

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). 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

⁴² (Burkle & Alarcón, 2011), but most studies under this framework have yet to account for how the scope
⁴³ of networks is influenced by the interaction types that define links. Existing methodologies for recon-
⁴⁴ structing interaction networks tend to emphasize structural patterns, while overlooking the functional
⁴⁵ outcomes of interactions that are critical for understanding how plant communities support pollinators
⁴⁶ (Quintero et al., 2022). In *Apidae* species, for example, plant interactions may have several outcomes.
⁴⁷ Bees consume plant material, including pollen, nectar, or even plant tissue (Pashalidou et al., 2020;
⁴⁸ Vaudo, 2015). They also collect pollen on their corbicula for transport to the nest for feeding drones
⁴⁹ and larvae (Leach & Drummond, 2018; Vaudo, 2015). Finally, visitation of the reproductive parts of
⁵⁰ flowers can have various outcomes for both the plant and pollinator, including pollen transfer (Emer
⁵¹ & Memmott, 2023) and pathogen transfer (Lignon et al., 2024) . Interaction networks generally repre-
⁵² sent only one of these outcomes, although each is important to understanding how plant taxa support
⁵³ pollinators.

⁵⁴ The importance of different outcomes in plant-pollinator interactions becomes clear when considering
⁵⁵ the biodiversity necessary to support pollinators across life stages. Because the resources needed for a
⁵⁶ foraging adult pollinator are different from those needed at the larval stage, or by other colony members
⁵⁷ (Leach & Drummond, 2018; Vaudo, 2015), transported pollen may not completely represent the inter-
⁵⁸ actions necessary to sustain adult pollinators. This is especially true for bumblebees (*Bombus spp.*),
⁵⁹ which are able to evaluate pollen resource quality to discern foraging choices (Leonhardt & Blüthgen,
⁶⁰ 2012; Timberlake, de Vere, et al., 2024). Bumblebees make trial-and-error floral visits in order to
⁶¹ find adequate forage (Selva et al., 2024), which may result in pollen transport without consumption.
⁶² Conversely, consumption, or simply visitation, may occur without resulting in transport (Popic et al.,

63 2012). Accounting for different interaction outcomes, such as visitation, transport, and consumption,
64 is a critical next step in representing the network of plant diversity used by pollinators.

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

73 Genetic tools can detect plant-pollinator interactions that may be unobserved in pollen microscopy and
74 traditional field surveys (Arstingstall et al., 2021; Bell et al., 2016; Lowe et al., 2022; Pornon et al.,
75 2017), and target specific interaction types. Metabarcoding of conserved gene regions from pollen
76 samples can complement field observations of visitation (Arstingstall et al., 2021; Bell et al., 2017),
77 increasing species detection by 9 - 144% (Baksay et al., 2022; Milla et al., 2022; Smart et al., 2017) and
78 network sampling completeness up to 30%, while reducing exaggeration of specialization (Arstingstall
79 et al., 2021) and revealing interactions beyond the traditionally surveyed floral community (de Vere et
80 al., 2017; Milla et al., 2022). Advances in the reliability and accessibility of sequencing technologies
81 have made these approaches more feasible for studying plant-pollinator interactions.

82 Most pollinator interaction network studies that apply metabarcoding focus on the external pollen loads
83 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 (Poronon 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. Corbicula 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 corbicula 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 corbicula 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

¹⁶⁶ described previously by Donald et al. (2022). Briefly, we performed a first step amplification using
¹⁶⁷ the Nex-F & Nex-R tagged internal transcribed spacer 2 primer pair, ITS-S2F (Chen et al., 2010) and
¹⁶⁸ ITS4R (White et al., 1990), with the following modifications: (1) 3-6 N-mers to increase sequence base
¹⁶⁹ diversity and (2) linker sequences that complement index linker. Amplified products were checked on
¹⁷⁰ a 1% agarose gel for successful amplification. The resulting amplicons were used as template in the
¹⁷¹ second-step PCR where unique 8-mer indices and illumina p5/p7 sequencing primers were attached.
¹⁷² Amplicons were checked for successful amplification as above, purified, normalised and size selected
¹⁷³ using SPRI-beads, and pooled equi-volume to generate the amplicon library. The resulting library was
¹⁷⁴ quantified using Qubit HS kit and functional library was estimated using Colibri qPCR assay. Final
¹⁷⁵ library was sequenced on the illumina MiSeq 3000 instrument for 300 cycles in paired-end mode at
¹⁷⁶ the Ecological Genetics laboratory, Bioeconomy Science Institute, Auckland, New Zealand. Detailed
¹⁷⁷ methods provided in Supporting Information

¹⁷⁸ *Bioinformatics: taxonomic assignment and contaminant analysis*

¹⁷⁹ Raw base call files (BCL) were converted to fastq using bcl2fastq2 (v2.20) tool. Demultiplexing was
¹⁸⁰ performed on the raw fastq files using index combinations and primer pair sequence simultaneously to
¹⁸¹ minimize non-target data, resulting in paired raw reads per sample. Raw reads were processed using
¹⁸² the DADA2 bioinformatics pipeline (Callahan et al., 2016). Reference ASVs were used to call taxo-
¹⁸³ nomic identities using the assign taxonomy method in dada2 in conjunction with the published ITS2
¹⁸⁴ reference database of Bell (2021). We selected the database due to high species coverage for our study
¹⁸⁵ area. All but 21 species were identified to species level, with the remaining 21 identified to genus level.
¹⁸⁶ 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

