RESEARCH ARTICLE

Ecological Solutions and Evidence | BRITISH ECOLOGICAL SOCIETY

Cacao flower visitation: Low pollen deposition, low fruit set and dominance of herbivores

Justine Vansynghel[1,2] | **Carolina Ocampo-Ariza[2,3]** | **Bea Maas[4]** |
Emily A. Martin[5] | **Evert Thomas[2]** | **Tara Hanf-Dressler[3]** |
Nils-Christian Schumacher[1] | **Carlos Ulloque-Samatelo[6]** | **Teja Tscharntke[3]** |
Ingolf Steffan-Dewenter[1]

[1]Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Würzburg, Germany

[2]Alliance of Bioversity International and CIAT, Lima Office, Lima, Peru

[3]Agroecology, Department of Crop Sciences, University of Göttingen, Göttingen, Germany

[4]Department of Botany and Biodiversity Research, University of Vienna, Vienna, Austria

[5]Zoological Biodiversity, Institute of Geobotany, Leibniz University Hannover, Hannover, Germany

[6]Facultad de Agronomía, Universidad Nacional de Piura, Piura, Peru

Correspondence
Justine Vansynghel, Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Am Hubland, 97074 Würzburg, Germany.
Email: justine.vansynghel@uni-wuerzburg.de

Abstract

1. Pollination services of cacao are crucial for global chocolate production, yet remain critically understudied, particularly in regions of origin of the species. Notably, uncertainties remain concerning the identity of cacao pollinators, the influence of landscape (forest distance) and management (shade cover) on flower visitation and the role of pollen deposition in limiting fruit set.

2. Here, we aimed to improve understanding of cacao pollination by studying limiting factors of fruit set in Peru, part of the centre of origin of cacao. Flower visitors were sampled with sticky insect glue in 20 cacao agroforests in two biogeographically distinct regions of Peru, across gradients of shade cover and forest distance. Further, we assessed pollen quantities and compared fruit set between naturally and manually pollinated flowers.

3. The most abundant flower visitors were aphids, ants and thrips in the north and thrips, midges and parasitoid wasps in the south of Peru. We present some evidence of increasing visitation rates from medium to high shade (40%–95% canopy closure) in the dry north, and opposite patterns in the semi-humid south, during the wet season.

4. Natural pollination resulted in remarkably low fruit set rates (2%), and very low pollen deposition. After hand pollination, fruit set more than tripled (7%), but was still low.

5. The diversity and high relative abundances of herbivore flower visitors limit our ability to draw conclusions on the functional role of different flower visitors. The remarkably low fruit set of naturally and even hand pollinated flowers indicates that other unaddressed factors limit cacao fruit production. Such factors could be, amongst others, a lack of effective pollinators, genetic incompatibility or resource limitation. Revealing efficient pollinator species and other causes of low fruit set rates is therefore key to establish location-specific management strategies and develop high yielding native cacao agroforestry systems in regions of origin of cacao.

1 | INTRODUCTION

Despite pollination services being central to successful fruit production of the cacao tree (*Theobroma cacao* L.), the underlying processes and limiting factors are still poorly understood (Klein et al., 2008; Toledo-Hernández et al., 2017). This is striking, considering that the tree is an important tropical cash crop used to manufacture chocolate and cacao cultivation sustains ∼6 million farmers globally, most of which are smallholders (Clay, 2004). While being an understorey tree native to the Amazon basin, cacao is nowadays mainly cultivated outside its native distribution range (Thomas et al., 2012). As a consequence, most research on cacao pollination services has been restricted to non-native countries (Toledo-Hernández et al., 2021). Yet, in recent years, cacao production in Amazonian countries has been on the rise (FAO, 2020), but yields of native cacao are often low (Romero & Vargas, 2016). Therefore, identifying limitations of pollination success (Figure 1) and closing the multiple knowledge gaps concerning fruit set in the native range of cacao is crucial for improving livelihoods of rural smallholders.

