.*, 2021 for few exceptions). As a result, contrary to what is happening for other pollinators such as butterflies (e.g. the Butterfly Monitoring Scheme project, eBMS), we sorely lack standardised, long-term and large-scale data for bees (Lebuhn *et al.*, 2013), cohesively collected across a large spatial scale, which could inform national and international policy needs (O'Connor *et al.*, 2019). This paper examines the implementation of the monitoring scheme based on fixed walking transects recently proposed in Italy by the Institute for Environmental Protection and Research (ISPRA), University of Turin in collaboration with other institutions (<http://www.parcocirceo.it/albOnline/2020/PNCIRdocumento53301-allegato3.pdf>), according to the European Pollinator Monitoring Scheme (EU-PoMS) (Potts *et al.*, 2021).

Italy hosts about 1000 Anthophila bee species (Pagliano, 1995) and it is one of the European countries which most depend from insect (and particularly bee) pollination services for its agricultural production (Lautenbach *et al.*, 2012; Leonhardt *et al.*, 2013). In order to implement the 2018 European Pollinators Initiative (https://ec.europa.eu/environment/nature/conservation/species/pollinators/policy_en.htm) and to collect data about the presence and abundance of pollinators, their threats and their possible decline, the Italian Ministry of the Ecological Transition has funded monitoring projects in all the Italian National Parks. Within this project, ISPRA, in collaboration with the University of Turin and other institutions, recently proposed a monitoring scheme comprising two different protocols for Papilionoidea and Anthophila to be adopted at the country level (Bonelli *et al.*, 2020; D'Antoni *et al.*, 2020). The protocol for Anthophila (hereafter called “the monitoring protocol”) is based on standard-fixed walking transects of 250 metres (explained in detail in the material and method section) and it has the main advantages of being simple, replicable, and to adapt to the observer taxonomic expertise. Indeed, identification at the species level is required for honey bees and bumblebees as adopted in other European monitoring schemes (Potts *et al.*, 2021, pages 73-74). As a further source of information, any other observed Anthophila individual has been recorded with the highest possible taxonomic resolution given the observer expertise. While the difficulty in identifying Anthophila in the field can clearly come with some limitations in the

information gathered (in particular the limited taxonomic resolution) this method has a great potential to be widely adopted as the standardised monitoring scheme in the country, and, at least for bumblebees, to be completely in line with most existing monitoring schemes. Finally, this method can represent a complementary tool to more detailed studies with higher taxonomic resolution.

With the aim of boosting the adoption of a simplified standardised bee monitoring scheme in Italy, we here perform a first assessment of the informative value of the monitoring protocol in a simplified ecosystem within a National Park, in order to assess the pros and cons of this monitoring scheme. For several reasons, we focused on the simplified ecosystem on the small island of Giannutri, which is part of the Tuscan Archipelago National Park. Firstly, a simplified insular ecosystem harbouring a limited set of pollinator and plant species represents a useful model where to test specific predictions that might be difficult to highlight in complex and well-connected ecosystems. Secondly, insular ecosystems are of huge ecological, biogeographical and conservation interest (Whittaker *et al.*, 2017), often hosting a disproportionate fraction of biodiversity (including many endemic taxa) which, due to isolation and limited island size, is often more at risk than continental one (Manes *et al.*, 2021). Indeed, the Tuscan Archipelago hosts a rich and peculiar invertebrate fauna with many endemic lineages, as a result of both historical and contemporary ecological as well as geographic determinants (Dapporto *et al.*, 2007; 2017; Fattorini, 2009; Barbato *et al.*, 2018; Ruzzier *et al.*, 2021). Moreover, Giannutri island has recently experienced a drastic change in its bee community, as since 2018 several honey bee hives are regularly moved every year to the island from winter to the end of the spring season for breeding purposes. Managed honey bees can have negative effects on native bee fauna (Mallinger *et al.*, 2017), especially in small and isolated ecosystems, as recently shown in the Aegean Archipelago (Lázaro *et al.*, 2021). Giannutri island represents a promising case study where to assess the monitoring protocol in a context of potential competitive dynamics at the very beginning of the managed-wild bees interaction process. Finally, the knowledge of bee fauna and its pollinating activity is virtually absent, which allows us to test to what extent the monitoring protocol provides significant information about potential plant-pollinators relationships.

In particular, we here aim at: (1) verifying that the monitoring protocol allows to recover known relationships between ecological features and pollinator abundance, by capitalising on the wide knowledge about many aspects of pollinators ecology. If the monitoring protocol is informative, predictions based on known extant relationships should be met by our data; (2) identifying the pattern of spatial and functional overlap among local pollinators, which could suggest potential insect-insect interaction dynamics, such as competition for floral resources, a known process driving pollinator guild spatial partitioning (e.g. Wojcik *et al.*, 2018; Jeavons *et al.*, 2020) and finally (3) inferring the most important floral resources for the pollinator guild in this insular ecosystem.

Materials and methods

The island

The study was carried out on Giannutri island (42°15'14"N 11°06'13"E), the southernmost and the easternmost island of the Tuscan Archipelago in the Tyrrhenian Sea, Italy (figure 1a). It is a calcareous island placed at 11.5 kilometres from the coast (Mount Argentario), it has a coastal perimeter of 11 km, a length of about 3 km, a width of about 500 m, an area of 2.6 km² and a maximum altitude of 89.4 m (Poggio di Capel Rosso). The entire island is within the Tuscan Archipelago National Park, with the southern part being protected as an integral reserve. The island is also part of Natura 2000 (site code IT51A0024) as SIC (Site of Community Interest) and ZPS (Zone of Special Protection) for birds.

A diachronic analysis in the last decades (1950–2008) highlighted an increase in surface of vegetation types with greater physiognomic development (high Mediterranean maquis) to the detriment of areas with a lower level of dynamism (open meadows). The current scrubland vegetation is dominated by *Teucrium fruticans* L., *Rosmarinus officinalis* L., *Juniperus turbinata* (Gussone), *Euphorbia dendroides* L., *Erica multiflora* L. and *Pistacia lentiscus* L., in particular the coastal vegetation is dominated by *Senecio cineraria* (Chater) (Foggi *et al.*, 2011). A single apiary is present on the island (figure 1b), consisting of 12 to 18 hives (according to the year, 18 hives in 2021) placed for the first time in 2018 in agreement with the National Park. Every year all hives are brought to the island around December and removed around mid-June.

