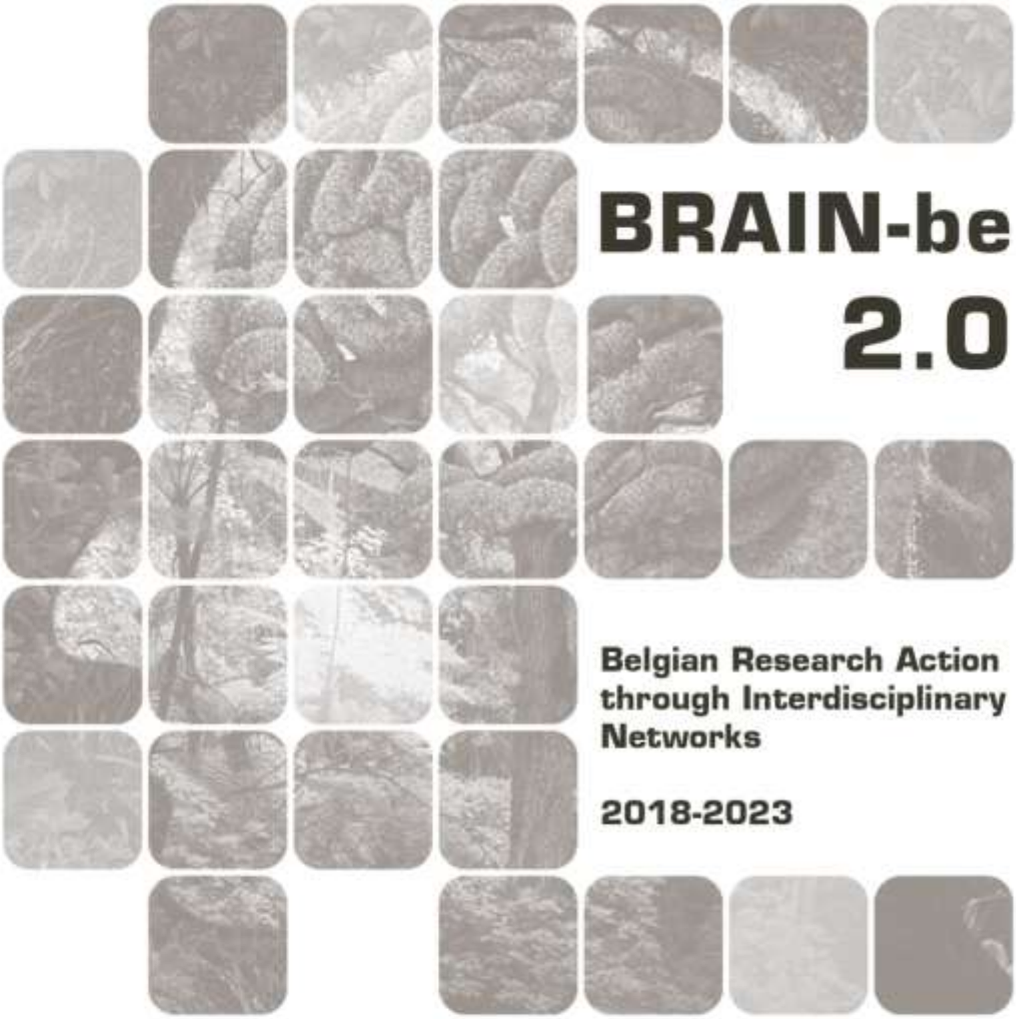

ISeBAF
Insect Service and Biodiversity in Agroecological Farming

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(SUA, International Cooperation)

NETWORK PROJECT

ISeBAF

Insect Service and Biodiversity in Agroecological Farming

Contract - B2/191/P1

FINAL REPORT

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CONTENTS*

(*) Color codes indicate the main contributing teams: RMCA–SUA (green), RBINS–SUA (blue), and ULB–SUA (violet). See Introduction.

ABSTRACT

The ISeBAF project examined biodiversity and pest dynamics in Tanzanian cucurbit agroecosystems, comparing agroecological and conventional family farming across contrasting landscapes. Field surveys, genetic and microbiome analyses, and socio-economic assessments documented bee and flower fly communities in cucurbit crops, assessed fruit fly infestations, and evaluated agroecological performance for smallholders. Results indicate that agroecology can improve pollinator diversity and profitability under favorable landscape conditions, while fruit fly control requires adaptive IPM strategies. Microbiome studies revealed species-specific patterns linked to environment and management, supporting integrated approaches that combine agroecology, pest management, and economic viability for family farming.

Keywords: Agroecology; Pollinator networks; Syrphidae; Apoidea; Tephritidae; Microbiome; Tanzania; Smallholder farming; Genetic diversity.

1. INTRODUCTION

Project Context and Rationale

The **ISeBAF project (Insect Service and Biodiversity in Agroecological Farming)** was developed within the **BRAIN-be 2.0 framework** to provide scientific support for the sustainable use of natural resources. Its overarching goal is to contribute to the **agroecological transition** and promote **nature-based solutions** for smallholder farming systems in sub-Saharan Africa.

Conventional pest control in the region often relies on inefficient and frequent application of synthetic pesticides, which negatively impact farmers' health, ecosystems, and beneficial insects such as pollinators. **Agroecology offers an alternative approach** that reduces chemical inputs while enhancing biodiversity and ecosystem services. However, its effects on insect diversity and crop production remain insufficiently documented.

Scope

ISeBAF addresses this knowledge gap by delivering **reference case studies** on the relationships between:

- **Agroecological farming practices**
- **Insect biodiversity** (pollinators and pests)
- **Cucurbit crop production** in East-Central Tanzania

The project focuses on two key functional groups:

- **Pollinators**: Bees (*Hymenoptera: Apoidea*) and flower flies (*Diptera: Syrphidae*)
- **Pests**: Fruit flies (*Diptera: Tephritidae*)

By comparing **agroecological and conventional family farms**, ISeBAF combines **ecological, genetic, and socio-economic analyses** to:

- Quantify insect diversity
- Evaluate pollination and pest dynamics
- Assess the cost-benefit performance of agroecological practices

This integrated approach aims to inform **policies that promote biodiversity conservation, sustainable agriculture, and improved livelihoods for smallholder farmers**.

Ethical and Administrative Framework

The implementation of this project was shaped by **ethical and administrative considerations**:

- **Field site access** was approved and regulated by **Sokoine University of Agriculture (SUA)** in collaboration with the Tanzanian authorities.
- As the **Nagoya Protocol on Access and Benefit-Sharing (ABS)** is *de facto* not implemented in Tanzania, the intellectual and physical property of samples collected in this study is governed by **Mutually Agreed Terms (MATs)** established between SUA and the Belgian partners. This agreement, inspired by and fully adhering to the principles of the Nagoya Protocol, ensures **fair and transparent use of genetic resources.** Copies of these agreements are provided in **Annexes 1–3**.

- The original project timeline (2019-2023) was delayed due to the **COVID-19 pandemic**, and an additional extension was granted to allow the ULB PhD student to complete her thesis.
- The report of the **final meeting of the project follow-up committee**, summarizing discussions and expert recommendations, is included as **Annex 4**.

1.4 Report Structure and Navigation

To facilitate navigation and highlight the collaborative nature of the project:

- headings and the Table of Contents use a color scheme that indicates the main contributing teams
    - **RMCA–SUA** (green)
    - **RBINS–SUA** (blue)
    - **ULB–SUA** (violet)

In addition, the report is organized into **tasks corresponding to the Work Packages (WP)** defined in the project's Annex 1. Each task number reflects its WP association (e.g., Task 1.x for WP1, Task 2.x for WP2, etc.), ensuring consistency with the original project design. This structure allows readers to easily trace scientific results, methodologies, and dissemination activities back to the specific objectives and deliverables outlined in the proposal.

2. STATE OF THE ART AND OBJECTIVES

Scientific Context

Pollination and pest control are critical ecosystem services underpinning global food security. Animal pollination accounts for nearly 30% of food production (Costanza et al., 1997), while pests such as tephritid fruit flies cause severe economic losses and threaten nutritional security in Africa (Aluja & Norrbom, 2001). Conventional pest management relies heavily on synthetic pesticides, which pose risks to human health, biodiversity, and ecosystem integrity. This has accelerated interest in **agroecology** as a nature-based solution that reduces chemical inputs and promotes biodiversity (Hilmi, 2017; Giraldo, 2019).

Agroecology is built on the principle that biodiversity and ecosystem services are interdependent (Wezel et al., 2009; Stassart et al., 2012). Yet, evidence on how agroecological practices influence biodiversity at multiple levels—species, genetic, and functional—and how these changes affect crop yields remains limited, particularly in African smallholder systems (Gaggiotti et al., 2018). Similarly, while integrated pest management (IPM) strategies have been widely advocated (Deguine et al., 2015), their performance under agroecological conditions in East Africa is poorly documented.

Recent advances in **molecular ecology** and **microbiomics** offer new opportunities to address these gaps. Phylogenomic approaches using Ultra-Conserved Elements (Bossert et al., 2019; Grab et al., 2019) and DNA barcoding enable robust assessments of genetic and phylogenetic diversity, while microbiome profiling provides insights into stress responses and functional traits (Zaneveld et al., 2017; De Cock et al., 2020). At the same time, socio-economic research emphasizes the need to align agroecological transitions with farmer livelihoods and policy frameworks (D'Annolfo et al., 2017; Bakengesa et al., 2023).

Strategic Choices

The ISeBAF project addresses these knowledge gaps through an integrated approach combining:

- **Ecological surveys** of pollinators (bees, Syrphidae) and pests (Tephritidae) in cucurbit agroecosystems.
- **Genomic and microbiome analyses** to quantify diversity and detect management-related patterns.
- **Socio-economic assessments** to evaluate profitability and adoption potential of agroecological practices.

Cucurbits were selected as a model system due to their high dependency on insect pollination and vulnerability to fruit fly infestations, making them ideal for studying interactions between biodiversity, ecosystem services, and farming practices.

Objectives

The project aimed to:

- Document pollinator and pest communities in cucurbit fields and analyze their ecological roles.
- Assess fruit fly infestation patterns under agroecological and conventional management.

- Quantify genetic and phylogenetic diversity of key insect species.
- Characterize microbiome composition in pollinators and pests under different farming systems.
- Evaluate the socio-economic performance of agroecological farming for smallholders.
- Provide evidence-based recommendations for biodiversity conservation, sustainable agriculture, and integrated pest management.

3. METHODOLOGY AND SCIENTIFIC RESULTS

Task 1.1 crop production and costs and farm characterization

The results pertaining to this task are reported below as part of Section Task 3.4 – *Synopsis and socio-economics of agroecological cucurbit farming*.

Task 1.2 insect community structure

Bee communities (Hymenoptera, Apoidea)

The identification efforts by the bee taxonomists, Alain Pauly (subcontractor) and Achik Dorchin, lead to some general conclusions, as follows. Tanzanian bees are potential pollinators of cucurbit crops, either as generalists, or specialists. Generalists (i.e., visiting flowers of many plant species) are *Apis mellifera, Dactylurina staudingeri, Plebeina armata, Ceratina* sp.*, Braunsapis* sp.*, Macrogalea candida*, *Amegilla* sp., and *Xylocopa* sp. In contrast, *Lasioglossum (Ipomalictus*) sp. belongs to the specialists, that is, visiting only cucurbits or some other plants with large spinose pollen like the Convolvulaceae and Malvaceae. Note that after analyzing all the field data, *Lasioglossum* seems to be the only genus with (morpho)species specialized in Cucurbitaceae. However, their relative importance in pollination may remain limited, due to the low number of individuals as compared with (morpho) species that are generalists but more abundant like the domestic bee. Furthermore, some taxa collected in the pan traps are non-visitors of cucurbits. Genera like *Lipotriches* are feeding exclusively on pollen of Poaceae (for example maize growing around the crops) while *Seladonia* are specialists of Asteraceae, this plant family including many weeds in cucurbit crops. For further eco-biological information about the Tanzanian wild bees, see the booklet "Harnessing pollinator diversity in cucurbit crop production in Tanzania", where RBINS's subcontractor is a coauthor. See also under Task 1.3 Genetic diversity, the results about "RBINS: microbial diversity in wild bee *Dactylurina*" that include a comparative approach to agroecological versus conventional farming.

As a reminder, the bees studied in ISeBAF were mainly collected via two methods, by using a hand net and by placing yellow pan traps on the ground. Four field trips are considered here: 2022 season 1 ("s1", i.e., April-May), 2022 season 2 ("s2", October), 2023 s1 (May-June), and 2023 s2 (September). These field trips were managed and performed by RMCA (2022 s1 & s2), ULB (2022 s1, 2023 s1) and/or SUA (2022 s2, 2023 s2).

To take advantage of data available about all bees collected and identified throughout the project, these data were pooled into a single table. However, this exercise of merging numerous spreadsheets into one general dataset most probably generated unwished duplicated records, some of them still needing to be detected, then discarded from further analyses. At the time of writing the present report, the general table contained specimens belonging to 176 (morpho)species (**Annex 6 - Annex allBees**). Note that in this annex *Braunsapis* sp. and *Ceratina* sp. may contain specimens belonging to listed congenerics. Each category ("AgroHigh", "AgroLow", "ConvHigh", "ConvLow") was represented by a similar total number of specimens as well as (morpho)species. This may indicate that, overall, the bees occurred quite homogeneously in the plots of each category. There are marked differences, however, at the species-specific level.

On each (morpho)species, a two-tailed Fisher exact probability test was performed (in 'R'), considering the number of specimens in each of the four categories (one row in **Annex 6 - Annex allBees**) as the four numbers in a 2x2 contingency table. The statistical results were significant for 14 out of 176 (morpho)species. This low proportion (8%) is due, for a majority of (morpho)species, to a too low number of specimens, rendering the statistical test per se non-significant. On the opposite side, among the 47 (morpho)species that were represented by at least 20 specimens, 13 showed a significant association between treatment and elevation (**Fig. beeHisto**). Note however that most of these overall results about bees (**Annex 6 - Annex allBees**; **Fig. beeHisto**) contain bio-ecological information that is probably worthwhile to be further discussed, even if the association is not significant. Here some examples of (morpho)species with a significant association: *Apis mellifera* was rarer in “AgroHigh“ than the other categories; *Dactylurina schmidti* was rarer at low altitude, especially in “ConvLow”; *Braunsapis* MOR3 was most abundant in “AgroHigh“ and “ConvLow“. Here some examples of (morpho)species with a non-significant association: *Plebeina armata*, *Lipotriches cinerascens* and *Macronomia natalensis* were only present at low altitudes and especially in the agroecological treatment; on the contrary, *Lasioglossum calliceras*, *Lipotriches usambarae*, *Thrinchostoma sjoestedti* and *Lasioglossum* (*Ipomalictus*) *ufiomicum* were present (almost) exclusively at high altitude. Such examples evidence the fact that generalizing results at a taxonomical level higher than the species should be avoided when discussing the bio-ecology of bees, their occurrence in a biotope being influenced by factors such as altitude but also foodplant association including diet breadth, landscape architecture, climatic conditions, etc. As observed in Morogoro, the variable geographic distribution of the bee (morpho)species, even among closely related ones, also indirectly emphasizes the importance of accurate identifications, to be performed by specialized taxonomists.

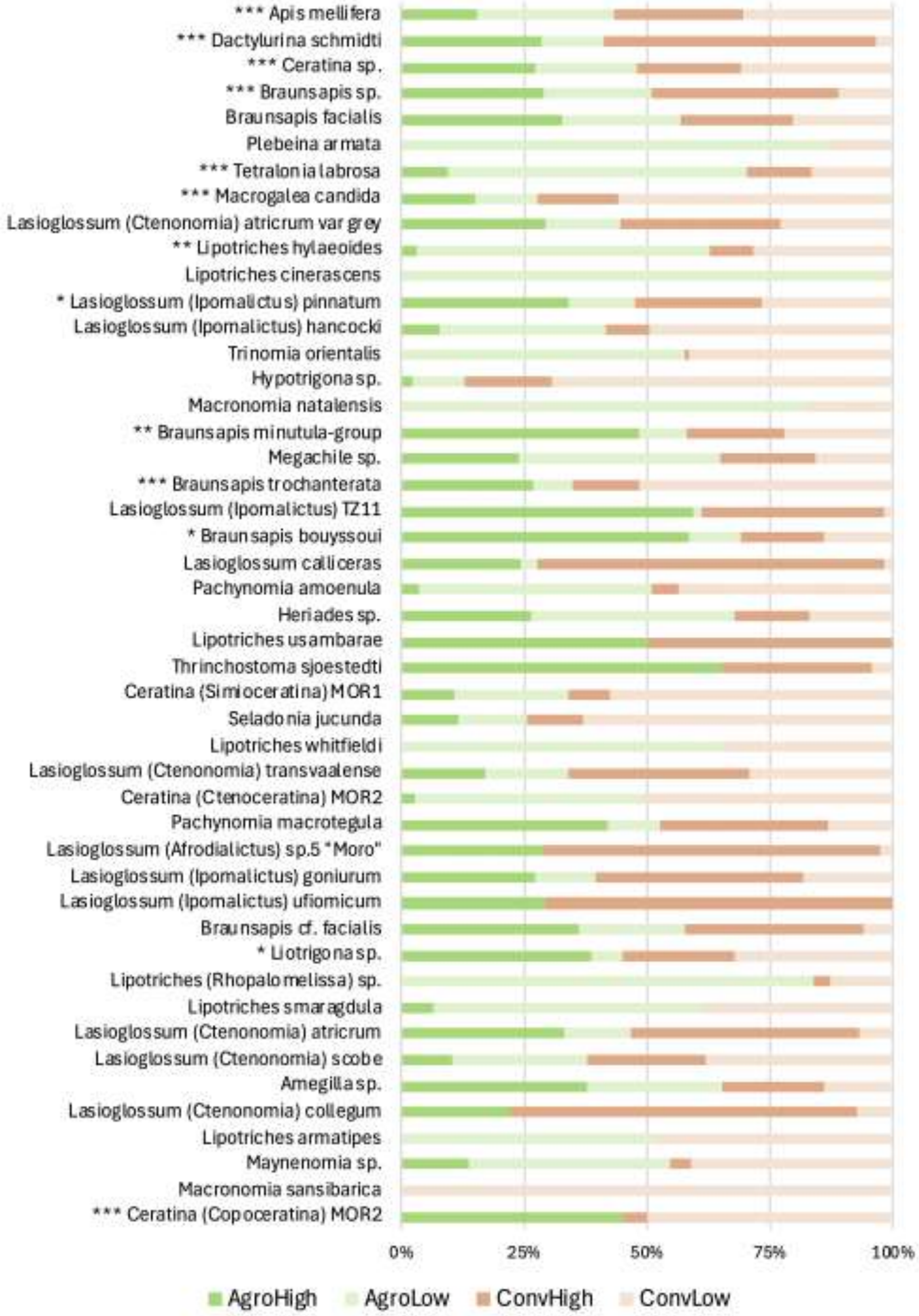


Figure beeHisto: From **Annex 6 - Annex allBees,** 50 (morpho)species are shown, each one being represented by at least 20 specimens. They are distributed in four categories obtained by crossing two categorical variables, treatment (i.e. management: agroecological “Agro“ versus conventional “Conv“) and elevation (i.e. altitude: “High“ versus “Low”): AgroHigh, AgroLow, ConvHigh, ConvLow. Asterisks preceding the name of a (morpho)species refer to a significant, two-tailed, P-value (at a = 0.05) obtained by the Fisher exact probability test: P < 0.05 (*), P < 0.01 (**), P < 0.001 (***). The (morpho)species are successively sorted by a decreasing number of specimens (given in percentages on the abscissa; see **Annex 6 - Annex allBees** for absolute values), an increasing P-value (see **Annex 6 - Annex allBees**), then alphabetically.

Flower fly communities (Diptera, Syrphidae)

Evaluating Sampling Methods for Flower Fly Diversity in East African Agroecosystems

These results have been submitted for publication to an international scientific journal with IF.

Methodological Context

Before analyzing flower fly community structure, we conducted a comparative study to evaluate two widely used sampling techniques—**hand netting** and **yellow pan trapping**—in cucurbit agroecosystems of Morogoro, Tanzania. The aim was to identify the most effective approach for capturing pollinator diversity and to inform the design of subsequent community-level analyses (O'Connor et al., 2019; Larson et al., 2024).

Study Design

Sampling was carried out during 2022–2023 across 20 plots in two agroecological zones (plateau and mountainous).

- **Hand netting:** Active sampling along three 15-m transects per plot for 45 min per session (Földesi & Kovács-Hostyánszki, 2014).
- **Pan traps:** Nine yellow pan traps per plot, exposed for 48 h (Berglund & Milberg, 2019).
- **Analysis:** Hill numbers (q = 0, 1, 2) for species richness and diversity; GLMs tested effects of method and zone.

Key Findings

- **Capture efficiency:** Hand netting accounted for 93.6% of individuals and recorded nearly all species (>94%), while pan traps captured ~6% of individuals and ~50% of species, mostly rare taxa.
- **Diversity:** All indices (species richness, Shannon, Simpson) were significantly higher for hand netting ($p < 0.0001$).
- **Landscape effect:** Both methods recorded higher diversity in mountainous zones, but hand netting remained superior across zones.
- **Implication:** Hand netting provides a more complete and cost-effective representation of flower fly assemblages, while pan traps offer limited complementary value for rare species.

Methodological Decision

Based on these results, we adopted a **total evidence approach** for the final analyses of flower fly communities, combining data from both hand netting and pan trapping. This strategy maximizes taxonomic coverage and ensures robust estimates of diversity and community composition across farming systems and landscapes (O'Connor et al., 2019).

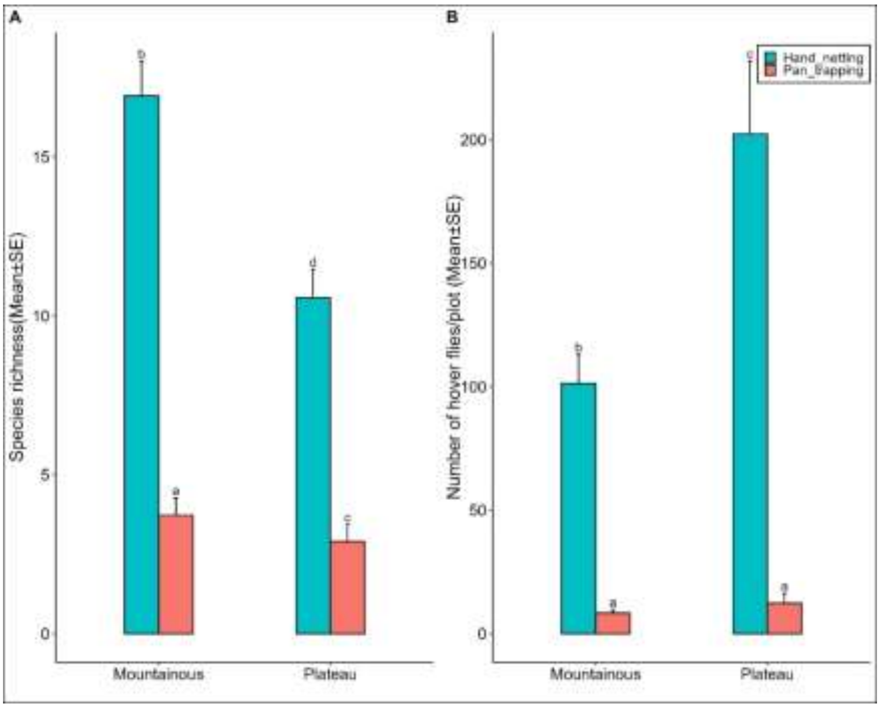


Figure T.1.1.2: Species richness and number of flies captured per plot by method and agroecological zone.

References

- Berglund, H.-L. & Milberg, P. (2019). Sampling of flower-visiting insects: Poor correspondence between the catches of colour pan-trap and sweep netting. *European Journal of Entomology*, 116:425–431.
- Földesi, R. & Kovács-Hostyánszki, A. (2014). Hoverfly community of cultivated arable field and adjacent hedgerow near Debrecen, Hungary. *Biologia*, 69:381–388.
- Larson, D.L. et al. (2024). Fewer bowl traps and more hand netting can increase effective number of bee species and reduce excessive captures. *Ecology and Evolution*, 14(2).
- O'Connor, R.S. et al. (2019). Monitoring insect pollinators and flower visitation: The effectiveness and feasibility of different survey methods. *Methods in Ecology and Evolution*, 10:2129–2140.

Characterizing Flower Fly Communities in East African Agroecosystems

Building on the preliminary evaluation of sampling methods, we integrated data from both hand netting and pan trapping into a **total evidence approach**. This combined dataset provided a comprehensive basis for analyzing flower fly communities across different farming systems and landscapes. These results were published in Kabota et al. (2025), "The impact of family farming on Afrotropical flower fly communities (*Diptera*, *Syrphidae*): A case study in Tanzania," PLoS ONE 20(7): e0327126. For a detailed overview of methods, results, bibliographic references, see https://doi.org/10.1371/journal.pone.0327126.

Methodology

Within the ISeBAF project framework, this study quantified the impact of family farming practices on Afrotropical flower fly communities in Tanzania. A large experimental setup was established in the

Morogoro region across two landscapes: plateau (300–600 m) and mountainous (600–900 m). Twenty small farms (10 per landscape) were selected, each managed either agroecologically or conventionally for at least four years. Experimental plots (45 × 45 m) were subdivided into subplots planted with cucurbit crops (*Cucumis sativus*, *Citrullus lanatus*, *Cucurbita moschata*). Sampling occurred weekly for eight weeks during four cropping seasons (2022–2023) using sweep netting and yellow pan traps. Collected specimens were preserved in ethanol and identified morphologically using Afrotropical Diptera keys. Diversity metrics (species richness, Shannon index, evenness) and abundance were analyzed via ANOVA and PERMANOVA, considering management practice, landscape, season, and plot as factors.

Scientific Results

Over two years, **12,969 flower flies** were collected, representing **55 species** across three subfamilies: *Eristalinae* (29 spp.), *Microdontinae* (2 spp.), and *Syrphinae* (24 spp.). The ten most abundant species accounted for **84.95%** of specimens, with *Toxomerus floralis* (51.3%), *Paragus borbonicus* (10.2%), and *Ischiodon aegyptius* (6.6%) dominating.

While **species richness and Shannon diversity did not show significant overall differences between agroecological and conventional farming**, the study revealed **clear agroecological effects when combined with other ecological processes** such as landscape and seasonality. Landscape explained approximately five times more variation than farming practice, confirming that spatial heterogeneity often outweighs management effects (Kennedy et al., 2013; Kremen & Miles, 2012).

Species-specific responses highlight these interactive effects:

- *T. floralis* was significantly more abundant in agroecological farms on the plateau, likely linked to its association with ruderal plants and cucurbit crops (Jordaens et al., 2015).
- *P. borbonicus* was significantly more abundant in agroecological farms in the mountains, consistent with its aphidophagous larval ecology and preference for cooler, structurally diverse habitats (Kaufmann, 1973; Azo'o Ela et al., 2021).
- *I. aegyptius* showed no consistent management effect but varied seasonally.

Alpha diversity was higher in mountainous landscapes, regardless of management, while **beta diversity** analyses showed significant differences between landscapes and a smaller but significant interaction between management and landscape (Baumann et al., 2021). These findings suggest that agroecological benefits for pollinators are **conditional on landscape context and species ecology rather than universal** (Henríquez-Piskulich et al., 2021).

This nuanced pattern aligns with previous evidence that dominant pollinator species often drive ecosystem services more than overall richness (Dainese et al., 2019). In our case, agroecological farming increased the abundance of dominant flower flies under favorable landscape conditions, which may enhance pollination efficiency even without a detectable increase in diversity.

Limitations: Strong spatiotemporal variability (season and plot effects) masked part of the farming practice effect; study covered only two years.

Scientific context: These results challenge simplistic assumptions about agroecology and emphasize the need for multi-year, landscape-aware studies to understand pollinator dynamics under climate variability (Reynolds et al., 2024).

Societal and decision-making relevance: Findings highlight the importance of integrating agroecological policies with landscape-level planning to optimize pollination services.

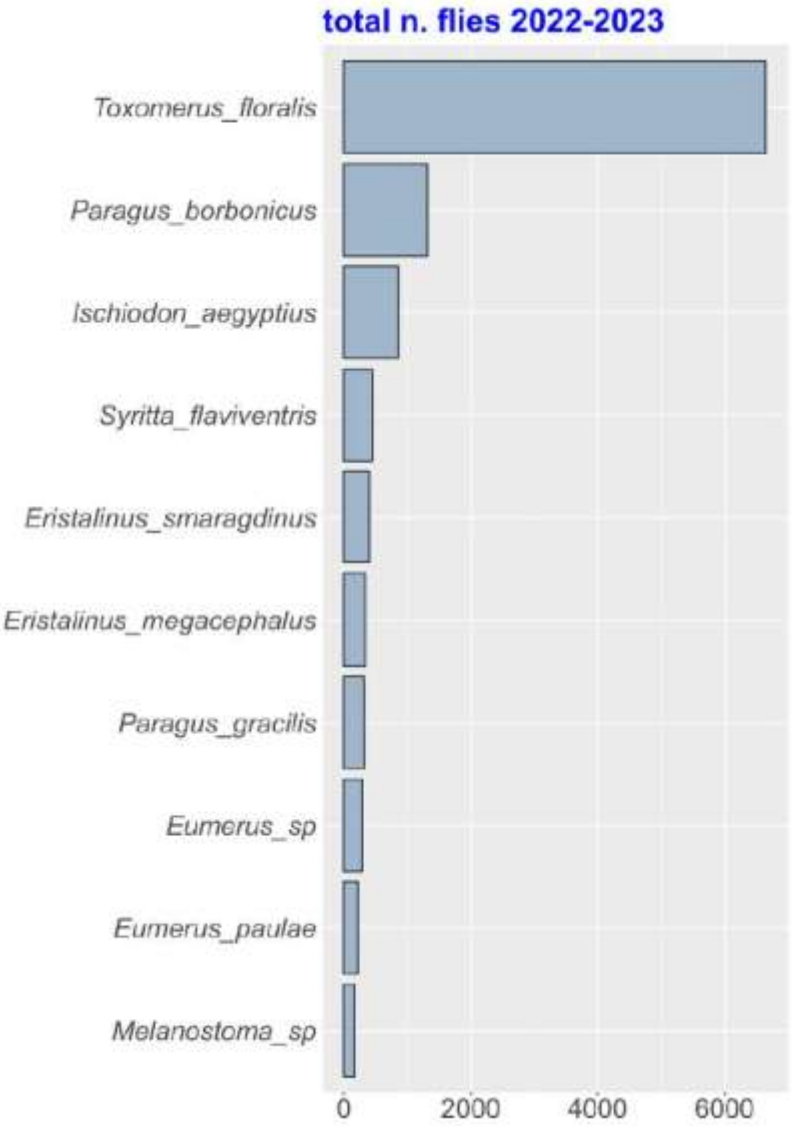


Figure T1.2.1: *Relative abundances of the ten most common Syrphidae species collected in Morogoro (2022–2023), showing dominance of Toxomerus floralis and Paragus borbonicus.*

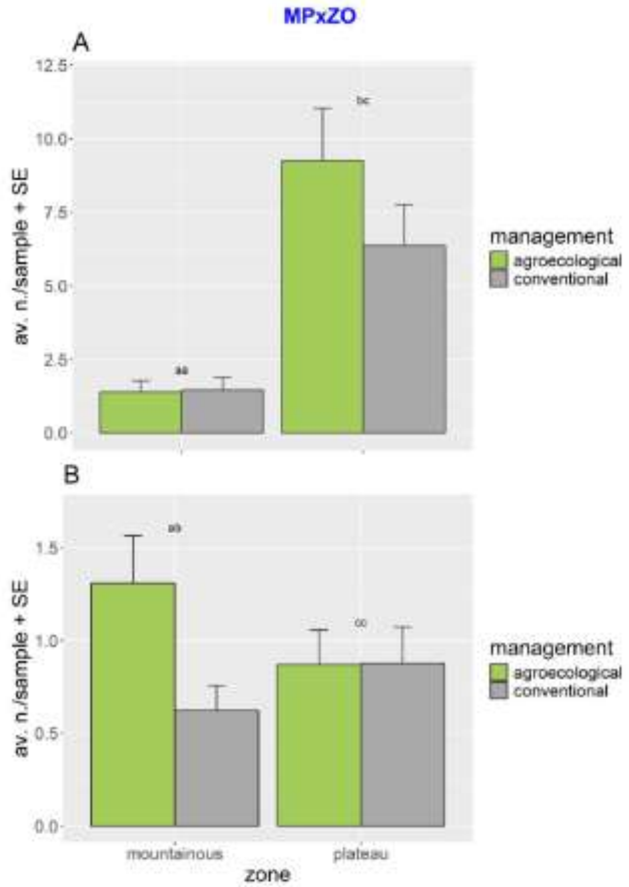


Figure T1.2.2: *Effect of agroecological vs conventional farming on the abundances of T. floralis (A) and P. borbonicus (B).*

References

- Kennedy CM et al. A global quantitative synthesis of local and landscape effects on wild bee pollinators in agroecosystems. *Ecol Lett*. 2013;16(5):584–99.
- Kremen C, Miles A. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. *Ecol Soc*. 2012;17.
- Jordaens K et al. A second New World hoverfly, *Toxomerus floralis* (Fabricius) (*Diptera*: Syrphidae), recorded from the Old World, with description of larval pollen-feeding ecology. *Zootaxa*. 2015;4044(4):567–76.
- Kaufmann T. Biology of *Paragus borbonicus* (*Diptera*: Syrphidae) as predator of *Toxoptera aurantii* (*Homoptera*: Aphididae) attacking cocoa in Ghana. *Am Midl Nat*. 1973;:252–6.
- Azo'o Ela M et al. Diversity of flower-visiting hoverflies (*Diptera*: Syrphidae) on ground cover vegetation from the market-gardening area of Meskine (Far-North Region, Cameroon). *Afr J Ecol*. 2021;60(1):58–66.
- Baumann K et al. Distribution and pollination services of wild bees and hoverflies along an altitudinal gradient in mountain hay meadows. *Ecol Evol*. 2021;11(16):11345–51.
- Henríquez-Piskulich PA et al. Agroecological strategies to safeguard insect pollinators in biodiversity hotspots: Chile as a case study. *Sustainability*. 2021;13:6728.

- Dainese M et al. A global synthesis reveals biodiversity-mediated benefits for crop production. *Sci Adv*. 2019;5(10):eaax0121.
- Reynolds SK et al. A comprehensive review of long-distance hover fly migration (*Diptera*: Syrphidae). *Ecological Entomology*. 2024;49(6):749–67.

Fruit fly communities (Diptera, Tephritidae).

These results are about to be submitted for publication by Bakengesa et al. (Fruit fly infestations in contrasting agricultural landscapes. A comparison between agroecological and conventional cucurbit farming in Central Eastern Tanzania).

Methodology

This study was conducted within the same experimental setup described for the Syrphidae research under the ISeBAF project—20 family farms in the Morogoro region (plateau and mountainous zones), managed either agroecologically or conventionally and planted with cucurbits (*Cucumis sativus*, *Citrullus lanatus*, *Cucurbita moschata*).

Unlike the Syrphidae study, which focused on pollinator diversity, this research targeted **infestation patterns of fruit flies (Tephritidae)**. Approximately 540 kg of fruits were sampled across four cropping seasons, incubated in controlled conditions, and monitored for adult emergence. Emerging flies were aspirated, preserved in ethanol, and identified using multi-entry keys (Virgilio et al., 2014). Infestation rates (flies/kg fruit) and diversity metrics were analyzed via ANOVA and PERMANOVA, considering management, altitude, crop, season, and field as factors.

Scientific Results

More than **22,000 adult fruit flies** emerged from the incubated fruits, representing eight species of Tephritidae. The community was dominated by *Zeugodacus cucurbitae* (melon fly, 69.3%), followed by *Dacus vertebratus* (14.6%), *D. ciliatus* (7.6%), and *D. bivittatus* (5.1%). Other species (*D. frontalis*, *D. punctatifrons*, *D. lounsburyii*, and *Bactrocera dorsalis*) were recorded at much lower frequencies.

Key findings:

- **Complex interactions:** Infestation patterns were not driven by a single factor but by **interactions among management, altitude, crop, and season**, as confirmed by ANOVA and PERMANOVA. These interactions explained more variation than any individual factor, highlighting the multi-layered nature of pest dynamics in smallholder systems.
- **Management effects:** Overall infestation rates tended to be higher in agroecological plots, particularly at high altitudes, mainly due to *Z. cucurbitae*. However, this effect was **not consistent across all species or zones**—for example, *D. ciliatus* and *D. bivittatus* sometimes showed higher infestations in conventional plots at low altitudes (Tarimo et al., 2023; De Meyer et al., 2015).
- **Altitude and crop interactions:**
    - *D. vertebratus* was more abundant at low altitudes, especially on watermelon (*C. lanatus*), confirming previous observations of host preference (Kambura et al., 2018).

  - *D. ciliatus* and *D. bivittatus* exhibited strong seasonal variability, with infestation peaks shifting between management systems depending on altitude and crop.
- **Alpha diversity:** Shannon diversity varied significantly across crops and altitudes, with lower diversity in cucumber plots dominated by *Z. cucurbitae*.
- **Beta diversity:** Multivariate analyses revealed that second- and third-order interactions (management × altitude × crop × season) contributed substantially to variation, underlining the complexity of agroecosystem dynamics.

Positive aspects of the agroecological approach:

Although agroecological plots showed higher infestations for some species, these systems **eliminate synthetic pesticide use**, reducing environmental and health risks (Deguine et al., 2015). Practices such as intercropping, mulching, and bio-fencing improve soil health and biodiversity, creating opportunities for **integrated pest management (IPM)** strategies that combine agroecology with targeted control measures (Amekawa et al., 2010; Wezel et al., 2009). Importantly, the observed complexity suggests that agroecological systems can be optimized to reduce pest pressure without compromising sustainability.

Scientific context: These findings challenge the assumption that agroecology alone reduces pest pressure and emphasize the need for adaptive strategies integrating ecological practices with effective monitoring and control (Deguine et al., 2012; Mokam et al., 2018).

Societal and decision-making relevance: Agroecology remains a viable alternative to chemical-intensive farming, offering socio-economic benefits and environmental sustainability, provided pest management is strengthened through IPM.

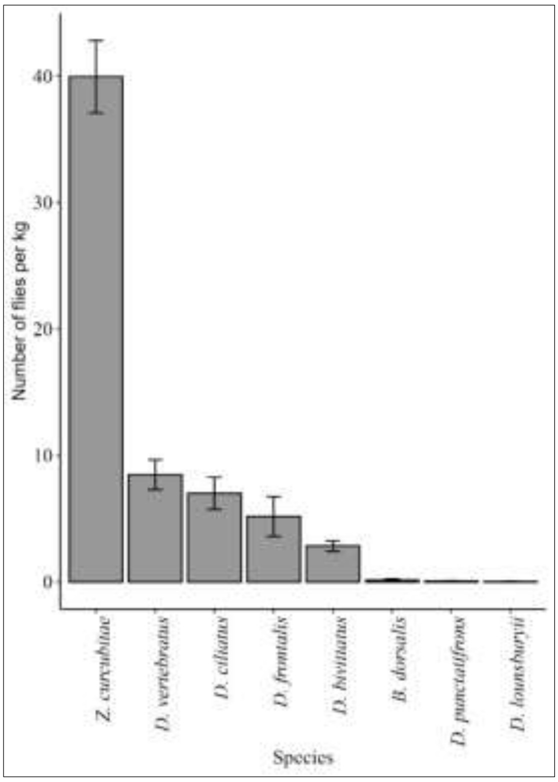


Figure T1.2.3: *Relative infestation rates of cucurbit crops by eight fruit fly species, dominated by Zeugodacus cucurbitae. (from draft manuscript)*

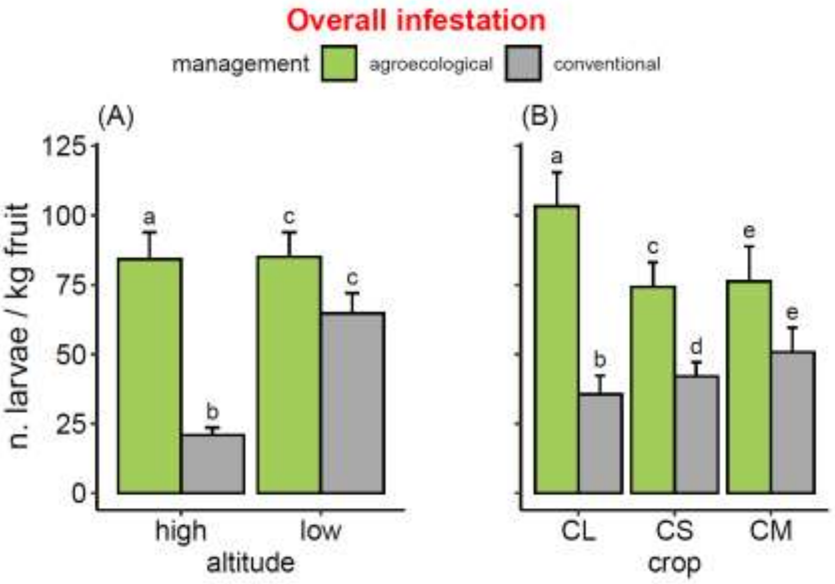


Figure T1.2.4: *Significant interactions showing higher infestations in agroecological plots at high altitudes and on watermelon and cucumber. (from draft manuscript)*

References

- Deguine J.-P. et al. (2015). Agroecological management of cucurbit-infesting fruit fly: a review. *Agronomy for Sustainable Development*, 35(3):937–965.
- Deguine J.-P. et al. (2012). Species diversity within a community of cucurbit fruit flies in Reunion Island. *Journal of Insect Science*, 12(1).
- Mokam D.G. et al. (2018). Host susceptibility and pest status of fruit flies attacking cucurbits in Cameroon. *African Entomology*, 26(2):317–332.
- Mwatawala M. et al. (2010). Incidence and host range of the melon fruit fly in Central Tanzania. *International Journal of Pest Management*, 56(3):265–273.
- Vayssières J.-F. et al. (2002). Demographic parameters and biotic factors of two Dacini species on Réunion Island. *Proceedings of the 6th International Fruit Fly Symposium*.
- Kambura C. et al. (2018). Composition and host range of vegetable-infesting tephritids on cucurbits in Kenya. *African Entomology*, 26(2):379–397.
- Tarimo P. et al. (2023). Host use preference of fruit flies among cucurbit vegetables in Morogoro. *bioRxiv*.
- De Meyer M. et al. (2015). A review of current knowledge on *Zeugodacus cucurbitae* in Africa. *ZooKeys*, 540:539–557.

