Pollen Metabarcoding and Pollination Networks of Insects of Agricultural Importance in Morogoro, Tanzania.

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Abstract

Pollination is essential for the productivity and sustainability of many tropical agroecosystems, however the relative role of different insect visitors remains poorly understood. Here, we used DNA metabarcoding of pollen loads to compare foraging patterns in honeybees (*Apis melifera*) and two hoverfly species (*Toxomerus floralis* and *Paragus borbonicus*) across highland and lowland farms managed either agroecologically or conventionally (pesticide-based) in Eastern Tanzania. Our pipeline recovered over 3,000 sequence variants covering 245 genera and 94 families, discovering that pollinator identity was the primary explanation of pollen-transport diversity—explaining about 4.9% of compositional variation—while landscape having a modest effect and farming practices a much smaller one. Honeybee carried the most divers and compositionally distinct pollen loads, with strong link to cucurbit crops, whereas hoverflies prefer open-flowered monocots and weeds. Sex differences in hoverflies were minor, except for a slight alteration in *P. borbonicus* at one elevation. In agroecological plots honeybees collect a more balanced mix of pollen types compared to conventional plots. Whereas hoverflies, which fly farther afield, showed no change in their pollen profiles between the two different farming systems. These patterns indicate the importance of comprehensive local and landscape-level pollinator management: enriching floral resources right around apiaries for honeybees and maintaining diverse habitats across the landscape for hoverflies.

Introduction

Pollination has an essential role in agricultural systems and is necessary for optimizing crop productivity and food security (Khalifa *et al.*, 2021; Klein *et al.*, 2007; Rader *et al.*, 2016) More than one third of the world's crops rely on animal pollinators, mainly insects such as bees, flies, beetles and wasps (Khalifa *et al.*, 2021). Among these, bees are the most effective and beneficial pollinators that contribute to the production and quality of crops, including fruits, vegetables, nuts and oilseed (Khalifa *et al.*, 2021). However, there is growing evidence that bees are not the only key pollinators and the importance of other pollinators such as hoverflies should not be overlooked (Rader *et al.*, 2016). These pollinators can account for up to 50% flower visits and provide additional services, particularly in areas impacted by habitat loss or intense farming (Rader *et al.*, 2016).

Recent global analyses show that, while roughly 60 % of crop species do not require animal pollination, about 35 % of total crop production by weight depends on animal pollinators (Ritchie, 2021). Nearly three-quarters of the world's flowering plant species and more than 75% of crop species rely on animal pollination to some extent, however cereals and other common crops (which make up the majority of volume) are wind- or self-pollinated (Ritchie, 2021). These findings have underlined how valuable pollinators are in sexual reproduction of many crop communities, with implication both in food security and agricultural resilience. Keeping bee and non-bee pollinators healthy is essential to sustain pollination services worldwide, since they are facing growing threats from climate change, pesticide use and habitat loss (Khalifa *et al.*, 2021).

This study offers a quantitative analysis of variation of pollen loads in honeybees (*Apis mellifera*) and flower flies (Syrphidae). Pollen analysis in honeybees has advanced rapidly in recent years with the development of DNA metabarcoding techniques, which allowed researchers to identify the botanical origin of pollen loads more accurately (Richardson *et al.*, 2015b, 2015a). While traditional microscopic palynology methods can be informative, they are limited since distinguishing morphologically similar pollen types is difficult and requires expertise (Hawkins *et al.*, 2015a). DNA metabarcoding has improved our ability to characterize the diversity of pollen carried by pollinators.

In the last decade, DNA metabarcoding has changed how we study pollen. Instead of microscope observation of pollen grains, we target markers such as ITS2 (Internal Transcribed Spacer 2), rbcL (Ribulose bisphosphate carboxylase large chain), and matK (maturase K), for taxonomic identification of pollen (Richardson *et al.*, 2015b). By extracting and sequencing plant DNA from mixed pollen loads, researchers can detect and assign many more plant taxa, even down to species level (Milla *et al.*, 2022). This high sensitivity can also reveal low-abundance or morphologically cryptic plants, which results in a more complete picture of a pollinator's foraging spectrum (Bell *et al.*, 2016; Encinas-Viso *et al.*, 2023).

The use of multi-locus barcoding (combining, for example, the nuclear ITS2 and chloroplast gene markers rbcL) helps to further improve the accuracy of plant identification by capturing a wider variety of plant species. This is because different genetic markers target different groups of plants. This allows researchers to detect a broader range of plant taxa that might not be identified by using only one marker alone (Encinas-Viso *et al.*, 2023; Milla *et al.*, 2022). For example, in tropical agroecosystems, DNA metabarcoding combined with a custom reference library allowed for accurate identification of pollen at the genus or species level in temperate grassland systems, revealing that bees and flies collect pollen from a wide variety of cultivated and wild plants (Lucas *et al.*, 2018).

By using DNA metabarcoding to identify pollen from bees and flies, ecologists have learned a lot about plant-pollinator networks and foraging behavior. Metabarcoding results show that one pollinator normally carries pollen from multiple plant species, which builds interaction networks that are more complex than those built through direct observation alone (Encinas-Viso *et al.*, 2023). Additionally, metabarcoding helped extend studies beyond honeybees to other pollinator families such as Syrphidae (hoverfly). Recent research shows that hoverflies can carry pollen from a wide variety of plant species, highlighting their potential importance alongside bees (Lucas *et al.*, 2018).

Metabarcoding analysis of pollen carried by hoverflies has shown that these flies can be major pollinators with diverse diets, and that pollination networks often involve both bees and flies interacting with overlapping sets of plants (Lucas *et al.*, 2018). These findings suggest that while most pollinators appear to be generalists, DNA-metabarcoding can still reveal small differences—such as how hoverflies may prefer certain blooms that bees tend to avoid (Lowe *et al.*, 2022). This molecular approach offers an effective way to explore how pollinator

communities distribute floral resources and how plant-pollinator interactions are structured over time and space.

Pollen metabarcoding itself has improved over its lifetime. Early studies were often limited by missing barcode data for local plants, but now, custom reference libraries built from voucher specimens in the study region can boost identification rates to over 90% of reads (Bell *et al.*, 2016). Improvements in reference-library availability and sequencing technology are broadening our knowledge further (Bell *et al.*, 2016). Certain limitations remain, particularly regarding the correlation between sequence abundance and actual pollen quality, which makes it necessary to carefully interpret the results (Bell *et al.*, 2016; Hawkins *et al.*, 2015).

The use of pollen DNA metabarcoding over the past decade has completely changed our knowledge of honeybee foraging ecology (Richardson *et al.*, 2015a, 2015b). Honeybees play an important role in pollination. They contribute to biodiversity and agricultural productivity through interactions with a variety of plants (Klein *et al.*, 2007). With the advances in DNA metabarcoding, researchers have been able to more efficiently study the diversity of floral resources used by honeybees, which has provided a more detailed understanding of their foraging behavior and pollen loads (Galimberti *et al.*, 2014).

Through the use of genetic markers such as ITS2 and rbcL, researchers have discovered new complexities of honeybee foraging behavior and plant-pollinator interactions. DNA metabarcoding has revealed that honeybees forage on a broader range of plants across different habitats and farming landscapes than was known from standard microscopic pollen examination in the past (Richardson *et al.*, 2015a, 2015b). This information is relevant to honeybee nutritional preferences, colony health, and responses to environmental changes such as habitat loss, changes in climate, and agricultural expansion (Goulson *et al.*, 2015; Smart *et al.*, 2016). As a result, pollen DNA metabarcoding has become an essential tool for current honeybee pollination research, and it has greatly enriched our ecological knowledge and conservation planning abilities (Pornon *et al.*, 2017).

Flower flies (Syrphidae), despite being globally widespread and ecologically important pollinators, have historically been understudied compared to bees (Bell *et al.*, 2016; Rader *et al.*, 2016a). Recent studies have started to apply DNA metabarcoding to characterize flower flies' pollen loads and foraging behaviors to reveal their interactions with diverse floral communities for the first time (Lucas *et al.*, 2018). For example, Lucas *et al.* (2018) used Illumina-based metabarcoding of the *rbcL* marker and found that hoverflies carry pollen from many plant taxa, with foraging patterns that vary by season and location. Another study showed that metabarcoding can uncover "invisible" plant-pollinator relationships, by comparing networks built from visitation data versus pollen DNA data, proving that Syrphidae often visit open-flower morphologies that are overlooked in field surveys (Pornon *et al.*, 2017). However, despite these innovative techniques, detailed metabarcoding studies that concentrate exclusively on Syrphidae are rare in comparison to hundreds of studies carried out on honeybees (Bell *et al.*, 2016).

This limited focus shows a significant knowledge gap: hoverfly nutrition choices, niche partitioning, and pollination contributions are still not well understood. To completely understand Syrphidae's ecological roles and potential as pollination service providers in both agroecological and natural settings, it is essential to expand DNA metabarcoding studies to them.

Pollinating Diptera, including Syrphidae, are now known as important supplementary pollinators alongside bees. Non-bee insects account for 25-50% of all flower visits in worldwide crops and can offer pollination services that are just as successful as those of bees, especially in environments where bee numbers fluctuate (Rader *et al.*, 2016). Particularly, hoverflies frequently visit small-flowered and open-flowered plants that bees ignore, which helps to fill the network gap and enhance the resilience of these systems to environmental change (Ssymank *et al.*, 2008).

Even though roughly 600 hoverfly species are recorded from the Afrotropical region (Jordaens *et al.*, 2015), their pollination roles remain poorly understood. Most of our knowledge comes from observation or morphological pollen-load studies rather than molecular techniques (Ssymank *et al.*, 2008). This points to a clear gap in this field for metabarcoding and ecological research as we still know very little about their foraging preferences, seasonal activity, and contributions to crop and wild plant pollination in tropical Africa.

This study contributes to the ISeBAF project, which aims to understand how insect biodiversity affects agricultural productivity in African agroecosystems (Royal Museum for Central Africa, n.d.). Parallel studies have focused on different aspects of biodiversity and productivity. Sija Kabota studied insect species diversity (Kabota *et al.*, 2025, unpublished manuscript), Nele Mullens studied soil and plant microbiome diversity (Mullens *et al.*, 2024), and here, we measure pollen load diversity among important pollinators. By integrating these perspectives, our work on pollen metabarcoding completes the picture of how elevation, farming technique, and pollinator identity all interact together to affect pollination networks and ultimately, crop quality.

