

1 **Key-words:** *Apidae*, Amplicon sequencing, Interaction, Metabarcoding, Network, Pollinator, Plant-  
2 pollinator interaction, Gut microbiome

### 3 **Abstract**

4 Our understanding of plant-pollinator interaction networks hinges on the methods used to describe their  
5 nodes and links. Most networks are built from field observations that may overlook many consumer-  
6 resource links, and these networks lack descriptive links that characterize interaction types and out-  
7 comes. Towards a more complete approach for building interaction networks, we compare plant inter-  
8 actions from the wild pollinator species, *Bombus pascuorum*, recorded by three methodologies with  
9 different implications for interaction outcomes. We compare floral visitation interactions obtained  
10 from field observations, plant consumption interactions revealed by metabarcoding of gut contents,  
11 and pollen transport interactions detected by metabarcoding of corbicular pollen loads. Our approach  
12 adds functional context to plant-pollinator network links and reveals new interactions. We show that  
13 both metabarcoding approaches increase the number of interactions and reveal links that were over-  
14 looked by field observations of visitation, highlighting plant taxa that are not pollinator-dependent, yet  
15 constitute important dietary resources. Paired with floral diversity surveys, gut-content results also  
16 reveal seasonal patterns in the spatial extent and functional diversity included in forage, which other  
17 methodologies fail to demonstrate. Metabarcoding data analyzed at the individual specimen level fur-  
18 ther contribute heterogeneity in plant resource use between pollen transport and consumption. Metabar-  
19 coding methodologies capture greater spatial, temporal, and taxonomic ranges, while field observations  
20 provide validating datasets with higher taxonomic precision. Our results show that integrating visita-  
21 tion, transport, and consumption data changes network topology and the roles of plant nodes, offering

22 a more nuanced and complete map of interactions with clearer priorities for management. We advocate  
23 for defining links explicitly by their functions and combining methods to account for hidden structure  
24 in ecological networks.

## 25 **Data and Code for peer-review**

26 All data and code used for the analyses in this manuscript are provided on an Anonymous GitHub repos-  
27 itory ([https://anonymous.4open.science/r/B\\_pascuorum\\_interaction\\_networks](https://anonymous.4open.science/r/B_pascuorum_interaction_networks)). All raw amplicon se-  
28 quencing data will be deposited in a project in the European Nucleotide Archive upon final manuscript  
29 acceptance.

## 30 **1. Introduction**

31 Pollination is a critical ecosystem service that is currently threatened by global anthropogenic change,  
32 including habitat loss, intensifying agriculture, pathogens, and invasive species (Klein et al., 2006).  
33 Pollinators crucially support the reproduction of 94% of wild flowering plants and 75% of crop species  
34 (Vanbergen & Insect Pollinators Initiative, 2013), contributing to 35% of global food production (Klein  
35 et al., 2006). Despite the clear importance of understanding plant-pollinator interactions, our knowledge  
36 of interaction diversity remains incomplete, as the methodological approach to studying plant-pollinator  
37 interactions has historically been biased towards the plants (Bosch et al., 2009; Evans & Kitson, 2020).  
38 As a consequence, the well-established relationship between pollinator diversity and the productivity of  
39 plant communities (Artamendi et al., 2025; Katumo et al., 2022; Woodcock et al., 2019) lacks an equally  
40 developed mirrored perspective, describing the floral diversity that supports pollinator populations.

41 Network theory provides a useful framework to summarize patterns of plant–pollinator interaction

(Burkle & Alarcón, 2011), but most studies under this framework have yet to account for how the scope of networks is influenced by the interaction types that define links. Existing methodologies for reconstructing interaction networks tend to emphasize structural patterns, while overlooking the functional outcomes of interactions that are critical for understanding how plant communities support pollinators (Quintero et al., 2022). In *Apidae* species, for example, plant interactions may have several outcomes. Bees consume plant material, including pollen, nectar, or even plant tissue (Pashalidou et al., 2020; Vaudo, 2015). They also collect pollen on their corbicula for transport to the nest for feeding drones and larvae (Leach & Drummond, 2018; Vaudo, 2015). Finally, visitation of the reproductive parts of flowers can have various outcomes for both the plant and pollinator, including pollen transfer (Emer & Memmott, 2023) and pathogen transfer (Lignon et al., 2024). Interaction networks generally represent only one of these outcomes, although each is important to understanding how plant taxa support pollinators.

The importance of different outcomes in plant-pollinator interactions becomes clear when considering the biodiversity necessary to support pollinators across life stages. Because the resources needed for a foraging adult pollinator are different from those needed at the larval stage, or by other colony members (Leach & Drummond, 2018; Vaudo, 2015), transported pollen may not completely represent the interactions necessary to sustain adult pollinators. This is especially true for bumblebees (*Bombus spp.*), which are able to evaluate pollen resource quality to discern foraging choices (Leonhardt & Blüthgen, 2012; Timberlake, de Vere, et al., 2024). Bumblebees make trial-and-error floral visits in order to find adequate forage (Selva et al., 2024), which may result in pollen transport without consumption. Conversely, consumption, or simply visitation, may occur without resulting in transport (Popic et al.,

2012). Accounting for different interaction outcomes, such as visitation, transport, and consumption, is a critical next step in representing the network of plant diversity used by pollinators.

Incorporating the pollinator-perspective by leveraging complimentary methodologies can produce a comprehensive understanding of the contexts within which plant-pollinator interactions occur. Microscopy and molecular analyses of pollen load samples sourced from insect specimens often identify greater plant species diversity within interaction networks compared to only field observations of floral visitation (Baksay et al., 2022; Bosch et al., 2009). Additionally, studies adopting a pollinator-centric view have revealed greater detail in forage preference trends, such as how pollinators use forage quality or quantity-based strategies (Selva et al., 2024; Timberlake, de Vere, et al., 2024), seasonal changes (Leponiemi et al., 2023), life cycle timing, and metabolic specialization (Vaudo, 2015).

Genetic tools can detect plant-pollinator interactions that may be unobserved in pollen microscopy and traditional field surveys (Arstingstall et al., 2021; Bell et al., 2016; Lowe et al., 2022; Pornon et al., 2017), and target specific interaction types. Metabarcoding of conserved gene regions from pollen samples can complement field observations of visitation (Arstingstall et al., 2021; Bell et al., 2017), increasing species detection by 9 - 144% (Baksay et al., 2022; Milla et al., 2022; Smart et al., 2017) and network sampling completeness up to 30%, while reducing exaggeration of specialization (Arstingstall et al., 2021) and revealing interactions beyond the traditionally surveyed floral community (de Vere et al., 2017; Milla et al., 2022). Advances in the reliability and accessibility of sequencing technologies have made these approaches more feasible for studying plant-pollinator interactions.

Most pollinator interaction network studies that apply metabarcoding focus on the external pollen loads of bees or pollen stored in nest reserves of honey and beebread (Baksay et al., 2022; Devriese et al.,

84 2024; Leontidou et al., 2021; Leponiemi et al., 2023; Selva et al., 2024), despite limitations of these  
85 sampling targets. Pollen in these samples can come from the environment, even including accumulation  
86 of windborne material (Negri et al., 2015). To account for this, past studies have ignored detections  
87 of wind-pollinated taxa (Pornon et al., 2017; Tanaka et al., 2020), although this may introduce bias  
88 to results, given that many plant taxa are both wind- and insect-pollinated (Saunders, 2018). A more  
89 fundamental issue with externally carried pollen and nest reserves is their restricted ability to represent  
90 interaction types. Corbicular pollen provides an easily obtained sample, containing pollen from one or  
91 more plant species collected for transport to the nest for brood feeding (Leach & Drummond, 2018;  
92 Vaudo, 2015), only directly representing pollen transport interactions (Arstingstall et al., 2021). Given  
93 the role of this pollen in bees' life cycles, it is often suggested as the central sample type reflecting diet,  
94 thus used to represent foraging networks (Shi et al., 2025), and considered synonymous with successful  
95 pollination interactions. This may overstate the function of corbicular pollen.

96 Pollinator intestinal tracts (hereafter: guts) represent an additional source for observing dietary inter-  
97 actions, specifically consumption of pollen and other plant material (Haag et al., 2023; Li et al., 2025;  
98 Mayr et al., 2021). Plant DNA detected in gut contents can reveal interactions with consumption as the  
99 exclusive outcome, which, in addition to flower visits, can include nectar robbing (Popic et al., 2012)  
100 and occasional herbivory (Pashalidou et al., 2020). The gut-content approach can also account for en-  
101 vironmental contamination in external pollen and nest stores by highlighting oversights resulting from  
102 the exclusion of interactions with the anemophilous and partially-anemophilous plant taxa in external  
103 pollen studies. There is an accumulating body of evidence supporting the idea that pollinators regularly  
104 search across functional groups of the plant community to meet their nutritional needs (de Vere et al.,

105 2017; Ibiyemi et al., 2025; Milla et al., 2022; Pojar, 1973; Selva et al., 2024; Tanaka et al., 2020; Ter-  
106 rell & Batra, 1984; Timberlake, de Vere, et al., 2024; Wood et al., 2022), although little attention has  
107 been given to these observations as a potentially important part of plant-pollinator networks (Saunders,  
108 2018). This understudied component of pollinator foraging together with the surprising lack of genetic  
109 analyses of pollinator gut contents, represents a clear knowledge gap and an opportunity to uncover  
110 finer detail in plant-pollinator interaction networks.

