

Article



Pollen Resource Repartition Between Managed Honey Bees (*Apis mellifera* L. 1758) and Unmanaged Bees in Three Italian National Parks

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Abstract: Despite its global importance for the maintenance of healthy ecosystems and its contribution in providing economic benefits to human society, the clade Anthophila is in severe decline worldwide. In this context, counteracting the decline in Apoidea is of fundamental importance. Honey bee density and beekeeping are believed to negatively impact wild bee populations, mostly through competition for food sources. In this study, interspecific trophic competition was investigated using the still seldom exploited approach of a palynological analysis combined with a metabarcoding analysis of the pollen gathered by both managed honey bees and wild bees in three Italian national parks. The entire trophic network was identified as highly specialized (H2' = 0.933). The results obtained suggest that, overall, wild bee species are sustained by different pollen sources than honey bees. This low sharing of resources could be due to the natural trend occurring in natural populations, where species tend to minimize the competitive overlap through niche differentiation or niche complementarity as a result of coevolution. National parks play a fundamental role in animal and plant species protection and conservation. Therefore, plans should focus on evaluating honey bee densities in the interests of achieving less intensive, more traditional, and sustainable beekeeping, as well as habitat restoration, to promote the survival and reproduction of wild bee populations.

Keywords: bee pastures; interspecific trophic competition; managed bees; palynological analysis; pollen repartitioning; protected areas

Academic Editor: Mário Gabriel Santiago dos Santos

Received: 19 December 2024 Revised: 17 January 2025 Accepted: 20 January 2025 Published: 24 January 2025

Citation: Boni, C.B.; Coppola, F.; Sagona, S.; Quaranta, M.; Flaminio, S.; Biella, P.; Tempesti, S.; Lazzeri, A.M.; Di Santo, M.; Felicioli, A. Pollen Resource Repartition Between Managed Honey Bees (*Apis mellifera* L. 1758) and Unmanaged Bees in Three Italian National Parks. *Conservation* **2025**, *5*, 5. https://doi.org/ 10.3390/conservation5010005

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1. Introduction

Insect pollinators are of global importance to ecosystems, as they provide fundamental ecological services and even contribute benefits related to their economic value to human society [1,2]. Among pollinators, bees are in severe decline worldwide [3]. Their decline is caused by different factors that act synergically and that can be individuated mainly into climate change factors [4], pollution (i.e., pesticides, fertilizers, urban and industrial pollutants) [5–7], modern agricultural practices, natural habitat losses, invasive species, and emerging diseases and parasites [8,9].

The Red List of European Bees assessed the status of 9.2% of the 1965 wild bee species analyzed as being at a lower risk of extinction (near-threatened (NT) status) or threatened (vulnerable (VU), endangered (EN), and critically endangered (CR) statuses). Conversely, for 55.6% of species, it was not possible to assess their threat status due to a lack of data, with data-deficient (DD) species primarily concentrated in the Mediterranean region [10]. Italy is one of the Mediterranean European countries with the highest bee species richness and the highest concentration of endemism [10]. The Red List of Italian Bees examined the status of 151 of the 1017 species occurring in Italy, indicating that 34 of these are at risk of extinction, or close to it [11]. Managed bees are considered a potential threat to wild bees because of the pathogen spillover risk [12–14], the genetic contamination of local bee populations [15–18], and interspecific trophic competition [12,19,20]. The occurrence of interspecific trophic competition between managed honey bees and wild bees is a widely debated topic in the scientific community [12,20]. According to some authors, honey bee density and beekeeping activities could affect different aspects of wild bee biology, such as the foraging behavior, fecundity, abundance, and survival of these populations [21–30]. Conversely, several authors reported the absence of negative effects of managed honey bees on wild bees [21,31–33]. A negative impact of managed honey bees on wild bees due to interspecific trophic competition was reported in 66% of studies, while the remainder reported the absence of competition or mixed effects, varying with the bee species or variables examined [20].