Together, these studies provide different perspectives on how farming practices and elevation affect biodiversity. In this project, we focus on pollen metabarcoding attempts describing the bioinformatic workflows and laboratory advances made to optimize taxonomic resolution and quantitative reproducibility.

This study intends to achieve three goals. First, to create a thorough reference DNA barcode library for the local flora, by sequencing standard markers (here rbcL) from plants sampled across highland and lowland locations in Morogoro, to ensure the exact taxonomic identification of pollen sequences. Next, by using high-throughput sequencing and a bioinformatic pipeline designed for maximum taxonomic resolution and reproducibility (Bell *et al.*, 2016), to optimize pollen isolation and characterize the pollen loads carried by three important pollinators: *Apis mellifera*, *Toxomerus floralis*, and *Paragus borbonicus*. Finally, we investigate how pollen diversity and composition are influenced by the three pollinator species, two elevation categories, and two farming practices, using a balanced experimental

design. In more detail, the studied factors are (a) pollinator identity, where the specific ecology and morphology of each insect species determines which plants they visit; (b) elevation between different agroecological zones, which compares the floral communities of highland and lowland sites; and (c) farming practice, where we expect that agroecological plots, which are managed without the use of synthetic chemicals, will support more abundant and diverse pollen mixtures than pesticide-based fields (Klein *et al.*, 2007). The goal of the research is to show how insect identity, elevation, and agroecological management interact together to construct pollination networks in African agroecosystems by combining laboratory advancements, bioinformatic refinement, and rigorous multivariate analysis.

Materials and Methods

Sample collection

Pollinators, including honeybees and hoverflies, were collected in April 2022 the Morogoro area, Eastern Central Tanzania (Supplementary Table S2). Hoverflies and honeybees were sampled once per week for eight consecutive weeks. Each weekly sampling included three sweep netting transects carried out for 15 minutes in each subplot. The insects were collected, preserved in 100% ethanol, and morphologically identified using the available identification keys, including the Manual of Afrotropical Diptera Volume 2. (Whittington, 2018). The collected species were deposited at Sokoine University of Agriculture (SUA, Tanzania) and Royal Museum for Central Africa (RMCA, Belgium).

Sampling was conducted in 12 sites (Supplementary Table S2), equally split between high altitude in the Uluguru mountains (~1000 m elevation), and low altitude (~500 m elevation) on the plains at the base of the mountains (Mullens *et al.*, 2024). At each site, two contiguous plots were established: one managed agroecologically and the other managed conventionally. Agroecological management involved manual weeding, mulching, composting, and intercropping, with no use of chemical treatments, whereas conventional crop management relied on the application of pesticides and fungicides for pest and disease control (Mullens *et al.*, 2024).

A multifactorial experimental design was implemented to test the effects of pollinator species, sex, agroecological zones (highland vs. lowland), and farming practices (agroecological vs. pesticide-based). We focused on the three most abundant pollinating insects sampled: *Apis mellifera* (69 female specimens in total), *Toxomerus floralis* (32 female and 33 male) and *Paragus borbonicus* (37 male and 32 female). *A. mellifera* included only female specimens, as they are the sole contributors to pollination, while both sexes were present in the hoverfly species. To account for this unbalanced experimental setup, a combined approach was used. For alpha diversity (linear modelling), the effect of sex was explicitly included in the model. For beta diversity, five insect groups were defined to reflect the uneven distribution of species and sexes (see details below).

Pollen Isolation

Pollen pellets—the external pollen loads removed from each insect (e.g., corbicular pellets of *Apis mellifera* or body-surface clumps on hoverflies)—were isolated by combining two techniques: manually by using sterile tweezers, and through mechanical dislodging by a bead beater to ensure thorough extraction. Each specimen was handled in a sterile environment to avoid cross-contamination. The pollen extraction process followed established protocols from the museum (supplementary Information SI1) to ensure the recovery of high-quality samples for further analysis.

DNA Extraction

After pollen grains were removed from the insect bodies, DNA extraction was performed using a Qiagen Plant DNA Mini Kit protocol (*DNeasy Plant Maxi and Mini Kits, Qiagen GmbH, Hilden, Germany*) to obtain the genetic material for downstream analysis. High-quality DNA was ensured through quality control steps, including quantification using Qubit 4 fluorometer (HS DNA Kit, Thermo Fisher Scientific) and purity assessment using Spectrophotometry

(Nanodrop: measuring the absorbance ratios A260/280 and A260/230 with an Implen NanoPhotometer N60 Touch).

For pollen metabarcoding, the *rbcL* (Bell *et al.*, 2016; Lucas *et al.*, 2018; Richardson *et al.*, 2015b) marker was amplified using universal plant primers.

Sequencing and Quality Control

Sequencing was performed on an Illumina platform, Illumina® NovaSeq™ 6000, and then subjected to a multi-stage quality control process (Figure 1). Sample QC ensured the integrity and purity of extracted DNA before library construction, while library QC checked fragment sizes and adaptor ligation efficiency. Finally, data QC removed low-quality reads, adapter remnants, and chimeric sequences, ensuring that only high-confidence sequences advanced to downstream bioinformatic and diversity analyzes (Novogene Co., 2024).

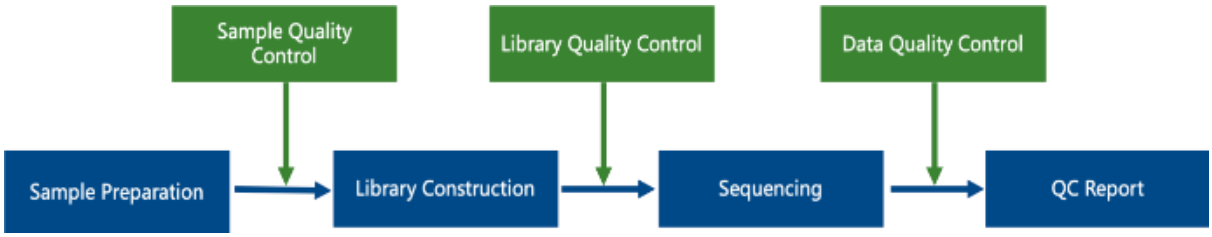

Figure 1. Overview of the library construction and sequencing pipeline with quality control checkpoints. Sample preparation is followed by library construction (with QC), high-throughput sequencing (with QC), and final QC reporting.

The library preparation involved PCR amplification of targeted region (in this case *rbcL*) and size selection which was done with 2% agarose gel electrophoresis. This is to remove shorter or longer fragments that are outside of the desired range so it would not interfere with sequence quality. After size selection, the fragments were end-repaired and A-tailed to ensure compatibility for sequencing and were subsequently ligated with Illumina P5 and P7 adapters (Magoč & Salzberg, 2011). Finally, the prepared library was quantified using qPCR with illumina P5 and P7 adapter primer.

During quality control steps low-quality bases, adapters, and chimeric sequences were removed using Fastp (Chen *et al.*, 2018) and UCHIME (Edgar *et al.*, 2011). Then raw sequencing data was evaluated using Phred scores (Q20, Q30), and only high-quality reads (>99% accuracy) were retained. The average read length was ~385 bp, with GC content ranging from 39–42% (Novogene Co., 2024).

Bioinformatics and Data Analysis

All sequence processing and statistical analyses were carried out in R (version 4.2.0; R Core Team, 2022) using the DADA2, phyloseq, and vegan packages, and Primer-E (CLARKE, 1993a) to examine pollen diversity across pollinators, agroecological zones, and farming practices. Raw paired-end reads were first trimmed and filtered (truncLen = c(240,200), maxN = 0, maxEE = c(2,2), truncQ = 2) using filterAndTrim(). Error rates were then learned per run with learnErrors(), followed by dereplication (derepFastq()) and sample inference via the core dada() algorithm. Forward and reverse reads were merged with mergePairs(), and a sequence table constructed using makeSequenceTable(). Chimeric sequences were removed by removeBimeraDenovo(method="consensus"). Finally, exact sequence variants were assigned

taxonomy against our custom rbcL reference library using assignTaxonomy() in DADA2 (Hendrycks *et al.*, 2025).

Differential Abundance Analysis (DAA): Since pollen metabarcoding data is inherently compositional, meaning the relative abundance of plant taxa sums to 1, we applied ANCOM (Analysis of Composition of Microbiomes) to detect differently abundant taxa across experimental groups (Mandal *et al.*, 2015). This method is particularly useful for analyzing microbiome or metabarcoding data, because the features are interdependent due to their relative abundance.

The statistical analyses were performed in R (v4.2.0), using phyloseq to organize genus-level counts tables (McMurdie & Holmes, 2013). To learn about community-level patterns and identify the specific plant taxa responsible for the observed differences, we turned to ANCOM-BC2 (the bias-corrected version of ANCOM) on our relative-abundance dataset (Lin & Peddada, 2020). This approach allowed us to account for the compositional nature of metabarcoding data while accurately estimating which taxa were considerably more or less abundant between groups.

The first step is data normalization where raw counts were converted to relative abundances by dividing each feature's count by the total count per sample. (If the data was already in relative abundance form, no further normalization was required). The ANCOM analysis used log-ratio tests to identify differentially abundant taxa between experimental groups, such as different insect species, sex, and farming practices. ANCOM allows for the compositional nature of the data by running tests that respect the interdependence of taxa. It also controls the false discovery rate (FDR) across multiple comparisons. The output included p-values and adjusted p-values (q-values) for each taxon, indicating the statistical significance of the observed differences in abundance, along with log-fold changes (lfc) to quantify the magnitude of differences between conditions.

