

MASTERARBEIT | MASTER'S THESIS

Titel | Title

"Differences in abundance and diversity of cacao flower visitors as well as early-fruit set between hybrid and native cacao varieties in Peruvian cacao agroforests"

verfasst von | submitted by

Marlene Erker BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien | Vienna, 2024

Studienkennzahl lt. Studienblatt | Degree programme code as it appears on the student record sheet: UA 066 879 Studienrichtung lt. Studienblatt | Degree programme as it appears on the student record sheet: Masterstudium Naturschutz und Biodiversitätsmanagement Betreut von | Supervisor: Assoz. Prof. Mag. Dr. Franz Essl

Acknowledgments

I would like to express my deepest appreciation to Carolina Ocampo-Ariza. Without her knowledge, expertise, patience and commitment, this thesis would not have been possible. Additionally, I am extremely grateful to the extraordinary cacao farmers I could meet in Peru. Thank you May, Calin, Seve, Charito, Pasiona, Gabriel, Gato, Will and Tuco, to only mention a few. I felt at home. Many thanks to Blanca Ivañez Ballesteros, Pablo Aycart Lazo, Ben Newstead und Daniel Ochterbeck, who contributed important parts to this work. Special thanks also to Bea Maas; without her informing me about this thesis opportunity, I might never have undertaken this adventure. Lastly, I'd like to mention my best friends, parents and sister who always listened attentively when I got lost in passionate narratives about the Peruvian jungle.

Abstract

Although cacao is one of the most widespread cash crops around the globe, surprisingly little is known about its pollinators. Cacao has many varieties and genotypes that differ greatly in taste and yield. Hybrid varieties are frequently favoured due to higher yield, but it is not clear yet to what extent the productivity differs from native varieties. Additionally, there is ongoing uncertainty about whether the ecology of varieties differs from each other. It is important to conserve the genetic diversity of cacao in order to promote resilience and ensure productivity in the eye of biodiversity loss and climate change. In this thesis, I investigated the abundance and diversity of cacao flower visitors and the early-fruit set of hybrid and native cacao varieties in San Martín, Northern Peru.

The study was conducted throughout 12 cacao agroforests, belonging to the Choba choba farmer's cooperative. The agroforests were selected to include agroforests dominated by hybrid cacao varieties, native varieties from the region, and combinations of hybrid varieties in one agroforest. I examined the diversity and abundance of flower visitors on cacao flowers in all selected agroforests using sticky glue on open flowers for 24 hours. Moreover, I quantified fruit set on four rounds of sampling covering ca. 2.5 months, by counting flower buds, open flowers and comparing these numbers with the small fruits found on the next round of sampling ca. 2 weeks after. I assessed how both arthropod diversity and early-fruit set varied according to agroforest management variables (shade cover, understory vegetation cover, leaf litter cover and leaf litter depth).

The analysis showed that the most abundant flower visitor group differed between varieties: aphids on hybrid and ants on native trees. This might indicate that they are either pollinated by different insects or exposed to different pests. Furthermore, I found significantly higher abundance and taxa richness of flower visitors on the native varieties, possibly indicating that insects are more adapted to native trees due to long-lasting co-evolution. I found that flower visitor abundance and richness increased with understory vegetation cover. Higher richness enables the cacao agroforest to be more resilient and robust to changing environmental factors. Early-fruit set of cacao was not significantly affected by any management variable, although the mean fruit set rate of native trees was more than twice that of the hybrid trees (not significant). This could either be attributed to the sensitivity of the mean to outliers or support the literature findings that the productivity of native varieties is highly variable, indicating that a larger sample size might have delivered different results. Additionally, studying not only early-fruit set but final yield could supply more meaningful results.

I conclude that more research on potential pollinators of native and hybrid cacao varieties is required to establish management practices that both promote higher biodiversity and higher yield in a sustainable way. My findings suggest that focussing on understory vegetation might be crucial.

Zusammenfassung

Obwohl Kakao eine der weitverbreitetsten Nutzpflanzen weltweit darstellt, ist der Kenntnisstand über die Bestäuber erstaunlich gering. Kakao weist viele verschiedene Varietäten und Genotypen, die in Geschmack und Ertrag stark voneinander abweichen, auf. Hybride Varianten werden häufig für ihre hohe Produktivität bevorzugt. Jedoch herrscht Uneindeutigkeit über das konkrete Ausmaß des gesteigerten Ertrages, verglichen mit einheimischen Varianten. Des Weiteren ist unklar, inwiefern sich die Ökologie der Varietäten unterscheidet. Es ist im Angesicht von Biodiversitätsverlust und Klimawandel von großer Wichtigkeit, die genetische Diversität von Kakao zu erhalten, um Resilienz zu fördern und Produktivität zu sichern. Im Zuge dieser Arbeit habe ich die Abundanz und Diversität der Kakaoblütenbesucher sowie die frühe Fruchtentwicklung hybrider und einheimischer Kakaovarietäten in San Martín, im Norden Perus, untersucht.

Die Studie wurde in 12 Kakao-Agroforsten, die der "Choba choba"-Bauernkooperative angehören, durchgeführt. Die Agroforste wurden so ausgewählt, dass sie entweder von hybriden bzw. einheimischen Varietäten dominiert wurden oder kombinierte Agroforste aus beiden Varianten darstellten. Ich untersuchte die Diversität und Abundanz der Kakaoblütenbesucher in allen selektierten Agroforsten, indem ich Insektenkleber auf die reproduktiven Blütenteile auftrug. Nach ca. 24 Stunden sammelte ich die noch vorhandenen bearbeiteten Blüten ein und analysierte diese. Darüber hinaus bestimmte ich die frühe Fruchtentwicklung in vier Stichprobenrunden, die ungefähr 2,5 Monate abdeckten, indem ich Blütenknospen sowie offene Blüten zählte und diese anschließend mit der Anzahl der neu entwickelten Früchte in der nächsten Runde, die ungefähr zwei Wochen darauffolgte, verglich. Zusätzlich untersuchte ich die Veränderung von Arthropoden-Diversität und früher Fruchtentwicklung im Zusammenhang mit Agroforst-Managementvariablen (Beschattung, Unterwuchsvegetationsbedeckung, Laubstreudecke und Laubstreutiefe).

Die Analyse zeigte, dass sich die häufigsten Blütenbesucher der beiden Varietäten unterschieden: Blattläuse auf hybriden und Ameisen auf einheimischen Bäumen. Möglicherweise deutet dies daraufhin, dass sie entweder von unterschiedlichen Insekten bestäubt werden oder unterschiedlichen Schädlingen ausgesetzt sind. Des Weiteren fand ich signifikant höhere Abundanz und Taxareichtum an Blütenbesuchern auf den einheimischen Varietäten. Daraus lässt sich eventuell ableiten, dass die Insekten aufgrund langandauernder Ko-Evolution mehr an die einheimischen als an die hybriden Bäume angepasst sind. Außerdem konnte ich feststellen, dass Blütenbesucherabundanz und -reichtum mit höherer Unterwuchsvegetationsbedeckung stieg. Größerer Artenreichtum ermöglicht Kakao-Agroforsten resilienter und robuster gegenüber veränderten Umweltbedingungen zu sein. Keine der Managementvariablen übte einen signifikanten Effekt auf die frühe

Fruchtentwicklung aus, obwohl die frühe Fruchtentwicklungsrate einheimischer Bäume mehr als doppelt so groß war wie jene der hybriden Bäume (nicht signifikant). Erklärungen hierfür lassen sich womöglich in der Anfälligkeit des arithmetischen Mittels gegenüber Ausreißern oder in der hohen Produktivitätsvariabilität einheimischer Varietäten finden. Dies deutet darauf hin, dass eine größere Stichprobemenge vielleicht andere Resultate geliefert hätte. Ferner könnte die Analyse des finalen Ertrags und nicht nur der frühen Fruchtentwicklung aussagekräftigere Ergebnisse bereitstellen.

Ich komme zu dem Schluss, dass mehr Forschung hinsichtlich potenzieller Bestäuber einheimischer und hybrider Kakao-Varietäten notwendig ist, um Managementpraktiken, die sowohl höhere Biodiversität als auch höhere Erträge auf nachhaltige Weise fördern, zu etablieren. Meine Ergebnisse legen nahe, dass dem Management der Unterwuchsvegetation eine bedeutende Rolle zustehen könnte.

Contents

1. Introduction

Cacao monocultures and cacao agroforestry systems provide two very different approaches concerning the cultivation of cacao. Existing literature has shown that yield in cacao monocultures is on average higher than in cacao agroforests (e.g., Armengot *et al.*, 2016; Mattalia *et al.*, 2022). However, this yield difference can be economically outbalanced by including the financial outcome of by-crops (Armengot *et al.*, 2016). Additionally, cacao monocultures hold significantly less animal diversity, whereas diversified systems as agroforests may hold high levels of biodiversity (e.g., Steffan-Dewenter *et al.*, 2007; Clough *et al.*, 2009; Ocampo-Ariza *et al.*, 2022). Preceding studies focused especially on arthropod diversity, suggesting that intensive or conventional management compared to organic or extensive farming significantly decreases the diversity of insect groups (e.g., Bisseleua *et al.*, 2009; Lichtenberg *et al.*, 2017; Akesse-Ransford *et al.*, 2021).