Several studies highlighting the presence of trophic competition have been performed in non-European countries, where the honey bee has only recently been introduced and can be considered an alien species [12,19,20,34]. Conversely, less studies have been performed in the native range of honey bees, where coevolution with other wild bee species may have led to a reduction in niche overlaps, limiting trophic competition [12,20,33,34].

Flowering plants mainly provide nectar and/or pollen to bees. Nectar is the main sugar source that allows adult bees to meet the energy needs required to fly, and it is a necessary source in *Apis mellifera* for honey storage [35,36]. A further sugar source for flight energy is available in the environment in the form of extrafloral nectars, produced by certain plants (e.g., Prunus *laurocerasus* and *Acacia dealbata*) and in the form of the honeydew produced by Rhynchota aphids, mealybugs, scale insects, whiteflies, and other plant parasites [37]. Conversely, pollen sources provide the proteins, lipids, carbohydrates, vitamins, and minerals necessary for brood rearing and development in both wild and managed bees, as well as for nurse honey bees, enabling their hypopharyngeal glands to produce royal jelly for feeding larvae [38–40]. Bees gather the pollen that is available in large amounts per unit of time [41]. A single wild honey bee colony will harvest 120 kg of nectar and 20 kg of pollen annually, and these amounts can be greatly exceeded in managed colonies [40].

The studies on trophic competition reported in the literature are based on several different direct and indirect methodologies, such as measurements of the volume of nectar gathered by bees [42], floral visitation rates, bee foraging behavior [43], wild bee population densities before and after the introduction of honey bees [44], etc. [12,20]. Pollinator–

flower interactions are often quantified as the flower visitation rates that are observed without discriminating between nectar or extrafloral nectar visitations from pollen visitations [30,45]. However, different bee species usually occupy different trophic niches [46] and when overlapping occurs, a single observation of flower visitation may not necessarily indicate competition [12]. To disentangle foraging dynamics related to flowers, resource overlaps or competition among species, palynological techniques applied to pollinator pollen loads are particularly useful [47].

The pivotal role of national parks in the protection of ecosystems is twofold. On the one hand, protected areas are fundamental for the conservation of pollinators such as bees, as they provide suitable habitat for bee survival, reproduction, and nesting [48]. On the other hand, national parks enhance and support the typical products, such as honey or other animal products, that are produced within the park territories and that characterize the park itself [49]. Nevertheless, national parks are desired destinations for sedentary and nomad beekeepers who manage a high density of hives in such natural landscapes, arousing concerns with regard to the negative impact of managed honey bees on natural and wild populations of wild bees [50–52]. In this context, the study of pollen resource repartition between honey bees and wild bees can provide useful data for a proper management of honey bees and for the identification of possible protection actions towards the most threatened bee species in national parks.

Therefore, within a wider project focused on increasing scientific knowledge of the ecology, biology, and conservation status of pollinators in three Italian national parks, this study aimed to (I) define which pollen resource supports different bee species populations, (II) detect pollen resource overlapping or repartition between managed honey bees and wild bees within the same area, in the native geographical region of the honey bee, (III) assess if palynological analysis may be an alternative suitable approach for studying interspecific trophic competition. We predicted that the trophic partitioning of the available pollen resources between managed honey bees and wild bees likely occurred as the result of a coevolution process leading to a niche differentiation or to a niche complementarity, with weak sharing of at least one dimension of the niche (i.e., a trophic or habitat-related or temporal niche).

2. Materials and Methods

2.1. Study Area and Pollen Sampling

The study was performed in three Italian national parks, from Northern to Southern Apennines: Foreste Casentinesi, Monte Falterona and Campigna National Park, Maiella National Park and Abruzzo, Lazio and Molise National Park (Figure 1). In each national park, pollen from both honey bees and wild bees was sampled from a pre-determined area (Foreste Casentinesi National Park: 43°92′5″ N, 11°79′3″ E; Maiella National Park: 42°04′7″ N, 14°18′9″ E; Abruzzo, Lazio and Molise National Park: 41°61′8″ N, 14°06′6″ E). Since the competition is expected to be highest when there is the greatest overlap in niche space [19] and at a distance of 0.6–1.1 km from the apiaries [28], the sampling areas were characterized by the presence of an apiary at a maximum distance of 1 km, set up in a mixed-heterogeneous and low-altitude (<1000 m) habitat. The locations of the sampling areas were chosen from those most representative of the habitats characterizing each national park.