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

209 *B. pascuorum*-plant interaction network metrics

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

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

222 **3. Results**

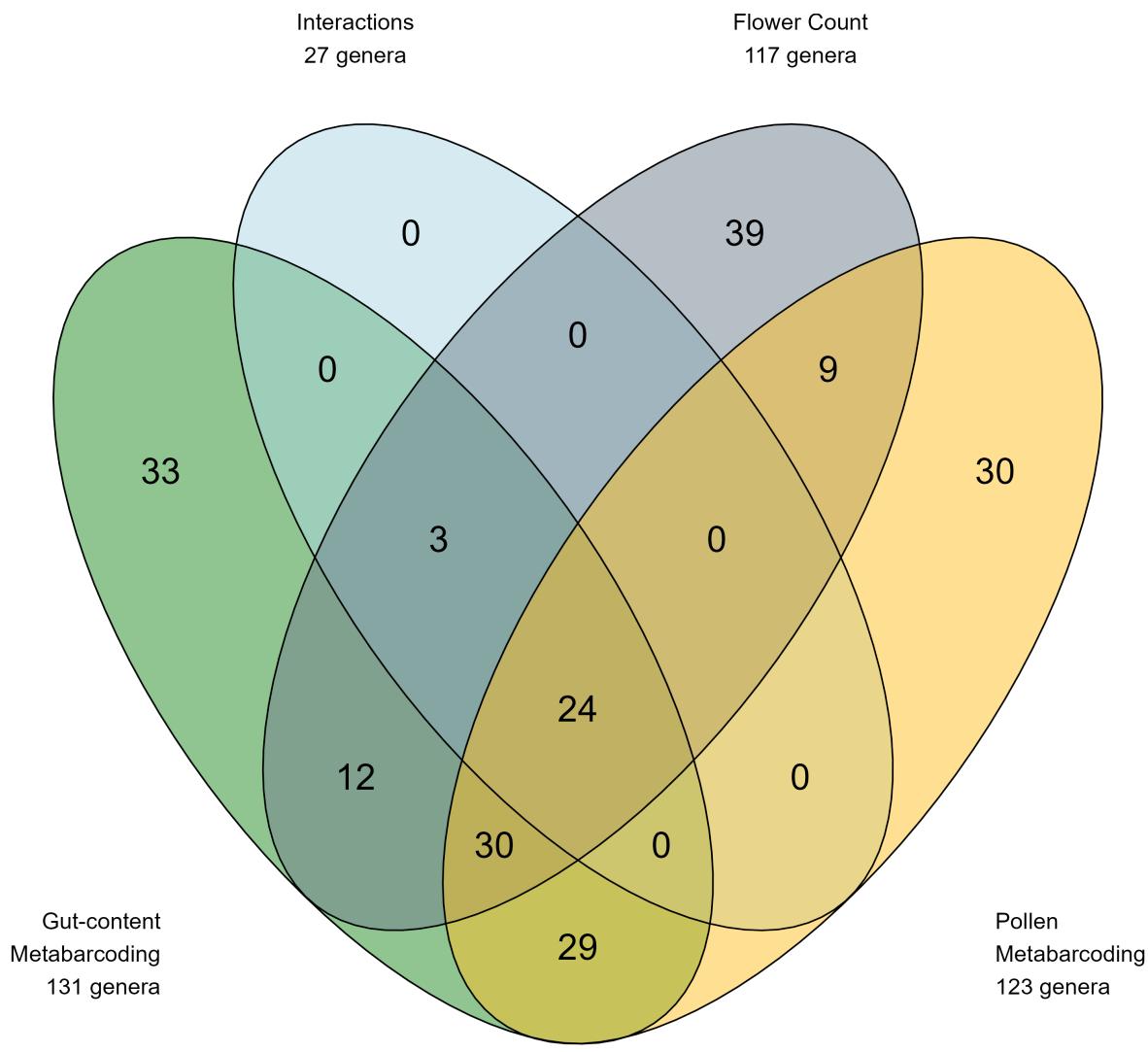
223 *Assessment of floral resource use relative to availability*

224 Within our flower count surveys we registered a total of 117 flowering herbaceous plant genera across
225 the sampling season, representing the pool of floral resources available to *B. pascuorum*, which in-
226 teracted with only a subset of this diversity (Fig. 1). In fact, 39 genera recorded in flower counts
227 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 corbiculair
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 corbiculair 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 corbiculair
²³⁴ pollen diversity and 63% of the gut content diversity.