The monitoring protocol

We performed simplified standardised transect walks following the guidelines of ISPRA (Bonelli *et al.*, 2020; D'Antoni *et al.*, 2020; the protocol is available at the following link: [\[ceo.it/albOnline/2020/PNCIRdocumento53301-allegato3.pdf\]\(http://ceo.it/albOnline/2020/PNCIRdocumento53301-allegato3.pdf\)\). These guidelines define the standard monitoring protocol for pollinators within National Parks and protected areas, which can fulfil several purposes such as evaluating the impact of phytosanitary products on agrosystems or monitoring the pollinator trends in natural and semi-natural environments \(Bonelli *et al.*, 2020; D'Antoni *et al.*, 2020; Giovanetti *et al.*, 2021\). The sampling design is based on Westphal *et al.* \(2008\) and Nielsen *et al.* \(2011\): transects are identified as fixed walks 250 m long and 4 m wide. Each transect is divided into 10 subunits, 25 m long, to be surveyed in 5 minutes \(for a total of 50 minutes\). The monitoring protocol specifies to record, during the walk, the number of Anthophila bee taxa together with the flower they are visiting. Differently to the standard monitoring protocol, in order to keep the information of all observed individuals, we scored the Anthophila bees recording two different activities: flying or visiting a flower. According to the protocol, the Anthophila bee species were identified at the species level both for the European honey bee \(*A. mellifera*, AM acronym\) and for the genus *Bombus* \(directly on the field or in the laboratory, B acronym\), while all the other Anthophila bee species were classified as “Other Anthophila bee species” \(AA in the Italian acronym\). However, the genus and species of the Anthophila bee species can be included in the notes when identification is possible. While performing the transects we used two approaches: a\) collection of specimens with a net and storage in tubes with ethyl acetate; b\) recording of bees, identified at genus or species level without capture. We also performed surveys on the island besides the transects in order to collect additional specimens. All captured bees have been identified with the aid of a taxonomist \(see acknowledgements\) and are stored in Marco Bonifacino and Simone Flaminio collections. We did not collect every bee we observed along the transects in agreement with the indications of the Tuscan Archipelago National Park, to limit our impact on bee populations of such a small island.](http://www.parcocir-</p></div><div data-bbox=)

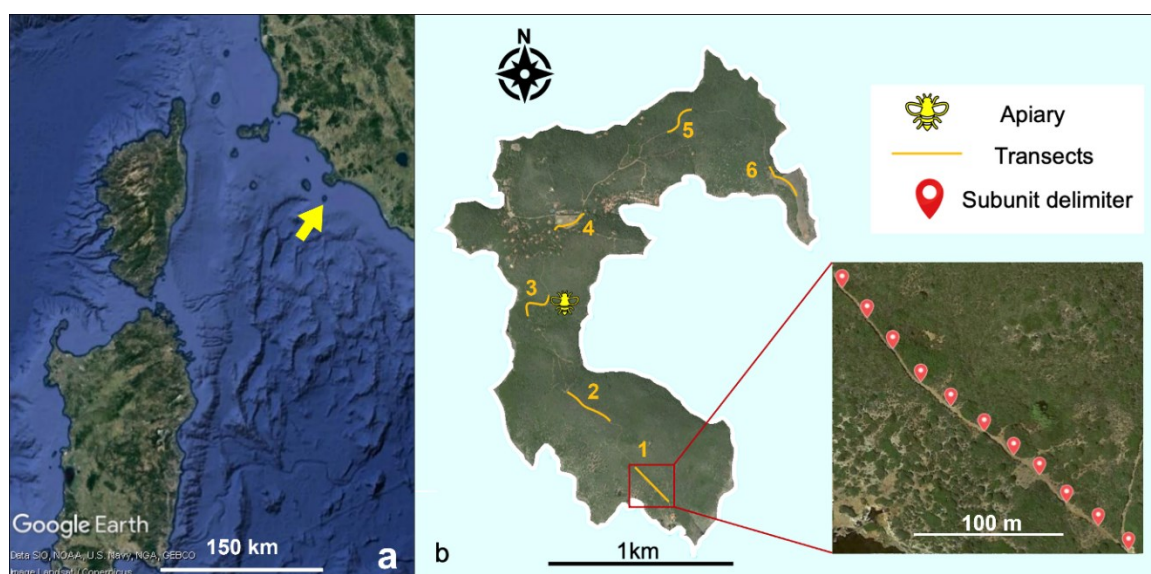


Figure 1. The location of Giannutri island (yellow arrow) in the context of the Tyrrhenian Sea (a); Giannutri island with the position of the six transects and the apiary, the inset shows the details of a transect with the eleven subunit delimiters (b).

According to the monitoring protocol, the flower visited by each observed insect is also recorded, at least at genus level. For each transect, the field-tables proposed by ISPRA and the University of Turin (see technical annex in D'Antoni *et al.*, 2020) must be compiled by entering information on biotic and abiotic factors. The recorder scores a percentage of the nectariferous floral coverage representing potential resources for bees in each subunit for three vegetational levels: grassland, shrub and arboreal. The percentages are then transformed in coverage abundance classes: 0 = 0-10%; 1 = 11-30%; 2 = 31-50%; 3 = 51-100%. For the grassland level, the average height (cm) is also measured. Four parameters were recorded at the beginning and at the end of the transect: time (standard time), temperature (°C), strength of the wind (according to the range 0-5 of Beaufort scale) and cloud coverage (converted in percentage classes: 0 = 0%, 1 = 1-10%, 2 = 11-30%, 3 = 30-50%, 4 = 50-70%, 5 = 71-100%). For wind, temperature and cloud coverage the mean value is also entered.

In our study we established six transects along the island, extending from one extremity to the other: four of them (1, 2, 3, 5) are characterised by Mediterranean maquis, the remaining two (4, 6) by grassy habitats, with a portion of transect 4 developing through an anthropised area (figure 1b, supplemental material geographical Keyhole Markup Language). We carried out a total of three field sessions: the first took place from the 23rd to the 27th of March 2021 and it was performed by five operators (AC, FB, MB, LD, VS), the second occurred on the 14th of April 2021, performed by two operators (AC, FB) and the third was carried out by AC, GS, and LP from the 8th to the 9th of May 2021. Overall, seven operators performed the monitoring for a total of 48 samplings, 8 replications for each transect.