Task 1.3 genetic diversity

Phylogenetic diversity of wild bees

To assess the phylogenetic diversity of wild bees in the Morogoro region, genomic data were generated for all (morpho)species (**Table PD**) identified among the specimens collected in April 2022 (season 1 of 2022). For additional phylogenetic diversity analyses, DNA barcodes corresponding to the 5'-end of the cytochrome oxidase subunit I gene (COI) were sequenced for all specimens collected in October 2022 (season 2 of 2022). These two analyses hereunder referred as “phylogenetic diversity of wild bees in April 2022” and “genetic diversity of wild bees in October 2022” provide different views on their molecular diversity because the first one relies on a large amount of genomic data (> 10^6 bp) from a selection of representative (morpho)species, while the second one relies on a short mitochondrial DNA sequence (689 bp) but from a maximum of specimens.

Material and Methods

UCE data acquisition for phylogenetic diversity of wild bees in April 2022

Ultra Conserved Elements (UCE) have been widely used to investigate bee phylogenetic diversity (Bossert et al. 2019; 2021; 2024a; Branstetter et al. 2017; 2021; Grab et al. 2019; Gueuning et al. 2019; Gueuning, Frey, and Praz 2020). To generate such a dataset, two methods are possible, either sequencing the whole genome and retrieving the UCEs from it, or proceeding to a UCE enrichment and sequencing the output. After a discussion with Silas Bossert, an expert in bee's phylogenetic at the Washington State University (USA), we decided to go for the first method. Indeed, when DNA is not too degraded, the whole genome method requires similar cost but less lab work and produces more genomic data than the enrichment method, offering more potential for further analyses. The generated data can be used to document genomes of wild bees in the Morogoro region and to investigate the influence of altitude and farming practices on wild bees' phylogenetic diversity. This metric quantifies the evolutionary history of wild bees captured by species assemblage in each field.

DNA was extracted from the tissue of three legs from each specimen using the NucleoSpin Tissue kit following the manufacturer's protocol with a final elution volume of 60 µL. The DNA concentration was then quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Inc.). When possible, 5 ng of genomic DNA was used from each sample for library preparation using NEBNext Ultra II FS DNA Library Prep Kit for Illumina combined with NEBNext Multiplex Oligos for Illumina (Dual Index Primer Set 1) following the manufacturer's protocol. To obtain DNA fragments around 300 bp, the 37°C fragmentation incubation phase was set at 6 min. The two cleanup steps were carried out with magnetic beads HighPrep PCR (MAGBIO). The PCR enrichment of adaptor-ligated DNA was done with eight cycles, and the final elution volume was 30 µL. DNA quality controls were performed after extraction and after PCR enrichment using Agilent High Sensitivity DNA Kit. The samples were then pooled at equimolar concentration. Sequencing of 150 bp paired end reads was carried out on an Illumina NovaSeq X Plus sequencer by Novogene Co. Ltd and that produced around 10 Gb of sequencing data per sample.

UCE data analysis for phylogenetic diversity of wild bees in April 2022

The bioinformatic processing followed a similar protocol as (Bossert et. al 2024b). Demultiplexed data were received from Novogene and Clumpify (BBtools) was used to remove duplicates and reorder the reads to reduce size and increase efficiency for subsequent steps. Adapter were removed using Fastp which also generated quality assessments then the reads were normalized with BBnorm (BBtools) targeting a coverage of maximum 20x and minimum 2x. Genomes were assembled with SPAdes implementing a 5x coverage cutoff and using the careful option to reduce the number of mismatches and short indels. Once the genomes were assembled into contigs, Phyluce (Faircloth 2016) was used to retrieve the Ultra Conserved Elements loci (UCEs). The genome of the wasp *Mimumesa dahlbomi* (NCBI assembly GCA_917499265.3) was added as an outgroup. The probes set 'bee-ant-specific hym-v2 bait set' (Grab et al. 2019) was matched against each genome and the sequences following the requirement of 80% overlap and 80% identity were extracted with 1,000 bp flanking regions. The sequences were grouped per UCE locus (2590 in total) then aligned with MAFFT and the L-INS-i method (Katoh and Standley 2013). They were then trimmed with Gblocks (Castresana 2000) using the relaxed setting (Talavera and Castresana 2007). Finally, the alignment with at least 75% (1272 UCEs) of the taxa were conserved and concatenated in one sequence matrix of 1,053,849 bp including 106 (morpho)species. Phylogenetic analyses were carried out using IQ-TREE2 with the best-fitting substitution models selected via ModelFinder Plus with partition merging (MFP+MERGE), branch support assessed using 1,000 bootstrap replicates and approximate likelihood ratio tests (aLRT) (**Fig. Phyl**). The resulting phylogenetic tree exhibited the expected topology based on the known taxonomy. An ultrametric tree was inferred using RelTime, a maximum likelihood approach implemented in MEGA (Tamura et al. 2018) following Bossert et al. (2024b) and with calibration points for families and subfamilies obtained from Almeida et al. (2023). The phylogenetic diversity of the wild bees present in one field was calculated in R using the package picante (Kembel et al. 2010) as the sum of the lengths of all those branches on the tree that span the species collected in that field (Faith 1992).

DNA barcode data acquisition for genetic diversity of wild bees in October 2022

DNA was extracted from the tissue of three legs from each specimen using the NucleoSpin Tissue kit following the manufacturer's protocol with a final elution volume of 60 µL. The DNA concentration was then quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific, Inc.). A fragment of the 5'-end of the cytochrome oxidase subunit I gene (COI) was amplified by polymerase chain reaction (PCR) using the primers BeeCox1F1 5'-TAGTCAACAAATCATAAAGATATTGG-3' and BeeCox1R2 5'-CCAAATCCTGGTAGAATTAAAATATA-3' (Bleidorn & Henze 2021). Each PCR reaction consists of a 20 µL aqueous solution containing final concentrations of Taq Platinum Buffer (1X), MgCl (3 mM), dNTP (0.2 mM), forward primer (0.2 µM), reverse primer (0.2 µM), Taq Platinum (0.03 units/µL), and 5 µL of DNA template. The PCR temperature profile started with an initial denaturation at 94°C for 3 min, continued with 40 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 45 s and elongation at 72°C for 60 s, and ended with a final extension at 72°C for 7 min. Amplification was checked by electrophoresis on a 1% agarose gel. PCR products were then purified using the ExoSAP-IT® method (Bell 2008) and sequenced bidirectionally at Macrogen Europe BV (Amsterdam, The Netherlands).

DNA barcode analysis for genetic diversity of wild bees in October 2022

Chromatograms and base calling were inspected and trimmed to remove bad quality sites using the program CodonCode Aligner v. 8.0.2 (CodonCode Corporation). COI sequences from this season (October 2022) were aligned with the COI sequences extracted from the whole genomes sequencing of the previous season (April 2022) using CLUSTALW (Thompson et al. 1994) and a distance neighbour joining tree based on uncorrected p-distances (number of substitutions divided by the number of sites being compared) was calculated using MEGA v. 11.0.13 (Tamura et al. 2021). Consistency between clusters of similar DNA barcodes and species identifications based on morphology were checked. Finally, genetic diversity values and cumulative curves of genetic diversity values were calculated in R using the package caper (Orme et al. 2023). Statistical tests were performed in R and comprised permutation tests and linear regressions.

Results

Phylogenetic diversity of wild bees in April 2022

The phylogenetic diversity values calculated for each of the 20 fields are based on the ultrametric tree representing the evolution of 106 of the 113 wild bee (morpho)species collected in April 2022. This evolution was inferred from an alignment of around 10^6 DNA characters. The phylogenetic diversity value calculated for each field is an estimation of the overall evolutionary history (the sum of the branch lengths) represented by the wild bees collected in that field (i.e. only part of the branches of the complete ultrametric tree). Phylogenetic diversity values (**Table PD_fields**) ranged from 493 million years (My) in a field called Kidokwe (high altitude and conventional management) to 1,386 My in a field called Kilangalanga (high altitude and agroecological management). The total phylogenetic diversity of all fields at low altitude was greater than that of all fields at high altitude (2,441 versus 2,099 My) but the permutation test (100 runs) did not reject the null hypothesis that altitude had no effect on phylogenetic diversity (**Table PD_altitude**). Similarly, the total phylogenetic diversity of all fields managed with agroecological practices was greater than that of all fields managed with more conventional practices (2,643 versus 2,417 My) but the permutation test (100 runs) did not reject the null hypothesis that management had no effect on phylogenetic diversity (**Table PD_management**). When considering both elevation and management, median phylogenetic diversity values were greater at low altitudes than at high altitudes (regardless of the management), and greater for fields managed agroecologically than those managed conventionally in the same altitude category (**Fig. PD_BoxPlot**). In the linear regression test, where phylogenetic diversity was set as the dependant variable and altitude and management as two independent variables, no significant effect of the variables taken separately or combined was observed (**Table PD_lm**).

Genetic diversity of wild bees in October 2022

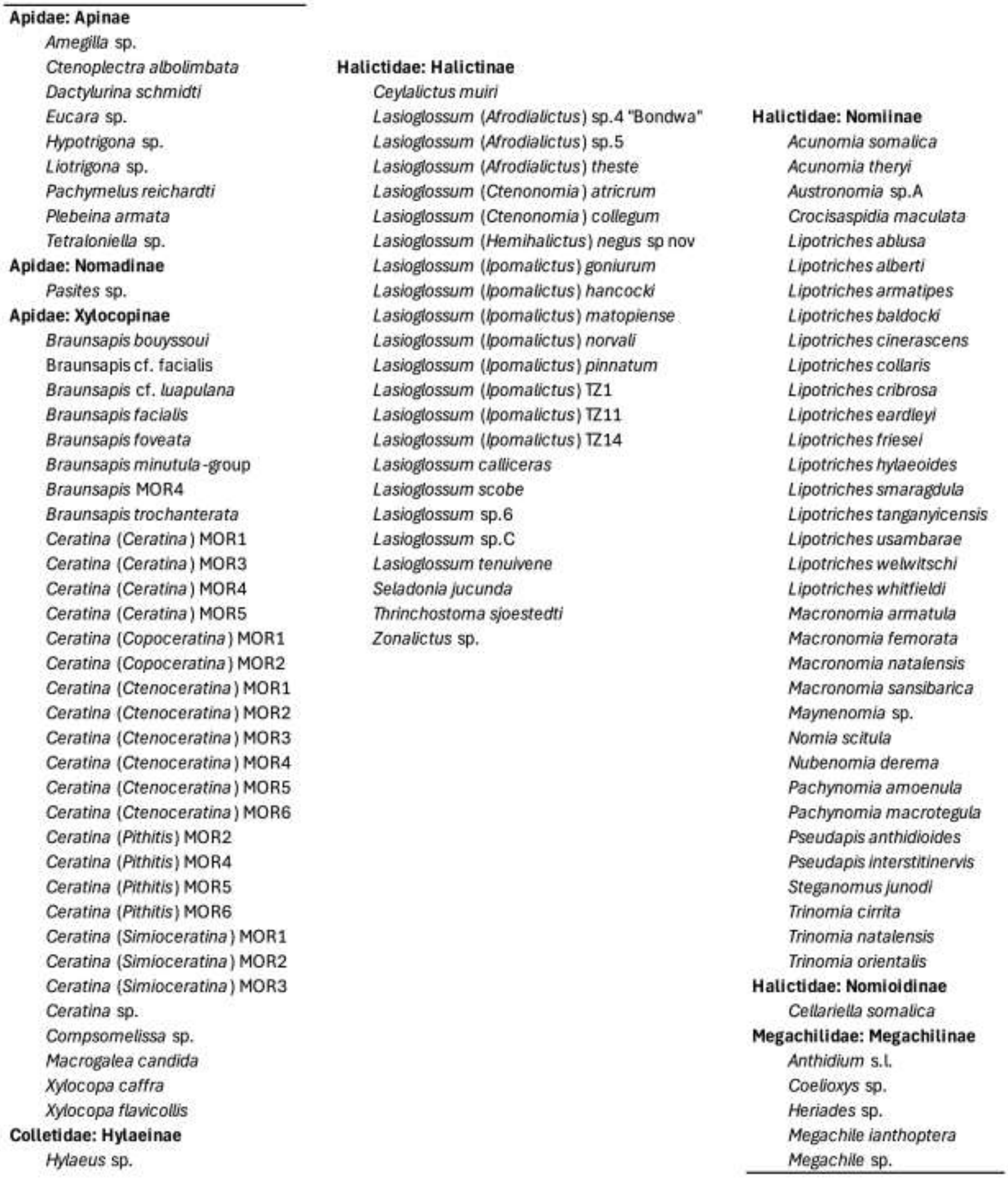
Genetic diversity measures were calculated on the basis of 348 COI sequences (DNA barcode data) successfully sequenced from the 407 wild bees collected in October 2022. In contrast to the phylogenetic diversity values calculated above using a calibrated phylogenetic tree, the genetic diversity values calculated here are based on a simple distance tree and therefore only represent a relative amount of genetic distance and not an estimation of evolutionary history expressed in number of years. The analysis was performed twice, once using only the bees collected on cucurbit flowers (288 sequences), once with all 348 sequences, including the bees collected on the borders of the fields. In the latter analysis, 18 of the 20 fields could be included because data was insufficient for two sites (bees from the borders only in the ”Morning Site” and no data from “Mafiga”). Genetic diversity values (**Table COI_fields**) including bees from the borders of the fields or not ranged from 0.23 in a field called Mgola A (high altitude and agroecological management) to 1.78 in a field called Shaulini (low altitude and agroecological management). Median genetic diversity values were greater for fields at low altitude and managed according to agroecological practices than for fields belonging to the three other categories (**Fig. COIBoxPlot**). In the linear regression test, low altitude had a significant positive effect on wild bee genetic diversity, and conventional farming at low altitude had a significant negative effect on wild bee genetic diversity. This was observed both when considering wild bees from the borders of the fields or not (**Table COI_lm)**. Noteworthy, wild bee abundances were also greater in low altitude agroecological fields than in the other fields (**Fig. COICountsBoxPlot**). Indeed, in the permutation tests, the null hypothesis – stating that samples randomly assigned to one field category show the same ranges of genetic diversity values as the samples collected in the field of that category – was never rejected (**Table COI_Perm**). Finally, all samples collected in fields of the same category (2 altitudes x 2 managements) were grouped together to calculate the cumulative genetic diversity for each category (**Fig. COICumul**). To compare genetic diversity at identical sample size, genetic diversity values were calculated for 40 or 30 random samples (1,000 replications) from each field category. The boxplot of these pseudo-replicates showed that the median of the genetic diversity values in low altitude agroecological fields was greater than in the three other field categories (**Fig. COIBoxPlotBal**). The genetic diversity values were significantly greater when comparing low altitude agroecological fields with all other field types when 40 samples were randomly sampled in each category, and with both conventional fields (low and high altitudes) when 30 samples were randomly sampled. The other comparisons did not reject the null hypothesis that genetic diversity values differ between fields from one versus another category (**Table COI_BootP**).

Discussion

Molecular diversity of wild bees on cucurbit crops

Phylogenetic diversity (Faith 1992) has been recommended as an interesting general measure of biodiversity, which maximises feature diversity (Lean & Maclaurin 2016). However, its reliability can be affected by phylogenetic uncertainty (the quality of the phylogenetic inference) and taxonomic sampling (the comprehensiveness of the species sampled) (Park et al. 2018). Here we applied two distinct approaches: one minimizing the phylogenetic uncertainty (UCE phylogenomics) and one minimizing the taxonomic sampling (DNA barcoding). The results of these two approaches cannot be compared because they were applied to two different seasons of 2022 but offer more contrasted measurements of phylogenetic diversity. In both approaches, median phylogenetic diversity was greater at low altitude than at high altitude, and also greater in agroecological fields compared to conventional fields, but these trends were only statistically significant for the approach based on COI and October 2022. The smaller values of phylogenetic diversity were generally obtained when smaller numbers of wild bees were collected. Even if equivalent sampling effort was made on all fields (in term of time of the day, duration and number of collectors), insufficient sampling may have affected the results because it did not capture the whole phylogenetic diversity that could be present on a longer timeframe. Hence, the values obtained here represent the situation at two specific moments of the year. Based on our experiments, phylogenetic diversity was not significantly affected when including or excluding the bees collected at the borders of the fields. When subsampling the four different categories (altitude & management) with equivalent numbers of samples (COI from October 2022), the phylogenetic diversity in low altitude agroecological fields was still significantly greater than in the other fields. To conclude, the values obtained here represent punctual indicators that could be used as a starting point to assess in more details and at the species level the diversity of wild bees present in the four categories of cucurbit fields studied in this project.

Table PD: List of wild bee's (morpho)species of April 2022 included in phylogenetic analyses



Apidae: Apinae
- *Amegilla* sp.
- *Ctenoplectra albolimbata*
- *Dactylurina schmidti*
- *Eucara* sp.
- *Hypotrigona* sp.
- *Liotrigona* sp.
- *Pachymelus reichardti*
- *Plebeina armata*
- *Tetraloniella* sp.

Apidae: Nomadinae
- *Pasites* sp.

Apidae: Xylocopinae
- *Braunsapis bouyssoui*
- Braunsapis cf. facialis
- *Braunsapis* cf. *luapulana*
- *Braunsapis facialis*
- *Braunsapis foveata*
- *Braunsapis minutula*-group
- *Braunsapis* MOR4
- *Braunsapis trochanterata*
- *Ceratina* (*Ceratina*) MOR1
- *Ceratina* (*Ceratina*) MOR3
- *Ceratina* (*Ceratina*) MOR4
- *Ceratina* (*Ceratina*) MOR5
- *Ceratina* (*Copoceratina*) MOR1
- *Ceratina* (*Copoceratina*) MOR2
- *Ceratina* (*Ctenoceratina*) MOR1
- *Ceratina* (*Ctenoceratina*) MOR2
- *Ceratina* (*Ctenoceratina*) MOR3
- *Ceratina* (*Ctenoceratina*) MOR4
- *Ceratina* (*Ctenoceratina*) MOR5
- *Ceratina* (*Ctenoceratina*) MOR6
- *Ceratina* (*Pithitis*) MOR2
- *Ceratina* (*Pithitis*) MOR4
- *Ceratina* (*Pithitis*) MOR5
- *Ceratina* (*Pithitis*) MOR6
- *Ceratina* (*Simioceratina*) MOR1
- *Ceratina* (*Simioceratina*) MOR2
- *Ceratina* (*Simioceratina*) MOR3
- *Ceratina* sp.
- *Compsomelissa* sp.
- *Macrogalea candida*
- *Xylocopa caffra*
- *Xylocopa flavicollis*

Colletidae: Hylaeinae
- *Hylaeus* sp.

Halictidae: Halictinae
- *Ceylalictus muiri*
- *Lasioglossum* (*Afrodialictus*) sp.4 "Bondwa"
- *Lasioglossum* (*Afrodialictus*) sp.5
- *Lasioglossum* (*Afrodialictus*) *theste*
- *Lasioglossum* (*Ctenonomia*) *atricrum*
- *Lasioglossum* (*Ctenonomia*) *collegum*
- *Lasioglossum* (*Hemihalictus*) *negus* sp nov
- *Lasioglossum* (*Ipomalictus*) *goniurum*
- *Lasioglossum* (*Ipomalictus*) *hancocki*
- *Lasioglossum* (*Ipomalictus*) *matopiense*
- *Lasioglossum* (*Ipomalictus*) *norvali*
- *Lasioglossum* (*Ipomalictus*) *pinnatum*
- *Lasioglossum* (*Ipomalictus*) TZ1
- *Lasioglossum* (*Ipomalictus*) TZ11
- *Lasioglossum* (*Ipomalictus*) TZ14
- *Lasioglossum calliceras*
- *Lasioglossum scobe*
- *Lasioglossum* sp.6
- *Lasioglossum* sp.C
- *Lasioglossum tenuivene*
- *Seladonia jucunda*
- *Thrinchostoma sjoestedti*
- *Zonalictus* sp.

Halictidae: Nomiinae
- *Acunomia somalica*
- *Acunomia theryi*
- *Austronomia* sp.A
- *Crocisaspidia maculata*
- *Lipotriches ablusa*
- *Lipotriches alberti*
- *Lipotriches armatipes*
- *Lipotriches baldocki*
- *Lipotriches cinerascens*
- *Lipotriches collaris*
- *Lipotriches cribrosa*
- *Lipotriches eardleyi*
- *Lipotriches friesei*
- *Lipotriches hylaeoides*
- *Lipotriches smaragdula*
- *Lipotriches tanganyicensis*
- *Lipotriches usambarae*
- *Lipotriches welwitschi*
- *Lipotriches whitfieldi*
- *Macronomia armatula*
- *Macronomia femorata*
- *Macronomia natalensis*
- *Macronomia sansibarica*
- *Maynenomia* sp.
- *Nomia scitula*
- *Nubenomia derema*
- *Pachynomia amoenula*
- *Pachynomia macrotegula*
- *Pseudapis anthidioides*
- *Pseudapis interstitinervis*
- *Steganomus junodi*
- *Trinomia cirrita*
- *Trinomia natalensis*
- *Trinomia orientalis*

Halictidae: Nomioidinae
- *Cellariella somalica*

Megachilidae: Megachilinae
- *Anthidium* s.l.
- *Coelioxys* sp.
- *Heriades* sp.
- *Megachile ianthoptera*
- *Megachile* sp.

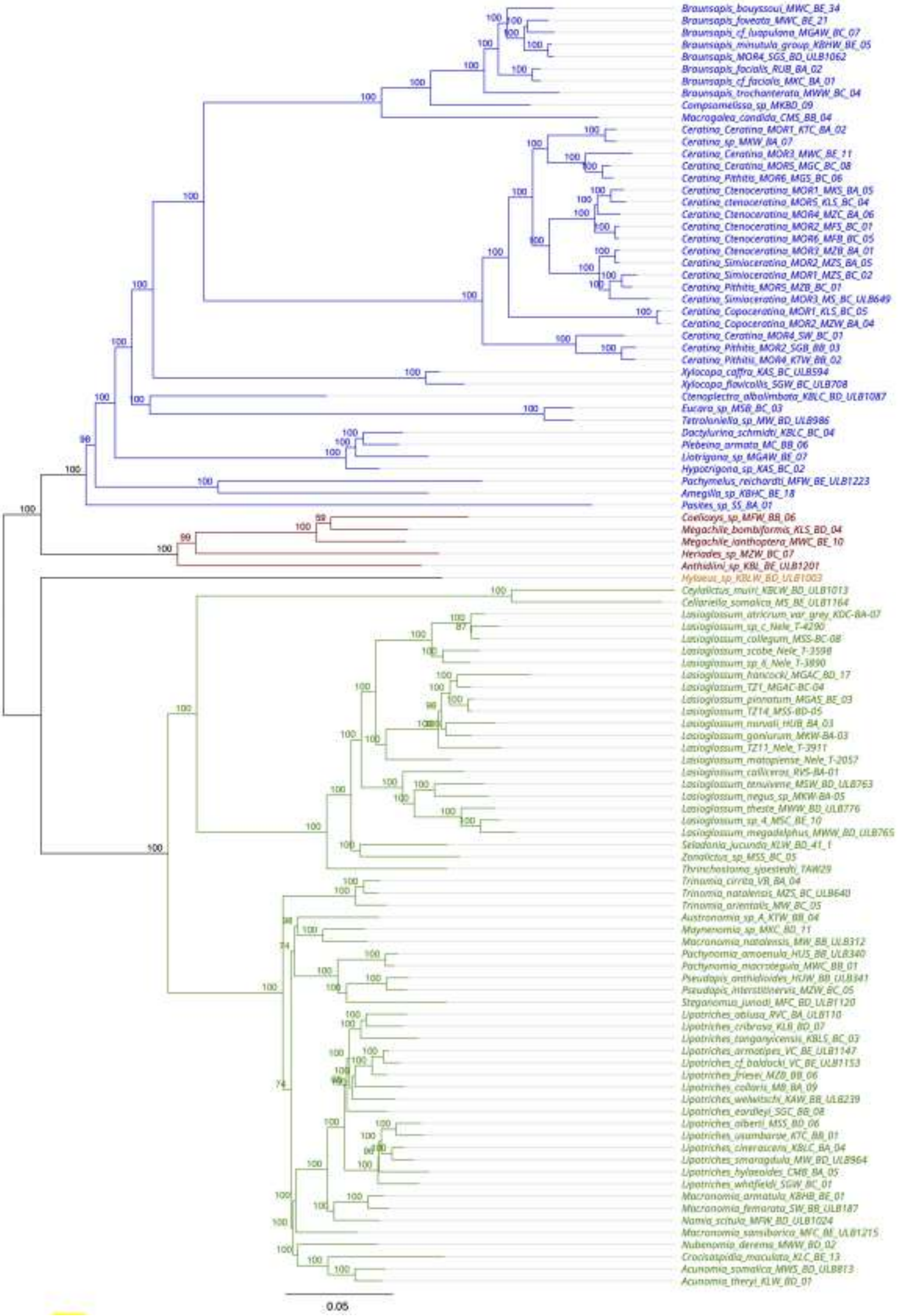
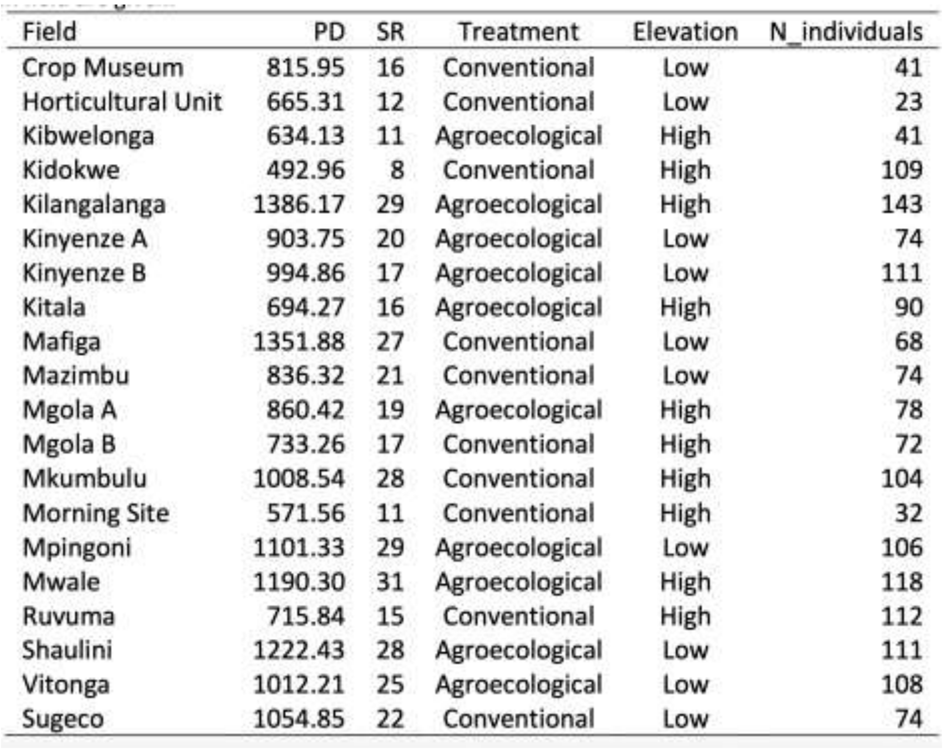


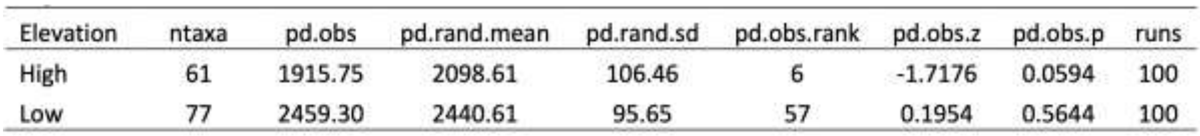
Figure Phyl: Phylogenomic tree generated on IQ-tree based on a ca. 1 Mb matrix including 1272 UCEs and 106 wild bee (morpho)species collected in April 2022. Node supports indicate bootstrap values.

Table PD_fields: Phylogenetic diversity (PD) of wild bees in April 2022 based on the phylogenomic tree constructed using the Ultra Conserved Elements (UCE). Treatment, elevation, species richness (SR) and total number of wild bees (N individuals) collected on each field are given.



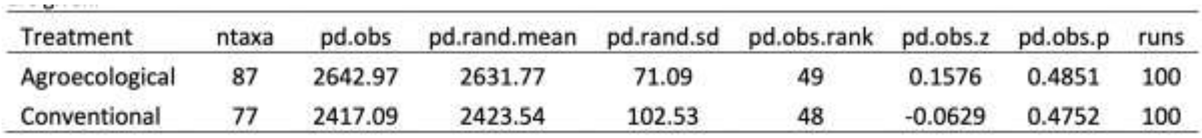
Field	PD	SR	Treatment	Elevation	N_individuals
Crop Museum	815.95	16	Conventional	Low	41
Horticultural Unit	665.31	12	Conventional	Low	23
Kibwelonga	634.13	11	Agroecological	High	41
Kidokwe	492.96	8	Conventional	High	109
Kilangalanga	1386.17	29	Agroecological	High	143
Kinyenze A	903.75	20	Agroecological	Low	74
Kinyenze B	994.86	17	Agroecological	Low	111
Kitala	694.27	16	Agroecological	High	90
Mafiga	1351.88	27	Conventional	Low	68
Mazimbu	836.32	21	Conventional	Low	74
Mgola A	860.42	19	Agroecological	High	78
Mgola B	733.26	17	Conventional	High	72
Mkumbulu	1008.54	28	Conventional	High	104
Morning Site	571.56	11	Conventional	High	32
Mpingoni	1101.33	29	Agroecological	Low	106
Mwale	1190.30	31	Agroecological	High	118
Ruvuma	715.84	15	Conventional	High	112
Shaulini	1222.43	28	Agroecological	Low	111
Vitonga	1012.21	25	Agroecological	Low	108
Sugeco	1054.85	22	Conventional	Low	74

Table PD_altitude: Results of the permutation test where phylogenetic diversity (pd) of wild bees of April 2022 was calculated for pseudo-replicates (100 runs) with random elevation labels (.rand) and compared with the observed (.obs) phylogenetic diversity. Number of bee species (ntaxa), mean (.mean), standard deviation (.sd), Z-score (.z) and P-value (.p) are given.



Elevation	ntaxa	pd.obs	pd.rand.mean	pd.rand.sd	pd.obs.rank	pd.obs.z	pd.obs.p	runs
High	61	1915.75	2098.61	106.46	6	-1.7176	0.0594	100
Low	77	2459.30	2440.61	95.65	57	0.1954	0.5644	100

Table PD_management: Results of the permutation test where phylogenetic diversity (pd) of wild bees of April 2022 was calculated for pseudo-replicates (100 runs) with random treatment labels (.rand) and compared with the observed (.obs) phylogenetic diversity. Number of bee species (ntaxa), mean (.mean), standard deviation (.sd), Z-score (.z) and P-value (.p) are given.



Treatment	ntaxa	pd.obs	pd.rand.mean	pd.rand.sd	pd.obs.rank	pd.obs.z	pd.obs.p	runs
Agroecological	87	2642.97	2631.77	71.09	49	0.1576	0.4851	100
Conventional	77	2417.09	2423.54	102.53	48	-0.0629	0.4752	100

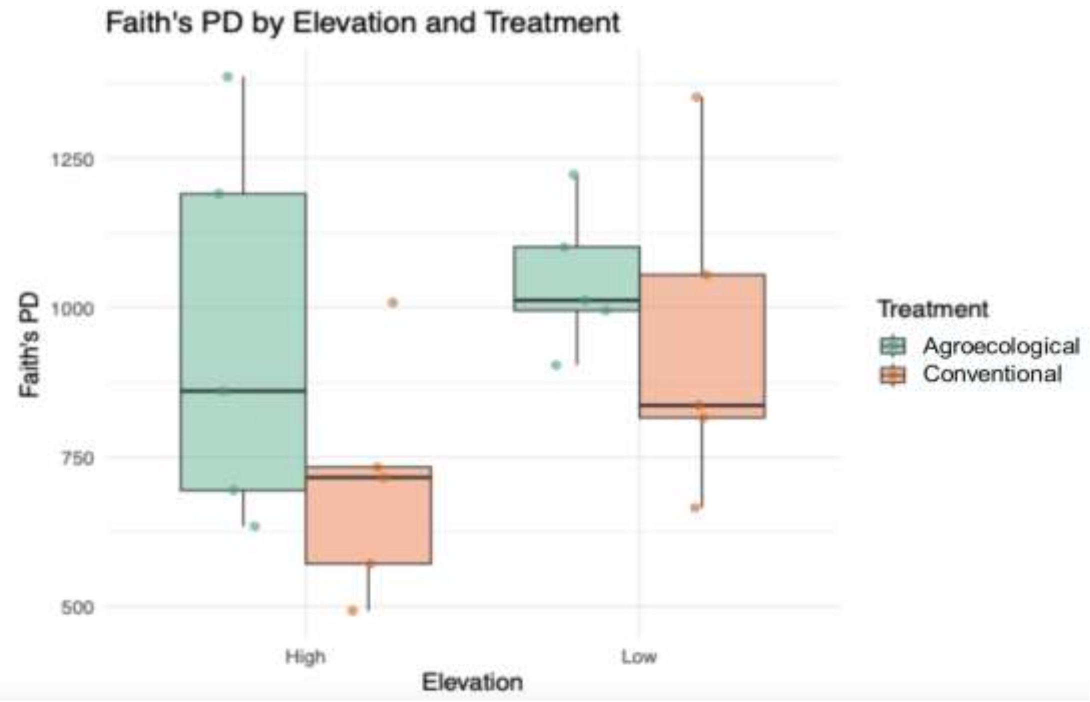
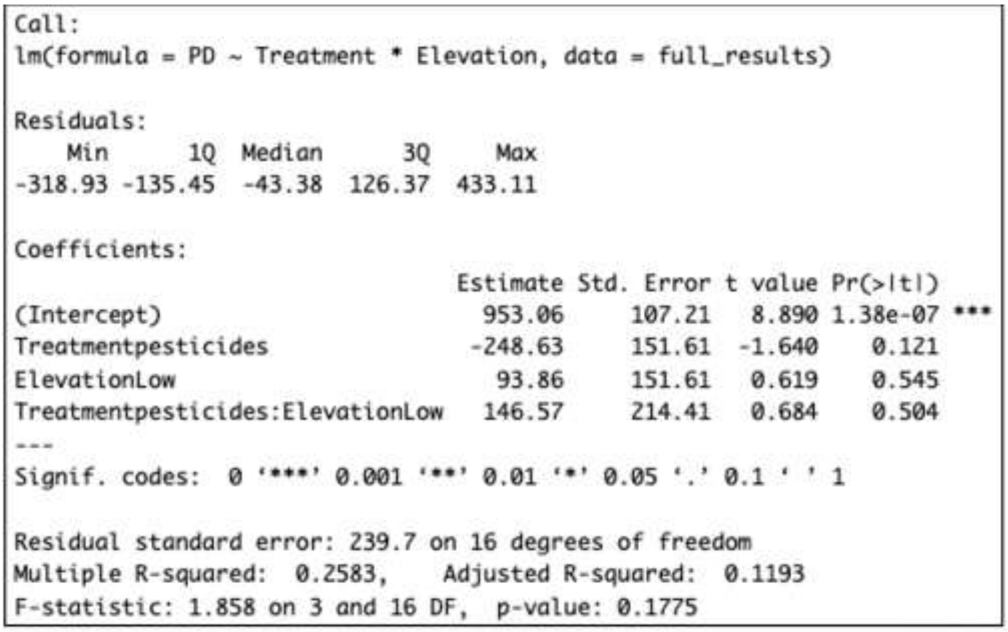


Figure PD_Boxplot: Phylogenetic diversity (Faith's PD) of wild bees of April 2022 obtained for each field and grouped according to the treatment and the elevation. Median (horizontal segment), interquartile (colored rectangle) and most extreme values within the range of 1.5 times the interquartile (vertical bars) are indicated.

Table PD_lm: Results of the linear regression testing if phylogenetic diversity of wild bees in April 2022 were dependent upon field elevation or treatment. ElevationLow: field at low altitude. Treatmentpesticides: field managed with conventional farming.



```
Call:
lm(formula = PD ~ Treatment * Elevation, data = full_results)

Residuals:
    Min      1Q  Median      3Q     Max 
-318.93 -135.45  -43.38  126.37  433.11 

Coefficients:
                                 Estimate Std. Error t value Pr(>|t|)    
(Intercept)                        953.06     107.21   8.890 1.38e-07 ***
Treatmentpesticides               -248.63     151.61  -1.640    0.121    
ElevationLow                        93.86     151.61   0.619    0.545    
Treatmentpesticides:ElevationLow   146.57     214.41   0.684    0.504    
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 239.7 on 16 degrees of freedom
Multiple R-squared:  0.2583,	Adjusted R-squared:  0.1193 
F-statistic: 1.858 on 3 and 16 DF,  p-value: 0.1775
```

Table COI_fields: Phylogenetic diversity (based on the COI uncalibrated tree) of wild bees in October 2022 including individuals collected on the borders of the crops (PD1) or not (PD2). N: number of wild bees collected on the crops (excluding those from the borders of the crops).

Field	Altitude	Treatment	N	PD1	PD2
Kibwelonga	high	agroecological	9	0.5704	0.5704
Kilangalanga	high	agroecological	12	0.7112	0.6274
Kitala	high	agroecological	12	0.9358	0.5995
Mgola A	high	agroecological	6	0.2280	0.2280
Mwale	high	agroecological	8	0.5337	0.4030
Kinyenze A	low	agroecological	18	1.1571	1.1571
Kinyenze B	low	agroecological	29	1.0681	1.0638
Mpingoni	low	agroecological	43	1.3103	1.2589
Shaulini	low	agroecological	36	1.7819	1.7819
Vitonga	low	agroecological	30	1.2407	1.1813
Kidokwe	high	conventional	6	0.6439	0.4714
Mgola B	high	conventional	10	0.5483	0.5483
Mkumbulu	high	conventional	5	0.2714	0.2714
Ruvuma	high	conventional	22	0.8999	0.7620
Crop Museum	low	conventional	4	0.4456	0.4456
Horticultural Unit	low	conventional	15	0.3967	0.3933
Mazimbu	low	conventional	18	0.6547	0.6056
Sugeco	low	conventional	5	0.5421	0.4887

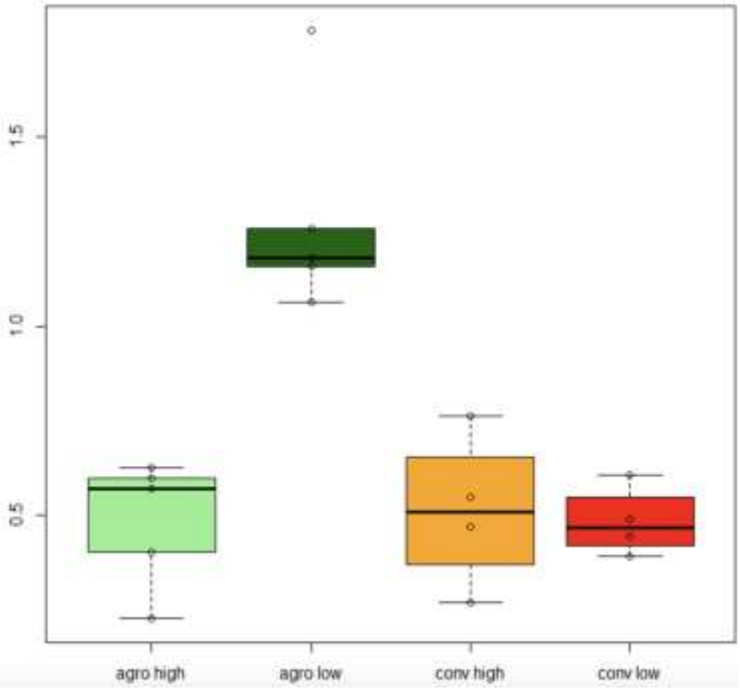
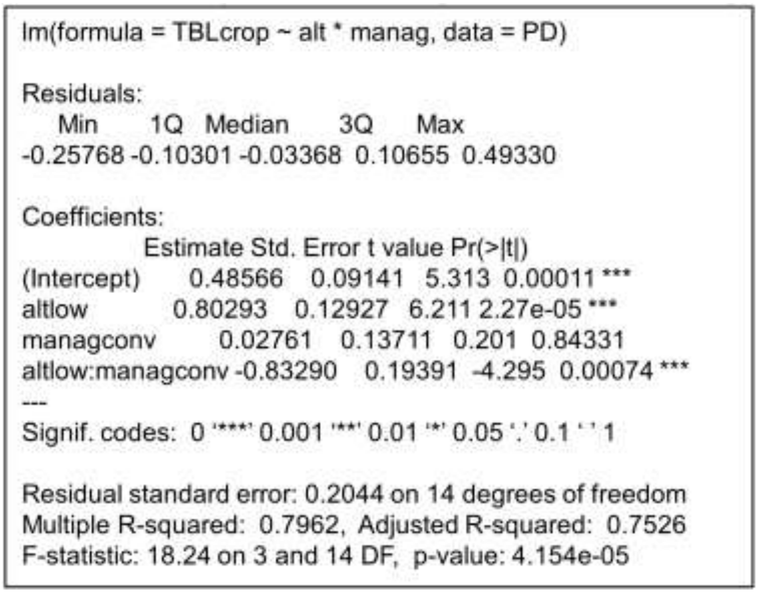


Figure COI_BoxPlot: Phylogenetic diversity (Faith's PD on the vertical axis) of wild bees of October 2022 calculated using the COI uncalibrated tree and excluding the bees from the borders of the crops. Each plot represents the phylogenetic diversity of one field, which was grouped according to the treatment and the elevation: agroecological fields at high altitude (agro high) and at low altitude (agro low), and conventional fields at high altitude (conv high) and at low altitude (conv low). Median (horizontal segment), interquartile (colored rectangle) and most extreme values within the range of 1.5 times the interquartile (whiskers) are indicated.

Table COI_LM: Results of the linear regression testing if phylogenetic diversity of wild bees in October 2022 (based on the uncalibrated COI tree and without the bees from the borders of the crops) were dependent upon field elevation or treatment. ElevationLow: field at low altitude. Treatmentpesticides: field managed with conventional farming.