Although cacao is one of the most widespread cash crops around the globe, with a production increase of 500 % from 1961 to 2021 (FAO, 2023), surprisingly little is known about its pollinators. Prior research has elucidated that the genus *Forcipomiya* (midges) is thought to be the main pollinator of cacao (e.g., Young, 2007; Monteiro *et al.*, 2009). However, the spectrum of flower pollinators appears to be much broader, varying strongly among the different regions of the world (e.g., Chumacero de Schawe *et al.*, 2018; Vansynghel *et al.*, 2022a).

Previous research has established that the diversity of cacao flower visitors especially in Peru is largely understudied and varies among regions of the country (Vansynghel *et al.*, 2022a). Studying flower visitor diversity in Peru, especially in the region of San Martín as part of the Amazon basin, is of outstanding interest since it is highly supported that cacao origins from the Amazon rainforest in South America (Zhang & Motilal, 2016). The management conditions of cacao crops are known to influence the diversity of insects and, therefore, potential pollinators (Toledo-Hernández *et al.*, 2021; Vansynghel *et al.*, 2022a). It is urgent to study abundance and diversity of cacao flower visitors in the context of sustainable cultivation forms such as agroforests in order to promote biodiversity-friendly farming and meet high socio-ecological standards – since cacao is not only one of the most traded commodities worldwide but also income provider for millions of farmers (Maas *et al.*, 2020).

Although there are still debates going on about the exact spatial and temporal origin of cacao (Motamayor *et al.*, 2022), it is highly supported that cacao origins from the Amazon rainforest in South America (Zhang & Motilal, 2016) or developed in South and Central America at the same time (Laurent *et al.*, 1994; Whitkus *et al.*, 1998). The distribution of cacao by mankind started in the Holocene (Thomas *et al.*, 2012) and the cultivation began more than 4.000 years ago in Mesoamerica (Powis *et al.*, 2011). *Theobroma cacao L.* is traditionally divided into three main groups: Forastero,

Criollo, and Trinitario. They differ in taste as well as yield (Laurent *et al.*, 1994) and are therefore attractive to different markets. Further research in the 2000s and 2010s classified the cacao germplasm into 10 genetic clusters, with each cluster filling a different niche (Motamayor *et al.*, 2008; Thomas *et al.*, 2012). This classification was further developed and adapted, resulting in 11 genetic groups (Thomas *et al.*, 2023). A cacao variety is considered native when it is located at its centre of origin where it developed naturally, in this case the Amazon basin, all other varieties are products of breeding and considered hybrid. Hybrid trees are created to improve their resistance against pests, diseases as well as pathogens, to achieve higher yields and to increase the level of selfincompatibility (Rodriguez-Medina *et al.*, 2019). Whereas native trees are planted primarily for their fine-flavour cacao beans. Peru as being one of the original cacao countries is home to many native fine-flavour cacao varieties (Tscharntke *et al.*, 2022). However, there is a huge knowledge gap about different cacao varieties that needs to be filled in order to gain better understanding about both sustainable and efficient ways of cultivating cacao.

In this thesis, I studied flower visitors and early-fruit set of hybrid and native cacao varieties in San Martín, Peru, to fill a knowledge gap about cacao flower visitors in Peru and to contribute to a biodiversity-friendlier cacao agroforest management. The hypotheses of this study were that (1) the main flower visitors, (2) the overall abundance and taxa richness of flower visitors as well as (3) the early-fruit set rate differ between hybrid and native cacao varieties and that (4) the agroforest management variables shade cover, understory vegetation cover, leaf litter cover and leaf litter depth affect the overall abundance und taxa richness of flower visitors. I glued the reproductive parts of cacao flowers to catch and identify flower visitors. Furthermore, I counted open flowers, flower buds (undeveloped stage of a flower before it blooms) and fruits to calculate an early-fruit set rate. Additionally, I looked at the previously mentioned agroforest management variables (shade cover, understory vegetation cover, leaf litter cover and leaf litter depth) and set them in relationship with the overall abundance as well as taxa richness of the flower visitors to find out if and how much the management variables affect them.

2. Material and Methods 2.1 Study area

The study was conducted in the region of San Martín, Northern Peru. The study area is located next to the Andes in the rainforest of the district Huicungo, next to the Huayabamba River [\(Figure 1\)](#page-8-2). Since cacao requires a humid and warm environment (Niether *et al.*, 2018), the climate of this region supports the growth of cacao trees with an intermediate to high average annual rainfall of 1 200 mm and average annual temperatures of 30 °C (Tuesta-Hidalgo *et al.*, 2017). Nowadays, the area is dominated by cacao and banana agroforests. However, the cacao agroforests are former coca agroforests that were only converted in the 1970/80s. The cacao agroforests are still mainly surrounded by coca agroforests.

FIGURE 1: (A) MAP SHOWING PERU (GREEN OUTLINE) AND STUDY REGION SAN MARTÍN (GREEN AREA) (B) MAP **SHOWING REGION SAN MARTÍN (GREEN AREA) WITH THE LOCATION OF THE STUDY AREA (BLACK POINT). (C) MAP SHOWING THE STUDY AGROFORESTS TO ASSESS THE DIVERSITY OF FLOWER VISITORS IN CACAO AGROFORESTS AROUND SANTA ROSA IN THE REGION OF SAN MARTÍN, PERU (D) MAP SHOWING THE STUDY AGROFORESTS TO ASSESS THE DIVERSITY OF FLOWER VISITORS IN CACAO AGROFORESTS AROUND PUCALPILLO AND PIZARRO IN THE REGION OF SAN MARTÍN, PERU.**

We selected 12 study agroforests from smallholding farmers belonging to the Choba choba farmers' cooperative – seven study agroforests in Pucalpillo, four in Santa Rosa and one in Pizarro. The cooperative comprises approximately 40 smallholding cacao farmers and dedicates its work to the sustainable production of high-quality cacao. "Choba choba" actually consists of three interlinked units, namely the Choba choba cooperative, the Choba choba AG and the Choba choba foundation. Through keeping all those units together, every working step from planting the cacao seed to selling the chocolate is also farmer-owned. The cacao agroforests differ in tree varieties, size, age, shade cover and other agroforest management variables such as herb cover, leaf litter cover and depth. Their sizes range from 0.2497 ha to 2.230315 ha. The land was formerly used for planting coca – planting cacao trees started approximately 40 years ago. Each selected agroforest showed hybrid trees (the vast majority type CCN-51, one agroforest CYP) and/or native trees.

2.2 Surveys 2.2.1 Cacao flower visitors

We selected up to four trees of each cacao variety (native or hybrid) per agroforest, for a total 77 study trees – 53 hybrid and 24 native trees. In the study area dominated the hybrid type "CCN". Only one agroforest showed the hybrid type "CYP" and another agroforest showed the types "CCN", "CYP" and "ICS" but only "CCN" was selected. If the agroforest showed at least eight native trees, four native and four hybrid trees were used for investigating insect diversity. If the number of native trees was less than eight, it was decided on a case-by-case basis how many trees were used for gluing. Each experimental tree was sampled 3 or 4 rounds. During the fourth round, only native trees were sampled in order to make the number of assessed flowers from native trees comparable to hybrids. During each experimental round, we placed a drop of sticky glue made of polybutene-n-hexane (polybutene adhesive) on the stigma of all open flowers found on the tree, using a syringe and a wooden stick, with the purpose of trapping flower visitors, following Vansynghel *et al.* (2022a). The flowers were collected after 24 hours and we registered whether any insects were glued on them or not. All flowers with insects on them were stored in plastic jars and refrigerated at 4 °C until the insects were identified. I used a general taxonomic key (Gibb & Oseto, 2006), family level keys (Brown *et al.*, 2009; Choate, 1999; Fernández, 2003; Fernández & Mason, 2006; Brothers *et al.*, 2006; Castillo, 2013) and a key for immature insects (Thyssen, 2010) to identify the collected insects to the lowest taxonomic level.

In total, we sampled 6358 flowers – 4309 flowers from hybrid and 2049 from native trees. Since unpollinated cacao flowers abscise after 24 to 36 hours (Almeida & Valle, 2007), only 1419 flowers (32.93%) from hybrid and 761 flowers (37.14%) from native trees could be collected afterwards.

We classified the collected arthropods in the following categories: Acariformes, Aphididae (Hemiptera), "Caterpillars", Cecidomyiidae (Diptera), Cicadellidae (Hemiptera), Coleoptera (including Coleoptera larvae), Formicidae (Hymenoptera), Isoptera, Neididae (Hemiptera), "Other Diptera" (including Diptera larvae), "Parasitic wasps", "Other Hymenoptera", Pseudococcidae (Hemiptera), Psocoptera, Reduviidae (Hemiptera), Siphonaptera, Thripidae (Hemiptera), "Unidentified insect larvae" and Vespoidea (Hymenoptera). Appendix A shows a list of all identified morphospecies and their finest level of classification.