Figure 1. The national parks (gray areas) and the respective sampling areas (black pots) in which the pollen samples from both managed honey bees and wild bees were obtained.

The pollen sampling was performed twice a year in summer (June–July–August) and in autumn (September–October) 2021. During each sampling session (each lasting 1 h, i.e., from 12:00 to 13:00), five wild bees carrying pollen were randomly captured with the entomological net, placed in a 50 mL plastic tube and killed by freezing (–20 °C). At the same time, 2 g of pollen gathered by a family of honey bees (*Apis mellifera*, hereafter referred to in tables and graphs as "HB") was collected from a pollen trap (Metalori type, for 10-frame Dadant-Blatt beehives) previously set up in the apiary, and then placed in a plastic tube. In order to achieve a sample that was as representative as possible, the use of pollen traps was preferred to a hand-netting capture of a large amount of honey bees. To facilitate the identification of the pollen types during the palynological analysis, a list of flowering species present in the surroundings of the sampling areas and the flower species on which wild bees were captured was also recorded. Each sample was labeled by using an individual alphanumeric code (reporting date, site, and progressive number of capture) and stored at –20 °C until analysis.

2.2. Palynological Analysis and Taxonomic Identification of Wild Bees

The pollen loads of each pollen sample gathered by honey bees were divided based on the color and each group was weighted. For each color, two pollen loads were suspended in 3 mL of sterile water and homogenized using a Pasteur pipette. A microscope slide for each color group was prepared by using 200 μ L of pollen suspension and glycerin jelly for permanent preparations. The identification of the pollen grains was performed using an optical microscope with total magnification of 400× and 1000×. The pollen grains were identified at the family, genus or species level by using a reference collection of the University of Pisa and pollen morphology guides [53–56]. The frequency of each pollen type in the blend was determined as a percentage of the 2 g analyzed mixture. The pollen gathered by wild bees was removed from every specimen using a steel spatula or by washing the insect in 3 mL of sterile water. A microscope slide for each pollen mixture gathered by wild bees was prepared using the same procedure described above. The pollen grains were counted to determine the relative abundance of each type for each slide. After the pollen analysis, all the captured wild bees were pinned and subjected to taxonomic recognition through the analysis of the diagnostic morphological traits in the entomological laboratory of CREA (Bologna, Italy) as well as using diagnostic keys [57].

A DNA-metabarcoding analysis was also performed on all the honey bee pollen samples to increase the identification of the pollen grain at a fine taxonomic resolution [47]. Honey bees feed on a wide range of plant taxa and, for this reason, conducting a DNAmetabarcoding analysis of honey bee pollen allowed us to obtain a detailed list of the available pollen resources present in the sampling area [58]. Following the protocols described by Tommasi et al. [59], the analysis focused on the ITS2 DNA region, and library preparation and samples sequencing were performed with Illumina MiSeq 600 V3 (2 × 300 bp paired-end sequencing). Firstly, primers were trimmed from the sequences, the DADA2 algorithm was applied to the forward strand to denoise, demultiplex and quality filter (expected error in forward: 2, quality score: 1), keeping final sequences in the range of 275–245 bp. The ESVs and representative sequences were taxonomically assigned to the lowest taxonomic rank using the BLAST algorithm and the MetaCurator reference dataset [60], and consensus taxonomy for each sequence was extracted. Moreover, the output of the taxonomic identification was further validated by visually inspecting the neighborjoining tree of all ESVs, and for the unidentified ESVs, we attempted to manually identify them using Basic Local Alignment Search Tool (BLAST) against the public NCBI Genbank online dataset based on the highest identity score and only if above 98%.