Productivity of cacao is, amongst others, limited by the plants' reproductive biology, for example entomophily and low abundances of presumed cacao pollinators reported by older studies (reviewed by Toledo-Hernández et al., 2017). Half of all cacao flower-visiting species worldwide are midges from the Ceratopogonidae and Cecidomyiidae families, yet, relative abundances observed on cacao flowers in Latin America can be as low as 2%, while other visitors such as thrips and ants have been found to be more abundant (Chumacero de Schawe et al., 2016; Toledo-Hernández et al., 2021). For example, in a study in Indonesia not a single Ceratopogonid was trapped visiting flowers (Toledo-Hernández et al., 2021). Owing to the variation in observed visitation patterns across study locations, the taxonomic identity of the main pollinators remains debated; it is likely that several arthropod taxa beyond midges contribute to pollination in cacao. Studying patterns of flower visitors across different cacao geographies is thus crucial to clarify pollination potential of different insects, as to improve pollination services.

Landscape properties and management features are known to drive pollination services of tropical agroforestry crops, including cacao, but patterns are still not fully understood. In Asia, flower visitation by potential coffee pollinators increased with forest proximity (Klein et al., 2008), but thus far, no such association has been detected for cacao (Toledo-Hernández et al., 2021). The integration of shade trees in cacao agroforests can provide multiple economic and ecological benefits (Blaser et al., 2018; Jezeer et al., 2017), such as increased Dipteran visitation rates under higher canopy closure detected in Indonesia (Toledo-Hernández et al., 2021). However, forest distance and shade cover patterns remain to be studied in cacao agroforestry outside of Asia.

Cacao yields also depend on characteristics of pollen deposition: Only a small fraction of the thousands of flowers receives a sufficient quantity of pollen to result in fruit set (Groeneveld et al., 2010). Because low pollen deposition can be linked to suboptimal cacao fruit set (Falque et al., 1996; Mena-Montoya et al., 2020), it is important to better understand the link between pollen deposition rates in the field and actual fruit setting rates. Limiting effects of pollen quantity and compatibility on yield can be alleviated by hand pollination (Toledo-Hernández et al., 2020), particularly so in self-incompatible cacao varieties . Manual pollen supplementation has been found to triple yields and increase cacao farmers' incomes by up to 69% (Toledo-Hernández et al., 2020). However, yield gains through hand pollination depend on environmental factors, cross-compatibility levels and timing (de Almeida & Valle, 2009; Forbes et al., 2019). Successes also might fluctuate locally, but no large-scale studies have addressed hand pollination gains in countries of origin of cacao.