About 85% of the records of AA belonged to the easily identifiable *Anthophora* genus, while the remaining part could be attributed to a mix of at least 5 taxa. We thus focused our analysis on the most abundant taxa which could be unequivocally identified in the field: *A. mellifera*, *Bombus terrestris* L. and *Anthophora* spp. The only bumblebee species found on the island was *B. terrestris*. We exclude the two similar species occurring in Italy, *Bombus cryptarum* (F.) and *Bombus lucorum* (L.), on the basis of morphological analysis of collected specimens (including queens and males) and their distribution (both species are reported in upland areas of mainland Italy, Intoppa *et al.*, 1995). Also, we did not record hybrids with *Bombus xanthopus* Kriechbaumer, the only species belonging to *Bombus* subgenus recorded for the Tuscan Archipelago (Rasmont and Quaranta, 1997; Forbicioni *et al.*, 2019). Morphological examination in the laboratory indicated the presence of two *Anthophora* species, *Anthophora senescens* (Lepeletier) and *Anthophora dispar* (Lepeletier). As it was not possible to distinguish them while performing the transects, we treated these two species as a single taxon in our analysis. The results which include also the other AA species are reported in the supplementary material (supplemental material table S1, figure S1).

As cloud coverage and height of grassland level showed a very limited variance in our sampling, we did not consider these factors when deriving predictions (see below),

in order to avoid lack of statistical power. Also, we did not include the effect of temperature in our predictions as it showed a very high significant correlation (multiple regression, $r^2 = 0.754$, $P < 0.001$) with a combination of day of sampling (estimate = 0.103, $t = 34.941$, $P < 0.001$) and time (estimate = 0.391, $t = 14.44$, $P < 0.001$).

Research aims

Aims 1. Verifying that the monitoring protocol allows to recover known relationships between ecological features and Anthophila abundance

We capitalise on the wide knowledge about pollinator ecology to derive specific predictions that, if the monitoring protocol is reliable, should be met by our data. We predict an influence of several time and ecological correlates on the abundance of different groups of pollinators (hour of the day, day of the year, flower resource abundance and, the distance from the nest for honey bees).

Firstly, nectariferous floral coverage, determining availability of flower resources, should clearly positively affect incidence of the three taxa. Secondly, in warm Mediterranean areas the flight period of most solitary *Anthophila* is narrowed to early spring (Potts *et al.*, 2005). As we sampled from March to May, we predicted to observe a decrease in the abundance of *Anthophora* bees from the first dates of monitoring to the last ones. This pattern is not expected in the two colonial species *A. mellifera* and *B. terrestris*, which show more extended phenologies, although with differences in colony dynamics. *A. mellifera* has multiannual colonies, while *B. terrestris* has annual ones, with at least two generations reported in Mediterranean areas (Rasmont *et al.*, 2008), thus both species are expected to be observed all year round. Thirdly, the hour of the day is known to affect pollinator foraging activity, depending on many factors such as local climate, vegetational composition and species life history (Herrera, 1990; Baldock *et al.*, 2011; Vaudo *et al.*, 2014). Finally, the abundance of pollinators is clearly dependent on the distance from their nests. While for wild pollinators a clear relationship might be hard to find, as nesting sites are unknown and possibly dispersed, an influence of distance from the nest should be highlighted in colonial central foragers such as the managed *A. mellifera*. Knowing the exact location of the single apiary present on Giannutri, we predicted a negative effect of distance from the apiary on *A. mellifera* abundance.

Wind strength is known to affect flying insects, thus influencing the spatio-temporal heterogeneity of pollinator visitation (Crall *et al.*, 2020). On one side wind may reduce manoeuvrability and impose energetic costs to flying insects, on the other side wind disperses chemical cues that mediate interactions between insects and plants (Ravi *et al.*, 2013; Crall *et al.*, 2017). The many and contrasting effects of wind on pollinators make it difficult to unequivocally predict its effect, so we included wind in the model without any specific predictions.

Most monitoring schemes do not distinguish between individuals visiting flowers (likely flower-visiting, even if we cannot exclude the presence of male bees looking for mating opportunities) and flying individuals, providing a

single count for both activities. However, we can expect the above relationships to be dependent on the different pollinator activity (flying, flower-visiting) performed by pollinators. For example, resource abundance is expected to affect more flower-visiting individuals than flying individuals ones, as pollinators can be frequent also in low flower areas, being involved in movements among sites or exploration flights. We thus performed the analyses separately for “visiting a flower” and “flying” to assess the importance of the above indicated factor on each of the two groups.

Aim 2. Assess potential spatial partitioning, i.e. the relationship between abundance of the three different groups

Spatial overlap among local pollinators is the first information needed to assess interaction dynamics, such as competition for floral resources, a known process driving pollinator guild spatial partitioning (Jeavons *et al.*, 2020). The beehives are moved each year from late winter to summer to Giannutri and might potentially have a strong impact on native wild pollinators. While a proper assessment would require experimental manipulation of bee presence and assessment of fitness effects, walking transect monitoring scheme could provide a first important information. We thus assessed the effect of *A. mellifera* on the abundance of the two main wild pollinator taxa, *Anthophora* spp. and *B. terrestris*.

Aim 3. Identify the most important plant resources for pollinators on Giannutri

Pollinator conservation actions need to be based on evidence about pollinator ecological requirements, especially the use of floral resources. While a comprehensive knowledge can be only achieved with detailed *ad hoc* studies, which require flower based observations and direct estimation of pollen transfer, walking transect monitoring scheme has been considered a rather efficient method for understanding plant-pollinator relationships, especially in simplified ecosystems with low pollinator richness (Westphal *et al.*, 2008; Hegland *et al.*, 2010). Indeed, transect data provide a rapid assessment of the main floral resources used by different pollinators, and a first estimation of flower resource partitioning among them. We thus recorded the plant taxa whose flowers were visited by the different bee taxa and drew flower-visitor networks to highlight the most used flower resources and provide a first assessment of the overlap in flower resource use among different bee taxa.

Data analysis

All analyses were performed using R version 4.0.5 using “fossil” (Vavrek, 2011), “mgcv” (Wood and Wood, 2015), and “bipartite” (Dormann *et al.*, 2008) packages.