2.3. Data Analysis

The Pianka index [61], applied to the relative abundance of pollen types gathered by each bee species and obtained from palynological analysis and taxonomically refined with metabarcoding, was used to assess the niche overlap between honey bees and wild bee species within the same sample session and in the same sampling area:

$$\alpha j k = \frac{\sum_{i}^{n} P i j \times P i k}{\sqrt{\sum_{i}^{n} P i j^{2} \sum_{i}^{n} P i k^{2}}}$$
(1)

where α_{ik} is the Pianka index of the niche overlap between the bee species *j* and bee species *k*, *Pij* is the proportion of the plant taxa *i*th on the total resources used by bee species *j*, and *Pik* is the proportion of the plant taxa *i*th on the total resources used by bee species *k*. The index ranges from 0 to 1, indicating a low and a high degree of niche overlap, respectively. The overall trophic niche overlap between honey bees and wild bee species was analyzed by combining the data resulting from palynological analysis of pollen gathered by all the detected wild bee species and honey bees sampled from all national parks and during both sampling sessions. The Pianka index was also used to calculate the overall trophic partitioning, comparing resources used by honey bees with resources used by each detected wild bee species. To determine the degree of specialization of the overall network, the H2' index, ranging from 0 to 1 (indicating generalization and specialization, respectively) was calculated [62].

For the generation of the pollen network graphs and calculation of H2', the R-package bipartite (v 4.1.2; R Core Team 2022) was used [63].

3. Results

Overall, 31 wild bees, of which 26 were females and 5 males, belonging to eight different genera were collected. The most representative genus was *Bombus*, with five identified species and three non-determined species of a total of sixteen individuals; followed by *Lasioglossum*, with two identified species and one non-determined species comprising a total of three individuals; *Andrena*, with two species and three individuals; *Megachile* and *Colletes*, with two species and two individuals; and then *Halictus*, *Heriades* and *Dasypoda*, each represented by only one species and one individual. Out of a total of 20 captured and identified species, 13 are not reported in any checklist of the entomofauna of the national parks investigated in this study (Table S1). The identified species are not reported in the threatened, or at-risk, or data-deficient categories of The Red List of Italian Bees.

The palynological analysis of wild bee pollen and honey bee pollen allowed us to identify a total of 27 plant taxa, of which 4 were identified at the species level, 17 at the genus level, and 6 at the family level, with four different shapes (i.e., s, t, h, m shape) (Table S2).

The DNA metabarcoding analysis identified a total of 47 plant taxa, of which 28 were identified at the species level, 18 at the genus level, and 1 at the family level. Metabarcoding allowed us to refine five plant taxa resulting from the palynological analysis at species level (i.e., Carduus nutans, Hedera helix, Picris hieracioides, Satureja montana and Diplotaxis tenuifolia). The correspondence between the metabarcoding analysis of honey bee pollen samples and the palynological analysis on honey bee pollen samples are reported in Table S3.

In the Abruzzo, Lazio and Molise National Park, in the summer session, no correspondence between the two methodologies was recorded: the metabarcoding on the honey bee pollen sample identified 40 plant taxa, while only *Cistus* sp. was identified by the palynological analysis. Conversely, in the autumn session, both metabarcoding and palynological analyses recorded only the occurrence of *Hedera helix*.

In the Foreste Casentinesi National Park in the summer sampling session, no plant species was shared between the four wild bees and honey bees and within the four wild bee species, a very low degree of trophic overlap (*M. centuncularis*—*H. scabiosae*: $\alpha = 0.170$; *M. centuncularis*—*M. melanopyga*: $\alpha = 0.038$) was recorded. Conversely, in the autumn sampling session, *Lasioglossum* sp. and *Colletes hederae* shared a low proportion of trophic resource with honey bees ($\alpha = 0.098$ and $\alpha = 0.337$, respectively), and *Lasioglossum* sp. and *Colletes hederae* showed a high degree of trophic overlap ($\alpha = 0.970$) for *Picris hieracioides* (Figure 2, Table S2).



Figure 2. The pollen network of bees sampled in the Foreste Casentinesi National Park in summer and autumn, 2021. The lower bar width indicates the relative abundance (%) of the pollen grains gathered per plant taxa across bee species (upper bar); underlined plant species have been refined at the species level through the metabarcoding analysis (HB: honey bees; s.: shape).