111 Our objective is to determine whether a combined methodological approach can provide further insights  
112 into pollinator forage ecology and plant-pollinator interaction networks by expanding interaction detec-  
113 tions and providing context to network links. We assess how metabarcoding of pollinator gut contents  
114 can complement or challenge the characterization of plant-pollinator interaction networks described  
115 by more common methodologies, including field surveys of plant-pollinator interactions and external  
116 pollen load metabarcoding. To this end, we compare interaction networks constructed from each of  
117 these methodologies for a single model pollinator, *Bombus pascuorum*, an easily identified bumblebee  
118 common to most of Europe (Lecocq et al., 2015). Our focus on a single pollinator species holds pol-  
119 linator identity constant and attributes differences in network structure to methodology, rather than to  
120 variation among pollinator species. We hypothesize that the consumption interactions detected in gut  
121 metabarcoding will include a network of plant taxa distinct from those detected by other methodolo-  
122 gies. Although we expect overlap between networks constructed by different methodologies, we expect  
123 to observe previously overlooked interaction network structure, including new links and significance  
124 of network links. Ideally, the resulting combination of observations will generate a network that will  
125 elevate our capacity to detect meaningful plant-pollinator interactions, and learn more about interaction

126 types and implications for pollinator health.

## 127 **2. Methods**

128 Our sample collection was conducted in Gorbeia Natural Park (coord: 43.068, -2.796) , a protected  
129 area in northern Spain. Within Gorbeia, we selected 16 sampling sites located within the mixed zones  
130 of meadows and shrublands found at higher elevations. We conducted fieldwork from early April to  
131 the end of July, 2023 covering the main flowering period and peak annual pollinator activity in Gor-  
132 beia. On each sampling day during this timeframe, we visited field sites in pairs. Sampling days were  
133 organized into six periods, in which we sampled each site pair once per period. We conducted three  
134 types of surveys during daily peaks of pollinator activity, including floral diversity surveys (“flower  
135 counts”), interaction transect surveys, and *Bombus pascuorum* specimen collection for amplicon se-  
136 quencing analyses.

### 137 *Interaction transects and floral resource availability surveys*

138 We used one 250 m transect at each site for both interaction transect and flower count surveys, recording  
139 observations within a ~2 m wide transect line. Interaction surveys were conducted three times per day,  
140 each lasting 1 h. All insects observed contacting the reproductive parts of herbaceous flowers within the  
141 transect were recorded; for this study, we retained only *Bombus pascuorum* interaction data. Surveys  
142 were spaced by ~2 hours (~11:00, ~13:00, ~15:00), and transects were walked at a constant pace to cover  
143 the full length within an hour. For each site and sampling period, one flower count was conducted by  
144 recording all of the flowering herbaceous species within the transect.

### 145 *Bombus pascuorum* specimens

146 For every period visit at each site, we collected up to five *B. pascuorum* specimens for molecular  
147 analyses (N = 126). We brought specimens back from the field and froze them at -20°C until processed.  
148 In the lab, we extracted the entire gut and honey stomach of *B. pascuorum* individuals. Additionally,  
149 if present, we collected pollen from the corbicula of specimens into sterile 1.5 mL centrifuge tubes.  
150 Pollen samples were stored individually by specimen sample at -20°C.

#### 151 *Gut-content DNA extraction*

152 Genomic DNA was extracted from *B. pascuorum* guts using the NucleoSpin® 96 Soil kit (Macherey-  
153 Nagel, Düren, Germany) and amplified in duplicate using the DFD forward and ASDFAS reverse  
154 primers. To avoid site and period bias, all samples were randomized before the DNA extraction. We  
155 followed the kit manufacturer protocol, only adjusting centrifuge spin duration to account for differing  
156 maximum velocity available within our centrifuge (See Supporting Information). Nanodrop spec-  
157 trophotometry was used to quantify DNA concentration and purity of the extracts by measuring re-  
158 flectance at 260/230 nm wavelengths.

#### 159 *DNA extraction from corbicular pollen pellets*

160 DNA was extracted from pollen pellets (N = 25) using the Machery-Nagel NucleoSpin® 8 Food kit,  
161 including additional initial steps recommended by the kit's supplementary protocol for pollen DNA ex-  
162 traction (See Supporting Information). The Qubit high sensitivity dsDNA kit (Thermo Fisher Scientific)  
163 was used to quantify DNA extract concentrations for randomly selected samples.

#### 164 *Amplicon sequencing*

165 We used a dual-indexed amplicon multiplexing approach to generate our metabarcoding library, as

described previously by Donald et al. (2022). Briefly, we performed a first step amplification using the Nex-F & Nex-R tagged internal transcribed spacer 2 primer pair, ITS-S2F (Chen et al., 2010) and ITS4R (White et al., 1990), with the following modifications: (1) 3-6 N-mers to increase sequence base diversity and (2) linker sequences that complement index linker. Amplified products were checked on a 1% agarose gel for successful amplification. The resulting amplicons were used as template in the second-step PCR where unique 8-mer indices and illumina p5/p7 sequencing primers were attached. Amplicons were checked for successful amplification as above, purified, normalised and size selected using SPRI-beads, and pooled equi-volume to generate the amplicon library. The resulting library was quantified using Qubit HS kit and functional library was estimated using Colibri qPCR assay. Final library was sequenced on the illumina MiSeq 3000 instrument for 300 cycles in paired-end mode at the Ecological Genetics laboratory, Bioeconomy Science Institute, Auckland, New Zealand. Detailed methods provided in Supporting Information

#### *Bioinformatics: taxonomic assignment and contaminant analysis*

Raw base call files (BCL) were converted to fastq using bcl2fastq2 (v2.20) tool. Demultiplexing was performed on the raw fastq files using index combinations and primer pair sequence simultaneously to minimize non-target data, resulting in paired raw reads per sample. Raw reads were processed using the DADA2 bioinformatics pipeline (Callahan et al., 2016). Reference ASVs were used to call taxonomic identities using the assign taxonomy method in dada2 in conjunction with the published ITS2 reference database of Bell (2021). We selected the database due to high species coverage for our study area. All but 21 species were identified to species level, with the remaining 21 identified to genus level. Taxonomy data were combined with sample x ASV matrix and sample metadata in phyloseq R package

187 (add citation) and downstream quality control and statistical analyses were performed in the R analysis  
188 environment. For contaminant and misidentified ASV removal, we used a three-step screening process.  
189 First, ASVs were analyzed for contaminants using the R package, decontam (Davis et al., 2018). Sec-  
190 ond, we conducted a BLAST search using ITS2 Database (Ankenbrand et al., 2015) to verify taxa that  
191 were identified by singleton ASVs within our results. Finally, the remaining list of taxa was screened  
192 against a locally specific herbarium (Agut & Hermosilla, 2025).

### 193 *Statistical analysis*

194 We compared the results of each methodology across methodology, time, and individual specimens.  
195 As an initial broad test of whether the methodologies detected interactions with different plant com-  
196 munities, we used binary presence-absence matrices to compare the communities detected by each  
197 methodology on each sampling day. Data were aggregated by sampling day for all sets of observations.  
198 Community composition was contrasted using the Raup-Crick dissimilarity index in a PERMANOVA  
199 test within the R package, vegan (Oksanen et al., 2024) with methodology as the independent vari-  
200 able. Further pairwise comparisons of these data were made by subsetting the dissimilarity matrix used  
201 in the first test by each unique methodology pair and using multiple pairwise PERMANOVAs. We  
202 also used vegan to observe beta dispersal of our data as a further means of testing the assumptions of  
203 PERMANOVA analysis.

204 Of the 126 *B. pascuorum* specimens, 25 yielded both pollen and gut samples. Using this subset of  
205 paired samples, we compared the plant communities detected by the two metabarcoding methodologies  
206 at the individual sample level without aggregation. As before, Raup-Crick dissimilarity matrices were  
207 calculated using binary detection data from pollen and gut detections. PERMANOVA compared both

methodologies' detected communities in strata defined by specimens of sample origin.

#### *B. pascuorum*-plant interaction network metrics

We used interaction frequencies from the three methodologies to build *B. pascuorum*-plant interaction networks and calculate species-level metrics for plant importance and specialization. Plant importance was defined as the proportion of all *B. pascuorum* interactions involving a given plant genus. For metabarcoding and pollen-load data, interactions were counted as the number of individual bee samples in which a plant genus was detected; for observational data, interactions corresponded to recorded visits. Species-level specialization ( $d'$ ) was calculated following Blüthgen et al. (2006), as implemented in the R package bipartite (Dormann et al., 2009).

We created a composite interaction network for *B. pascuorum*, incorporating the data of each methodology and the interaction outcome types as network metadata. Network nodes included *B. pascuorum* and the list of plant genera detected across the three interaction datasets. Single plant genera were assigned between one and three links corresponding to interaction type, depending on their detection across methodologies.