2.2.2 Cacao early-fruit set

To investigate early-fruit set rates, the number of flower buds, flowers and just fertilized fruits as well as grown fruits were counted. 11 out of the 12 cacao agroforests that were also used for flower visitor diversity assessment were included. The number of experimental trees depended on the availability of native trees. If the agroforest showed at least eight native trees, four native and four hybrid trees were counted, as the other four native trees were used for the previously mentioned assessment of flower visitors. If the number of native trees was less than eight, <4 trees per variety were assessed (always an equal number per variety er agroforest). Each experimental tree was sampled three to four times. During the fourth round, only native trees were sampled in order to make the number of assessed flowers from native trees comparable to hybrids. The time interval between the first and the second round was 12 days, between the second and the third round 13 days and between the third and the fourth round 14 days. In total, 67 trees were counted – 44 hybrid and 23 native trees. 33 hybrid and 4 native trees were sampled three times, whereas 11 hybrid and 19 native trees were sampled four times. Additionally, the number of cacao pods affected by certain insect groups – harmful as well as harmless – was counted each time.

The flower buds were grouped into "small buds" (maximum length of 0.5 cm incl. bud stalk), "grown buds" (minimum length of 0.5 cm incl. bud stalk) and "large buds" (buds that showed already open slits). The fruits were grouped into "just fertilized fruits" (maximum length of 5 cm), "small fruits" (length ranging between 5 and 10 cm), "medium fruits" (length ranging between 10 and 20 cm) and "large fruits" (minimum length of 20 cm).

2.2.3 Agroforest management variables

To calculate the canopy cover of shade trees for each agroforest, I used drone pictures from each cacao agroforest collected by Benjamin Newstead (unpublished results). Using the pictures, we built orthomosaics and digital surface models (DSM) in Agisoft Metashape – professional edition (Agisoft LLC, 2023). With the resulting maps, we calculated shade cover percentage in QGIS

Desktop 3.28.4 (QGIS Development Team, 2023). The average value of percentage of shade cover among the 12 agroforests was 30.62 ± 11.62%.

To measure the percentage cover of understory vegetation, we used four pictures of 1 $m²$ areas in each agroforest. The pictures were taken by Benjamin Newstead ca. 25 m away from the center of the agroforest in each of the four cardinal directions (North, South, East, West). We analyzed the pictures within the software ImageJ (Rasband, 1997-2018) to measure the number of pixels covered by understory vegetation, leaf litter or bare ground in each picture. The sliders of the threshold function within ImageJ were adjusted to the following settings: "Hue" 30 – 130, "Saturation" 0 – 150 and "Brightness" according to the light conditions that were present when the photo was taken. If red dots appeared in the analysis, we brought the bottom of the saturation slider up until most of the dots have disappeared. We then transformed these numbers into percentages and calculated an average of the four pictures for each agroforest. The average value of percentage of understory vegetation cover among the 12 agroforests was 13.99% ± 10.88%. The average value of percentage of leaf litter cover among the 12 agroforests was 85.73% ± 11.05%. The percentage of bare ground cover within the 12 agroforests was neglectable. Since the percentages of plant and leaf litter cover were highly correlated (0.16), the leaf litter cover was excluded as predictor variable.

To measure leaf litter depth, I laid a grid measuring 80 m x 80 m upon each agroforest in GAIA-GPS. I took away 10 metres from each side to exclude the edges that may show extremes. The grid included 9 points that were all the same distance (30 m) from each other. If the agroforest had such a size that the 80 m x 80 m grid did not cover at least seven measurement points, the distance between the points was reduced to 10 metres. The depth of the leaf litter was measured with a conventional tape measure. This resulted in a different number of measured points per agroforest. The average number of points per agroforest was 8.5 ± 1.98. The average value of leaf litter varied among the 12 agroforests between 4.76 cm \pm 4.47 cm.

2.3 Statistical analysis

RStudio was used for statistical analysis (Posit team, 2023; R Core Team, 2023).

We calculated overall abundance of all insects, species (taxa) richness and early-fruit set rate for each tree within RStudio. With the package "vegan" (Oksanen *et al.*, 2022), we calculated the Shannon diversity index. The applied function uses the Shannon-Wiener diversity index by default. Whereas species richness gives us simply the number of species – in our case, taxa – we have, Shannon diversity accounts for the abundance of the species (taxa), as well (Magurran, 2004).

We used Generalized linear mixed effect models (GLMM) within the package "glmmTMB" (Brooks *et al.*, 2017) to evaluate whether cacao variety and agroforest management variables

(shade cover, understory vegetation cover, leaf litter depth) significantly affected the abundance and diversity of flower visitors as well as early-fruit set rate in the study area. The sampling unit was the cacao tree. Therefore, we added the gathered information of flower visitors and early-fruit set per tree and across all rounds. Given the limited sample size, we were unable to combine all variables into one single model. Therefore, we ran 14 separate models for abundance and taxa richness as well as 7 separate models for early-fruit set rate, combining cacao variety with each of the agroforestry management variables, and the interaction between them. All models included a random effect that accounted for the presence of multiple trees per variety and agroforests

(1|Agroforest/Variety). Furthermore, the overall abundance and taxa richness data was weighted accordingly to the flower rate (sum of the collected flowers divided by the number of visits). This function considers that the collected flowers and visits per tree differed among the trees. We used a Poisson or Generalised Poisson distribution for modelling both overall abundance as well as species (taxa) richness and checked residual fit with the package DHARMa (Hartig, 2022). Whereas for modelling early-fruit set rate, we used a hurdle model with a zero-inflated gamma family and checked residual fit again with the package DHARMa (Hartig, 2022). No significant issues with the KS (Kolmogorov-Smirnov), dispersion or outlier test were identified. All deviations between model residuals and predicted values were not statistically significant.

We used non-metric multidimensional scaling (NMDS) with the package "vegan"

(Oksanen *et al.*, 2022) to visualize and compare how the taxa composition varied between the two varieties. Since stress was (nearly) zero when using only the two varieties as sites, indicating that the provided data offers too little information, and since the "metaMDS"-function within the package "vegan" cannot deal with an excessive quantity of zeros, the agroforest codes were used instead (e.g., C01 H, C03 N). A stress value below 0.2 is considered good/okay (the lower the better, excluding 0), above 0.2 might deliver results that are dangerous to interpret and above 0.35 is considered bad (Clarke, 1993).

We used the package "ggagroforest2" (Wickham, 2016) to create bar agroforests and graphs that show the predicted counts of overall abundance and species (taxa) richness in RStudio.

3. Results

3.1 Cacao flower visitors

I collected a total of 427 insects on 385 cacao flowers (6% of all sampled flowers), including 151 native and 234 hybrid flowers. However, the average number of flowers that were glued resp. collected per tree hardly varies between hybrid and native trees [\(Table 1\)](#page-13-2). 17.66% of the collected flowers had at least one insect on them – 4.93% of the flowers with insects had two or more. Hence, every 5.66 collected flower showed at least one visitor. In six hybrid and one native out of the 77 trees, no insect was found in any of the sampling rounds.

TABLE 1: AVERAGE NUMBER OF GLUED RESP. COLLECTED FLOWERS PER TREE FOR BOTH VARIETIES (HYBRID AND NATIVE TREES)

| Variety | Average number of glued flowers per tree | SD | Average number of collected flowers per tree | SD |
|---------|---|-------|---|-----------|
| Hybrid | 24.91 | 16.33 | 8.18 | 6.87 |
| Native | 22.27 | 15.98 | 8.27 | 6.9 |

I found that the most abundant flower visitor group differed between varieties: Whereas aphids (Hemiptera: Aphididae) were most abundant on flowers from hybrid cacao (1.55 ± 5.43 individuals) than on native trees (0.58 \pm 1.1), ants (Hymenoptera: Formicidae) were most abundant on native cacao flower (1.17 \pm 2.32) than on hybrids (0.55 \pm 0.87; [Figure 2a](#page-14-0), 2b).

Since a high percentage (82.3%) of the collected flowers had no visitors on them, the average number of visitors per tree was low, even below one [\(Figure 2b](#page-14-0)). Native trees had significantly more visitors than hybrid trees, when taking the number of sampled trees and visits into account (Appendix B). Among the insect groups that were only present as visitors on hybrid trees, were Acariformes (3 individuals), Isoptera (1 individual) and unidentified insect larvae (2 individuals). Among the insect groups that were only present as visitors on native trees, were Siphonaptera (1 individual) and Vespoidea (1 individual).