In the Maiella National Park summer sampling session, a high degree of *Rubus* pollen overlap between the three *Bombus* species and honey bee (*B. terrestris*—honey bees: $\alpha = 0.918$; *B. pascuorum*—honeybees: $\alpha = 0.919$; *B. lapidarius*—honeybee: $\alpha = 0.918$) and within the three *Bombus* species (*B. terrestris*—*B. pascuorum*: $\alpha = 0.999$; *B. terrestris*—*B. lapidarius*: $\alpha = 1.00$; *B. pascuorum*—*B. lapidarius*: $\alpha = 0.999$) was recorded. Conversely, *Andrena decipiens* and honey bees showed a little overlap for *T. pratense* pollen ($\alpha = 0.096$). In autumn, a very low pollen-use overlap between *B. pascuorum* and *B. ruderatus* with honey bees ($\alpha = 0.107$, $\alpha = 0.015$,



respectively) was detected. *B. pascuorum* and *B. ruderatus* showed a little pollen use overlap (α = 0.017), while *Dasypoda hirtipes* shared no plant with any bee (Figure 3, Table S2).

Figure 3. The pollen network of bees sampled in the Maiella National Park in summer and in autumn, 2021. The lower bar width indicates the relative abundance (%) of the pollen grains gathered per plant taxa across bee species (upper bar); underlined plant species have been refined at the species level through the metabarcoding analysis (HB: honey bees; s.: shape; gr.: group).

In the Abruzzo, Lazio and Molise National Park, the amount of pollen source overlap between wild bees and honey bees, as well as within the wild bees species in both sampling sessions, was recorded (Figure 4, Table S2).



Figure 4. The pollen network of bees sampled in the Abruzzo, Lazio and Molise National Park in summer and in autumn, 2021. The lower bar width indicates the relative abundance (%) of pollen grains gathered per plant taxa across bee species (upper bar); underlined plant species have been refined at the species level through the metabarcoding analysis (HB: honey bees; s: shape; gr: group).

Overall, the analysis of pollen source utilization in both the sampling sessions and in all national parks (Figure 5), showed a highly specialized trophic network (H₂' = 0.933) and high trophic partitioning between each wild bee species and honey bees. The Pianka index of all the pairwise "honey bees-wild bees species" ranged between 0 and 0.193, except for honey bees–*B. pascuorum* (α = 0.537), honey bees–*B. lapidarius* (α = 0.558) and honey bees–*B. terrestris* (α = 0.385).



Figure 5. The pollen network of the combined data regarding the pollen use of all bee species sampled in the three national parks in both sampling sessions in 2021. The lower bar width indicates the relative abundance (%) of the pollen grains gathered per plant taxa across bee species (upper bar); underlined plant species have been refined at the species level through the metabarcoding analysis (HB: honey bees; s: shape; gr.: group).

4. Discussion

Although most of the wild bee species captured and identified in this study are quite common and are not included in the Red List of Italian Threatened Bees [11], the results obtained provide new data for updating the checklist of the wild bee fauna occurring in national parks. The report of *Bombus bisiculus* in the National Park of Abruzzo, Lazio and Molise is particularly relevant, as it has been only recently recognized as a new species in South Italy due to the elevation of the *decipiens*-like subspecies of *B. l. lapidarius* [64].