### **3. Results**

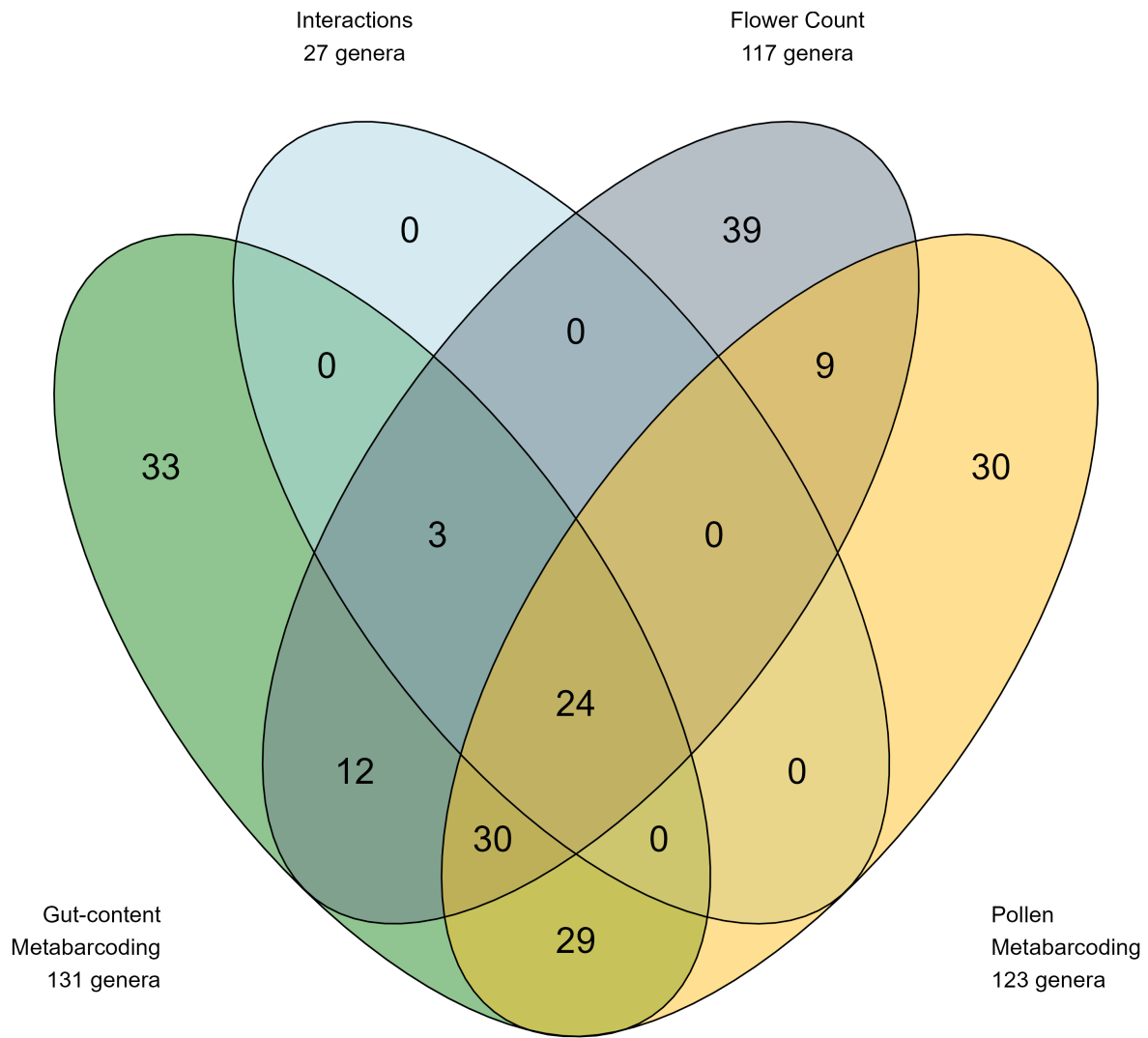
#### *Assessment of floral resource use relative to availability*

Within our flower count surveys we registered a total of 117 flowering herbaceous plant genera across the sampling season, representing the pool of floral resources available to *B. pascuorum*, which interacted with only a subset of this diversity (Fig. 1). In fact, 39 genera recorded in flower counts were absent from the interaction networks generated by any of the methodologies. Interaction transects

228 revealed interactions with 27 genera (23% of total floral diversity), while gut-content and corbicular  
229 pollen metabarcoding revealed interactions with 58% and 53% of available taxa, respectively.

230 *Comparison of interaction detections by methodology*

231 Both metabarcoding methodologies detected multiple unique taxa (33 taxa for gut contents and 30 for  
232 corbicular pollen), while interaction transects did not detect any unique interactions (Fig. 1). The two  
233 metabarcoding methodologies shared 83 common plant genera, representing 68% of the total corbicular  
234 pollen diversity and 63% of the gut content diversity.

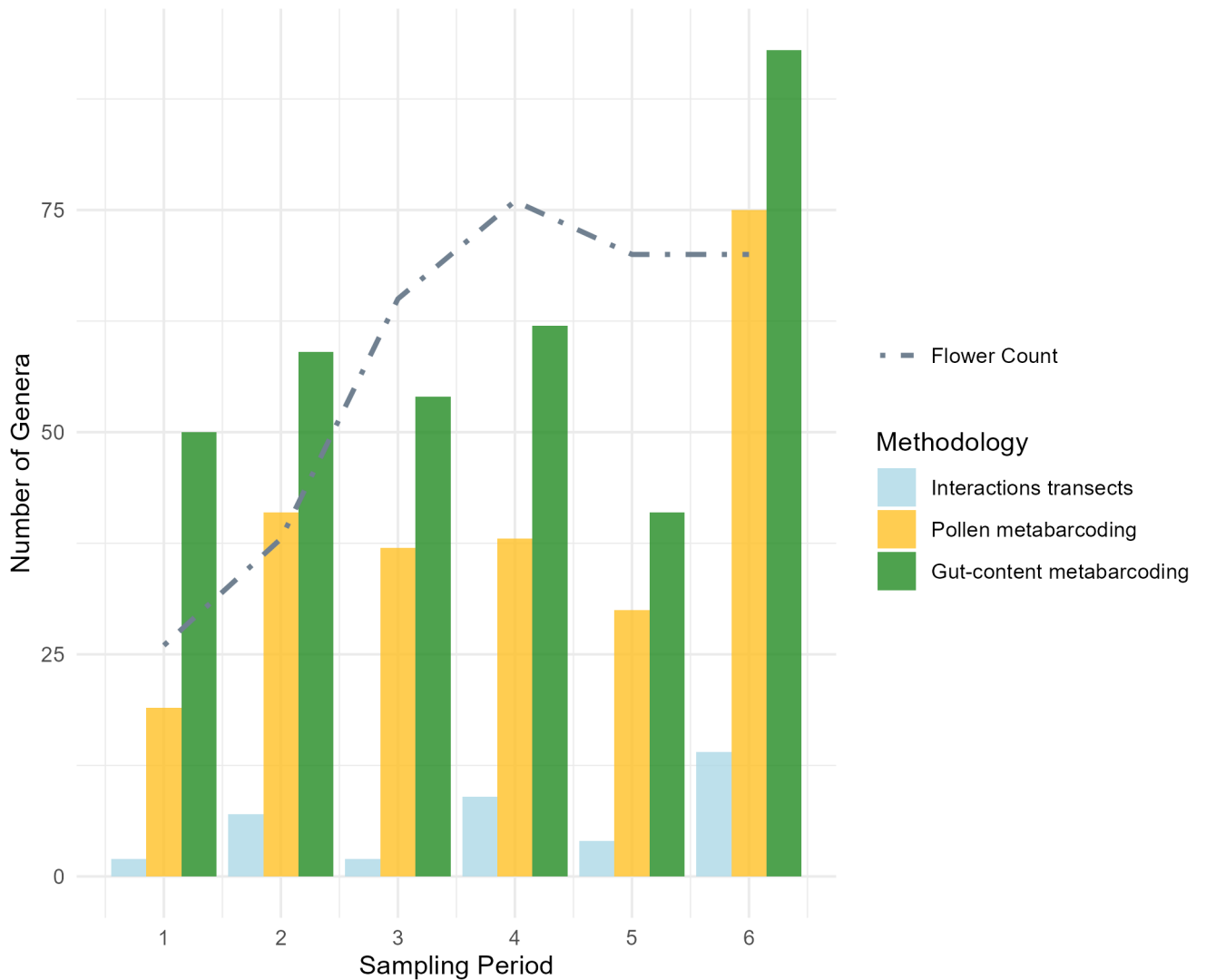


235

236 **Figure 1:** Total diversity and overlap of plant genera observed by four observation methodologies:  
 237 transect surveys of floral diversity ("flower counts") and *Bombus pascuorum* - flower interactions,  
 238 and metabarcoding of plant DNA in corbicular pollen and gut contents of *B. pascuorum*.

239 Taxonomic diversity varied across sampling periods, revealing distinct temporal patterns in flower-  
 240 ing taxa and interactions (Fig. 2). Although floral and interaction diversity increased overall from

the first to the last period, flowering taxa peaked in period four, whereas interactions peaked in period six. Metabarcoding consistently detected more taxa than interaction transects, with gut-content metabarcoding outperforming all other methods. In periods one, two, and six—before and after peak flowering—gut metabarcoding detected 59% more taxa than were recorded in flower counts on average, while in periods three to five floral diversity exceeded gut-content diversity.



246

247 **Figure 2:** Taxonomic diversity in *Bombus pascuorum* interaction networks over six sampling periods

248 *(April - August, 2023) observed through floral visitation surveys and ITS2 metabarcoding of DNA*  
249 *extracted from bumblebee gut contents and corbicular pollen loads. The results of each methodology*  
250 *correspond to samples or surveys taken across the same 48 sampling days. The number of plant genera*  
251 *indicated is a cumulative raw value for each methodology and period, with no standardization for*  
252 *sampling effort. Interaction diversity for transects is represented by the total number of plant taxa*  
253 *observed over each transect and sampling day, for each period. For metabarcoding methodologies,*  
254 *interaction diversity is the total number of plant genera observed across all samples collected during*  
255 *the given period.*

#### 256 *Functional diversity observations*

257 The design of interaction transects only included taxa from the entomophilous community, while both  
258 metabarcoding methodologies detected taxa from the anemophilous community as well, representing  
259 28% (N = 41) of the total identified plant genera between the two methodologies. Among these were 20  
260 genera from *Poaceae*, nine tree or woody plant genera, and 12 other herbaceous genera (See Supporting  
261 Information Fig. S2). During periods one, two, and six—when gut-content metabarcoding detected  
262 more taxa than the entomophilous community recorded in transects—an average of 13% of those taxa  
263 were anemophilous or partially anemophilous (See Supporting Information).

