

¹ **Key-words:** interaction, metabarcoding, network, pollinator

² **Abstract**

³ Our understanding of plant-pollinator interaction networks hinges on the methods used to describe their
⁴ nodes and links. Most networks are built from field observations that may overlook many consumer–
⁵ resource links, and these networks lack descriptive links that characterize interaction types and out-
⁶ comes. Towards a more complete approach for building interaction networks, we compare plant inter-
⁷ actions from the wild pollinator species, *Bombus pascuorum*, recorded by three methodologies with
⁸ different implications for interaction outcomes. We compare floral visitation interactions obtained
⁹ from field observations, plant consumption interactions revealed by metabarcoding of gut contents,
¹⁰ and pollen transport interactions detected by metabarcoding of corbicular pollen loads. Our approach
¹¹ adds functional context to plant–pollinator network links and reveals new interactions. We show that
¹² both metabarcoding approaches increase observed interactions and reveal links left unobserved by field
¹³ observations of visitation, highlighting plant taxa that are not pollinator-dependent, yet constitute im-
¹⁴ portant dietary resources. Paired with floral diversity surveys, gut content results also reveal seasonal
¹⁵ patterns in the spatial extent and functional diversity included in forage, which other methodologies fail
¹⁶ to demonstrate. Metabarcoding data analyzed at the individual specimen level further reveal hetero-
¹⁷ geneity in plant resource use between pollen transport and consumption. Metabarcoding methodologies
¹⁸ capture greater spatial, temporal, and taxonomic ranges, while field observations provide validating
¹⁹ datasets with greater taxonomic precision. Our results show that integrating visitation, transport, and
²⁰ consumption data changes network topology and the roles of plant nodes, offering a more nuanced
²¹ and complete map of interactions with clearer priorities for management. We advocate for defining

²² links explicitly by their functions and combining methods to account for hidden structure in ecological
²³ networks.

²⁴ **Data and Code for peer-review**

²⁵ All amplicon sequencing data will be deposited in the European Nucleotide Archive project: PR-
²⁶ JEB105453. Other data and code will be available in the GorBEEa GitHub repository, Compari-
²⁷ son_interaction_methodologies, which will be made available to peer-reviewers upon acceptance for
²⁸ review.

²⁹ **1. Introduction**

³⁰ Pollination is a critical ecosystem service that is currently threatened by different global changes, in-
³¹ cluding habitat loss, intensifying agriculture, pathogens, and invasive species (Klein et al., 2006). Pol-
³² linators critically support the reproduction of 94% of wild flowering plants and 75% of crop species
³³ (Vanbergen & Insect Pollinators Initiative, 2013), contributing to 35% of global food production (Klein
³⁴ et al., 2006). Despite the clear importance of understanding plant-pollinator interactions, our knowl-
³⁵ edge of interaction diversity remains incomplete, as the methodological approach to studying plant-
³⁶ pollinator interactions has historically been biased towards the plant side of interactions (Bosch et al.,
³⁷ 2009; Evans & Kitson, 2020). As a consequence, the well-established relationship between pollina-
³⁸ tor diversity and the productivity of plant communities (Artamendi et al., 2025; Katumo et al., 2022;
³⁹ Woodcock et al., 2019) lacks an equally developed mirrored perspective, describing the floral diversity
⁴⁰ that supports pollinator populations.

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

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

⁵³ The importance of different outcomes in plant-pollinator interactions becomes clear when consider-
⁵⁴ ing the biodiversity necessary to support pollinators across life stages. Because the resources needed
⁵⁵ for foraging adult pollinator nutrition can be different from those needed at the larval stage, or by other
⁵⁶ colony members (Leach & Drummond, 2018; Vaudo, 2015), transported pollen may not completely rep-
⁵⁷ resent the interactions necessary to sustain adult pollinator diets. This is especially true for bumblebees
⁵⁸ (*Bombus spp.*), which are able to evaluate pollen resource quality to make discerning forage choices
⁵⁹ (Leonhardt & Blüthgen, 2012; Timberlake, de Vere, et al., 2024). Bumblebees make trial-and-error
⁶⁰ floral visits in order to find adequate forage (Selva et al., 2024), which may result in pollen transport
⁶¹ without consumption. Conversely, consumption, or simply visitation, may occur without resulting in
⁶² transport (Popic et al., 2012). Accounting for different interaction outcomes, such as visitation, trans-

63 port, and consumption, is a critical next step in representing the network of plant diversity used by
64 pollinators.

65 Shifting network studies to incorporate the pollinator perspective and leveraging the contributions of
66 different methodologies can produce a more complete image of interaction networks. Research based
67 on microscopy and molecular analyses of pollen load samples sourced from insect specimens can iden-
68 tify greater plant species diversity within interaction networks compared to studies based solely on field
69 observations of floral visitation (Baksay et al., 2022; Bosch et al., 2009). Additionally, studies adopt-
70 ing a pollinator-centered view have revealed greater detail in forage preference trends, such as how
71 pollinators use forage quality or quantity-based strategies (Selva et al., 2024; Timberlake, de Vere, et
72 al., 2024), seasonal changes (Leponiemi et al., 2023), life cycle timing, and metabolic specialization
73 (Vaudo, 2015).

74 Genetic tools can detect plant-pollinator interactions that may be unobserved in pollen microscopy and
75 traditional field surveys (Arstingstall et al., 2021; K. L. Bell et al., 2016; Lowe et al., 2022; Pöron et
76 al., 2017), and target specific interaction types. Amplicon sequence metabarcoding of pollen samples
77 complements the visitation interactions observed by field studies (Arstingstall et al., 2021; K. L. Bell et
78 al., 2017), increasing species detection by 9 - 144% (Baksay et al., 2022; Milla et al., 2022; Smart et al.,
79 2017) and network sampling completeness up to 30%, while reducing exaggeration of specialization
80 (Arstingstall et al., 2021) and revealing interactions beyond the traditionally surveyed floral community
81 (de Vere et al., 2017; Milla et al., 2022). Advances in the reliability and accessibility of amplicon
82 sequencing have made these approaches more feasible for studying plant-pollinator interactions. Field
83 surveys of visitation can now be effectively complemented by genetic tools (Milla et al., 2022) targeting

84 specific interaction types, enhancing our understanding of interaction diversity.