Hypothesis testing

Because no male *A. mellifera* were sampled, our design was inherently unbalanced with respect to sex. To fully characterize community-level and composition-level responses, we therefore applied two complementary approaches:

1. α-Diversity

Within-sample diversity was quantified using both Shannon's H and Simpson's D indices calculated for each individual sample. In this approach to deal with the unbalanced design caused by lack of male *A. melifera*, first, the effect of sex was tested only in the two syrphid species (Toxomerus *floralis* and *Paragus borbonicus*) by fitting separate ANOVAs on Shannon and Simpson values with sex as the sole factor. We fitted separate ANOVAs for each index including sex and its interactions with other factors; neither the main effect of sex nor any sex-involving interaction reached significance (all $p > 0.05$), so males and females were pooled. We then fitted full three-way ANOVA models for each diversity metric with pollinator species (Apis mellifera, T. floralis, P. borbonicus), elevation (highland vs. lowland), and farming practice (agroecological vs. conventional) as fixed factors. We simplified each model by sequentially removing non-significant terms ($p > 0.05$). Post-hoc pairwise *t*-tests with FDR correction then

identified which species, elevations, or farming systems differed in α-diversity. (Supplementary Table S3 and Figure 2)

2. β-diversity

As explorative Permutational Analysis of Variance (PERMANOVA) (Anderson, 2017) suggested that the effects of sex on pollen load composition was not consistently negligible, we decided to treat male and female specimens as separate groups for further analysis. Specifically, PERMANOVA as implemented in Primer-e 7.0.21 (CLARKE, 1993), was used to test differences in pollen profiles between five *a priori* defined insect groups (GR: *Apis mellifera* (Am), *Toxomerus floralis* males (TfM), *Toxomerus floralis* females (TfF), *Paragus borbonicus* males (PbM), and *Paragus borbonicus* females (PbF)), farming practices (FA: agroecological, conventional) and landscapes (LA: mountaineous, plateau). Before PERMANOVA, data were 4^{th} root-transformed to modulate the weight of dominant pollen taxa and better detect possible changes in the abundance of rare taxa (Clarke, 1993). Effects on pollen β diversity were tested using Euclidean Distances and 999,999 permutations of residuals under a reduced model. *Posteriori* pairwise comparisons were implemented via permutational t-statistics (Anderson, 2017). However, PERMANOVA only tells us that the multivariate centroids shift; it does not indicate *which* plant taxa are driving those shifts. Therefore, we turned to ANCOM-BC2—a bias-corrected differential-abundance test designed for compositional metabarcoding data—to pinpoint the specific families and genera whose relative abundances differ significantly between our insect-sex-landscape-farming combinations. ANCOM-BC2's log-ratio framework and built-in FDR control make it particularly well suited to unbalanced designs and to identify the taxa most responsible for the community-level changes detected by PERMANOVA.

We then explored two key interaction effects. For the group-by-landscape (GR×LA) interaction, we compared each Syrphidae subgroup (PbF, PbM, TfF and TfM) against *A. mellifera* within the plateau and mountainous zones, using Am as our reference. For the group-by-farming (GR×FA) interaction, we tested which pollen genera differed across insect GR (PbF, PbM, TfF, TfM, Am) in agroecological compared to conventional farming.

To ensure our findings were robust across taxonomic scales, we repeated all differential-abundance analyses at the ASV, genus, and family levels.

All scripts, raw sequencing data, and metadata used in this study are available in a single downloadable archive at: Scripts for the master thesis.zip

This includes R scripts for data cleaning, DADA2 processing, and statistical analyses, as well as the reference barcode library and raw ASV table.

Result

A total of 3,006 high-quality ASVs were confidently assigned to 94 plant families and 245 genera. At the family level, the ten most abundant families—led by Cucurbitaceae (54.1 %), Asteraceae (8.0 %), Solanaceae (5.0 %), Poaceae (5.0 %) and Fabaceae (4.0 %)—together accounted for 86 % of all ASVs; extending to the top thirteen families captured over 90 % of the data. At the genus level, three cucurbit genera dominated, with Cucumis (23 %), Lagenaria (22 %) and Cucurbita (9 %) alone representing 54 % of sequences, and the ten most common genera (adding Solanum, Persea, Musa, Urtica, Galinsoga, Desmanthus and Digitaria) encompassing 75 % of the ASVs (Supplementary Table S4 and S5).

1. α-Diversity

Sex had no detectable effect on Shannon's H or Simpson's D in either hoverfly species (all $p > 0.05$; Supplementary Table S3a), so hoverfly male and female data were pooled in each species. In the full three-way ANOVAs, pollinator species was highly significant for both Shannon's H' ($F= 55.12$, $p < 2.2 \times 10^{-16}$ ***) and Simpson's D ($F = 68.93$, $p < 2 \times 10^{-16}$ ***). Farming practice had a modest but significant effect on Shannon's H' ($F = 4.25$, $p = 0.0406$ *) and on Simpson's D ($F= 4.72$, $p = 0.0311$ *). The species × elevation × farming interaction was also significant for both metrics (Shannon's H': $F = 6.49$, $p = 0.00188$ **; Simpson's D: $F = 4.77$, $p = 0.00954$ **). Post-hoc, FDR-corrected pairwise t-tests showed that *Apis mellifera* carried significantly more diverse pollen loads than both syrphid species (for both Shannon's H' and Simpson's D), and that diversity patterns varied with farming system and elevation in a species-specific manner. This pattern is illustrated in Figure 2, which reveals that while agroecological plots generally supported higher Simpson diversity than conventional ones across all pollinators and elevations, T. floralis at low elevation exhibited the reverse pattern (Figure 2). The result is qualitatively the same as the Shannon index (Supplementary Table 3).

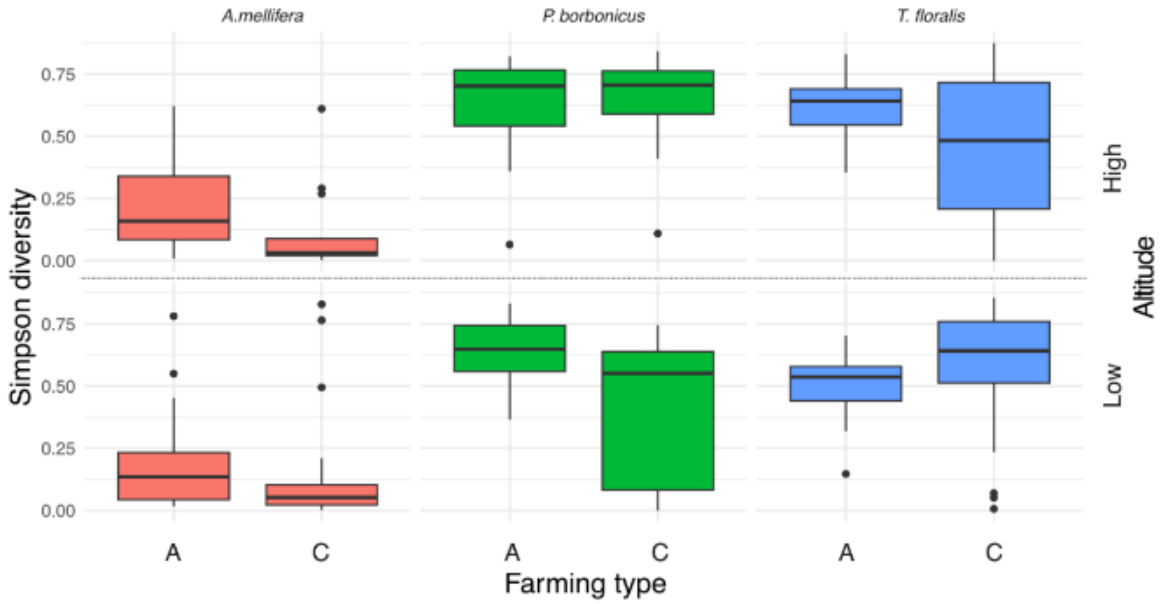

Figure 2. ***Alpha diversity****: Simpson's D across pollinator species, farming system (A = agroecological, C = conventional), and altitude (high vs. low) (a) the effects of pollinator species (gen_sp), sex, altitude, farming practice, and their interactions on* ***Simpson diversity****. Significant p-values are indicated (*** p < 0.001; * p < 0.05). Residuals and total sums of squares are also shown. (b) Simpson diversity of pollen assemblages by farming practice and pollinator species, faceted by altitude (high vs. low). Boxplots show the distribution of Simpson values for Apis mellifera (red), Paragus borbonicus (green), and Toxomerus floralis (blue) under agroecological and conventional farming at each elevation.*

2. β-diversity

Beta-diversity of pollen communities, as measured by Euclidean distances on fourth-root–transformed data, varied significantly with pollinator groups, landscape, farming practice and their interactions (Table 1).

Pollinator group effects: PERMANOVA revealed significant differences across 203 samples (*Apis mellifera*, *Paragus borbonicus* M & F, *Toxomerus floralis* M & F), for pollen ASVs, genera and families. Insect groups explained 4.9 % of the variance in ASV composition ($F = 2.22$, $p < 0.001$), 4.9 % at the genus level ($F = 2.71$, $p = 0.000$), and 20.7 % at the family level ($F = 13.18$, $p = 0.000$). The *posthoc* tests revealed that at ASVs and pollen genera recovered from *Apis mellifera* were different from other insect groups, while there were no significant differences between male and female hoverflies. Conversely, pollen composition differed not only between the two flower-fly species (*Paragus borbonicus* vs. *Toxomerus floralis*) but also between sexes in *P. borbonicus* (but not in *T. floralis*).

Landscape effects: Significant landscape-driven differences were observed at for pollen ASVs and genera. This factor accounted for 1.3 % of the total variance observed for both pollen ASVs ($F = 2.34$, $p = 0.000$) and genera ($F = 2.89$, $p = 0.000$). While these differences disappeared when pollen identifications were aggregated to the family level.

Farming-practice effects: In the pollen analysis at ASV-level, farming (agroecological vs. conventional) provided a significant effect and explained 0.7 % of the total variance ($F = 1.33$, $p = 0.046$). While no significant effects were observed when pollen genera or families were considered.

Landscape × Farming (LA×FA) interaction: Both ASV- and genus-level pollen compositions exhibited significant LA×FA interactions. The post-hoc tests revealed that ASVs patterns differed between farming-practice in both the plateau (lowland) and mountainous (highland) landscapes. For genera, however, farming-practice differences arose only in the mountainous landscape.

Group × Landscape (GR×LA) interaction: At pollen ASV- and genus-level, landscape effects varied across insect groups: significant highland vs. lowland *a posteriori* contrasts were observed for female hoverflies and *A. mellifera*, but not for male flies (Figure4).

Group × Farming (GR×FA) interaction: Significant differences were observed for the interaction of GRxFA for pollen ASVs and genera. The post-hocs showed that farming practices significantly affected the pollen composition of *A. mellifera* (Figure 3).

Three-way interaction (GR×LA×FA): We also observe a third-degree interaction at ASV and genus levels. ASV patterns were relatively simple: farming-practice effects occurred only in *A. mellifera* and only at the lowland plateau. Genus-level responses displayed more complex, pollinator- and landscape-specific patterns.

Together, these results indicate that pollen-transport β-diversity is shaped not only by individual factors (pollinator identity, landscape, management) but also by their interactions—and that the level of taxonomic resolution (ASV vs. genus vs. family) influences which effects can be observed (Table1).