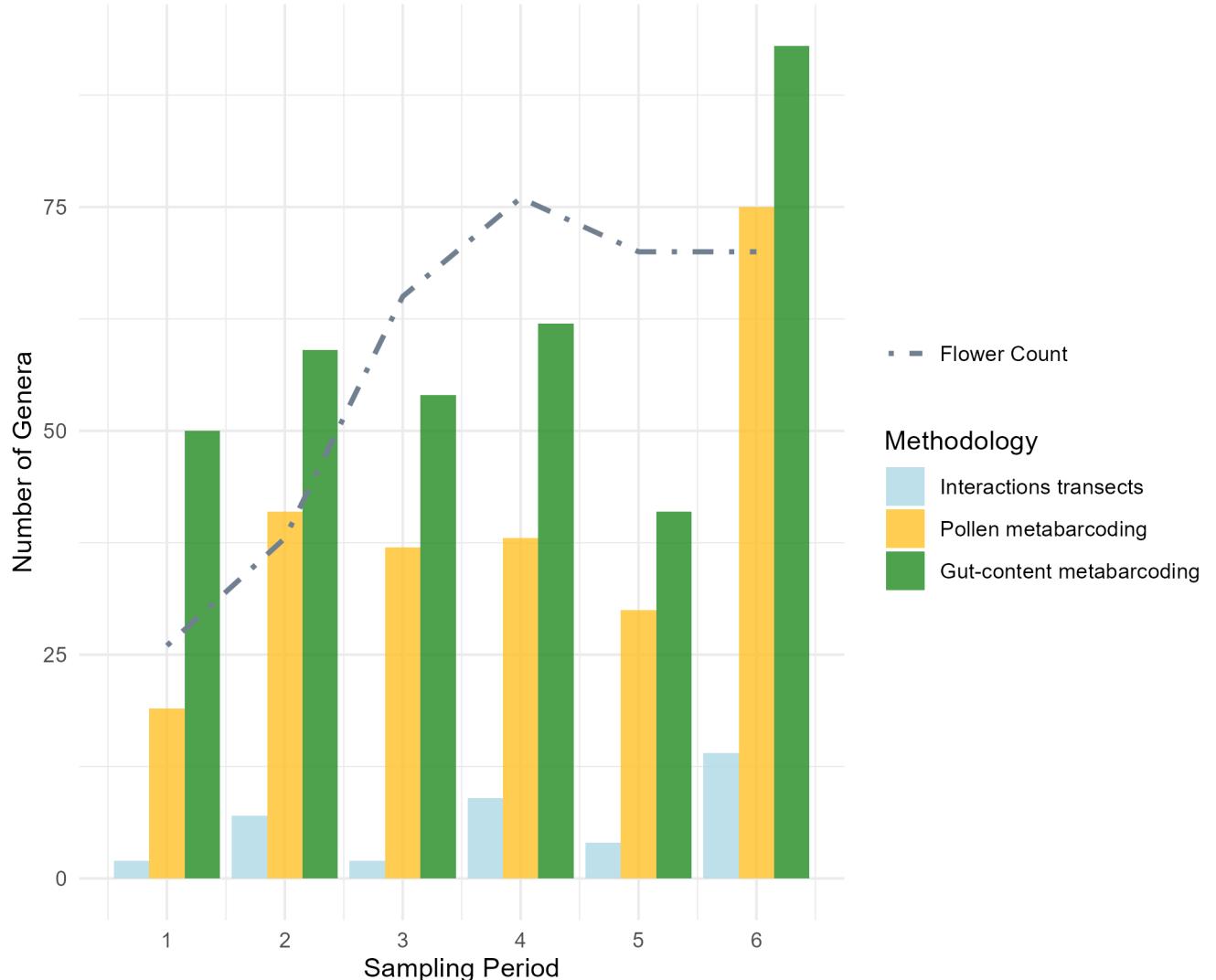


²³⁵

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

²⁴¹ the first to the last period, flowering taxa peaked in period four, whereas interactions peaked in pe-
²⁴² riod six. Metabarcoding consistently detected more taxa than interaction transects, with gut-content
²⁴³ metabarcoding outperforming all other methods. In periods one, two, and six—before and after peak
²⁴⁴ flowering—gut metabarcoding detected 59% more taxa than were recorded in flower counts on average,
²⁴⁵ 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