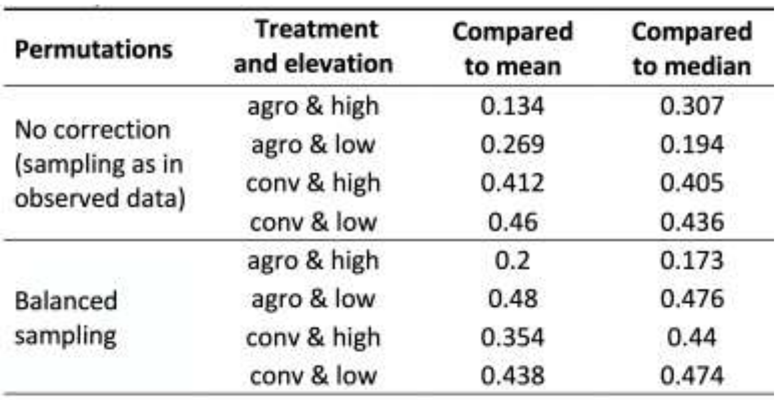
```
lm(formula = TBLcrop ~ alt * manag, data = PD)

Residuals:
    Min       1Q   Median       3Q      Max
-0.25768 -0.10301 -0.03368  0.10655  0.49330

Coefficients:
                  Estimate Std. Error t value Pr(>|t|)
(Intercept)        0.48566    0.09141   5.313  0.00011 ***
altlow             0.80293    0.12927   6.211 2.27e-05 ***
managconv          0.02761    0.13711   0.201  0.84331
altlow:managconv -0.83290    0.19391  -4.295  0.00074 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.2044 on 14 degrees of freedom
Multiple R-squared:  0.7962,  Adjusted R-squared:  0.7526
F-statistic: 18.24 on 3 and 14 DF,  p-value: 4.154e-05
```

Table COI_Perm: P-values obtained from the permutation tests (1,000 replicates) where same number (higher part) or equivalent number (lower part) of the samples of October 2022 were randomly relabelled as one of the four field categories based on treatment and elevation. Phylogenetic diversity values were based on the uncalibrated COI tree and without the bees from the borders of the crops.



Permutations	Treatment and elevation	Compared to mean	Compared to median
No correction (sampling as in observed data)	agro & high	0.134	0.307
	agro & low	0.269	0.194
	conv & high	0.412	0.405
	conv & low	0.46	0.436
Balanced sampling	agro & high	0.2	0.173
	agro & low	0.48	0.476
	conv & high	0.354	0.44
	conv & low	0.438	0.474

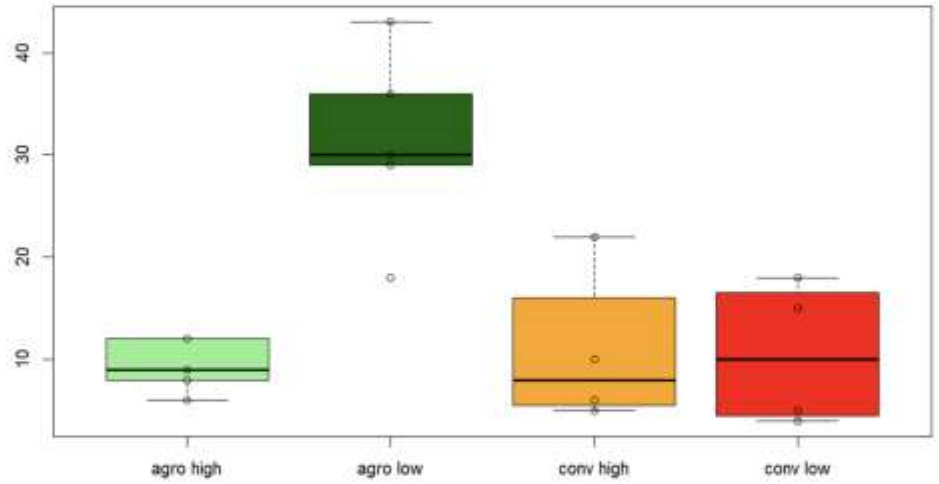


Figure COICountsBoxPlot: Number of wild bees (vertical axis) collected in October 2022 in each field (excluding those collected on the borders of the crops) and grouped according to the treatment and the elevation: agroecological fields at high altitude (agro high) and at low altitude (agro low), and conventional fields at high altitude (conv high) and at low altitude (conv low). Median (horizontal segment), interquartile (colored rectangle) and most extreme values within the range of 1.5 times the interquartile (whiskers) are indicated.

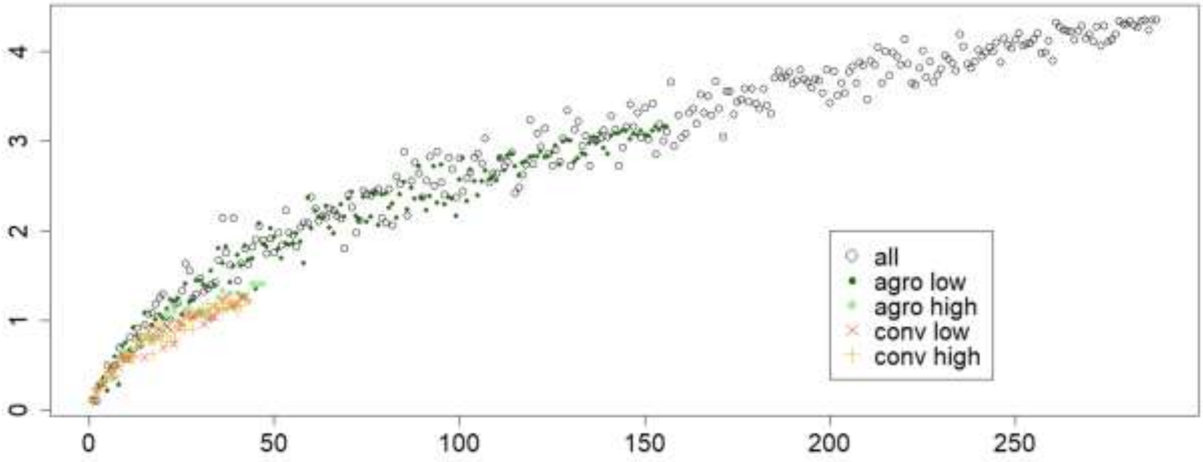


Figure COICumul: Random subsampling of phylogenetic diversity (Faith's PD on the vertical axis) of wild bees of October 2022 (excluding those collected at the borders of the crops) calculated using the COI uncalibrated tree. Each plot represents the phylogenetic diversity calculated for an increasing size (horizontal axis) of random samples selected from the whole set of bees (all) or from each field category: agroecological fields at high altitude (agro high) and at low altitude (agro low), and conventional fields at high altitude (conv high) and at low altitude (conv low).

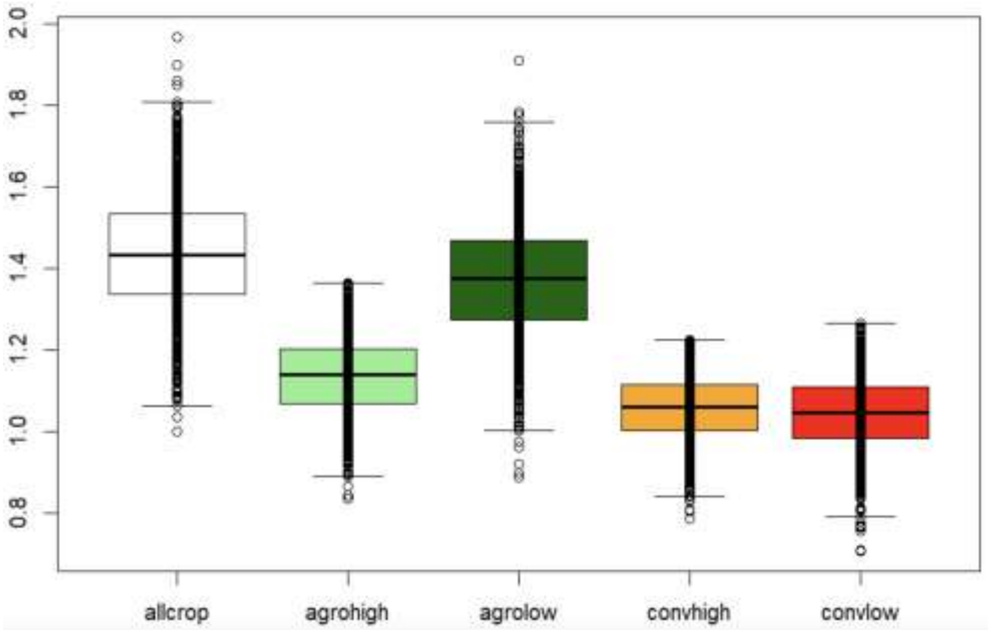
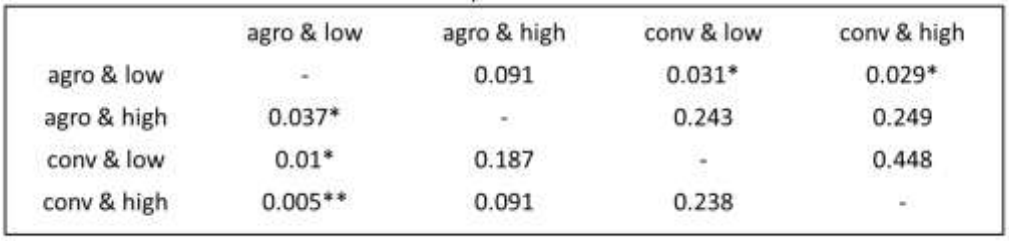


Figure COIBoxPlotBal: Pseudo-replicates of phylogenetic diversity of wild bees of October 2022 (vertical axis) calculated using the uncalibrated COI tree and excluding bees collected on the borders of the crop. Each plot represents the phylogenetic diversity calculated for 40 bees randomly sampled (1,000 replicates) from all samples grouped together (allcrop) or each field category: agroecological fields at high altitude (agrohigh), at low altitude (agrolow), and conventional fields at high altitude (convhigh) and at low altitude (convlow). Median (horizontal segment), interquartile (colored rectangle) and most extreme values within the range of 1.5 times the interquartile (whiskers) are indicated.

Table COI_BootP: P-values of the permutation tests (1,000 pseudo-replicates) involving 30 (upper triangle) or 40 (lower triangle) bees of October 2022 randomly sampled from each field category. agro: agroecological treatment; conv: conventional treatment; low: low altitude; high: high altitude. Phylogenetic diversity values were based on the uncalibrated COI tree and without the bees from the borders of the crops.



	agro & low	agro & high	conv & low	conv & high
agro & low	-	0.091	0.031*	0.029*
agro & high	0.037*	-	0.243	0.249
conv & low	0.01*	0.187	-	0.448
conv & high	0.005**	0.091	0.238	-

References

Almeida EAB, Bossert S, Danforth BN, Porto DS, Freitas FV, Davis CC, Murray EA, Blaimer BB, Spasojevic T, Ströher PR, Orr MC, Packer L, Brady SG, Kuhlmann M, Branstetter MG, Pie MR

(2023). The evolutionary history of bees in time and space. Current Biology 33(16): 3409-3422.e6. https://doi.org/10.1016/j.cub.2023.07.005

Bell JR (2008). A Simple Way to Treat PCR Products Prior to Sequencing Using ExoSAP-IT®. BioTechniques, 44(6): 834. https://doi.org/10.2144/000112890

Bleidorn C, Henze K (2021). A new primer pair for barcoding of bees (Hymenoptera : Anthophila) without amplifying the orthologous coxA gene of *Wolbachia* bacteria. BMC Research Notes, 14(1): 427. https://doi.org/10.1186/s13104-021-05845-9.

Bossert S, Murray EA, Almeida EAB, Brady SG, Blaimer BB, Danforth BN (2019). Combining Transcriptomes and Ultraconserved Elements to Illuminate the Phylogeny of Apidae. Molecular Phylogenetics and Evolution 130: 121-131. https://doi.org/10.1016/j.ympev.2018.10.012.

Bossert S, Murray EA, Pauly A, Chernyshov K, Brady SG, Danforth BN (2021). Gene Tree Estimation Error with Ultraconserved Elements: An Empirical Study on *Pseudapis* Bees. Systematic Biology 70 (4): 803–821. https://doi.org/10.1093/sysbio/syaa097.

Bossert S, Hung K-L, Neff J (2024a). Evolutionary History and Ecology of *Andrena* (*Foveoandrena*) *androfovea*: A New Nearctic Mining Bee (Hymenoptera, Andrenidae) Species and Subgenus. Ecology and Evolution 14: e70453. https://doi.org/10.1002/ece3.70453

Bossert S, Pauly A, Danforth BN, Orr MC, Murray EA (2024b). Lessons from Assembling UCEs: A Comparison of Common Methods and the Case of *Clavinomia* (Halictidae). Molecular Ecology Resources n/a: e13925. https://doi.org/10.1111/1755-0998.13925

Branstetter MG, Longino JT, Ward PS, Faircloth BC (2017). Enriching the Ant Tree of Life: Enhanced UCE Bait Set for Genome-Scale Phylogenetics of Ants and Other Hymenoptera. Methods in Ecology and Evolution 8 (6): 768–776. https://doi.org/10.1111/2041-210X.12742

Branstetter MG, Müller A, Griswold TL, Orr MC, Zhu C-D (2021). Ultraconserved Element Phylogenomics and Biogeography of the Agriculturally Important Mason Bee Subgenus *Osmia* (*Osmia*). Systematic Entomology 46(2): 453-472. https://doi.org/10.1111/syen.12470

Castresana J (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17(4): 540-552.

Faith DP (1992). Conservation evaluation and phylogenetic diversity. Biological Conservation 61: 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Faircloth BC (2016). "PHYLUCE is a software package for the analysis of conserved genomic loci." Bioinformatics 32(5): 786-788.

Grab H, Branstetter MG, Amon N, Urban-Mead KR, Park MG, Gibbs J, Blitzer EJ, Poveda K, Loeb G, Danforth BN (2019). Agriculturally Dominated Landscapes Reduce Bee Phylogenetic Diversity and Pollination Services. Science 363(6424): 282-284. https://doi.org/10.1126/science.aat6016

Gueuning M, Frey JE, Praz C (2020). Ultraconserved yet Informative for Species Delimitation: Ultraconserved Elements Resolve Long-Standing Systematic Enigma in Central European Bees. Molecular Ecology 29(21): 4203–4220. https://doi.org/10.1111/mec.15629.

Gueuning, M, Ganser D, Blaser S, Albrecht M, Knop E, Praz C, Frey JE (2019). Evaluating Next-Generation Sequencing (NGS) Methods for Routine Monitoring of Wild Bees: Metabarcoding, Mitogenomics or NGS Barcoding. Molecular Ecology Resources 19(4): 847–62. https://doi.org/10.1111/1755-0998.13013.

Katoh K, Standley DM (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772-780.

Lean C, Maclaurin J (2016). The Value of Phylogenetic Diversity. In: Pellens R, Grandcolas P (eds) Biodiversity Conservation and Phylogenetic Systematics. Topics in Biodiversity and Conservation, vol 14. Springer, Cham. https://doi.org/10.1007/978-3-319-22461-9_2

Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W (2023). *caper: Comparative Analyses of Phylogenetics and Evolution in R*. R package version 1.0.3. https://github.com/davidorme/caper

Park DS, Worthington S, Xi Z (2018). Taxon sampling effects on the quantification and comparison of community phylogenetic diversity. *Mol Ecol*. 27: 1296–1308. https://doi.org/10.1111/mec.14520

Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO (2010). Picante: R tools for integrating phylogenies and ecology. Bioinformatics 26(11): 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Talavera G, Castresana J (2007). Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Systematic Biology 56(4): 564-577.

Tamura K, Stecher G, Kumar S (2021). MEGA11: Molecular Evolutionary Genetics Analysis version 11. Molecular Biology and Evolution. 38(7): 3022–3027.

Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680.

Tamura K, Tao Q, Kumar S (2018). Theoretical Foundation of the RelTime Method for Estimating Divergence Times from Variable Evolutionary Rates. Molecular Biology and Evolution 35(7): 1770–1782. https://doi.org/10.1093/molbev/msy044

Microbial diversity in wild bee *Dactylurina schmidti*

Metabarcoding was carried out on 96 specimens of *Dactylurina schmidti*, a slender stingless bee, to evaluate the impact of treatments (agroecological versus conventional) and altitudes on this wild bee’s microbiome. This wild bee species was selected because it was the most abundant wild bee observed in April 2022. First, the alpha-diversity was tested, and no significant impact of the treatment or the altitude was found (**Fig. alpha-diversity**).

Regarding the beta-diversity, the influence of the treatment and the altitude were assessed using a PERMANOVA. Micro-organisms communities were significantly different between bees collected in conventional and agroecological plots and between bees collected in high and low altitude (**Table Permanova**).

To visualize the microbiome community structure (dis)similarities, a PcoA (Principal Coordinates Analysis) was generated (**Fig. PCA**). Based on that plot, a group distance analysis (Wilcoxon rank sum test) was performed to compare the microbiome variability within groups (**Fig. groupDistance**). The specimens collected in lowland exhibited overall a higher variability than those collected in highland and the agroecological group was more diverse than the conventional one.

To summarise the microbiomics results, no significant difference was observed in the apha-diversity (i.e. species richness) between bees collected in different altitudes or in plots with different farming management. The beta-diversity (i.e. the microbiome community structure) was significantly influenced by treatment and altitude. When comparing within groups variations, the agroecological group showed higher variation than the conventional group and lowland showed more variation than highland.

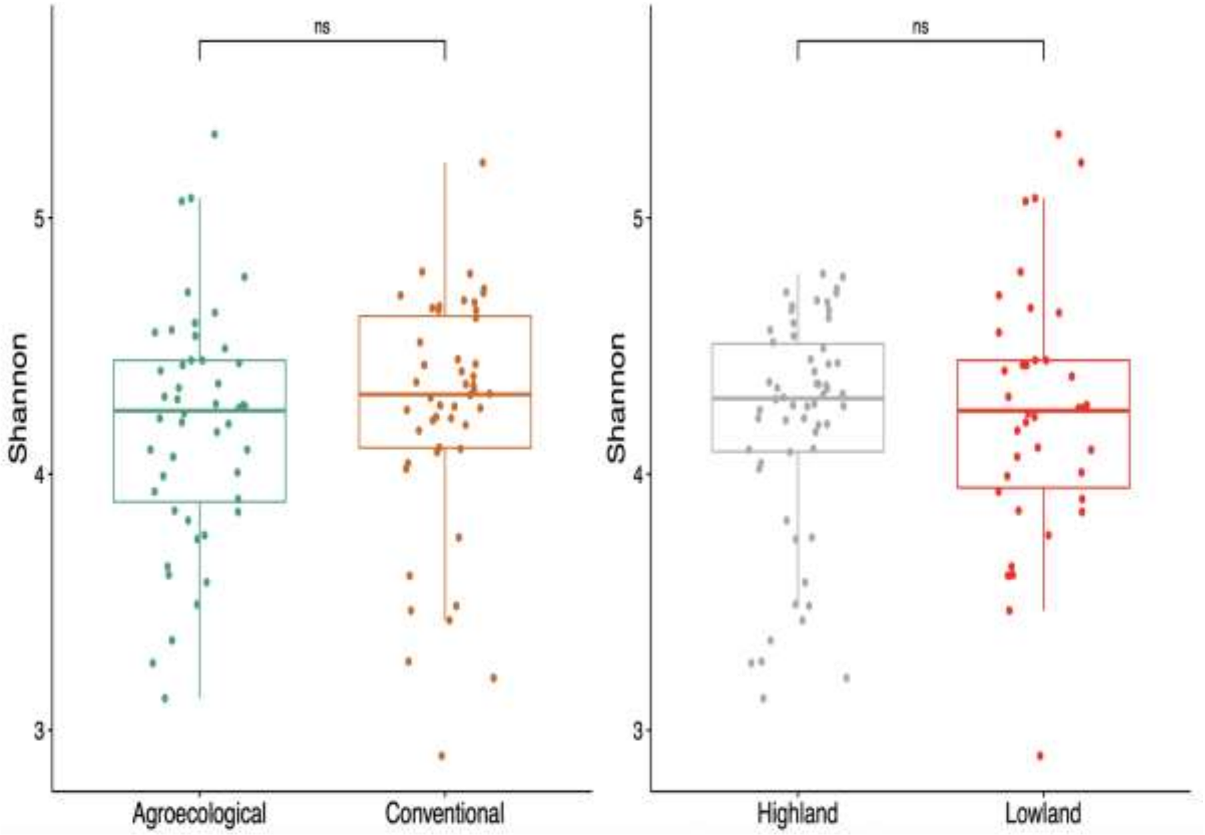


Figure alpha-diversity: Microbiome alpha-diversity comparison using Shannon index of 96 *Dactylurina schmidti* specimens in different altitudes and treatments (i.e. agricultural management).

Table Permanova: Permanova for the effect of treatment, altitude (and their interaction) on the microbiome community structure of 96 specimens of *Dactylurina schmidti*.

	Df	MS	F	P
Treatment	1	1.148	3.157	0.001***
Altitude	1	1.026	1.446	0.001***
Treatment:Altitude	1	0.526	0.015	0.005**
Residual	92	33.446		

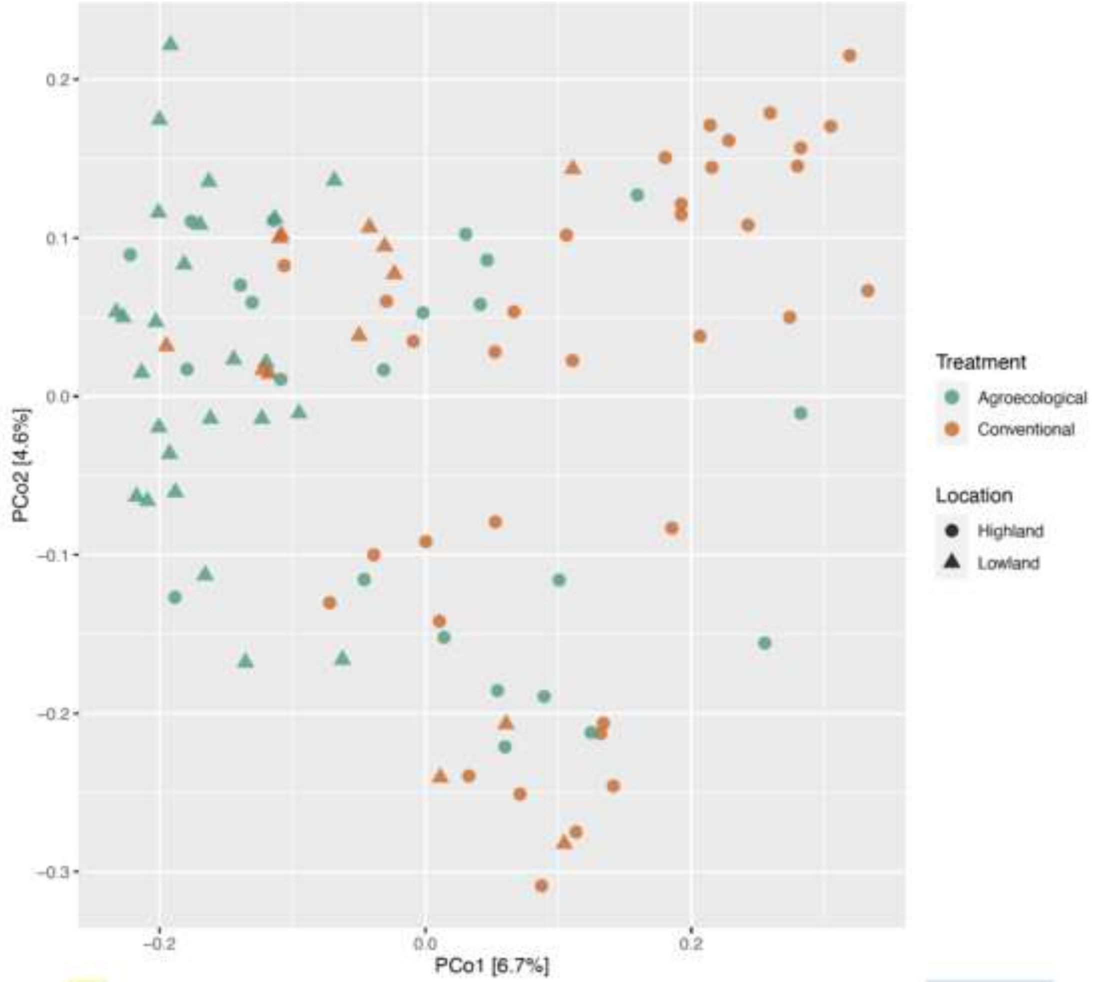


Figure PCA: Principal Coordinates Analysis for the microbiome community structure of 96 *Dactylurina schmidti* specimens collected in sites with different treatment (Agroecological and Conventional) and altitudes (Highland and Lowland).

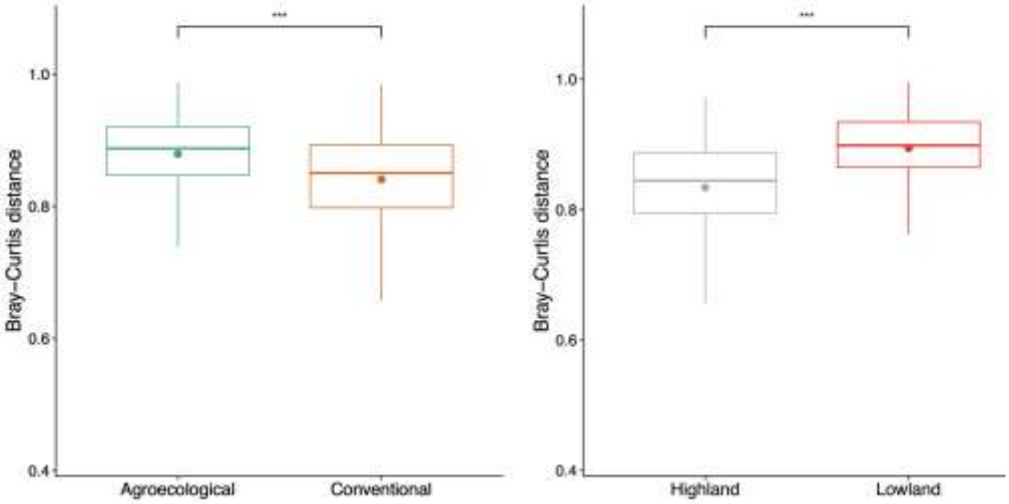


Figure groupDistance: Group distance comparison using Wilcoxon rank sum test between altitude (Highland-Lowland) and treatment (Agroecological-Conventional); significant difference p <0.005 (***).

Microbiomes of the honeybee *Apis mellifera* (Apidae) and the hoverfly *Paragus borbonicus* (Syrphidae).

These results were published as Chapter 3 of the PhD thesis "Microbiomes, Management and Phylogeny: A genomic investigation of African insects of agricultural importance" by Nele Mullens (University of Antwerp, 2025) and have been submitted for publication to an international scientific journal with IF. For a detailed overview of methods, results, bibliographic references, see the open access document available at https://repository.uantwerpen.be/docstore/d:irua:30409.

Methodology

Honeybee (*Apis mellifera*) and hoverfly (*Paragus borbonicus*) adults were collected from experimental sites at two altitudes (~500 m and ~1000 m) under agroecological and conventional farming. Microbial profiles were characterized using 16S rRNA metabarcoding (V3–V4 regions) and analyzed via DADA2. Alpha diversity was estimated using ACE, Faith's PD, Shannon, and Inverse Simpson indices; beta diversity was assessed via PERMANOVA and PERMDISP on Bray–Curtis dissimilarity matrices. Differential abundance was tested using ALDEx2.

Scientific Results

The microbiomes of *A. mellifera* and *P. borbonicus* showed contrasting responses to farming practices and altitude:

- **General patterns:**

  P. borbonicus exhibited a highly diverse microbiome (18,457 ASVs, dominated by Cyanobacteria and Cyanobiaceae), while *A. mellifera* had a less diverse but more structured microbiome (1,060 ASVs, dominated by Proteobacteria and Acetobacteraceae). Honeybees displayed lower alpha diversity and dispersion compared to hoverflies, likely reflecting their eusocial lifestyle and stable microbial transmission among nestmates.

- **Altitude effects:**

  Both species showed compositional shifts with altitude. For *P. borbonicus*, this effect was significant only in females, which also exhibited higher phylogenetic diversity at high altitude. In *A. mellifera*, altitude influenced beta diversity but not alpha diversity metrics, suggesting changes driven by rare taxa rather than overall richness.

- **Farming practices:**

  Management practices affected *A. mellifera* but not *P. borbonicus*. In honeybees, differences were detected at the ASV level, indicating subtle shifts within dominant genera rather than wholesale changes in genus composition. Agroecological farming, by avoiding synthetic pesticides and promoting habitat diversity, may help maintain microbiome stability in honeybees, reducing stress-related disruptions. Conventional farming, while not inducing Anna Karenina Effects (AKEs), still caused detectable compositional shifts, consistent with previous studies linking pesticide exposure to microbiome changes and reduced functional capacity.

  For hoverflies, no significant differences were observed between agroecological and conventional farming, suggesting resilience to localized stressors—possibly due to their solitary lifestyle and broader foraging range, which reduces pesticide exposure compared to honeybees.

- **Anna Karenina Effect:**

  Unlike previous findings in *Zeugodacus cucurbitae*, neither pollinator species exhibited increased microbial dispersion under conventional farming. This suggests that stress levels were insufficient to destabilize microbiomes or that pollinators possess physiological or behavioral traits buffering against such effects.

Interpretation:

Agroecological practices appear beneficial for honeybee microbiome stability, even if effects are subtle. For hoverflies, resilience to farming practices may reflect ecological traits rather than absence of impact. These species-specific responses highlight the complexity of microbiome–environment interactions and the need for tailored strategies to safeguard pollinator health.

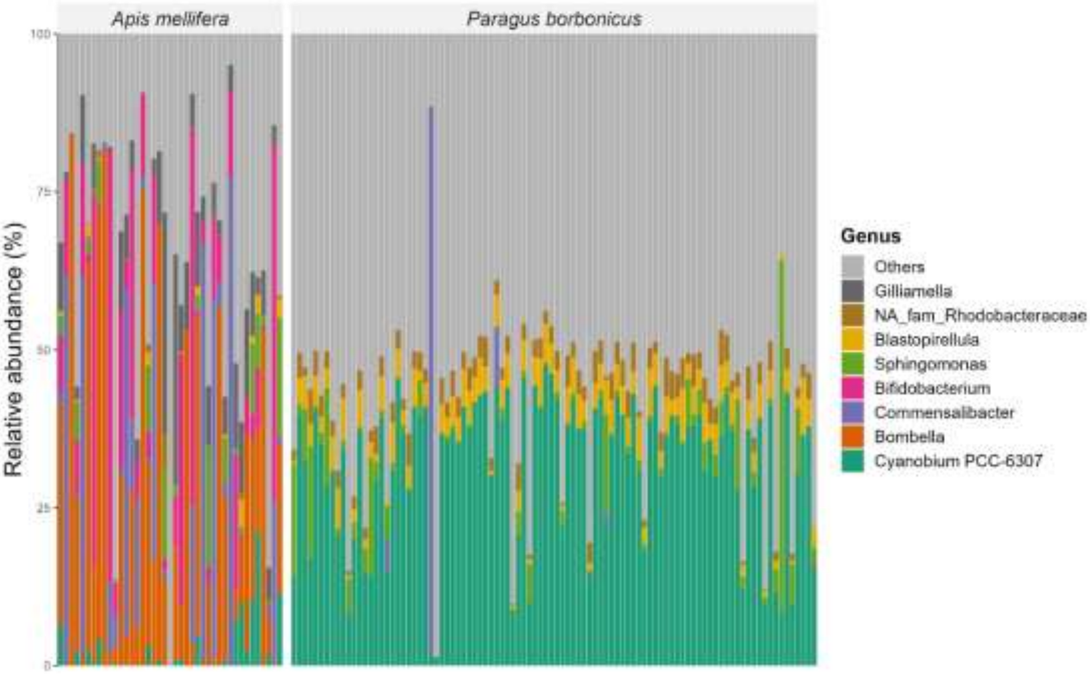


Figure 3.2: Comparison of microbial composition of *A. mellifera* and *P. borbonicus* at genus level. *Caption: Distinct profiles dominated by Proteobacteria in honeybees and Cyanobacteria in hoverflies.*

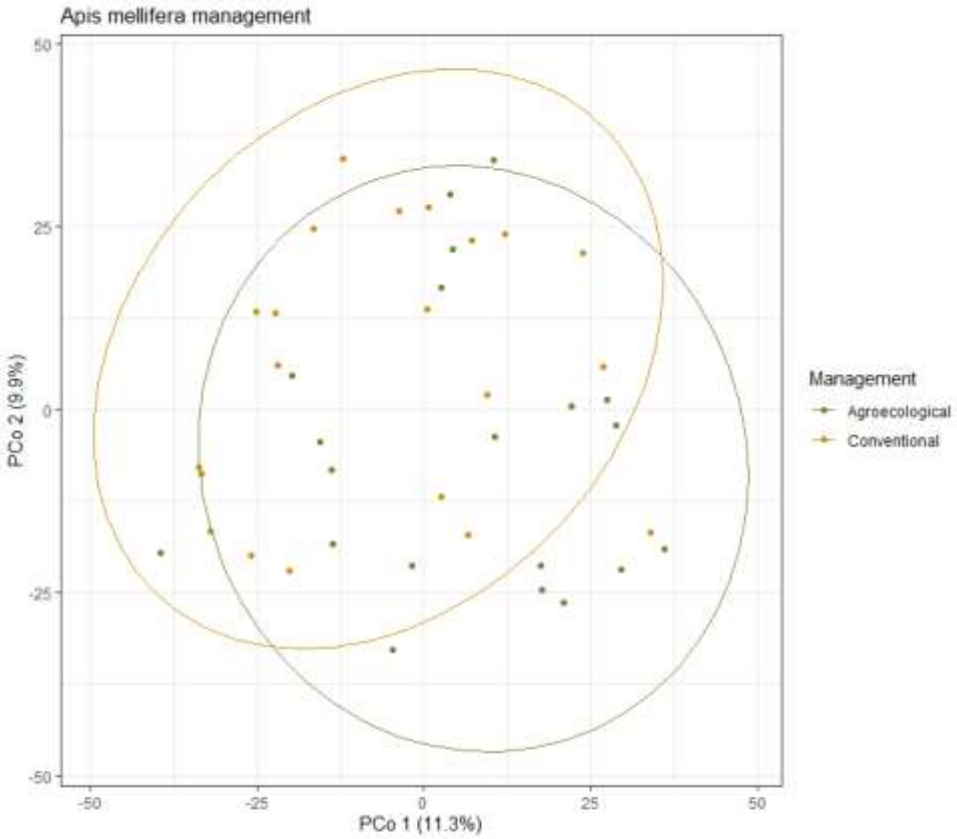


Figure 3.3: Principal Coordinates Analysis (PCoA) of *A. mellifera* microbiomes under conventional vs agroecological management.

References

- Mullens N et al. (2024). Anna Karenina as a promoter of microbial diversity in *Zeugodacus cucurbitae*. *PLoS ONE*, 19(4):e0300875.
- Hendrycks W et al. (2022). Dietary shifts affect the gut microbiome of the melon fly. *Microbiol Open*, 11:e1307.
- Kwong WK & Moran NA. (2016). Gut microbial communities of social bees. *Nat Rev Microbiol*, 14:374–384.
- Kakumanu ML et al. (2016). Pesticide exposure alters honeybee gut microbiome. *Sci Rep*, 6:22572.
- Zaneveld JR et al. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol*, 2.

Microbiomes of key pollinator flower flies

These results were published as Chapter 4 of the PhD thesis "Microbiomes, Management and Phylogeny: A genomic investigation of African insects of agricultural importance" by Nele Mullens (University of Antwerp, 2025) and have been submitted for publication to an international scientific journal with IF. For a detailed overview of methods, results, bibliographic references, see the open access document available at https://repository.uantwerpen.be/docstore/d:irua:30409.

Methodology

Hoverflies (*Paragus borbonicus*, *Toxomerus floralis*, *Ischiodon aegyptius*) and honeybees (*Apis mellifera*) were collected from experimental sites at two altitudes (~500 m and ~1000 m). Microbial profiles were characterized using 16S rRNA metabarcoding (V3–V4 regions) and analyzed via DADA2. Alpha diversity was estimated using Shannon, Inverse Simpson, ACE, and Faith's PD; beta diversity was assessed via PERMANOVA and PERMDISP on Bray–Curtis dissimilarity matrices. Differential abundance was tested using ALDEx2 (Mullens et al., 2024).

Scientific Results

This study provides the first comparative characterization of hoverfly microbiomes and their differences from honeybees:

- **General patterns:**

  Hoverflies exhibited higher alpha diversity than honeybees, except in female *I. aegyptius*, which showed low evenness and dominance by Proteobacteria (similar to honeybees). Honeybee microbiomes were dominated by a few core genera (*Bombella*, *Commensalibacter*, *Bifidobacterium*), while hoverflies harbored more diverse communities, including genera rarely reported in insects (*Blastopirellula*, *Corynebacterium*, *Cyanobium PCC-6307*, *Micrococcus*).
- **Altitude effects:**

  Significant compositional shifts (beta diversity) were detected in female *P. borbonicus* and in *A. mellifera* across altitudes, though no specific genera were linked to altitude. These differences likely reflect environmental factors such as floral diversity and temperature rather than altitude per se.
- **Sex-specific patterns:**

  All three hoverfly species exhibited sex-related microbiome differences, but patterns varied:
  - *I. aegyptius* showed the strongest sexual differentiation, with females having more heterogeneous microbiomic profiles and higher dispersion. *Asaia* was significantly more abundant in females, while *Lawsonella* dominated in males.
  - *T. floralis* males displayed greater dispersion than females, though overall composition did not differ by sex.
  - *P. borbonicus* showed minimal sex-related differences, except for altitude-driven variation in females.
- **Interspecific contrasts:**

  Honeybees had the least diverse microbiome and lowest dispersion, consistent with their eusocial lifestyle and horizontal microbial transmission among nestmates (Engel & Moran, 2013; Kwong & Moran, 2016). Flower flies, being solitary, exhibited broader diversity and variability. This pattern may confer greater ecological resilience to flower flies, as elevated microbiome diversity can function as a buffering mechanism, enhancing stability and adaptive capacity under environmental stressors (Mullens et al., 2024).

- **Bacterial genera associations:**

  Six genera were specific to honeybees (including core symbionts *Gilliamella*, *Snodgrassella*, *Bombella*), linked to functions such as pectin degradation, nutritional support, and pesticide tolerance (Engel & Moran, 2013; Kakumanu et al., 2016). Four genera were common across flower flies but rare in honeybees (*Blastopirellula*, *Corynebacterium*, *Cyanobium PCC-6307*, *Micrococcus*), with potential roles in nutrient provision.

Interpretation:

Flower flies and honeybees differ markedly in microbiome diversity and composition, reflecting ecological differences and contrasting life-history strategies. Honeybee microbiomes are specialized and stable, while flower flies harbor diverse, environmentally influenced communities. These findings highlight the importance of considering species-specific microbiome dynamics in pollinator conservation and agroecological strategies (Engel & Moran, 2013; Kwong & Moran, 2016; Mullens et al., 2024).

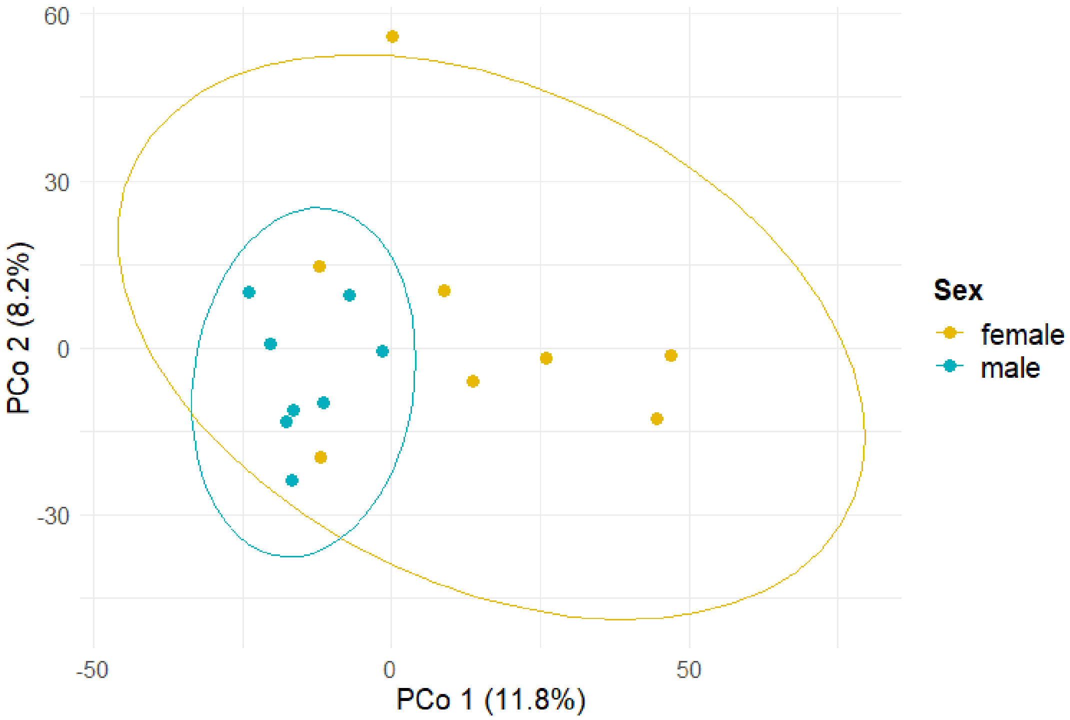


Figure 4.3: Principal Coordinates Analysis (PCoA) of *I. aegyptius* microbiomes by sex. *Separation between female and male microbiomes, with females showing higher dispersion.*

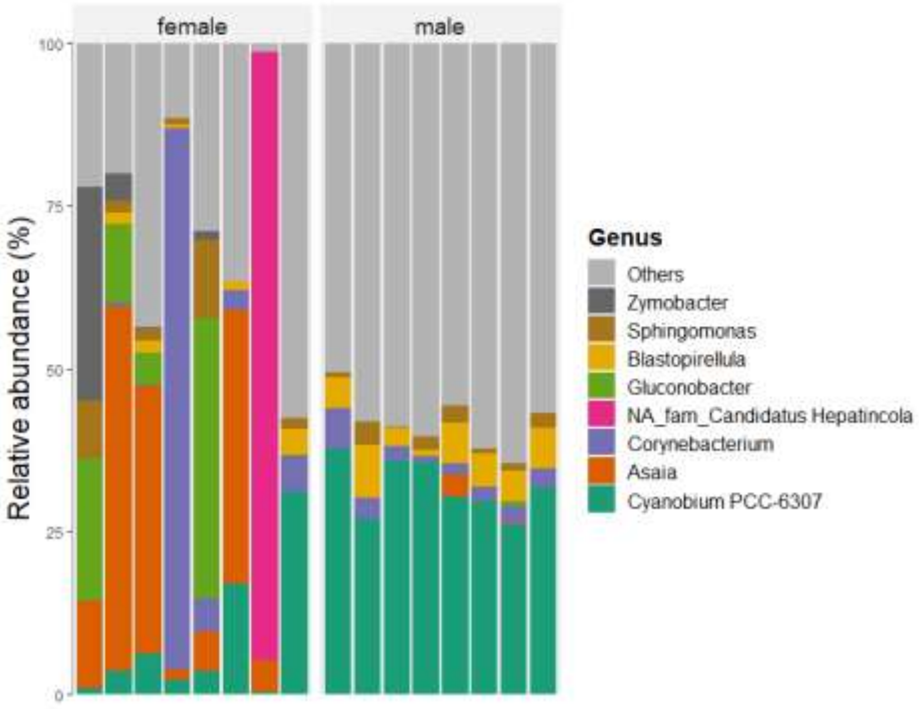


Figure 4.6: Comparison of bacterial genera between female and male *I. aegyptius*. *Sex-specific associations: Asaia enriched in females, Lawsonella in males.*

References

- Engel P & Moran NA. (2013). Functional roles of gut microbiota in bees. *Nat Rev Microbiol*, 11:735–745.
- Kwong WK & Moran NA. (2016). Gut microbial communities of social bees. *Nat Rev Microbiol*, 14:374–384.
- Mullens N et al. (2024). Anna Karenina as a promoter of microbial diversity in *Zeugodacus cucurbitae*. *PLoS ONE*, 19(4):e0300875.
- Kakumanu ML et al. (2016). Pesticide exposure alters honeybee gut microbiome. *Sci Rep*, 6:22572.