This study investigated the interspecific trophic competitive interactions between managed honey bees and wild bee species in three Italian national parks using the still seldom-exploited approach of a pollen diet analysis [21,51,65–70]. Compared to other methodologies, the analysis of the pollen gathered by bees allowed us to obtain useful data on bees' feeding habits per unit of time and space and was not limited to what the operator was able to observe during field experiments. Moreover, Allen and Davies [71] highlighted the underestimated importance of woodlands and canopies, where a significant proportion of bee abundance and diversity was found. In this context, the palynological analysis could address the lack of information on the interaction and on the pollen use of tree species by bees. Many studies that use the floral visitation rates to detect the overlap of resource use do not discriminate between whether bees visit the flowers for nectar or pollen gathering [26,30,72–74]. Consequently, the plant–pollinator networks resulting from the floral visitation rates may appear rich in interactions that do not provide actual data on the use of the trophic source, which ensures the survival and the reproduction of bees. In addition, the palynological analysis can provide information on the foraging activity of bees on plant species that are not in the immediate vicinity of the operator and/or that are not included in the list of botanical species exploited by bees. The limit of the identification of the plant taxa at species level through the palynological analysis has been overcome in this study with the application of the metabarcoding analysis, which provides a supplementary tool for the characterization of the pollen gathered by bees [47].

Nevertheless, some of the results reported in this study underline that the correspondence between the two methodologies is not always complete. Such a mismatch, which is difficult to interpret, is particularly evident in the results obtained from the summer sampling session in Abruzzo, Lazio and Molise National Park, where the palynological analysis detected the presence of only *Cistus* sp., while the metabarcoding detected a total of 40 different plant taxa. In this case, a plausible reason for the failure to detect the same plant sources of palynological analysis and metabarcoding may be the degraded status of the honey bee pollen that was gathered, sampled and analyzed. New efforts to optimize the combination of these two techniques are desirable.

Authors are aware of the small size of the wild bee samples; nevertheless, the results obtained in this investigation showed a breakdown of the pollen sources that were available within the same time-space conditions and were accessed by managed honey bees and wild bees in three national parks in the Northern-Central Apennines. A breakdown of the trophic resources used by managed honey bees and unmanaged non-Apis bees has also been previously reported in other studies performed in different countries [65,66,68,75,76]. Moreover, this study focused on the potential impact of A. mellifera on a variety of wild bee species by examining and comparing the use of pollen resources by managed honey bees and at least 17 wild bee species. Overall, the bee species sampled in this study represented a high trophic specialized network. By comparing the pollen gathered by honey bees to that gathered by every wild bee species, only a weak sharing of resources between honey bees and *B. pascuorum*, *B. lapidarius* and *B. terrestris* was found. The resource overlap between A. mellifera and Bombus sp. has already been reported by several studies [77–81] and it could be attributed to their generalist diets that tend to overlap or, alternatively, to the imbalance in the number of studies focusing on the interspecific trophic competition between bumblebees and honey bees, which may have created a bias [20]. The highly specialized trophic network combined with the low degree of dietary overlap within bee species may strongly suggest that in natural areas, such as national parks, wild bee species are sustained by different pollen resources than honey bees.

The trophic partitioning was particularly evident in Abruzzo, Lazio and Molise National Park in both summer and autumn, where the honey bees foraged exclusively on *Cistus* sp. and on *Hedera* sp., respectively. For both sampling sessions, a complete partitioning of the pollen resources was recorded among wild bee species, too (B. bisiculus, B. terrestris, Bombus sp., H. rubicola, A. pellucens, A. nitidiuscola and H. truncorum). In the Maiella National Park, in autumn, moderate sharing of the pollen use was recorded between honey bees and three species of *Bombus* (*B. pascuorum*, *B. lapidarius*, and *B. ruderatus*). Pollen resource partitioning was also detected in the Foreste Casentinesi National Park in summer, where the pollen gathered by honey bees (*Hedera* sp.) completely differed from that gathered by B. pascuorum, M. centuncularis, H. scabiosae, and M. melanopyga (Lythrum salicaria, Compositae T shape, Compositae H shape, Centaurea f., Labiatae M, and Compositae S shape). These results indicate the presence of a strong or a moderate pollen resource repartition between honey bees and wild bees. The strong repartition could be the effect of a reduced sample size, or the outcome of a natural trend occurring in natural populations. In this latest case, species tend to minimize the competitive overlap by a niche differentiation [46], or share, with a low degree, at least one dimension of the niche (i.e., trophic or habitat or temporal niche), following the hypothesis of the niche complementarity. Many Apoidea species display flexible and generalist feed habits and can shift their diet in the presence of other potentially competitive Apoidea species [66,75,82,83]. In this way, an alternative hypothesis supporting the presence of trophic repartitioning is that this breakdown could be the consequence of ongoing interspecific trophic competition, which leads species to differentiate their diets. Furthermore, although the diet of honey bee is commonly reported as super-generalist [9,84], the results in this study

suggest that the actual pollen use is represented by a relative small number of plant taxa, as already reported by Hawkins et al. [85]. This selection of the pollen source by honey bees is evident when the amount of plant taxa resulting from the metabarcoding are compared with the few plant taxa resulted from the palynological analysis.