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

Functional diversity observations

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

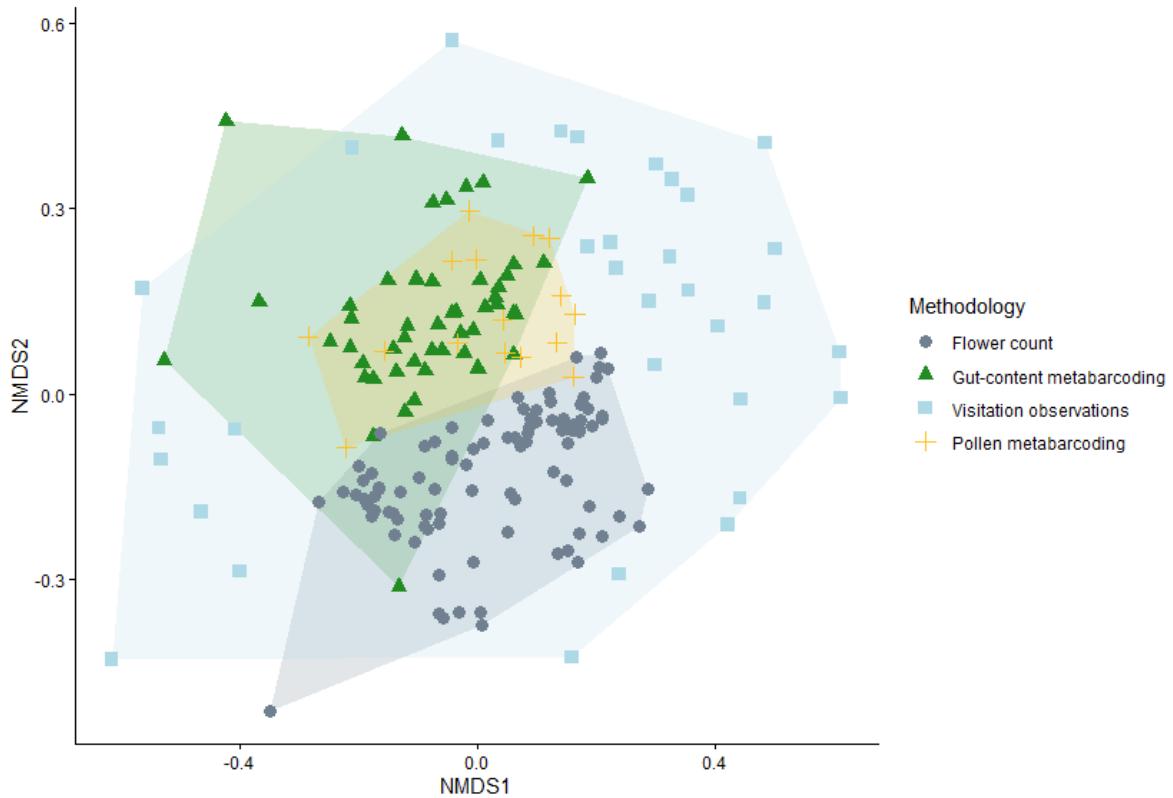
Plant community composition across methodologies

A PERMANOVA test comparing taxonomic composition of interaction plant communities between methodologies indicated a significant effect of methodology on the observed community ($P < 0.001$, $R^2 = 0.28$). In this analysis, interaction transects showed high beta-dispersal (distance to centroid = 0.62) compared to the more centered metabarcoding and flower count results (distance to centroid ≤ 0.10),

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

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

Methodology 1	Methodology 2	DF1	DF2	R^2	F	$\langle i \rangle p \langle /i \rangle$	Adjusted $\langle i \rangle p \langle /i \rangle$
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



281

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

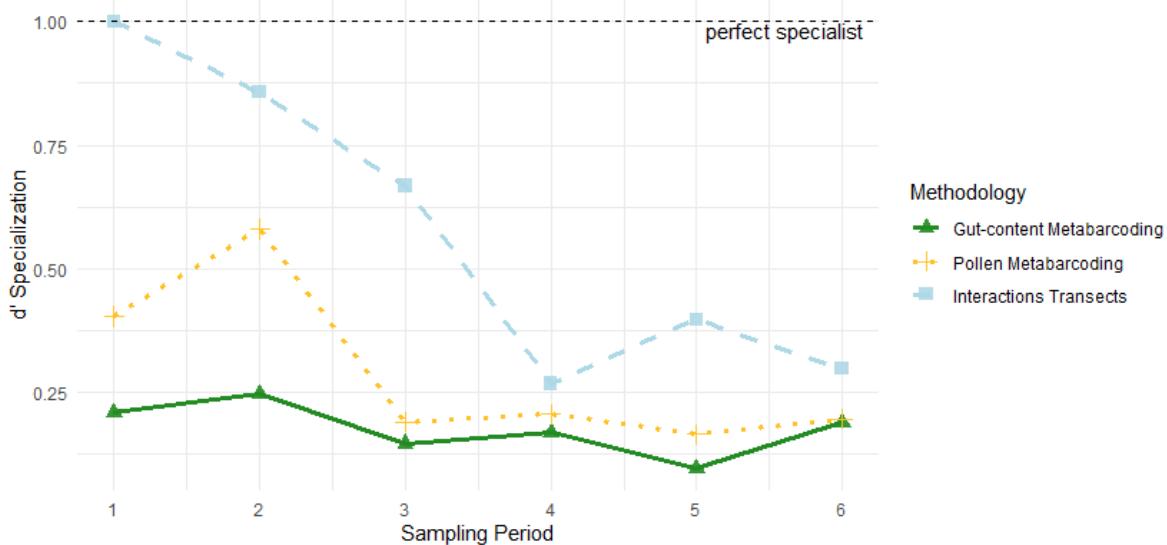
286 *Gut-content vs. pollen-derived plant communities from paired samples*

287 Comparing metabarcoding results from the same specimens, gut contents yielded fewer taxa (mean =
 288 12 genera, $sd = 9$) than pollen samples (mean = 18 genera, $sd = 7$). On average, only 20% of taxa (mean
 289 = 6 genera, $sd = 3$) were shared between the two sample types. A PERMANOVA with specimen as a
 290 blocking factor indicated a difference in the plant community observed by both sample types ($P < 0.01$,