Aims 1 and 2. *Anthophila* abundance, relationships with ecological features and the potential interaction with *A. mellifera*

We assessed the influence of biotic and abiotic factors

(see above) on the abundance of the three bee taxa with Generalised Additive Mixed Models (GAMM). We carried out separated models for each species and for the two different activities: flying and visiting a flower. As unit of analysis we considered the single replicate of each subunit of each transect, in order to account for the different ecological features within and among the transects. The count data of individuals recorded in each subunit have been modelled using the “Poisson” family. Spatial autocorrelation was considered in the model by including a covariance structure with distances based on midpoints of each subunit. We included the following predictors as smoothed terms: starting time of each transect subunit (hereafter “hour”), wind strength (hereafter “wind”), total abundance of floral coverage (hereafter “flowers”), obtained by summing, for each subunit, the scores for the three vegetation levels, day of year of the sampling (hereafter “day”), distance from the apiary (hereafter “dist”), and, for bumblebees (B) and AA, the recorded abundance of *A. mellifera* (hereafter “AMtot”). “Dist” and “AMtot” were used to investigate a potential interaction between the wild *Anthophila* and *A. mellifera*. Operator identity was included as a random factor.

Aim 3. Identifying the most important plant resources for the pollinators on Giannutri

In order to provide a rapid assessment of the main floral resources visited by the different *Anthophila* and to detect potential resource partitioning among them, we constructed bipartite flower-visitor networks. First, we built an overall network by pooling all flower visit data from all sampling periods, then we computed individual networks for each of the three sampling periods (March, April and May) in order to detect seasonal changes in flower-visitor networks. As this paper is focused on the three main *Anthophila* taxa detected (*A. mellifera*, *B. terrestris*, *Anthophora* spp.), we report here networks built only using these taxa. Networks including the other bee species (not identified and thus pooled into a single group: other AA) are included in the supplemental material (table S1, figure S1).

For each network we also computed the main network and species-level indices considered to provide ecologically-relevant information about the structure and functioning of pollination networks (Prendergast and Ollerton, 2021). At the network-level we computed: network size (the sum of nodes, i.e. number of taxa of pollinators and plants), connectance (the proportion of possible links actually recorded); interaction evenness (which quantifies how balanced the distribution of interactions is across species, based on Shannon’s diversity); H2 (a specialisation index, which ranges from 0-highly generalised- to 1-highly specialised); nestedness (i.e. the extent to which specialised species interact with a subset of the species that also the generalists interact with; it ranges from 0 to 100, with increasing values representing an increase in nestedness); niche overlap which indicates the average similarity in interaction patterns between species of the same level (values near 0 indicate no common use of niches, while 1 indicates perfect niche overlap); robustness (which indicates the robustness of species of a given level to removal of

species of the other level; e.g. when computed for the pollinator level indicates whether, if many plant species are lost, most of the pollinators will still survive -high R-, or if even a small fraction of the plants in the network are removed many secondary extinctions of pollinators will occur -low R-, vice versa for the plant level).

At the species-level we computed: normalised degree (the sums of the links per species, scaled by the number of possible interaction targets); pollination service index (which attempts to quantify the pollination services of a given flower-visitor to all plants in the network); push-pull index (which measures dependence asymmetry and ranges from -1 to +1, with positive values indicating that a plant has a higher dependence on the flower-visitors, and negative values indicating that a flower-visitor is, on average, more dependent upon the plants); Bluthgen's d' (which measures the specialisation of each species and ranges from 0 (no specialisation) to 1 (full specialisation) based on frequency of visits).

While a proper characterisation of the pollination networks requires an extensive sampling across multiple years, detailed assessment of visit duration, visitor behaviours and eventually direct measures of pollinator effectiveness (King *et al.*, 2013), we here aim at assessing whether it is possible to gather a first understanding of flower-visitor networks using the information collected through the monitoring protocol. Index computation and network visualisation were performed using the package "bipartite".

Results

During our surveys we recorded 9 Anthophila taxa: *A. mellifera*, *A. dispar*, *A. senescens*, *B. terrestris*, *Nomada goodeniana* (Kirby), *Nomada succincta* Panzer,

Xylocopa sp., *Lasioglossum transitorium* (Schenk) and *Andrena nigroaenea* (Kirby).

Aim 1 and Aim 2

The six GAMM analyses carried out separately for the three taxa showed that bee occurrence was correlated with several abiotic and biotic variables measured during the monitoring, in overall agreement with our predictions.

First, nectariferous floral coverage positively affected the occurrence of the three taxa (table 1). Both flower visiting and flying honey bees and bumblebees were linearly and positively correlated with local flower abundance (honey bees: table 1a, 1b; figure 2a, 2f; bumblebees: table 1c, 1d; figure 2a, 2f), showing an average increase of about one individual ranging from flower abundance class 0 to class 5. Flower abundance also had a linear and positive correlation with *Anthophora* spp. occurrence, but the relationship was non-linear (in "flower visiting individuals", table 1e; figure 2a) and linear (in "flying individuals", table 1f; figure 2f).

Occurrence of the three taxa was also affected by the day of sampling, which showed different effects according to the taxon, differentiating in particular honey bees from both bumblebees and *Anthophora* spp. For honey bees visiting flowers, sampling day showed a significant and non-linear positive correlation, with fewer individuals at the beginning of the season (Julian day 84-85) (table 1a; figure 2b), while sampling day did not affect the occurrence of flying bees (table 1b; figure 2g). For both bumblebees and *Anthophora* spp. and in both flower-visiting and flying individuals, on the contrary, sampling day showed a non-linear negative correlation with a maximum in April and a decline towards late spring for "flying" activity (bumblebees: table 1c, 1d; figure 2b, 2g; *Anthophora* spp.: table 1e, 1f; figure 2b, 2g).