85 Most studies applying metabarcoding to pollinator-sourced samples for constructing interaction net-
86 works analyze the external pollen loads of bees or pollen stored in nest reserves of honey and bee bread
87 (Baksay et al., 2022; Devries et al., 2024; Leontidou et al., 2021; Leponiemi et al., 2023; Selva et al.,
88 2024), despite limitations of these sampling targets. Pollen in these samples can come from the envi-
89 ronment, even including accumulation of windborne material (Negri et al., 2015). To account for this,
90 past studies have ignored detections of wind pollinated taxa (Pornon et al., 2017; Tanaka et al., 2020),
91 although this may introduce bias to results, given that many plant taxa have partial identities as wind
92 or insect pollinated taxa (Saunders, 2018). A more fundamental issue with externally carried pollen
93 and nest reserves is present in their restricted ability to represent interaction types. Studies of external
94 pollen carried by eusocial bees, for example, have generally sequenced the DNA of pollen from the
95 corbicula (e.g. Shi et al. (2025)). Corbicula pollen provides an easily obtained sample, containing
96 a mixture of pollen collected for transport to the nest for brood feeding (Leach & Drummond, 2018;
97 Vaudo, 2015), which only directly observes interactions where pollen is transported (Arstingstall et al.,
98 2021). Given the role of this pollen in bees' life cycles, it is easy to overstep the interpretative capacity
99 of these sample types when characterizing forage networks to describe diet, or successful pollination
100 interactions.

101 Pollinator intestinal tracts (hereafter: guts) represent an additional source for observing interactions,
102 specifically those related to consumption of pollen and other plant material (Haag et al., 2023; Li et al.,
103 2025; Mayr et al., 2021). Plant DNA detected in gut contents can reveal interactions with consumption
104 as the exclusive outcome, which, aside from flower visits, can include nectar robbing (Popic et al.,

¹⁰⁵ 2012) and plant damage (Pashalidou et al., 2020). The gut content approach can also account for en-
¹⁰⁶ vironmental contamination in external pollen and nest stores by highlighting oversights resulting from
¹⁰⁷ the exclusion of interactions with the anemophilous and partially-anemophilous plant taxa in external
¹⁰⁸ pollen studies. There is an accumulating body of evidence supporting the idea that pollinators must
¹⁰⁹ regularly search across functional groups of the plant community to meet their nutritional needs (de
¹¹⁰ Vere et al., 2017; Ibiyemi et al., 2025; Milla et al., 2022; Pojar, 1973; Selva et al., 2024; Tanaka et al.,
¹¹¹ 2020; Terrell & Batra, 1984; Timberlake, de Vere, et al., 2024; Wood et al., 2022), although little atten-
¹¹² tion has been given to these observations as a potentially important part of plant-pollinator networks
¹¹³ (Saunders, 2018). This understudied component of pollinator forage together with the surprising lack
¹¹⁴ of genetic analyses of pollinator gut contents, represents a clear knowledge gap and an opportunity to
¹¹⁵ uncover finer detail in pollinator interaction networks.

¹¹⁶ Our objective is to determine whether a combined methodological approach can provide further insights
¹¹⁷ into pollinator forage ecology and plant-pollinator interaction networks by expanding interaction detec-
¹¹⁸ tions and providing context to network links. We assess how metabarcoding of pollinator gut contents
¹¹⁹ can complement or challenge the characterization of plant-pollinator interaction networks described
¹²⁰ by more common methodologies, including field surveys of plant-pollinator interactions and external
¹²¹ pollen load metabarcoding. To this end, we compare interaction networks constructed from each of
¹²² these methodologies for a single model pollinator, *Bombus pascuorum*, an easily identified bumblebee
¹²³ common to most of Europe (Lecocq et al., 2015). Our focus on a single pollinator species holds pol-
¹²⁴ linator identity constant and attributes differences in network structure to methodology, rather than to
¹²⁵ variation among pollinator species. We hypothesize that the consumption interactions detected in gut

₁₂₆ metabarcoding will include a network of plant taxa distinct from those detected by other methodolo-
₁₂₇ gies. Although we expect overlap between networks constructed by different methodologies, we expect
₁₂₈ to observe previously overlooked interaction network structure, including new links and significance
₁₂₉ of network links. Ideally, the resulting combination of observations will generate a network that will
₁₃₀ elevate our capacity to detect meaningful plant-pollinator interactions, and learn more about interaction
₁₃₁ types and implications for pollinator health.

₁₃₂ **2. Methods**

₁₃₃ Our sample collection was conducted in Gorbeia Natural Park, a protected area in Spain. Within Gor-
₁₃₄ beia, we selected 16 sampling sites located within the mixed zones of meadows and shrublands found
₁₃₅ at higher elevations within the park. We conducted fieldwork from early April to the end of July, 2023
₁₃₆ covering the main flowering period and peak annual pollinator activity in Gorbeia. On each sampling
₁₃₇ day during this timeframe, we visited field sites in pairs. Sampling days were organized into six periods,
₁₃₈ in which we sampled each site pair once per period. We conducted three types of surveys during daily
₁₃₉ peaks of pollinator activity, including floral diversity surveys (“flower counts”), interaction transect
₁₄₀ surveys, and *Bombus pascuorum* specimen collection for amplicon sequencing analyses.

₁₄₁ *Interaction transects and floral resource availability surveys*

₁₄₂ We used the one 250 m transect at each site for both interaction transect and flower count surveys,
₁₄₃ recording observations within ~2 m of the transect line. Interaction surveys were conducted three times
₁₄₄ per day, each lasting 1 h. All insects observed contacting the reproductive parts of herbaceous flowers
₁₄₅ within the transect were recorded; for this study, we retained only *Bombus pascuorum* interaction data.
₁₄₆ Surveys were spaced by ~2 hours (~11:00, ~13:00, ~15:00), and transects were walked at a constant

¹⁴⁷ pace to cover the full length within an hour. For each site and sampling period, one flower count was
¹⁴⁸ conducted by recording all of the flowering herbaceous species within the transects.