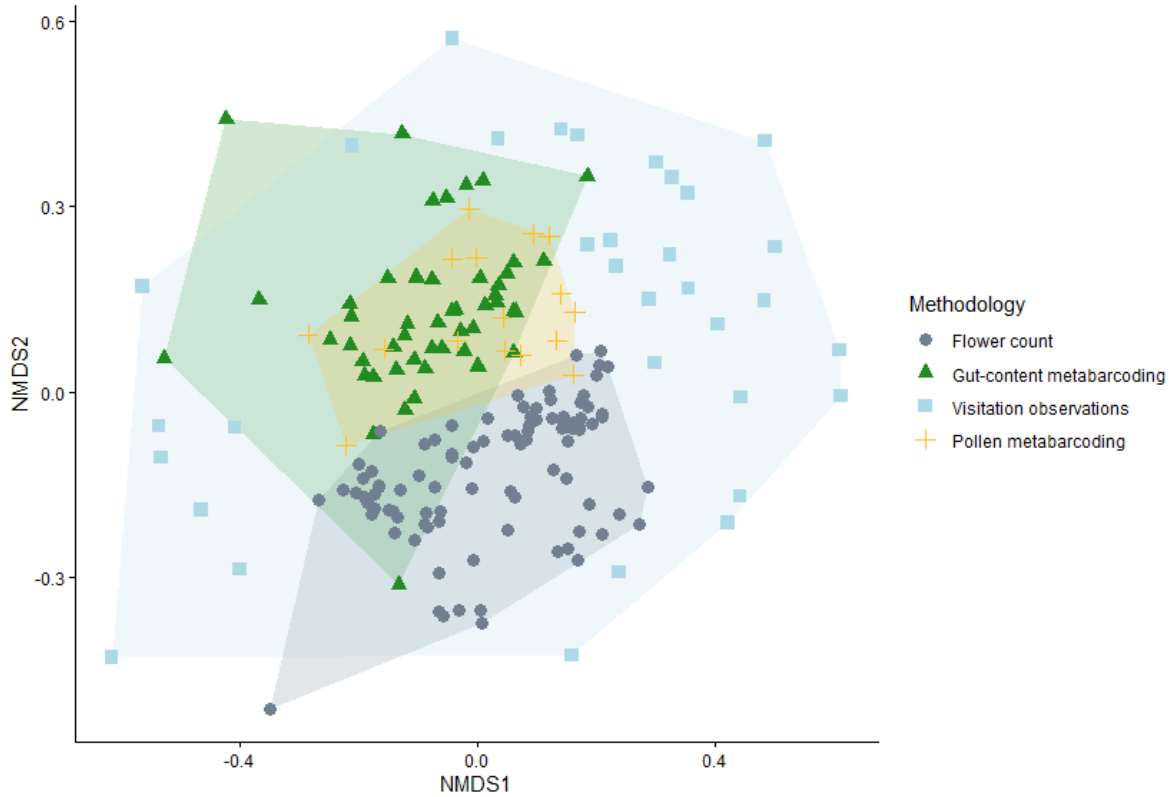
#### 264 *Plant community composition across methodologies*

265 A PERMANOVA test comparing taxonomic composition of interaction plant communities between  
266 methodologies indicated a significant effect of methodology on the observed community ( $P < 0.001$ ,  $R$   
267  $= 0.28$ ). In this analysis, interaction transects showed high beta-dispersal (distance to centroid = 0.62)  
268 compared to the more centered metabarcoding and flower count results (distance to centroid  $\leq 0.10$ ),

and an ANOVA test of mean dispersal by methodology indicated different levels of dispersal ( $P < 0.001$ ) for each methodology. The communities detected by each of the methodologies were also visualized using non-metric Multidimensional Scaling (nMDS, stress = 0.17, Fig. 3). Pairwise comparisons (Table 1) showed that the plant communities detected by flower counts were different from those of all other methodologies ( $P < 0.001$ , Holm–Bonferroni), although between pairs of interaction methodologies, no differences were observed.

**Table 1.** Pairwise tests comparing the community composition of plant taxa detected by four methodologies. Detected communities were compared by repeating PERMANOVA tests for each methodology pair. Tests applied the Raup–Crick dissimilarity index with 9999 permutations, and adjusted  $p$ -values were calculated using the Holm–Bonferroni method. The summarized test statistics include degrees of freedom for each methodology (DF),  $R^2$ , test  $F$ -statistics ( $F$ ) and associated  $p$ -value ( $p$ ), as well as the adjusted  $p$ -value.

| Methodology 1        | Methodology 2        | DF1 | DF2 | $R^2$  | $F$    | $p$    | Adjusted $p$ |
|----------------------|----------------------|-----|-----|--------|--------|--------|--------------|
| flower count         | gut metabarcoding    | 1   | 1   | 0.534  | 161.69 | <0.001 | <0.001       |
| flower count         | pollen metabarcoding | 1   | 1   | 0.376  | 64.95  | <0.001 | <0.001       |
| flower count         | interaction          | 1   | 1   | 0.230  | 37.64  | <0.001 | <0.001       |
| gut metabarcoding    | pollen metabarcoding | 1   | 1   | 0.130  | 9.38   | 0.038  | 0.114        |
| gut metabarcoding    | interaction          | 1   | 1   | 0.010  | 0.80   | 0.55   | 1            |
| pollen metabarcoding | interaction          | 1   | 1   | -0.024 | -1.13  | 0.997  | 1            |



**Figure 3:** Non-metric multi-dimensional scaling plot of plant communities detected by three methodologies for observing *Bombus pascuorum* floral interactions and a flower diversity survey. Observations from each methodology are aggregated by sampling day, reduced to binary presence/absence data, and compared in ordination using the Raup-Crick dissimilarity index (ordination stress = 0.17).

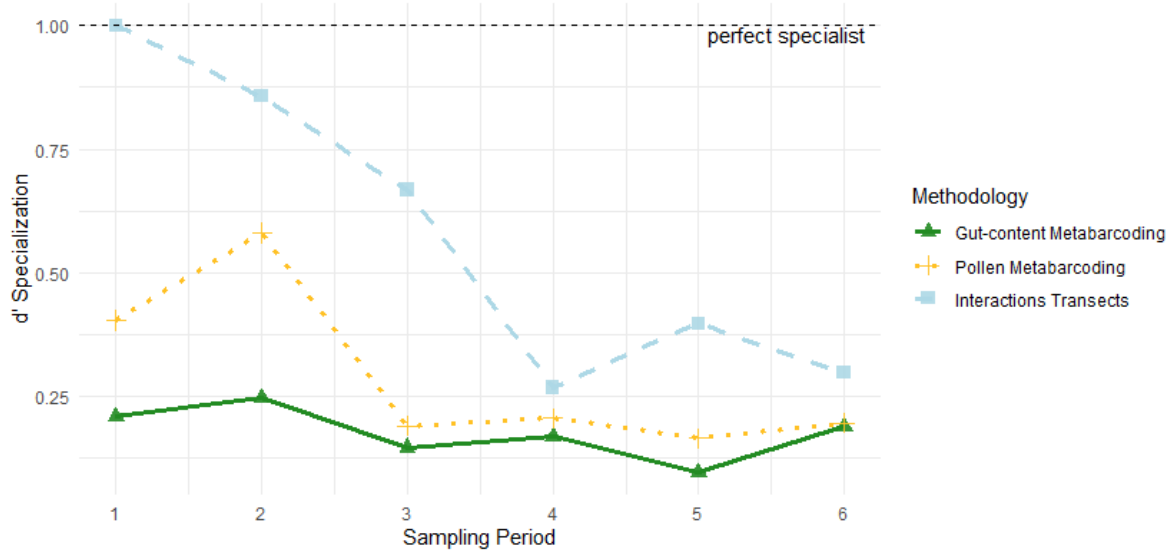
#### Gut-content vs. pollen-derived plant communities from paired samples

Comparing metabarcoding results from the same specimens, gut contents yielded fewer taxa (mean = 12 genera, sd = 9) than pollen samples (mean = 18 genera, sd = 7). On average, only 20% of taxa (mean = 6 genera, sd = 3) were shared between the two sample types. A PERMANOVA with specimen as a blocking factor indicated a difference in the plant community observed by both sample types ( $P < 0.01$ ,

See Supporting Information Table S2) explaining 18% of the variation between gut- and pollen-based detections. Data used in this comparison were similarly dispersed (distance to centroid = 0.08), with no difference between the two groups observed by a permutest.

#### Species level interaction network

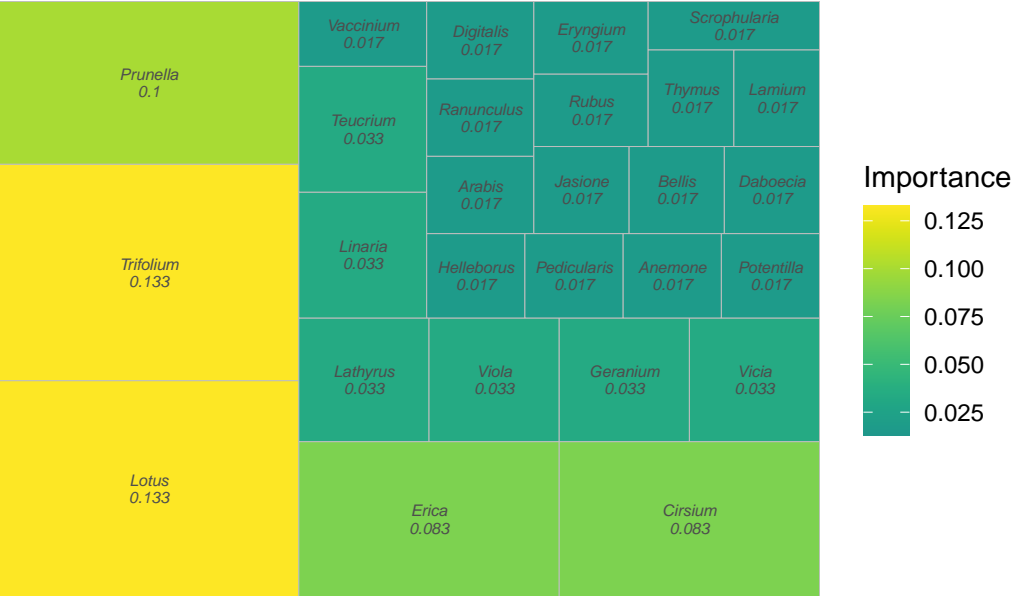
We calculated interaction specialization of *B. pascuorum* and an importance metric for the plant taxa within interaction networks. Specialization [ $d'$ ; Blüthgen et al. (2006)] declined over the season for transect and pollen-metabarcoding data but remained relatively stable for gut-content metabarcoding (Fig. 4), with transects indicating complete specialization in the first period. Across all methods, *Lo-tus* emerged as the most important plant genus, though the structure of importance differed: the two metabarcoding networks showed more evenly distributed importance values (Fig. 5b-c), whereas the transect network was dominated by a few top taxa (Fig. 5a).