In spite of decades of research on cacao pollination, our general understanding of flower visitation rates, pollen quantity effects on fruiting success and hand pollination gains remains limited. Patterns differ among and within continents and remain to be unravelled in understudied parts of the world. Here, we combined flower visitor surveys in two biogeographically contrasting regions with quantification of pollen deposition and hand pollination experiments in Peru, part of the centre of origin and domestication of cacao. Specifically, we asked: (Q1) What are the visitation rates of cacao flower visitors across
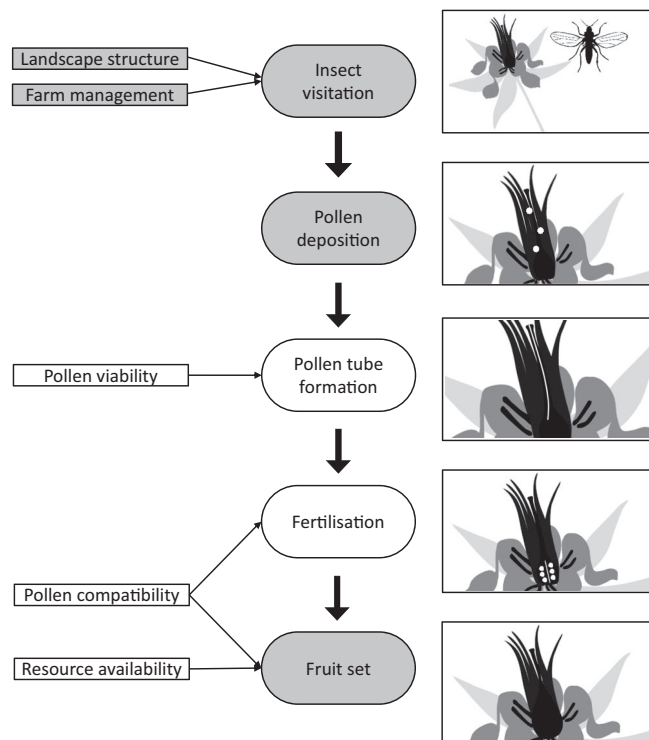
FIGURE 1 Conceptual overview of the cacao pollination process, depicting several steps preceding fruit set (ovals), including relevant drivers and limitations (rectangles). Variables addressed in this study are highlighted in grey. Insect visitation is necessary for pollen deposition and may depend on a plethora of factors, such as farm and landscape-level management such as canopy closure and forest distance (Toledo-Hernández et al., 2021). Pollen deposition can be influenced by visitation rates of insects and the amount and quality of pollen carried by different visitor species. When sufficient viable and compatible pollen is deposited on the style of a cacao flower, pollen tubes are formed, and the sperm nuclei migrate to the ovary for fertilization (Claus et al., 2018; Falque et al., 1995). Finally, pollen compatibility and resource availability can affect setting of fruits even until after fertilization (de Almeida & Valle, 2009; Ford & Wilkinson, 2012)

gradients of forest distance and shade cover in biogeographically distinct regions; (Q2) how much pollen is deposited during natural pollination and how does this affect fruit set rates in the field; and (Q3) to what extent does hand pollination improve cacao fruit set rates. Drawing on our findings, we discuss next steps to improve knowledge on pollination services in smallholder agroforestry systems in cacao's native range.

2 | MATERIAL AND METHODS

Research was developed under permit number 0519-2019-MINAGRI-SERFOR-DGGSPFFS.

2.1 | Study regions

We conducted our research in two cacao-growing areas in Peru with a distinct climate, vegetation type and biogeography: the dry north-ern lowlands, west of the Andes, and the humid south-eastern Andean slopes. The study area in the north was located around the farmer community of La Quemazón, in the department of Piura, in the coastal northwest of Peru (S5.312249°, W79.718996°, 240 m.a.s.l.; Figure S1a) where the local variety, Piura white cacao, is cultivated under irrigation. The area is characterized by the dominance of seasonally dry tropical forest vegetation and the climate is hot and semi-arid (SENAMHI, 2020a). Annual rainfall averages to 235 mm per year. Most of the annual rainfall (235 mm) occurs during the short, wet season from December until March. In the dry months, rainfall is close or equal to 0 mm.

The southern study area was located in the lowlands of the Cusco department, near Echarati (S12.768999°, W72.578451°, 987 m.a.s.l.; Figure S1b). The landscape is dominated by wet and humid montane forest vegetation (Rodríguez & Young, 2000) and the climate is moderate and humid (SENAMHI, 2020b). During the wet season, from November until April, rainfall is about 100–200 mm per month and during the dry season, about 50 mm per month (Merma & Julca, 2012; SENAMHI, 2020b). In the southern agroforests, introduced hybrid clones are cultivated alongside the local native variety, called chuncho cacao.

2.2 | Site selection and characterization

In the northern study region, 12 smallholder organic cacao agroforests were selected, between 0.2 and 2 ha in size, consisting of 5- to 10-year-old trees mainly from the native Piura white cacao. During the dry season, these agroforests are irrigated every 15–20 days by means of gravity-fed flood canals. In the southern study region, we selected eight organic smallholder agroforests, smaller than 3 ha and ranging between 5 and 65 years old. Here, gravity-fed flood canals and aspersion were used for irrigation, mainly during the dry season.