ANCOM-BC allowed for a more detailed investigation of the observed second- and third-degree interactions by identifying how specific pollen families and genera that varied across treatments. This approach provided taxon-level resolution that complemented the broader patterns revealed by the PERMANOVA-based beta diversity analysis (which necessarily could

only capture overall multivariate differences in community composition. ANCOM-BC allowed pinpointing the specific pollen taxa driving these compositional changes, and helped disentangling the combined effects of landscape, farming, and insect group on pollen composition.

Table 1: *PERMANOVA (a) and a posteriori comparisons (b) on 203 pollen samples testing the effects of insect group (A. mellifera F, P. borbonicus M and F, T. floralis M and F), landscape (plateau, mountainous), farming practices (agroecological, conventional) on the multivariate patterns of pollen ASVs, genera and families. PERMANOVA - standardized, fourth root transformed data, Euclidean distance*

(a)

		3,006 pollen ASVs					247 pollen genera					94 pollen families				
	df	MS	% var	F	P		MS	% var	F	P		MS	% var	F	P	
Insect Group (GR)	4	42.38	4.9%	2.22	0.000	***	34.15	4.9%	2.71	0.000	***	39701.0	20.7%	13.18	0.000	***
Landscape (LA)	1	44.66	1.3%	2.34	0.000	***	36.45	1.3%	2.89	0.000	***	6025.7	0.8%	2.00	0.058	n.s.
Farming practices (FA)	1	25.44	0.7%	1.33	0.046	*	17.38	0.6%	1.38	0.084	n.s.	5034.4	0.7%	1.67	0.103	n.s.
LA x FA	1	27.19	0.8%	1.43	0.023	*	20.32	0.7%	1.61	0.025	*	2862.3	0.4%	0.95	0.420	n.s.
GR x LA	4	38.19	4.4%	2.00	0.000	***	29.17	4.2%	2.31	0.000	***	3798.7	2.0%	1.26	0.164	n.s.
GR x FA	4	24.06	2.8%	1.26	0.007	**	16.31	2.4%	1.29	0.023	*	3938.2	2.1%	1.31	0.134	n.s.
GR x LA x FA	4	26.43	3.0%	1.39	0.000	***	19.06	2.7%	1.51	0.001	***	2712.3	1.4%	0.90	0.591	n.s.
Residual	183	19.08	84.8%				12.60	83.1%				3012.5	72%			

Table 1 (b) A posteriori comparisons - permutational t-tests

		Am	Pb F	Pb M	Tf F
Insect Group (GR)	Pb F	***			
	Pb M	***	n.s.		
	Tf F	***	n.s.	n.s.	
	Tf M	***	n.s.	n.s.	n.s.

		Am	Pb F	Pb M	Tf F
	Pb F	***			
	Pb M	***	n.s.		
	Tf F	***	n.s.	n.s.	
	Tf M	***	n.s.	n.s.	n.s.

		Am	Pb F	Pb M	Tf F
	Pb F	***			
	Pb M	***	*		
	Tf F	***	n.s.	n.s.	
	Tf M	***	n.s.	*	n.s.

Landscape (LA)	plateau ≠ mountainous	
Farming practices (FA)	agroecological ≠ conventional	

Landscape (LA)	plateau ≠ mountainous	
Farming practices (FA)	agroecological = conventional	

LA x FA	plateau	agroecological ≠ conventional
	mountainous	agroecological ≠ conventional

LA x FA	plateau	agroecological = conventional
	mountainous	agroecological ≠ conventional

GR x LA	Am F	plateau ≠ mountainous
	Pb M	plateau = mountainous
	Pb F	plateau ≠ mountainous
	Tf M	plateau = mountainous
	Tf F	plateau ≠ mountainous

GR x LA	Am F	plateau ≠ mountainous
	Pb M	plateau = mountainous
	Pb F	plateau ≠ mountainous
	Tf M	plateau = mountainous
	Tf F	plateau ≠ mountainous

GR x FA	Am F	agroecological = conventional
	Pb M	agroecological = conventional
	Pb F	agroecological = conventional
	Tf M	agroecological = conventional
	Tf F	agroecological = conventional

GR x FA	Am F	agroecological ≠ conventional
	Pb M	agroecological = conventional
	Pb F	agroecological = conventional
	Tf M	agroecological = conventional
	Tf F	agroecological = conventional

GR x LA x FA	Am F	plateau
		mountainous
	Pb M	plateau
		mountainous
	Pb F	plateau
		mountainous
	Tf M	plateau
		mountainous
	Tf F	plateau
		mountainous

Am F	plateau	agroecological ≠ conventional
	mountainous	agroecological = conventional
Pb M	plateau	agroecological = conventional
	mountainous	agroecological = conventional
Pb F	plateau	agroecological = conventional
	mountainous	agroecological = conventional
Tf M	plateau	agroecological = conventional
	mountainous	agroecological = conventional
Tf F	plateau	agroecological = conventional
	mountainous	agroecological = conventional

Am F	plateau	agroecological ≠ conventional
	mountainous	agroecological = conventional
Pb M	plateau	agroecological ≠ conventional
	mountainous	agroecological = conventional
Pb F	plateau	agroecological = conventional
	mountainous	agroecological = conventional
TfM	plateau	agroecological ≠ conventional
	mountainous	agroecological = conventional
Tf F	plateau	agroecological = conventional
	mountainous	agroecological ≠ conventional

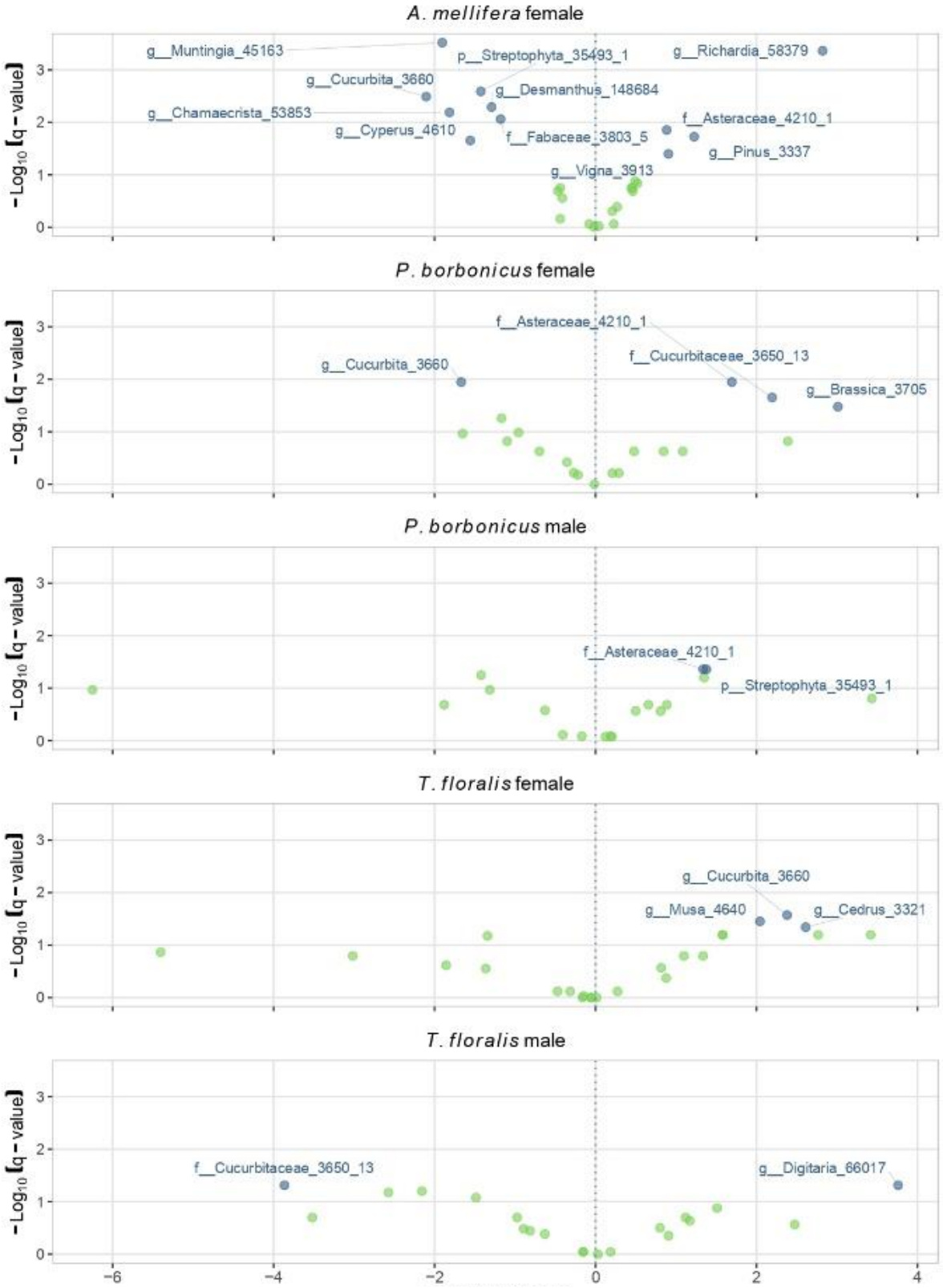

Figure 3. ***Differential abundance volcano plots for the top taxa driving differences between agroecological and conventional farming within each pollinator group.*** *Each panel shows log_2 fold-changes (x–axis) versus $–log_{10}$ q–values (y–axis) from ANCOM-BC2 contrasts comparing agroecological to conventional farming for (from top to bottom): Apis mellifera females, Paragus borbonicus females, P. borbonicus males, Toxomerus floralis females, and T. floralis males. Points in blue denote taxa significantly enriched under agroecological farming (q < 0.05), while green points are non-significant. Selected genera (g__) and families (f__) with the largest effect sizes are labeled. Vertical dotted line at log_2 fold-change = 0 indicates no change in relative abundance between farming practices.*