Table 1. Smoothing variables and their effect on the frequencies of the tree Anthophila groups derived by GAMM analysis. The significant variables are in bold. The biotic and abiotic variables are in white background while interplay variables are highlighted in grey.

a	<i>A. mellifera</i> visiting flowers			c	<i>B. terrestris</i> visiting flowers			e	<i>Anthophora</i> spp. visiting flowers		
	edf	F	p		edf	F	p		edf	F	p
s(flowers)	1.000	53.48	<0.001	s(flowers)	1.000	12.218	<0.001	s(flowers)	1.874	18.01	<0.001
s(day)	1.986	75.84	<0.001	s(day)	1.964	21.613	<0.001	s(day)	1.939	23.057	<0.001
s(hour)	1.684	30.87	<0.001	s(hour)	1.95	26.46	<0.001	s(hour)	1.941	8.411	<0.001
s(wind)	1.978	110.12	<0.001	s(wind)	1.000	1.668	0.197	s(wind)	1.000	0.043	0.837
s(dist)	1.996	128.19	<0.001	s(dist)	1.695	3.009	0.125	s(dist)	1.534	2.617	0.183
	R-sq.(adj) = 0.305			s(AMtot)	1.000	57.161	<0.001	s(AMtot)	1.000	13.342	<0.001
					R-sq.(adj) = 0.17				R-sq.(adj) = 0.331		
b	<i>A. mellifera</i> flying			d	<i>B. terrestris</i> flying			f	<i>Anthophora</i> spp. flying		
	edf	F	p		edf	F	p		edf	F	p
s(flowers)	1.000	7.877	0.005	s(flowers)	1.000	3.932	0.048	s(flowers)	1.000	26.836	<0.001
s(day)	1.000	2.547	0.111	s(day)	1.899	17.892	<0.001	s(day)	1.907	16.648	<0.001
s(hour)	1.000	7.267	0.007	s(hour)	1.724	2.272	0.218	s(hour)	1.000	0.01	0.919
s(wind)	1.666	2.475	0.211	s(wind)	1.000	5.551	0.019	s(wind)	1.000	0.563	0.453
s(dist)	1.888	5.345	0.018	s(dist)	1.912	12.642	<0.001	s(dist)	1.871	5.881	0.006
	R-sq.(adj) = 0.0363			s(AMtot)	1.000	4.824	0.029	s(AMtot)	1.000	0.233	0.629
					R-sq.(adj) = 0.245				R-sq.(adj) = 0.266		

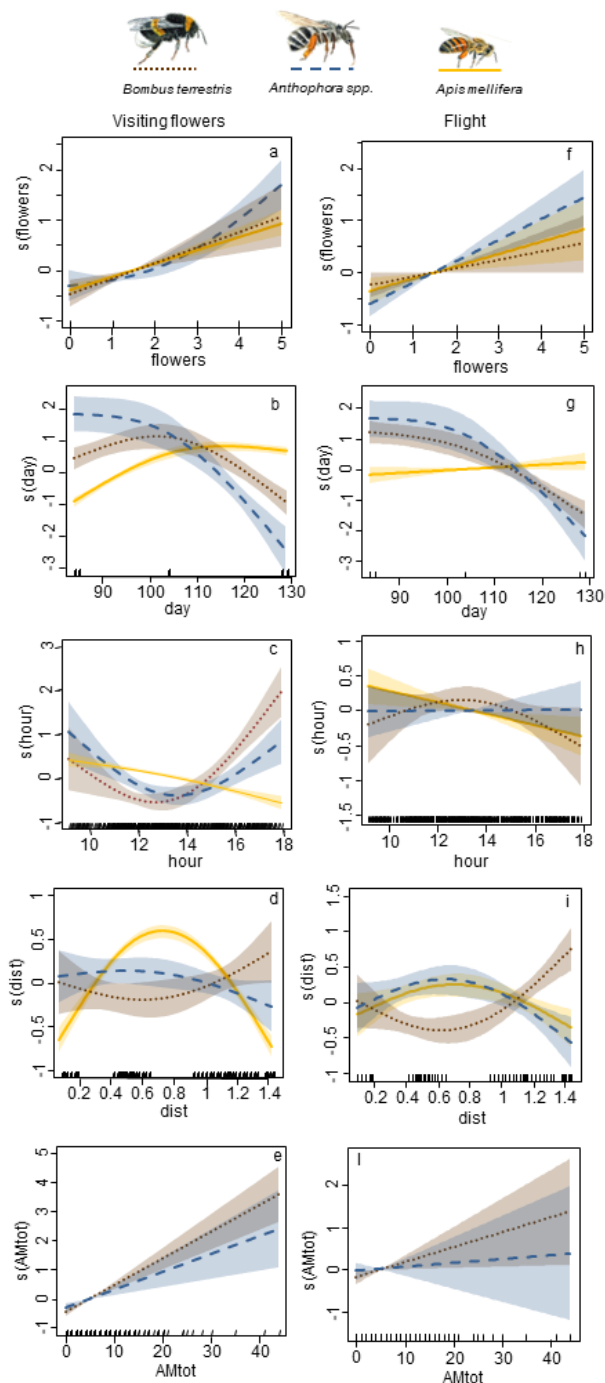


Figure 2. Smoothing curves of the abiotic and biotic variables, obtained through GAMM analysis and plotted together for the three bee taxa and divided by activity: a-e for “visiting flowers” and f-l for “flying”. Yellow lines represent *A. mellifera* relations, brown lines represent *B. terrestris* relations and blue lines represent *Anthophora* spp. relations.

The hour of sampling significantly affected bee occurrence as well, even if with different patterns among the three taxa. Honey bee abundance was negatively affected, even if slightly, by the hour of sampling, with both flower-visiting and flying individuals decreasing at later hours of the day. The relationship was nonlinear for flower-visiting individuals (table 1a; figure 2c) while linear for flying ones (table 1b; figure 2h). In bumblebees,

on the contrary, the sampling hour showed a curvilinear relation with an opposite trend in the two activities: in flower-visiting individuals occurrence had a maximum value in late afternoon (6:00 pm) and a minimum in the warmer hours of the day (12:00 pm - 2:00 pm) (table 1c; figure 2c), while in “flying” the maximum of the curve corresponded to this central moment of the day (table 1d; figure 2h). Occurrence of flower-visiting *Anthophora* spp. matched the pattern of bumblebees, with a curvilinear pattern with a maximum occurrence in the coolest hours of the day, early in the morning or late in the evening, and a minimum presence between 12:00 pm and 2:00 pm (table 1e; figure 2c). Occurrence of flying *Anthophora* spp. individuals was, on the contrary, not significantly affected by the hour of sampling, as shown by the horizontal trend, which means that the presence of flying wild bees was independent from the hour of the day (table 1f; figure 2h).