Microbiome assemblies in cucurbit feeding fruit flies: deterministic vs. stochastic processes

These results were published in Hendrycks et al. (2025), PLoS ONE, 20(1): e0313447. For a detailed overview of methods, results, and references, see https://doi.org/10.1371/journal.pone.0313447.

Context and Relevance

While ISeBAF primarily investigates biodiversity and ecosystem services in agroecological farming, understanding the **microbial ecology of key pests** is essential for predicting their adaptability and resilience under changing management systems. *Zeugodacus cucurbitae*, a dominant cucurbit pest in East Africa, harbors complex gut microbiomes that may influence host plant specialization and pest fitness. This study provides a **mechanistic insight into microbial assembly processes**, generating data that can inform applied strategies for pest management and agroecological transitions.

Methodological Approach

Third-instar larvae of *Z. cucurbitae* and two other cucurbit feeders (*Dacus bivittatus*, *D. ciliatus*) were collected from cucurbit crops (*Cucumis sativus*, *Citrullus lanatus*, *Cucurbita pepo*) at 20 sites in Morogoro, Tanzania. Microbiomes were profiled using 16S rRNA metabarcoding (V3–V4 regions) and analyzed through:

- **Null model analysis** to detect phylogenetic clustering vs. repulsion.
- **Sloan neutral model** to quantify the role of stochastic processes and identify taxa under positive or negative selection.

Key Findings

- **Deterministic filtering dominates:** SES.MNTD values were significantly lower than zero, indicating strong phylogenetic clustering and host-driven selection (Fig. 1).
- **Taxonomic vs. phylogenetic turnover:** High taxonomic turnover without phylogenetic turnover suggests substitutions among closely related taxa.
- **Neutral model fit:** Poor overall fit (RMSE = 0.116 for *Z. cucurbitae*) confirmed deterministic effects, though 69–79% of ASVs conformed to neutral expectations, highlighting stochastic contributions.
- **Selection signatures:** 18–28% of ASVs were overrepresented, including Enterobacterales and Pseudomonadales, taxa potentially linked to cucurbitacin detoxification.
- **Dispersal limitation:** Extremely low migration rates (m = 0.0002–0.0007) indicate strong spatial constraints on microbial acquisition.

These findings support the **"microbiome on a leash"** model (Foster et al., 2017), where host filtering interacts with local environmental sampling and stochastic processes. Understanding these dynamics is crucial for predicting pest responses to agroecological practices and for designing microbiome-informed IPM strategies.

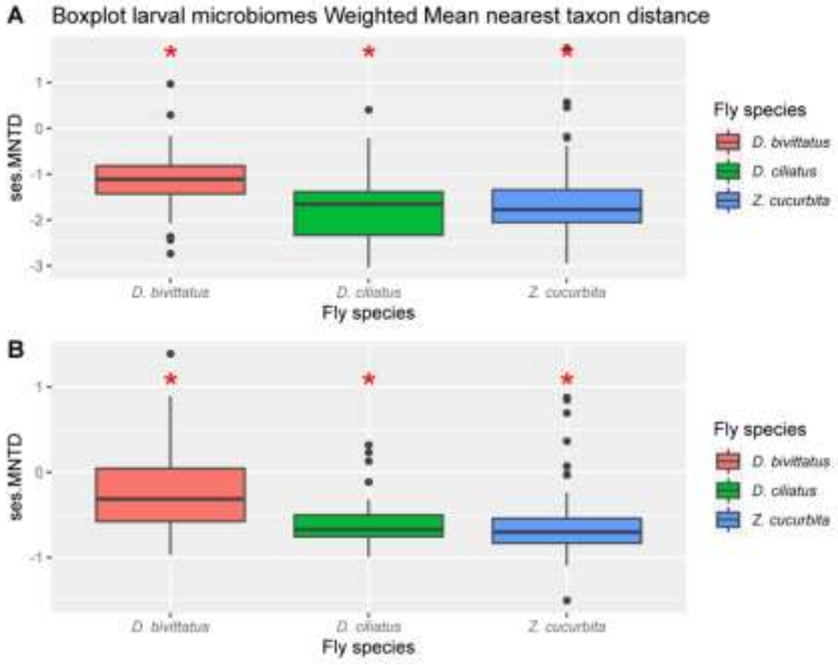


Fig. 1: Standardized effect sizes (SES.MNTD) showing phylogenetic clustering across fly species. Upper panel are estimated using the taxa.labels null model while lower panel are estimated with the independent swap model. * indicate which treatments differed significantly from 0.

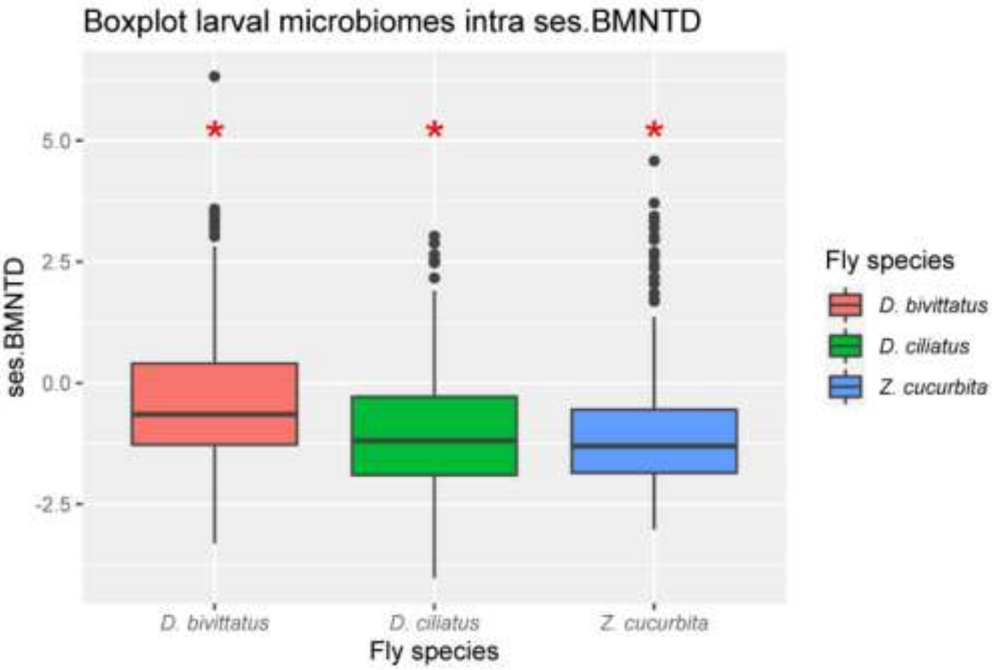


Fig. 2: Neutral model partitioning of ASVs: proportion fitting neutral expectations vs. over-/underrepresented taxa. Calculated for third instar larvae ofdifferent fly species *(D. ciliatus, D. bivittatus, Z. cucurbitae*). * indicate which treatments differed significantly from 0.

References

1. Hendrycks W, Mullens N, Bakengesa J, et al. (2025). *Deterministic and stochastic effects drive the gut microbial diversity in cucurbit-feeding fruit flies (Diptera, Tephritidae)*. PLoS ONE, 20(1): e0313447.
2. Sloan WT et al. (2007). Modeling taxa-abundance distributions in microbial communities. *Microbial Ecology*, 53:443–455.
3. Burns AR et al. (2015). Contribution of neutral processes to gut microbial assembly in zebrafish. *ISME J*, 10:655–664.
4. Zhou J & Ning D. (2017). Stochastic community assembly: Does it matter in microbial ecology? *Microbiol Mol Biol Rev*, 81:1–32.
5. Foster KR et al. (2017). The evolution of the host microbiome as an ecosystem on a leash. *Nature*, 548:43–51.

Microbial diversity in the cosmopolitan agricultural pest *Zeugodacus cucurbitae* (Diptera, Tephritidae)

These results were published in Mullens et al. (2024). PLoS ONE 19(4): e0300875. For a detailed overview of methods, results, bibliographic references, see *https://doi.org/10.1371/journal.pone.0300875*.

Methodology

This study was conducted within the same experimental setup described for previous sections under the ISeBAF project in Morogoro (Tanzania), comparing agroecological and conventional farming at two altitudes (low and high). This research targeted **gut microbial communities of *Z. cucurbitae* larvae**.

Infested cucumbers and watermelons were collected from eight sites (four per altitude), and third-instar larvae were dissected, rinsed, and preserved in ethanol. Larval identity was confirmed via DNA barcoding (Virgilio et al., 2012). Microbial profiles were characterized using 16S rRNA metabarcoding (V3–V4 regions) and analyzed through a consensus pipeline combining DADA2, microDecon, and ALDEx2. Alpha diversity was estimated using ACE, Faith's Phylogenetic Diversity, Shannon, and Inverse Simpson indices; beta diversity was assessed via compositional data analysis (CLR transformation) and PERMANOVA. Multivariate dispersion was tested using PERMDISP to detect Anna Karenina effects (AKEs).

Scientific Results

Sequencing yielded **2,973 unique ASVs**, assigned to **22 phyla, 221 families, and 590 genera**. The most abundant phyla were Proteobacteria (35.4%), Bacteroidota (32.2%), and Firmicutes (22.4%). Dominant genera included *Romboutsia* (16.2%) and *Leadbetterella* (13.0%).

Key findings:

- **Complex interactions:** Microbial diversity was shaped by interactions among management, altitude, crop, and site rather than by any single factor.
- **Management × altitude effect:** At high altitude, microbial communities from conventional farming showed significantly higher species and phylogenetic richness (ACE, PD) and greater multivariate dispersion compared to agroecological farming, indicating stronger Anna Karenina effects under stressful conditions (Zaneveld et al., 2017). No significant differences were observed at low altitude.
- **Crop effect:** Weak and inconsistent across sites, confirming previous observations of strong random variability in fruit fly microbiomes (De Cock et al., 2020; Hendrycks et al., 2022).
- **Anna Karenina principle:** Higher dispersion in conventional farming at high altitude suggests that environmental stress (pesticide exposure, suboptimal temperatures) promotes stochastic microbial shifts, increasing diversity and potentially adaptive potential (Ma, 2020; Lavrinienko et al., 2020).
- **Differential taxa:** *Romboutsia* was significantly more abundant in conventional farming at high altitude, along with rare genera such as *Lysinibacillus* and *Empedobacter*, which may be linked to stress responses (Liu et al., 2021; Yang et al., 2022).

Scientific context: These findings highlight that microbial diversity in *Z. cucurbitae* is not solely crop-driven but strongly influenced by environmental stressors and management practices. The Anna

Karenina principle provides a framework to explain stochastic microbial shifts under stress, which may enhance the adaptive capacity of polyphagous pests.

Societal and decision-making relevance: Understanding microbiome dynamics under different farming systems can inform sustainable pest management strategies and predict pest adaptability under climate and land-use changes.

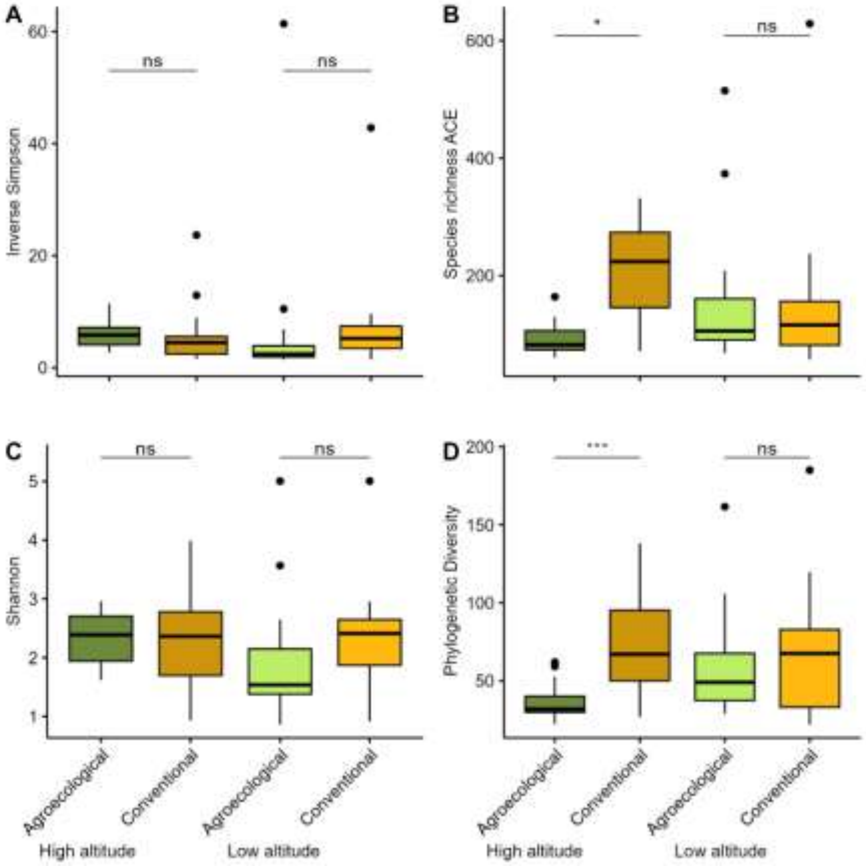


Figure 1.3.1: Differences in microbial alpha diversity between agroecological and conventional farming at low and high altitude. *ACE and Faith's PD indicate higher richness in conventional farming at high altitude; no significant differences at low altitude.*

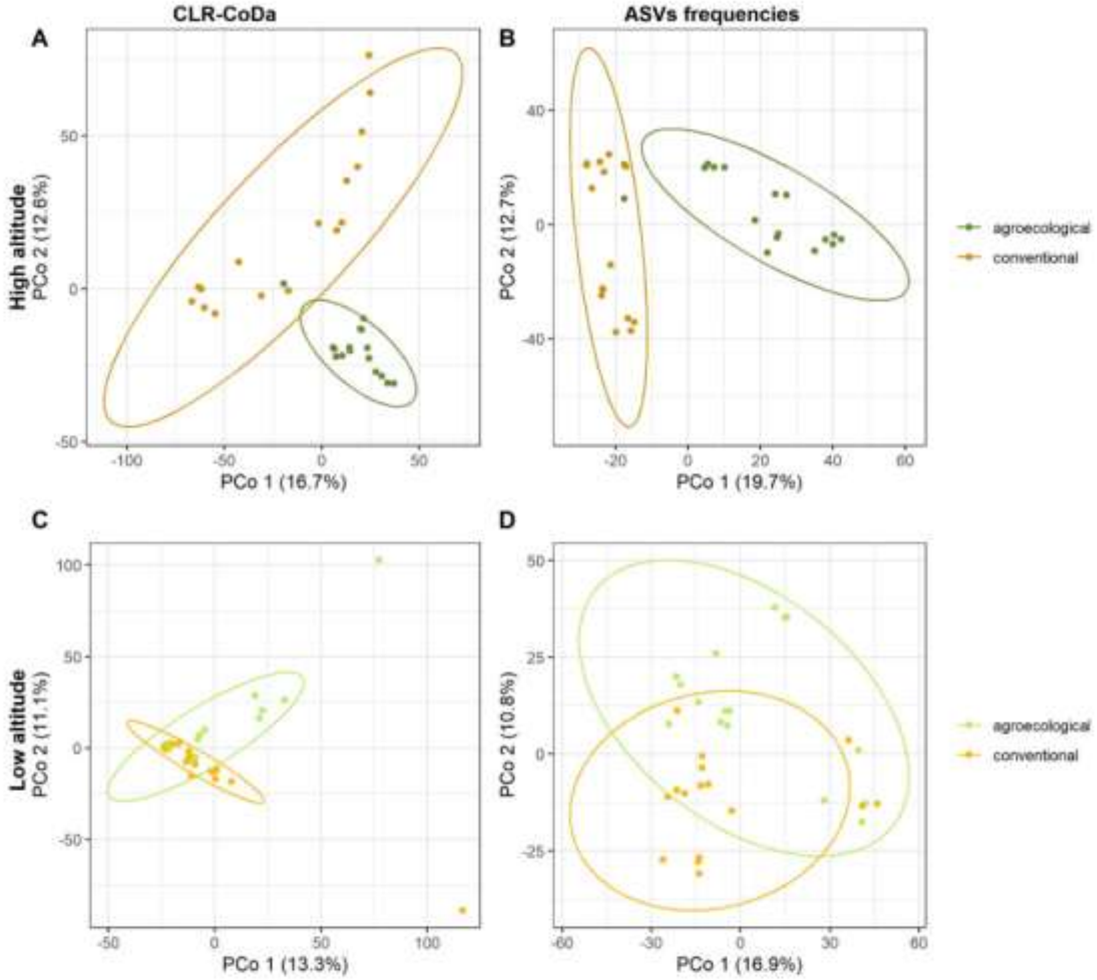


Figure 1.3.2: Principal Coordinates Analysis (PCoA) of microbial communities. *Beta diversity patterns showing greater dispersion in conventional farming at high altitude (Anna Karenina effect).*

References

- Zaneveld JR et al. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol*, 2.
- Ma Z (2020). Testing the Anna Karenina principle in human microbiome-associated diseases. *iScience*, 23.
- Lavrinienko A et al. (2020). Applying the Anna Karenina principle for wild animal gut microbiota. *J Anim Ecol*, 89:2617–2630.
- De Cock M et al. (2020). Comparative microbiomics of tephritid frugivorous pests. *Front Microbiol*, 11:1890.
- Hendrycks W et al. (2022). Dietary shifts affect the gut microbiome of the melon fly. *Microbiol Open*, 11:e1307.
- Liu J et al. (2021). Gut flora-mediated metabolic health under pesticide exposure. *Foods*, 10.
- Yang Y et al. (2022). Effect of Deltamethrin on gut microbiota. *Front Immunol*, 13.

Microbiome and bacterial metabolic functions of *Dacus vertebratus* (Diptera, Tephritidae) in agroecological and conventional farming

These results were published in Tairo et al. (2025). Journal of Applied Entomology. For a detailed overview of methods, results, bibliographic references, see *https://doi.org/10.1111/jen.13450*.

Methodology

Larvae of *Dacus vertebratus* were collected from infested watermelons in plots managed under agroecological or pesticide-based farming. Species identity was confirmed via DNA barcoding (Virgilio et al., 2012). Microbial communities were characterized using 16S rRNA metabarcoding (V3–V4 regions) and analyzed through the DADA2 pipeline. Beta diversity was tested via PERMANOVA on fourth-root transformed data, and predictive functional profiling was inferred using PICRUSt2, mapping ASVs to Enzyme Commission numbers. Functional pathways were visualized using ggpicrust2 and BURRITO.

Scientific Results

The analysis of **43 larvae of *D. vertebratus*** produced **2,552 ASVs**, assigned to **22 phyla, 212 families, and 465 genera**. The microbiome was dominated by *Bacteroidota* (50.1%) and *Proteobacteria* (33.5%). The most abundant families were *Rhizobiaceae* (30.8%) and *Weeksellaceae* (20.6%), while dominant genera included *Sphingobacterium* (15.2%) and *Flaschrobactrum* (14.2%).

Key findings:

- No significant differences were detected between agroecological and pesticide-based farming in microbial composition or predicted metabolic functions (PERMANOVA, $p > 0.05$).
- Strong interspecific differences were observed between *D. vertebratus* and other cucurbit feeders (*D. bivittatus*, *D. ciliatus*, *Zeugodacus cucurbitae*), confirmed by PERMANOVA and PCoA clustering.
- About 14% of predicted functions were metabolic, dominated by amino acid metabolism (3.1%), carbohydrate metabolism (2.9%), and metabolism of cofactors and vitamins (2.3%). Key contributors included peptidases, oxidative phosphorylation, and purine metabolism.
- Larger differences in enzyme commission profiles were detected between *D. vertebratus* and *Z. cucurbitae* (251 ECs) than between *D. vertebratus* and other *Dacus* species, suggesting evolutionary divergence in metabolic pathways.

Scientific context: These results provide the first insight into the microbiome of *D. vertebratus*, highlighting its functional potential and interspecific variability. While farming practices did not show strong effects in this study, previous research suggests that subtle differences may emerge under broader spatial or environmental gradients.

Societal and decision-making relevance: Understanding microbial functions in fruit fly pests can inform integrated pest management strategies and predict adaptive responses to agroecological transitions.

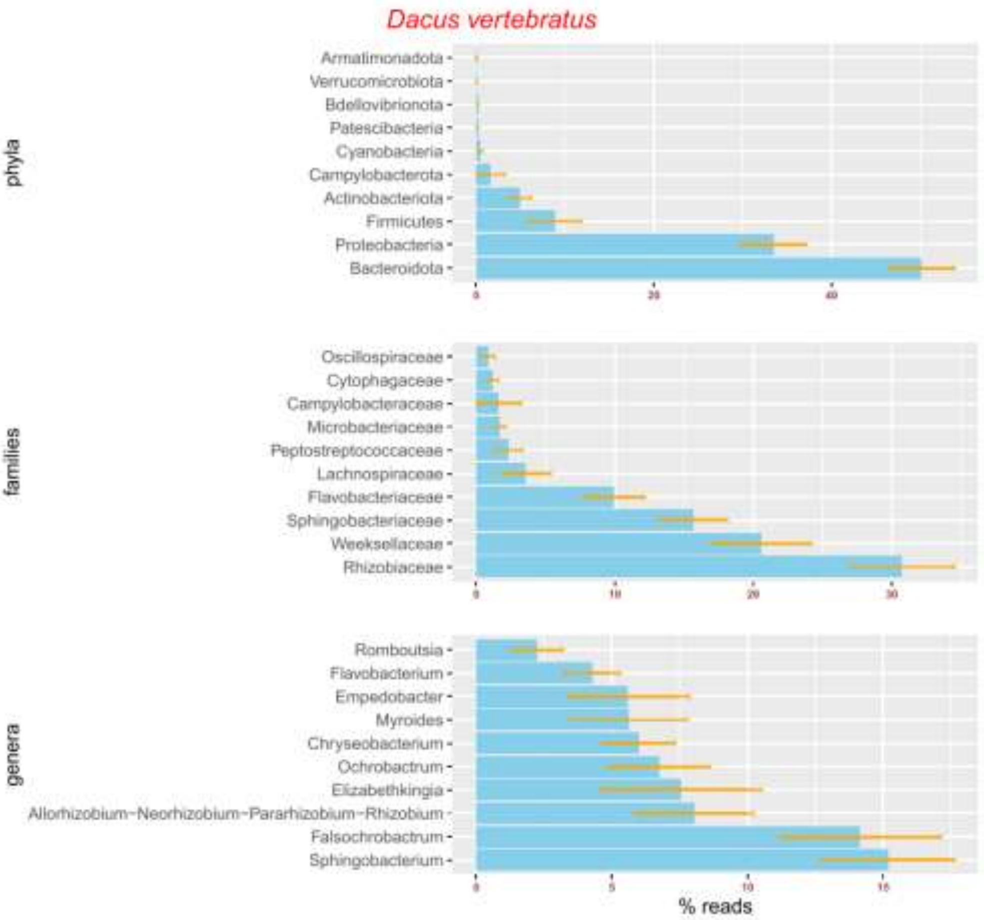


Figure 1.3.3: *Relative abundances of major taxa in the microbiome of D. vertebratus larvae collected from watermelon plots.*

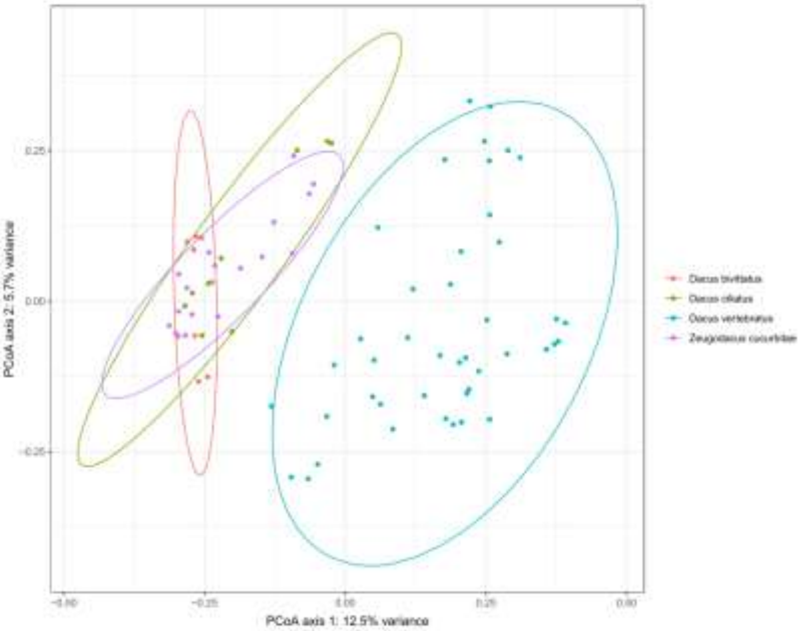


Figure 1.3.4: Principal Coordinates Analysis (PCoA) of microbial communities. *Distinct clustering of D. vertebratus compared to other cucurbit-feeding fruit flies.*

References

- Hendrycks W et al. (2022). Dietary shifts affect the gut microbiome of the melon fly. *Microbiol Open*, 11:e1307.
- Mullens N et al. (2024). Anna Karenina as a promoter of microbial diversity in *Zeugodacus cucurbitae*. *PLoS ONE*, 19(4):e0300875.
- De Cock M et al. (2020). Comparative microbiomics of tephritid frugivorous pests. *Front Microbiol*, 11:1890.
- Deguine J-P et al. (2015). Agroecological management of cucurbit-infesting fruit fly: a review. *Agron Sustain Dev*, 35:937–965.
- Starkie ML et al. (2022). Evolutionary history of host breadth and lure response in Dacini fruit flies. *Mol Phylogenet Evol*, 172:107481.

Task 2.1: field experiments on pollination service

The results pertaining to this task are reported below as part of Section Task 3.2 – *Crop pollination service*.

Task 3.1 comparisons between agroecological and conventional farms in the Morogoro region

Community Structure of Bee Visitors to Cucurbit Crops in Morogoro, Tanzania

Those results are some of the results that will be published

Materials and methods

Study site:

During the cucurbit flowering season of 2023, we surveyed 20 experimental sites across the Morogoro region (Tanzania). Sites were stratified by altitude (10 lowland, 10 highland) and by agricultural management: agroecological (organic fertilization, biopesticides, cucurbit–legume intercropping) or conventional (chemical fertilizers and pesticides). Manual weeding was practiced at all sites.

Sampling Methods:

Sampling took place from mid-May to early June 2023. Five sites were sampled per day by two collectors per site (a 10-person team including SUA students and ULB researchers). Sites of similar altitude and management were sampled on the same day. Lowland plots were surveyed first due to delayed crop phenology in the highlands. Sampling was avoided on rainy days since bees are inactive under such conditions (Westphal et al., 2008). Each session ran from 07:00 to 12:00 h, corresponding to peak pollination activity (Bomfin et al., 2016; Sawe et al., 2020). We combined active and passive sampling methods to capture the widest range of bee taxa : flower visitors by sweep netting and circulating entomofauna by Yellw Pan Traps + Blue Vane traps (Packer et al., 2020). Active Netting:

Bees visiting flowers were captured using sweep nets during repeated 15–20 min surveys per crop throughout the morning. Specimens were placed in tubes with paper to prevent nectar regurgitation. Although effective for flower visitors, netting may underestimate small or fast-flying species (Nielsen et al., 2011; Wilson et al., 2016). Passive Pan Traps: Two sets of three yellow plastic000 bowls ("Belton Molotow 400 ml – 0003 Cadmium Yellow") were placed in each crop (18 bowls per site). Bowls, spaced 1–2 m apart, were filled halfway with soapy water (non-toxic "Tarmol" detergent) and left for approximately 4 h (07:00–12:00 h). Colour selection followed A. Pauly (pers. comm., 2023), as multicolour sets are less efficient in tropical regions. Pan traps reduce observer bias but may under- or overrepresent certain taxa (Gezon et al., 2015; Packer et al., 2020).

Statistical analysis

All analyses were performed using pooled data obtained by both sampling techniques (i.e., pan traps and netting) because these techniques can be complementary since they help assess the circulating entomofauna. For the flower-visitor communities we pooled only specimens collected with sweep netting. We then characterized the entire bee community associated with each crop in total 20 plots conducted in contrasted landscapes (Highlands Vs Lowlands), with the following diversity metrics: Hill-Shannon diversity (Hill-Shannon), partitioning of beta diversity among the three cucurbits and between plots conducted in Highland and Lowland (and phylogenetic diversity (PD)). Hill numbers, also called "the effective number of species" represent an efficient way of computing the species richness of a community, based on abundance matrices. Hill-Shannon was chosen because it gives a stronger leverage to both very common and very rare species, thanks to its geometric mean. PD have been used previously in studies as a proxy to assess community characteristics and are also relevant to measure the efficiency of ecosystem services provided by pollinators. We assessed the phylogenetic diversity based on the hierarchical Linnaean taxonomic classification proposed by Danforth et al. (2006), using the 'ape' package (Paradis and Schliep, 2018). We used the following ranks: superfamily, position, family, subfamily, tribe, genus, subgenus, and species. Each rank represents a separation in the tree. Phylogenetic diversity was calculated as the sum of branch lengths separating each pair of species collected from one of the three cucurbit species crops. Two bee species belonging to the same subgenus will be closest to each other and assigned the smallest branch length. For some species and morphospecies lacking subgenus information, we categorize them as "unknown." The Linnaean approach can serve as a proxy for multi-gene molecular phylogeny (Vereecken et al., 2021). To estimate the multiple-site variation in species composition we computed beta diversity metrics. We used the ``betapart'' package (Baselga & Orme, 2012), with which it is possible to partition the Sørensen index of beta diversity (βsor : measure of total dissimilarity) (i) species replacement (i.e., βsim : species turnover) and (ii) species loss/or gain (i.e., nestedness: βnes). The Sørensen index (βsor) ranges from 0 (identical species assemblages) to 1 (different species assemblages). Using this approach with our dataset allowed testing (i) differences in the values of total dissimilarity (βsor) first between different crops within a plot, on the other hand among plots conducted in two different farming systems, but also (ii) the relative contribution of species turnover (βsim) and nestedness-resultant dissimilarity (βnes) in different crop, altitude and management.

Results:

The sampling of bees collected by netting and pan trapping during the April-May 2022 and May-June & Sept-Oct 2023 sampling campaign yielded **4930 bee specimens** . The bee specimens collected belonged to three bee families (Apidae, Megachilidae, Halictidae), comprising **32 genera** and **117 species**. The Halictidae was the most diverse family represented among our samples, with **37 species** (5.6% of the total captured specimens), followed by the Apidae with **19 species** (87.2%) and the Megachilidae with **5 species** (1.1%). Out of the 3,404 bees, 1932 (56.75%) were honey bees (*Apis mellifera*).

Net sampling resulted in the capture of 4000 bees, representing at least **54 species**, while yellow pan traps collected **233 bees** belonging to at least **20 species**. A total of **12 species** were captured by both sampling methods (Fig. 2).

1- Abundance and observed species richness:

When comparing the mean abundance and observed species richness of flower visitors between fields conducted under two different intercropping management “ Agroecological Vs. Conventional”, we found that, although Agroecological fields hosted lower abundance of flower visitors, the mean observed species richness was higher than in Conventional fields (cf. Table 1). **Table 1** indicates that, although fields conducted in higher altitude exhibited higher abundance of flower visitors, they were less taxonomically diverse (lower observed SR) and *vice versa* in lowland fields. Regarding the three crops, we can see clearly in **Table 1** that squash hosted lower abundance and observed species richness of flower visitors. Cucumber and watermelon, on the other hand, exhibit relatively higher and similar average values.

2- Alpha diversity indices & Species evenness:

Alpha diversity refers to species diversity at a local level. Three diversity indices (Shannon, Simpson, and Pielou's Evenness/J' Index) were computed for each of the 20 study sites to gain insights into the structure and composition of the three cucurbits flower visitor communities. The Shannon Index measures diversity by considering both species richness and evenness, providing a more comprehensive understanding of diversity. The Simpson Index places more importance on the dominance or abundance of species, giving more weight to dominant species. Lastly, Pielou's Evenness index measures the extent to which species are numerically balanced in each community.

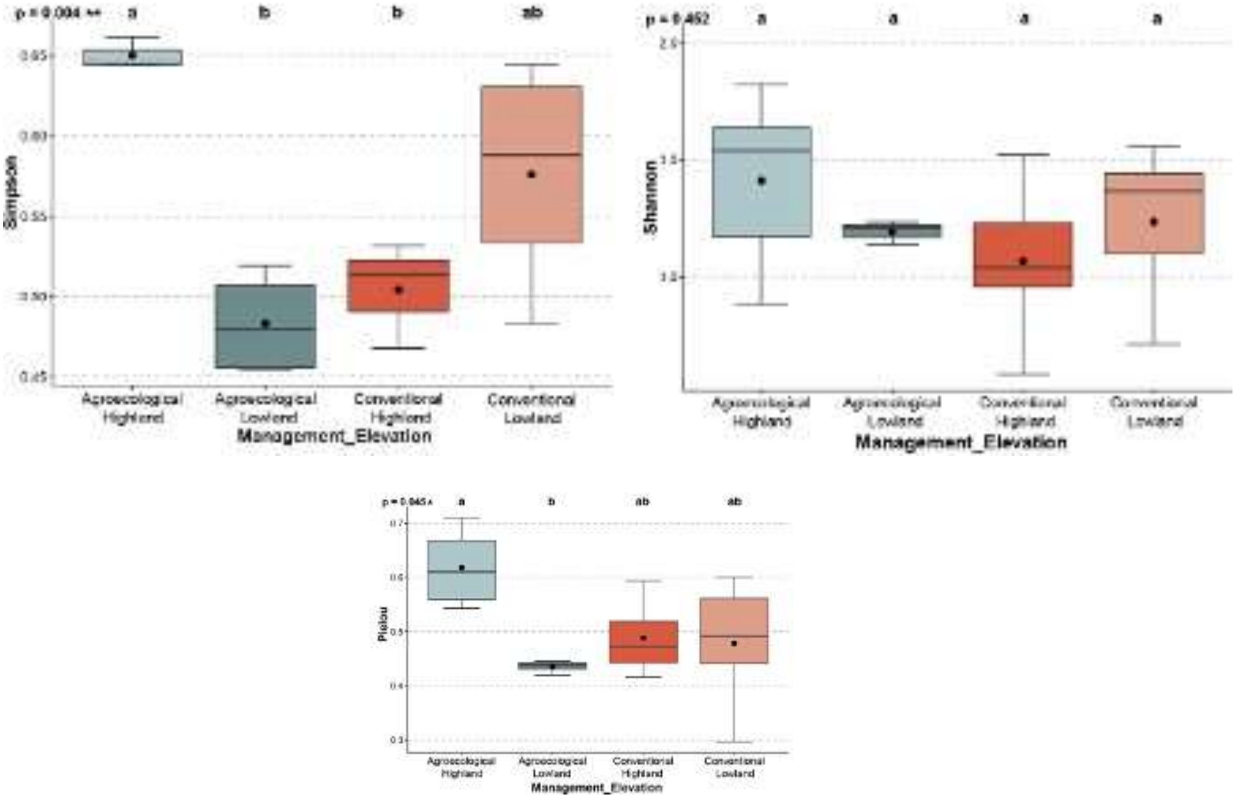


Fig. 5: Boxplots illustrating the three alpha diversity indices (Simpson, Shannon, and Pielou) based on agricultural management and altitude. The average values of alpha diversity indexes are shown as bold black points, while the median values are represented by black horizontal lines. The resulting p-values from an ANOVA at a significance level of α = 0.05 are indicated on the Fig 7 All assumptions of the ANOVA are met, including normal distribution of residuals, homogeneity of variances, and independence of observations. When the p-value provided by the ANOVA is below the α threshold of 0.05, it indicates that the means are significantly different. In the Fig., α = 0.05 is represented by "*", and α = 0.01 is represented by "**". Different letters are used to indicate groups with significantly different means. If two groups have different letters (or groups of letters), it means that their means are significantly different.

There are contrasting results across different sites. For instance, AE sites show Simpson alpha diversity ranging from 0.337 to 0.729. The various boxplots presented reveal a significant difference in Simpson index between altitudes for both AE and conventional sites. Regarding the Pielou index, there is a significant difference in altitudes for AE sites only. Disparities in alpha diversity appear to be more pronounced with altitude among AE sites than among conventional sites. The results of an ANOVA between alpha diversity indices (Simpson, Shannon, Pielou) and the combination of agricultural management/altitude factors are as follows: p = 0.004, p = 0.452, p = 0.045, respectively.

3- Heat maps:

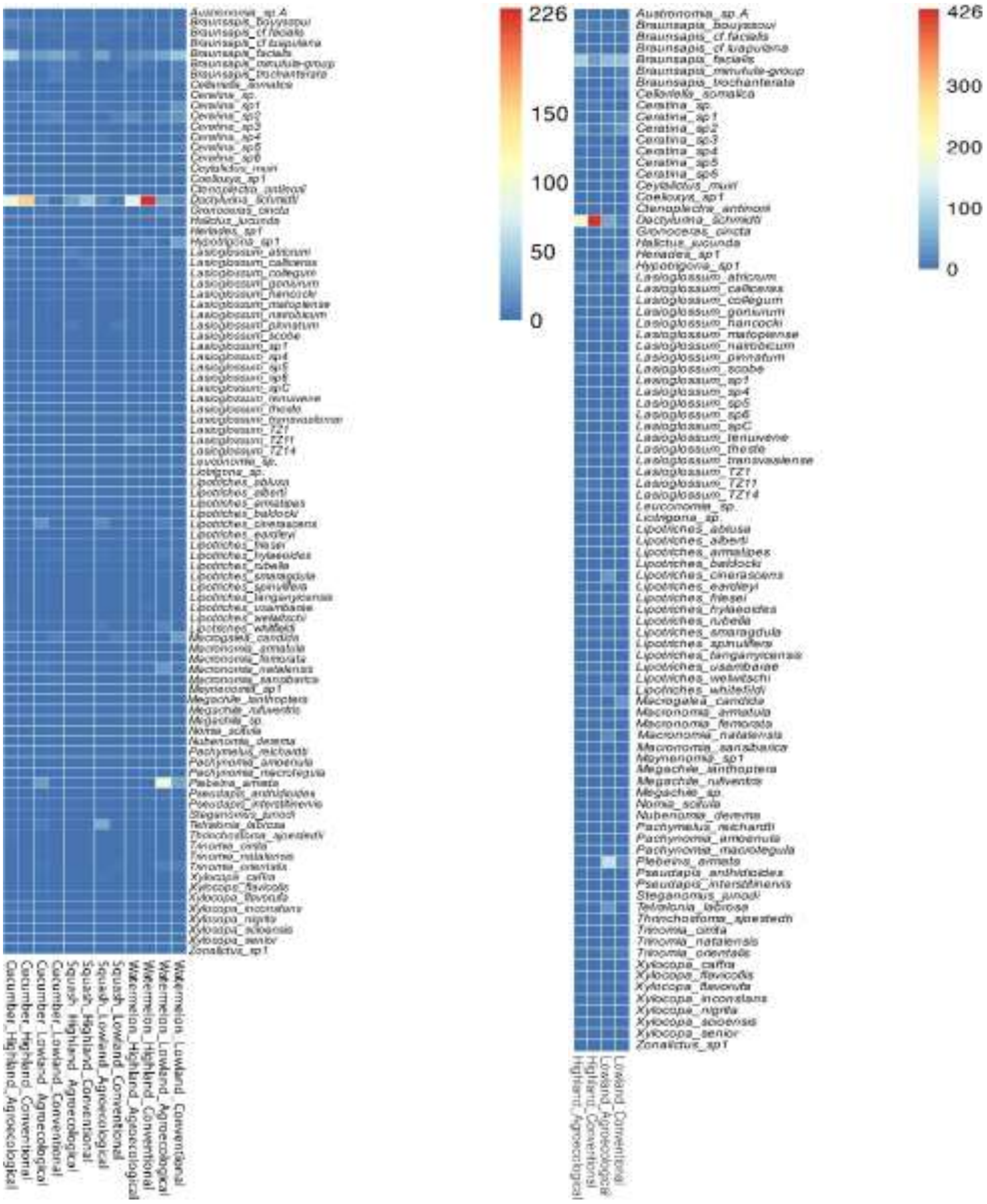


Fig. 6: Two Heatmaps illustrating qualitatively the abundance and species richness of floral visitors (wild and managed bees) collected by active and passive sampling methods (net, pan traps and vane traps) in three cultivated cucurbits species through 20 plots conducted in two different intercropping management (Agroecological vs. Conventional) and two different altitudes.

The dual-panel heat maps depict the abundance of various flower visitor species based on agricultural management, altitude, and the three cucurbit crops. The closer a box is to red, the higher the specimen abundance for any given species (max 426 specimens). The Western Honey Bee, *Apis mellifera*, was deliberately omitted from the analysis due to its high abundance (2244 specimens). Other bees collected only by nets like *Dactylurina schmidti* demonstrated greater abundance on watermelon in conventional fields followed by cucumber, particularly at high altitudes. *Braunsapis facialis* was abundant, but with a lower abundance than *D. schmidti*, in agroecological fields only in highlands and in conventional fields conducted in both altitudes. *Plebeina armata* was predominantly found on watermelon in agroecological fields in lower altitudes watermelon flowers.