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

294 *Species level interaction network*

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



302
 303 **Figure 4:** Specialization of plant interactions for *Bombus pascuorum* as indicated by networks con-
 304 structed from three interaction observation methodologies. Specialization was calculated as d' using

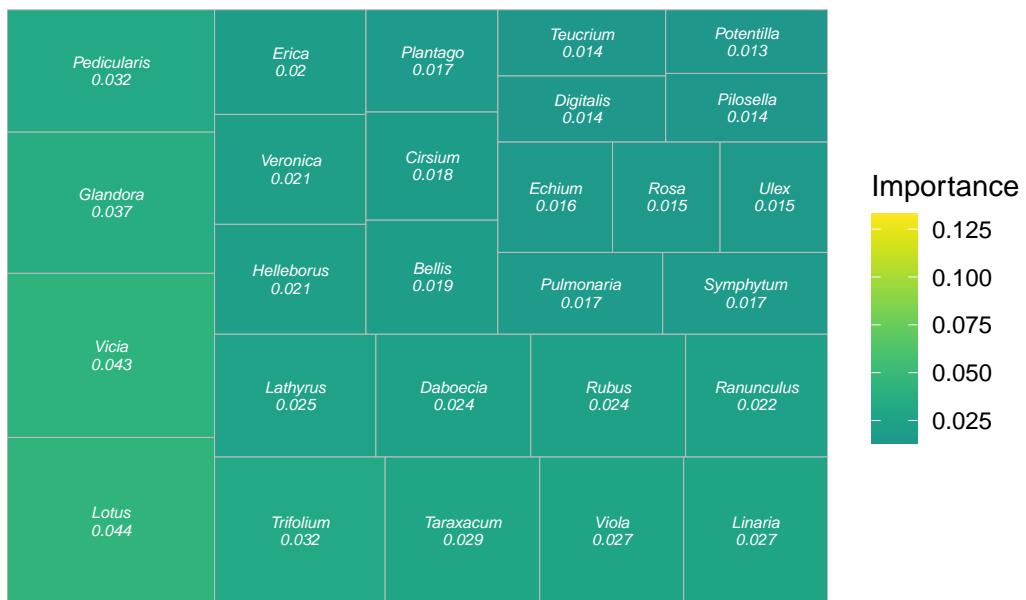
305 the methodology of Blüthgen et al. (2006), with $d' = 1$ representing perfect specialist behavior. Spe-
 306 cialization of *B. pascuorum* for each period was calculated relative to interaction data from the same
 307 species in other periods, rather than other pollinator species.