FIGURE 2: (A) TOTAL NUMBER OF INSECT INDIVIDUALS AND (B) AVERAGE NUMBER OF INSECT INDIVIDUALS FOUND ON FLOWERS FROM CACAO TREES OF HYBRID ("H", GREEN, N = 53) OR NATIVE ("N", BROWN, N = 24) VARIETIES IN **THE REGION OF SAN MARTIN, PERU. THE LINES ON TOP OF BARDS IN FIGURE (B) DISPLAY STANDARD ERROR FROM THE MEAN.**

I found significantly higher abundance of flower visitors on the native variety (8.76 ± 3.6 insects) than on the hybrid variety (5.6 \pm 4.4 insects; [Figure 3a](#page-15-0)). Moreover, I found that flower visitor abundance significantly increased with understory vegetation cover [\(Figure 3c](#page-15-0)). However, I did not find a significant effect of shade cover or leaf litter depth on the abundance of flower visitors (Appendix B).

I found significantly higher taxa richness of flower visitors on the native variety (4.54 \pm 1.45 taxa per tree) than on the hybrid variety (2.84 ± 1.28 taxa pe tree[; Figure 3b](#page-15-0)). Moreover, I found that taxa richness significantly increased with understory vegetation cover [\(Figure 3d](#page-15-0)). However, I did not find a significant effect of shade cover or leaf litter depth on taxa richness (Appendix C).

FIGURE 3: (A) OVERALL ABUNDANCE OF FLOWER VISITORS (ONE OUTLIER FOR EACH BOXPLOT EXCLUDED FROM THE FIGURE) AND (B) TAXA RICHNESS OF FLOWER VISITORS FOUND ON FLOWERS FROM CACAO TREES OF HYBRID ("H", GREEN, $N = 53$) OR NATIVE ("N", BROWN, $N = 24$) VARIETIES IN THE REGION OF SAN MARTIN, PERU. (C) EFFECT OF **THE PERCENTAGE OF UNDERSTORY VEGETATION COVER (%) ON OVERALL ABUNDANCE OF FLOWER VISITORS ON CACAO** TREES OF BOTH HYBRID (N = 53) AND NATIVE (N = 24) VARIETIES IN THE REGION OF SAN MARTIN, PERU. THE GREY **AREA DISPLAYS THE CONFIDENCE INTERVAL. THREE DATA POINTS ARE ABOVE 25. (D) EFFECT OF THE PERCENTAGE OF UNDERSTORY VEGETATION COVER (%) ON TAXA RICHNESS OF FLOWER VISITORS ON CACAO TREES OF BOTH HYBRID** (N = 53) AND NATIVE (N = 24) VARIETIES IN THE REGION OF SAN MARTIN, PERU. THE GREY AREA DISPLAYS THE **CONFIDENCE INTERVAL. THREE DATA POINTS ARE ABOVE 8.**

Overall, the variability in flower visitor community composition across samples (all native resp. hybrid trees of an agroforest grouped into an artificial native resp. hybrid "agroforest") assessed with NMDS was higher in hybrids than in natives, but there were no large differences in the concrete composition of flower visitor communities [\(Figure 4\)](#page-16-0). Instead, the community of flower visitors present on native cacao flowers was a subset of the one observed on hybrids, having native resp. hybrid "agroforests" as sampling units. However, I found no evidence that the Shannon diversity index was significantly affected by variety, shade cover, understory vegetation cover or leaf litter depth (Appendix D).

FIGURE 4: A NON-METRIC MULTI-DIMENSIONAL SCALING (NMDS) PLOT SHOWING THE DIFFERENCES BETWEEN THE FLOWER VISITOR COMMUNITIES OF HYBRID AND NATIVE TREES.

3.2 Cacao early-fruit set

Early-fruit set of cacao was neither significantly affected by variety, shade cover, understory vegetation cover nor leaf litter depth (Appendix E). Although the mean fruit set of native trees with 1.9% \pm 6.6% is more than twice that of the hybrid trees with 0.9% \pm 1.4%, the standard deviation is too high to provide a significant difference between the varieties. The median of the fruit set rate is higher for the hybrid than for the native trees [\(Figure 5\)](#page-17-1).

FIGURE 5: (A) MEAN FRUIT SET RATE FROM CACAO TREES OF HYBRID ("H", GREEN, N = 44) OR NATIVE ("N", **BROWN, N = 23) VARIETIES IN THE REGION OF SAN MARTIN, PERU. THREE DATA POINTS OF THE HYBRID TREES ARE** ABOVE 4. (B) MEDIAN FRUIT SET RATE FROM CACAO TREES OF HYBRID ("H", GREEN, N = 44) OR NATIVE ("N", **BROWN, N = 23) VARIETIES IN THE REGION OF SAN MARTIN, PERU. THREE DATA POINTS OF THE NATIVE TREES ARE ABOVE 4.**

4. Discussion

The aim of this study was to compare the diversity and abundance of flower visitors on hybrid and native cacao trees as well as early fruit set. Additionally, the effects of different agroforest management variables (shade cover, understory vegetation cover, leaf litter cover and leaf litter depth) on abundance and taxa richness of flower visitors were analysed. This is the first time that the effects of understory vegetation on cacao flowers have been investigated. Addressing this knowledge gap will pave the way for sustainable and simultaneously efficient cacao production. The findings of our study reveal that the main flower visitors differ between hybrid and native trees. Furthermore, the analysis of our data indicates consistent effects of understory vegetation on abundance and taxa richness of cacao flower visitors. However, our results did not show any significant effect of understory vegetation on early-fruit set.

A remarkable difference in the taxa composition of flower visitors regarding the frequency of occurrence between hybrid and native trees was observed, likely indicating that they are either pollinated by different insects or exposed to different pests. The most dominant insects on hybrids were aphids. Due to their settling behaviour, they can be only considered as pollinators for selfcompatible cacao varieties (Tarmadja, 2015). However, a study conducted in Hawaii could prove that there was no pollen found on any caught or photographed aphid individual (O'Doherty & Zoll, 2012). According to this and other related studies (Billes, 1941; Chumacero de Schawe *et al.*, 2018), it is rather unlikely that aphids play an important role in pollinating cacao flowers. Yet, it might be that if another insect is already taking over the role of pollination, the aphids will not function as pollinators in the first place. Another possibility would be that aphids are actually harming the flowers due to their phytophagous lifestyle (e.g., Kennedy & Stroyan, 1959; Lombaert *et al.*, 2006), causing the hybrid varieties to achieve a lower mean fruit set. Still, the contribution of aphids to cacao pollination cannot be clearly defined within this study. The most dominant insects on natives were ants. Recently, Vansynghel *et. al* (2022b) could show that more ants resulted in higher yields – but only if the cacao agroforest was close to a forest. In contrast, ants were also associated with increased fruit loss. However, two studies conducted in Central Sulawesi, Indonesia, could demonstrate that ant exclusion lead to lower yields (Wielgloss *et al.*, 2014; Gras *et al.*, 2016), suggesting that the presence of ants has positive effects on fruit production. Since ants show very diverse diets (Tillberg *et al.*, 2006), it is difficult to determine their role in complex ecosystems or understudied insect communities such as the arthropod community in cacao agroforests. Ants are known to be able to pollinate flowers (Gómez *et al.*, 1996; Delnevo *et al.*, 2020) and reduce herbivory as well as pest damage due to the displacement of pest provoking insects (Wielgloss *et al.*, 2014). However, the function of ants as pollinators in cacao has been doubted (Leston, 1970). A more recent study

suggests that ants rather play a role in facilitating cacao flower pollination than directly pollinating cacao flowers (Wielgloss *et al.*, 2014). Since ants were the most abundant insect group on native trees and since the mean early-fruit set was higher on native than on hybrid trees (not significant), we might conclude that ants indeed contribute in a positive way to cacao pollination. As with aphids, the function of ants in cacao agroforests cannot be fully explained, yet.

The second most abundant insects for both native and hybrid trees were thrips. Several authors suggest that thrips might play a role in pollination (Chumacero de Schawe *et al.*, 2018; Vansynghel *et. al*, 2022a; Billes, 1941). Since thrips occurred on both varieties in relatively high abundances (46 individuals on hybrid and 25 individuals on native trees), this could be the case for cacao agroforests in Peru, too.

The third most abundant insects for both native and hybrid trees were parasitic wasps. Whereas the majority of the cacao pollination related literature deals with aphids, ants and thrips, less is known about the function of parasitic wasps. Sperber *et al.* (2012) state that parasitic wasps in Brazilian cacao agroforests play an important role in pest control, e.g., due to their parasitic lifestyle, but there was no experiment conducted specifically related to their potential pollination behaviour. However, several studies demonstrate that parasitic wasps are known pollinators of other plants, especially fig species (Jousselin *et al.*, 2003; Suleman *et al.*, 2013). In contrast, parasitic wasps are also known to feed on nectar and pollen of cotton (Geng *et al.*, 2006; Röse *et al.*, 2006), resulting in potential negative effects on cacao pollination if they fed on cacao pollen, as well. Since parasitic wasps were found both in native and hybrid trees on the reproductive parts of the cacao flower in relatively high abundances, (32 individuals on hybrid and 19 individuals on native trees), their potential function as pollinators is not unlikely. However, the possibility that they are affecting the cacao pollination in a bad way due to their potential cacao pollen diet is not to be excluded, either. Moreover, a high abundance of parasitic wasps should lead to a high fruit set if they fulfil their potential function as cacao pest controllers.