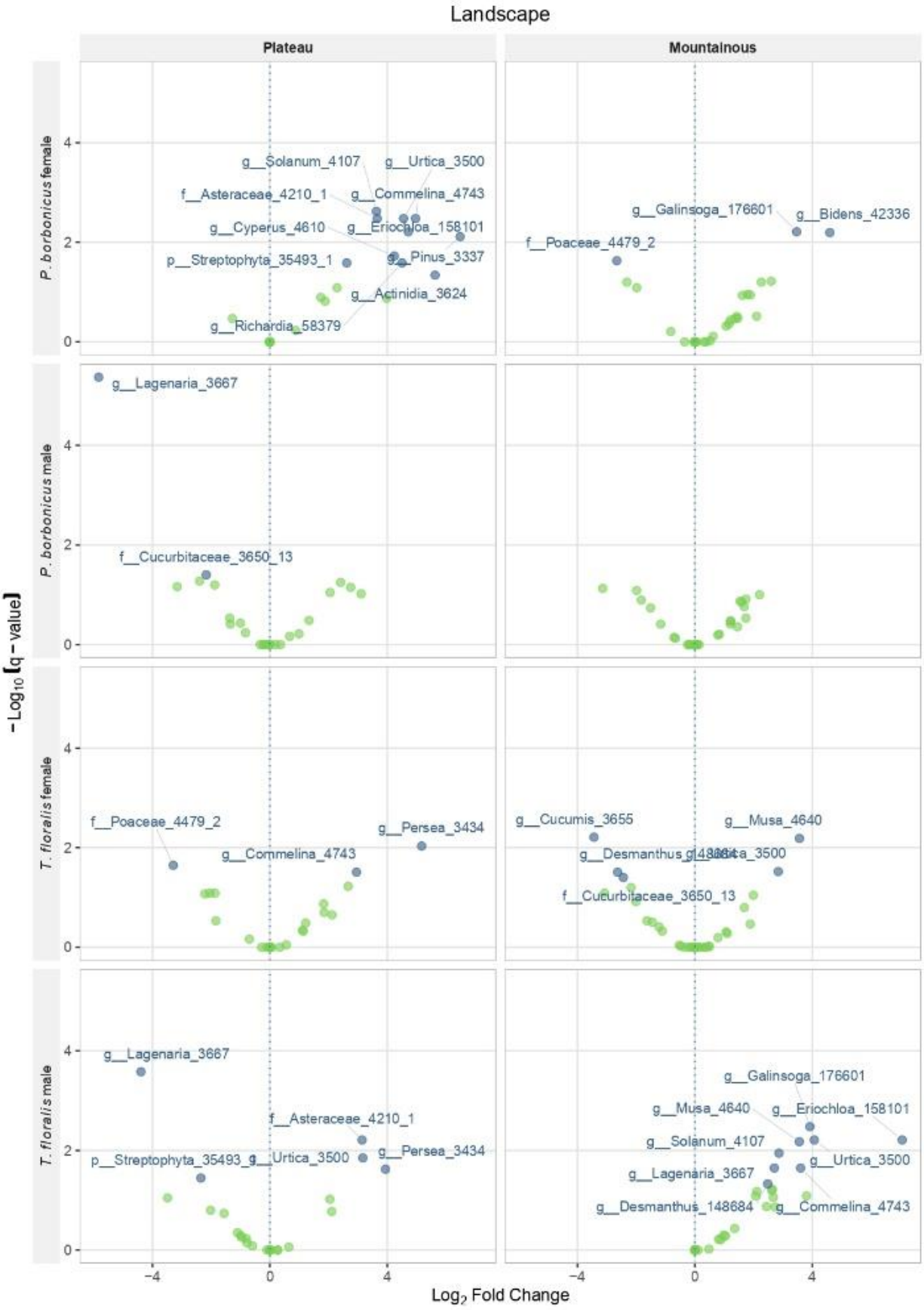

Figure 4. ***Volcano plots of taxa enriched in hoverflies relative to Apis mellifera, stratified by landscape (Plateau vs. Mountainous), species, and sex.*** *Each panel displays log_2 fold-changes (x–axis) of taxon relative abundance in Paragus borbonicus or Toxomerus floralis (males and females separately) compared to A. mellifera, against $–log_{10}$ q-values (y–axis) from ANCOM-BC2 analyses. Blue points denote taxa significantly over-represented in the hoverfly group ($q < 0.05$), while green points are non-significant. Labels highlight the most strongly differentiated taxa (g__ = genus; f__ = family; p__ = phylum). Vertical dashed lines mark zero fold-change. Panels are arranged with Plateau landscape results on the left and Mountainous landscape results on the right, showing how landscape context interacts with pollinator identity and sex to shape pollen profiles.*

Discussion

In this study, we investigated how pollinator identity (*Apis mellifera*, *Toxomerus floralis*, *Paragus borbonicus*), landscape (highland vs. lowland), and farming practice (agroecological vs. conventional) shape pollen-transport diversity in an African agroecosystem using DNA metabarcoding. Our metabarcoding pipeline recovered 3,006 pollen ASVs across 94 families and 245 genera, demonstrating the high taxonomic resolution achievable with high-throughput sequencing and a custom *rbcL* reference library. Multivariate analyses (PERMANOVA) revealed that pollinator identity was the strongest single factor, explaining nearly 5% of the variation in pollen composition at all three, the ASV, genus and family levels ($F = 2.222$, $p < 0.001$). Landscape and farming practice also had significant—but smaller—effects, and all two- and three-way interactions were significant at the ASV scale. We look at these patterns in the context of pollinator ecology, landscape diversity, and agricultural management, and discuss what they mean for pollination services in tropical African agroecosystems.

Sex-based differences

Overall there is no indication of sex differences in α-diversity of hoverfly pollen loads. This is consistent with previous metabarcoding studies in Eristalis hoverflies (Lucas *et al.* 2018). In contrast, male P. borbonicus carried a higher proportion of pollen from *Lagenaria* and *Cucurbitaceae* pollen, as measured by β-diversity —but only at highland sites. This elevational, sex-specific shift mirrors earlier microscope observation that male syrphids sometimes specialize on particular open-flower resources in certain habitats, likely reflecting nutritional needs for mate-search flights (Ssymank *et al.*, 2008).

Species-level differences

Pollinator identity was the strongest driver of pollen-transport patterns, explaining ~4.9% (Table 1a) of variation at both ASV and genus scales ($p < 0.001$). *Apis mellifera* carried considerably more diverse and compositionally distinct pollen loads than either hoverfly species, reflecting bees' corbiculae and floral fidelity versus hoverflies' sponging mouthparts and preference for open-flower morphologies. Although *T. floralis* and *P. borbonicus* overlapped in many plant genera, they exhibited species-specific foraging patterns, underscoring niche differentiation among Syrphidae. *T. floralis* showed a clear enrichment in pollen from open-flowered monocots—most notably *Poaceae* (e.g. *Digitaria*) and *Musa*—relative to honeybees. This pattern suggests a morphological preference for easily accessible, grassy resources, consistent with hoverflies' well-documented tendency to exploit open-flower morphologies that bees often ignore (Lucas *et al.*, 2018). Moreover, this monocot-biased signature aligns with *T. floralis*'s common occurrence in lowland, grassland-dominated agricultural habitats, where small-flowered cereals and grasses prevail (Mullens *et al.*, 2024).

Farming-practice effects

Farming practice accounted for only 0.7 % of ASV-level variation (PERMANOVA, $F = 1.334$, $p = 0.046$) and had no detectable effect at the genus or family scales ($p > 0.05$) with β-diversity

analyses. However, α-diversity analyses (Simpson's D) did reveal a modest but significant effect of management on within-sample richness and evenness (ANOVA, F = 4.25, p = 0.041; Supplementary Table S3b). Post-hoc comparisons showed that *Apis mellifera* in agroecological fields carried significantly richer and more even pollen loads than in conventional fields, while neither *Toxomerus floralis* nor *Paragus borbonicus* exhibited consistent management-driven shifts in α-diversity (in *T. floralis* the trend even flips at low altitude).

This species-specific agroecological effect likely reflects differences in foraging biology. Honeybees, as central-place foragers with limited flight ranges, depend heavily on the immediate floral neighborhood; thus, local enhancements—such as flowering intercrops, cover-crops, and uncultivated margins—translate directly into more diverse pollen diets in agroecological plots. Because honeybees mainly forage close to their hives, they immediately capitalize on the extra flowers planted nearby, resulting in more even pollen loads (Danner *et al.*, 2016). In contrast, hover flies are capable of longer-distance movements across adjacent patches, and so their pollen profiles don't change noticeably between the two farming systems (Lucas *et al.*, 2018).

Overall, these results suggest that agroecological versus conventional practices can subtly shape pollen-load diversity at the sample level, but such effects remain small. This minimal impact aligns with Mullens et al. (2024) findings that management influences on pollen diversity are smaller than those of elevation or species identity.

Pollination networks and cucurbit crops

Metabarcoding-derived interaction networks revealed that honeybees form dense, central links to Cucurbitaceae taxa, indicating *A. mellifera* as the primary pollinator of cucurbits in our system, while hoverflies exhibit fewer and more peripheral links to these crops, suggesting that they may have a supplementary but resilient role in these crops. Maintaining both bee and hoverfly diversity thus supports effective pollination of cucurbits and other crop communities. Having both bees and hoverflies in the pollinator community provides a form of ecological insurance: if honeybee populations decline in a given year (due to disease, weather, or management issues), hoverflies can pick up some of the pollination slack, helping maintain fruit set. This functional redundancy ensures more consistent cucurbit yields across variable environmental conditions.

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Appendix

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Supplementary Table S1. Field-scale pest and soil-fertility management practices by growth stage. This table summarizes the timing, agronomic activities, and the corresponding pest-control or soil-fertility treatments applied at each stage of the crop cycle across experimental plots in Morogoro. Treatments include pre-planting bio-fencing, field preparations for high- and low-altitude terraces, mulching, bio-insecticides, bio-fungicides, bio-fertilizers, and intercropping regimes.

Time	Activities	Pest and soil fertility management
During field preparations	Terraces were prepared for all fields in high altitude and ridges for low altitude	0.5kg per cow manure was applied per hole before planting
After planting	A 15 cm thickness of mulching from dried straw materials was applied in all fields under agroecology.	A bio-insecticide made from 700g neem leaves + 200g of chill + 100g garlic + two aloe vera leaves were grided together to make a 1kg mixture followed by soaking the mixture into10L of water for 12 hours followed by adding 4 spoons of cooking oil. The obtained mixture was applied twice a week.
After planting	Throughout crop growth stages	Bio-fungicide made from; - ▪ 1L of fresh milk was diluted into 10L of water, and 100g baking powder was added to the mixture. The mixture was then applied twice a week. ▪ 1kg of grided pawpaw leaves mixed with 10L of water, fermented for 24 hours, sieved and applied once after two weeks
After planting		Biofertilisers made from 50kg of fresh cow dung were sacked into a 50kg sack and soaked in 200L of water for 21 days. The obtained liquid fertiliser was applied at 250ml per week per plant.
Before planting		Bio-fence made from pigeon peas was planted at the border surrounding all fields.
After planting		Intercropping using green gram was also done in each ridge or terrace at 50cm spacing.

Supplementary Table S2. Geographic coordinates and elevations of the 12 sampling sites in Morogoro, Eastern Central Tanzania. Each site is classified as highland (~1000 m) or lowland (~500 m) based on its elevation.