A. Interaction transect-based network



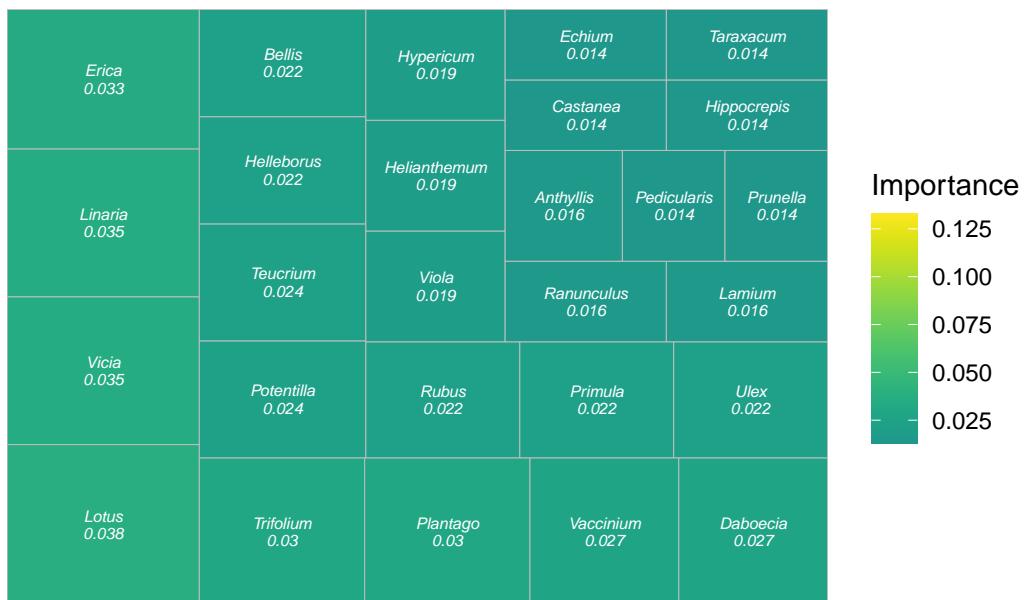
308

B. Gut–content metabarcoding



309

C. Corbicular pollen metabarcoding



310

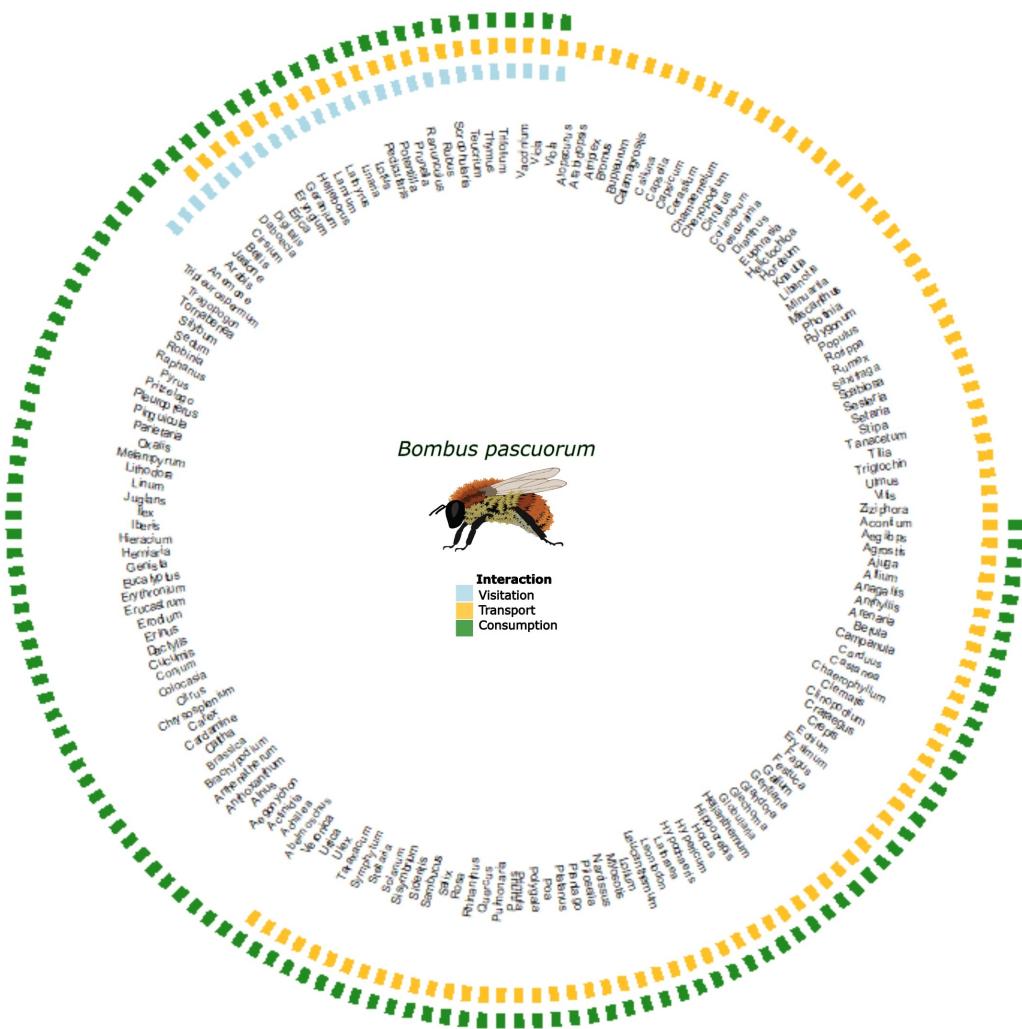
³¹¹ **Figure 5.** Plant “importance” within *Bombus pascuorum* interaction networks constructed from three ³¹² interaction observation methodologies: (A) interaction transects, (B) gut-content metabarcoding, and

(C) corbicula pollen metabarcoding. Importance was calculated as the proportion of total plant interactions observed by the given methodology represented by interactions with the specific plant genus.

Importance is visualized with block size proportional to importance, and color scaled to minimum and maximum values observed by each methodology.

Combined interaction network

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



324

Figure 6. Combined interaction network for *Bombus pascuorum* including all interaction plant taxa detected by three methodologies. Interaction transect observations are represented by visitation, corbicular pollen metabarcoding observation by transport, and gut-content metabarcoding observations by consumption. The network includes 169 plant genera, each with up to three links describing the 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

³⁵¹ capabilities, the results from each methodology allowed for network-level cross-validation.

³⁵² Network topology and specialization patterns differed markedly across methodologies. Interaction tran-
³⁵³ sects tended to overstate both the degree of specialization and the dominance of the most frequently
³⁵⁴ visited plant taxa. Although *B. pascuorum* is known to form strong early-season associations with cer-
³⁵⁵ tain plant species (Artamendi et al., unpublished dataset), the metabarcoding approaches indicated much
³⁵⁶ lower specialization and produced more evenly distributed network structures. These results mirrored
³⁵⁷ previous interaction networks constructed for individual pollinator species, which also have shown a
³⁵⁸ tendency towards representing pollinators as specialists when using field observation data versus the
³⁵⁹ generalist behavior indicated by metabarcoding data (Arstingstall et al., 2021). Overall, the combined
³⁶⁰ datasets across methodologies suggested a more diverse foraging niche than visitation data alone would
³⁶¹ have implied.

³⁶² The three methodologies showed complementary patterns in network composition. Flower counts and
³⁶³ interaction transects overlapped as expected from the study design, yet differed statistically, likely due
³⁶⁴ to the much larger number of taxa detected by the former. No statistical differences were found among
³⁶⁵ the three interaction-focused methods, although their dispersion differed, reflecting variation in spa-
³⁶⁶ tial and taxonomic coverage. Interaction transects are shaped by local habitat and plant-community
³⁶⁷ differences, whereas metabarcoding integrates interactions across the broader landscape, producing
³⁶⁸ more consistent results. Metabarcoding approaches overlapped minimally with the floral community
³⁶⁹ detected by flower counts, indicating that interaction networks include taxa not captured within tran-
³⁷⁰ sects. This is unsurprising given that flower counts reflect potential, not actual, interactions and are
³⁷¹ constrained by spatial and temporal limits that do not restrict metabarcoding.