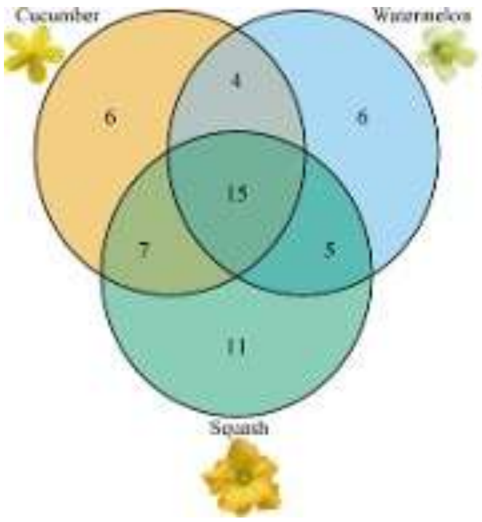
4- Differentiation among sites: beta diversity and its partitioning

The results of the Sørensen dissimilarity index calculation, representing beta diversity and its two components, "turnover" (βsim) and "nestedness" (βnes), are presented in Table 9. The overall value of βsor ($\beta Sor = \beta sim + \beta nes$) diversity indicates a significant difference in composition between the sites. This difference is primarily explained by the turnover factor "βsim", accounting for over 95% of the variation. When considering agricultural management and altitude criteria, there is a substantial difference in composition between the sites belonging to these categories. Moreover, there are even more pronounced differences in composition among the various communities associated with different crops.

Table 3: The values of beta diversity ($\beta Sor = \beta sim + \beta nes$) and its components, "turnover" ($\beta siim$) and "nestedness" (βnes), were calculated for combined agricultural management and altitudes, as well as crops. The βSor value indicates the degree of difference in composition between communities, with a value closer to 1 indicating a greater difference. The communities were captured using various sampling methods (nets, bowls, wing traps), except for crops, which were sampled using nets only.

Management	**β_sim**	**β_nes**	**β_Sor**
Agroecological	0.7600	0.0544	0.8144
Conventional	0.7892	0.0297	0.8188
Highlands	0.7086	0.0719	0.7805
Lowlands	0.7459	0.0495	0.7954
Cucumber	0.8380	0.0549	0.8929
Watermelon	0.8315	0.0519	0.8834
Squash	0.8652	0.0625	0.9277
General	**0.8514**	**0.0327**	**0.8842**

A- Venn Diagram:



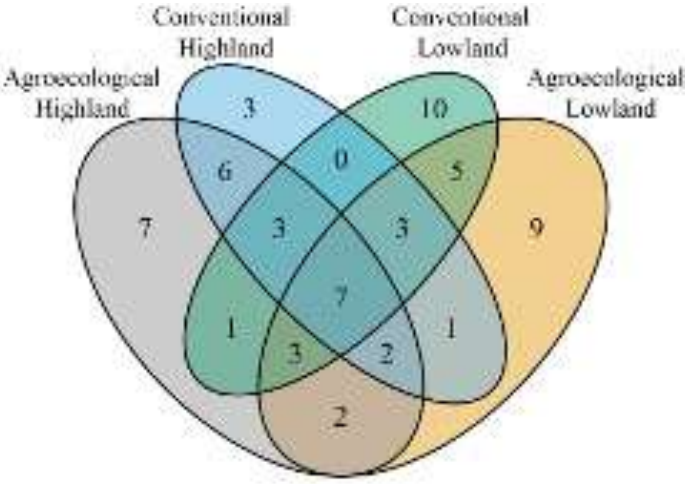


Fig. 7: Venn diagrams illustrating species overlaps between crops (specimens captured by nets) on the left and agricultural practices/altitudes on the right (combining all sampling methods: nets, bowls, wing traps). The numbers of shared species within these different factors are displayed at the intersections of all present circles, i.e., in the center of each diagram.

On the left diagram of Fig. 6, the species overlaps for different sampled crops in different alitutdes and conducted under different management are presented. Fifteen species were common between all three crops at both altitudes ("highlands" and "lowlands"). These species and their abundances are detailed in Table 4. Additionally, the number of unique species for each crop is indicated outside the circle: six for cucumber, eleven for squash, and six for watermelon. The intersection between two circles indicates the number of species shared between two crops: seven between cucumber and squash, five between watermelon and squash, and four between watermelon and cucumber. Whereas, in the right diagram of Fig. 5, the overlaps of species for the two agricultural management practices are shown. This diagram displays four factors and indicates that there are seven species found in all sites, altitudes, and management practices combined. Additionally, the sites with different altitudes and agricultural practices only share two species. For conventional sites, this number is reduced to zero. However, when comparing the agricultural practices based on altitude, there are six and five species shared between them.

Table 4: List of 10 most abundanct common species occurring in combinations of Management/Altitude, along with their corresponding abundances and sampling methods (Net, yellow pan traps).

Agroecological			**Conventional**		
Species	AgroHigh	AgroLow	**Species**	ConvHigh	ConvLow
Dactylurina_schmidti	190	50	*Dactylurina_schmidti*	426	14
Braunsapis_facialis	90	6	*Braunsapis_facialis*	39	75
Ceratina_sp2	23	17	*Braunsapis_bouyssoui*	10	9
Braunsapis_minutula-group	21	2	*Ceratina_sp.*	9	3
Lasioglossum_atricrum	21	6	*Braunsapis_minutula-group*	8	17
Braunsapis_bouyssoui	20	11	*Lasioglossum_atricrum*	8	3
Lasioglossum_pinnatum	13	2	*Hypotrigona_sp1*	7	27
Macrogalea_candida	11	1	*Lasioglossum_collegum*	7	2
Ceratina_sp.	8	3	*Ceratina_sp2*	6	35
Ceratina_sp1	8	13	*Macrogalea_candida*	5	36

Table 5: List of the 10 most abundant common species between the same crop conducted in two different altitude crop/Altitude combination along with their corresponding abundances

Watermelon			**Cucumber**			Squash		
Species	**WatHigh**	**WatLow**	**Species**	**CucuHigh**	**Cuculow**	**Species**	**SquaHigh**	**SquaLow**
Dactylurina_schmidti	307	33	*Dactylurina_schmidti*	245	16	*Dactylurina_schmidti*	64	15
Braunsapis_facialis	31	67	*Braunsapis_facialis*	76	49	*Braunsapis_facialis*	22	30
Ceratina_sp2	16	27	*Braunsapis_bouyssoui*	19	7	*Lasioglossum_atricrum*	10	2
Braunsapis_minutula-group	13	11	*Lasioglossum_atricrum*	13	1	*Lasioglossum_pinnatum*	8	7
Hypotrigona_sp1	8	24	*Braunsapis_minutula-group*	11	10	*Braunsapis_minutula-group*	5	2
Braunsapis_bouyssoui	7	7	*Ceratina_sp.*	11	1	*Ceratina_sp2*	5	9
Lasioglossum_atricrum	6	6	*Macrogalea_candida*	11	9	*Braunsapis_bouyssoui*	4	6
Lasioglossum_collegum	6	2	*Ceratina_sp2*	8	16	*Ceratina_sp.*	4	2
Ceratina_sp1	4	19	*Lasioglossum_pinnatum*	8	1	*Ceratina_sp4*	4	5
Macrogalea_candida	4	22	*Braunsapis_cf.luapulana*	6	1	*Lipotriches_hylaeoides*	4	3

5- Three-way ANOVA:

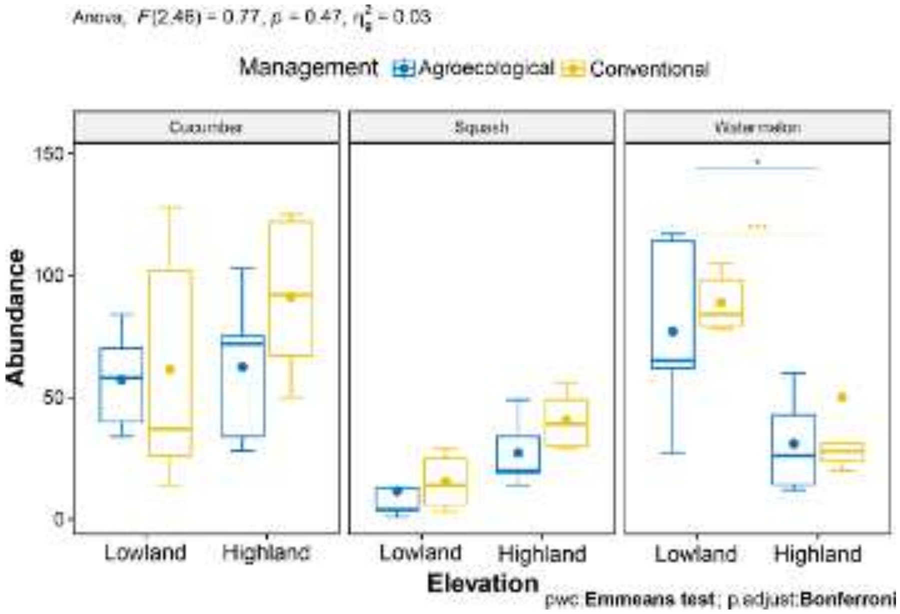


Fig. 8: Boxplots panel illustrating three-Way ANOVA analysis showing interaction between three cucurbits conducted in two intercropping systems (Agroecological vs. Conventional) and in two contrasting landscape contexts (low vs. high altitude) on explaining the abundance of flower visitor (wild and managed bees) communities. All ANOVA assumptions are met (normal distribution of residuals, homogeneity of variances, independence of observations). Colored points and lines respectively depict the means and medians of each boxplot. **F** and **P** values are provided to indicate the statistical significance of the inter-factor interaction. Significance thresholds are denoted by asterisks: "*" $p < 0.05$, "**" $p < 0.01$, "***"$p < 0.00$.

The boxplots panel above Fig. 6 illustrate a Three-Way ANOVA analysis that aim to appraise a significant three-way interaction between three cucurbits conducted in two intercropping systems (Agroecological vs. Conventional) and in two contrasting landscape contexts (low vs. high altitude) on explaining the abundance of flower visitor communities. An interaction effect occurs when the effect of one independent variable, on an outcome variable, depends on the level of the other independent variables.

The residuals have a normal distribution, as confirmed by the Shapiro-Wilk normality test (p-value: 0.594 > 0.05), and the Levene's test confirms homogeneity of variances (p-value: 0.1 > 0.05). There is no statistically significant interaction among the three factors (F(2,45) = 0.77; p-value = 0.47). In terms of abundance, a statistically significant difference was only observed in contrasted altitude, specifically in watermelon conducted in both management. The primary effect of altitude on abundance is statistically significant for both watermelon in agroecological fields (p-value: 0.01 < 0.05) and conventional ones (p-value: 0.0005 < 0.05). Therefore, on average, the abundance is significantly higher at low altitude than at high altitude for watermelon, regardless of the agricultural management practices. We hypothesize that the diversity and abundance of flower-visitor communities in highlands are less attracted by the cucurbit crops/fields within their heterogenous, semi-natural landscape.

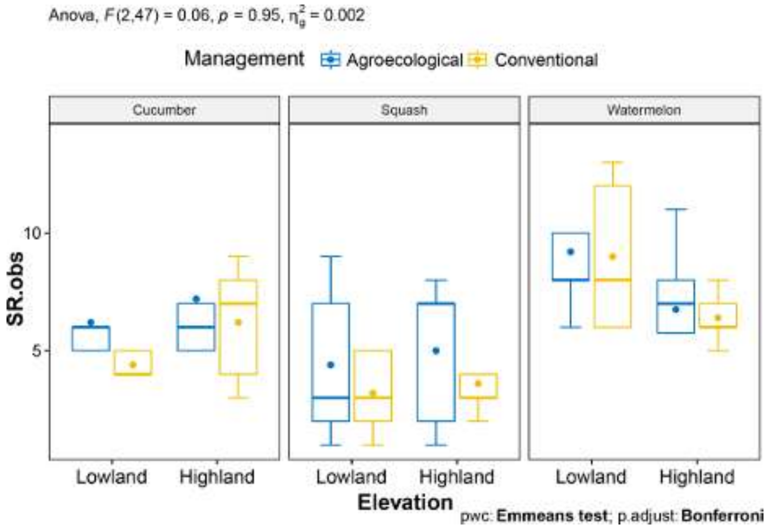


Fig. 8: Boxplots panel illustrating three-Way ANOVA analysis showing interaction between three cucurbits conducted in two intercropping systems (Agroecological vs. Conventional) and in two contrasting landscape contexts (low vs. high altitude) on explaining the observed species richness of flower visitor (wild and managed bees) communities. All ANOVA assumptions are met (normal distribution of residuals, homogeneity of variances, independence of observations). Colored points and lines depict the means and medians of each boxplot, respectively. **F** and **P** values are provided to indicate the statistical significance of the inter-factor interaction.

The Fig. 7 presents the same analysis as described above, this time focusing on the observed species richness (RS). No extremely abnormal values were observed. The residuals show a normal distribution (Shapiro-Wilk test, p-value: 0.325 > 0.05), and the homogeneity of variances is confirmed (Levene's test, p-value: 0.859 > 0.05). There is no statistically significant interaction among the three factors (F(2,47) = 0.06; p-value = 0.95), and there are also no significant interactions between two factors.

The main effect of crop on species richness was statistically significant for all combined managements but only in the lowlands (AE, p-value: 0.022 < 0.05; conventional, p-value: 0.003 < 0.05).

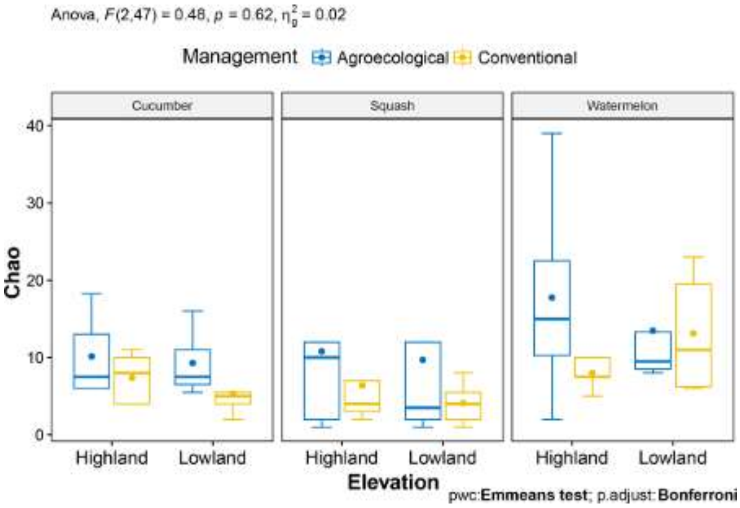


Fig. 9: Boxplots panel illustrating three-Way ANOVA analysis showing interaction between three cucurbits conducted in two intercropping systems (Agroecological vs. Conventional) and in two contrasting landscape contexts (low vs. high altitude) on explaining the extrapolated species richness of flower visitor (wild and managed bees) communities. All ANOVA assumptions are met (normal distribution of residuals, homogeneity of variances, independence of observations). Colored points and lines respectively depict the means and medians of each boxplot. **F** and **P** values are provided to indicate the statistical significance of the inter-factor interaction.

The Fig. 8 shows the results of the three-factor ANOVA for extrapolated species richness (Chao) using the Chao1 indicator. The data were square root transformed to ensure normality of residuals. The Shapiro-Wilk test resulted in a p-value of 0.00003, indicating significant deviation from normality. However, after the square root transformation, the p-value increased to 0.161, suggesting that the normality assumption is met. There are no extreme values observed after the transformation. The homogeneity of variances is confirmed, as shown by the Levene's test result of 0.400, which is greater than the critical p-value of 0.05.

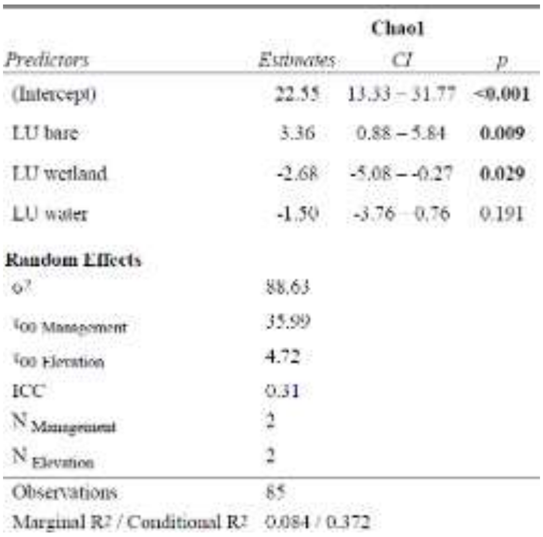
The analysis reveals no statistically significant interaction among the three factors (F(2,47) = 0.48, p-value = 0.62), and there are no significant interactions between any two factors. However, crop type has a significant influence on extrapolated species richness, with a p-value of 0.032. The main effect of crop type on extrapolated species richness (Chao) is not statistically significant for either agricultural management or altitude.

6- Quantitative description of land use in agricultural and semi-natural areas:

For each study site, we computed the Shannon's landscape diversity index at the following radius: 250m, 500m, 1000m, 1500m and 2000m. This index was used to choose a preferred buffer area around the 20 sites to extract landscape characteristics. Here we show the correlation between the Shannon index and two diversity metrics (species richness, Hill-Shannon), calculated at different radius. The highest correlation scores happen at the 250m radius around the study sites at different altitudes. According to these results, we chose to consider only 250m radius around study sites for further extractions of landscape characteristics.

		Shannon div. 250m	**Shannon div. 500m**	**Shannon div. 1000m**	**Shannon div. 1500m**	**Shannon div. 2000m**
1	Hill-Shannon index	0.440	0.338	0.349	0.301	0.287
2	SR	0.093	-0.047	-0.054	-0.075	-0.064

Ps: Hill-Shannon Index: Uses a logarithmic scale to calculate diversity. It provides leverage to both rare and common species.



	Chao1		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	22.55	13.33 – 31.77	**<0.001**
LU bare	3.36	0.88 – 5.84	**0.009**
LU wetland	-2.68	-5.08 – -0.27	**0.029**
LU water	-1.50	-3.76 – 0.76	0.191
Random Effects			
σ^2	88.63		
$\tau_{00\ \mathrm{Management}}$	35.99		
$\tau_{00\ \mathrm{Elevation}}$	4.72		
ICC	0.31		
$N_{\mathrm{Management}}$	2		
$N_{\mathrm{Elevation}}$	2		
Observations	65		
Marginal R^2 / Conditional R^2	0.084 / 0.372		

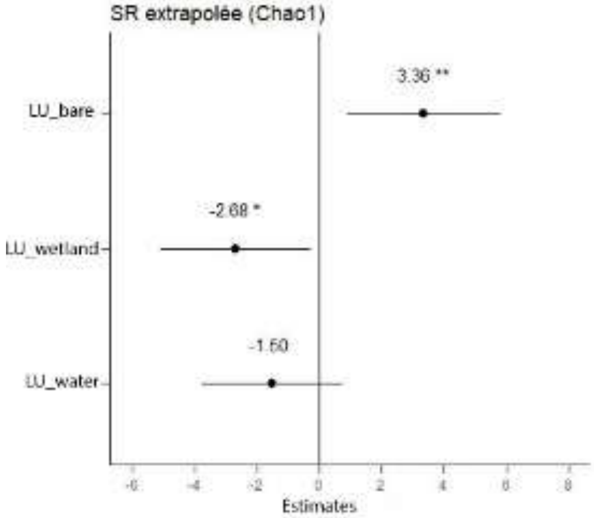
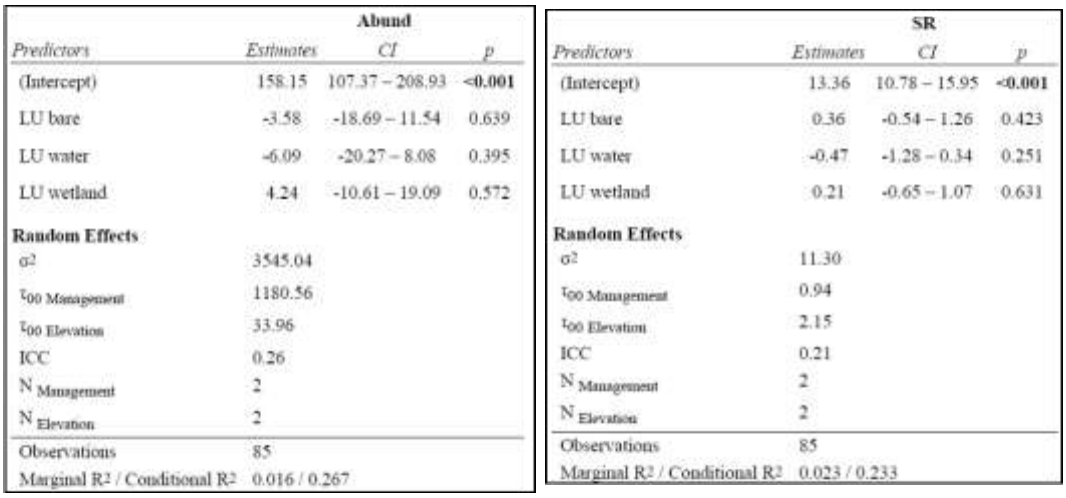


Fig. 10: Results of a Generalized Linear Model (GLM) - Influence of Landscape Components on Extrapolated Species Richness. A landscape component is considered to have a significant influence when its p-value is below the threshold α = 0.05. Significance thresholds are indicated by asterisks: "*" for $p < 0.05$, "**" for $p < 0.01$. The Intraclass Correlation Coefficient (ICC) quantifies the proportion of total variance explained by random effects such as "Altitude" and "Agricultural Management". The landscape components are categorized as follows: LU_wetland represents Herbaceous Wetlands, LU_bare corresponds to Bare/Sparsely Vegetated areas, and LU_water represents Permanent Water Bodies.

The influence of the landscape was assessed using Generalized Linear Models (GLMs) for three response variables: abundance, observed species richness (Chao1), and extrapolated species richness of both domestic and wild bees, considering all sampling methods. Altitude and agricultural management are treated as random variables.

For abundance, observed species richness, and extrapolated species richness, the landscape components excluded from the model are land cover classes such as "Trees," "Shrubs," "Grassland," "Croplands," and "Built-up." Their Variance Inflation Factor (VIF) values all exceeded the acceptability threshold of 5. Therefore, the components included in the simplified GLMs are the land cover classes "Bare/sparse vegetation," "Permanent water bodies," and "Herbaceous Wetland," with VIF values below 5.

Among the three models conducted (Influence of Landscape Components on 1. Abundance, 2. Observed species richness, 3. Extrapolated species richness, Chao1), only the one evaluating the influence of landscape components on extrapolated species richness revealed a significant influence of one or more factors (Fig. 9). The results for models with no significant influence are in Fig. 10. All assumptions (residual uniformity and dispersion, homogeneity of variances, non-significant overdispersion) are met for all three models. There is a statistically significant influence of the land cover class "Bare/sparse vegetation" (p-value: 0.009 < 0.05) and "Herbaceous Wetlands" (p-value: 0.029 < 0.05) on extrapolated species richness (Chao1). Additionally, the random effects "Agricultural Management" and "Altitude" explain 31% of the total variance in the model (ICC). The model suggests that as the proportion of Bare/Sparsely Vegetated lands in the landscape increases, extrapolated species richness also increases. Furthermore, the model indicates that with an increase in the proportion of Herbaceous Wetlands in the landscape, there is a decrease in extrapolated species richness (cf. Fig. 9).



	Abund		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	158.15	107.37 – 208.93	**<0.001**
LU bare	-3.58	-18.69 – 11.54	0.639
LU water	-6.09	-20.27 – 8.08	0.395
LU wetland	4.24	-10.61 – 19.09	0.572
Random Effects			
σ^2	3545.04		
$\tau_{00\ \text{Management}}$	1180.56		
$\tau_{00\ \text{Elevation}}$	33.96		
ICC	0.26		
$N_{\text{Management}}$	2		
$N_{\text{Elevation}}$	2		
Observations	85		
Marginal R^2 / Conditional R^2	0.016 / 0.267		

	SR		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	13.36	10.78 – 15.95	**<0.001**
LU bare	0.36	-0.54 – 1.26	0.423
LU water	-0.47	-1.28 – 0.34	0.251
LU wetland	0.21	-0.65 – 1.07	0.631
Random Effects			
σ^2	11.30		
$\tau_{00\ \text{Management}}$	0.94		
$\tau_{00\ \text{Elevation}}$	2.15		
ICC	0.21		
$N_{\text{Management}}$	2		
$N_{\text{Elevation}}$	2		
Observations	85		
Marginal R^2 / Conditional R^2	0.023 / 0.233		

Fig. 11: Results of GLM models – Influence of Landscape Components on Abundance (Abund) and Observed Species Richness (SR). A p-value below the threshold α = 0.05 is considered statistically significant. ICC indicates the total variance explained by random variables (Agricultural Management and Altitude).

Table 6 list of the bee species collected during the 2022 and 2023 campaigns, including occurrence data across different crops, managements, altitudes, and catching methods (Net and yellow pan traps).

Tribe	**Genus**	**Subgenus**	***Species***	**Cucu**	**Wat**	**Squa**	**HL**	**LL**	**Agro**	**Conv**	**Net**	**Pan trap**
Nomiini	Acunomia		*Acunomia somalica*	x		x	x		x		x	
	Austronomia		*Austronomia spB*					x	x			x
	Austronomia		*Austronomia spA*			x	x			x	x	
	Leuconomia		*Leuconomia sp*		x			x		x	x	
	Lipotriches		*Lipotriches ablusa*	x			x			x	x	
	Lipotriches		*Lipotriches alberti*	x		x	x		x	x	x	
	Lipotriches		*Lipotriches armatipes*	x	x			x	x	x	x	
	Lipotriches		*Lipotriches baldocki*	x	x			x	x		x	
	Lipotriches		*Lipotriches cinerascens*	x	x	x		x	x	x	x	
	Lipotriches		*Lipotriches eardleyi*	x	x			x	x	x	x	x
	Lipotriches		*Lipotriches friesei*	x				x	x		x	
	Lipotriches		*Lipotriches hylaeoides*	x	x	x	x	x	x	x	x	
	Lipotriches		*Lipotriches natalensis*					x	x	x		x
	Lipotriches		*Lipotriches rubella*	x				x		x	x	
	Lipotriches		*Lipotriches smaragdula*	x	x	x	x	x	x	x	x	x
	Lipotriches		*Lipotriches spinulifera*	x	x		x	x	x	x	x	
	Lipotriches		*Lipotriches tanganyicensis*			x		x	x		x	
	Lipotriches		*Lipotriches usambarae*	x	x		x		x	x	x	
	Lipotriches		*Lipotriches welwitschi*		x			x	x		x	
	Lipotriches		*Lipotriches whitfieldi*	x	x	x		x	x	x	x	
	Macronomia		*Macronomia armatula*		x			x		x	x	
	Macronomia		*Macronomia femorata*		x			x	x		x	
	Macronomia		*Macronomia natalensis*	x	x	x		x	x	x	x	
	Macronomia		*Macronomia sansibarica*	x	x			x		x	x	
	Maynenomia		*Maynenomia sp1*	x			x	x	x	x	x	

	Nomia		*Nomia scitula*		x			x		x	x	
	Nubenomia		*Nubenomia derema*		x		x		x		x	
	Pachynomia		*Pachynomia amoenula*	x	x	x	x	x	x	x	x	x
	Pachynomia		*Pachynomia macrotegula*	x	x	x	x	x	x	x	x	x
	Pseudapis		*Pseudapis anthidioides*		x			x	x	x	x	
	Pseudapis		*Pseudapis interstitinervis*	x		x		x	x	x	x	
	Steganomus		*Steganomus junodi*	x				x		x	x	
	Trinomia		*Trinomia cirrita*	x	x	x		x	x	x	x	
	Trinomia		*Trinomia natalensis*	x		x	x	x	x	x	x	x
	Trinomia		*Trinomia orientalis*	x	x	x	x	x	x	x	x	
Halicitni	Lasioglossum	Afrodialictus	*Lasioglossum bellulum*				x	x	x	x		x
	Lasioglossum	Afrodialictus	*Lasioglossum sp4*		x		x			x	x	x
	Lasioglossum	Afrodialictus	*Lasioglossum sp5*	x	x		x	x	x	x	x	
	Lasioglossum	Afrodialictus	*Lasioglossum theste*		x		x		x		x	
	Eupetersia		*Eupetersia emini*				x	x	x	x		x
	Halictus haasi		*Halictus haasi*					x	x			x
	Halictus jucunda		*Halictus jucunda*	x	x		x	x	x	x	x	x
	Lasioglossum	Ctenonomia	*Lasioglossum atricrum*	x	x	x	x	x	x	x	x	x
	Lasioglossum	Ctenonomia	*Lasioglossum collegum*	x	x	x	x	x	x	x	x	
	Lasioglossum	Ctenonomia	*Lasioglossum scobe*	x	x	x	x	x	x	x	x	x
	Lasioglossum	Ctenonomia	*Lasioglossum sp5*				x		x			x
	Lasioglossum	Ctenonomia	*Lasioglossum sp6*	x			x		x		x	
	Lasioglossum	Ctenonomia	*Lasioglossum spC*	x	x	x	x		x	x	x	
	Lasioglossum	Ctenonomia	*Lasioglossum transvaalense*	x			x	x	x	x	x	x
	Lasioglossum	Ipomalictus	*Lasioglossum bowkeri*					x	x	x		x
	Lasioglossum	Ipomalictus	*Lasioglossum goniurum*		x	x	x	x	x	x	x	x
	Lasioglossum	Ipomalictus	*Lasioglossum hancocki*	x	x	x	x	x	x	x	x	x
	Lasioglossum	Ipomalictus	*Lasioglossum matopiense*		x			x	x	x	x	x

	Lasioglossum	Ipomalictus	*Lasioglossum norvali*					x	x			x
	Lasioglossum	Ipomalictus	*Lasioglossum pinnatum*	x	x	x	x	x	x	x	x	x
	Lasioglossum	Ipomalictus	*Lasioglossum TZ1*	x			x		x		x	
	Lasioglossum	Ipomalictus	*Lasioglossum TZ11*	x	x	x	x		x	x	x	x
	Lasioglossum	Ipomalictus	*Lasioglossum TZ14*	x	x	x	x		x	x	x	x
	Lasioglossum	Ipomalictus	*Lasioglossum TZ8*				x		x			x
	Lasioglossum	Oxyhalictus	*Lasioglossum acuiferum*				x	x	x	x		x
	Lasioglossum	Oxyhalictus	*Lasioglossum calliceras*	x	x	x	x	x	x	x	x	x
	Lasioglossum	Oxyhalictus	*Lasioglossum nairobicum*	x		x	x	x	x	x	x	x
	Lasioglossum	Oxyhalictus	*Lasioglossum sp1*	x	x		x	x	x	x	x	x
	Lasioglossum	Oxyhalictus	*Lasioglossum sp2*					x	x			x
	Zonalictus		*Zonalictus sp1*		x	x	x		x	x	x	
Nomioidini	Cellariella		*Cellariella somalica*	x		x		x	x	x	x	
	Ceylalictus		*Ceylalictus muiri*		x	x		x	x		x	
Trinchostomini	Thrinchostoma		*Thrinchostoma sjoestedti*	x	x	x	x	x	x	x	x	x
Anthiidini	Anthidium		*Anthidium sp*			x	x	x	x	x	x	x
Megachilini	Coelioxys		*Coelioxys sp1*			x	x		x		x	
	Megachile		*Megachile ianthoptera*		x		x		x		x	
	Megachile		*Megachile rufiventris*	x	x			x	x		x	
	Megachile		*Megachile sp*	x	x		x	x	x	x	x	x
	Gronoceras		*Gronoceras cinctum*	x	x	x		x	x		x	
Osmiini	Heriades		*Heriades sp1*		x			x	x	x	x	
Apini	Apis		*Apis mellifera*	x	x	x	x	x	x	x	x	x
Meliponini	Dactylurina		*Dactylurina schmidti*	x	x	x	x	x	x	x	x	x
	Hypotrigona		*Hypotrigona sp1*	x	x	x	x	x	x	x	x	
	Liotrigona		*Liotrigona sp*	x			x		x		x	
	Plebeina		*Plebeina armata*	x	x			x	x	x	x	x
Anthophorini	Amegilla		*Amegilla sp*			x	x	x	x	x	x	x

	Pachymelus		*Pachymelus reichardti*		x			x		x	x	
Eucerini	Tetralonia		*Tetralonia labrosa*	x	x	x	x	x	x	x	x	x
Allodapini	Braunsapis		*Braunsapis bouyssoui*	x	x	x	x	x	x	x	x	
	Braunsapis		*Braunsapis cf.facialis*	x	x	x	x	x	x	x	x	x
	Braunsapis		*Braunsapis cf.luapulana*	x	x	x	x	x	x	x	x	
	Braunsapis		*Braunsapis facialis*	x	x	x	x	x	x	x	x	
	Braunsapis		*Braunsapis minutula-group*	x	x	x	x	x	x	x	x	
	Braunsapis		*Braunsapis trochanterata*	x	x	x	x	x	x	x	x	
	Macrogalea		*Macrogalea candida*	x	x	x	x	x	x	x	x	x
	Ceratina		*Ceratina sp1*	x	x	x	x	x	x	x	x	
	Ceratina	Pithitis	*Ceratina sp2*	x	x	x	x	x	x	x	x	x
	Ceratina		*Ceratina sp3*	x	x	x	x	x	x	x	x	
	Ceratina		*Ceratina sp4*	x	x	x	x	x	x	x	x	
	Ceratina		*Ceratina sp5*	x	x	x	x	x	x	x	x	
	Ceratina		*Ceratina sp6*		x			x	x		x	
Xylocopini	Xylocopa		*Xylocopa caffra*	x	x	x	x	x	x	x	x	x
	Xylocopa		*Xylocopa flavicollis*	x	x	x	x	x	x	x	x	
	Xylocopa		*Xylocopa flavorufa*	x	x	x	x	x	x	x	x	
	Xylocopa		*Xylocopa inconstans*	x	x	x	x	x	x	x	x	
	Xylocopa		*Xylocopa nigrita*	x		x	x		x	x	x	
	Xylocopa		*Xylocopa scioensis*		x	x	x	x	x	x	x	
	Xylocopa		*Xylocopa senior*	x	x	x	x	x	x	x	x	
Ctenoplectrini	Ctenoplectra		*Ctenoplectra antinorii*	x			x	x		x	x	x

Agroecological farming practices for fruit fly management in Central Eastern Tanzania.

Published in Bakengesa et al. (2023). Fruits, 78(3). https://doi.org/10.17660/th2023/010.

Methodology

A baseline survey was conducted in 2022 across three districts (Morogoro, Mvomero, Kilosa) in Tanzania's Central Eastern Zone.

- **Sample:** 138 smallholder cucurbit farmers (<2.5 ha) interviewed using semi-structured questionnaires, focus groups, and key informant interviews.
- **Analysis:** Descriptive statistics, chi-square tests, and GLMs identified predictors of awareness and adoption of agroecological practices.

Scientific Results

- **Crops:** Cucumber dominated (48.6%), followed by pumpkin (24.6%), watermelon (21.7%), and squash (5.1%).
- **Pests:** Fruit flies were the most reported pest (31.2%), followed by aphids (22.5%). Damage was perceived as increasing by 55.6% of farmers.
- **Awareness:** 63% of farmers knew about agroecology, but only 27.5% applied it for fruit fly control. Adoption was strongly linked to farming experience ($p<0.01$).
- **Practices:** Most common were crop rotation (56.5%), intercropping (56.5%), orchard sanitation (23.7%), weeding (26.3%), and bait stations (26.3%). Bio-pesticides were rarely used (5.3%).
- **Willingness:** 82.6% of farmers expressed readiness to adopt agroecology if trained.

Interpretation:

Agroecological practices remain underutilized despite clear interest. Heavy reliance on synthetic pesticides (63%) reflects knowledge gaps and accessibility issues. Training and extension programs are critical to scale adoption and reduce pesticide dependency.

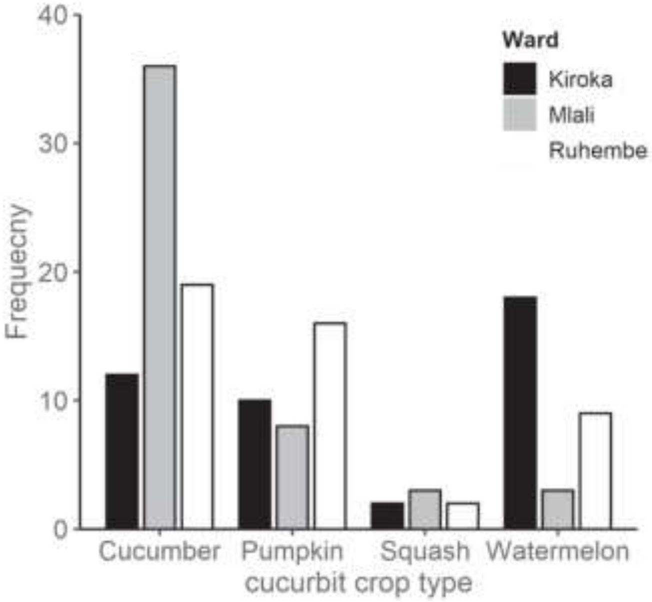


Figure 2: Distribution of cucurbit crops grown.

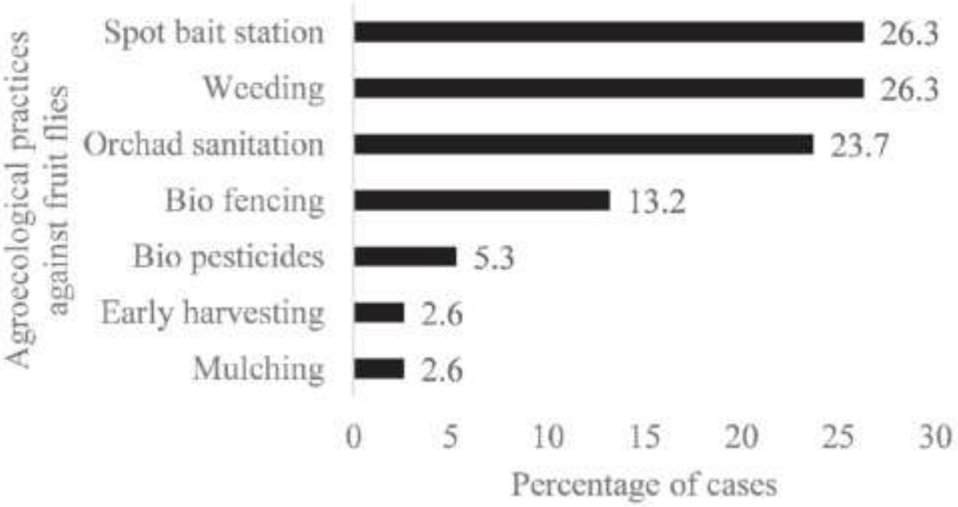


Figure 5: Agroecological practices used for fruit fly control.

References

1. Bakengesa JA et al. (2023). Agroecological farming practices against fruit flies in Central Eastern Tanzania. *Fruits*, 78(3). https://doi.org/10.17660/th2023/010
2. Deguine J-P et al. (2015). Agroecological management of cucurbit-infesting fruit fly: A review. *Agron Sustain Dev*, 35:937–965.
3. Mwatawala M et al. (2010). Incidence and host range of melon fruit fly in Tanzania. *Int J Pest Manag*, 56:265–273.
4. Canhanga L et al. (2021). Perception of fruit farmers on fruit fly occurrence and economic impact. *Fruits*, 76:295–302.
5. Ainembabazi JH & Mugisha J. (2014). Role of farming experience on adoption of agricultural technologies. *J Dev Stud*, 50:666–679.

Task 3.2. crop pollination service

Field experiments on pollination service

In a subset of six fields (three conventional and three agroecological) located in highlands, we randomly selected 60 female flower buds per plot (20 flowers per crop : cucumber, squash and watermelon). Ten female flowers were assigned to receive hand-pollination (supplementary pollination), and the remaining ten received only natural/entomophilous pollination (control). On the evening before the hand-pollinations were performed, we identified male and female blossoms that were beginning to show a yellow flush of colour. Cucurbits flowers (cucumber, watermelon and squash) are unisexual, with male and female occurring on the same plant (monoecious). The flowers have sticky pollen grains and are highly dependent on insect pollination (Bomfim et al., 2015). Female flowers remain receptive for only one day after opening. Moreover, fruit initiation requires numerous pollen grains to be deposited on the stigma otherwise the flower bud will abort. Each hand-pollinated female flower was marked beneath the blossom, around the stem, with coloured tie wraps, and we added pollen to their stigmas once the flowers (♀ & ♂) opened. The stigmas were saturated with pollen by gently rubbing pollen-loaded anthers uniformly across all stigma lobes (using a paintbrush for watermelon and cucumber; for squash, we used the male flowers themselves as brushes and swabbed the pollen-covered anthers onto each of the three lobes of the female flower's stigma). We conducted hand-pollination between 7:00 and 10:30 AM, which correspond to the period of maximum stigma receptivity (Bomfim et al., 2015) and pollen maximum viability (Nepi and Pacini, 1993). From a crop production perspective, commercial output and the quality of pollination services in cucumber, squash and watermelon production are generally computed through measurements of seed set, fruit weight and malformation, or more broadly through the overall crop yield (Garratt et al., 2014, 2021). To measure the relative impact of bee community and land use on fruit quality, we collected cucumber, squash and watermelons fruits in a subset of 6 sites, two weeks after early fruit set . The three parameters can, therefore, be used to reflect pollination efficiency (Grab et al., 2019). Levels of pollination service and pollination deficit were assessed for each crop across the experimental plots (conventional Vs agroecological) for cucurbits response metric. These response metrics are divided into two broad categories: "pollination" and "production". Seed number is a proxy that represent "pollination" as they reflected the level of compatible pollen delivery to cucurbits flowers but are not intrinsically of value to farmers. Final fruit set at harvest (four weeks after hand and open pollination)(Delapane and Mayer, 2000; Sawe et al., 2020), yield (fruit set x fruit weight), and size of ripe fruits (Squash, Cucumber, Watermelon) were measured (size in cm2, Size (cm2) = Length (cm) x Average girth (cm)) represent final crop outputs for farmers and are considered as "production" metrics. "Pollination deficit" represent a shortfall in output due to a lack of pollination and was calculated by subtracting outputs from open pollination treatments from those achieved under supplementary pollination.