¹⁴⁹ *Bombus pascuorum specimens*

¹⁵⁰ For every period visit at each site, we collected up to five *B. pascuorum* specimens for molecular
¹⁵¹ analyses (N = 126). We brought specimens back from the field and froze them at -20°C until processed.
¹⁵² In the lab, we extracted the entire gut and honey stomach of *B. pascuorum* individuals. Additionally,
¹⁵³ if present, we collected pollen pellets from the corbicula of specimens into sterile 1.5 mL centrifuge
¹⁵⁴ tubes. Pollen samples were stored individually by specimen sample at -20°C.

¹⁵⁵ *Gut Content DNA extraction*

¹⁵⁶ Genomic DNA was extracted from *B. pascuorum* guts using the NucleoSpin® 96 Soil kit (Macherey-
¹⁵⁷ Nagel, Düren, Germany) and amplified in duplicate using the DFD forward and ASDFAS reverse
¹⁵⁸ primers. To avoid site and period bias, all samples were randomized before the DNA extraction. We fol-
¹⁵⁹ lowed the kit manufacturer protocol, only adjusting centrifuge times to account for the lower maximum
¹⁶⁰ velocity of the large centrifuge used to process large sample numbers simultaneously (See Supporting
¹⁶¹ Information). To confirm successful DNA extraction, Nanodrop tests were performed on random sam-
¹⁶² ples.

¹⁶³ *DNA extraction from corbicular pollen pellets*

¹⁶⁴ DNA was extracted from pollen pellets (N = 25) using the Machery-Nagel NucleoSpin® 8 Food kit,
¹⁶⁵ including additional initial steps recommended by the kit's supplementary protocol for pollen DNA

₁₆₆ extraction (See Supporting Information). Qubit (Thermo Fisher Scientific) fluorometry tests using
₁₆₇ random samples confirmed successful DNA extractions.

₁₆₈ *Amplicon Sequencing*

₁₆₉ Our metabarcoding sequence libraries were built by amplifying and sequencing the internal transcribed
₁₇₀ spacer (ITS2) region of the ribosomal DNA in our extract samples. For all samples, we used existing
₁₇₁ primers for amplification of the ITS2 region (See Supporting Information). Libraries were sequenced
₁₇₂ on an Illumina platform to generate paired-end raw reads. We used demultiplexed raw sequence data,
₁₇₃ with primer and adapter sequences removed, in further bioinformatic analyses.

₁₇₄ *Bioinformatics: taxonomic assignment and contaminant analysis*

₁₇₅ Raw Illumina sequences were processed using the DADA2 bioinformatics pipeline (Callahan et al.,
₁₇₆ 2016). Taxonomy was added to the ASVs using an existing reference sequence database (K. Bell, 2021),
₁₇₇ which provided reference sequences at the species level for all but 21 of the species present in the study
₁₇₈ area, all of which were identifiable to the genus level in the database. We removed likely contaminants
₁₇₉ and misidentified ASVs from our bioinformatics results using a three-step screening process. First,
₁₈₀ ASVs were analyzed for contaminants using the R package, decontam (Davis et al., 2018). Second,
₁₈₁ we conducted a BLAST search using ITS2 Database (Ankenbrand et al., 2015) to verify taxa that were
₁₈₂ identified by only one ASV within our results. Finally, the remaining list of taxa was screened by a
₁₈₃ local botanist.

₁₈₄ *Statistical analysis*

₁₈₅ We analyzed the results of each methodology together using statistical tools for comparing interac-

186 tion plant communities across methodology, time, and individual specimens. As an initial broad test
187 of whether the methodologies detected interactions with different plant communities, we used binary
188 presence-absence matrices to compare the communities detected by each methodology on each sam-
189 pling day. Data were aggregated by sampling day for all sets of observations. Community composition
190 was contrasted using the Raup-Crick dissimilarity index in a PERMANOVA test within the R package,
191 vegan (Oksanen et al., 2024) with methodology as the independent variable. Further pairwise compar-
192 isons of these data were made by subsetting the dissimilarity matrix used in the first test by each unique
193 methodology pair and using multiple PERMANOVAs to test the pairs. We also used vegan to observe
194 beta dispersal of our data as a further means of understanding PERMANOVA results.

195 Among our *B. pascuorum* specimens, 25 provided both pollen and gut samples. Using the data from this
196 subset of samples, we compared the plant communities detected by the two metabarcoding methodolo-
197 gies at the individual sample level without aggregation. As before, Raup-Crick dissimilarity matrices
198 were calculated using binary detection data from pollen and gut detections. PERMANOVA compared
199 both methodologies' detected communities in strata defined by specimens of sample origin.

200 *B. pascuorum* - plant interaction network metrics

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

207 bipartite (Dormann et al., 2009).

208 We created a composite interaction network for *B. pascuorum*, incorporating the data of each method-

209 ology and the interaction outcome types as network metadata. Network nodes included *B. pascuorum*

210 and the list of plant genera detected across the three interaction datasets. Single plant genera were

211 assigned between one and three links corresponding to interaction type, depending on their detection

212 across methodologies.

213 3. Results

214 *Assessment of floral resource use relative to availability*

215 Within our flower count surveys we registered a total of 117 flowering herbaceous plant genera across

216 the sampling season, representing the pool of floral resources available to *B. pascuorum*, which in-

217 teracted with only a subset of this diversity (Fig. 1). In fact, 39 genera recorded in flower counts

218 were absent from the interaction networks generated by any of the methodologies. Interaction transects

219 revealed interactions with 27 genera (23% of total floral diversity), while gut content and corbiculair

220 pollen metabarcoding revealed interactions with 58% and 53% of available taxa, respectively.