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

383 *Metabarcoding observes forage across functional groups*

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

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

393 et al., 2024; Tanaka et al., 2020; Terrell & Batra, 1984; Timberlake, de Vere, et al., 2024; Wood et al.,
394 2022), and have especially intriguing implications for bumblebee forage behavior. Previous studies
395 using external pollen metabarcoding have removed wind-pollinated taxa from their analyses under the
396 argument that wind-borne pollen in samples may represent false positive interactions (Negri et al., 2015;
397 Pornon et al., 2017; Tanaka et al., 2020). Our gut-content results, however, caution against the practice
398 of removing these taxa as contaminants, especially if using external pollen loads as standalone proxies
399 for forage networks.

400 The presence of DNA from anemophilous taxa within gut samples suggests that interactions with these
401 taxa may be more than coincidental interactions with pollen in the environment. Indeed, beyond con-
402 sumption for adult nutrition, there are previous indications that pollen from flowering trees supports
403 colony establishment success and low larval mortality (Wood et al., 2022). Our results support the hy-
404 pothesis that most bumblebees forage selectively for consumption and transport of high quality pollen
405 (Ruedenauer et al., 2016; Timberlake, de Vere, et al., 2024), adapting their forage to take advantage of
406 the best available resources as they change with environmental variability (Selva et al., 2024). While
407 it is possible that some plant material may be transported or consumed incidentally (Arstingstall et al.,
408 2021), the taxa detected within *B. pascuorum* gut contents and corbicular pollen form part of the web
409 of biodiversity that supports the species and possibly other pollinators. Our detection of anemophilous
410 plant DNA in both metabarcoding methodologies indicates that *B. pascuorum*, and perhaps other bum-
411 blebee species, may intentionally forage on these taxa to meet nutritional needs at various life stages.

412 Existing hypotheses for pollinator forage adaptations in response to environmental changes have sug-
413 gested 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 underestimate 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 corbiculate 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-

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

460 **References**

- 461 Agut, A., & Hermosilla, B. (2025). *Herbario del Jard?n Bot?nico de Olarizu (Vitoria-Gasteiz)/Olar-*
462 *izuko Lorategi Botanikoaren Herbarioa (Vitoria-Gasteiz)*. Jard?n Bot?nico de Olarizu (Vitoria-
463 Gasteiz)/Olarizuko Lorategi Botanikoa (Vitoria-Gasteiz). <https://doi.org/10.15470/R7IFMA>
- 464 Ankenbrand, M. J., Keller, A., Wolf, M., Schultz, J., & F?rster, F. (2015). ITS2 Database V: Twice
465 as Much: Table 1. *Molecular Biology and Evolution*, 32(11), 3030–3032. <https://doi.org/10.1093/molbev/msv174>
- 466 Arstingstall, K. A., DeBano, S. J., Li, X., Wooster, D. E., Rowland, M. M., Burrows, S., & Frost, K.
467 (2021). Capabilities and limitations of using DNA metabarcoding to study plant–pollinator inter-
468 actions. *Molecular Ecology*, 30(20), 5266–5297. <https://doi.org/10.1111/mec.16112>
- 469 Artamendi, M., Martin, P. A., Bartomeus, I., & Magrach, A. (2025). Loss of pollinator diversity con-
470 sistently reduces reproductive success for wild and cultivated plants. *Nature Ecology & Evolution*,
471 9(2), 296–313. <https://doi.org/10.1038/s41559-024-02595-2>
- 472 Baksay, S., Andalo, C., Galop, D., Burrus, M., Escaravage, N., & Poron, A. (2022). Using metabar-
473 coding to investigate the strength of plant-pollinator interactions from surveys of visits to DNA
474 sequences. *Frontiers in Ecology and Evolution*, 10. <https://doi.org/10.3389/fevo.2022.735588>
- 475 Becher, M. A., Twiston-Davies, G., Osborne, J. L., & Lander, T. A. (2024). Resource gaps pose the