The distance from the apiary significantly affected bee occurrence as well, again with different patterns among taxa. In honey bees, a significant, bell-shaped relationship between distance from the apiary and occurrence revealed a maximum incidence of *A. mellifera* at a distance of 600-800 m from the apiary, for both flower-visiting and flying individuals (table 1a, 1b; figure 2d, 2i). The two transects located at this distance pass through an area of Mediterranean shrubs and in the area close to the village (figure 1b), making it difficult to link this high incidence of bees with the occurrence of a peculiar environment. Bumblebees showed an opposite pattern, with a reversed bell-shaped relationship which shows a minimum occurrence at a distance of 600-800 m from the apiary. This relationship was significant for flying bumblebees while it was not for flower-visiting ones (table 1c, 1d; figure 2d, 2i). In *Anthophora* spp. the distance from the apiary showed a similar pattern for both flower-visiting and flying individuals: a curvilinear pattern with maximum values in the range of 700-800 m, even if this variable was not significant for flower-visiting individuals (table 1e, 1f; figure 2d, 2i).

Finally, our data did not provide any evidence of spatial segregation, but, rather, they showed that bumblebee and *Anthophora* spp. abundance was overall positively correlated with honey bee presence. In both flying and flower-visiting bumblebees the occurrence had a linear and positive correlation with *A. mellifera* abundance (AMtot, figure 2e, 2l). A significant positive relationship was found for flower-visiting *Anthophora* spp. as well, with individuals increasing with the abundance of *A. mellifera* (table 1e; figure 2e). On the contrary, no significant relationship was found between *A. mellifera* abundance and flying *Anthophora* spp. (table 1f; figure 2l).

Aim 3

Flower-visitor network size (considering the visits of the three bee taxa across the whole sampling period, from March to May, pooled into a single visit network) included 20 nodes (the three bee taxa and 17 plant taxa). The network showed a high connectance (i.e. the realised proportion of possible links, $c = 0.706$), and moderate nestedness (NODF = 66.042), which suggests a relevant amount of overlap in visited plant taxa among the three

pollinator groups. This is confirmed also by the niche overlap index (0.801), the low network specialisation (0.158) and a moderate interaction evenness (0.487) (table 2). The network appears rather robust, i.e. the extinction curve would only decrease mildly until almost all species of a given level are lost, with a moderate robustness for pollinators (0.657) (table 2).

The network was dominated by *A. mellifera*. Honey bees were involved in 81.4% of the 2332 bee-flower interactions recorded (figure 3). Honey bees also visited a drastically greater number of plant species than both *Anthophora* spp. (16) and bumblebees (9) (table 3). The dominant role of *A. mellifera* is supported by PSI (pollination service index), higher for *A. mellifera* (0.849) than *Anthophora* spp. and bumblebees (respectively 0.170 and 0.212). While bumblebees visited a smaller number of plant species than honey bees and *Anthophora* spp., they tended to distribute their visits more equitably among the plant species (d' , bumblebees: 0.236, for honey bees: 0.127, *Anthophora* spp.: 0.115).

When analysing the bee flower-visitor networks of the three different periods, the overall network characteristics remained stable, with honey bees dominating the network (table 2). Some tendencies however emerged: the network became smaller and less generalised, mainly due

to a shrink in the importance of both *Anthophora* spp. and bumblebees, clearly paralleled by an increase in the dominance of *A. mellifera* from March to May (figure 3b and table 3). At the network level, this is confirmed by a decrease in network size and connectance and an increase in network specialisation (table 2). At the species level this is supported by the increase and decrease in pollination service index for, respectively, *A. mellifera* and *Anthophora* spp. from March to May (table 3).

Table 2. Network-level indices providing ecologically relevant information about the structure and functioning of flower-visitor networks along the three spring months; definition of each index in material and methods section.

Measures	Total	March	April	May
Network size	20	13	13	10
Connectance	0.706	0.733	0.633	0.524
Interaction evenness	0.487	0.670	0.465	0.322
H2	0.158	0.137	0.102	0.237
Nestedness	66.042	59.821	75.000	50.000
Niche overlap	0.801	0.730	0.860	0.678
Robustness	0.657	0.676	0.654	0.531