Since the fruit set rates are extremely low, both for natives and hybrids, the question remains if insects might not affect fruit set at all. Another possibility would be that fruit set is rather affected by the availability of different nutrients. Iglesias *et al.* (2003) showed that fruit set in citrus trees depended heavily on the availability of carbohydrate, Erel *et al.* (2013) observed that the availability of nitrogen had a great impact on the fruit set in olive trees. As a consequence, we might conclude that hybrid and native varieties exhibit differences in their mechanisms of nutrient uptake, leading therefore to different results in fruit set. Again, further research is urgently needed to be able to support or neglect these assumptions.

All stress values regarding NMDS range between 0.21 and 0.3. They are possibly still valid to be interpreted although the reduction of dimensionality certainly caused information loss (e.g., Hammer & Harper, 2006; Shen & Shi, 2004). At first glance, the results of NMDS and taxa richness seem contradictory, but diving deeper into the meaning of these techniques and the sampling units that were used offer a valid explanation. Firstly, taxa richness reflects the total number of taxa without considering composition or distribution, whereas NMDS focuses on the variability in taxa composition across samples (Clarke, 1993). Secondly, the sampling units differ from each other since single trees were used for calculating taxa richness, whereas "artificial" agroforests (consisting only of hybrid resp. native trees) were used for NMDS. Overall, this might indicate that the variability in flower visitor community composition across agroforests was higher in hybrids than in natives but not across trees. However, the results of the NMDS should be interpreted with caution since the stress values are not very reliable.

Against my expectations, I found no effect of shade cover or leaf litter depth on either overall abundance, taxa richness or Shannon diversity. Since the visitor's rate per flower is very low (1 out of 5.66 flowers had at least one insect), a remarkably larger number of glued flowers might have delivered different results. On the other hand, other comparable studies (Vansynghel *et. al*, 2022a; Chumacero de Schawe *et al.*, 2018) did not collect unusually more (631) or less (304) insects. However, neither of these two studies investigated the effects of shade cover, understory vegetation cover and leaf litter depth on overall abundance or species richness. The only agroforest management variable that had an impact on the overall abundance and taxa richness of the flower visitors was the percentage of understory vegetation cover. Whereas strong evidence exists that more understory vegetation leads to higher understory bird richness (Wilsey & Temple, 2011), the effects of understory vegetation on cacao flower visitors have not been studied yet.

Since the percentages of understory vegetation and leaf litter cover were highly correlated (0.16), previous research about leaf litter cover might help to interpret the analysis outcome. Our results show that the abundance of flower visitors on both hybrid and native trees increases with an ascending percentage of understory vegetation cover. In contrast to our results, taking the high correlation between understory vegetation and leaf litter cover into account, the findings of Toledo-Hernández *et al.* (2021) established that the amount of leaf-litter in the field positively correlated with an increased abundance of ants, although not with Diptera. However, increasing the amount of leaf litter by actively managing it on-site did not significantly increase the abundance neither of ants nor Diptera. The authors suggest that more extended sampling periods might be required to detect differences in the abundance of visitors. Furthermore, leaf litter is known to be a potential breeding habitat for cacao pollinators (Vandromme *et al.*, 2023) as well as a habitat for cacao flower visitors in general (Toledo-Hernández *et al.*, 2021). Additionally, previous research on

coffee suggests that clearing understory vegetation decreases species diversity (Schmitt *et al.*, 2009; Arai *et al.*, 2023). Based on these previous results and my current study, it seems possible that understory vegetation cover is more relevant for promoting cacao flower visitors than leaf litter. Therefore, maintaining high levels of understory cover within agroforest may be a valuable management technique to maintain arthropod diversity and their beneficial ecosystem services (e.g., pollination) for cacao. While understory vegetation plays an important role in providing essential nesting and food resources for insects, it is noteworthy that canopy as well offers crucial nesting and food resources (Sobek *et al.*, 2009). However, the canopy offers less humidity than the understory and shows higher predator abundance and temperature extremes (Hirao *et al.*, 2009; Sobek *et al.*, 2009). Accordingly, insects might profit from a high availability of understory vegetation. To meaningfully interpret these results, further research in the field of cacao agroforest management is needed since the importance of the individual management measures is not sufficiently understood yet.

Although our study revealed consistent effects of understory vegetation cover on overall abundance and taxa richness of cacao flower visitors, we did not find a significant effect on early-fruit set. One possible explanation for this might be that a higher cover of understory vegetation increases not specifically the abundance of potential pollinators but of arthropods in general. In this case, however, questions regarding their activities around the reproductive parts would arise. Existing literature from the late $20th$ century mentions pollen theft as a reason for certain bee species to visit the cacao flower (Young, 1985) but no recent literature deals with pollen thieves affecting cacao. Another reason could be that a longer sampling period of the early-fruit set is required to observe significant differences. It is also worth considering that not only the cacao pollinators but certain species damaging the young fruits become more abundant when understory vegetation cover is increased. In general, the mean early-fruit set rate was low $(1.9% \pm 6.6%$ for native trees, 0.9% $\pm 1.4%$ for hybrid trees) but in line with other studies (1.7 ± 0.2%) in the same area (Vansynghel *et al.*, 2022b). Fruit set can be influenced by the interaction of temperature and humidity (Vansynghel *et al.*, 2023), general resource limitations (Ayre & Whelan, 1989; Groeneveld *et al.*, 2010) or increased pest and disease abundance (Bos *et al.*, 2007), to only mention a few. Conducting a humidity assessment in the study region to find out if the air is too humid compared to the high temperatures for the cultivation of cacao could reveal yield-related issues. Nature in general but especially tropical rainforests are threatened by rising humidity due to climate change (Perez & Feeley, 2018). A recent study also showed that increased temperatures provoked more pollination failures in cacao (Vansynghel *et al.*, 2022a). If a certain region was suitable for cultivating cacao in the past, it does not automatically mean that it will stay suitable in the future, bearing the still ongoing change of climate in mind. Surprisingly, the median of the fruit set rate and the mean

fruit set rate show different patterns. Mean values are sensitive to outliers as well as small sample sizes (Osborne, 2004) and could therefore be less reliable.

A limitation of this study is the uneven amount of native and hybrid experimental trees. The large difference in the number of trees per agroforest and variety resulted in a more difficult statistical analysis. The second limitation concerns the amount of available data. Since only a low percentage of cacao flowers becomes actually pollinated, the number of sampled flowers has to be very large to cover a decent number of insects. A higher number of collected flowers might have delivered more significant results.

The differences in the amount of flower visiting insects comparing hybrid and native trees had not been investigated in this study area before. Our research shows that the predicted overall abundance as well as taxa richness of flower visitors is higher in native than in hybrid trees. To meaningfully interpret this result, again further research is needed since the function of the insects that were found is not nearly understood yet. One possible reason for this could be that the native trees attract more insects, since the co-evolution of plant and pollinator happened long before hybrid varieties were established. Pollination syndromes are the result of long-lasting co-evolution, ending up in a coadaptation of varying extent between pollinators and plants (Pyke, 2016; Barrett, 2010). Focusing more on native varieties in the field of cacao production provides new opportunities concerning the diversification of the cacao agroforestry. In the long run, this will lead to the establishment of cacao agroforests that show a higher resistance to threats like diseases and climate change (Maas *et al.*, 2020). In general, planting different varieties within one agroforest should increase pollen diversity and therefore enhance pollination success since (pollen) diversity promotes the resilience and stability of an ecosystem (Paschke *et al.*, 2002). Furthermore, overall abundance as well taxa richness of flower visitors significantly increases with higher understory vegetation cover. Conducting further research on these findings will lead the way to a more sustainable, efficient and biodiversity-friendlier cacao production.

5. Conclusion

Although cacao is one of the most important crops worldwide, the main pollinators of cacao remain unknown. Additionally, the reasons for the low fruit set rate are not solved, either. My results show that the main flower visitors differ between hybrid and native varieties. Furthermore, the effects of understory vegetation on the abundance and diversity of flower visitors were studied for the first time, resulting in detecting a significant increase of flower visitors with increased understory vegetation cover. However, no effects of any assessed management variable on fruit set rate were found. This leads to the assumption that further research on cacao agroforest management is urgently needed to make way for a both sustainable and efficient cacao cultivation. It is important to find out if the findings of this study hold true in other geographical locations that possibly provide different conditions concerning humidity, temperature, age of the agroforests, managing practices through the farmers, to only mention a few examples. Since the methods that were used in this specific field study can be applied in the same manner in other regions as well, it is important to seize the chance and extend this research to more areas. If the results keep showing that increased understory vegetation leads to more flower visitors and if further research proves that final yield is rising, too, the way of managing agroforests should be reconsidered. The low fruit set and the reasons behind it remain a crucial clue. This leads to the assumption that further research on cacao agroforest management is urgently needed to make way for a both sustainable and efficient cacao cultivation, focusing on finding alternative ways of managing understory vegetation and conducting more research in the field of native varieties. This would not only promote biodiversity and productivity but might also provide a better income for millions of cacao farmers worldwide.