- 477 greatest threat for bumblebees during the colony establishment phase. *Insect Conservation and*
478 *Diversity*, 17(4), 676–689. <https://doi.org/10.1111/icad.12736>
- 479 Bell, K. (2021). *ITS2 july 2021*. figshare. <https://doi.org/10.6084/M9.FIGSHARE.14936004.V1>
- 480 Bell, K., de Vere, N., Keller, A., Richardson, R. T., Gous, A., Burgess, K. S., & Brosi, B. J. (2016).
481 Pollen DNA barcoding: Current applications and future prospects. *Genome*, 59(9), 629–640. <https://doi.org/10.1139/gen-2015-0200>
- 482 Bell, K., Fowler, J., Burgess, K. S., Dobbs, E. K., Gruenewald, D., Lawley, B., Morozumi, C., & Brosi,
483 B. J. (2017). Applying pollen DNA metabarcoding to the study of plant-pollinator interactions.
484 *Applications in Plant Sciences*, 5(6), 1600124. <https://doi.org/10.3732/apps.1600124>
- 485 Blüthgen, N., Menzel, F., & Blüthgen, N. (2006). Measuring specialization in species interaction net-
486 works. *BMC Ecology*, 6(1), 9. <https://doi.org/10.1186/1472-6785-6-9>
- 487 Bosch, J., Martín González, A. M., Rodrigo, A., & Navarro, D. (2009). Plant-pollinator networks:
488 adding the pollinator's perspective. *Ecology Letters*, 12(5), 409–419. <https://doi.org/10.1111/j.1461-0248.2009.01296.x>
- 489 Burkle, L. A., & Alarcón, R. (2011). The future of plant-pollinator diversity: Understanding interaction
490 networks across time, space, and global change. *American Journal of Botany*, 98(3), 528–538.
491 <https://doi.org/10.3732/ajb.1000391>
- 492 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016).
493 DADA2: High resolution sample inference from illumina amplicon data. *Nature Methods*, 13(7),
494 581–583. <https://doi.org/10.1038/nmeth.3869>
- 495 Chen, S., Yao, H., Han, J., Liu, C., Song, J., Shi, L., Zhu, Y., Ma, X., Gao, T., Pang, X., Luo, K., Li, Y.,
496 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.pone.0008613>
- 500
- 501 Cullen, N., Fetters, 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/srep42838>
- 509
- 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.cois.2020.01.005>
- 523
- 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 Strat-
530 egies. *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
- 537 Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., &
538 Tscharntke, 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.2006.3721>
- 540
- 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., Valterovà, 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 Goeve, 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. [023-42102-4](https://doi.org/10.1038/s41598-
556 023-42102-4)
- 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
- 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/ece3.7605>
- 571
- 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. *Ecological
574 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.org/10.1371/journal.pone.0132491>
- 580
- 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*,

- 587 368(6493), 881–884. <https://doi.org/10.1126/science.aay0496>
- 588 Pojar, J. (1973). Pollination of typically anemophilous salt marsh plants by bumble bees, *bombus*
589 *terricola occidentalis* grne. *The American Midland Naturalist*, 89(2), 448–451. [https://doi.org/10.](https://doi.org/10.2307/2424049)
590 [2307/2424049](https://doi.org/10.2307/2424049)
- 591 Popic, T. J., Wardle, G. M., & Davila, Y. C. (2012). Flower-visitor networks only partially predict the
592 function of pollen transport by bees. *Austral Ecology*, 38(1), 76–86. <https://doi.org/10.1111/j.1442-9993.2012.02377.x>
- 593 Pornon, A., Andalo, C., Burrus, M., & Escaravage, N. (2017). DNA metabarcoding data unveils in-
594 visible pollination networks. *Scientific Reports*, 7(1), 16828. <https://doi.org/10.1038/s41598-017-16785-5>
- 595 Quintero, E., Isla, J., & Jordano, P. (2022). Methodological overview and data-merging approaches in
596 the study of plant-frugivore interactions. *Oikos*, 2022(2). <https://doi.org/10.1111/oik.08379>
- 597 Ruedenauer, F. A., Spaethe, J., & Leonhardt, S. D. (2016). Hungry for quality-individual bumble-
598 bees forage flexibly to collect high-quality pollen. *Behavioral Ecology and Sociobiology*, 70(8),
599 1209–1217. <https://doi.org/10.1007/s00265-016-2129-8>
- 600 Saunders, M. E. (2018). Insect pollinators collect pollen from wind-pollinated plants: implications for
601 pollination ecology and sustainable agriculture. *Insect Conservation and Diversity*, 11(1), 13–31.
602 <https://doi.org/10.1111/icad.12243>
- 603 Selva, S., Moretti, M., Ruedenauer, F., Keller, A., Fournier, B., Leonhardt, S. D., Eggenberger, H. A.,
604 & Abella, J. C. (2024). *Urban bumblebees diversify their foraging strategy to maintain nutrient*
605 *intake*. <https://ecoenvrxiv.org/repository/view/7812/>
- 606 Shi, H., Ratering, S., Schneider, B., & Schnell, S. (2025). Microbiome of honey bee corbicular pollen:
607

- 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/10.1002/2688-8319.12360>
- 623 Timberlake, T. P., Tew, N. E., & Memmott, J. (2024). Gardens reduce seasonal hunger gaps for farmland
624 pollinators. *Proceedings of the Royal Society B: Biological Sciences*, 291(2033). <https://doi.org/10.1098/rspb.2024.1523>
- 625 Vanbergen, A. J., & Insect Pollinators Initiative, the. (2013). Threats to an ecosystem service: pressures
626 on pollinators. *Frontiers in Ecology and the Environment*, 11(5), 251–259. <https://doi.org/10.1890/120126>
- 627 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_Brun_s_B_Lee_and_J_W_Taylor_Amplification_and_direct_sequencing_of_fungal_ribosomal_RNA_Genes_for_phylogenetics
- 635
- 636
- 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>