In this study, the highest degree of sharing of the pollen resources was recorded in summer in Maiella National Park of *Rubus* sp. pollen, between honey bees and wild bees and within three *Bombus* species, probably due to the high abundance of this pollen resource in the sampling area. This hypothesis could be supported by previous studies reporting high exploitation of the same flower resource by different bee species, when it is abundantly present in the environment [65,66,75]. Conversely, when floral resources are variable and heterogeneous, different species within the same genus tend to differentiate their trophic niches by repartitioning of the trophic sources [86,87].

Although there is still a clear degree of uncertainty about the effect of honey bee trophic competition on wild bees, a change in wild bee foraging behavior may not necessarily have negative impacts at the population level [12]. In support of this, from the studies present in the literature analyzing the use of pollen, at least four different scenarios could be highlighted: (i) there is a high overlap of the pollen use between honey bees and wild bees, but there are clear differences in their dietary preferences [51]; (ii) honey bees and wild bees gather the same pollen type with different peak gathering times, i.e., there is a differentiation of the temporal niche [88]; (iii) honey bees and wild bees share the same pollen sources, but there are no negative impacts on wild bee progeny body mass [21]; (iv) the overlap of pollen use between honey bees and native bees is rather low, and different bee populations are supported by different pollen resources [65–67,70].

Particularly interesting are the results concerning the foraging behavior of *Colletes hederae*. This ubiquitous solitary bee has always been reported as an oligoleptic species, with a strict foraging preference for *Hedera* sp. [89]. However, in the Foreste Casentinesi National Park, during the autumn sampling session, *C. hederae* showed more flexible for-aging behavior, almost exclusively gathering pollen from *Picris hieracioides*. This result could allow us to speculate on the foraging preference of *C. hederae*, considering it a pseudo-oligoleptic species, with binding to *Hedera* sp. due the typical lack or insufficient presence of other attractive nectar and pollen sources during the late season, rather than a species with a foraging preference [90]. However, further investigations on *C. hederae* feeding habits are needed to confirm this hypothesis.

This study provides an insight into the complex discussion on the threats beekeeping poses to biodiversity conservation and, in order to deepen scientific knowledge of the dynamics of interspecific interactions between honey bees and wild bees, some of the limitations encountered in this study must be overcome. Firstly, the niche overlap between two bee species can be better supported by an adequate sample size, which, in this preliminary study, was limited to five wild bee specimens for each sampling session and, additionally, was affected by the inadvertent collection of male individuals. Also, it would be of great interest to investigate bees' diets during the spring seasons and with a higher sampling frequency. Such limitations highlight clearer directions for future research, suggesting the need for a larger sample size, an extension of the investigation into bees' competition for nectar, and an outline of the sustainability of the beekeeping practices in national parks [28].

The results obtained in this study suggest that the beekeeping practiced in the national parks investigated here does not pose a threat to wild bees through interspecific pollen competition. Although strategies for the protection of insects and wild bees are still poorly developed in protected areas [91], national parks are crucial for the conservation of wild bees [48] and, at the same time, are needed to enhance local and traditional production activities, such as beekeeping [49]. In this scenario, there is a need to identify solutions for the coexistence of the beekeeping and the protection of wild bee species [92] by regulating and mitigating, as precautional principles, some intensive bee management practices in national parks [28]. Some of these could include the adoption of more traditional beekeeping practices, such as the exclusion of large-scale nomadism, reducing the apiaries consisting of excessive bee colony densities, and excluding the management of non-native subspecies of honey bee.