References

Agisoft LLC. (2023). *Agisoft Metashape 1.6.5—Professional edition*. Agisoft LLC software.

Akesse-Ransford, G., Owusu, E. O., Kyerematen, R., & Adu-Acheampong, S. (2021). Arthropod diversity of cocoa farms under two management systems in the Eastern and Central regions of Ghana. *Agroforestry Systems, 95*(5), 791–803. https://doi.org/10.1007/s10457-020-00568-5

Almeida, A.-A. F. de, & Valle, R. R. (2007). Ecophysiology of the cacao tree. *Brazilian Journal of Plant Physiology, 19*, 425–448. https://doi.org/10.1590/S1677-04202007000400011

Arai, Y., Hundera, K., & Yoshikura, T. (2023). Challenges in conserving forest ecosystems through coffee certification: A case study from southwestern Ethiopia. *Frontiers in Environmental Science, 11*. https://www.frontiersin.org/articles/10.3389/fenvs.2023.1193242

Armengot, L., Barbieri, P., Andres, C., Milz, J., & Schneider, M. (2016). Cacao agroforestry systems have higher return on labor compared to full-sun monocultures. *Agronomy for Sustainable Development, 36*(4), 70. https://doi.org/10.1007/s13593-016-0406-6

Ayre, D. J., & Whelan, R. J. (1989). Factors controlling fruit set in hermaphroditic plants: Studies with the Australian proteaceae. *Trends in Ecology & Evolution, 4*(9), 267–272. https://doi.org/10.1016/0169-5347(89)90197-3

Barrett, S. C. H. (2010). Darwin's legacy: The forms, function and sexual diversity of flowers. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1539), 351–368. https://doi.org/10.1098/rstb.2009.0212

Billes, D. J. (1941). *POLLINATION OF THEOBROMA CACAO L. IN TRINIDAD*, B.W.I. Tropical Agriculture.

Bisseleua, D. H. B., Missoup, A. D., & Vidal, S. (2009). Biodiversity Conservation, Ecosystem Functioning, and Economic Incentives under Cocoa Agroforestry Intensification. *Conservation Biology, 23*(5), 1176–1184. https://doi.org/10.1111/j.1523-1739.2009.01220.x

Bos, M. M., Veddeler, D., Bogdanski, A. K., Klein, A.-M., Tscharntke, T., Steffan-Dewenter, I., & Tylianakis, J. M. (2007). Caveats to Quantifying Ecosystem Services: Fruit Abortion Blurs Benefits from Crop Pollination. *Ecological Applications, 17*(6), 1841–1849. https://doi.org/10.1890/06-1763.1

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., & Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal, 9*(2), 378-400. https://doi.org/10.32614/RJ-2017-066

Brothers, D. J., Finnamore, A. T., & Fernández, F. (2006). Superfamilia Vespoidea. In Fernández, F. & Sharkey, M. J. (Eds.), *Introducción a los Hymenoptera de la Región Neotropical* (pp. 505–514). Sociedad Colombiana de Entomología & Universidad Nacional de Colombia, Bogota.

Brown, B. V., Borkent, A., Cumming, J. M., Wood, D. M., Woodley, N. E., & Zumbado, M. A. (2009). *Manual of Central American Diptera*. NRC Research Press.

Castillo, P. (2013). lnsectos plagas y sus enemigos naturales en el cultivo de Theobroma cacao L. (cacao) en los valles de Tumbes y Zarumilla, Perú. *Revista Manglar 10*(2): 3–16. Revista de Investigación Científica Universidad Nacional de Tumbes.

Chao, A., Chiu, C.-H., & Jost, L. (2014). Unifying Species Diversity, Phylogenetic Diversity, Functional Diversity, and Related Similarity and Differentiation Measures Through Hill Numbers. *Annual Review* *of Ecology, Evolution, and Systematics, 45*, 297–324. https://doi.org/10.1146/annurev-ecolsys-120213-091540

Choate, P. M. (1999). Introduction to the Identification of Beetles (Coleoptera). *Dichotomous keys to some families of Florida Coleoptera*, 23-33.

Chumacero de Schawe, C., Kessler, M., Hensen, I., & Tscharntke, T. (2018). Abundance and diversity of flower visitors on wild and cultivated cacao (Theobroma cacao L.) in Bolivia. *Agroforestry Systems, 92*(1), 117–125. https://doi.org/10.1007/s10457-016-0019-8

Clarke, K. R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology, 18*(1), 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x

Clough, Y., Barkmann, J., Juhrbandt, J., Kessler, M., Wanger, T. C., Anshary, A., Buchori, D., Cicuzza, D., Darras, K., Putra, D. D., Erasmi, S., Pitopang, R., Schmidt, C., Schulze, C. H., Seidel, D., Steffan-Dewenter, I., Stenchly, K., Vidal, S., Weist, M., Wielgoss, A., Tscharntke, T. (2011). Combining high biodiversity with high yields in tropical agroforests. *Proceedings of the National Academy of Sciences, 108*(20), 8311–8316. https://doi.org/10.1073/pnas.1016799108

Delnevo, N., van Etten, E. J., Clemente, N., Fogu, L., Pavarani, E., Byrne, M., & Stock, W. D. (2020). Pollen adaptation to ant pollination: A case study from the Proteaceae. *Annals of Botany, 126*(3), 377–386. https://doi.org/10.1093/aob/mcaa058

Erel, R., Yermiyahu, U., Van Opstal, J., Ben-Gal, A., Schwartz, A., & Dag, A. (2013). The importance of olive (Olea europaea L.) tree nutritional status on its productivity. *Scientia Horticulturae, 159*, 8–18. https://doi.org/10.1016/j.scienta.2013.04.036

Fernández, F. (2003). *Introducción a Las Hormigas de la Región Neotropical*. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. https://doi.org/10.5281/zenodo.11738

Fernández, F. & Mason, W. (2006). Clave para las superfamilias neotropicales de Hyemnoptera. In Fernández, F. & Sharkey, M. J. (Eds.), *Introducción a los Hymenoptera de la Región Neotropical* (pp. 177–202). Universidad Nacional de Colombia & Socolen.

Food and Agriculture Organization of the United Nations (FAO). (2023). *Cocoa bean production by region, 1961 to 2021*. Our World in Data. Retrieved 24 June 2023, from https://ourworldindata.org/grapher/cocoa-beans-production-by-region

Geng, J.-H., Shen, Z.-R., Song, K., & Zheng, L. (2006). Effect of Pollen of Regular Cotton and Transgenic Bt+CpTi Cotton on the Survival and Reproduction of the Parasitoid Wasp Trichogramma chilonis (Hymenoptera: Trichogrammatidae) in the Laboratory. *Environmental Entomology, 35*(6), 1661– 1668. https://doi.org/10.1093/ee/35.6.1661

Gibb, T. J., & Oseto, C. Y. (2006). *Arthropod Collection and Identification: Laboratory and Field Techniques*. Academic Press.

Gómez, J. M., Zamora, R., Hódar, J. A., & García, D. (1996). Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia, 105*(2), 236–242. https://doi.org/10.1007/BF00328552

Gras, P., Tscharntke, T., Maas, B., Tjoa, A., Hafsah, A., & Clough, Y. (2016). How ants, birds and bats affect crop yield along shade gradients in tropical cacao agroforestry. *Journal of Applied Ecology, 53*(3), 953–963. https://doi.org/10.1111/1365-2664.12625

Greenberg, R., Bichier, P., & Angón, A. C. (2000). The conservation value for birds of cacao plantations with diverse planted shade in Tabasco, Mexico. *Animal Conservation Forum, 3*(2), 105– 112. https://doi.org/10.1111/j.1469-1795.2000.tb00235.x

Groeneveld, J. H., Tscharntke, T., Moser, G., & Clough, Y. (2010). Experimental evidence for stronger cacao yield limitation by pollination than by plant resources. *Perspectives in Plant Ecology, Evolution and Systematics, 12*(3), 183–191. https://doi.org/10.1016/j.ppees.2010.02.005

Hammer, Ø., & Harper, D. A. T. (Eds.). (2005). *Paleobiogeography and Paleoecology*. In Paleontological Data Analysis (1st ed., pp. 183–253). Wiley. https://doi.org/10.1002/9780470750711.ch6

Hartig, F. (2022). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models*. R package version 0.4.6. Retrieved from https://CRAN.R-project.org/package=DHARMa

Hirao, T., Murakami, M., & Kashizaki, A. (2009). Importance of the understory stratum to entomofaunal diversity in a temperate deciduous forest. *Ecological Research, 24*(2), 263–272. https://doi.org/10.1007/s11284-008-0502-4

Hsieh, T. C., Ma, K. H., & Chao, A. (2016a). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution, 7*(12), 1451–1456. https://doi.org/10.1111/2041-210X.12613

Hsieh, T. C., Ma, K. H., & Chao, A. (2016b). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution, 7*(12), 1451–1456. https://doi.org/10.1111/2041-210X.12613

Iglesias, D. J., Tadeo, F. R., Primo-Millo, E., & Talon, M. (2003). Fruit set dependence on carbohydrate availability in citrus trees. *Tree Physiology, 23*(3), 199–204. https://doi.org/10.1093/treephys/23.3.199

Jousselin, E., Rasplus, J.-Y., & Kjellberg, F. (2001). Shift to mutualism in parasitic lineages of the fig/fig wasp interaction. *Oikos, 94*(2), 287–294. https://doi.org/10.1034/j.1600-0706.2001.940209.x

Kaonga, M. (2012). *Agroforestry for Biodiversity and Ecosystem Services: Science and Practice*. BoD – Books on Demand.