Data analysis:

All statistical analyses were implemented using the lme4, glmmTMB, ordinal, and emmeans packages in RStudio Version (R 4.4.3). We evaluated the effects of pollination treatment (hand- vs. open-pollination), management system (agroecological vs. conventional), and crop species (cucumber, squash, watermelon) on fruit-production and pollination metrics using generalized and linear mixed-effects models. Flower-visitors bee abundance and species richness (scaled) were included as covariates to assess their contribution in providing pollination service. Seed number was analysed using a negative binomial GLMM (glmmTMB), while fruit set was analysed using a binomial GLMM restricted to cucumber and squash due to incomplete treatment combinations in watermelon. Fruit weight and fruit size were analysed using linear mixed-effects models (lmer) fitted to log-transformed responses, and fruit malformation (ordinal scores 0–3) was analysed using a cumulative link mixed model (clmm). Field identity was included as a random intercept in all models to account for non-independence among observations. Pollination deficits were calculated per field and crop as the difference between supplementary (hand) and open pollination for seed number, fruit-set proportion, fruit weight and fruit size, and subsequently analysed using linear mixed-effects models with management system and crop as fixed effects.

Results:

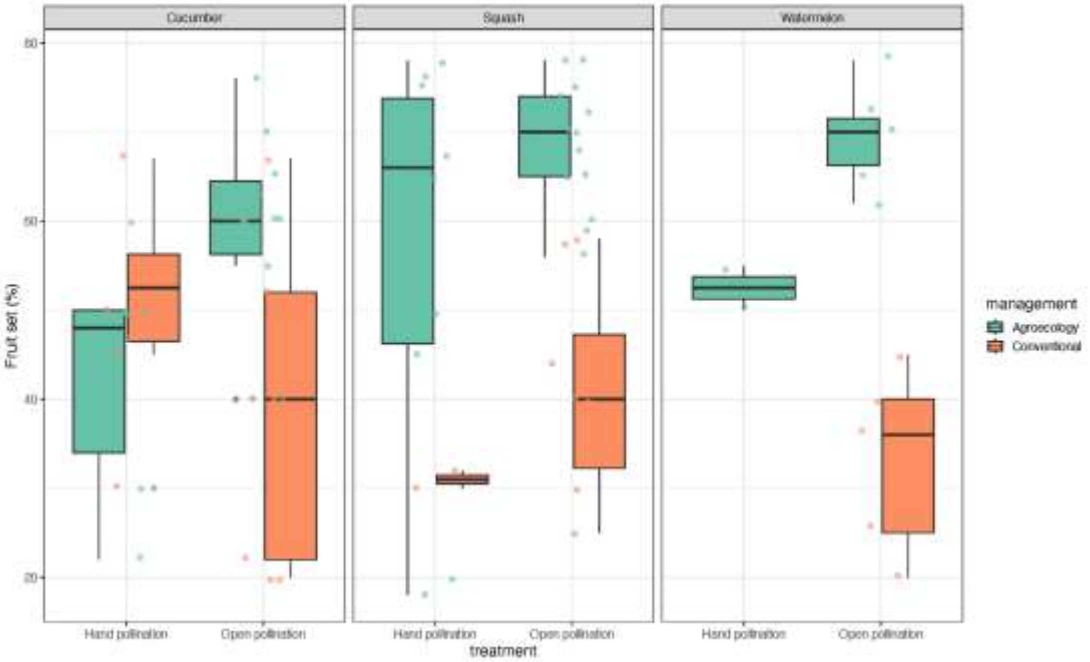


Fig. 12: Early fruit-set (a successful pollination proxy) for cucumber and squash. The binomial GLMM revealed a strong positive effect of open pollination (*p* < 0.0001). A strong negative management × treatment interaction (*p* < 0.0001) showed that open pollination increased fruit set in agroecological fields but reduced it in conventional fields.

Fruit set (Fig. 12), used as a direct measure of successful pollination, varied significantly across crops, management systems, and pollination treatments, as shown by the binomial GLMM (AIC = 387.9, logLik = –181.9). The model indicated that open pollination strongly increased the probability of fruit set overall compared with hand pollination ($\beta = 0.84 \pm 0.25$, $z = 3.41$, $p = 0.00065$), suggesting that in many conditions, entomophilous natural pollination is effective. However, the effect of pollination treatment depended strongly on the management system. The management and treatment interaction was highly significant ($\beta = -1.34 \pm 0.33$, $z = -4.04$, $p < 0.001$), showing that the positive effect of open pollination was largely restricted to agroecological fields, while in conventional fields hand and open pollination produced similar or lower fruit-set probabilities. Crop identity also had a significant effect, with squash exhibiting higher fruit set than cucumber ($\beta = 0.62 \pm 0.24$, $z = 2.62$, $p = 0.0088$), while watermelon showed intermediate values. Bee abundance and bee species richness had no detectable effect on fruit set ($p > 0.10$). Random variation among fields was modest (SD = 0.16). In agroecological cucumber, open pollination increased fruit-set probability from 0.39 to 0.60, whereas in conventional cucumber fruit set decreased slightly under open pollination (0.50 → 0.38). In agroecological squash, open pollination also produced higher fruit set (0.54 → 0.68), while conventional squash maintained the lowest fruit-set probabilities overall (0.31 → 0.42). In watermelon, fruit set increased under open pollination in agroecology (0.53 → 0.70) but remained very low under conventional management (0.34). Overall, the combined statistical and visual evidence demonstrates that pollination success is strongly shaped by agricultural management, with agroecological fields supporting substantially higher fruit set, especially in squash, while conventional fields experience pollination limitation.

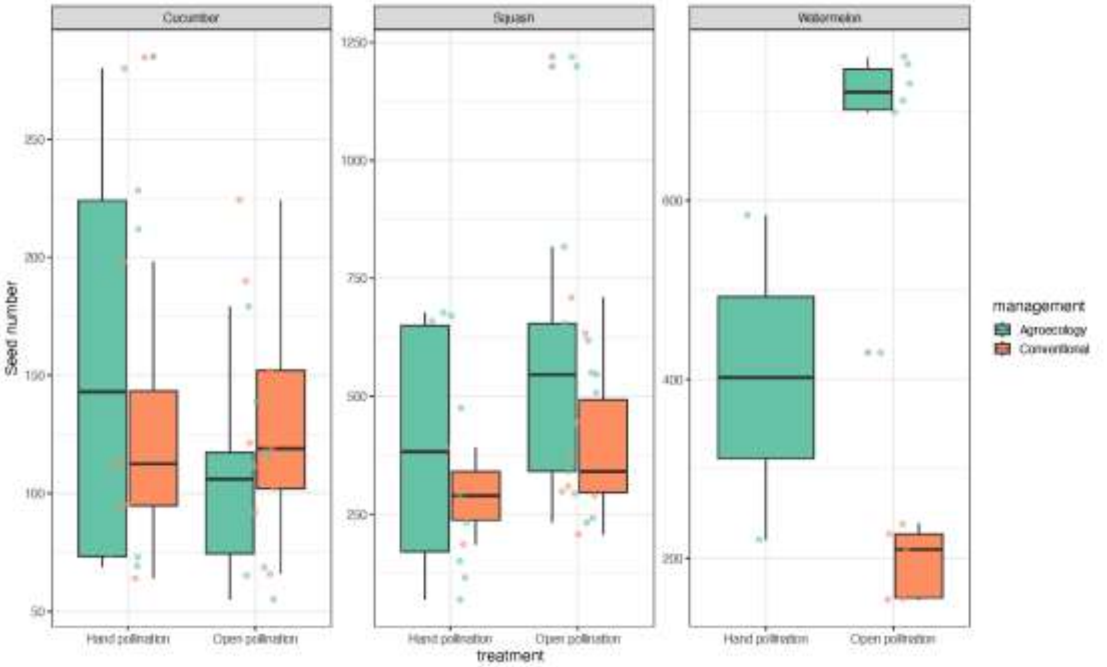


Fig. 13: Seed number under hand vs. open pollination across agricultural management for cucumber, squash, and watermelon. The negative binomial mixed model (GLMM; n = 155, 5 fields) showed strong crop effects (squash: $p < 0.001$; watermelon: $p< 0.001$) and significant treatment × crop interactions, with open pollination increasing seed number in squash ($p = 0.0026$) and watermelon ($p = 0.013$).

Seed number (Fig. 13) varied significantly across crops, management systems, and pollination treatments, as shown by the negative-binomial GLMM. The model indicated that open pollination produced substantially more seeds overall compared with hand pollination in certain crops, with strong treatment × crop interactions (squash: $\beta = 0.63 \pm 0.21$, $p = 0.003$; watermelon: $\beta = 0.76 \pm 0.31$, $p = 0.013$), demonstrating that the effect of pollination treatment depended strongly on crop identity. However, no three-way interaction between management, treatment, and crop was detected, indicating that management did not modify the crop-specific responses to pollination treatment. Bee abundance and bee species richness had no detectable effect on seed number ($p > 0.62$), and random variation among fields was modest (SD = 0.15). Overdispersion was not detected (dispersion ratio = 1.09, $p = 0.584$). In agroecological cucumber, hand pollination produced slightly higher seed numbers (140 vs. 110 seeds), whereas in conventional cucumber both treatments generated similar seed sets (126 vs. 125 seeds). In squash, open-pollinated flowers produced markedly more seeds than hand-pollinated flowers in both management systems (agroecological: 533 vs. 362; conventional: 394 vs. 208). In watermelon, agroecological fields supported very high natural pollination (open pollination = 577 seeds), while conventional watermelon produced no hand-pollinated fruits, preventing model estimation for that combination. Overall, the combined statistical and visual evidence demonstrates that pollination success in terms of seed number is strongly crop-specific, with particularly pronounced pollination deficits in conventional watermelon fields.

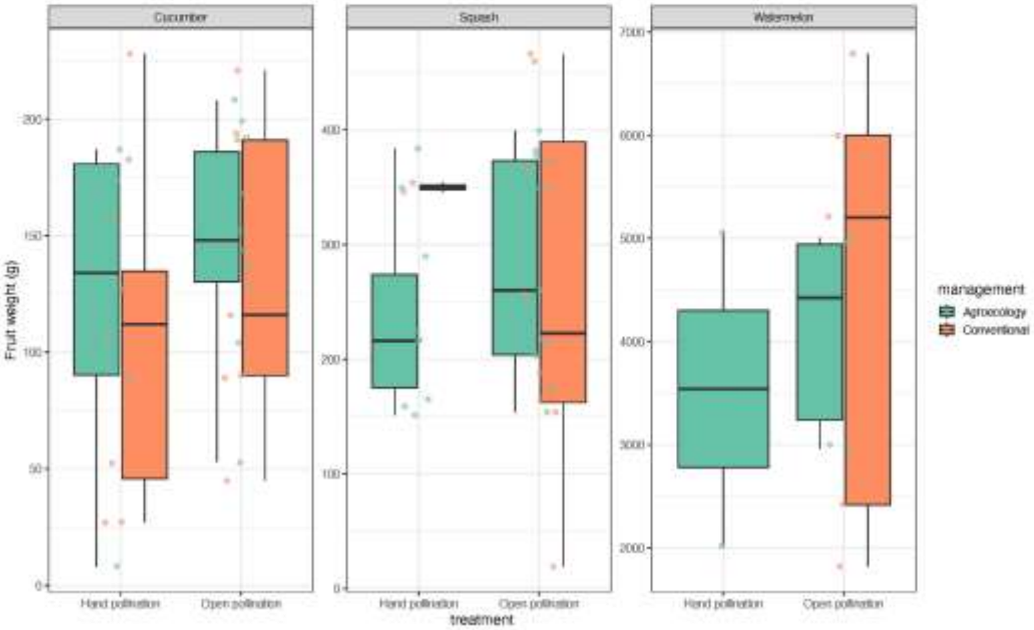


Fig. 14: Fruit weight responses to pollination treatment and agricultural management. The linear mixed model (*n* = 155) showed large crop differences (squash lighter, $p < 0.001$; watermelon much heavier, $p < 0.001$). Open pollination significantly increased fruit weight in squash ($p < 0.001$). Management had no overall effect ($p = 0.77$), although conventional fields produced heavier watermelon ($p = 0.032$).

Fruit weight (Fig. 14) varied strongly among crops and management systems, with additional differences between pollination treatments, as indicated by the linear mixed model. Across all crops, management alone had **no significant main effect on fruit weight** (β = –0.053 ± 0.358, t = –0.15, p = 0.88), nor did pollination treatment (β = 0.397 ± 0.326, t = 1.22, p = 0.23). Similarly, bee abundance and bee richness had no detectable influence on fruit weight (p > 0.75). There were also evident interaction effects between pollination treatment and crop: in squash, open-pollinated fruits tended to be heavier than hand-pollinated fruits (β = –0.27 ± 0.41, t = –0.66), although this effect was not statistically significant, and no such pattern occurred in cucumber or watermelon. Management interactions were similarly weak, and no three-way interaction could be reliably estimated due to rank deficiency in the model matrix.Visual inspection of the boxplots confirmed these patterns. In cucumber, fruit weights varied moderately across management systems and pollination treatments, with no consistent directional differences. In squash, agroecological fields produced relatively larger fruits in both treatments, aligning with the higher seed numbers observed in this system. In watermelon, the expected pollination effect was overshadowed by crop size differences: fruits from conventional fields were generally the heaviest, regardless of treatment, suggesting that factors unrelated to pollination—such as crop vigor, resource availability, or management inputs—dominated fruit-weight outcomes in this crop. Random variation among fields was modest (SD = 0.14), and the model showed no major convergence issues aside from a non-estimable coefficient in the full interaction.

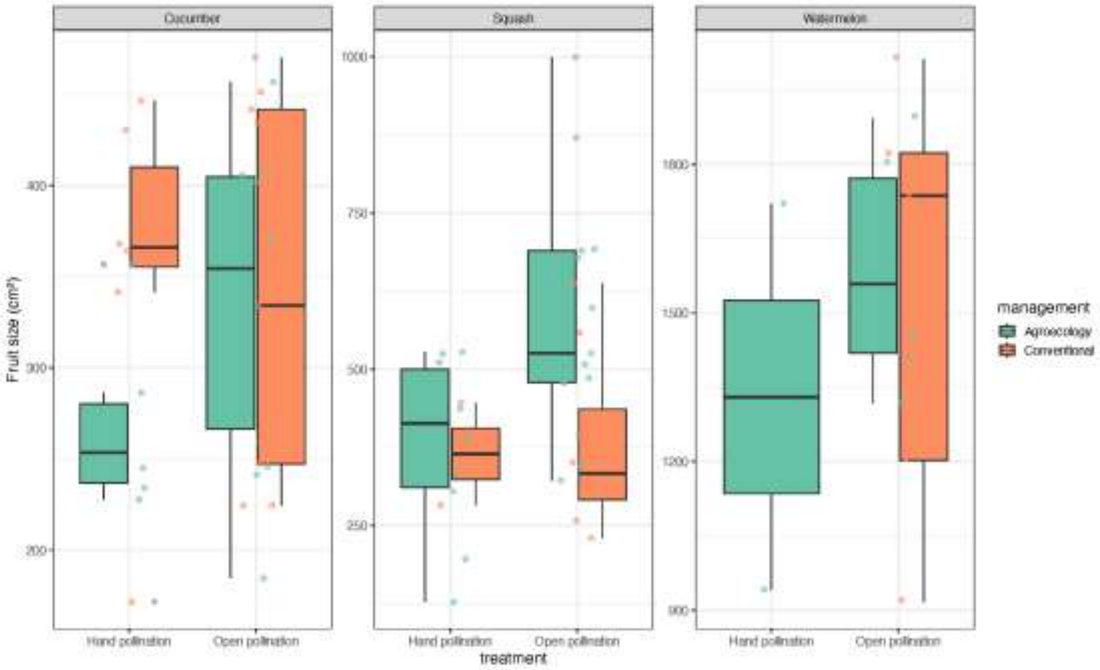


Fig. 15: Fruit size across crops, pollination treatments, and agricultural management. The linear mixed model (*n* = 155) was dominated by crop differences, with watermelon producing far larger fruits ($p < 0.001$). Open pollination increased fruit size in squash ($p = 0.0006$).

Fruit size (Fig. 15) varied across crops and pollination treatments, as confirmed by the linear mixed-effects model based on log-transformed size. Pollination treatment also significantly influenced fruit size depending on crop identity: in squash, open-pollinated fruits were considerably larger than hand-pollinated fruits (treatment × squash: $\beta = 0.26 \pm 0.22$, $t = 1.20$), while the treatment effect was negligible in cucumber and watermelon. No main effect of management was detected ($\beta = 0.27 \pm 0.18$, $t = 1.54$, $p > 0.12$). In agroecological squash, open pollination produced noticeably larger fruits than hand pollination, whereas conventional squash showed only modest differences. Cucumber displayed a mild tendency toward larger fruits under open pollination in both management systems, though the effect size was small. Watermelon produced consistently large fruits across all treatments and management systems, reflecting the negligible influence of pollination treatment on size in this crop. Overall, both the model and the figure indicate that pollination contributes strongly to fruit size in squash, moderately in cucumber, and minimally in watermelon, with little evidence that management influences fruit size once crop identity and treatment are accounted for.

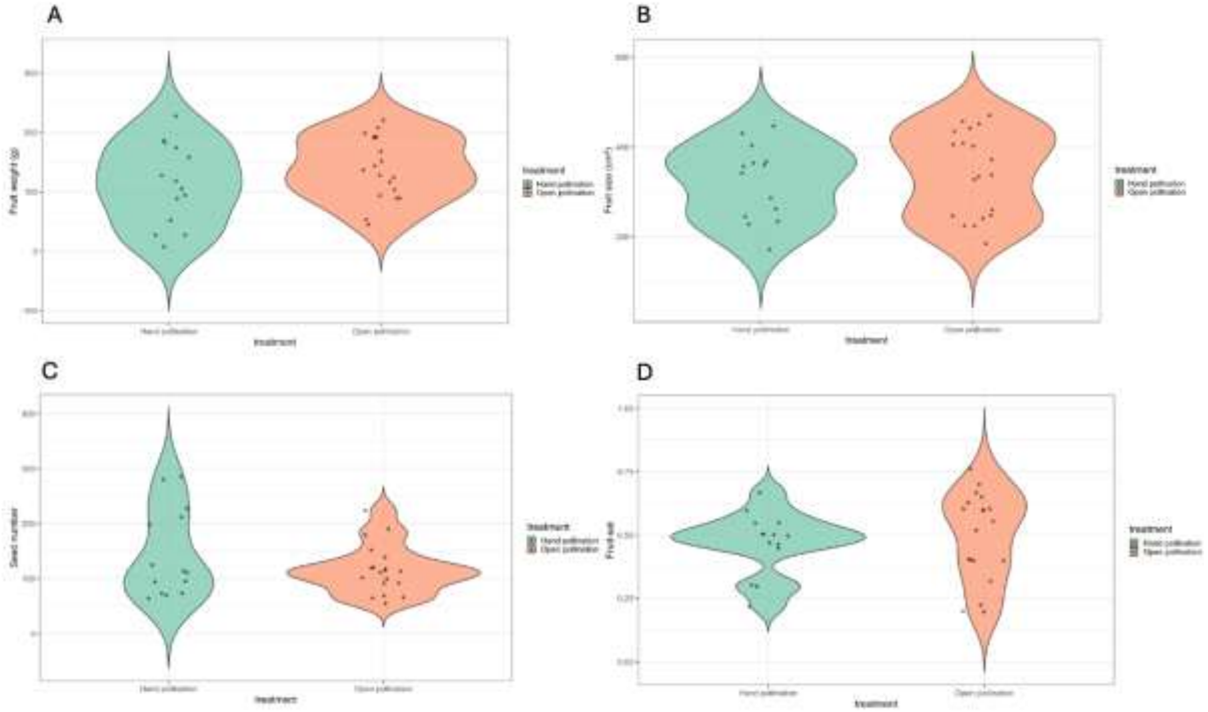


Fig. 16: Violon plots displaying: A) the distribution fruit weights, B) differences in fruit size (cm²), C) distribution of seed number and D) proportion of fruit set of cucumber across pollination treatments (open Vs hand pollination)

All violin plots (Fig. 16) show very similar distributions between hand pollination and open pollination in cucumber flowers. Seed number does not differ between treatments (p = 0.16), and fruit weight shows no significant treatment effect (p = 0.22). Fruit set is actually similar under open pollination and under hand pollination (p = 0.60), and fruit size is also not significantly greater for open-pollinated fruits (p = 0.53). The statistics confirm the absence of a pollination deficit: hand pollination did not improve fruit production, and natural pollination is equal to or superior to it.

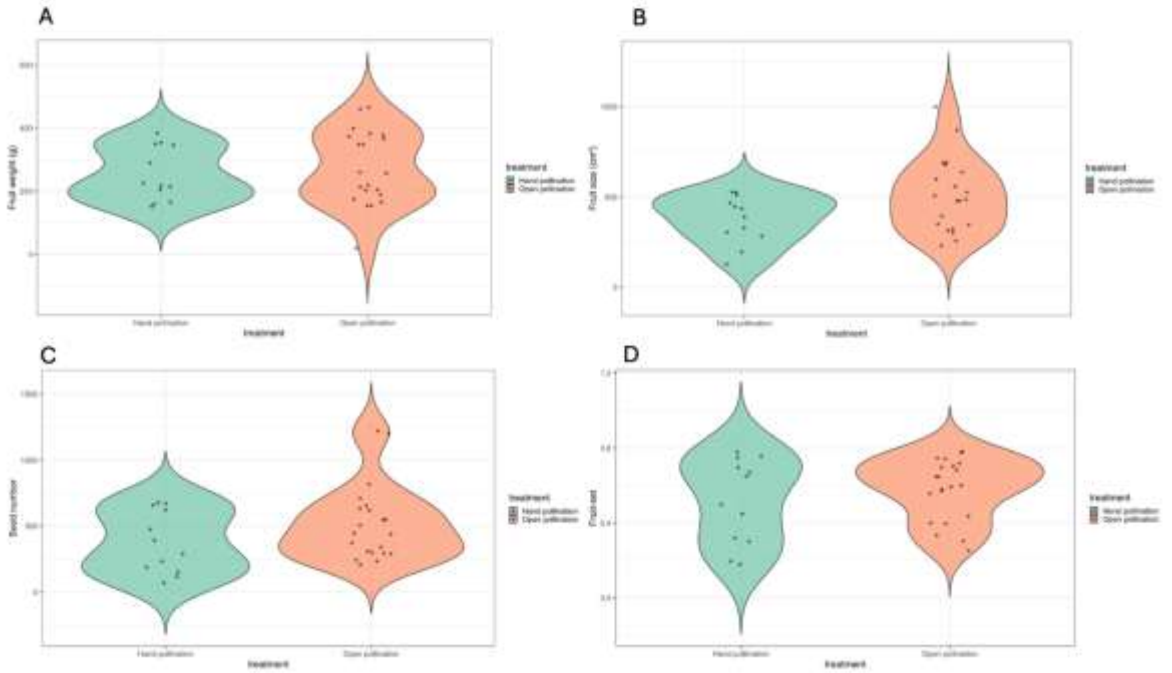


Fig. 17: Violon plots displaying: A) the distribution fruit weights, B) differences in fruit size (cm²), C) distribution of seed number and D) proportion of fruit set of squash across pollination treatments (open Vs hand pollination).

The results (Fig. 17) indicate that open-pollinated squash flowers outperform hand-pollinated flowers across all metrics. The violin plots show that the number of seeds is significantly higher under open pollination ($p = 0.043$), fruit set is much higher for open pollination than for hand pollination ($p = 0.0013$), and both fruit weight ($p = 0.90$) and fruit size ($p = 0.048$) are greater under open pollination. These results demonstrate that squash female flowers receive sufficient pollen through natural pollination, indicating no pollination deficit for this crop.

References:

Bomfim, I.G.A. *et al.* (2015) “Pollination requirements of seeded and seedless mini watermelon varieties cultivated under protected environment,” *Pesquisa Agropecuaria Brasileira*, 50(1), pp. 44–53. Available at: https://doi.org/10.1590/S0100-204X2015000100005.

Delapane, K.S. and Mayer, D.F. (2000) *CROP POLLINATION BY BEES*.

Garratt, M.P.D. *et al.* (2014) “The identity of crop pollinators helps target conservation for improved ecosystem services,” *Biological Conservation*, 169, pp. 128–135. Available at: https://doi.org/10.1016/j.biocon.2013.11.001.

Garratt, M.P.D. *et al.* (2021) “Opportunities to reduce pollination deficits and address production shortfalls in an important insect-pollinated crop,” *Ecological Applications*, 31(8). Available at: https://doi.org/10.1002/eap.2445.

Grab, H. *et al.* (2019) "Agriculturally dominated landscapes reduce bee phylogenetic diversity and pollination services," *Science*, 363(6424), pp. 282–284. Available at: https://doi.org/10.1126/science.aat6016.

Nepi, M. and Pacini, E. (1993) "Pollination, Pollen Viability and Pistil Receptivity in Cucurbita pepo," *Annals of Botany*, 72, pp. 527–536.

Sawe, T. *et al.* (2020) "Inadequate pollination services limit watermelon yields in northern Tanzania," *Basic and Applied Ecology*, 44, pp. 35–45. Available at: https://doi.org/10.1016/j.baae.2020.02.004.

Pollen loads in flower flies and honey bees

These results are based on the manuscript "Fields of influence: how agricultural landscapes shape pollen loads in flower flies (Diptera: Syrphidae)" (Kabota et al.) have been submitted for publication to an international scientific journal with IF.

Methodology

Pollen loads were characterized from 245 specimens of honeybees (Apis mellifera) and hoverflies (Paragus borbonicus, Toxomerus floralis) collected across 20 family farms in Morogoro, Tanzania. Farms were managed under agroecological or conventional practices and located in two contrasting landscapes (plateau vs. mountainous). Pollen adhering to insect bodies and honeybee corbicular pellets was isolated and profiled using rbcL DNA metabarcoding. Taxonomic assignment was performed against a composite reference database integrating a public rbcL reference library (Bell et al., 2017), locally generated plant barcodes, and GenBank sequences. Pollination networks were constructed at genus and family levels using variance-stabilized interaction matrices. Community-level differences were tested via PERMANOVA, and differential abundances of pollen taxa were assessed using ANCOM-BC2.**Scientific Results**

General pollen patterns:

- Thirteen pollen families accounted for ~95% of reads, dominated by Cucurbitaceae (75.8%), followed by Fabaceae (6.9%) and Asteraceae (5.4%). At genus level, *Cucumis* (34.8%), *Lagenaria* (29.1%), and *Cucurbita* (11.9%) were most abundant, confirming strong crop association in pollen loads.

Pollination networks:

- Network metrics indicated moderate nestedness and low connectance, suggesting a structure dominated by generalist species but with some specialized interactions.
- *Apis mellifera*, *T. floralis*, and *P. borbonicus* were central connectors, showing high species strength and low specialization scores (0.00–0.29), confirming their generalist roles.
- Families such as Cucurbitaceae, Asteraceae, Brassicaceae, Poaceae, and Solanaceae acted as network hubs, supporting numerous insect taxa.

Differences among insect groups:

- PERMANOVA revealed insect group as the strongest driver of pollen composition (explaining 7.6–15.8% of variation), followed by interactions with landscape and farming practices.

- ANCOM showed that both hoverfly species carried significantly less crop-associated pollen (Cucurbitaceae, Fabaceae) than honeybees. *T. floralis* also showed higher proportions of Streptophyta pollen.

Sex-specific differences:

- Females of both hoverfly species carried more pollen from Asteraceae and Commelinaceae, while males had higher proportions of grasses and cucurbits.
- In *P. borbonicus*, Musaceae pollen was lower in females; in *T. floralis*, Musaceae and Myrtaceae were higher in females, while Asteraceae and Rutaceae were lower.

Landscape effects:

- Pollen assemblages differed between mountainous and plateau sites. Musaceae was consistently higher in mountainous landscapes for syrphid females, while Poaceae and Streptophyta were lower. Honeybees showed increased Fabaceae and Pinaceae pollen at higher altitudes.

Farming practices:

- Farming effects were subtle but detectable. In honeybees, Asteraceae and Rubiaceae were more abundant in agroecological farms, while Muntingiaceae and Streptophyta were lower. Hoverflies showed limited responses, with only a few taxa (e.g., *Lagenaria* in *T. floralis* females) differing between farming systems.

Interpretation:

These findings show the complementary roles of bees and wild hoverflies in pollination networks. Honeybees concentrate on crop pollen, while hoverflies exploit a broader range of plant resources, including wild taxa. Agroecological practices influenced honeybee pollen profiles more than hoverflies, suggesting that hoverflies may be less sensitive to farm-level management and more responsive to landscape-scale floral diversity. This functional complementarity underscores the importance of maintaining diverse pollinator communities to ensure resilient pollination services.

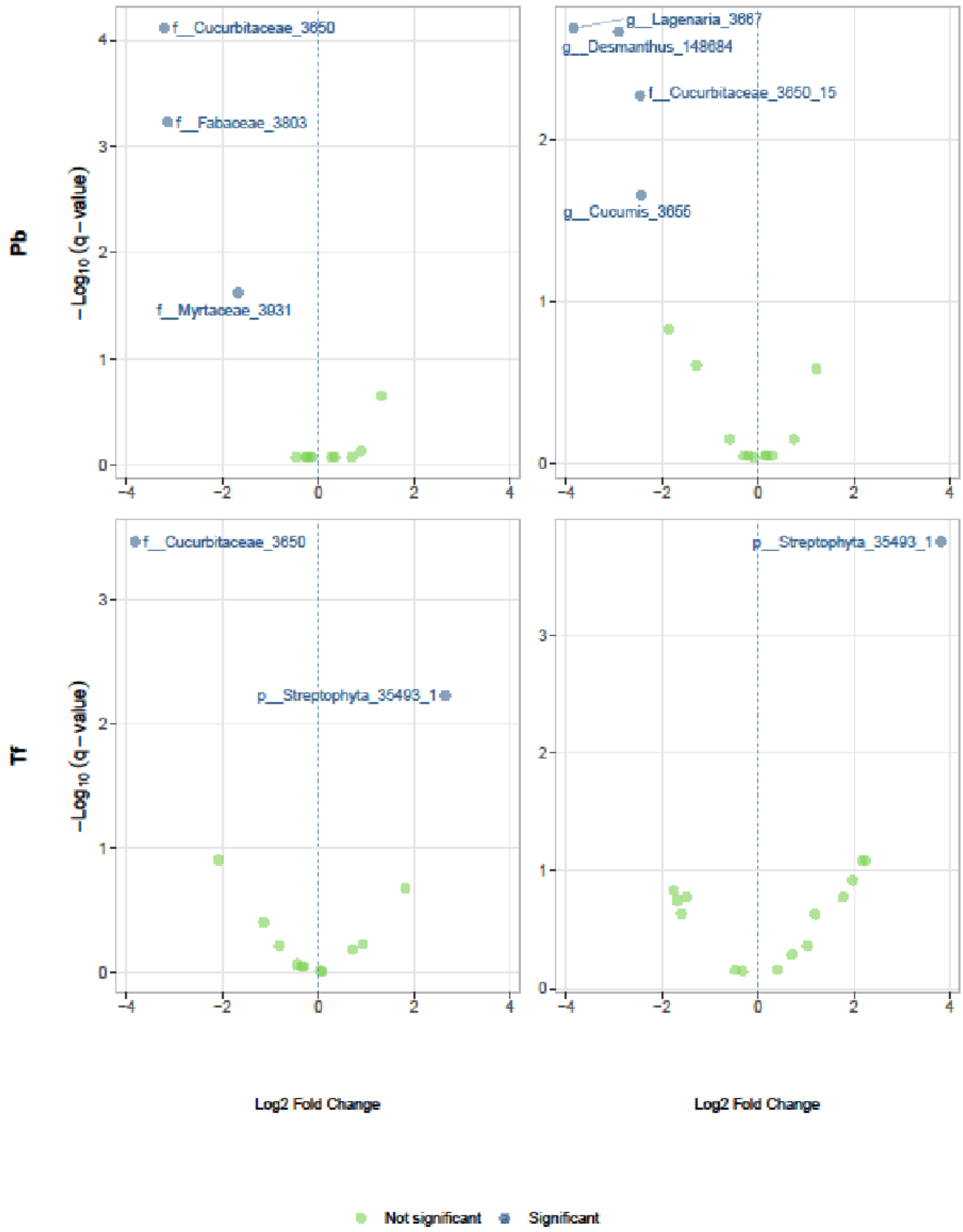


Figure 3.2.1: Volcano plots of ANCOM results comparing pollen composition in hoverflies (Pb: *P. borbonicus*, Tf: *T. floralis*) vs. Honeybees (*A. mellifera*).

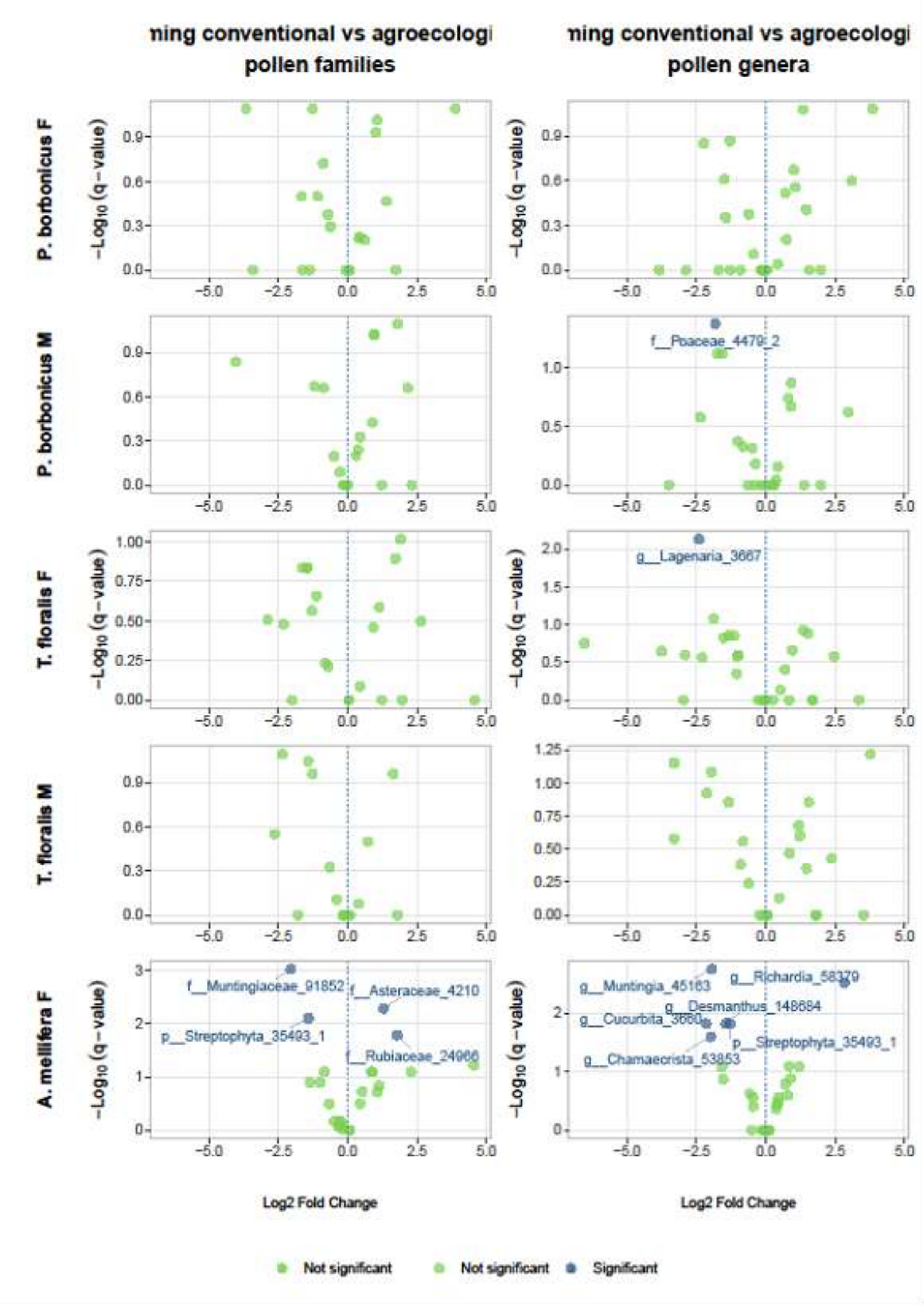


Figure 3.2.2: Volcano plots showing pollen taxa differing between agroecological and conventional farms. *Honeybee pollen profiles were more responsive to farming practices than hoverflies.*

References

- Bell KL, Loeffler VM, Brosi BJ. (2017). An rbcL reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Applications in Plant Sciences*, 5(3): apps.1600110.
- Kabota S et al. (2025). Diversity of hoverflies and their floral visitation patterns in cultivated cucurbit crops. *Entomol Exp Appl*, 1–14.
- Rader R et al. (2016). Non-bee insects are important contributors to global crop pollination. *PNAS*, 113:146–151.
- Cullen N et al. (2021). Diversity and composition of pollen loads carried by pollinators. *Oecologia*, 196:131–143.
- Ponisio LC et al. (2015). Diversification practices reduce organic-to-conventional yield gap. *Proc R Soc B*, 282:20141396.
- Fantinato E et al. (2019). Network robustness and pollination redundancy. *Sci Rep*, 9:33652.

Pollen loads in wild bees

Methodology

- A total of 133 specimens of wild bees were sampled to characterize pollen loads of wild bees visiting cucurbits. They represented 28 (morpho)species collected in October 2022 (season 2 of 2022). They included five to ten specimens per species for the most abundant species, and a few representatives of some less abundant genera (not all species could be sampled). One negative control was included in the analysis and gave no results as expected. All bees were collected in farms managed under agroecological or conventional practices and located in two contrasting landscapes (plateau vs. mountainous). Pollen adhering to insect bodies was isolated and profiled using rbcL DNA metabarcoding. Data cleaning and taxonomic assignment was performed in R v4.4.0, using the package DADA2 v1.34.0 (Callahan et al., 2016) against a composite reference database integrating a public rbcL reference library (Bell et al., 2017), locally generated plant barcodes, and GenBank sequences. Statistical tests included Mann–Whitney U tests and permutational multivariate analysis of variance (PERMANOVA). They were based pollen rbcl amplicon sequence variants (ASV) and on assigned plant families and were performed using Shannon and Simpson measures of species richness (alpha diversity), and Bray-Curtis and Jaccard indices (beta diversity), respectively, using R packages Phyloseq v1.50.0 (McMurdie & Holmes 2013) and vegan v2.7.2 (Oksanen et al. 2025). Network analyses based on Jaccard indices were constructed using Phyloseq v1.50.0 (McMurdie & Holmes 2013).

Scientific Results

General pollen patterns:

- Rbcl amplification and good quality DNA data were obtained from 103 of the 133 specimens sampled, representing 25 (morpho)species from 12 genera. Pollen DNA data of nine of these (morpho)species were obtained from five to ten wild bees. Thirteen plant families accounted for ca. 95% of the reads (**Figure 3.2.3**), with the top three family being Cucurbitaceae (45.2%), Asteraceae (28.6%) and Fabaceae (3.8%). At genus level, *Cucumis* (26.8%), *Lagenaria* (23.4%), Bidens (9.0%) and *Cucurbita* (6.4%) were most abundant, confirming a strong crop association in pollen loads, where Cucurbitaceae are dominant and where other taxa are also present, for example Asteraceae, which are preferentially visited by the wild bee *Seladonia*, and in particular the genus *Bidens* that is very common in crops.

Differences among wild bee taxa:

- Proportions of plant families (including proportions of Cucurbitaceae) detected in wild bees varied both within and among species (**Figures 3.2.4 and 3.2.5**). Among nine morphospecies represented by five to ten wild bees, proportions of DNA reads assigned to Cucurbitaceae were considerably higher in the pollen loads of *Lasioglossum* (*Ipomalictus*) *pinnatum*, and lower in those of *Seladonia jucunda* (morphospecies 1 and 2) (**Figure 3.2.5**).

Determinants of pollen loads composition:

- No significant differences were detected in overall pollen loads richness between fields managed with agroecological and conventional farming, or between fields at lower or higher altitudes (Shannon and Simpson measures).
- In contrast, significant differences in pollen loads compositions were detected among wild bee species, bee genera, altitudes, fields, and collection dates, but not among farming managements or types of cucurbit crop. Combined analyses showed that:
    - field and wild bee species (more than genus) significantly explained most of the variation among pollen loads (>35% together, with respective part impossible to determine due to the unbalanced sampling).
    - altitude alone significantly explained ca. 4.5% of the variation among pollen loads (p=0.001), while farming management seemed to have a small (ca. 1.5%) non-negligible (p=0.043) effect when considered alongside altitude with Bray-Curtis index (not with Jaccard index).
    - the contribution of the collection dates to the variation among pollen loads is not significant anymore when combined with the field variable (probably meaning that the significant effect observed above for the collection date was biased by the fact that specific fields were sampled at specific dates, and therefore rejecting a strong effect of contamination during sampling and sample manipulation).
- In the network analyses, pollen loads showing more similar compositions (connected in the network with a maximum Jaccard value of 0.5) were not determined by one single variable (wild bee species, genus, altitude, farm management, field). Connections corresponded most of the time to altitude, bee species (or genus) and fields (**Figures 3.2.6-9**).

Interpretation:

- Even if pollen loads are not representing pollination efficiency, these results represent useful resources to evaluate the potential importance of wild bee pollination for cucurbit cultures. Preliminary knowledge about the visiting behaviour of some wild bees was confirmed, with more specialized species like *Lasioglossum (Ipomalictus) pinnatum* carrying a larger proportion of cucurbit pollen compared to more generalist species like *Plebeina armata*, *Braunsapis fascialis*, *Braunsapis trochanterata* or *Ceratina (Copoceratina*) (morphospecies "MOR1"). In contrast *Seladonia jucunda* (morphospecies 1 and 2) carried generally lower proportion of cucurbit pollen. These findings provide a first detailed picture of pollen loads for some generalist and specialist wild bee species occurring in Tanzania. This specific study also provides preliminary data about individual differences in pollen loads, which represent part of the intraspecific variation, and may reflect differences in the flora around the cultures.
- Based on the current sampling, pollen loads compositions were mainly influenced by the field where the bee was caught and the bee species. However, small but significant contribution of the altitude was observed on pollen loads composition, and possible non-negligible effect of farm management.
- The cucurbit pollen profiles observed here suggest that wild bees considerably contribute to cucurbit crop pollination. The diversity of the pollen-load compositions across bee species

confirms that not only bee abundance but also wild bee diversity is a key factor for efficient and resilient pollination. These conclusions suggest clear yield benefits to Tanzanian crop farmers as far as their agrological practices are promoting biodiversity in general, and particularly the one of wild bee communities.