Country	Region	Exact Site	Latitude (DMS)	Longitude (DMS)	Latitude (DD)	Longitude (DD)	Elevation
Tanzania	Morogoro	Kibwelonga	S6°_50'_56"	E37°_39'_18"	6.848888889	37.655	high
Tanzania	Morogoro	Mkumbulu	S6°_50'_56"	E37°_39'_18"	6.848888889	37.655	high
Tanzania	Morogoro	Ruvuma	S6°_47'_25"	E37°_38'_10"	6.790277778	37.63611111	high
Tanzania	Morogoro	Mpingoni	S6°_52'_30"	E37°_39'_49"	6.875	37.66361111	low
Tanzania	Morogoro	Vitonga	S6°_52'_29"	E37°_40'_06"	6.874722222	37.66833333	low
Tanzania	Morogoro	Mazimbu	S6°_55'_55"	E37°_31'_45"	6.931944444	37.52916667	low
Tanzania	Morogoro	Kilangalanga	S6°_50'_24"	E37°_37'_48"	6.84	37.63	high
Tanzania	Morogoro	Horticultural	S6°_47'_25"	E37°_38'_10"	6.790277778	37.63611111	low
Tanzania	Morogoro	Kidokwe	S6°_50'_20"	E37°_38'_34"	6.838888889	37.64277778	high
Tanzania	Morogoro	Crop_museum	S6°_47'_25"	E37°_38'_10"	6.790277778	37.63611111	low
Tanzania	Morogoro	Kinyenze_A	S6°_50'_24"	E37°_37'_48"	6.84	37.63	low
Tanzania	Morogoro	Mgola_A	S6°_56'_41"	E37°_31'_17"	6.944722222	37.52138889	high

*Supplementary Table S3. ANOVA of alpha-diversity metrics. (a) Simpson's D for the two hoverflies (T. floralis, P. borbonicus) tested against species, sex, altitude, farming and their interactions. (b) Shannon's H' for all three pollinators (A. mellifera, T. floralis, P. borbonicus) tested against species, altitude, farming and interactions. (c) Simpson's D for all three pollinators, showing the same significant terms as Shannon's H'.Significance codes: n.s.: Not significant; * p < 0.05; ** p < 0.01; *** p < 0.001.*

a) *T. floralis/P. borbonicus*

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Genus_species	1	0.086	0.08604	1.5708	0.212542	
sex	1	0.0002	0.00016	0.0029	0.956903	
Altitude	1	0.0797	0.07973	1.4555	0.230043	
Farming	1	0.1502	0.15024	2.7427	0.100333	
Genus_species:Sex	1	0.0381	0.03807	0.695	0.406137	
Genus_species:Altitude	1	0.1893	0.18927	3.4554	0.065516	
Genus_species:Farming	1	0.0098	0.00981	0.179	0.672995	
Sex:Altitude	1	0.0428	0.04282	0.7818	0.378389	
Sex:Farming	1	0.0185	0.01854	0.3385	0.561804	
Altitude:Farming	1	0.0082	0.00821	0.1498	0.699394	
Genus_species:Sex:Altitude	1	0.0235	0.02347	0.4285	0.513972	
Genus_species:Sex:Farming	1	0.0679	0.06795	1.2405	0.26762	
Genus_species:Altitude:Farming	1	0.4377	0.43767	7.9901	0.005519	**
Sex:Altitude:Farming	1	0.0019	0.00193	0.0352	0.851415	
Residuals	119	6.5184	0.05478			

b) SHANNON *A. melifera/T. floralis/P. borbonicus*

Term	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
Genus_species	2	25.607	12.8033	55.1161	<2.2e-16	***
Altitude	1	0.653	0.6527	2.8099	0.09532	
Farming	1	0.987	0.987	4.249	0.040628	*
Genus_species:Altitude	2	1.134	0.567	2.4409	0.089792	
Genus_species:F*arming*	2	0.382	0.1908	0.8213	0.441409	
Altitude:Farming	1	0.012	0.0117	0.0505	0.822512	
Genus_species:Altitude:Farming	2	3.013	1.5066	6.4855	0.001883	**
Residuals	191	44.369	0.2323			

C) SIMPSON *A. MELIFERA/T. FLORALIS/P. BORBONICUS*

TERM	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Significance
GEN_SP	2	6.8058	3.4029	68.927	<2e-16	***
ALTITUDE	1	0.0462	0.0462	0.9349	0.334826	
FARMING	1	0.2329	0.2329	4.7185	0.031072	*
GEN_SP:ALTITUDE	2	0.2494	0.1247	5.2622	0.082635	
GEN_SP:FARMING	2	0.0118	0.0059	0.1192	0.887669	
ALTITUDE:FARMING	1	0	0	0.0002	0.989592	
GEN_SP:ALTITUDE:FARMING	2	0.4707	0.2353	4.7671	0.009543	**
RESIDUALS	191	9.4296	0.0494			

Supplementary Information 1 (SI1): Pollen isolation Protocol from RMCA.

Pollen isolation

- Prepare a bead beater tube for each sample
- Take out label from sample tube
- Try to remove as much pollen / pollen sack from bee and transfer to bead beater tube
- Also transfer the bee and the remaining ethanol to bead beater tube
- Shake the bead beater tube twice for 5 minutes on a bead beater at 9Hz (as a compromise between vigorously shaking the pollen and avoiding damaging the vouchers).
- Put the label back in sample tube
- Remove the bee from the bead beater tube and transfer back to sample tube with the label and add a solution of 100% EtOH
- Centrifuge the bead beater tube with pollen at 13,000 rpm for 5 min.

Pollen DNA extraction

- remove the paper label from the tube (if there is one present)
- centrifuge the tube at 13,000 rpm for 15 min
- remove most of the EtOH but avoid pipetting pollen
- pool 3 samples in an empty bead beater tube – preferably by pipetting! make sure to transfer all pollen
- dry the pellet in an Eppendorf® Concentrator:
    - 60 mins (can vary -> make sure the pollen are dry)
    - V-AL (concentrator, alcoholic solutions, so that it spins at 1400 rpm)
    - Temp: 30°C
- add 3 metal beads and disrupt the pellet in a bead beater (VWR® Star Beater) for 2 minutes at 22.5 Hz
- Proceed to DNA extraction (kit protocol)

Beads re-use

- Empty the contents of tubes/jars into a sieve and rinse the beads thoroughly with water.
- Incubate beads in 0.4 M HCl* for 1 min at room temperature to degrade any DNA and avoid cross-contamination.
- Rinse beads thoroughly with distilled water to remove the HCl.
- Dry beads before use.

DNA quantification

❖ Qubit
    - Follow kit protocol

❖ Nanodrop
    - Clean with special tissue
    - Blank: add 1ul of elution buffer and press “blank”
    - Clean
    - Re-measure blank, below 2ng/ul OK; absorbance should be almost 0
    - Start measuring samples

Supplementary Table S4. Full genus-level pollen metabarcoding results. Raw read counts, relative proportions, and cumulative proportions are shown for each of the 245 genera detected across all samples (n = 3006 ASVs total). Genera are ordered by descending relative abundance; those below 0.01 % are included for completeness.

GENUS	Raw Counts	Relative Proportion	Cumulative Proportion
g__Cucumis_3655	3441758	0.23	0.23
g__Lagenaria_3667	3314120	0.22	0.45
g__Cucurbita_3660	1333955	0.09	0.54
g__Solanum_4107	739350	0.05	0.59
f__Asteraceae_4210_1	659361	0.04	0.64
g__Persea_3434	449537	0.03	0.67
g__Musa_4640	442698	0.03	0.69
g__Urtica_3500	324083	0.02	0.72
g__Galinsoga_176601	285397	0.02	0.74
g__Desmanthus_148684	242924	0.02	0.75
g__Digitaria_66017	186465	0.01	0.76
g__Remusatia_189454	165757	0.01	0.78
g__Richardia_58379	164563	0.01	0.79
g__Eriochloa_158101	162571	0.01	0.80
g__Oceanopapaver_210146	140172	0.01	0.81
g__Amphineurion_792595	139562	0.01	0.82
g__Commelina_4743	135548	0.01	0.83
g__Cedrus_3321	126802	0.01	0.83
k__Viridiplantae_33090_1	120313	0.01	0.84
g__Cajanus_3820	96206	0.01	0.85
g__Oryza_4527	95331	0.01	0.85
f__Poaceae_4479_2	94879	0.01	0.86
g__Pinus_3337	89078	0.01	0.87
g__Laggera_313966	87332	0.01	0.87
g__Chamaecrista_53853	81454	0.01	0.88
p__Streptophyta_35493_1	80574	0.01	0.88
g__Brassica_3705	73331	0.00	0.89
g__Passiflora_3684	71201	0.00	0.89
f__Zingiberaceae_4642_3	67876	0.00	0.90
g__Nepeta_39172	64836	0.00	0.90
f__Fabaceae_3803_5	63684	0.00	0.91
g__Echinochloa_45618	60130	0.00	0.91
g__Manihot_3982	58787	0.00	0.91
g__Cynodon_15437	56383	0.00	0.92
g__Actinidia_3624	55361	0.00	0.92
g__Juniperus_13100	54873	0.00	0.93
g__Bidens_42336	51666	0.00	0.93
g__Citrus_2706	48929	0.00	0.93
g__Cyperus_4610	48505	0.00	0.94
g__Oxalis_4034	47486	0.00	0.94
g__Euphorbia_3990	40325	0.00	0.94
g__Conyza_41552	40143	0.00	0.94
g__Vachellia_468162	37391	0.00	0.95
f__Sapindaceae_23672_6	36670	0.00	0.95
g__Picea_3328	32566	0.00	0.95
g__Castanea_21019	28978	0.00	0.95
g__Eugenia_119950	27909	0.00	0.95
g__Mollugo_3591	27632	0.00	0.96
g__Heteromeles_36611	23513	0.00	0.96
g__Daucus_4038	22672	0.00	0.96
g__Vigna_3913	22611	0.00	0.96