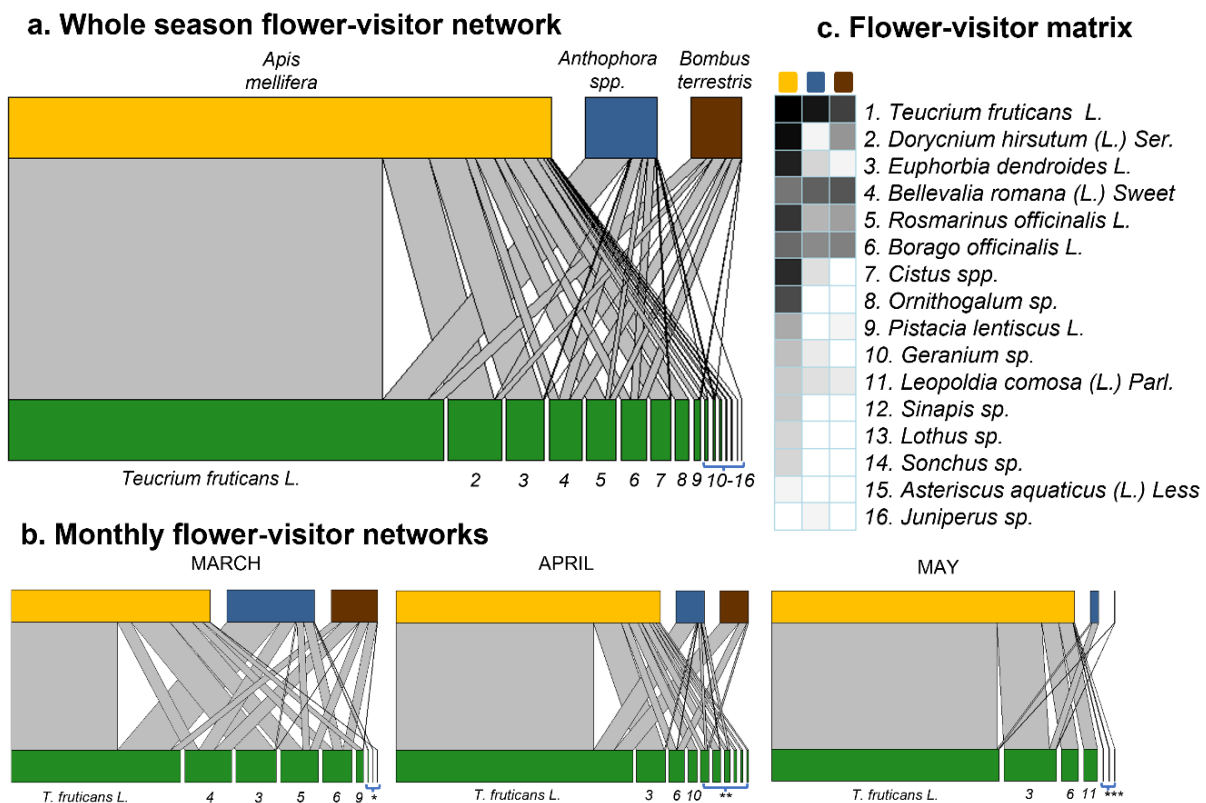


Figure 3. Flower-visitor networks on Giannutri island show the predominance of honey bees on both *Anthophora* spp. and *B. terrestris*, both considering the whole season (a) and each month separately (b). The overall network shows a moderate nestedness (c) suggesting a relevant amount of overlap in visited plant taxa among the three pollinator groups; * indicates *Geranium* sp., *Lothus* sp. and *Juniperus* sp.; ** indicates *Leopoldia comosa*, *Rosmarinus officinalis*, *Cistus* sp., *Sonchus* sp., *Pistacia lentiscus* and *Sinapis* sp; *** indicates *Lotus* sp., *Sinapis* sp., *Asteriscus aquaticus* (all in order from left to right in the network).

Table 3. Individual-level indices providing ecologically-relevant information about the role of each taxon in the flower-visitor network along the three spring months; definition of each index in material and methods section.

Sampling period	Total			March			April			May		
	AM	ANT	B	AM	ANT	B	AM	ANT	B	AM	ANT	B
Normalised degree	0.941	0.647	0.529	0.800	0.700	0.700	1.000	0.600	0.300	1.000	0.286	0.286
Pollination service index	0.849	0.170	0.212	0.662	0.316	0.263	0.841	0.134	0.191	0.973	0.003	0.104
Push pull index	0.730	0.140	0.087	0.533	0.315	0.076	0.725	-0.001	-0.082	0.837	-0.496	-0.432
Specialisation (Bluthgen's d')	0.127	0.115	0.236	0.110	0.102	0.210	0.056	0.072	0.169	0.04	0	0.273

Discussion

The analysis of the Anthophila community on Giannutri island revealed a strong influence of both abiotic and biotic variables on the abundance of the three main taxa, as in accordance with most of our predictions. Notably, we found no evidence for spatial and flower-resource segregations between managed *A. mellifera* and the two other wild bee taxa, *B. terrestris* and *Anthophora* spp. Indeed, the three taxa showed a great overlap in visited flower resources: *Anthophora* spp. and bumblebees showed higher abundance where also *A. mellifera* were noticeably abundant. This is not surprising since the three taxa show similar functional features (mid-large size and mid-long ligula) thus increasing the possibility for the use of similar resources. We also depicted the first flower-visitor network for the three pollinator taxa on Giannutri island, thus highlighting the most important floral resources that sustain the Anthophila community during spring. Crucially, this work provides a first assessment of the informative value of the monitoring protocol. Indeed, in this simplified insular ecosystem, the main predicted relationships between ecological features and pollinator abundance could be recorded using this monitoring protocol.

Anthophila abundance, relationships with ecological features and the potential influence of *A. mellifera*

As a first evidence, the abundance of the three taxa was positively influenced by the nectariferous floral coverage. While this relationship is rather predictable, not all methods are similarly able to recover it (O'Connor *et al.*, 2019). The hour of the day influenced foraging activity in the three taxa as well. We found that *Anthophora* spp. and *B. terrestris* show a daily variation in abundance with peaks in early morning and late afternoon and a decrease around midday. *A. mellifera* shows a different trend, with a linear decrease during the day, starting from a peak early in the morning, as already demonstrated (Abou-Shaara, 2014).

While honey bees can fly several kilometres away from their apiary in order to forage (Ratnieks, 2007; Couvillon *et al.*, 2014) and could easily cover the entire surface of Giannutri island (maximum distance from the apiary about 1.5 km), at the same time many studies confirmed their preference to forage close to the beehive in order to reduce energetic costs and time-investment (Couvillon *et al.*, 2014; 2015). This pattern is partially mirrored by our data, which depicted a bell-shaped abundance curve, with the highest abundance of *A. mellifera* at a distance about 600 metres from the apiary.

As it is suggested by Thomson (2004), spatial overlap among pollinators, such as the negative correlation between honey bees and native bee abundance, could be seen as indirect evidence of competition. Concerning Giannutri, the data do not provide evidence of spatial segregation, since the abundance of honey bees is positively correlated with the abundance of the other two Anthophila taxa. Moreover, the large majority of the Anthophila we recorded belong to mid-large species with a mid to long ligula (*Apis*, *Bombus*, *Anthophora*), which increases the possibility for resource use overlap as revealed by network analysis. However, more reliable assessment of the honey bee vs local wild bees competition would require more accurate and dedicated methods, such as the observation of behavioural interaction on flower resources thanks to observation plots, hopefully coupled with the experimental introduction and removal of bee-hives (Thomson, 2004; Shavit *et al.*, 2009).

Finally, the occurrence of the three taxa confidently mirrors the phenology of the investigated species. Despite our sampling effort covered just the late winter and spring months, a decrease in the abundance of *Anthophora* spp. in the last month of sampling (May) was evident, consistently with its phenology (Ballantyne *et al.*, 2017). Also, as expected in a human-managed social species, *A. mellifera* abundance did not decrease in late spring (Ornai and Keasar, 2020). *B. terrestris* showed a decrease in its abundance from March to May. This might appear counter-intuitive for a social species, and it is in contrast with previous studies overviewed by Rasmont *et al.*, (2008), where *B. terrestris* is found active all year with peaks in May and October. However, the decrease could be potentially explained by a reduced survival of colonies in this wild social species (compared to the human-managed *A. mellifera*), due to the variation in food availability that could affect, and slow down, the colony cycle and by the process of aestivation, well known in insular population of bumblebees (Gurel *et al.*, 2008). In this respect, the absence of spatial partition with honey bees and the consequent potential for competition for food resources could have exacerbated this phenomenon.