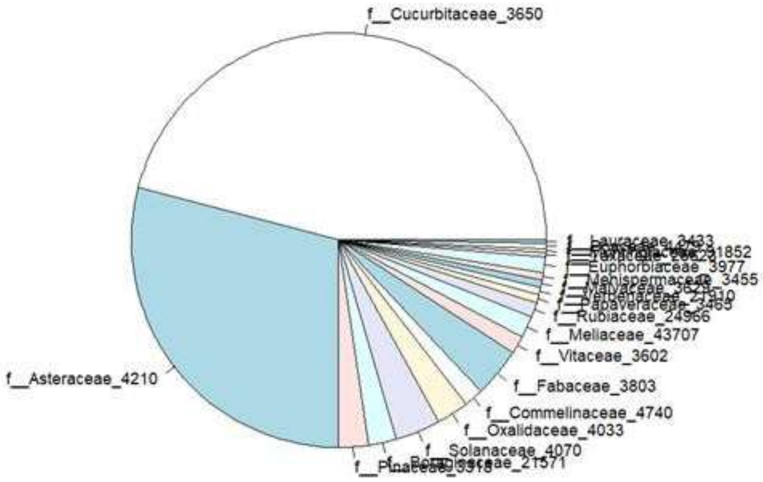


Figure 3.2.3 Pie chart representing the relative abundances of rbcl plant reads detected on all wild bees investigated here.

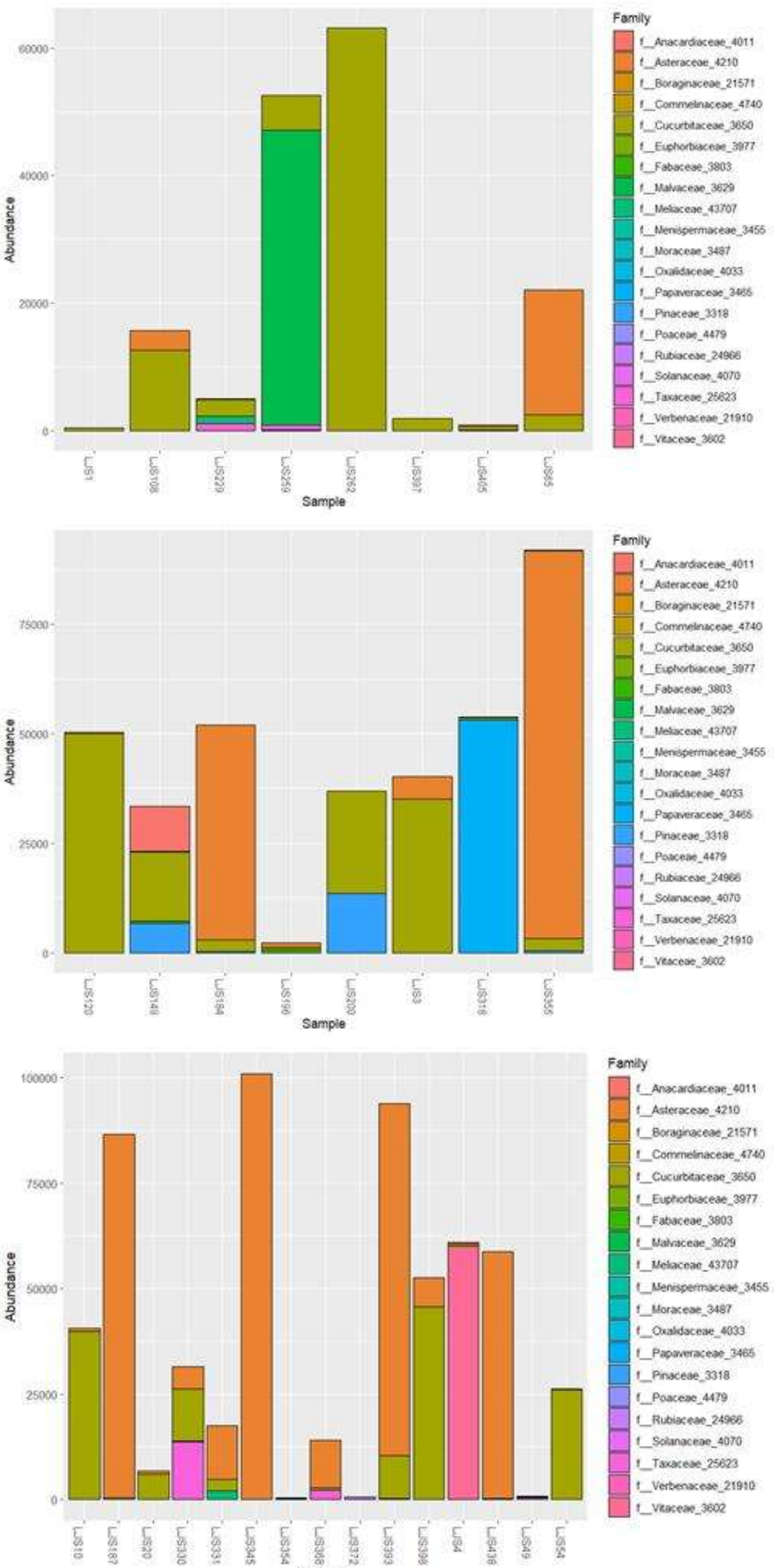


Figure 3.2.4 Bar plots representing the proportions of the 20 most represented plant families (in term of rbcl read abundances) detected in specimens of *Lasioglossum* (*Ipomalictus*) *pinnatum* (top), *Plebeina armata* (middle) and *Seladonia jucunda* (bottom).

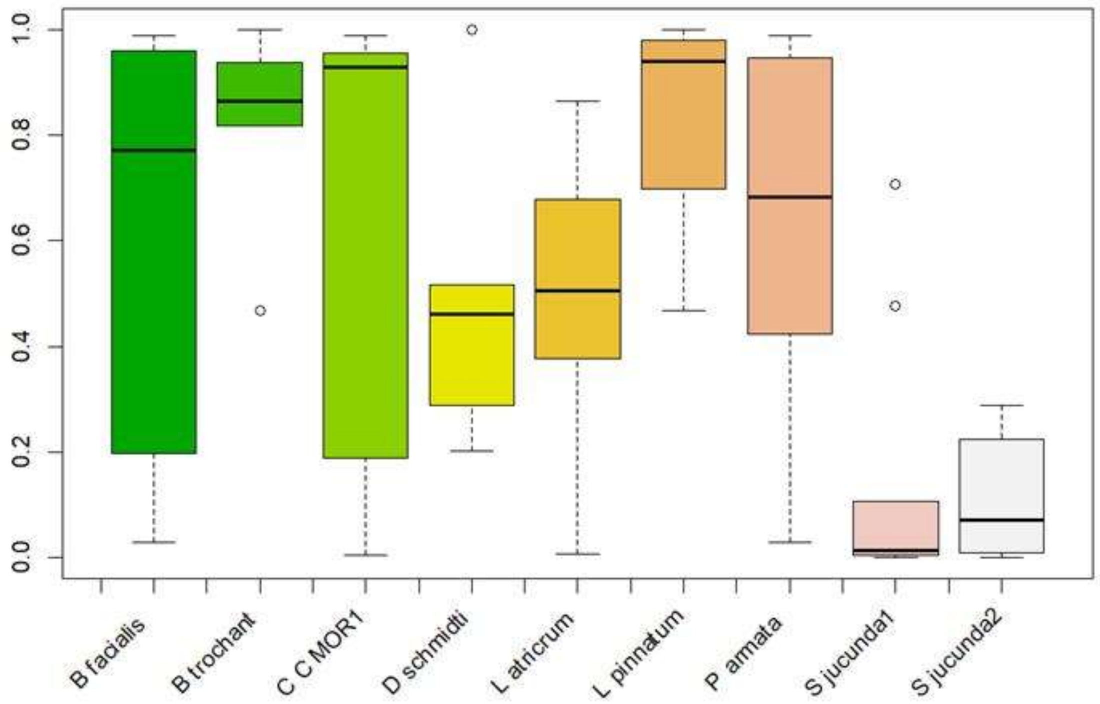


Figure 3.2.5 Box plots representing the proportion of cucurbit DNA detected in nine wild bee morphospecies for which more than five specimens were sampled (*Braunsapis facialis*, *B trochanterata*, *Ceratina (Copoceratina)* morphospecies “MOR1”, *Dactylurina schmidti*, *Lasioglossum (Ctenonomia) atricrum*, *Lasioglossum (Ipomalictus) pinnatum*, *Plebeina armata*, *Seladonia jucunda* morphospecies “1” and *S. jucunda* morphospecies “2").

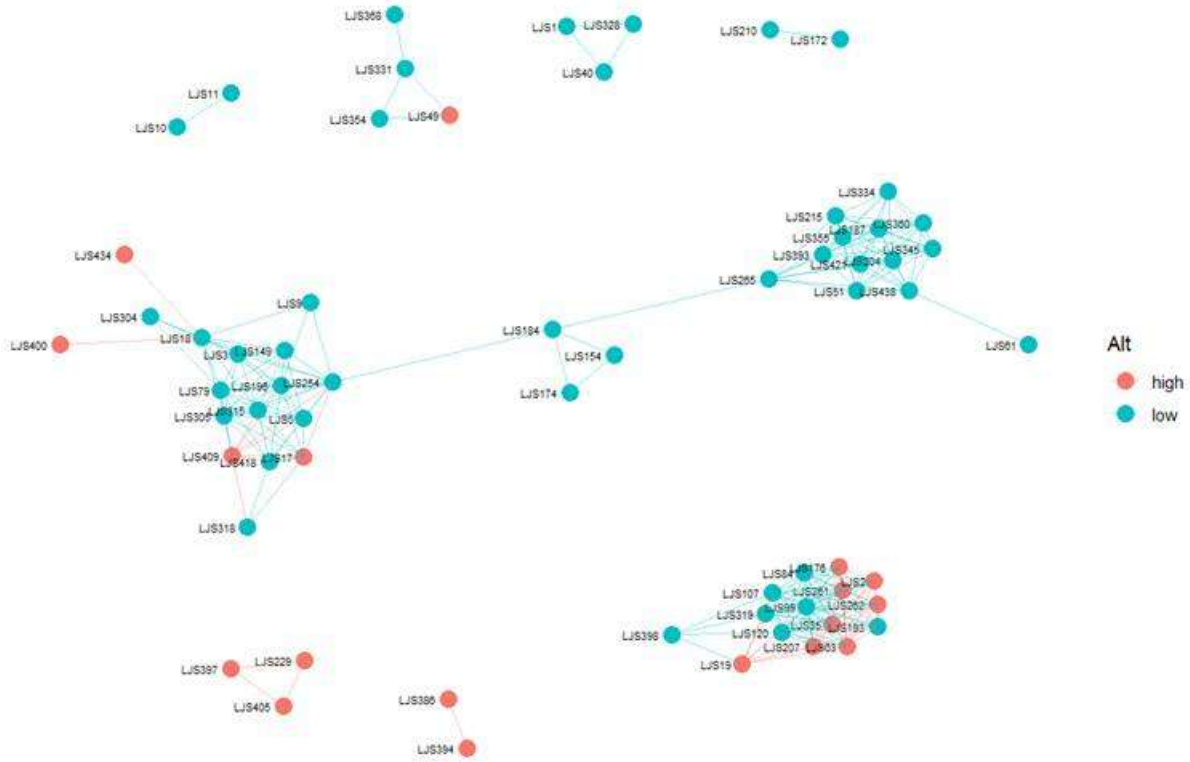


Figure 3.2.6 Network showing connections between samples with more similar pollen loads compositions (Jaccard index ≤ 0.5), Samples are coloured according to altitude.

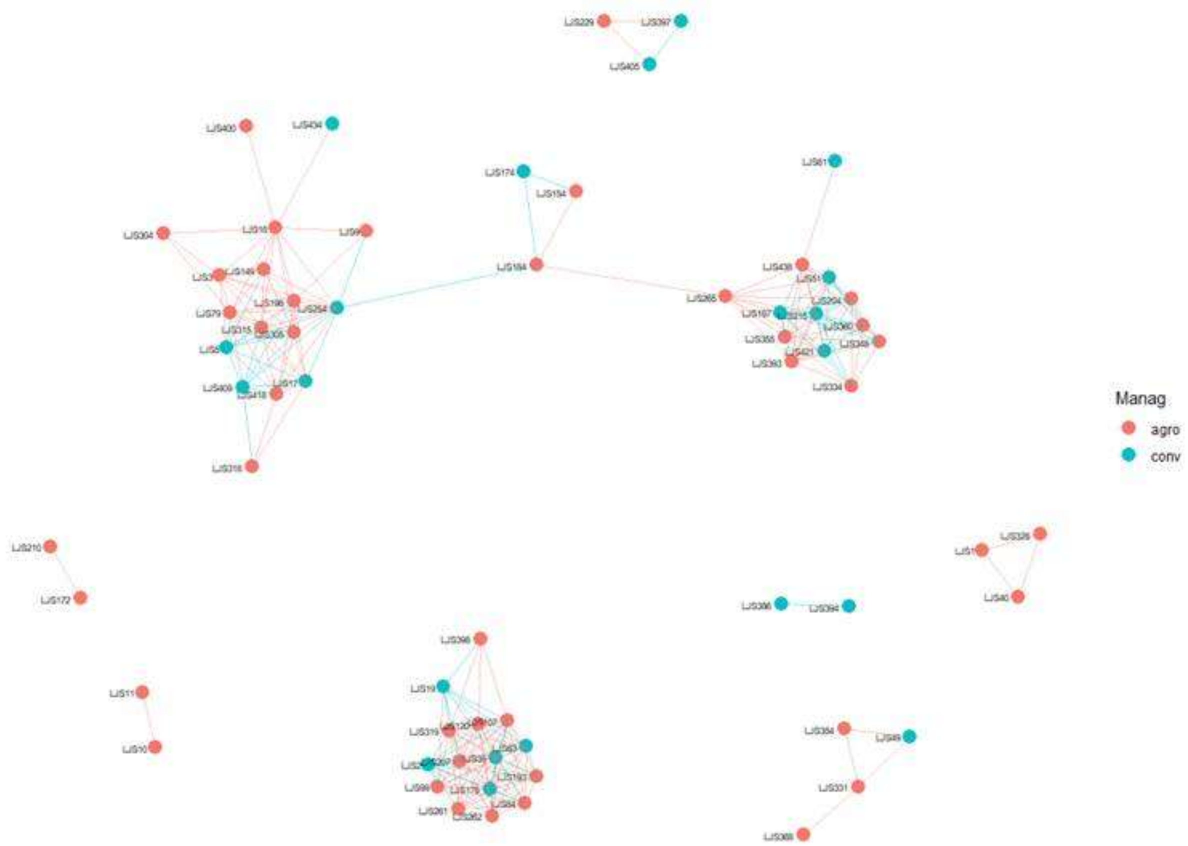


Figure 3.2.7 Network showing connections between samples with more similar pollen loads compositions (Jaccard index ≤ 0.5), Samples are coloured according to the type of farm management (agro: agroecological practices; conv: conventional practices).

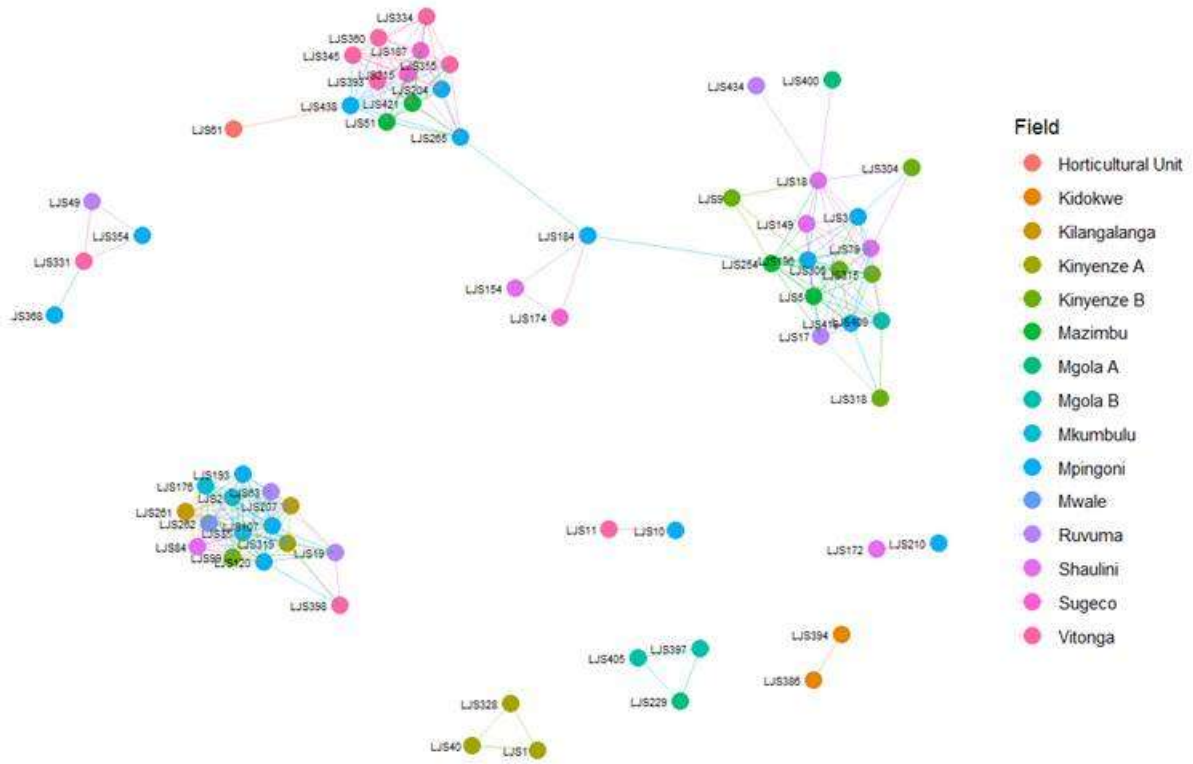


Figure 3.2.8 Network showing connections between samples with more similar pollen loads compositions (Jaccard index ≤ 0.5), Samples are coloured according to the field where it was collected.

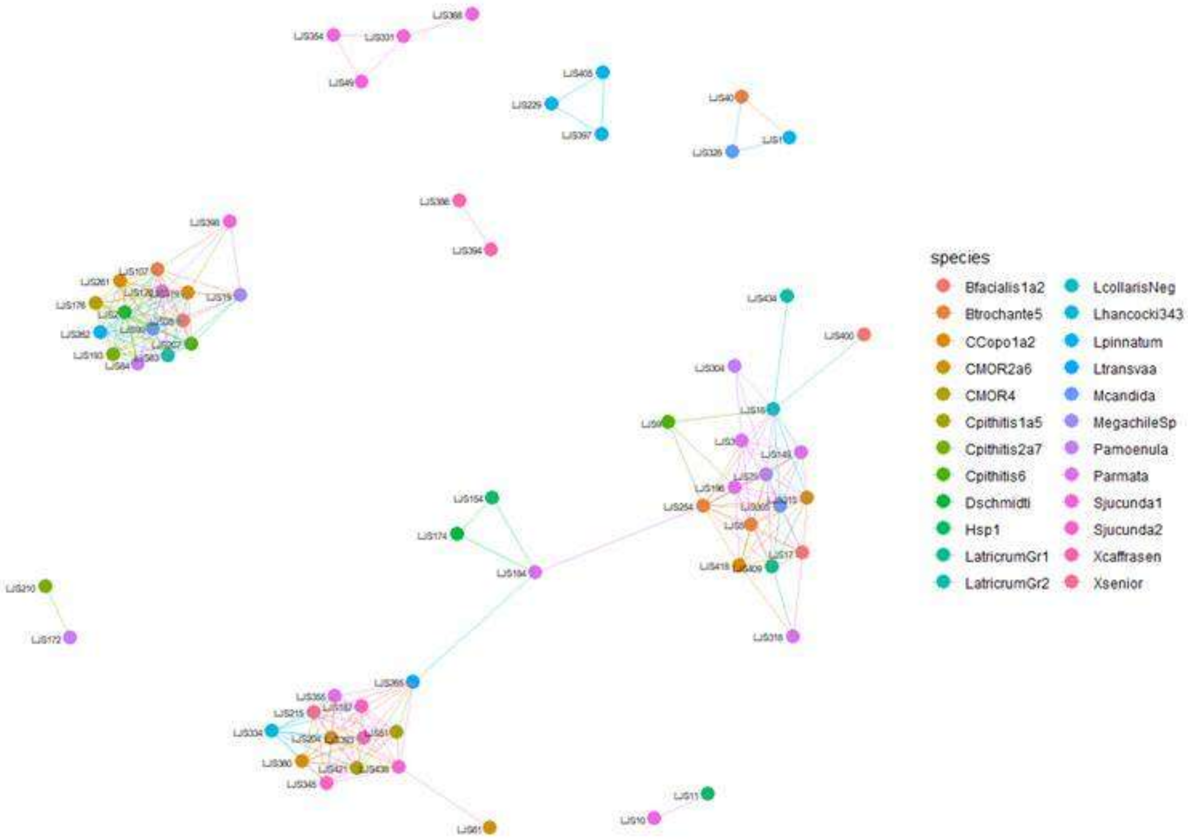


Figure 3.2.9 Network showing connections between samples with more similar pollen loads compositions (Jaccard index ≤ 0.5), Samples are coloured according to the bee species.

References

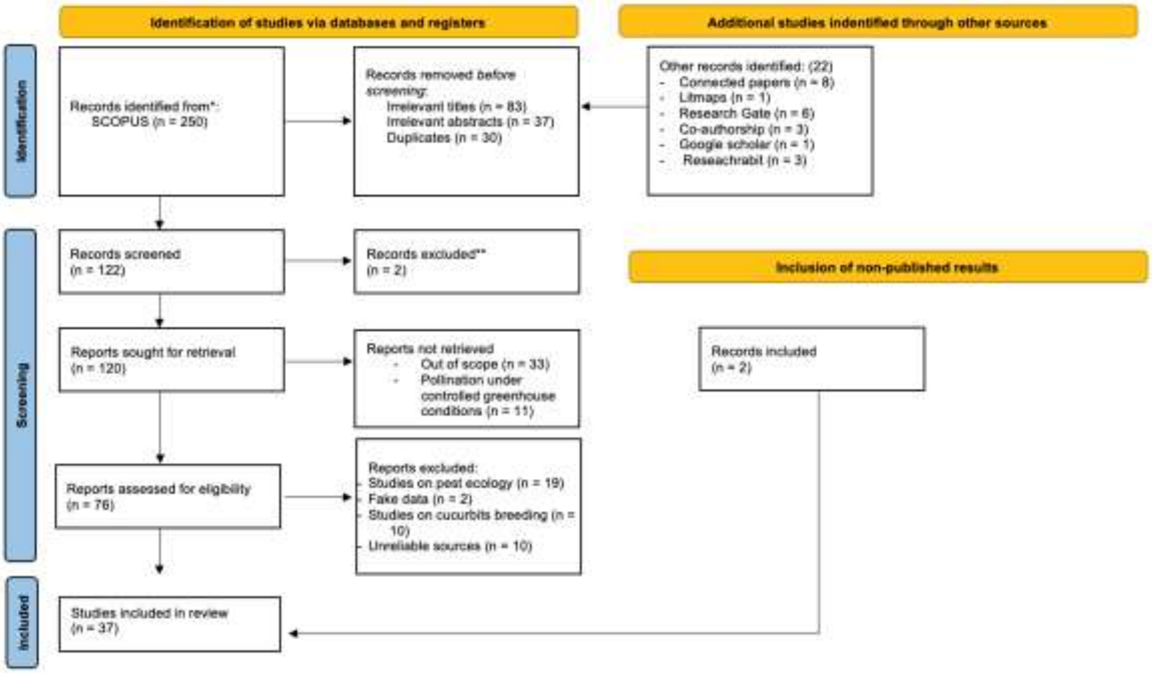
- Callahan BJ et al. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7): 581–583.
- Bell KL et al. (2017). An *rbcL* reference library to aid in the identification of plant species mixtures by DNA metabarcoding. *Applications in plant sciences*. 5(3): apps.1600110
- McMurdie & Holmes. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*. 8(4): e61217.
- Oksanen J et al. (2025). vegan: Community Ecology Package, https://CRAN.R-project.org/package=vegan.

Task 3.3. Review of crop pollination research in Africa with a focus on cucurbits

Literature review and data collection:

Materials & Methods

Search protocol and data collection:
A comprehensive literature search was conducted in scientific journals to identify flower visitors of cucurbits across all 54 African Countries, with a focus on both common cucurbit food crops and wild species in Sub-Saharan region. Relevant terminology related to crops, cucurbit species, pollination, pollinators, and insect flower visitors, as well as country names in various languages, was employed during the search. From the articles selected in this search, we also consulted the literature cited within them, referred to as "Reference Retrieval in Additional Studies Identified Through Other Sources" in Fig. 1. Keyword searches and their combinations were utilized. These included "cucurbit*" OR "citrullus" OR "cucumis" OR "courge" OR "luffa" OR "Momordica" OR "Lagenaria" AND "Insects" OR "bees" OR "abeille*" OR "apis*" OR "abejas*" OR "abelha*" OR "poliniza*" OR "pollinat*" OR "pollinisa*" OR "flower visitor*" OR "visiteur flor*" OR "beetle" OR "bats" OR "butterflies" OR "ants" OR "hummingbirds" OR "hoverflies" OR "wasps" AND "Afri*" OR "Congo" OR "RDC*" OR "Ethiopia" OR "Gabon" OR "Ghana" OR "Guinea" OR "Madagascar" OR "Mozambique" OR "Rwanda" OR "Botswana" OR "Cameroon" OR "Comoros" OR "Mali" OR "Mauritius" OR "Namibia" OR "Senegal" OR "Somalia" OR "South Africa" OR "Sudan" OR "Zimbabwe" OR "Egypt" OR "Malawi" OR "Niger" OR "Nigeria" OR "Ivory Coast" OR "Kenya" OR "Algeria" OR "Tanzania" OR "Morocco" OR "Maroc" OR "Uganda" OR "Angola" OR "Central African Republic" OR "Chad" OR "Seychelles" OR "Sierra Leone" OR "Togo" OR "Zambia" OR "Lesotho" OR "Liberia" OR "Burkina Faso" OR "Burundi" OR "Equatorial Guinea" OR "Eritrea" OR "Eswatini" OR "Gambia" OR "Guinea-Bissau" OR "São Tomé and Príncipe" OR "Benin" OR "Cabo Verde" OR "Djibouti" OR "Libya" OR "Mauritania" OR "South Sudan" OR "Tunisia". The slected taxa of flower visitors were chosen based on their known importance as insect pollinators in natural entomophilous pollination. Articles used in our study were selected through an intensive search in the public databases Web of Science, Scopus, ResearchGate, and digitized bibliographic tools such as Connected Papers, Litmaps, and ResearchRabbit. Selection criteria: After conducting the literature search, article titles and abstracts were reviewed to determine whether they met the inclusion criteria (a. studies that assessed cultivated or wild cucurbit species flower visitors/pollinators and identified them to species level, b. taxonomic keys or checklist that reported the host plants of bees species listed and c. Shared Biodiversity-Knowledge Infrastructure that documented the bee-plant interaction) for the review. Subsequently, articles meeting these criteria were read in their entirety. After this, only articles that: 1- identified cultivated and wild cucurbit species pollinators or flower visitors to the species level or at least the morphospecies level; 2- specified which cucurbit species were visited or at least at the genus level; and, last but not least, mentioned the name of the country where the study was conducted were included. All the studies that met the inclusion criteria were therefore chosen for the analyses, while those not meeting the criteria were excluded (see Fig. 1).



Source: Page MJ, et al. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

Fig. 1 *:* PRISMA flow diagram illustrating the data compilation process for the systematic review. In the flowchart, the articles sourced from other references are retrieved via bibliographic digitized tools and from social networking sites where scientists and researchers share publications. The excluded articles are those that did not meet the evaluation criteria for our review.

The final database we obtained includes occurrences cleaned using spatial and taxonomic filtering. The species names were harmonised using the Discover Life online portal (Ascher & Pickering, 2024), a peer-reviewed global bee taxonomy framework available online integrated with the ITIS World Bee Checklist (National Museum of Natural History, Smithsonian Institution, 2024).

Data Analysis:

We started by combining published data on cucurbit flower visitors with FAO statistics on average cucurbit crop yields, visualizing the results as bivariate plots. Cucurbit yield data for cucumbers, melons, watermelons, and squashes was sourced from FAOSTAT. For each country, the total cucurbit area was calculated as the sum of the harvested areas across these four crops. To normalize yield values and mitigate biases from unequal land allocation, we computed a weighted cucurbit yield by multiplying each crop's yield by its land proportion and summing the results. To evaluate differences in flower visitor assemblage composition across biogeographic regions in the Sub-Saharan region and the status of cucurbits (food crop vs. non-food crop), we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using the adonis2 function in the vegan package (Oksanen et al., 2024). The analysis was based on Bray–Curtis dissimilarity matrices derived from presence/absence data of bee species visiting cucurbit (cultivated "food crop" and wild "non-food crop") flowers. This approach allowed us to test whether the multivariate centroids of bee assemblages differed significantly among biogeographic regions, crop usage types (food vs. non-food cucurbits), and their interaction. The significance of each term was assessed using 999 permutations under a reduced model, and the R^2 (Pearson coefficient) values were used to estimate the proportion of total variance explained by each factor. All analyses were complemented by non-metric multidimensional scaling (NMDS) ordination and β-diversity partitioning (turnover and nestedness) to visualize and interpret community patterns. To visualize and compare the structure of pollination networks across space, we constructed chord diagrams that depict the associations between flower visitor species and cucurbit species in the Sub-Saharan region, Zambezian region, and Tanzania with published and unpublished data. Each chord diagram connects a flower visitor species to the cucurbit species it visitsThis visualization approach complements quantitative β-diversity and PERMANOVA analyses by providing a clear graphical synthesis of how flower visitor–cucurbit associations vary both within and between geographic regions.

Results:

From a total of 36 articles published between 1993 and 2024, a total of 193 species of insects under four Orders (Hymenoptera (159), Lepidoptera (16), Diptera (12) and Coleoptera (5) (Figure2)

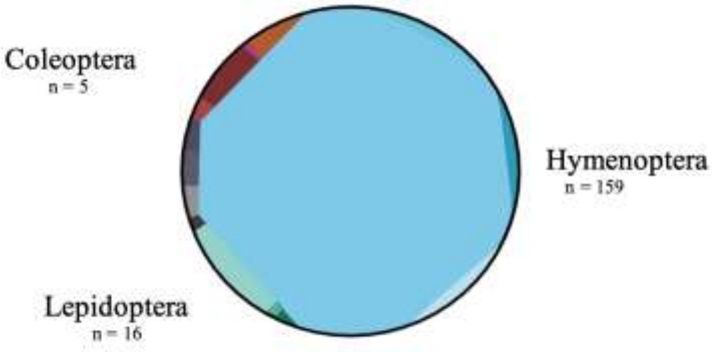


Fig. 2: Voronoi diagram showing the most important flower visitors among cucurbit flower visitors; Order: Hymenoptera subdivided in four Superfamilies (Apoidea, Chrysidoidea, Formicoidea and Vespoidea) with 629 records in the whole African continent.

Bivariate maps (Fig. 3) show geographical contrasts in weighted cultivated cucurbit yields (FAO 2021) and the known cucurbit flower visitors across Africa. Countries differ in agricultural performance and the availability of published information on cucurbit flower visitors. A cluster of West and Central African countries—such as Mali, Niger, Burkina Faso, Chad, Guinea, Sierra Leone, Liberia, and the Central African Republic—exhibits low cucurbit yields and few or no known flower visitors. This overlap indicates gaps in both pollination knowledge and yield. Notably, the Democratic Republic of the Congo and South Sudan show similar patterns. In contrast, several North African countries—particularly Egypt, Morocco, Tunisia, and Algeria—display higher cucurbit yields while still having limited published information on flower visitors. Similarly, parts of Southern Africa, including Botswana, Namibia, and Angola, show moderate to high yields but low data on flower visitors. A smaller group of countries combines higher yields with well-documented flower visitors, including South Africa and Kenya, where cucurbit agriculture and pollination research are more advanced. These patterns highlight the need for targeted ecological studies, especially in regions where cucurbit production is economically significant but knowledge about flower visitors is scarce.

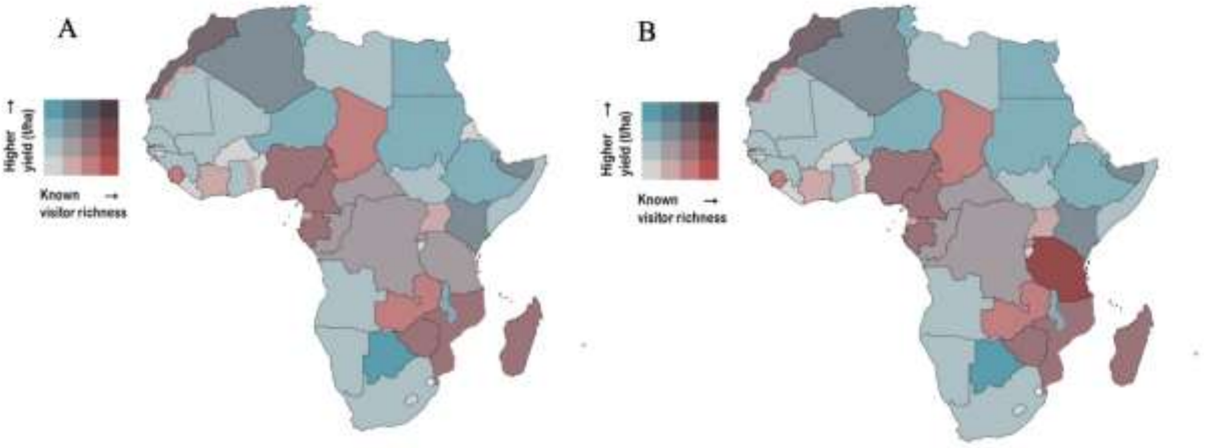


Fig. 3: Bivariate plots of per country changes in published data on insect cultivated cucurbit flower visitors and four groups of cucurbits (Cucumbers & Guerkins, Melons & Cantaloupes, Watermelons, Squashes & Gourds production statistics. Knowledge Gap of flower visitors of the four cucurbit groups in Africa vs. cucurbits national cucurbit yield in tons per hectare. A) Bivariate plots of per country changes of only published data on insect cultivated cucurbit flower visitors, B) Bivariate plots of per country changes of published data on insect cultivated cucurbit flower visitors and unpublished data collected during our trials in central eastern Tanzania.

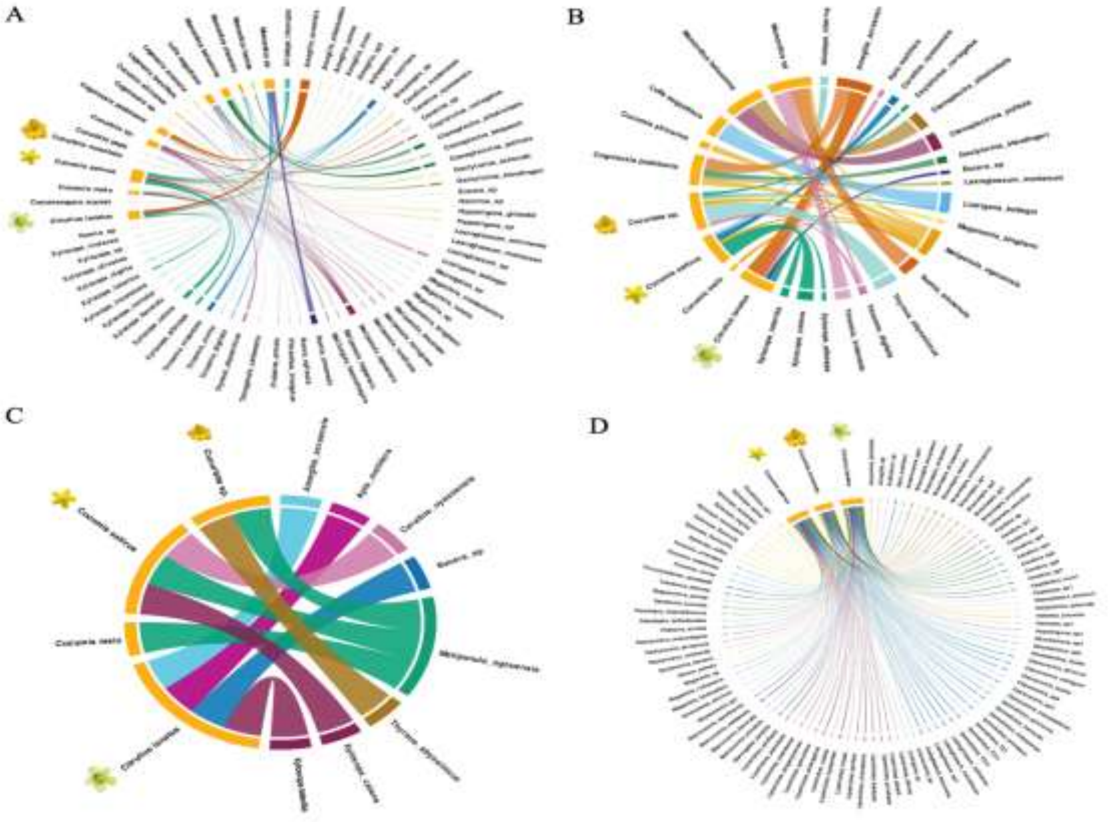


***Figure 4** : Chord diagram illustrating the plant-bee interactions between the six cultivated cucurbit species and eight wild species and bee species recorded visiting both cucurbit food and non-crop species in (A) Sub-saharan region from 1993 to 2023, (B) Zambezian region between 2010-2020 (Mozambique, Botswana, Namibia, Zimbabwe, Malawi, Tanzania and Zambia), (C) Tanzania 2010-2020 and (D) unpublished bee species recorded during twoyears trial 2022-2023 of our study conducted in eastern-central part of Tanzania*

Fig. 4: Chord diagram illustrating the plant-bee interactions between the six cultivated cucurbit species and eight wild species and bee species recorded visiting both cucurbit food and non-crop species in (A) Sub-saharan region from 1993 to 2023, (B) Zambezian region between 2010-2020 (Mozambique, Botswana, Namibia, Zimbabwe, Malawi, Tanzania and Zambia), (C) Tanzania 2010-2020 and (D) unpublished bee species recorded during two years trial 2022-2023 of our study conducted in eastern-central part of Tanzania

In Panel A, the chord diagram shows that there were only 58 bee species recorded over 30 years in the Sub-Saharan region. Additionally, it reveals a highly generalized interaction structure linking six cultivated and eight wild cucurbit species to a broad array of bee taxa. Cultivated cucurbits—particularly *Cucurbita pepo*, *Citrullus lanatus*, and *Cucumis sativus*—are each connected to numerous bee genera. These species were recorded receiving visits from diverse groups, including the following genera: *Apis*, *Amegilla*, *Xylocopa*, *Lasioglossum*, and some genera from the *Halictidae* family. Wild cucurbits exhibit fewer links but maintain taxonomically diverse visitors. Overall, the broad distribution of chord widths highlights a regionally generalized plant–pollinator system, where a small number of widespread bee groups serve as repeated visitors across the cucurbit community.

In Panel B, only 20 bee species were recorded over 10 years in the Zambezian biogeographic region. Dominant visitors include large carpenter bees (*Xylocopa*), *Amegilla*, *Ceratina*, and multiple Halictidae, which all form thick, central chords linking to both cultivated and wild cucurbit species. The structure suggests that Zambian cucurbit systems rely on a small set of abundant and efficient generalist pollinators, which act as ecological keystones within the network.

In Panel C, the Tanzanian dataset displays only 8 bee species recorded from 2010 to 2020. Three cultivated species—*Cucurbita pepo*, *Cucumis melo*, and *Citrullus lanatus*—form particularly strong links with *Amegilla*, *Xylocopa*, and several Halictidae genera. The scarcity of published data on cucurbits (both crop and wild species) is evident in this chord diagram. In Panel D, 104 bee species were collected during two years of trials conducted in central-eastern Tanzania.

Beta diversity across biogeographic regions:

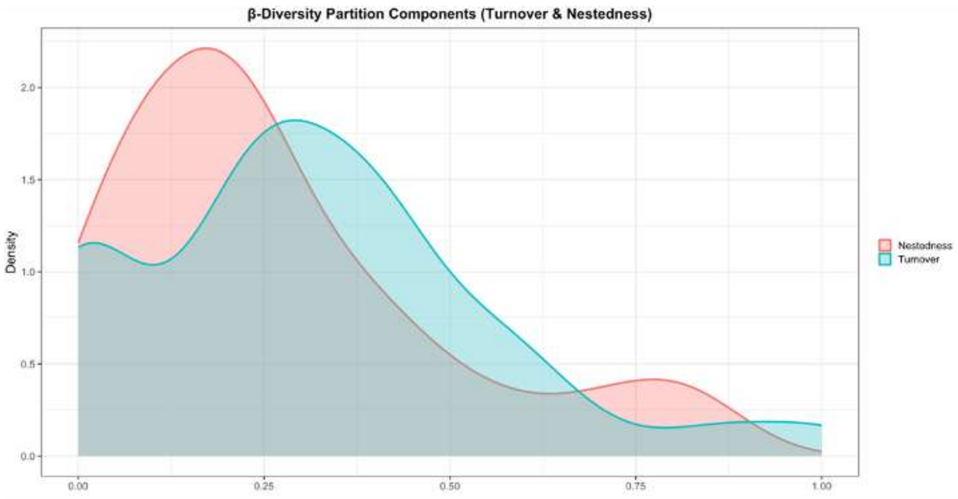


Fig. 5: β-diversity: density distributions of nestedness and turnover derived from all pairwise comparisons of bee communities across food and non-food cucurbit sites.

The turnover curve peak at (~0.25–0.35), it indicates that there are distinct bee assemblages between biogeographic regions. On the other hand, nestedness curve peaks at lower value (~0.10–0.20) and declines steadily. Together, these patterns reveal cucurbit flower visitors assemblages are distinct across biogeographic regions rather than hierarchically nested, reflecting strong biogeographic structuring and environmental heterogeneity across Sub-saharan regions.

PERMANOVA test revealed significant differences in community structure across cucurbits flower visitors assemblages across biogeographic regions (F = 2.82, $R^2 = 0.612$, p = 0.001). It explained 61.2% *of the variation in* cucurbits flower visitors *community composition (PERMANOVA, F = 2.82, p = 0.001),* indicating strong spatial and cucurbit status (food-crop versus non-food crop) structuring of Apoidea assemblages on cucurbit flowers. Only 38.8% of the variation remained unexplained by the tested predictors. The p-value (p = 0.001), highly significant, demonstrates that the observed differences in community composition means that biogeographic context and cucurbit status both influence flower visitors species visiting cucurbit crops across the biogeographic regions in Sub-Saharan Africa.