g__Malvaviscus_93787	21851	0.00	0.96
g__Pisum_3887	20393	0.00	0.96
g__Elaeis_51952	20324	0.00	0.97
g__Robinia_35937	20231	0.00	0.97
g__Leersia_35711	19844	0.00	0.97
f__Cucurbitaceae_3650_13	18633	0.00	0.97
g__Jacobaea_405757	18600	0.00	0.97
g__Amaranthus_3564	18354	0.00	0.97
g__Ostrya_13621	15462	0.00	0.97
o__Fagales_3502_1	15458	0.00	0.97
g__Phaulopsis_440907	15195	0.00	0.98
g__Muntingia_45163	15109	0.00	0.98
g__Kyllinga_76461	14618	0.00	0.98
g__Juglans_16718	14548	0.00	0.98
f__Asteraceae_4210_10	13904	0.00	0.98
g__Quercus_3511	12154	0.00	0.98
g__Thecorchus_353855	10727	0.00	0.98
g__Tapirira_43857	10671	0.00	0.98
g__Prunus_3754	10448	0.00	0.98
g__Sporobolus_38730	10014	0.00	0.98
f__Malvaceae_3629_7	9635	0.00	0.98
g__Desmodium_53866	9217	0.00	0.98
g__Lantana_87005	9035	0.00	0.98
f__Myrtaceae_3931_8	8758	0.00	0.98
g__Tsuga_3358	8168	0.00	0.99
g__Alocasia_4455	8135	0.00	0.99
g__Alnus_3515	7692	0.00	0.99
g__Betula_3504	7558	0.00	0.99
g__Synedrella_183084	7529	0.00	0.99
g__Sorghum_4557	6882	0.00	0.99
g__Dombeya_82530	6741	0.00	0.99
g__Holcus_15560	6655	0.00	0.99
g__Kalopanax_46398	6599	0.00	0.99
g__Sesamum_4181	6526	0.00	0.99
g__Megathyrsus_649763	6201	0.00	0.99
g__Glycine_3846	6034	0.00	0.99
g__Ageratum_BGM	5970	0.00	0.99
g__Humulus_3484	5715	0.00	0.99
g__Salvia_21880	5701	0.00	0.99
c__Magnoliopsida_BGM_2	5390	0.00	0.99
g__Eperua_162768	4769	0.00	0.99
g__Arachis_3817	4580	0.00	0.99
g__Cenchrus_4583	4507	0.00	0.99
g__Emilia_189212	4406	0.00	0.99
c__sub__asterids_71274_1	4263	0.00	0.99
g__Glechoma_21766	4191	0.00	0.99
g__Taxus_25628	4078	0.00	0.99
g__Phyllanthus_58880	3964	0.00	0.99
g__Eucalyptus_3932	3928	0.00	0.99
g__Ficus_3493	3860	0.00	1.00
g__Setaria_4554	3838	0.00	1.00
g__Heliotropium_21621	3695	0.00	1.00
f__Rosaceae_3745_14	3535	0.00	1.00
g__Ipomoea_4119	3119	0.00	1.00
g__Allophylus_201004	2868	0.00	1.00
g__Echinacanthus_1204777	2663	0.00	1.00

g__Delairea_407160	2519	0.00	1.00
g__Acer_4022	2470	0.00	1.00
g__Ulmus_24735	2360	0.00	1.00
g__Veronica_4173	2319	0.00	1.00
f__Polygonaceae_3615_16	2316	0.00	1.00
g__Plantago_26867	2296	0.00	1.00
g__Momordica_3671	2283	0.00	1.00
g__Rubus_23216	1976	0.00	1.00
g__Smilax_49656	1854	0.00	1.00
g__Aeschynomene_48134	1836	0.00	1.00
f__Arecaceae_4710_15	1768	0.00	1.00
g__Erythronium_49641	1474	0.00	1.00
f__Rutaceae_23513_9	1458	0.00	1.00
g__Impatiens_35939	1412	0.00	1.00
g__Gossypium_3633	1405	0.00	1.00
g__Morus_3497	1234	0.00	1.00
g__Platanus_4402	1223	0.00	1.00
g__Neea_427808	1127	0.00	1.00
g__Rhoicissus_178806	1059	0.00	1.00
g__Vaccinium_13749	1049	0.00	1.00
f__Cupressaceae_3367_17	1002	0.00	1.00
g__Allium_4678	972	0.00	1.00
g__Fagus_21024	877	0.00	1.00
c__sub__rosids_71275_3	842	0.00	1.00
g__Mimosa_21013	691	0.00	1.00
g__Okenia_335911	678	0.00	1.00
g__Centrosema_167616	664	0.00	1.00
f__Annonaceae_22140_20	617	0.00	1.00
f__Combretaceae_3954_21	608	0.00	1.00
g__Solidago_59293	526	0.00	1.00
g__Phaseolus_3883	495	0.00	1.00
f__Heliotropiaceae_1561072_22	482	0.00	1.00
g__Cynoglossum_181188	479	0.00	1.00
g__Acalypha_20025	471	0.00	1.00
p__Tracheophyta_BGM_2	444	0.00	1.00
g__Cercidiphyllum_13412	436	0.00	1.00
g__Adenia_56632	429	0.00	1.00
g__Ranunculus_3445	417	0.00	1.00
f__Boraginaceae_21571_23	411	0.00	1.00
g__Capsicum_4071	394	0.00	1.00
f__Vitaceae_3602_24	342	0.00	1.00
g__Lannea_289715	335	0.00	1.00
g__Coccinia_213598	327	0.00	1.00
g__Flacourtia_112818	320	0.00	1.00
g__Psorospermum_999533	286	0.00	1.00
g__Petroselinum_4042	274	0.00	1.00
g__Polygala_4275	246	0.00	1.00
g__Fragaria_3746	241	0.00	1.00
g__Xanthium_36590	231	0.00	1.00
g__Bridelia_283086	203	0.00	1.00
g__Aneilema_75413	202	0.00	1.00
g__Youngia_89038	202	0.00	1.00
g__Teramnus_45682	202	0.00	1.00
f__Araliaceae_4050_28	194	0.00	1.00
g__Guizotia_4229	189	0.00	1.00
g__Trifolium_3898	182	0.00	1.00

g__Spermacoce_35916	180	0.00	1.00
f__Anacardiaceae_4011_29	174	0.00	1.00
g__Panicum_4539	166	0.00	1.00
g__Cirsium_41549	164	0.00	1.00
g__Heteropogon_79848	163	0.00	1.00
f__Acanthaceae_4185_31	154	0.00	1.00
g__Gaillardia_55623	152	0.00	1.00
g__Trianthema_3547	146	0.00	1.00
g__Lolium_4520	142	0.00	1.00
g__Gymnocladus_53882	140	0.00	1.00
f__Betulaceae_3514_30	139	0.00	1.00
g__Nymphaea_4418	132	0.00	1.00
g__Helianthus_4231	127	0.00	1.00
g__Festuca_4605	105	0.00	1.00
g__Sophora_3896	103	0.00	1.00
g__Eriosema_109225	99	0.00	1.00
g__Atropa_24609	89	0.00	1.00
g__Ligustrum_13596	85	0.00	1.00
g__Ananas_4614	75	0.00	1.00
f__Commelinaceae_4740_32	75	0.00	1.00
g__Corylus_13450	75	0.00	1.00
g__Tribulus_66647	74	0.00	1.00
o__Malpighiales_3646_4	71	0.00	1.00
g__Fraxinus_38871	61	0.00	1.00
g__Neonotonia_103822	57	0.00	1.00
g__Physalis_24663	54	0.00	1.00
g__Sida_108335	54	0.00	1.00
o__Asterales_4209_3	52	0.00	1.00
g__Grevillea_83716	50	0.00	1.00
g__Foeniculum_48037	47	0.00	1.00
g__Poa_4544	45	0.00	1.00
g__Taraxacum_49743	45	0.00	1.00
g__Pistacia_55512	38	0.00	1.00
g__Paspalum_147271	38	0.00	1.00
g__Geum_3761	37	0.00	1.00
g__Callistemon_73722	36	0.00	1.00
g__Ruellia_13659	35	0.00	1.00
g__Dunbaria_553673	25	0.00	1.00
g__Artocarpus_3488	24	0.00	1.00
g__Sorghastrum_79856	24	0.00	1.00
g__Jacquemontia_112276	21	0.00	1.00
g__Ricinus_3987	0	0.00	1.00
f__Meliaceae_43707_4	0	0.00	1.00
g__Ruttya_4194	0	0.00	1.00
f__Theaceae_27065_11	0	0.00	1.00
f__Rubiaceae_24966_12	0	0.00	1.00
g__Tectona_41395	0	0.00	1.00
g__Lapsana_268079	0	0.00	1.00
g__Plectranthus_BGM	0	0.00	1.00
g__Arabidopsis_3701	0	0.00	1.00
g__Vicia_3904	0	0.00	1.00
g__Primula_49647	0	0.00	1.00
g__Maesa_59979	0	0.00	1.00
f__Araceae_4454_18	0	0.00	1.00
g__Scleria_76510	0	0.00	1.00
f__Brassicaceae_3700_19	0	0.00	1.00

g__Xanthosoma_15105	0	0.00	1.00
f__Amaranthaceae_3563_25	0	0.00	1.00
f__Cyperaceae_4609_26	0	0.00	1.00
f__Verbenaceae_21910_27	0	0.00	1.00
g__Hydrostachys_37820	0	0.00	1.00
g__Zizania_15949	0	0.00	1.00
o__Lamiales_4143_2	0	0.00	1.00
g__Mitracarpus_60380	0	0.00	1.00
g__Hibiscus_47605	0	0.00	1.00
f__Fagaceae_3503_33	0	0.00	1.00
g__Cicer_3826	0	0.00	1.00
g__Pelargonium_4030	0	0.00	1.00
g__Moringa_3734	0	0.00	1.00
f__Cannabaceae_3481_34	0	0.00	1.00
g__Mangifera_23461	0	0.00	1.00
g__Aspilia_BGM	0	0.00	1.00
f__Moraceae_3487_35	0	0.00	1.00
f__Bignoniaceae_24079_36	0	0.00	1.00
g__Calamagrostis_15376	0	0.00	1.00
g__Fagopyrum_3616	0	0.00	1.00
o__Zingiberales_4618_5	0	0.00	1.00
g__Coix_4504	0	0.00	1.00
o__Laurales_3432_6	0	0.00	1.00
o__Rosales_3744_7	0	0.00	1.00
g__Trema_3479	0	0.00	1.00
g__Geranium_4028	0	0.00	1.00
o__Caryophyllales_3524_8	0	0.00	1.00
g__Thecacoris_283109	0	0.00	1.00

Supplementary Table S5; A total of 3,006 filtered exact sequence variants (ASVs) assigned to 94 plant families. Just ten families accounted for 87.0 % of all ASVs, led by Cucurbitaceae (54.0 %) and Asteraceae (8.0 %), followed by Solanaceae (5.0 %), Poaceae (5.0 %), Fabaceae (4.0 %), Lauraceae (3.0 %), Musaceae (3.0 %), Urticaceae (2.0 %), Pinaceae (2.0 %) and Rubiaceae (1.0 %). At the genus level, the ten most abundant taxa represented 74.0 % of the total, with Cucumis (23.0 %) and Lagenaria (22.0 %) dominating the profile and the top two genera alone comprising nearly half of all pollen ASVs.