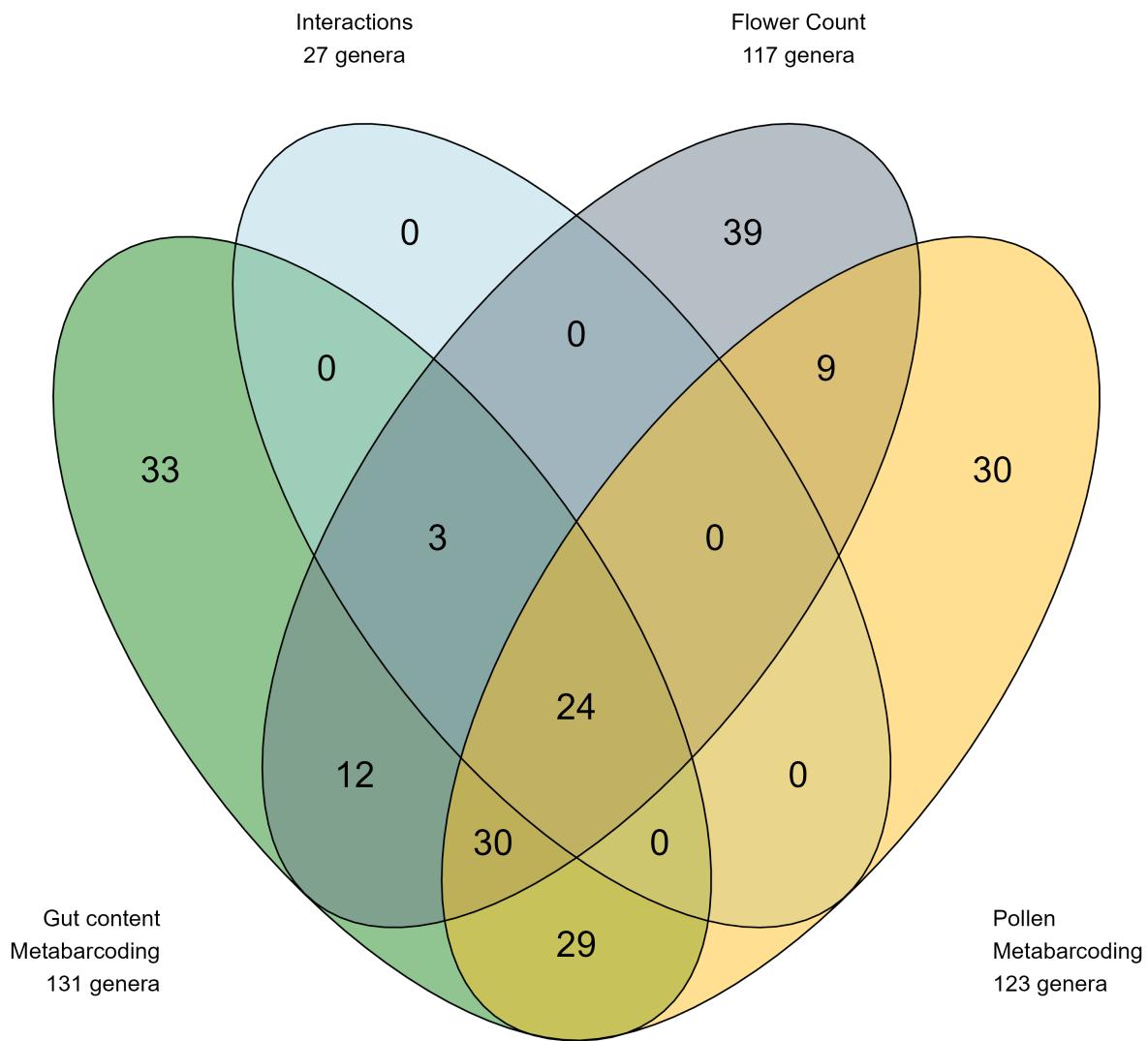
221 *Comparison of interaction detections by methodology*

222 Both metabarcoding methodologies detected multiple unique taxa (33 taxa for gut contents and 30 for

223 corbiculair pollen), while interaction transects did not detect any unique interactions (Fig. 1). The two

224 metabarcoding methodologies shared 83 common plant genera, representing 67% of the total corbiculair

225 pollen diversity and 63% of the gut content diversity.

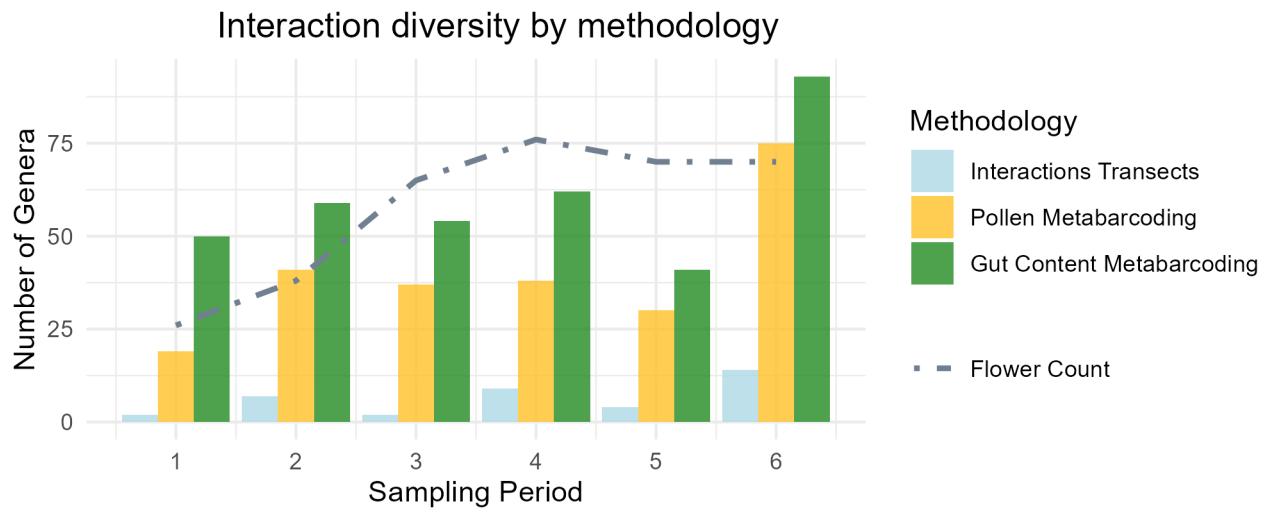


226

227 **Figure 1:** Total diversity and overlap of plant genera observed by four observation methodologies:
 228 transect surveys of floral diversity (“flower counts”) and *B. pascuorum* - flower interactions, and
 229 metabarcoding of plant DNA in corbiculate pollen and gut contents of *B. pascuorum*.