Kennedy, J. S., & Stroyan, H. L. G. (1959). Biology of Aphids. *Annual Review of Entomology, 4*(1), 139– 160. https://doi.org/10.1146/annurev.en.04.010159.001035

Laurent, V., Risterucci, A. M., & Lanaud, C. (1994). Genetic diversity in cocoa revealed by cDNA probes. *Theoretical and Applied Genetics, 88*(2), 193–198. https://doi.org/10.1007/BF00225897

Leston, D. (1970). Entomology of the Cocoa Farm. *Annual Review of Entomology, 15*(1), 273–294. https://doi.org/10.1146/annurev.en.15.010170.001421

Lichtenberg, E. M., Kennedy, C. M., Kremen, C., Batáry, P., Berendse, F., Bommarco, R., Bosque-Pérez, N. A., Carvalheiro, L. G., Snyder, W. E., Williams, N. M., Winfree, R., Klatt, B. K., Åström, S. A., Benjamin, F., Brittain, C., Chaplin-Kramer, R., Clough, Y., Danforth, B., Diekötter, T., Eigenbrode, S. D., Ekroos, J., Elle, E., Freitas, B. M., Fukuda, Y., Gaines-Day, H. R., Grab, H., Gratton, C., Holzschuh, A., Isaacs, R., Isaia, M., Jha, S., Jonason, D., Jones, V. P., Klein, A.-M., Krauss, J., Letourneau, D. K., Macfadyen, S., Mallinger, R. E., Martin, E. A., Martinez, E., Memmott, J., Morandin, L., Neame, L., Otieno, M., Park, M. G., Pfiffner, L., Pocock, M. J. O., Ponce, C., Potts, S. G., Poveda, K., Ramos, M., Rosenheim, J. A., Rundlöf, M., Sardiñas, H., Saunders, M. E., Schon, N. L., Sciligo, A. R., Sidhu, C. S., Steffan-Dewenter, I., Tscharntke, T., Veselý, M., Weisser, W. W., Wilson, J. K., Crowder, D. W. (2017).

A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes. *Global Change Biology, 23*(11), 4946–4957. https://doi.org/10.1111/gcb.13714

Lombaert, E., Boll, R., & Lapchin, L. (2006). Dispersal strategies of phytophagous insects at a local scale: Adaptive potential of aphids in an agricultural environment. *BMC Evolutionary Biology, 6*(1), 75. https://doi.org/10.1186/1471-2148-6-75

Maas, B., Thomas, E., Ocampo-Ariza, C., Vansynghel, J., Steffan-Dewenter, I., & Tscharntke, T. (2020). Transforming Tropical Agroforestry towards High Socio-Ecological Standards. *Trends in Ecology & Evolution, 35*(12), 1049–1052. https://doi.org/10.1016/j.tree.2020.09.002

Magurran, A. E. (2004). *Measuring Biological Diversity*. Blackwell Science Ltd.

Mattalia, G., Wezel, A., Costet, P., Jagoret, P., Deheuvels, O., Migliorini, P., & David, C. (2022). Contribution of cacao agroforestry versus mono-cropping systems for enhanced sustainability. A review with a focus on yield. *Agroforestry Systems, 96*(7), 1077–1089. https://doi.org/10.1007/s10457-022-00765-4

Monteiro, W. R., Lopes, U. V., & Clement, D. (2009). Genetic Improvement in Cocoa. In S. M. Jain & P. M. Priyadarshan (Eds.), *Breeding Plantation Tree Crops: Tropical Species* (pp. 589–626). Springer New York. https://doi.org/10.1007/978-0-387-71201-7_16

Motamayor, J. C., Lachenaud, P., Mota, J. W. da S. e, Loor, R., Kuhn, D. N., Brown, J. S., & Schnell, R. J. (2008). Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (Theobroma cacao L). *PLOS ONE, 3*(10), e3311. https://doi.org/10.1371/journal.pone.0003311

Motamayor, J. C., Risterucci, A. M., Lopez, P. A., Ortiz, C. F., Moreno, A., & Lanaud, C. (2002). Cacao domestication I: The origin of the cacao cultivated by the Mayas. *Heredity, 89*(5), Article 5. https://doi.org/10.1038/sj.hdy.6800156

Niether, W., Armengot, L., Andres, C., Schneider, M., & Gerold, G. (2018). Shade trees and tree pruning alter throughfall and microclimate in cocoa (Theobroma cacao L.) production systems. *Annals of Forest Science, 75*(2), Article 2. https://doi.org/10.1007/s13595-018-0723-9

Ocampo-Ariza, C., Maas, B., Castro-Namuche, J. P., Thomas, E., Vansynghel, J., Steffan-Dewenter, I., & Tscharntke, T. (2022). Trait-dependent responses of birds and bats to season and dry forest distance in tropical agroforestry. *Agriculture, Ecosystems & Environment, 325*, 107751. https://doi.org/10.1016/j.agee.2021.107751

O'Doherty, D. C., Zoll, J. J. K. (2012). Forcipomyia hardyi (Diptera: Ceratopogonidae), a Potential Pollinator of Cacao (Theobroma cacao) Flowers in Hawaii. *Proceedings of the Hawaiian Entomological Society (2012) 44*, 79–81.

Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos, P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro Cunha, E., Smith, T., Stier, A., Ter Braak, C., Weedon, J. (2022). *vegan: Community Ecology Package*. R package version 2.6-4. Retrieved from https://CRAN.R-project.org/package=vegan

Osborne, J. W. (2004). The power of outliers (and why researchers should ALWAYS check for them). *Practical Assessment, Research, and Evaluation, 9*(1), Article 1. https://doi.org/10.7275/qf69-7k43

Paschke, M., Abs, C., & Schmid, B. (2002). Effects of population size and pollen diversity on reproductive success and offspring size in the narrow endemic Cochlearia bavarica (Brassicaceae). *American Journal of Botany, 89*(8), 1250–1259. https://doi.org/10.3732/ajb.89.8.1250

Perez, T. M., & Feeley, K. J. (2018). Increasing Humidity Threatens Tropical Rainforests. *Frontiers in Ecology and Evolution, 6*(68).

Perfecto, I., Vandermeer, J., Mas, A., & Pinto, L. S. (2005). Biodiversity, yield, and shade coffee certification. *Ecological Economics, 54*(4), 435–446. https://doi.org/10.1016/j.ecolecon.2004.10.009

Posit team (2023). *RStudio: Integrated Development Environment for R*. Posit Software, PBC, Boston, MA.

Powis, T. G., Cyphers, A., Gaikwad, N. W., Grivetti, L., & Cheong, K. (2011). Cacao use and the San Lorenzo Olmec. *Proceedings of the National Academy of Sciences, 108*(21), 8595–8600. https://doi.org/10.1073/pnas.1100620108

Pyke, G. H. (2016). Plant–pollinator co-evolution: It's time to reconnect with Optimal Foraging Theory and Evolutionarily Stable Strategies. *Perspectives in Plant Ecology, Evolution and Systematics, 19*, 70– 76. https://doi.org/10.1016/j.ppees.2016.02.004

QGIS Development Team. (2023). *QGIS Geographic Information System*. Open Source Geospatial Foundation Project.

R Core Team (2023). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rasband, W. S. (1997-2018). *ImageJ*. U. S. National Institutes of Health, Bethesda, Maryland, USA.

Rodriguez-Medina, C., Arana, A. C., Sounigo, O., Argout, X., Alvarado, G. A., & Yockteng, R. (2019). Cacao breeding in Colombia, past, present and future. *Breeding Science, 69*(3), 373–382. https://doi.org/10.1270/jsbbs.19011

Röse, U. S. R., Lewis, J., & Tumlinson, J. H. (2006). Extrafloral Nectar from Cotton (Gossypium hirsutum) as a Food Source for Parasitic Wasps. *Functional Ecology, 20*(1), 67–74.