We calculated forest proximity, that is the shortest distance from each study site to the nearest forest (km) using ArcMap 10.5.1. To this end, we used updated versions of land-use map of Piura in the north (Otivo Barreto, 2010) and the vegetation cover map of Cusco in the south (MINAM, 2015). Canopy closure, assessed with a spherical densitometer, was used as measure for shade cover. For the northern agroforests, we averaged canopy closure over 25 readings spread out over an area of about ~0.2 ha, and in the southern agroforests, we averaged 20 readings over ~0.15 ha, to account for slightly larger subplot sizes in the north. Cacao tree density and abundance were comparable throughout the study: in most of the agroforests, trees were planted following a 3×3 m grid, with few exceptions of 3.5 m grids.

2.3 | Surveys

2.3.1 | Flower visitors

To trap arthropods visitors of cacao flowers, we applied non-drying, odourless and colourless insect adhesive (*Schacht Raupenleim*) on the

reproductive parts of cacao flowers (mainly around the style), between 5:15 AM and 11:30 AM. We retrieved the flowers about 24 h later. In the north, we sampled flowers during the dry season (Oct–Dec), and in the south, during the rainy season (Jan–Feb) in 2018/2019.

All agroforests were sampled three times, with minimum 4 and maximum 40 days between sampling rounds. During each sampling round, we selected 50 flowers distributed among 10 trees and covered the reproductive parts with glue, totalling to 150 flowers per agroforest. Upon flower retrieval, 24 h after glue application, most of the flowers had abscised, a process that is normal in cacao (24–36 h; Toledo-Hernández et al., 2017). Therefore, not all flowers could be recollected and numbers of retrieved flowers differed among trees and farms (Table S1). Arthropod specimens were retrieved from the flowers, and sorted into morphological and functional groups, based on general taxonomic keys (Gibb & Oseto, 2006) and keys to family level for Diptera (Brown et al., 2009). Cecidomyiidae and Ceratopogonidae were lumped, representing potential cacao-pollinating midges, hereafter referred to as midges. Other dipteran families were categorized as other Diptera; Hymenopterans were either classified as parasitoid wasps, ants or other Hymenoptera.

2.3.2 | Pollen quantity

To study how pollen deposition affects fruiting success in northern Peru, we took ultra-macro photographs of flowers directly on the tree and estimated the amount of pollen grains deposited on the style, following Macinnis and Forrest (2017).

Pollen deposition is usually quantified destructively, that is by removing pollinated flowers or flower parts. Here, flowers were monitored whilst developing further on the tree and as such, we avoided the risk of interfering with pollination success. We used a DSLR camera with ultra-macro lens (LAOWA, five times magnification) and a LED lamp and ring to increase light intensity. Photographs were taken at ISO 400 with shutter speed 1:40 and aperture F8. Of each flower, two series of photographs with different focusing depth were used for capturing the two opposite sides of the style (Figure S2).

We took 7704 macro photographs of 518 flowers, spread over five agroforests and different shooting days. Data of two consecutive years were included (Table S2). Normal cacao flower lifetime is about 24–36 h (Toledo-Hernández et al., 2017). Cacao buds show a slit between petals in the late afternoon when they are about to open the next day, early in the morning. To standardize the time flowers were exposed to visitors, we marked flower buds about to open by checking for the petal slit in the afternoon. These marked flowers were receptive for pollen from the next morning onwards, and the photographs were taken between 7 and 11 AM, 24–28 h after opening. To protect the styles from pollen deposition after photographing, flowers were isolated with caps covered with fine mesh adhered to the stem with modelling clay. Two days later, isolation caps were removed. We assessed fruiting success 7 days after photographing and counted the number of flowers that abscised (fruiting failure) and set fruit (fruiting success).