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

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



237
 238 **Figure 2:** Taxonomic diversity in *Bombus pascuorum* interaction networks over six sampling periods
 239 (April - August, 2023) observed through floral visitation surveys and ITS2 metabarcoding of DNA ex-
 240 tracted from bumblebee gut contents and corbiculae pollen loads. The results of each methodology
 241 correspond to samples or surveys each taken across the same 48 sampling days. The number of plant
 242 genera indicated is a cumulative raw value for each methodology and period, with no standardiza-
 243 tion for sampling effort. Interaction diversity for transects is represented by the total number of taxa
 244 observed over each transect and sampling day, for each period. For metabarcoding methodologies,
 245 interaction diversity is the total number of plant genera observed across all samples collected during
 246 the given period.

²⁴⁷ *Functional diversity observations*

²⁴⁸ The design of interaction transects only included taxa from the entomophilous community, while both
²⁴⁹ metabarcoding methodologies detected taxa from the anemophilous community as well, representing
²⁵⁰ 28% ($N = 41$) of the total identified plant genera between the two methodologies. These genera in-
²⁵¹ cluded 20 genera from *Poaceae*, nine tree or woody plant genera, and 12 other herbaceous genera (See
²⁵² Supporting Information). During periods one, two, and six—when gut-content metabarcoding detected
²⁵³ more taxa than the entomophilous community recorded in transects—an average of 13% of those taxa
²⁵⁴ were anemophilous or partially anemophilous (See Supporting Information).

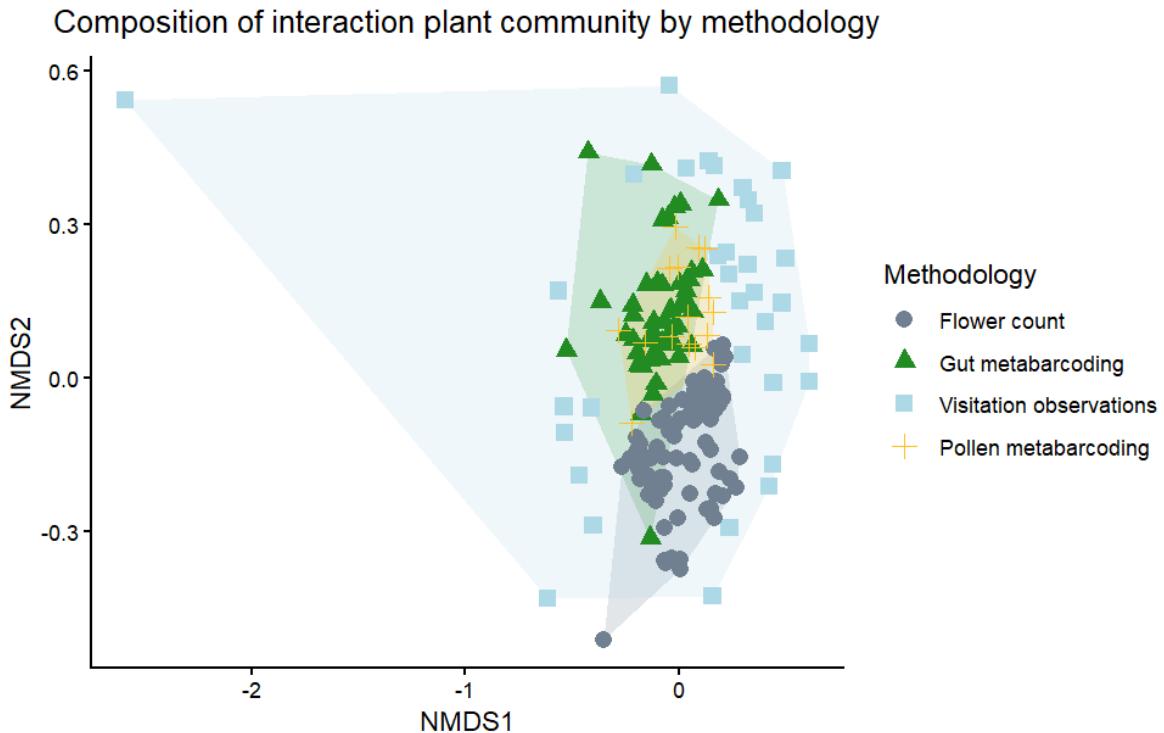
²⁵⁵ *Plant community composition across methodologies*

²⁵⁶ A PERMANOVA test comparing taxonomic composition of interaction plant communities between
²⁵⁷ methodologies indicated a significant effect of methodology on the observed community ($P < 0.001$, R
²⁵⁸ $= 0.28$). In this analysis, interaction transects showed high beta-dispersal (distance to centroid = 0.62)
²⁵⁹ compared to the more centered metabarcoding and flower count results (distance to centroid ≤ 0.10),
²⁶⁰ and an ANOVA test of mean dispersal by methodology indicated different levels of dispersal ($P < 0.001$)
²⁶¹ for each methodology. The communities detected by each of the methodologies were also visualized
²⁶² using non-metric Multidimensional Scaling (nMDS, stress = 0.17, Fig. 3). Pairwise comparisons (Table
²⁶³ 1) showed that the plant communities detected by flower counts were different from those of all other
²⁶⁴ methodologies ($P < 0.001$, Holm-Bonferroni), although between pairs of interaction methodologies, no
²⁶⁵ differences were observed.