5. Conclusions

Palynological analysis was found to be an efficient quantitative method for studying the interspecific trophic competition between managed honey bees and wild bees. The main limit of this approach is that it performs pollen identification at the species level. However, combined use of palynological and metabarcoding analysis allows us to achieve a fine taxonomic resolution in pollen species identification, facilitating highly effective quantification of the resource overlap. The high amount of resource partitioning found in this investigation indicates that the trophic resources sustaining and providing feed for the reproduction of honey bees differ from those of wild bee species, while the sharing of pollen sources occurs mainly when such sources are abundantly available in the environment. These results are most likely the outcome of coevolutionary processes that regulate the population dynamics (i.e., reduced niche overlap and niche complementarity) and they also suggest that interspecific trophic competition by exploitation has a lateral role in the impact of beekeeping on wild bee populations. Conversely, further investigations focusing on the potential negative impact of the pathogen spillover and the genetic introgression managed honey bees impose on unmanaged bees are desirable. In this context, further studies on the competition dynamics occurring within bee species that aim to compensate for the lack of data on the threat state of populations are desirable and necessary to delineate conservation action plans related to wild bee species and ecosystems. Conservation plans within the investigated national parks should focus on maintaining less intensive beekeeping and protecting habitats in order to optimize the availability of resources useful for the survival and the reproduction of wild bee populations.

Supplementary Materials: The following supporting information can be downloaded at www.mdpi.com/article/10.3390/conservation5010005/s1, Table S1: Species of wild bees captured during the summer and autumn pollen sampling session in the Habitat Spots of Foreste Casentinesi National Park, Maiella National Park and Abruzzo, Lazio and Molise National Park. Sex, number (N) of individuals for each identified species and presence in checklists (1: Comba. 2016; 2: I-Naturalist; 0: none) are also reported. (* = reported in the checklist as *Bombus lapidarius*). Table S2: Composition (%) of pollen samples collected from both honey bees and wild bees species in each National Park and in each sampling session. (HB: honey bees; s.: shape; gr.: group). Table S3: Correspondence between metabarcoding analysis and palynological analysis (%) of honey bee samples in summer and autumn sampling sessions in Foreste Casentinesi National Park, Maiella National Park and Abruzzo, Lazio and Molise National Park (HB: honey bee).

Author Contributions: Conceptualization, C.B.B., F.C. and A.F.; methodology, C.B.B., F.C., A.F., S.S. and P.B.; data collection, M.D.S., A.M.L. and S.T.; formal analysis and investigation, C.B.B., F.C., A.F., S.S., P.B., M.Q. and S.F.; writing—original draft preparation, C.B.B., F.C., A.F. and S.S.; writing—review and editing, C.B.B., F.C., A.F., S.S., P.B., M.Q., S.F., M.D.S., A.M.L. and S.T.; supervision, A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Foreste Casentinesi National Park, Majella National Park, Abruzzo, Lazio and Molise National Park, under the project name "Ricerca e conservazione sull'apoideofauna nei Parchi Nazionali dell'Appennino centro-settentrionale" within the Direttiva Biodiversità 2019–2020 of Italian Ministry of Ecological Transition. Author PB is supported by the

Italian Ministry of Universities and Research (with resources from the PONRI FSE REACT-EU 2014–2020—"Azione IV.4—Dottorati e contratti di ricerca su tematiche dell'innovazione, Azione IV.6—Contratti di ricerca su tematiche Green").

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors wish to thank Cinzia Sulli (Parco Nazionale d'Abruzzo, Lazio and Molise), Luciano Di Martino, Giuseppe Marcantonio (Parco Nazionale della Majella), and Davide Alberti (Parco Nazionale delle Foreste Casentinesi) for their technical support and Piotr Medrzycki for his valuable suggestions on the first draft. The authors also thank the beekeepers (Luigi Noviello and Paolo Mainetti) who allowed us to sample honey bee pollen from their hives.

Conflicts of Interest: The authors declare no conflicts of interest.

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