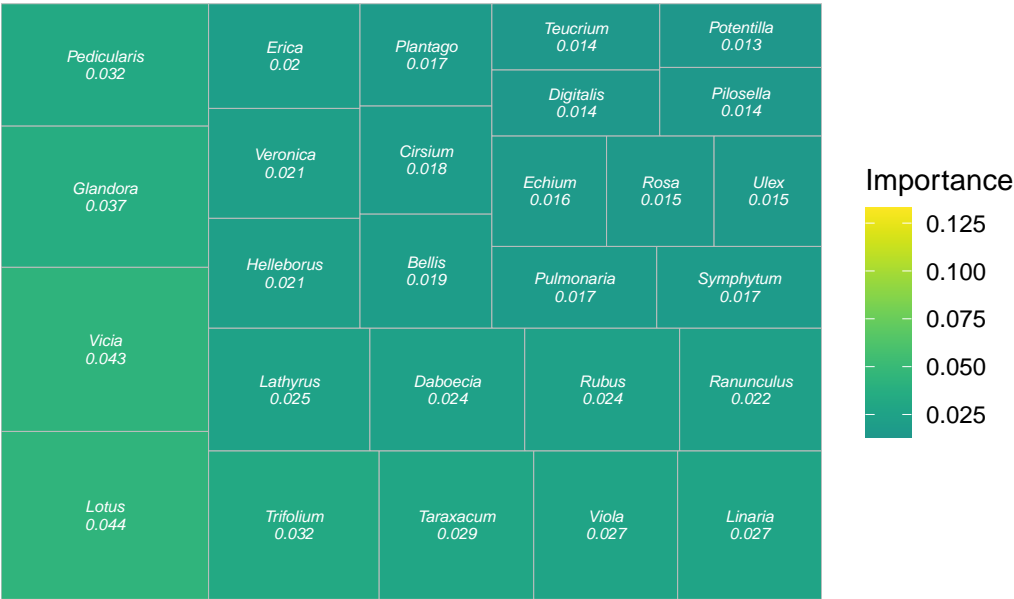
**Figure 4:** Specialization of plant interactions for *Bombus pascuorum* as indicated by networks constructed from three interaction observation methodologies. Specialization was calculated as  $d'$  using

the methodology of Blüthgen et al. (2006), with  $d' = 1$  representing perfect specialist behavior. Specialization of *B. pascuorum* for each period was calculated relative to interaction data from the same species in other periods, rather than other pollinator species.

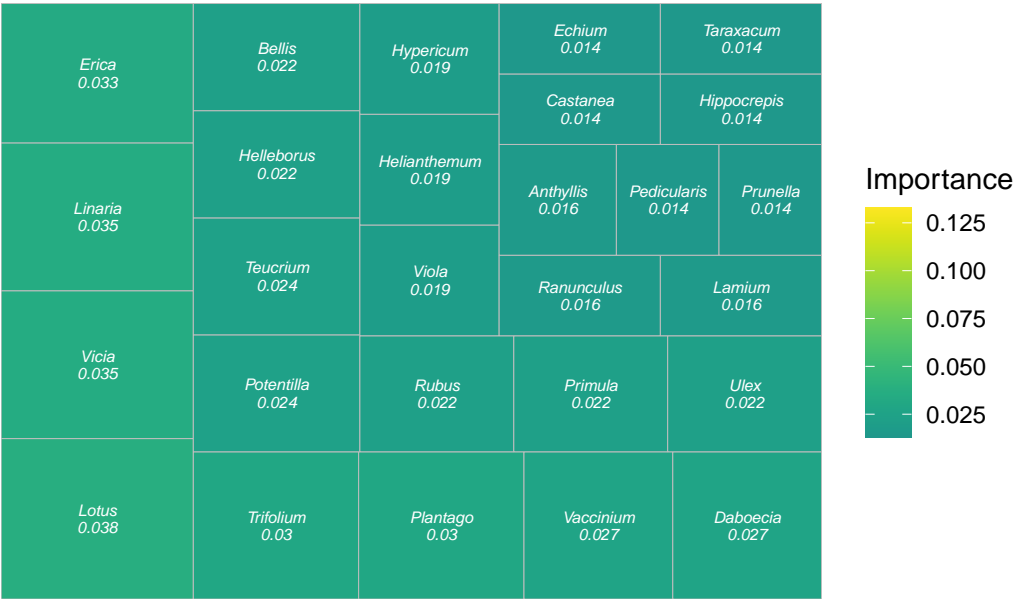
### A. Interaction transect–based network



B. Gut-content metabarcoding



C. Corbicular pollen metabarcoding

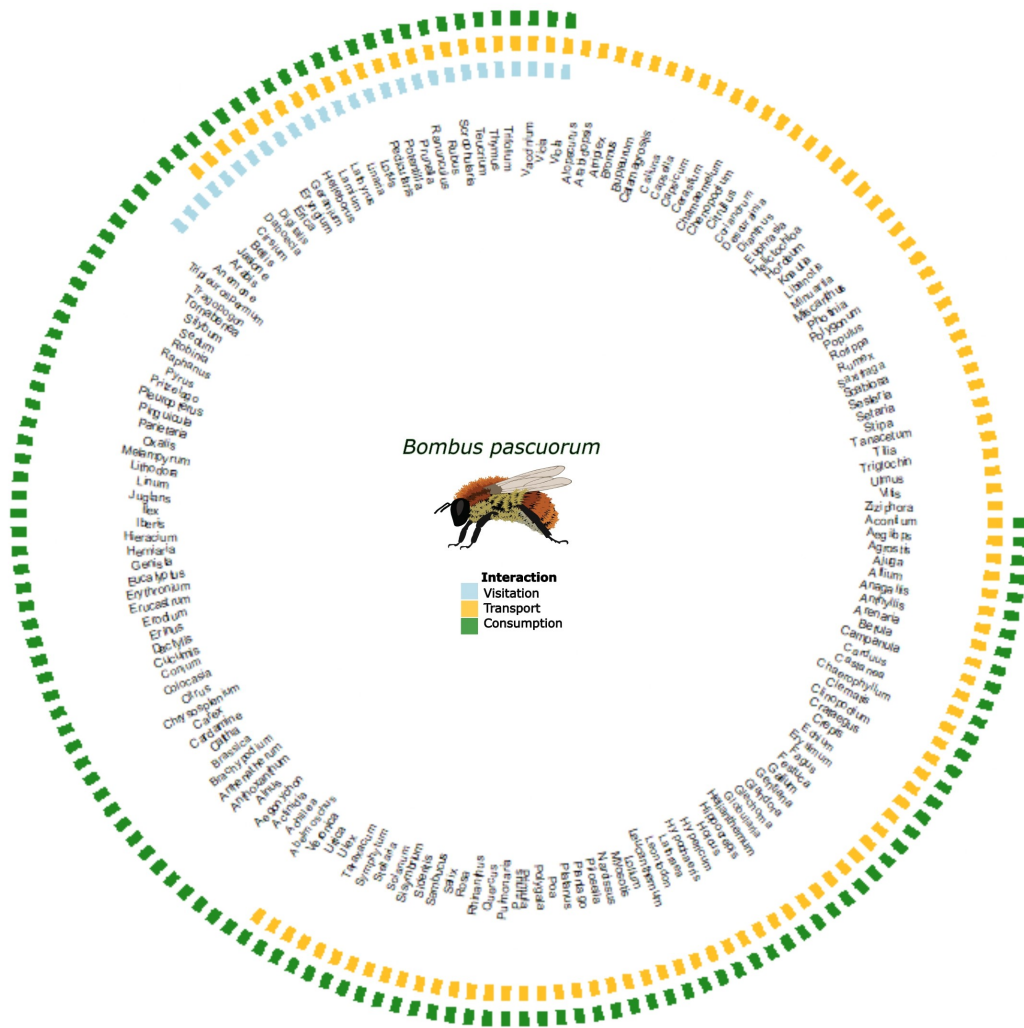


**Figure 5.** Plant “importance” within *Bombus pascuorum* interaction networks constructed from three interaction observation methodologies: (A) interaction transects, (B) gut-content metabarcoding, and

313 *(C) corbicular pollen metabarcoding. Importance was calculated as the proportion of total plant inter-*  
314 *actions observed by the given methodology represented by interactions with the specific plant genus.*  
315 *Importance is visualized with block size proportional to importance, and color scaled to minimum and*  
316 *maximum values observed by each methodology.*

#### 317 *Combined interaction network*

318 We combined the results from each interaction methodology to create an interaction network for *B.*  
319 *pascuorum* with links defined by interaction outcomes, including consumption, transport, and visitation  
320 (Fig. 6). This single species network included 169 nodes, increasing the number of taxa included in  
321 the network compared to individual methodology constructed networks. Additionally, each plant taxa  
322 received up to three links, including link metadata for interaction outcomes in the network. In total, the  
323 network contained 281 descriptive links.



324

325 **Figure 6.** Combined interaction network for *Bombus pascuorum* including all interaction plant taxa  
 326 detected by three methodologies. Interaction transect observations are represented by visitation, cor-  
 327 bicular pollen metabarcoding observation by transport, and gut-content metabarcoding observations  
 328 by consumption. The network includes 169 plant genera, each with up to three links describing the  
 329 outcomes of interactions with the single pollinator species. Interactions providing links represent the

330 *presence or absence of any interaction observation within the dataset of a given methodology.*

#### 331 **4. Discussion**

332 Plant-pollinator interaction networks are complex. We show that using functionally informa-  
333 tive network links and combining methodologies yields a comprehensive and strongly validated  
334 plant-pollinator interaction network. Notably, the two metabarcoding approaches revealed shared in-  
335 teractions with anemophilous and partially anemophilous plants for pollen consumption and transport,  
336 highlighting the complementarity of their data. Although each interaction methodology overlapped  
337 statistically at the aggregated level, the combined network resulting from each methodology increased  
338 the total nodes, and each methodology provided context to network links. Metabarcoding alone  
339 also proved effective at capturing a broad range of links and providing detailed, specimen-level  
340 data. Important information from the function of links is missing under the current approach to  
341 characterizing interaction networks, but using multiple methodologies helps to fill these gaps.