²⁶⁶ **Table 1.** *Pairwise tests comparing the community composition of plant taxa detected by four method-
ologies. Detected communities were compared by repeating PERMANOVA tests for each methodology*

268 pair. Tests applied the Raup-Crick dissimilarity index with 9999 permutations, and adjusted p-values
 269 were calculated using the Holm–Bonferroni method. The summarized test statistics include degrees of
 270 freedom for each methodology (DF), R^2 , test F-statistics (F) and associated p-value (p), as well as the
 271 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



272

273 **Figure 3:** Non-metric dimensional scaled visualization of plant communities detected by three method-
 274 ologies for observing *B. pascuorum* floral interactions and a flower diversity survey. Observations from
 275 each methodology are aggregated by sampling day, reduced to binary presence/absence data, and com-
 276 pared in ordination using the Raup-Crick dissimilarity index (ordination stress = 0.17).

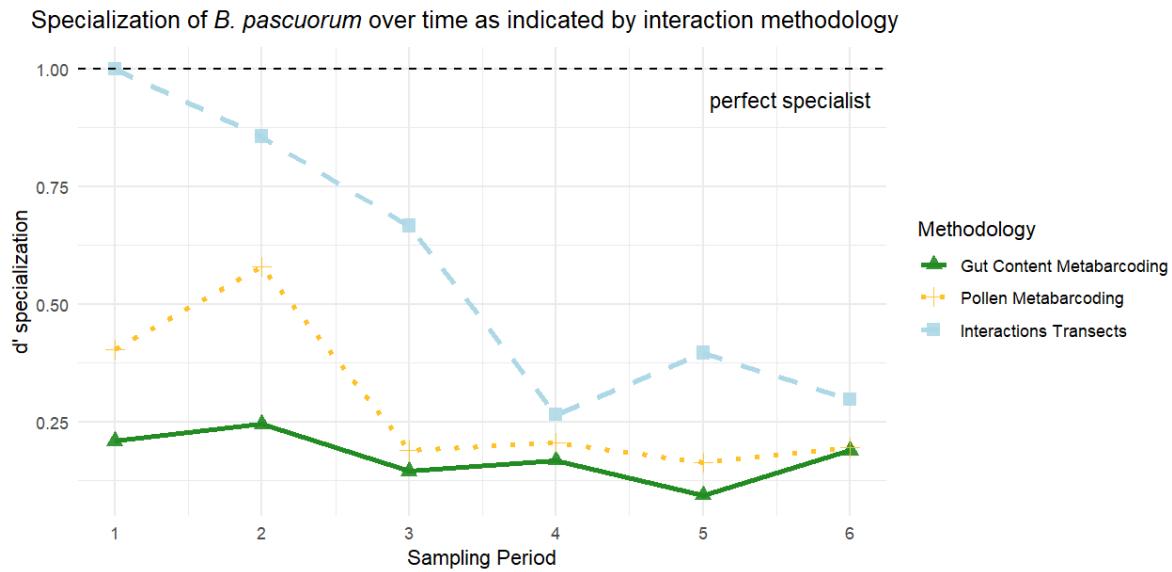
277 Specimen level metabarcoding results

278 Comparing metabarcoding results from the same specimens, gut contents yielded fewer taxa (mean =
 279 12 genera, sd = 9) than pollen samples (mean = 18 genera, sd = 7). On average, only 20% of taxa
 280 (mean = 6 genera, sd = 3) were shared between the two sample types. A PERMANOVA with specimen
 281 as a blocking factor indicated a difference in the plant community observed by both sample types ($P <$
 282 0.01, See Supporting Information) explaining 17% of the variation between gut- and pollen-based de-

283 tectors (See Supporting Information). Data used in this comparison were similarly dispersed (distance
284 to centroid = 0.08), with no difference between the two groups observed by a permute test.

285 *Species Level Interaction Network*

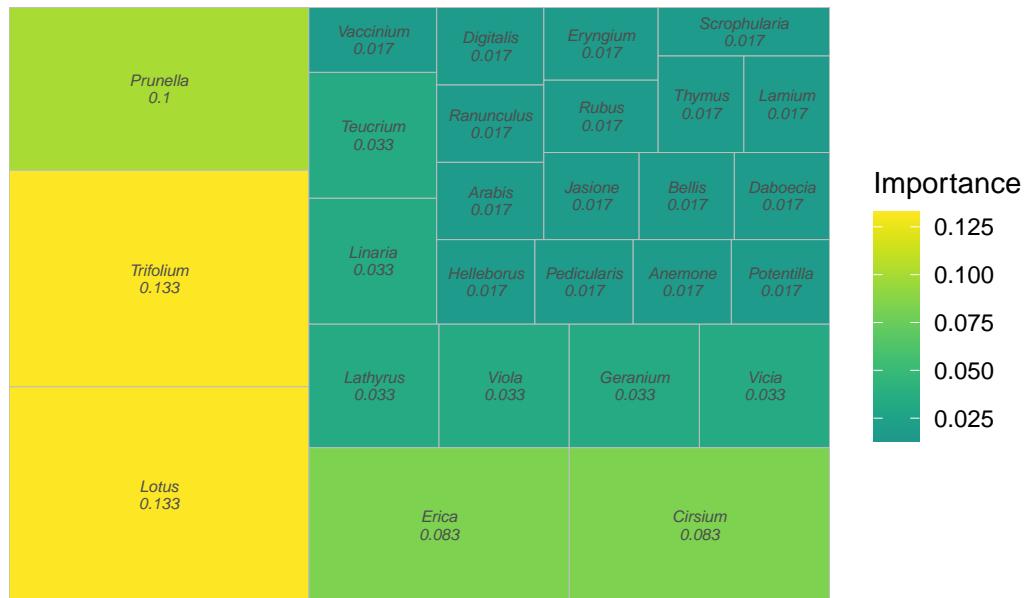
286 We calculated interaction specialization of *B. pascuorum* and an importance metric for the plant taxa
287 within interaction networks. Specialization [d' ; Blüthgen et al. (2006)] declined over the season for
288 transect and pollen-metabarcoding data but remained relatively stable for gut-content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