The most important plant resources for the pollinators on Giannutri

The data gathered through the monitoring protocol provided a first indication of the degree of overlap in visits to flower resources among the three taxa, as well as it allowed a rapid assessment of the main floral resources visited by the different Anthophila bees on the island. The flower-visitor network on Giannutri is overall well connected, robust and poorly specialised. The three taxa

indeed showed a relevant overlap in the flower resources they visited, thus producing a rather nested network with no evidence of resource partitioning. *A. mellifera* visited the highest number of different plant taxa, while *Anthophora* spp. and *B. terrestris* visited less plant taxa (figure 3). However, the flower visit profile is rather similar between *A. mellifera* and *Anthophora* spp. when looking at the distribution of visits among plant taxa, as both strongly relied on one single species (*T. fruticans*) for almost half of their visits and then allocated their visits to the remaining flower species in a more equitable way. Contrastingly, bumblebees look less specialised on particular flower resources. Overall, the network is clearly dominated by the very abundant *A. mellifera*, on the visitor side, and by *T. fruticans*, on the plant side. The visit network is rather stable along the season, showing as a main tendency only the marked increase in dominance by *A. mellifera*, likely due to the decrease in abundance of the two other taxa.

Our results suggest no clear partitioning among the three taxa in flower visiting. The evidence presented here suggests that at the current stage there is no evidence for a spatial exclusion of a given taxon as a consequence of the presence of other taxa. Long term monitoring data would be instrumental to assess the possible decline in the abundance of a given bee taxon, thus highlighting possible negative effects of the coexistence on the island. However, without direct manipulation of the abundance of given pollinator taxa (i.e. exclusion studies such as Wignall *et al.*, 2020) it would be rather difficult to assess the direction and the magnitude of the interaction among taxa.

Concerning the importance of different flower resources, our study highlighted the importance of *T. fruticans* as a main visited flower all along the spring season for all the three investigated taxa, and highlighted that other flowering species have a more time-limited influence on the visiting network, likely because of their narrower flowering seasons, (such as *E. dendroides* and *Bellevalia romana* L. in March and April, or *Dorycnium hirsutum* L. and *Cistus* sp. in later spring). Here again we highlight that the monitoring protocol provides a rapid way to gather information about the most important resources, but does not allow to infer pollinator flower preferences, as the abundance of flower resource taxa is not taken into account. A proper understanding of the nature and consequences of the interaction on both partners (plants and pollinators) will necessarily require more in-depth studies (such as observation plot, measurements of pollination transfer and fitness effects), especially considering the different relative abundance and energy-reward of the flower resources.

Advantages and disadvantages of the monitoring protocol

The main advantages of the monitoring protocol are its simplicity as well as the large amount of ecological information gathered. These transects are fast to be set up and could be performed by non-expert people with a minimum of training, as the protocol does not necessarily require extensive knowledge in recognising Anthophila species. Given the rising engagement of the public in

pollinator-correlated citizen science initiatives (Domrose and Johnson, 2017; Koffler *et al.*, 2021), the use of the monitoring protocol could provide an unprecedented contribution to the Anthophila community monitoring (Mason and Arathi, 2019; Fontaine *et al.*, 2021), similarly to the case of the eBMS project (Dennis *et al.*, 2017). Indeed, we have shown here that even a single season sampling can provide data to recover the main interactions among different Anthophila species and the relationships between their abundance and environmental variables. Therefore, the monitoring data provided by the monitoring protocol transects look promisingly informative to detect alterations of bee pollinators abundance and potential negative interactions between managed honey bees and wild Anthophila with a relatively simple monitoring protocol. Moreover, the monitoring protocol also allowed us to identify the most and the least influencing ecological variables for each taxon, a basic knowledge to establish conservation actions.

However, it must be noted that Giannutri is a very simplified ecosystem and the small dimension of the island and its relatively isolated position in the Tuscan Archipelago has determined the development of a very simplified ecosystem with a moderately low species richness (Whittaker and Fernández-Palacios, 2007). Future studies should thus test the informative value of the monitoring protocol in more complex ecosystems, where the Anthophila communities are richer. Moreover, the easiness of the method, due to the categorisation of Anthophila bees in few groups (*A. mellifera*, bumblebees at the species level and all other Anthophila grouped together), could also be its Achilles' heel. Due to the complex taxonomy and functional diversity of wild bees, merging most Anthophila into a few or a single category would likely result in losing any possibility to link overall bee incidence with environmental settings. The use of lower taxonomic categories, such as morphospecies based on the main genera and the coupling of the transect method with other techniques that guarantee a higher taxonomic resolution (such as pan traps) could solve this potential main weakness.

Conclusions

Despite the temporal limitation of this study (one season, March - May 2021), the information obtained with the basic version of the Italian Anthophila monitoring scheme provided interesting results. We found that the relations with abiotic and biotic variables and pollinator abundance reflected literature-based predictions. The monitoring data pointed out that currently there is no evidence for a spatial segregation between managed honey bees and wild bees. The flower-visitor network showed a great overlap between *A. mellifera*, *B. terrestris* and *Anthophora* spp. resources preferences suggesting that neither a resource-segregation is occurring. These results demonstrate the informative value of the monitoring protocol: it provides reliable monitoring data (allowing to clearly distinguish between managed honey bees and wild bee abundance trends) and at the same time it provides important ecological information for pollinators

communities conservation (such as the pivotal role of *T. fruticans* during the spring for Anthophila species on Giannutri island). In conclusion, the monitoring protocol meets the needs of a national and standardised, even though very simplified, monitoring scheme for pollinators and, due to its flexibility, it also could be used as a starting point for more in-depth studies, other than directly monitoring Anthophila pollinators abundance, for which taxonomic expertise remains crucial. Indeed, the long-term implementation of the monitoring protocol represents a first step to highlight species-species interactions, pollinator community dynamics and thus paving the way to a better understanding, and conservation, of this highly threatened group of pollinators, especially in such fragmented, isolated and small-size insular Mediterranean ecosystems.

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The authors declare no conflict of interest.

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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