342 We compared each methodology in terms of the diversity of detected interactions, assignment of relative  
343 importance of plant taxa and specialization of *B. pascuorum* within the resulting network, and observed  
344 plant community composition. Consistent with previous comparisons between field and metabarcod-  
345 ing observation of plant-pollinator interactions, metabarcoding increased observed interaction diversity  
346 (Baksay et al., 2022; Milla et al., 2022; Smart et al., 2017), in our case by more than six-fold compared  
347 to interaction transect results. Considering this, and the time dedicated to data collection for both types  
348 of methodologies, metabarcoding was a more efficient approach. Interaction transects did provide the  
349 advantage of greater taxonomic resolution, as we were able to detect interactions at the species-species  
350 level, whereas metabarcoding provided species-genus level interactions. Beyond taxonomic detection

capabilities, the results from each methodology allowed for network-level cross-validation.

Network topology and specialization patterns differed markedly across methodologies. Interaction transects tended to overstate both the degree of specialization and the dominance of the most frequently visited plant taxa. Although *B. pascuorum* is known to form strong early-season associations with certain plant species (Artamendi et al., unpublished dataset), the metabarcoding approaches indicated much lower specialization and produced more evenly distributed network structures. These results mirrored previous interaction networks constructed for individual pollinator species, which also have shown a tendency towards representing pollinators as specialists when using field observation data versus the generalist behavior indicated by metabarcoding data (Arstingstall et al., 2021). Overall, the combined datasets across methodologies suggested a more diverse foraging niche than visitation data alone would have implied.

The three methodologies showed complementary patterns in network composition. Flower counts and interaction transects overlapped as expected from the study design, yet differed statistically, likely due to the much larger number of taxa detected by the former. No statistical differences were found among the three interaction-focused methods, although their dispersion differed, reflecting variation in spatial and taxonomic coverage. Interaction transects are shaped by local habitat and plant-community differences, whereas metabarcoding integrates interactions across the broader landscape, producing more consistent results. Metabarcoding approaches overlapped minimally with the floral community detected by flower counts, indicating that interaction networks include taxa not captured within transects. This is unsurprising given that flower counts reflect potential, not actual, interactions and are constrained by spatial and temporal limits that do not restrict metabarcoding.

Between the two metabarcoding approaches, gut-content metabarcoding captured greater overall taxonomic diversity and was more efficient, given that every specimen provided a gut sample, but not necessarily a pollen sample. Pollen samples detected more taxa per individual, however, and hypothetically offered an advantage as a non-lethal sampling option. The combination of both methodologies' results broadened the interaction network greatly, and incorporated contextualized interaction links. These links showed which plant genera were consumed for adult bee nutrition, and which provided pollen for transport to the nest. In our case, gut-content metabarcoding was particularly informative for revealing seasonal foraging patterns, detecting more consumed taxa than were flowering in the early and late parts of the season, and showing relatively stable specialization over time. Together, these results indicated that the plant community represented in consumption-based interactions differs from the floral community captured by field- and pollen-based surveys.

### *Metabarcoding observes forage across functional groups*

The diversity of plant groups observed within our metabarcoding data, especially the temporal changes in diversity observed by gut-content metabarcoding, indicated that *B. pascuorum* forages on different plant taxa than previously expected. Our reference database for metabarcoding allowed us to identify taxa from functional groups beyond the floral community sampled in our transects (See Supporting Information). Through metabarcoding, we observed interactions with a variety of taxa outside of the entomophilous meadow and shrubland plant community, including trees and shrubs, grasses, and other herbaceous plants.

Our observations of anemophilous plant interactions are supported by previously documented records for *Bombus* species (de Vere et al., 2017; Ibiyemi et al., 2025; Milla et al., 2022; Pojar, 1973; Selva

et al., 2024; Tanaka et al., 2020; Terrell & Batra, 1984; Timberlake, de Vere, et al., 2024; Wood et al., 2022), and have especially intriguing implications for bumblebee forage behavior. Previous studies using external pollen metabarcoding have removed wind-pollinated taxa from their analyses under the argument that wind-borne pollen in samples may represent false positive interactions (Negri et al., 2015; Pornon et al., 2017; Tanaka et al., 2020). Our gut-content results, however, caution against the practice of removing these taxa as contaminants, especially if using external pollen loads as standalone proxies for forage networks.

The presence of DNA from anemophilous taxa within gut samples suggests that interactions with these taxa may be more than coincidental interactions with pollen in the environment. Indeed, beyond consumption for adult nutrition, there are previous indications that pollen from flowering trees supports colony establishment success and low larval mortality (Wood et al., 2022). Our results support the hypothesis that most bumblebees forage selectively for consumption and transport of high quality pollen (Ruedenauer et al., 2016; Timberlake, de Vere, et al., 2024), adapting their forage to take advantage of the best available resources as they change with environmental variability (Selva et al., 2024). While it is possible that some plant material may be transported or consumed incidentally (Arstingstall et al., 2021), the taxa detected within *B. pascuorum* gut contents and corbicular pollen form part of the web of biodiversity that supports the species and possibly other pollinators. Our detection of anemophilous plant DNA in both metabarcoding methodologies indicates that *B. pascuorum*, and perhaps other bumblebee species, may intentionally forage on these taxa to meet nutritional needs at various life stages.

Existing hypotheses for pollinator forage adaptations in response to environmental changes have suggested that bees expand forage diversity beyond the flowering community and across habitats in order

414 to survive annual “hunger gaps” (Becher et al., 2024; Timberlake, Tew, et al., 2024), when blooming  
415 floral species are limited (Morozumi et al., 2022; Wood et al., 2022). Our observation of high forage  
416 diversity in gut contents before and after the floral peak, distinct interaction and flowering taxa network  
417 topologies, and consumption of taxa across functional groups, all together support these hypotheses.  
418 While the floral community beyond the immediate area of our transects likely played a large role in  
419 these observations, the detection of anemophilous taxa in gut contents during the periods where forage  
420 diversity was higher than flowering diversity provide evidence for a community driven component as  
421 well. These observations show how the broader taxonomic detection capacity of metabarcoding allows  
422 for detection of interactions that otherwise would go unobserved by flower visitation surveys. This  
423 advantage is extended when working with metabarcoding data at the individual sample level, where  
424 greater resolution for interactions is obtainable.

#### 425 *Metabarcoding offers individual level analysis*

426 Our comparative analyses understate the resolution of the metabarcoding derived data. We aggregated  
427 detections by sampling day to balance effort across methods, overlooking the individual-level detail that  
428 metabarcoding can provide. When we compared taxa detected from paired pollen and gut samples at the  
429 individual level, overlap was low, revealing a difference between sample sources that was not apparent  
430 in comparisons of aggregated data. This difference likely reflects the different roles of corbicular pollen  
431 and immediately consumed pollen in the nutrition needed for different life-cycle stages (Vaudo, 2015).  
432 Taxa repeatedly detected by both methods increased confidence in their importance. For instance, the  
433 consistent appearance of *Vicia* in both sample types early in the season supports field observations  
434 of a strong association between *B. pascuorum* and *Vicia* species (Artamendi et al., unpublished data),

underscoring the value of integrating field surveys with laboratory-based methods.

## *Conclusions*

The similarities between interaction data suggest robustness between each methodology, and the inherent implications of the sample sources of each provide varied means of interpreting different interactions. Interaction transects provide a valuable field-based perspective, although given their lower sampling efficiency, incorporating them as a validation of other surveys may be the best way to integrate this methodology into future studies. Field observations can fill gaps left by metabarcoding methodologies, such as confirmation of pollination efficacy, interaction frequency, and species-level resolution. As a direct observation of the pollen transported to the nest, corbicular pollen may also be a good starting point for identifying plants that may provide pollen with optimal macronutrients for larval development. Similarly, gut-content metabarcoding provides an important perspective on the nutritional needs of actively foraging pollinators, identifying taxa that provide pollen as food for supporting this activity (Li et al., 2025). Knowing which taxa are actually ingested by pollinators is especially useful for identifying taxa that facilitate microbiota exchange and acquisition during plant interactions (Cullen et al., 2021; Keller et al., 2021), including parasite and disease transfer (Lignon et al., 2024). Although they are not equal, our research highlights overall that each methodology offers advantages and disadvantages in terms of sensitivity, sampling effort, and perspective.

While most of the methodologies we applied, aside from gut-content metabarcoding, have previously been used independently to characterize plant–pollinator networks (e.g., Devriese et al., 2024; Magrach et al., 2023), our findings highlight the synergistic value of integrating them. Gut-content metabarcoding emerges as a promising approach, but its greatest potential is realized when combined with estab-

lished approaches. A key next step is improving our ability to quantify interaction frequencies at the individual level using metabarcoding, whether from gut contents or pollen. Overall, methodological advances are likely to come from linking complementary data sources to fill the informational gaps left by any single approach.