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

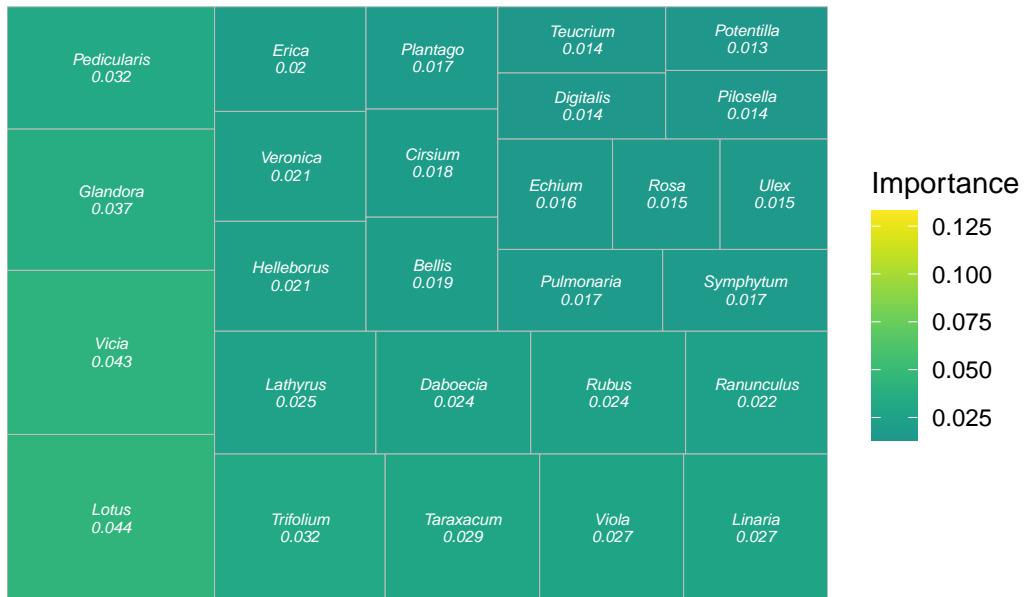
296 methodology of Blüthgen et al. (2006), with $d' = 1$ representing perfect specialist behavior. Specializa-
 297 tion of *B. pascuorum* for each period was calculated relative to interaction data from the same species
 298 in other periods, rather than other pollinator species.

A. Importance of plant taxa in interaction network



299

B. Gut Content Metabarcoding



300

C. Corbicular Pollen Metabarcoding



301

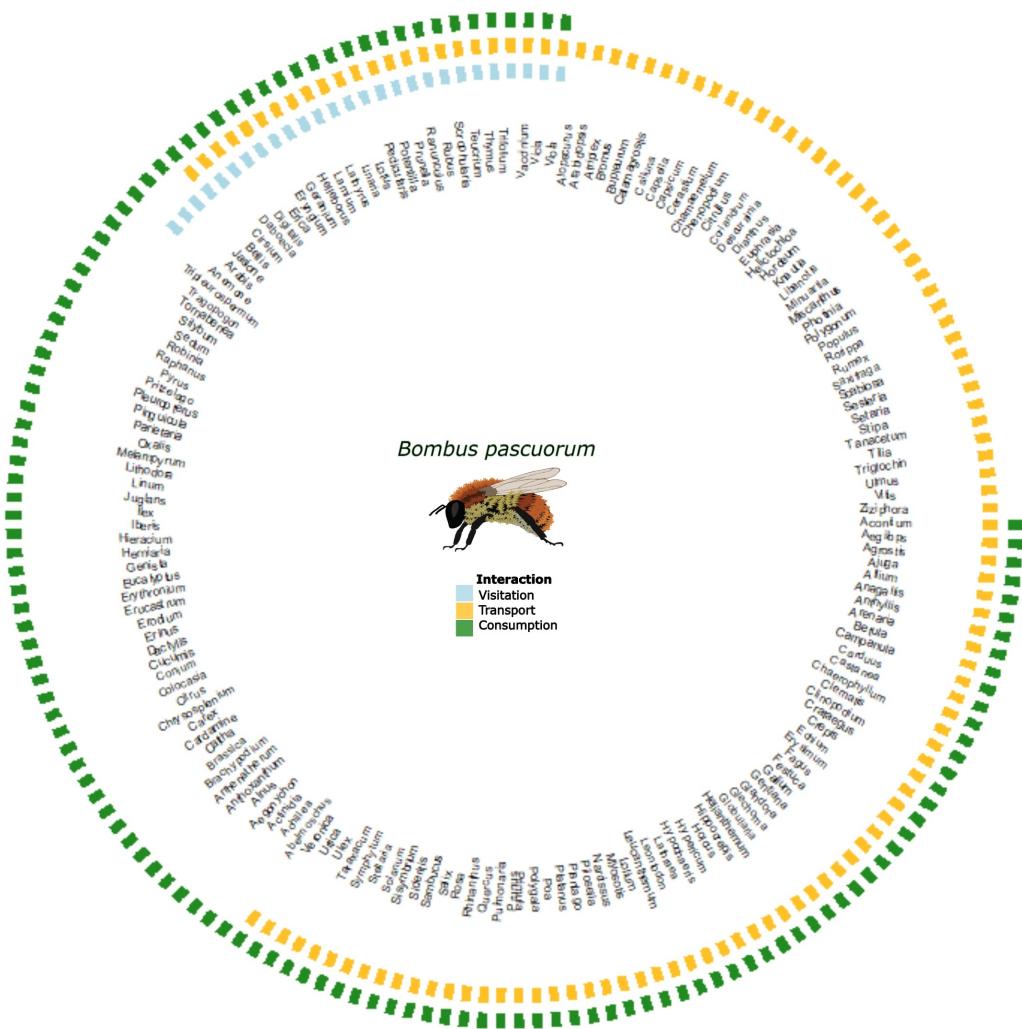
³⁰² **Figure 5.** Plant “importance” within *B. pascuorum* interaction networks constructed from three in-
³⁰³ teraction observation methodologies (A) interaction transects, (B) gut content metabarcoding, and (C)

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.