Roswell, M., Dushoff, J., & Winfree, R. (2021). A conceptual guide to measuring species diversity. *Oikos, 130*(3), 321–338. https://doi.org/10.1111/oik.07202

Schmitt, C. B., Senbeta, F., Denich, M., Preisinger, H., & Boehmer, H. J. (2010). Wild coffee management and plant diversity in the montane rainforest of southwestern Ethiopia. *African Journal of Ecology, 48*(1), 78–86. https://doi.org/10.1111/j.1365-2028.2009.01084.x

Shen, S., & Shi, G. R. (2004). Capitanian (Late Guadalupian, Permian) global brachiopod palaeobiogeography and latitudinal diversity pattern. *Palaeogeography, Palaeoclimatology, Palaeoecology, 208*, 235–262. https://doi.org/10.1016/j.palaeo.2004.03.009

Sobek, S., Tscharntke, T., Scherber, C., Schiele, S., & Steffan-Dewenter, I. (2009). Canopy vs. understory: Does tree diversity affect bee and wasp communities and their natural enemies across forest strata? *Forest Ecology and Management, 258*(5), 609–615. https://doi.org/10.1016/j.foreco.2009.04.026

Steffan-Dewenter, I., Kessler, M., Barkmann, J., Bos, M. M., Buchori, D., Erasmi, S., Faust, H., Gerold, G., Glenk, K., Gradstein, S. R., Guhardja, E., Harteveld, M., Hertel, D., Höhn, P., Kappas, M., Köhler, S., Leuschner, C., Maertens, M., Marggraf, R., Migge-Kleian, S., Mogea, J., Pitopang, R., Schaefer, M., Schwarze, S., Sporn, S. G., Steingrebe, A., Tjitrosoedirdjo, S. S., Tjitrosoemito, S., Twele, A., Weber, R., Woltmann, L., Zeller, M., & Tscharntke, T. (2007). Tradeoffs between income, biodiversity, and ecosystem functioning during tropical rainforest conversion and agroforestry intensification. *Proceedings of the National Academy of Sciences, 104*(12), 4973–4978. https://doi.org/10.1073/pnas.0608409104

Suleman, N., Raja, S., & Compton, S. G. (2013). Parasitism of a pollinator fig wasp: Mortalities are higher in figs with more pollinators, but are not related to local densities of figs*. Ecological Entomology, 38*(5), 478–484. https://doi.org/10.1111/een.12041

Tarmadja, S. (2015). THE CACAO FLOWER VISITOR INSECTS DIVERSITY AND THEIR POTENTIALITIES AS POLLINATORS. *KnE Life Sciences*, 540–543. https://doi.org/10.18502/kls.v2i1.212

Thomas, E., Lastra, S., & Zavaleta, D. (2023). *Catálogo de cacaos de Perú*. Bioversity International y MOCCA.

Thomas, E., Zonneveld, M. van, Loo, J., Hodgkin, T., Galluzzi, G., & Etten, J. van. (2012). Present Spatial Diversity Patterns of Theobroma cacao L. in the Neotropics Reflect Genetic Differentiation in Pleistocene Refugia Followed by Human-Influenced Dispersal. *PLOS ONE, 7*(10), e47676. https://doi.org/10.1371/journal.pone.0047676

Thyssen, P. (2010). Keys for Identification of Immature Insects. In Amendt, J., Goff, M. L., Campobasso, C. P., & Grassberger M. (Eds.), *Current Concepts in Forensic Entomology* (pp.25-42). Springer Science+Business Media B.V. https://doi.org[/10.1007/978-1-4020-9684-6_2](http://dx.doi.org/10.1007/978-1-4020-9684-6_2)

Tillberg, C. V., McCarthy, D. P., Dolezal, A. G., & Suarez, A. V. (2006). Measuring the trophic ecology of ants using stable isotopes. *Insectes Sociaux, 53*(1), 65–69. https://doi.org/10.1007/s00040-005-0836-7

Toledo-Hernández, M., Tscharntke, T., Tjoa, A., Anshary, A., Cyio, B., & Wanger, T. C. (2021). Landscape and farm-level management for conservation of potential pollinators in Indonesian cocoa agroforests. *Biological Conservation, 257*, 109106. https://doi.org/10.1016/j.biocon.2021.109106

Tscharntke, T., Ocampo-Ariza, C., Vansynghel, J., Ivañez-Ballesteros, B., Aycart, P., Rodriguez, L., Ramirez, M., Steffan-Dewenter, I., Maas, B., & Thomas, E. (2023). Socio-ecological benefits of fineflavor cacao in its center of origin. *Conservation Letters, 16*(1), e12936. https://doi.org/10.1111/conl.12936

Tuesta-Hidalgo, O., Santistevan-Méndez, M., Borjas, R., Castro Cepero, V., & Julca Otiniano, A. (2017). Sustainability of cacao farms in the district of Huicungo San Martín Perú. *Peruvian Journal of Agronomy, 1*. https://doi.org/10.21704/pja.v1i1.1062

Vandromme, M., Van de Sande, E., Pinceel, T., Vanhove, W., Trekels, H., & Vanschoenwinkel, B. (2023). Resolving the identity and breeding habitats of cryptic dipteran cacao flower visitors in a neotropical cacao agroforestry system. *Basic and Applied Ecology, 68*, 35–45. https://doi.org/10.1016/j.baae.2023.03.002

Vansynghel, J., Ocampo-Ariza, C., Maas, B., Martin, E. A., Thomas, E., Hanf-Dressler, T., Schumacher, N.-C., Ulloque-Samatelo, C., Tscharntke, T., & Steffan-Dewenter, I. (2022a). Cacao flower visitation: Low pollen deposition, low fruit set and dominance of herbivores. *Ecological Solutions and Evidence, 3*(2), e12140. https://doi.org/10.1002/2688-8319.12140

Vansynghel, J., Ocampo-Ariza, C., Maas, B., Martin, E. A., Thomas, E., Hanf-Dressler, T., Schumacher, N.-C., Ulloque-Samatelo, C., Yovera, F. F., Tscharntke, T., & Steffan-Dewenter, I. (2022b). Quantifying services and disservices provided by insects and vertebrates in cacao agroforestry landscapes.

Proceedings of the Royal Society B: Biological Sciences, 289(1982), 20221309. https://doi.org/10.1098/rspb.2022.1309

Vansynghel, J., Thomas, E., Ocampo-Ariza, C., Maas, B., Ulloque-Samatelo, C., Zhang, D., Tscharntke, T., & Steffan-Dewenter, I. (2023). Cross-pollination with native genotypes improves fruit set and yield quality of Peruvian cacao. *Agriculture, Ecosystems & Environment, 357*, 108671. https://doi.org/10.1016/j.agee.2023.108671

Whitkus, R., de la Cruz, M., Mota-Bravo, L., & Gómez-Pompa, A. (1998). Genetic diversity and relationships of cacao (Theobroma cacao L.) in southern Mexico. *Theoretical and Applied Genetics, 96*(5), 621–627. https://doi.org/10.1007/s001220050780

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wielgoss, A., Tscharntke, T., Rumede, A., Fiala, B., Seidel, H., Shahabuddin, S., & Clough, Y. (2014). Interaction complexity matters: Disentangling services and disservices of ant communities driving yield in tropical agroecosystems. *Proceedings of the Royal Society B: Biological Sciences, 281*(1775), 20132144. https://doi.org/10.1098/rspb.2013.2144

Wilsey, C. B., & Temple, S. A. (2011). The Effects of Cropping Systems on Avian Communities in Cacao and Banana Agro-Forestry Systems of Talamanca, Costa Rica. *Biotropica, 43*(1), 68–76. https://doi.org/10.1111/j.1744-7429.2010.00640.x

Wurz, A., Tscharntke, T., Martin, D. A., Osen, K., Rakotomalala, A. A. N. A., Raveloaritiana, E., Andrianisaina, F., Dröge, S., Fulgence, T. R., Soazafy, M. R., Andriafanomezantsoa, R., Andrianarimisa, A., Babarezoto, F. S., Barkmann, J., Hänke, H., Hölscher, D., Kreft, H., Rakouth, B., Guerrero-Ramírez, N. R., Ranarijaona, H. L. T., Randriamanantena, R., Ratsoavina, F. M., Ravaomanarivo, L. H. R., & Grass, I. (2022). Win-win opportunities combining high yields with high multi-taxa biodiversity in tropical agroforestry. *Nature Communications, 13*(1), Article 1. https://doi.org/10.1038/s41467-022-30866-8

Young, A. M. (1985). Pollen-collecting by stingless bees on cacao flowers. *Experientia, 41*(6), 760– 762. https://doi.org/10.1007/BF02012584

Zhang, D., & Motilal, L. (2016). Origin, Dispersal, and Current Global Distribution of Cacao Genetic Diversity. In B. A. Bailey & L. W. Meinhardt (Eds.), *Cacao Diseases* (pp. 3–31). Springer International Publishing. https://doi.org/10.1007/978-3-319-24789-2_1

Appendix A – List of all identified morphospecies

Appendix B – Overall abundance

Appendix C – Taxa richness

Appendix D – Shannon diversity

Appendix E – Early-fruit set