## References

- Agut, A., & Hermosilla, B. (2025). *Herbario del Jard?n Bot?nico de Olarizu (Vitoria-Gasteiz)/Olarizuko Lorategi Botanikoaren Herbarioa (Vitoria-Gasteiz)*. Jard?n Bot?nico de Olarizu (Vitoria-Gasteiz)/Olarizuko Lorategi Botanikoa (Vitoria-Gasteiz). <https://doi.org/10.15470/R7IFMA>
- Ankenbrand, M. J., Keller, A., Wolf, M., Schultz, J., & Förster, F. (2015). ITS2 Database V: Twice as Much: Table 1. *Molecular Biology and Evolution*, 32(11), 3030–3032. <https://doi.org/10.1093/molbev/msv174>
- Arstingstall, K. A., DeBano, S. J., Li, X., Wooster, D. E., Rowland, M. M., Burrows, S., & Frost, K. (2021). Capabilities and limitations of using DNA metabarcoding to study plant–pollinator interactions. *Molecular Ecology*, 30(20), 5266–5297. <https://doi.org/10.1111/mec.16112>
- Artamendi, M., Martin, P. A., Bartomeus, I., & Magrach, A. (2025). Loss of pollinator diversity consistently reduces reproductive success for wild and cultivated plants. *Nature Ecology & Evolution*, 9(2), 296–313. <https://doi.org/10.1038/s41559-024-02595-2>
- Baksay, S., Andalo, C., Galop, D., Burrus, M., Escaravage, N., & Pornon, A. (2022). Using metabarcoding to investigate the strength of plant-pollinator interactions from surveys of visits to DNA sequences. *Frontiers in Ecology and Evolution*, 10. <https://doi.org/10.3389/fevo.2022.735588>
- Becher, M. A., Twiston-Davies, G., Osborne, J. L., & Lander, T. A. (2024). Resource gaps pose the

greatest threat for bumblebees during the colony establishment phase. *Insect Conservation and Diversity*, 17(4), 676–689. <https://doi.org/10.1111/icad.12736>

Bell, K. (2021). *ITS2 july 2021*. figshare. <https://doi.org/10.6084/M9.FIGSHARE.14936004.V1>

Bell, K., de Vere, N., Keller, A., Richardson, R. T., Gous, A., Burgess, K. S., & Brosi, B. J. (2016). Pollen DNA barcoding: Current applications and future prospects. *Genome*, 59(9), 629–640. <https://doi.org/10.1139/gen-2015-0200>

Bell, K., Fowler, J., Burgess, K. S., Dobbs, E. K., Gruenewald, D., Lawley, B., Morozumi, C., & Brosi, B. J. (2017). Applying pollen DNA metabarcoding to the study of plant-pollinator interactions. *Applications in Plant Sciences*, 5(6), 1600124. <https://doi.org/10.3732/apps.1600124>

Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *BMC Ecology*, 6(1), 9. <https://doi.org/10.1186/1472-6785-6-9>

Bosch, J., Martín González, A. M., Rodrigo, A., & Navarro, D. (2009). Plant-pollinator networks: adding the pollinator’s perspective. *Ecology Letters*, 12(5), 409–419. <https://doi.org/10.1111/j.1461-0248.2009.01296.x>

Burkle, L. A., & Alarcón, R. (2011). The future of plant-pollinator diversity: Understanding interaction networks across time, space, and global change. *American Journal of Botany*, 98(3), 528–538. <https://doi.org/10.3732/ajb.1000391>

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High resolution sample inference from illumina amplicon data. *Nature Methods*, 13(7), 581–583. <https://doi.org/10.1038/nmeth.3869>

Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y., Li, X., Jia, X., Lin, Y., & Leon, C. (2010). Validation of the ITS2 Region as a Novel DNA Barcode

499 for Identifying Medicinal Plant Species. *PLOS ONE*, 5(1), e8613. [https://doi.org/10.1371/journal.](https://doi.org/10.1371/journal.pone.0008613)  
500 [pone.0008613](https://doi.org/10.1371/journal.pone.0008613)

501 Cullen, N., Fethers, A., & Ashman, T.-L. (2021). Integrating microbes into pollination. *Current Opinion*  
502 *in Insect Science*, 44, 48–54. <https://doi.org/10.1016/j.cois.2020.11.002>

503 Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statis-  
504 tical identification and removal of contaminant sequences in marker-gene and metagenomics data.  
505 *Microbiome*, 6(1), 226. <https://doi.org/10.1186/s40168-018-0605-2>

506 de Vere, N., Jones, L. E., Gilmore, T., Moscrop, J., Lowe, A., Smith, D., Hegarty, M. J., Creer, S., &  
507 Ford, C. R. (2017). Using DNA metabarcoding to investigate honey bee foraging reveals limited  
508 flower use despite high floral availability. *Scientific Reports*, 7(1), 42838. [https://doi.org/10.1038/](https://doi.org/10.1038/srep42838)  
509 [srep42838](https://doi.org/10.1038/srep42838)

510 Devriese, A., Peeters, G., Brys, R., & Jacquemyn, H. (2024). The impact of extraction method and  
511 pollen concentration on community composition for pollen metabarcoding. *Applications in Plant*  
512 *Sciences*, 12(5), e11601. <https://doi.org/10.1002/aps3.11601>

513 Donald, M. L., Galbraith, J. A., Erastova, D. A., Podolyan, A., Miller, T. E. X., & Dhami, M. K. (2022).  
514 Nectar resources affect bird-dispersed microbial metacommunities in suburban and rural gardens.  
515 *Environmental Microbiology*, 24(12), 5654–5665. <https://doi.org/10.1111/1462-2920.16159>

516 Dormann, C. F., Fruend, J., Bluethgen, N., & Gruber, B. (2009). *Indices, graphs and null models:*  
517 *Analyzing bipartite ecological networks*. 2, 7–24.

518 Emer, C., & Memmott, J. (2023). Intraspecific variation of invaded pollination networks – the role  
519 of pollen-transport, pollen-transfer and different levels of biological organization. *Perspectives in*  
520 *Ecology and Conservation*, 21(2), 151–163. <https://doi.org/10.1016/j.pecon.2023.03.003>

521 Evans, D., & Kitson, J. (2020). Molecular ecology as a tool for understanding pollination and other  
 522 plant-insect interactions. *Current Opinion in Insect Science*, 38, 26–33. [https://doi.org/10.1016/j.](https://doi.org/10.1016/j.cois.2020.01.005)  
 523 [cois.2020.01.005](https://doi.org/10.1016/j.cois.2020.01.005)

524 Haag, K. L., Caesar, L., Silveira Regueira-Neto, M. da, Sousa, D. R. de, Montenegro Marcelino, V.,  
 525 Queiroz Balbino, V. de, & Torres Carvalho, A. (2023). Temporal Changes in Gut Microbiota Com-  
 526 position and Pollen Diet Associated with Colony Weakness of a Stingless Bee. *Microbial Ecology*,  
 527 85(4), 1514–1526. <https://doi.org/10.1007/s00248-022-02027-3>

528 Ibiyemi, D., Harris-Shultz, K., Jespersen, D., & Joseph, S. V. (2025). Understanding the Foraging Be-  
 529 havior of Sweat Bees, Bumble Bees, and Honey Bees on Centipedegrass for Conservation Strate-  
 530 gies. *Journal of Insect Behavior*, 38(2), 29. <https://doi.org/10.1007/s10905-025-09893-y>

531 Katumo, D. M., Liang, H., Ochola, A. C., Lv, M., Wang, Q.-F., & Yang, C.-F. (2022). Pollinator  
 532 diversity benefits natural and agricultural ecosystems, environmental health, and human welfare.  
 533 *Plant Diversity*, 44(5), 429–435. <https://doi.org/10.1016/j.pld.2022.01.005>

534 Keller, A., McFrederick, Q., & Leonhardt, S. (2021). (More than) hitchhikers through the network:  
 535 The shared microbiome of bees and flowers. *Current Opinion in Insect Science*, 44, 8–15. <https://doi.org/10.1016/j.cois.2020.09.007>  
 536 [//doi.org/10.1016/j.cois.2020.09.007](https://doi.org/10.1016/j.cois.2020.09.007)

537 Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., &  
 538 Tscharnkte, T. (2006). Importance of pollinators in changing landscapes for world crops. *Proceed-*  
 539 *ings of the Royal Society B: Biological Sciences*, 274(1608), 303–313. [https://doi.org/10.1098/rspb.](https://doi.org/10.1098/rspb.2006.3721)  
 540 [2006.3721](https://doi.org/10.1098/rspb.2006.3721)

541 Leach, M. E., & Drummond, F. (2018). A Review of Native Wild Bee Nutritional Health. *International*  
 542 *Journal of Ecology*, 2018(1), 9607246. <https://doi.org/10.1155/2018/9607246>

- 543 Lecocq, T., Brasero, N., Martinet, B., Valterová, I., & Rasmont, P. (2015). Highly polytypic taxon com-  
544 plex: interspecific and intraspecific integrative taxonomic assessment of the widespread pollinator  
545 ombus pascuorum Scopoli 1763 (Hymenoptera: Apidae). *Systematic Entomology*, 40(4), 881–890.  
546 <https://doi.org/10.1111/syen.12137>
- 547 Leonhardt, S. D., & Blüthgen, N. (2012). The same, but different: pollen foraging in honeybee and  
548 bumblebee colonies. *Apidologie*, 43(4), 449–464. <https://doi.org/10.1007/s13592-011-0112-y>
- 549 Leontidou, K., Vokou, D., Sandionigi, A., Bruno, A., Lazarina, M., De Groeve, J., Li, M., Varotto,  
550 C., Girardi, M., Casiraghi, M., & Cristofori, A. (2021). Plant biodiversity assessment through  
551 pollen DNA metabarcoding in Natura 2000 habitats (Italian Alps). *Scientific Reports*, 11(1), 18226.  
552 <https://doi.org/10.1038/s41598-021-97619-3>
- 553 Leponiemi, M., Freitak, D., Moreno-Torres, M., Pferschy-Wenzig, E.-M., Becker-Scarpitta, A., Tiusa-  
554 nen, M., Vesterinen, E. J., & Wirta, H. (2023). Honeybees' foraging choices for nectar and pollen  
555 revealed by DNA metabarcoding. *Scientific Reports*, 13(1), 14753. <https://doi.org/10.1038/s41598-023-42102-4>
- 557 Li, Y., Liu, C., Wang, Y., Li, M., Zou, S., Hu, X., Chen, Z., Li, M., Ma, C., Obi, C. J., Zhou, X., Zou,  
558 Y., & Tang, M. (2025). Urban wild bee well-being revealed by gut metagenome data: A mason bee  
559 model. *Insect Science*, 32(6). <https://doi.org/10.1111/1744-7917.70051>
- 560 Lignon, V. A., Mas, F., Jones, E. E., Kaiser, C., & Dhami, M. K. (2024). The floral interface: a play-  
561 ground for interactions between insect pollinators, microbes, and plants. *New Zealand Journal of*  
562 *Zoology*, 1–20. <https://doi.org/10.1080/03014223.2024.2353285>
- 563 Lowe, A., Jones, L., Witter, L., Creer, S., & de Vere, N. (2022). Using DNA Metabarcoding to Identify  
564 Floral Visitation by Pollinators. *Diversity*, 14(4), 236. <https://doi.org/10.3390/d14040236>