2.3.3 | Hand pollination

To compare natural pollination with manual pollination, we hand-pollinated flowers of eight receptor trees in each of the 12 northern agroforests and monitored the subsequent appearance of young fruits, hereafter referred to as cherelles. On each of the 96 experimental trees that served as pollen receptors, we selected sections of 35 cm on two branches per tree and assigned a natural or hand pollination treatment to these sections. Once a week, we manually pollinated all open flowers on the respective 35 cm branch section on each tree and followed the development of all open flowers on the other branch section over a period of 7 weeks during the dry season, which is the typical flowering period of *Piura white cacao*.

Flowers were pollinated between 6:30 AM and 1 PM. At 6 AM, freshly opened pollen donor flowers were collected from five genotypes of the native variety *Piura white cacao* established in a clonal garden managed by the cooperative Norandino. These genotypes were different from the ones present in the agroforests, thus lowering potential cross-incompatibility issues between donor and receptor of pollen. First, the petal hoods were removed from donor flowers before pollinating. Next, each of the five anthers were rubbed onto the stigma of the receptor flower. By rubbing multiple times with several anthers, we ensured that large pollen quantities were transferred onto the style of the receptor flowers. Before starting the experiments, we visually confirmed that pollen deposition was over 100 grains with a microscope (Figure S3). Following similar study designs used in Asia, flowers were not isolated from flower visitors before or after hand pollination (Groeneveld et al., 2010; Toledo-Hernández et al., 2020).

Six days after manual pollination, we counted the young fruits smaller than 1 cm (hereafter cherelles), as this size corresponds with ∼7 days old cherelles. Weekly fruit set rates were defined as cherelles observed 6 days after pollination, divided by the number of open flowers recorded 6 days earlier. In the natural pollination treatment, we did not intervene, and simply recorded open flowers and cherelles during the same visits to trees. Fruit set rates (cherelles/open flowers) were calculated based on pooled observations over the 7 weeks of the experiment.

2.4 | Statistical analyses

All statistical analyses were performed with R (R Core Team, 2020); plots were built with the package ggplot2 (Wickham, 2016). Spatial analyses and maps were performed and created with ArcMap 10.5.1.

2.4.1 | Flower visitors

We used generalized linear mixed effect models (GLMM) with the package lme4 (Bates et al., 2015) to investigate the effect of region, distance from forest (km) and canopy closure (decimal percentage) on three groups of flower visitors (based on visitation frequencies): thrips, aphids and all other flower visitors. The model for thrips and other

visitors included the interactions of region with canopy closure and region with forest distance. The model for aphid visitation included only data from the north and thus no interactions, as very few specimens were detected in the south (Table S1). Because surveys were conducted during the dry season in the north, and during the wet season in the south, seasonality is implicitly included in region.

In all three models, identity of agroforest was included as random effect variable to account for multiple sampling in each agroforest. Data from one southern agroforest were excluded from all models, because of incomplete canopy closure assessments (Q14; Table S1; Figure S1b). Aphid visits were modelled with a Poisson distribution. Due to over-dispersion in the models constructed for thrips and other visitors, we used a negative binomial distribution. All model residuals were inspected with package "DHARMa" (Hartig, 2018); no significant deviations were detected.

In our models, we integrated the differences in retrieved flowers per agroforest by including this value as offset, which is a good way to standardize count data of visits per flower (Reitan & Nielsen, 2016). For plotting, we used visitation rates (i.e. total visitors/retrieved flowers) instead of total visitors, and held the offset held constant at one to obtain predictions that are easy to compare.

2.4.2 | Pollen quantity

We recorded extremely low fruit sets during the experiment: the proportion of successes and failures was unbalanced (1:128). Although unbalanced data is a common phenomenon in ecological data (Salas-Eljatib et al., 2018), the success events were too rare to perform any meaningful statistical analysis.

2.4.3 | Hand pollination

To examine differences in fruit set rates (proportion ranging from 0 to 1) between naturally and hand pollinated flowers, we used a generalized linear mixed model (package "lme4"). Fruit set rates were pooled over seven counting rounds and compared between pollination treatment (fixed effect variable) using a binomial distribution, whereby the total number of open flowers was included as weights argument. DHARMa residual plots signalled no model violations. Since counts of cherelles and flowers were performed on eight trees per farm (Table S3), we included trees nested in farms as random effect variables. Trees with incomplete counts were excluded: only 93 were considered in this analysis ($N_{Manual} = 90$, $N_{Natural} = 91$; Table S3).