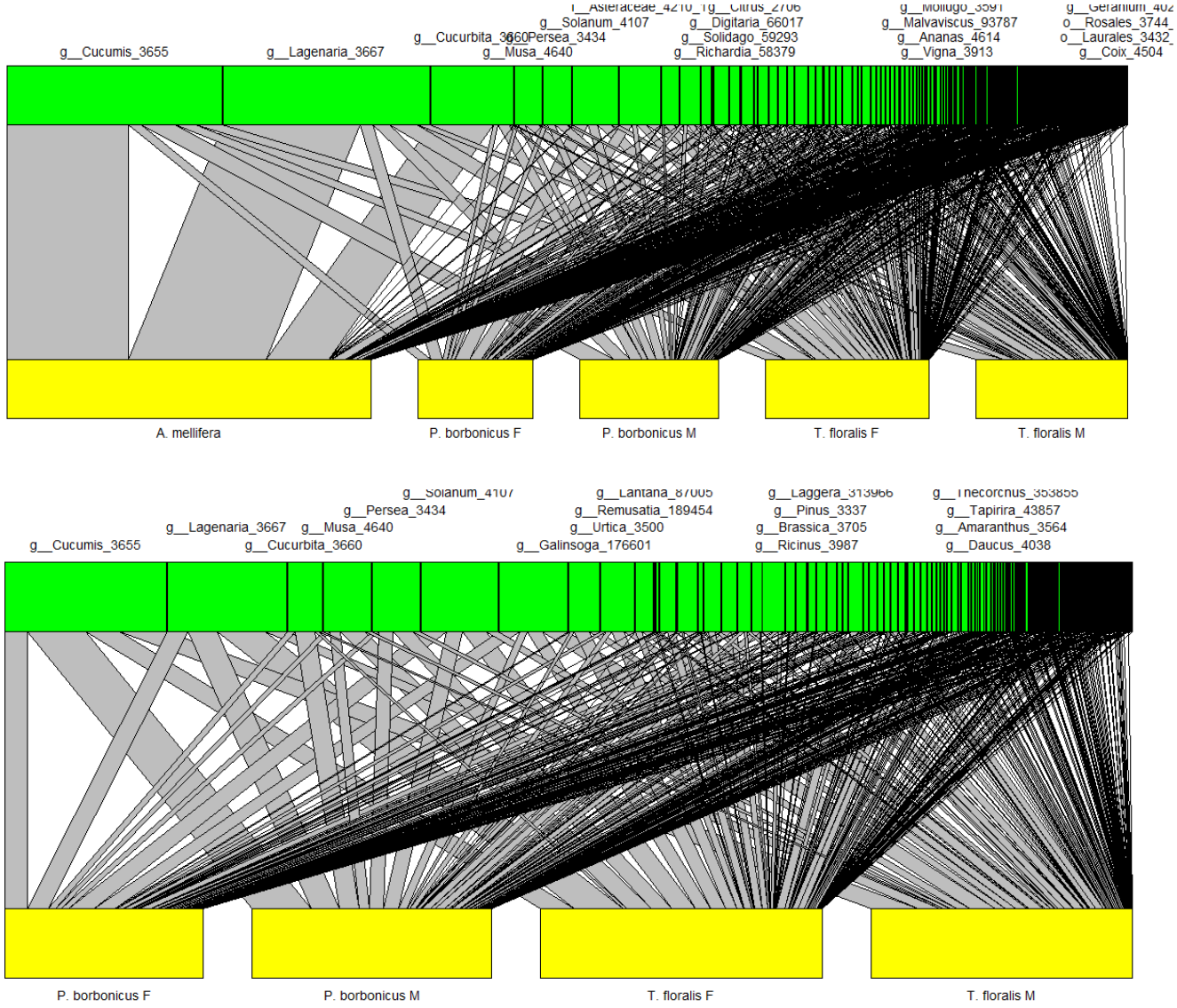
Family	Raw Counts	Relative Proportion	Cumulative Proportion
f__Cucurbitaceae_3650	8111285	0.54	0.54
f__Asteraceae_4210	1178582	0.08	0.62
f__Solanaceae_4070	739887	0.05	0.67
f__Poaceae_4479	714515	0.05	0.72
f__Fabaceae_3803	614120	0.04	0.76
f__Lauraceae_3433	449537	0.03	0.79
f__Musaceae_4637	442698	0.03	0.82
f__Urticaceae_3499	324083	0.02	0.84
f__Pinaceae_3318	256620	0.02	0.86
f__Rubiaceae_24966	175470	0.01	0.87
f__Araceae_4454	173892	0.01	0.88
f__Papaveraceae_3465	140172	0.01	0.89
f__Apocynaceae_4056	139562	0.01	0.90
f__Commelinaceae_4740	135843	0.01	0.91
k__Viridiplantae_33090_1	120313	0.01	0.92
f__Euphorbiaceae_3977	99583	0.01	0.92
p__Streptophyta_35493_1	80574	0.01	0.93
f__Lamiaceae_4136	74728	0.01	0.94
f__Brassicaceae_3700	73335	0.00	0.94
f__Passifloraceae_3683	71630	0.00	0.94

f__Zingiberaceae_4642	67876	0.00	0.95
f__Cyperaceae_4609	63123	0.00	0.95
f__Cupressaceae_3367	55875	0.00	0.96
f__Actinidiaceae_3623	55361	0.00	0.96
f__Rutaceae_23513	50387	0.00	0.96
f__Oxalidaceae_4033	47486	0.00	0.97
f__Fagaceae_3503	42009	0.00	0.97
f__Myrtaceae_3931	40648	0.00	0.97
f__Rosaceae_3745	39750	0.00	0.98
f__Malvaceae_3629	39686	0.00	0.98
f__Sapindaceae_23672	39538	0.00	0.98
f__Betulaceae_3514	30926	0.00	0.98
f__Molluginaceae_3590	27632	0.00	0.98
f__Apiaceae_4037	22993	0.00	0.99
f__Arecaceae_4710	22092	0.00	0.99
f__Amaranthaceae_3563	18354	0.00	0.99
f__Acanthaceae_4185	18047	0.00	0.99
o__Fagales_3502_1	15458	0.00	0.99
f__Muntingiaceae_91852	15109	0.00	0.99
f__Juglandaceae_16714	14548	0.00	0.99
f__Anacardiaceae_4011	11218	0.00	0.99
f__Verbenaceae_21910	9035	0.00	0.99
f__Araliaceae_4050	6793	0.00	1.00
f__Pedaliaceae_4180	6526	0.00	1.00
f__Cannabaceae_3481	5715	0.00	1.00
c__Magnoliopsida_BGM_2	5390	0.00	1.00
f__Moraceae_3487	5118	0.00	1.00
f__Plantaginaceae_156152	4615	0.00	1.00
c__sub__asterids_71274_1	4263	0.00	1.00
f__Heliotropiaceae_1561072	4177	0.00	1.00
f__Phyllanthaceae_233880	4170	0.00	1.00
f__Taxaceae_25623	4078	0.00	1.00
f__Convolvulaceae_4118	3150	0.00	1.00
f__Aceraceae_910345	2470	0.00	1.00
f__Ulmaceae_3474	2360	0.00	1.00
f__Polygonaceae_3615	2316	0.00	1.00
f__Smilacaceae_4703	1854	0.00	1.00
f__Nyctaginaceae_3536	1805	0.00	1.00
f__Liliaceae_4677	1474	0.00	1.00
f__Balsaminaceae_25692	1412	0.00	1.00
f__Vitaceae_3602	1401	0.00	1.00
f__Platanaceae_4401	1223	0.00	1.00
f__Ericaceae_4345	1049	0.00	1.00
f__Amaryllidaceae_4668	972	0.00	1.00
f__Boraginaceae_21571	890	0.00	1.00
c__sub__rosids_71275_3	842	0.00	1.00
f__Annonaceae_22140	617	0.00	1.00
f__Combretaceae_3954	608	0.00	1.00
p__Tracheophyta_BGM_2	444	0.00	1.00
f__Cercidiphyllaceae_16711	436	0.00	1.00
f__Ranunculaceae_3440	417	0.00	1.00
f__Salicaceae_3688	320	0.00	1.00
f__Hypericaceae_629714	286	0.00	1.00
f__Polygalaceae_4274	246	0.00	1.00
f__Aizoaceae_3542	146	0.00	1.00
f__Oleaceae_4144	146	0.00	1.00

f__Nymphaeaceae_4410	132	0.00	1.00
f__Bromeliaceae_4613	75	0.00	1.00
f__Zygophyllaceae_43873	74	0.00	1.00
o__Malpighiales_3646_4	71	0.00	1.00
o__Asterales_4209_3	52	0.00	1.00
f__Proteaceae_4328	50	0.00	1.00
f__Meliaceae_43707	0	0.00	1.00
f__Theaceae_27065	0	0.00	1.00
f__Primulaceae_4335	0	0.00	1.00
f__Hydrostachyaceae_235902	0	0.00	1.00
o__Lamiales_4143_2	0	0.00	1.00
f__Geraniaceae_4027	0	0.00	1.00
f__Moringaceae_3733	0	0.00	1.00
f__Bignoniaceae_24079	0	0.00	1.00
o__Zingiberales_4618_5	0	0.00	1.00
o__Laurales_3432_6	0	0.00	1.00
o__Rosales_3744_7	0	0.00	1.00
o__Caryophyllales_3524_8	0	0.00	1.00

Supplementary Information S2

To analyze the structure of plant-pollinator interactions, we constructed pollination networks based on the pollen metabarcoding data. The network was built by linking pollinators to the plant taxa identified from their pollen loads. Each insect sample was assigned to a unique interaction node based on the taxonomic identity of the pollen it carried.



Supplementary Figure 1. ***Bipartite pollination networks at the plant-genus–pollinator level.*** *(a) All three pollinators (Apis mellifera, P. borbonicus F/M, T. floralis F/M). (b) Only the two syrphid species (P. borbonicus female, P. borbonicus male, T. floralis female, T. floralis male).– Upper (green) bars are pollen-genus nodes, lower (yellow) bars are pollinator nodes. Ribbon thickness is proportional to the number of pollen grains carried (interaction strength).*