565 Magrach, A., Artamendi, M., Lapido, P. D., Parejo, C., & Rubio, E. (2023). Indirect interactions  
 566 between pollinators drive interaction rewiring through space. *Ecosphere*, 14(6), e4521. <https://doi.org/10.1002/ecs2.4521>  
 567 <https://doi.org/10.1002/ecs2.4521>

568 Mayr, A. V., Keller, A., Peters, M. K., Grimmer, G., Krischke, B., Geyer, M., Schmitt, T., & Steffan-  
 569 Dewenter, I. (2021). Cryptic species and hidden ecological interactions of halictine bees along  
 570 an elevational gradient. *Ecology and Evolution*, 11(12), 7700–7712. [https://doi.org/10.1002/ece3.](https://doi.org/10.1002/ece3.7605)  
 571 [7605](https://doi.org/10.1002/ece3.7605)

572 Milla, L., Schmidt-Lebuhn, A., Bovill, J., & Encinas-Viso, F. (2022). Monitoring of honey bee floral  
 573 resources with pollen DNA metabarcoding as a complementary tool to vegetation surveys. *Ecolog-  
 574 ical Solutions and Evidence*, 3(1), e12120. <https://doi.org/10.1002/2688-8319.12120>

575 Morozumi, C., Loy, X., Reynolds, V., Schiffer, A., Morrison, B., Savage, J., & Brosi, B. (2022). Si-  
 576 multaneous niche expansion and contraction in plant–pollinator networks under drought. *Oikos*,  
 577 2022(11), e09265. <https://doi.org/10.1111/oik.09265>

578 Negri, I., Mavris, C., Prisco, G. D., Caprio, E., & Pellecchia, M. (2015). Honey Bees (*Apis mellifera*,  
 579 L.) as Active Samplers of Airborne Particulate Matter. *PLOS ONE*, 10(7), e0132491. [https://doi.](https://doi.org/10.1371/journal.pone.0132491)  
 580 [org/10.1371/journal.pone.0132491](https://doi.org/10.1371/journal.pone.0132491)

581 Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B.,  
 582 Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B.,  
 583 Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., ... Weedon, J. (2024). *Vegan:*  
 584 *Community ecology package*. <https://CRAN.R-project.org/package=vegan>

585 Pashalidou, F. G., Lambert, H., Peybernes, T., Mescher, M. C., & De Moraes, C. M. (2020). Bum-  
 586 ble bees damage plant leaves and accelerate flower production when pollen is scarce. *Science*,

368(6493), 881–884. <https://doi.org/10.1126/science.aay0496>

Pojar, J. (1973). Pollination of typically anemophilous salt marsh plants by bumble bees, *bombus*  
*terricola occidentalis* grne. *The American Midland Naturalist*, 89(2), 448–451. <https://doi.org/10.2307/2424049>

Popic, T. J., Wardle, G. M., & Davila, Y. C. (2012). Flower-visitor networks only partially predict the  
function of pollen transport by bees. *Austral Ecology*, 38(1), 76–86. <https://doi.org/10.1111/j.1442-9993.2012.02377.x>

Pornon, A., Andalo, C., Burrus, M., & Escaravage, N. (2017). DNA metabarcoding data unveils in-  
visible pollination networks. *Scientific Reports*, 7(1), 16828. <https://doi.org/10.1038/s41598-017-16785-5>

Quintero, E., Isla, J., & Jordano, P. (2022). Methodological overview and data-merging approaches in  
the study of plant–frugivore interactions. *Oikos*, 2022(2). <https://doi.org/10.1111/oik.08379>

Ruedenauer, F. A., Spaethe, J., & Leonhardt, S. D. (2016). Hungry for quality-individual bumble-  
bees forage flexibly to collect high-quality pollen. *Behavioral Ecology and Sociobiology*, 70(8),  
1209–1217. <https://doi.org/10.1007/s00265-016-2129-8>

Saunders, M. E. (2018). Insect pollinators collect pollen from wind-pollinated plants: implications for  
pollination ecology and sustainable agriculture. *Insect Conservation and Diversity*, 11(1), 13–31.  
<https://doi.org/10.1111/icad.12243>

Selva, S., Moretti, M., Ruedenauer, F., Keller, A., Fournier, B., Leonhardt, S. D., Eggenberger, H. A.,  
& Abella, J. C. (2024). *Urban bumblebees diversify their foraging strategy to maintain nutrient*  
*intake*. <https://ecoevorxiv.org/repository/view/7812/>

Shi, H., Ratering, S., Schneider, B., & Schnell, S. (2025). Microbiome of honey bee corbicular pollen:

609 Factors influencing its structure and potential for studying pathogen transmission. *Science of The*  
610 *Total Environment*, 958, 178107. <https://doi.org/10.1016/j.scitotenv.2024.178107>

611 Smart, M. D., Cornman, R. S., Iwanowicz, D. D., McDermott-Kubeczko, M., Pettis, J. S., Spivak, M.  
612 S., & Otto, C. R. V. (2017). A comparison of honey bee-collected pollen from working agricultural  
613 lands using light microscopy and ITS metabarcoding. *Environmental Entomology*, 46(1), 38–49.  
614 <https://doi.org/10.1093/ee/nvw159>

615 Tanaka, K., Nozaki, A., Nakadai, H., Shiwa, Y., & Shimizu-Kadota, M. (2020). Using pollen DNA  
616 metabarcoding to profile nectar sources of urban beekeeping in Kōtō-ku, Tokyo. *BMC Research*  
617 *Notes*, 13(1), 515. <https://doi.org/10.1186/s13104-020-05361-2>

618 Terrell, E. E., & Batra, S. W. T. (1984). Insects collect pollen of eastern wildrice, zizania aquatica  
619 (poaceae). *Castanea*, 49(1), 31–34. <https://www.jstor.org/stable/4033059>

620 Timberlake, T. P., de Vere, N., Jones, L. E., Vaughan, I. P., Baude, M., & Memmott, J. (2024). Ten-  
621 a-day: Bumblebee pollen loads reveal high consistency in foraging breadth among species, sites  
622 and seasons. *Ecological Solutions and Evidence*, 5(3), e12360. [https://doi.org/10.1002/2688-8319.](https://doi.org/10.1002/2688-8319.12360)  
623 [12360](https://doi.org/10.1002/2688-8319.12360)

624 Timberlake, T. P., Tew, N. E., & Memmott, J. (2024). Gardens reduce seasonal hunger gaps for farmland  
625 pollinators. *Proceedings of the Royal Society B: Biological Sciences*, 291(2033). [https://doi.org/](https://doi.org/10.1098/rspb.2024.1523)  
626 [10.1098/rspb.2024.1523](https://doi.org/10.1098/rspb.2024.1523)

627 Vanbergen, A. J., & Insect Pollinators Initiative, the. (2013). Threats to an ecosystem service: pressures  
628 on pollinators. *Frontiers in Ecology and the Environment*, 11(5), 251–259. [https://doi.org/10.1890/](https://doi.org/10.1890/120126)  
629 [120126](https://doi.org/10.1890/120126)

630 Vaudo, A. D. (2015). Bee nutrition and floral resource restoration. *Current Opinion in Insect Science*,

631 10, 133–141. <https://doi.org/10.1016/j.cois.2015.05.008>

632 White, T. J., Bruns, T. D., Lee, S. B., & Taylor, J. W. (1990). *Amplification and direct sequencing*  
633 *of fungal ribosomal RNA Genes for phylogenetics* (1st ed., Vol. 18, pp. 315–322). Academic  
634 Press. [https://www.researchgate.net/publication/223397588\\_White\\_T\\_J\\_T\\_D\\_Bruns\\_S\\_B\\_Lee\\_](https://www.researchgate.net/publication/223397588_White_T_J_T_D_Bruns_S_B_Lee_and_J_W_Taylor_Amplification_and_direct_sequencing_of_fungal_ribosomal_RNA_Genes_for_phylogenetics)  
635 [and\\_J\\_W\\_Taylor\\_Amplification\\_and\\_direct\\_sequencing\\_of\\_fungal\\_ribosomal\\_RNA\\_Genes\\_](https://www.researchgate.net/publication/223397588_White_T_J_T_D_Bruns_S_B_Lee_and_J_W_Taylor_Amplification_and_direct_sequencing_of_fungal_ribosomal_RNA_Genes_for_phylogenetics)  
636 [for\\_phylogenetics](https://www.researchgate.net/publication/223397588_White_T_J_T_D_Bruns_S_B_Lee_and_J_W_Taylor_Amplification_and_direct_sequencing_of_fungal_ribosomal_RNA_Genes_for_phylogenetics)

637 Wood, T. J., Vanderplanck, M., Vastrade, M., Vaudo, A. D., & Michez, D. (2022). Trees for bees: could  
638 woody plant pollen be used as a consistent resource in bee-focused agri-environment schemes?  
639 *Entomologia Generalis*, 42(3), 361. <https://doi.org/10.1127/entomologia/2021/1241>

640 Woodcock, B. A., Garratt, M. P. D., Powney, G. D., Shaw, R. F., Osborne, J. L., Soroka, J., Lindström,  
641 S. a. M., Stanley, D., Ouvrard, P., Edwards, M. E., Jauker, F., McCracken, M. E., Zou, Y., Potts, S.  
642 G., Rundlöf, M., Noriega, J. A., Greenop, A., Smith, H. G., Bommarco, R., ... Pywell, R. F. (2019).  
643 Meta-analysis reveals that pollinator functional diversity and abundance enhance crop pollination  
644 and yield. *Nature Communications*, 10(1), 1481. <https://doi.org/10.1038/s41467-019-09393-6>