315

Figure 6. Combined interaction network for *B. 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. 169 plant genera are included within the network, each with up to three links describing the outcomes of interactions with the single pollinator species. Interactions providing links represent

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

322 **4. Discussion**

323 Our results show that combining methodologies yields stronger validation of plant–pollinator interac-
324 tions and deeper insight into network structure. Notably, the two metabarcoding approaches revealed
325 shared interactions with anemophilous and partially anemophilous plants for pollen consumption and
326 transport, highlighting the complementarity of their data. Although each interaction methodology over-
327 lapped statistically at the aggregated level, the combined network resulting from each methodology in-
328 creased the total nodes, and each methodology provided context to network links. Metabarcoding alone
329 also proved effective at capturing a broad range of links and providing detailed, specimen-level data.
330 Important information from the function of links is missing under the current approach to characterizing
331 interaction networks, but using multiple methodologies helps to fill these gaps.

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

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

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

362 Between the two metabarcoding approaches, gut-content metabarcoding captured greater overall tax-

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

373 *Metabarcoding observes forage across functional groups*

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

381 Our observations of interactions with the anemophilous community are supported by previously doc-
382 umented interactions (de Vere et al., 2017; Ibiyemi et al., 2025; Milla et al., 2022; Pojar, 1973; Selva
383 et al., 2024; Tanaka et al., 2020; Terrell & Batra, 1984; Timberlake, de Vere, et al., 2024; Wood et

³⁸⁴ al., 2022), and have especially intriguing implications for bumblebee forage behavior. Previous stud-
³⁸⁵ ies using external pollen metabarcoding have removed wind-pollinated taxa from their analyses under
³⁸⁶ the argument that wind-borne pollen in samples may represent false positive interactions (Negri et al.,
³⁸⁷ 2015; Pernon et al., 2017; Tanaka et al., 2020). Our gut content results, however, suggest that the
³⁸⁸ practice of removing these taxa as contaminants could be a large oversight, especially if using external
³⁸⁹ pollen loads as standalone proxies for forage networks.

³⁹⁰ The presence of DNA from anemophilous taxa within gut samples suggests that interactions with these
³⁹¹ taxa may be more than coincidental interactions with pollen in the environment. Indeed, beyond con-
³⁹² sumption for adult nutrition, there are previous indications that pollen from flowering trees supports
³⁹³ colony establishment success and low larval mortality (Wood et al., 2022). Our results support the
³⁹⁴ hypothesis that bumblebees forage selectively for consumption and transport of high quality pollen
³⁹⁵ (Ruedenauer et al., 2016; Timberlake, de Vere, et al., 2024), adapting their forage to take advantage of
³⁹⁶ the best available resources as they change with environmental variability (Selva et al., 2024). While
³⁹⁷ it is possible that some plant material may be transported or consumed incidentally (Arstingstall et al.,
³⁹⁸ 2021), the taxa detected within *B. pascuorum* gut contents and corbicular pollen form part of the web
³⁹⁹ of biodiversity that supports the species and possibly other pollinators. Our detection of DNA from
⁴⁰⁰ anemophilous pollen sources across the metabarcoding methodologies indicates the potential for inten-
⁴⁰¹ tional forage interactions with these taxa as a means of meeting the nutritional needs for bumblebees at
⁴⁰² various lifecycle stages.

⁴⁰³ Existing hypotheses for pollinator forage adaptations in response to environmental changes have sug-
⁴⁰⁴ gested that bees expand forage diversity beyond the flowering community and across habitats in order

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

416 *Metabarcoding offers individual level analysis*

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

⁴²⁶ underscoring the value of integrating field surveys with laboratory-based methods.

⁴²⁷ *Conclusions*

⁴²⁸ The similarities between interaction data suggest robustness between each methodology, and the in-
⁴²⁹ herent implications of the sample sources of each provide varied means of interpreting different inter-
⁴³⁰ actions. Interaction transects provide a valuable field-based perspective, although given their lower
⁴³¹ sampling efficiency, incorporating them as a validation of other surveys may be the best way to in-
⁴³² tegrate this methodology into future studies. Field observations can fill gaps left by metabarcoding
⁴³³ methodologies, such as confirmation of pollination efficacy, interaction frequency, and species-level
⁴³⁴ resolution. As a direct observation of the pollen transported to the nest, corbiculate pollen may also
⁴³⁵ be a good starting point for identifying plants that may provide pollen with optimal macronutrients
⁴³⁶ for larval development. Similarly, gut content metabarcoding provides an important perspective on
⁴³⁷ the nutritional needs of actively foraging pollinators, identifying taxa that provide pollen as food for
⁴³⁸ supporting this activity (Li et al., 2025). Knowing which taxa are actually ingested by pollinators is
⁴³⁹ especially useful for identifying taxa that facilitate microbiota exchange and acquisition during plant
⁴⁴⁰ interactions (Cullen et al., 2021; Keller et al., 2021), including parasite and disease transfer (Lignon et
⁴⁴¹ al., 2024). Although they are not equal, our research highlights overall that each methodology offers
⁴⁴² advantages and disadvantages in terms of sensitivity, sampling effort, and perspective.

⁴⁴³ While most of the methodologies we applied, aside from gut-content metabarcoding, have previously
⁴⁴⁴ been used independently to characterize plant–pollinator networks (e.g., Devriese et al., 2024; Magrach
⁴⁴⁵ et al., 2023), our findings highlight the added value of integrating them. Gut-content metabarcoding
⁴⁴⁶ emerges as a promising new tool, but its greatest potential is realized when combined with established

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

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