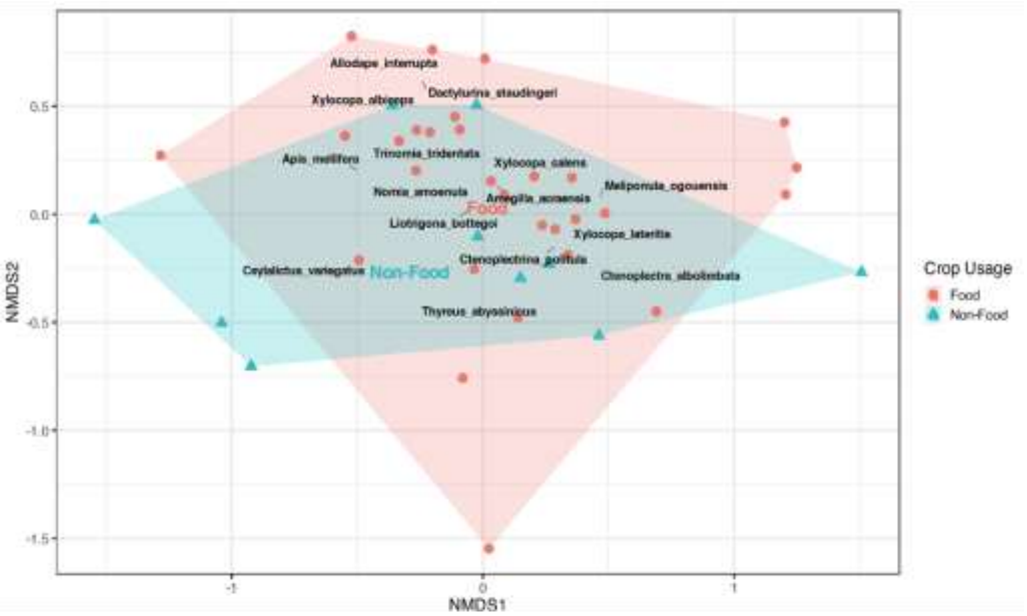


Fig. 6: Non-metric multidimensional scaling (NMDS) ordination of the 15 most-frequent bee species recorded on food cucurbits versus non-food cucurbits in Sub-saharan Africa. Points represent species (labelled), with red circles for those associated with “Food” cucurbit crops and triangles for those associated with “Non-Food” cucurbits.

The NMDS axes (NMDS1 and NMDS2) represent patterns of community dissimilarities. Species that are closer to each other share more similar occurrence patterns across food and non-food cucurbit crops, appear to use both food-crop and non-food crop cucurbits without a clear preference. We can clearly assume that there is a community turnover between food and non-food systems, with some bee species that were recorded visiting more cultivated cucurbits and others more non-food cucurbit species. The overlap of the polygons shows that several species occur in both groups. Certain species, such as *Allodape interrupta*, cluster strongly with food cucurbits, whereas others, like *Ctenoplectrina albolimbata*, are more characteristic of non-food cucurbits. Some central species (e.g., *Liotrigona bottegoi*) appear to use both food-crop and non-food crop cucurbits without a clear preference. These distinctions imply that food crops may depend on a somewhat different subset of bee flower visitors than non-food cucurbits, highlighting the importance of identifying which species cluster with each cucurbit status (cultivated or wild species) to guide targeted management or habitat support strategies.

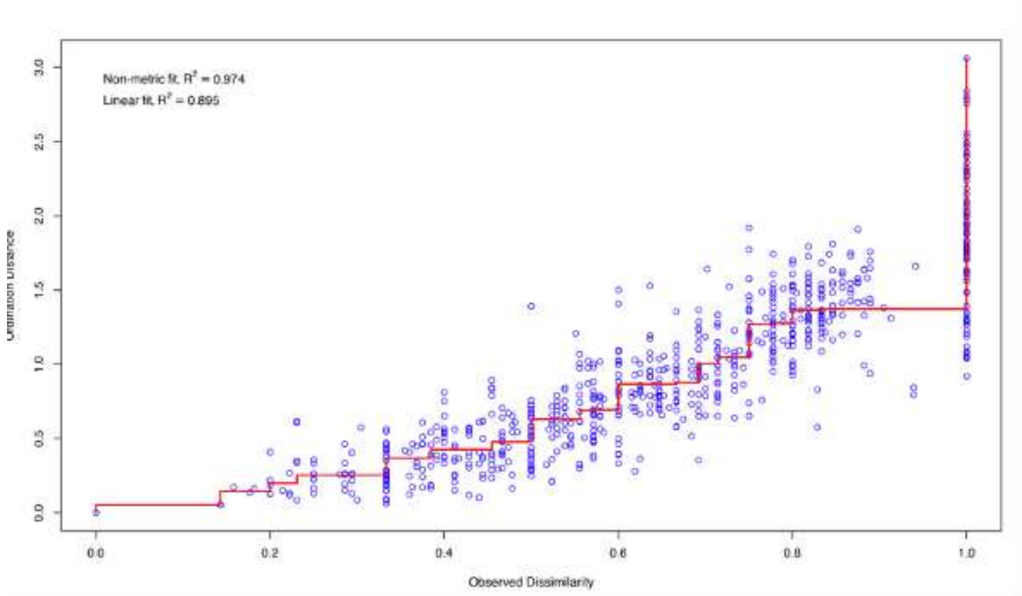


Fig. 7: NMDS Stress plot assesses the goodness of the NMDS ordination conducted

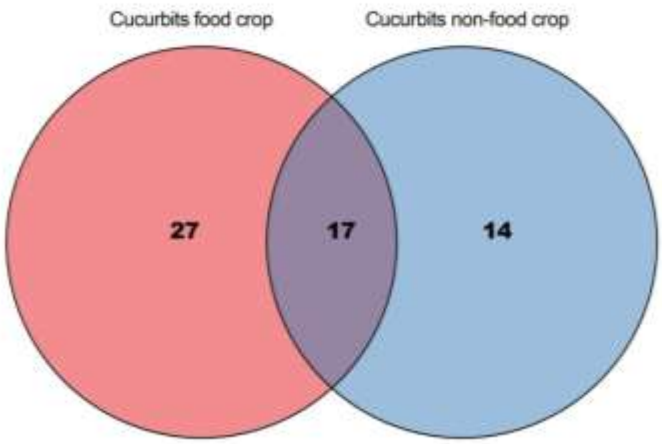


Fig. 8: Venn diagram showing the number of shared and unique cucurbit species (food crop Vs non-food crop) flower visitors bee species

A total of 44 bee species were recorded visiting cucurbit food crops (fig.8) , comprising 27 unique species. In contrast, 31 bee species were identified as visitors to non-food crop (wild species) flowers, with only 14 unique species. The two groups shared 17 species.

References used in the review :

Adja, N. A., Danho, M., Alabi, T. A. F., Gnago, A. J., Zimmer, J. Y., Francis, F., ... & Zoro, B. I. A. (2014, October). Entomofaune associée à la culture de cucurbites oléagineuses africaines (Lagenaria siceraria Molina (Standl. 1930) et Citrullus lanatus Thumb (Matsum & Nakai 1916)) et impact des ravageurs sur la production. In Annales de la Société entomologique de France (NS) (Vol. 50, No. 3-4, pp. 301-310). https://doi.org/10.1080/00379271.2014.937104

Apie, A., Marina, N., Louis, A., Nondenot, R., Landry, C., Djè, T., & Christian, K. (2018). Entomofauna of cucumber *Cucumis sativus* (L.), damage assessment caused by insect pests in Dabou in South Côte d'Ivoire. *International Journal of Fauna and Biological Studies,* *5*(6), 27–34. https://doi.org/10.35410/IJAEB.2020.5529

Ayoub, L., Hanif, R., Yaqoob, M., Rasool, J., & War, W. A. (2024). *Exploring the rich diversity of insect pollinators in cucumber, Cucumis sativus L.* https://doi.org/10.21203/rs.3.rs-4552574/v1

Azo'o Ela, M., & Messi, J. (2012). Yield responses of *Cucumeropsis mannii* (Cucurbitaceae) to the presence or absence of insect foraging activity at Nkolbisson in Cameroon. *Journal of Animal & Plant Sciences, 13*. http://www.m.elewa.org/JAPS

Azo'o Ela, M., Messi, J., Tchuenguem Fohouo, F.-N., Tamesse, J. L., Kekeunou, S., & Pando, J. B. (2010). Foraging behaviour of *Apis mellifera adansonii* and its impact on pollination, fruit, and seed yields of *Citrullus lanatus* at Nkolbisson (Yaoundé, Cameroon). *Cameroon Journal of Experimental Biology, 6.* http://www.ajol.info/browse-journals.php

Sentil, A., Lhomme, P., Michez, D. et al. "Farming with Alternative Pollinators" approach increases pollinator abundance and diversity in faba bean fields. J Insect Conserv 26, 401–414 (2022). https://doi.org/10.1007/s10841-021-00351-6

Bodlah, I., & Waqar, M. (2013). Pollinators visiting summer vegetables ridge gourd (*Luffa acutangula*), bitter gourd (*Momordica charantia* L.), and brinjal (*Solanum melongena*). *Asian Journal of Agriculture and Biology, 1*(1). https://doi.org/10.1007/S42853-023-00189-7

Duchen, P., & Renner, S. S. (2010). The evolution of *Cayaponia* (Cucurbitaceae): Repeated shifts from bat to bee pollination and long-distance dispersal to Africa 2–5 million years ago. *American Journal of Botany, 97*(7), 1129–1141. https://doi.org/10.3732/ajb.0900385

Eardley, C. D. (2003). Revision of the Afrotropical Ctenoplectrini (Hymenoptera: Apidae). African Plant Protection, 9(1), 5-18.

Eardley, C. D. (2004). Taxonomic revision of the African stingless bees (Apoidea: apidae: apinae: meliponini). African plant protection, 10(2), 63-96.

Eardley, C. D., & Urban, R. P. (2010). *Catalogue of Afrotropical bees (Hymenoptera: Apoidea: Apiformes).* Magnolia Press.

Emmanuel, O., Oludele, O. E., & Unwabunne, U. M. (2019). Diversity, spatial and temporal distribution of above-ground arthropods associated with watermelon in the Nigerian southern guinea savanna. *Journal of Insect Biodiversity and Systematics*, *5*(1), 11-32.

Fikadu, Z. (2020). The contribution of managed honey bees to crop pollination, food security, and economic stability: Case of Ethiopia. *The Open Agriculture Journal, 13*(1), 175–181. https://doi.org/10.2174/1874331501913010175

Heard, T. A. (1999). The role of stingless bees in crop pollination. Annual review of entomology, 44(1), 183-206. WILLS, O. U. H. (2019). Pollination and guttation in sweet melon Cucumis melo l. grown under field and greenhouse conditions in Nairobi county, Kenya (Doctoral dissertation, Kenyatta University).

Kiatoko, N., Pozo, M. I., Van Oystaeyen, A., Van Langevelde, F., Wäckers, F., Kumar, R. S., Hundt, B., & Jaramillo, J. (2022). Effective pollination of greenhouse Galia muskmelon (*Cucumis melo* L. var. reticulatus). *Journal of Apicultural Research, 61*(5), 664–674. https://doi.org/10.1080/00218839.2021.2021641

Kiatoko, N., Pozo, M. I., Kasiera, W., Kariuki, S. K., van Langevelde, F., Hundt, B., & Jaramillo, J. (2023). Body size as a proxy of probing time and visitation rates on cucumber by two African stingless bees increase fruit quality and seed quantity. *Scientia Horticulturae*, *309*, 111671. https://doi.org/10.1016/j.scienta.2022.111671.

Kiatoko, N., Pozo, M. I., Van Oystaeyen, A., Musonye, M., Kika, J., Wäckers, F., ... & Jaramillo, J. (2023). African endemic stingless bees as an efficient alternative pollinator to honey bees in greenhouse cucumber (Cucumis sativus L). Journal of Apicultural Research, 62(5), 1017-1029.

Mensah, B. A., & Kudom, A. A. (2010). Relating bee activity to pollination of *Luffa aegyptiaca* Mill. in Ghana. *Journal of Apicultural Research, 49*(2), 192–196. https://doi.org/10.3896/IBRA.1.49.2.08

Mensah, B., & Kudom, A. (2011). Foraging dynamics and pollination efficiency of *Apis mellifera* and *Xylocopa olivacea* on *Luffa aegyptiaca* Mill. (Cucurbitaceae) in southern Ghana. *Journal of Pollination Ecology*, 34–38.

Messellem, I., Aguib, S., Abed, R., & Abderrezak, S. (2024). Comparative study of pollination efficiency and yield components of melon crops by three bee species in Algeria. *Journal of the Kansas Entomological Society, 97*(1), 1 12. https://doi.org/10.2317/0022-8567-97.1.1

Morimoto, Y., Gikungu, M., & Maundu, P. (2004). Pollinators of the bottle gourd (*Lagenaria siceraria*) observed in Kenya. *International Journal of Tropical Insect Science, 24*(1), 79–86. https://doi.org/10.1079/IJT20046

Nchang, E. C., Nkontcheu, D. B. K., Taboue, G. C. T., Bonwen, F. R., & Fokam, E. B. (2024). Pesticides drive patterns of insect visitors and pollination-related attributes of four crops in Buea, Southwest Cameroon. *International Journal of Tropical Insect Science, 44*(1), 117–128. https://doi.org/10.1007/s42690-023-01138-9

Njeri, D. S. (2020). Seasonal diversity and abundance of insect pollinators as influenced by farming practices in cowpea and cucumber cropping systems in Kikome, Makueni County, Kenya (Doctoral dissertation, JKUAT-AGRICULTURE).

Njoroge, N., Gemmill, B., Bussmann, R., Newton, L. E., & Ngumi, V. W. (2010). Diversity and efficiency of wild pollinators of watermelon (Citrullus lanatus (Thunb.) Mansf.) at Yatta (Kenya). *Journal of Applied Horticulture*, *12*(1), 35-41.

Roubik, D. W. (Ed.). (1995). Pollination of cultivated plants in the tropics (Vol. 118). Food & Agriculture Org..

Sawe, T., Eldegard, K., Totland, Ø., Macrice, S., & Nielsen, A. (2020). Enhancing pollination is more effective than increased conventional agriculture inputs for improving watermelon yields. *Ecology and Evolution, 10*(12), 5343–5353. https://doi.org/10.1002/ece3.6278

Sawe, T., Nielsen, A., & Eldegard, K. (2020). Crop pollination in small-scale agriculture in Tanzania: Household dependence, awareness and conservation. Sustainability, 12(6), 2228.

Sivaram, V., Jayaramappa, K. V., Menon, A., & Ceballos, R. M. (n.d.). Use of bee-attractants in increasing crop productivity in Niger (*Guizotia abyssinica* L.). *Brazilian Archives of Biology and Technology, 56*(3), 365–370.

Taha, E. K. A., & Bayoumi, Y. A. (2009). The value of honey bees (Apis mellifera, L.) as pollinators of summer seed watermelon (Citrullus lanatus colothynthoides L.) in Egypt. Acta Biologica Szegediensis, 53(1), 33-37.

Taha, E. K. A., Taha, R. A., & Al-Kahtani, S. N. (2019). Nectar and pollen sources for honeybees in Kafrelsheikh province of northern Egypt. Saudi Journal of Biological Sciences, 26(5), 890-896.

Fomekong, A., Messi, J., Kekeunou, S., Tchuenguem-Fohouo, F. N., & Tamesse, J. L. (2008). Entomofauna of Cucumeropsis mannii Naudin, its impact on plant yield and some aspects of the biology of Dacus bivitattus (Diptera: Tephritidae). African Journal of Agricultural Research, 3(5), 363-370.

Michener, C. D. (2007). The bees of the world. JHU press.

Vaissiere, B. E., & Froissart, R. (1996). Pollination of cantaloupes under spunbonded row cover by honey bees (Hymenoptera: Apidae) in West Africa. *Journal of Economic Entomology, 89*(5), 1213–1218. https://academic.oup.com/jee/article/89/5/1213/791463

Yao, K. A. G., Koffi, K. K., Ondo-Azi, S. A., Baudoin, J. P., & Zoro, B. I. A. (2014). Seed yield component identification and analysis for exploiting recombinative heterosis in bottle gourd. *International Journal of Vegetable Science, 21*(5), 441–453. https://doi.org/10.1080/19315260.2014.895791

Zoro Bi, I. A., Koffi, K. K., Djè, Y., Malice, M., & Baudoin, J.-P. (2006). Genetic resources of indigenous cucurbits from Côte d'Ivoire. *Sciences & Nature, 3.*

Task 3.4. synopsis and socio economics of agroecological cucurbit farming

Socioeconomic sustainability of agroecological farming: a quantitative analysis of cucurbit crop production in Eastern Central Tanzania

These results were published in Bakengesa et al. (2024). Agroecology and Sustainable Food Systems. https://doi.org/10.1080/21683565.2024.2357643.

Methodology

The study combined **baseline surveys** and **field experiments** to assess the socioeconomic performance of agroecological versus conventional cucurbit farming in Morogoro, Tanzania.

- **Survey:** 138 farmers were interviewed using semi-structured questionnaires to capture perceptions of fruit fly impacts, benefits, and constraints of agroecological practices.
- **Field experiments:** Conducted over two years (2021–2022) in 20 experimental fields across plateau and mountainous zones. Plots (15 × 15 m) were assigned to agroecological management (biofertilizers, biopesticides, mulching, bio-fencing, intercropping) or conventional management (synthetic fertilizers and pesticides). Crops included cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), and squash (*Cucurbita moschata*).
- **Data analysis:** Yield, fruit set, abortion, and damage were analyzed via ANOVA; economic metrics included gross revenue, gross margin, production costs, cost-benefit ratio (CBR), and return on investment (ROI%).

Scientific Results

Farmer perceptions:

- Fruit flies were consistently identified as the most damaging cucurbit pests, causing income loss through fruit rot and reduced market value.

- Benefits of agroecology cited by farmers included improved soil fertility, moisture retention, and local availability of inputs. Constraints included higher labor demands and limited technical skills.

Crop performance:

- **Fruit set and abortion:** Agroecological plots showed higher fruit set in squash and watermelon during the second year, while cucumber favored conventional plots in the first year. Aborted fruits were more frequent in agroecological plots early on but declined over time.
- **Fruit damage:** Patterns varied by crop and year; overall, damage decreased in agroecological plots during the second year, suggesting cumulative benefits of soil and ecosystem improvements.

Yield and economic metrics:

- **Yields:** Comparable between systems overall, with cucumber and squash showing improved yields under agroecology in the second year.
- **Production costs:** Agroecological farming consistently reduced costs (e.g., cucumber: $939/ha vs. $1,139/ha conventional).
- **Gross margin and revenue:** Higher for cucumber and squash under agroecology in the second year; watermelon showed no significant differences.
- **Cost-benefit ratio (CBR) and ROI:** Agroecological cucumber achieved CBR = 0.9 and ROI = 90%, versus 0.3 and 30% under conventional management. Squash showed strong gains in the second year (CBR = 2.0; ROI = 200%).

Interpretation:

Agroecological farming offers clear economic advantages through reduced input costs and improved profitability for certain crops, particularly cucumber and squash. While short-term yield differences were minimal, positive trends in fruit set, gross margins, and ROI during the second year suggest long-term sustainability benefits. These findings support agroecology as a viable alternative to pesticide-based systems, aligning with SDGs on poverty reduction, food security, and environmental health.

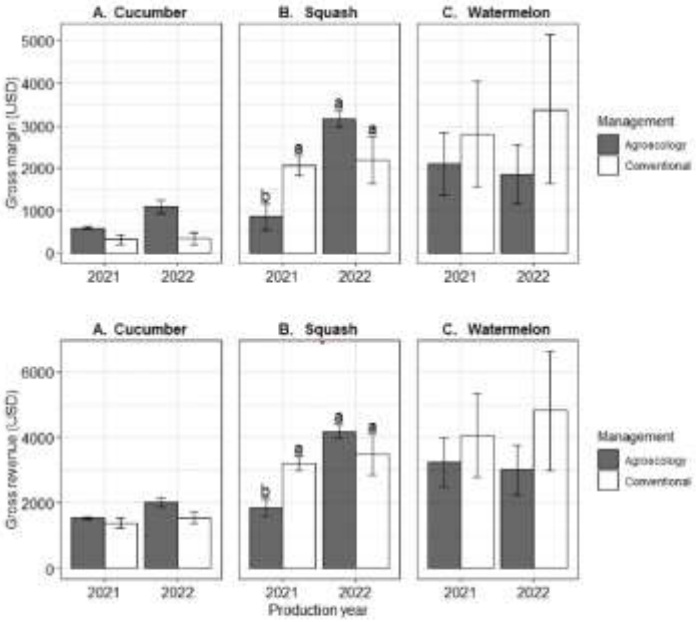


Figure 3.4.1: Gross margin and revenue trends for cucumber, squash, and watermelon under agroecological vs. conventional farming.

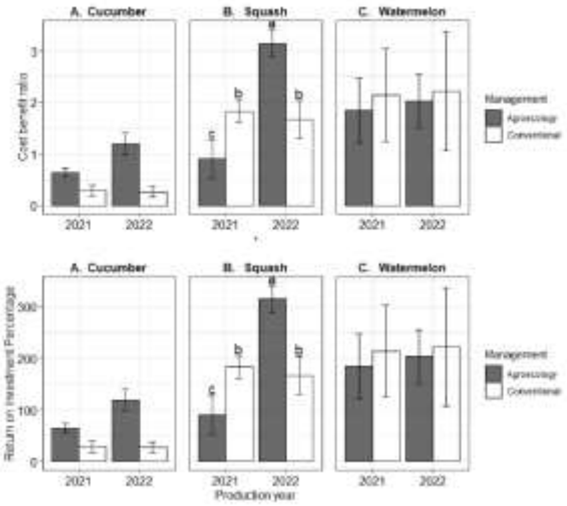


Figure 3.4.2: Cost-benefit ratio and ROI across crops and management systems.

References

- Bakengesa JA et al. (2024). Socioeconomic sustainability of agroecological farming: a quantitative analysis of cucurbit crop production in Eastern Central Tanzania. *Agroecology and Sustainable Food Systems*. https://doi.org/10.1080/21683565.2024.2357643
- Deguine J-P et al. (2015). Agroecological management of cucurbit-infesting fruit fly: A review. *Agron Sustain Dev*, 35:937–965.
- D'Annolfo R et al. (2017). Social and economic performance of agroecology: A review. *Int J Agric Sustain*, 15:632–644.
- Mwatawala M et al. (2010). Incidence and host range of melon fruit fly in Tanzania. *Int J Pest Manag*, 56:265–273.

Economic benefits of agroecology in smallholder cucurbit production in Tanzania

These results are based on a manuscript submitted for publication to an international scientific journal with IF.

Methodology

Field experiments were conducted in Morogoro Region (plateau and mountainous zones) over four cropping periods (2021–2022) to compare **agroecological and conventional cucurbit farming**.

- **Crops:** Cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), squash (*Cucurbita moschata*).
- **Agroecological practices:** Mass trapping, spot baiting (GF120), mulching, orchard sanitation, organic fertilization.
- **Conventional practices:** Synthetic insecticides (Dimethoate), fungicides (Chlorothalonil), industrial fertilizers.
- **Data collected:** Marketable fruit weight (kg/ha), production costs, total revenue, net profit, cost-benefit ratio (CBR), and return on investment (ROI).
- **Analysis:** ANOVA and farm budget analysis in R.

Scientific Results

- **Marketable fruit weight:** Squash showed significantly higher weight under agroecology (6,247 kg/ha) vs. conventional (4,622 kg/ha). Cucumber and watermelon weights were comparable between agroecology and conventional but far higher than control plots.
- **Production costs:** Agroecology reduced costs by 20–25% compared to conventional (e.g., cucumber: $1,073 vs. $1,309).
- **Profitability:** Net profit and CBR were consistently higher in agroecological plots (CBR: squash 1.6 vs. 0.6 conventional; cucumber 0.5 vs. 0.09). ROI followed the same trend.

Interpretation

Agroecology offers clear economic advantages through lower input costs and improved profitability, particularly for squash. While yield gains were crop-specific, overall economic performance strongly favored agroecology over conventional systems.

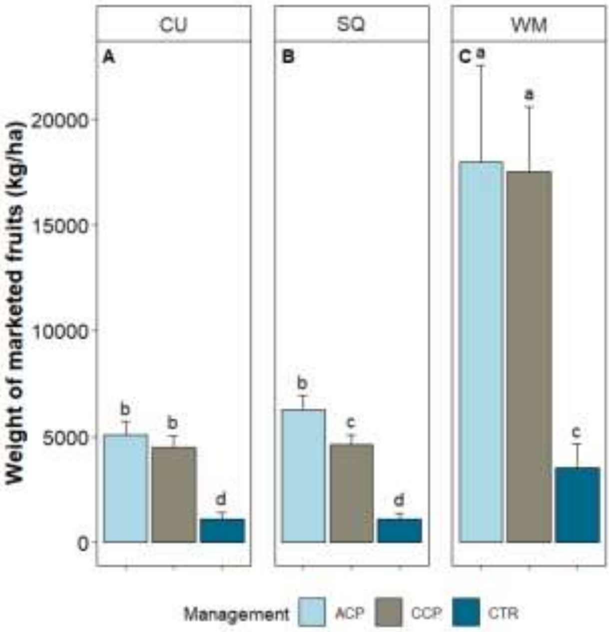


Figure 3.4.3: Marketable fruit weight of cucumber, squash, and watermelon under agroecology, conventional, and control.

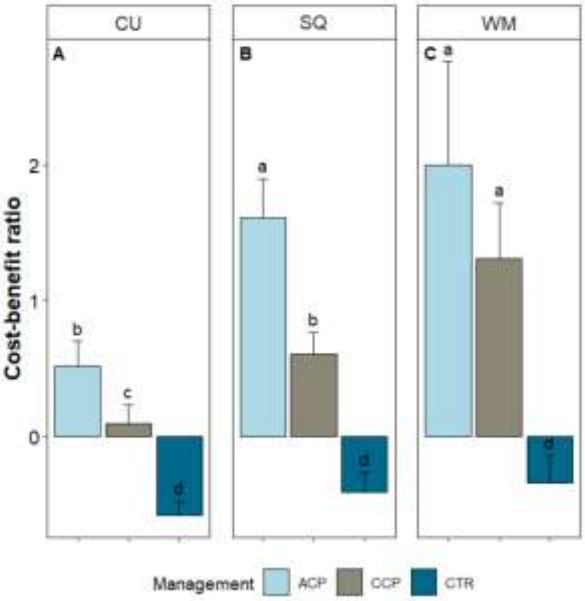


Figure 3.4.4: Cost-benefit ratio across management systems for the three cucurbit crops.

References

1. Bakengesa JA et al. (2024). Socioeconomic sustainability of agroecological farming: a quantitative analysis of cucurbit crop production in Eastern Central Tanzania. *Agroecology and Sustainable Food Systems*. https://doi.org/10.1080/21683565.2024.2357643
2. Bakengesa JA et al. (2023). Agroecological farming practices against fruit flies in Central Eastern Tanzania. *Fruits*, 78(3). https://doi.org/10.17660/th2023/010
3. Deguine J-P et al. (2015). Agroecological management of cucurbit-infesting fruit fly: A review. *Agron Sustain Dev*, 35:937–965.

4. Bwire PM et al. (2025). Impact of agroecological protection practices against cucurbit-infesting fruit flies in Morogoro, Tanzania. *J Appl Entomol*.
5. Mouratiadou I & Wezel A et al. (2024). The socio-economic performance of agroecology: A review. *Agroecology and Sustainable Food Systems*, 48:125–152.

Project added value

The ISeBAF research delivered significant added value beyond its core scientific outputs. It strengthened **local capacity** through the training of Tanzanian field assistants in insect sampling, identification, and molecular techniques, and fostered **long-term collaborations** between RMCA, Sokoine University of Agriculture, and European partners. Methodologically, it introduced **innovative approaches**, including the integration of multiple sampling techniques for pollinator monitoring, metabarcoding for pollen and microbiome profiling, and phylogenomic analyses using Ultra-Conserved Elements (UCEs). These advances generated **baseline datasets** on Afrotropical pollinators (bees and flower flies), fruit fly pests, and their microbiomes—resources that will support future agroecological research and biodiversity assessments. The project also developed **bioinformatic pipelines** for microbiome and functional profiling. By linking ecological, genomic, and socio-economic data, the work provided **evidence-based insights** into the benefits and limitations of agroecological farming. Finally, the project result contributed to **knowledge transfer and outreach**, including the preparation of didactic materials and the establishment of reusable cooperation networks, ensuring that its impact extends well beyond the project timeline.

4. RECOMMENDATIONS

The ISeBAF project provides actionable insights for decision-making at multiple levels—local, national, and international—by linking agroecological practices, biodiversity conservation, and socio-economic performance. Below are the key recommendations:

Scientific Support to Policy

- **Pollinator Conservation and Agroecology:** Evidence from bee and flower fly studies shows that agroecological farming can enhance pollinator abundance and stability under favorable landscape conditions. Policies should integrate **pollinator-friendly practices** (e.g., intercropping, reduced pesticide use) into national agroecology strategies and biodiversity action plans.
- **Landscape-Level Planning:** Results demonstrate that landscape heterogeneity influences pollinator diversity and pest dynamics. Agroecological policies should be coupled with **landscape management measures** (e.g., maintaining semi-natural habitats, hedgerows) to optimize ecosystem services.
- **Integrated Pest Management (IPM):** Fruit fly research highlights that agroecology alone does not guarantee reduced pest pressure. Decision-makers should promote **adaptive IPM strategies** that combine agroecological practices with targeted control measures, reducing reliance on synthetic pesticides while safeguarding yields. As part of these strategies, the **push-pull approach developed by ICIPE** offers an affordable and proven improvement. This technique uses **repellent intercrops such as *Desmodium* species** to "push" pests away from the main crop and **trap crops like Napier grass (*Pennisetum purpureum*) or Brachiaria** to "pull" them toward designated areas for control. Push-pull can be complemented by **male annihilation techniques (MAT)** using species-specific lures and toxicants to suppress fruit fly populations. Incorporating these measures into agroecological protocols strengthens pest management while maintaining sustainability.
- **Microbiome-Based Insights:** Comparative microbiome analyses of pollinators and pests reveal species-specific stress responses. These findings can inform **risk assessment frameworks** for pesticide regulation and guide **pollinator health monitoring** under climate and land-use change scenarios.
- **Socio-Economic Viability:** Economic analyses confirm that agroecology improves profitability through lower input costs and higher ROI for certain crops. Policies should support **financial incentives, training, and extension services** to scale adoption among smallholders.

Operational Recommendations

- **Capacity Building:** Expand farmer training programs on agroecological practices, pollinator management, and IPM, leveraging the networks and didactic materials developed by the project.
- **Monitoring Systems:** Establish **long-term biodiversity and microbiome monitoring** in agroecosystems to track pollinator health, pest adaptation, and ecosystem service delivery.

- **Data Integration:** Use the project’s baseline datasets (pollinator diversity, fruit fly profiles, microbiome data) to inform open access **databases** and support evidence-based agricultural planning.
- **Research Continuity:** Encourage multi-year, landscape-aware studies to refine agroecological strategies under climate variability, building on the methodological innovations introduced (e.g., total evidence approach, genetic, genomic tools).
- **Policy Alignment:** Align agroecological initiatives with **SDGs on food security, biodiversity, and climate resilience**, ensuring coherence between agricultural, environmental, and health policies.
- **Improving Agroecological Protocols:** Strengthen agroecological pest management by integrating **affordable and practical enhancements**—such as **push-pull strategies** and **male annihilation techniques**—without overcomplicating protocols. Improvements should remain **operationally simple and economically viable for smallholders**, ensuring adoption at scale without increasing labor or input costs. This effort is being **continued at RMCA in collaboration with African partners**, mainly through **project proposals targeting affordable, nature-based solutions in line with EU and BELSPO research program requirements**, and within the **DGD Framework Agreement 2024–2029**, which prioritizes sustainable agriculture and capacity building in Africa.

5. DISSEMINATION AND VALORISATION

Task 3.5: Outreach to stakeholders

Stakeholder engagement and farmer interaction

The project actively supported **knowledge transfer and participatory approaches** through regular engagement with smallholder farmers in the Morogoro region. **Sokoine University of Agriculture (SUA)**, as part of its institutional mandate, organized frequent meetings and field demonstrations in collaboration with the NGO **Sustainable Agriculture Tanzania (SAT)**. These sessions provided practical training on agroecological practices, integrated pest management, and pollinator-friendly farming techniques (see **Annex 5** as an example). Demonstration plots and workshops enabled farmers to observe and adopt methods that improve productivity while reducing reliance on synthetic inputs.

International workshop and policy dialogue

In synergy with the project **AGROVEG (RAAC 2019–2023)**, an **international workshop** was organized in **Dodoma in 2020**, bringing together stakeholders from Tanzania and Mozambique.

- **63 stakeholders and end-users** were reached, including governmental officials from both countries and representatives of non-governmental organizations.

Communication tools and materials

- **Policy brief** produced in **English and Kiswahili**, presented to governmental and other stakeholders during the Dodoma meeting.
- **Manual on agroecological farmer practices**, produced in **English and Kiswahili**, presented to **more than 200 farmers** during demonstration events in Morogoro.
- Dissemination through SUA's and RMCA's institutional channels and social media:
    - **National TV**: https://www.youtube.com/live/48jZwFR9YE8
    - **Social media channels** (Instagram, Facebook) and **radio broadcasts**.
- https://www.africamuseum.be/en/research/news/agroecology
- https://www.africamuseum.be/en/staff/896/project_detail_view?prjid=714

Digital resources and data valorisation

In line with BELSPO's Open Science policy, all digital resources developed under ISeBAF comply with open access and FAIR data principles. Publications are deposited in institutional repositories, and genomic datasets generated through co-financed studies (see below) are archived in trusted platforms linked to RMCA's and RBINS's DaRWIN system. Specifically, the project contributed additional information to existing online databases and developed new resources to enhance the accessibility and traceability of insect biodiversity and genomic data. These efforts were complemented by a feasibility study on **genomic data mining and curation of Diptera collections** hosted at RMCA, co-financed by ISeBAF and published as Esselens et al. (2025) in *Biodiversity Data Journal* (https://doi.org/10.3897/BDJ.13.e157274). This study established standardized workflows for Illumina short-read whole genome sequencing of Syrphidae and Tephritidae and integrated genomic metadata into the DaRWIN collection management system, ensuring linkage between physical vouchers, DNA samples, and sequence data. By integrating genomic data into collection management systems and linking them to digital platforms, the project advances **open science**, supports **data-driven biodiversity research**, and provides a foundation for **future museomic initiatives**. These resources are critical for **taxonomic validation, phylogenetic studies, and population genomics**, aligning with ISeBAF's objectives to strengthen scientific infrastructure for biodiversity and agroecological research.

The project contributed additional information to other existing online databases and developed new resources:

- **Digit03 Virtual Collections**: https://virtualcol.africamuseum.be/providence/pawtucket/ (additional images).
- **Pollinator Database**: https://www.pindip.org.
- **Dedicated RMCA Website** hosting all fruit fly research outputs: https://fruitflies.africamuseum.be/.

Outreach report

As part of Task 3.5, the project produced an illustrated outreach report entitled **"Harnessing Pollinator Diversity in Cucurbit Crop Production in Tanzania"**. This guide translates scientific findings into practical recommendations for farmers, cooperatives, and NGOs, emphasizing the economic and ecological importance of pollinators in cucurbit farming. It provides strategies for pollinator-friendly practices, integrated pest and pollinator management, and sustainable agriculture. The report is openly accessible via Zenodo: https://doi.org/10.5281/zenodo.16085951. **Pollinator fact sheets for students/NPPO officers.** RBINS's subcontractor collaborated on the implementation of the booklet by Vereecken et al. (2025) and that illustrates the main pollinators visiting cucurbit crops.

More specialized documents written by the subcontractor:

- "Illustrated guide of Tanzanian bee genera" (**Annex 7 - Annex beeGenera**) that remains to be improved by adding pictures taken in the field.
- "Illustrated keys for African species of *Ipomalictus*" (see list of species in **Annex 8 - Annex *Ipomalictus***), as a basis for an ongoing publication on a systematic revision. RBINS's contractor established a species catalogue by recording all available data, that is, from the literature and field observations, as well as by a reexamination of his own manuscript diagnoses (see exemplative data for *Lasioglossum* (*Ipomalictus*) *bowkeri* in **Annex 8 - Annex *Ipomalictus***).

6. PUBLICATIONS

Scientific articles published on international journals with IF (with link to open access pdf)

Bakengesa, J. A. A., Kudra, A. B. B., De Meyer, M., Virgilio, M., Mwatawala, M. W. W., Majubwa, R. O. O., Meyer, M. De, Virgilio, M., Mwatawala, M. W. W., & Majubwa, R. O. O. (2023). Agroecological farming practices against fruit flies in Central Eastern Tanzania. *Fruits*, *78*(3), 1–12. https://doi.org/10.17660/TH2023/010

Bakengesa, J. A., De Meyer, M., Kudra, A. B., Virgilio, M., Mwatawala, M. W., & Majubwa, R. O. (2024). Socioeconomic sustainability of agroecological farming: a quantitative analysis of cucurbit crop production in Eastern Central Tanzania. *Agroecology and Sustainable Food Systems*, 1–24. https://doi.org/10.1080/21683565.2024.2357643

Esselens, L., Addison, P., Bakengesa, J., Bota, L., Canhanga, L., Cugala, D., Daniel, B., De Meyer, M., Delatte, H., Herpers, J.-M., Jordaens, K., Kabota, S., Kudra, A., Majubwa, R., Manrakhan, A., Mussumbe, M., Mwatawala, M., Theeten, F., Van den Spiegel, D., … Virgilio, M. (2025). Navigating uncertainty in museum workflows: genomic data mining and curation of the Diptera collections hosted at RMCA. *Biodiversity Data Journal*, *13*. https://doi.org/10.3897/BDJ.13.E157274

Hendrycks, W., Mullens, N., Bakengesa, J., Kabota, S., Tairo, J., Backeljau, T., Majubwa, R., Mwatawala, M., De Meyer, M., & Virgilio, M. (2025). Deterministic and stochastic effects drive the gut microbial diversity in cucurbit-feeding fruit flies (Diptera, Tephritidae). *PLOS ONE*, *20*(1), e0313447. https://doi.org/10.1371/JOURNAL.PONE.0313447

Kabota et al. (2025) The impact of family farming on afrotropical flower fly communities (Diptera, Syrphidae). A case study in Tanzania. PlosONE.

Mullens, N., Hendrycks, W., Bakengesa, J., Kabota, S., Tairo, J., Svardal, H., Majubwa, R., Mwatawala, M., De Meyer, M., & Virgilio, M. (2024). Anna Karenina as a promoter of microbial diversity in the cosmopolitan agricultural pest *Zeugodacus cucurbitae* (Diptera, Tephritidae). *PloS One*, 1–20. https://doi.org/10.1371/journal.pone.0300875

Tairo, J., Hendrycks, W., Kabota, S., Zinga, M., Majubwa, R., Kudra, A., Mwatawala, M., De Meyer, M., & Virgilio, M. (2025). A first characterisation of the microbiome and bacterial metabolic functions of *Dacus vertebratus* (Diptera, Tephritidae) in Agroecological and Conventional Farming. *Journal of Applied Entomology*, 1–10. https://doi.org/10.1111/jen.13450

PhD Theses (with link to open access pdf)

Nele Mullens, Hannes Svardal, Massimiliano Virgilio (2025). Microbiomes, management and phylogeny : a genomic investigation of African insects of agricultural importance. Antwerpen, University of Antwerp, Faculty of Science, Department of Biology, 193 p. https://hdl.handle.net/10067/2164120151162165141

Scientific articles submitted to international journals with IF

Bwire et al. Economic benefits of agroecology in smallholder cucurbit production in Tanzania

Kabota et al. Comparison of hand netting and pan trapping methods for estimating hover fly (Diptera: Syrphidae) diversity in the tropical agroecosystems of East Africa.

Mullens et al. Effect of altitude and management practices on the microbiome of the honeybee *Apis mellifera* (Apidae) and the hoverfly *Paragus borbonicus (Syrphidae)* in Morogoro, Tanzania.

Mullens et al. Exploring pollinator microbiomes: a comparative study of hover flies (Diptera: Syrphidae) and honeybees (Hymenoptera: Apidae).

Advanced manuscript drafts to be submitted to international journals with IF

Kabota, Marzie et al. The impact of family farming on pollen recovered from Afrotropical flower flies (Diptera, Syrphidae). A case study in Tanzania.

Bakengesa et al. The impact of family farming on Afrotropical fruit fly communities (Diptera, Syrphidae). A case study in Tanzania.

Tairo et al. The impact of family farming on the microbial functional diversity of Afrotropical pest and pollinator communities (Diptera, Tephritidae, Syrphidae, honeybees).

7. ACKNOWLEDGEMENTS

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- **Laura Canhanga**, Eduardo Mondlane University, Mozambique
- **Nkoba Kiatoko**, International Centre for Insect Physiology and Ecology (ICIPE), Kenya
- **Achik Dorchin**, FED-tWIN researcher, University of Mons - RMCA
- **Denis Michez**, University of Mons, Belgium
- **Steven Janssens**, Botanic Garden Meise (BGM), Belgium
- **Marc De Meyer**, Project collaborator and taxonomist, RMCA
- **Kurt Jordaens**, Project collaborator and taxonomist, RMCA

We also extend our sincere thanks to **Sustainable Agriculture Tanzania (SAT)** (https://www.kilimo.org), the farmers of the Morogoro area, and all individuals who contributed to this project. In particular, we acknowledge the dedication of **students, field assistants, and administrative personnel at Sokoine University of Agriculture (SUA)**, whose efforts were essential for research conceptualisation, data collection and analysis, and local coordination.

We also thank Lukas Jara Sanchez and Denisa Magda, BSc. students at Institut Roger Lambion, for the DNA barcoding of wild bees and the metabarcoding of wild bee pollen loads.

Finally, we gratefully acknowledge the support of Hannes Svardal (University of Antwerp) for his valuable scientific insights and for providing financial support to cover a student’s costs when unexpected funding issues arose.

ANNEXES

Annex 1 – Mutually Agreed Terms (MAT) between SUA and RMCA

Annex 2 – Mutually Agreed Terms (MAT) between SUA and RBINS

Annex 3 – Mutually Agreed Terms (MAT) between SUA and ULB

Annex 4 – Final Follow-up Committee Report (June 26, 2025). Minutes of the 4th meeting of the project follow-up committee, including expert recommendations and discussion points on outreach, data integration, and analytical strategies.

Annex 5 – Minutes of a Farmer Engagement Meeting (April 16, 2021). Summary of discussions between SAT, SUA, and farmer groups on agroecological practices, experimental plot management, and farmer participation strategies under the ISeBAF project.

Annex 6 – Annex allBees

Annex 7 – Annex beeGenera

Annex 8 – Annex Ipomalictus