3 | RESULTS

3.1 | Flower visitor sampling

In total, 304 flower visitors were collected from 1179 flowers (1 visitor per 3.88 flowers); 7% of the entire visitor community were midges
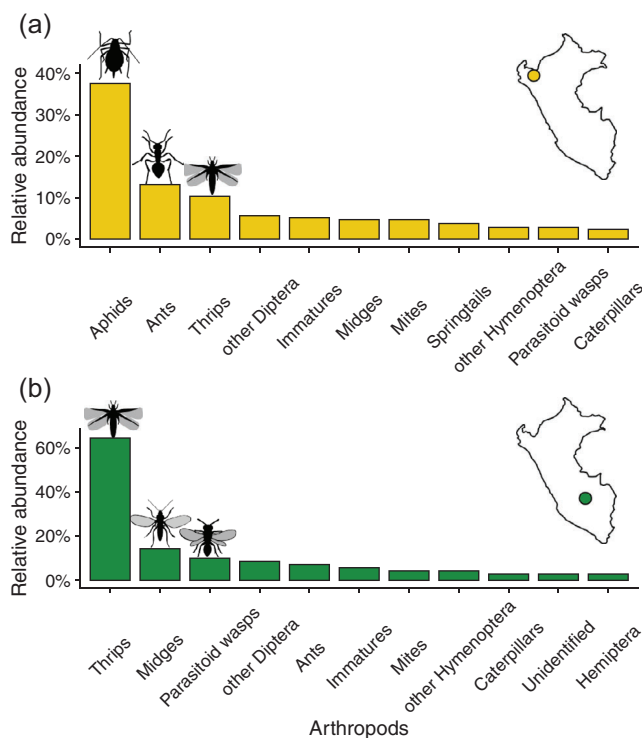

FIGURE 2 Relative abundances of top 11 arthropod groups per region (maps with circles) trapped with insect sticky glue from flowers in northern (a) and southern (b) Peru. Only groups with relative abundances >2% are shown

(Ceratopogonidae + Cecidomyiidae), the assumed cacao pollinators. We sampled 213 visitors from 885 flowers in the north (1 visitor per 4.15 flowers), and 70 visitors from 294 flowers (1 per 3.23 flowers) in the south. In the north (Figure 2a), the most abundant visitor groups were aphids (38%), ants (13%), thrips (10%), other Diptera (6%), immature arthropods such as larvae, pupae and nymphs (5%) and midges (5%). In the south (Figure 2b), the dominant visitors were thrips (65%), followed by midges (14%), parasitoid wasps (10%), other Diptera (9%), ants (7%) and immature arthropods (6%).

Overall, visitation rates of flower-visiting arthropods increased along higher canopy closure in the north and decreased in the south, whereas forest distance did not play an important role in flower visitation patterns (Table S4). Thrip visitations increased with canopy closure in the north and decreased along this gradient in the south (GLMM: $z = 5.74$, $P = 0.028$; Figure 3a), although patterns might be influenced by outliers. Further from forest, thrip visitations appeared to increase in the south and decrease in the north, but this is supported by weak evidence only (GLMM: $z = -1.91$, $P = 0.056$; Figure 3b). Neither canopy closure nor forest distance influenced visitation rates of aphids, which was the most abundant visitor in the north (Figure 3c,d). Visits by all other arthropods (excluding thrips and aphids) seemed to increase with higher canopy closure in northern Peru. In the south, visitations decreased along the canopy closure gradient, but this trend could only be weakly confirmed by analyses (GLMM: $z = 1.87$, $P = 0.062$; Figure 3e). Finally, visits by other arthropods did not seem to be affected by increasing forest distance (Figure 3f).
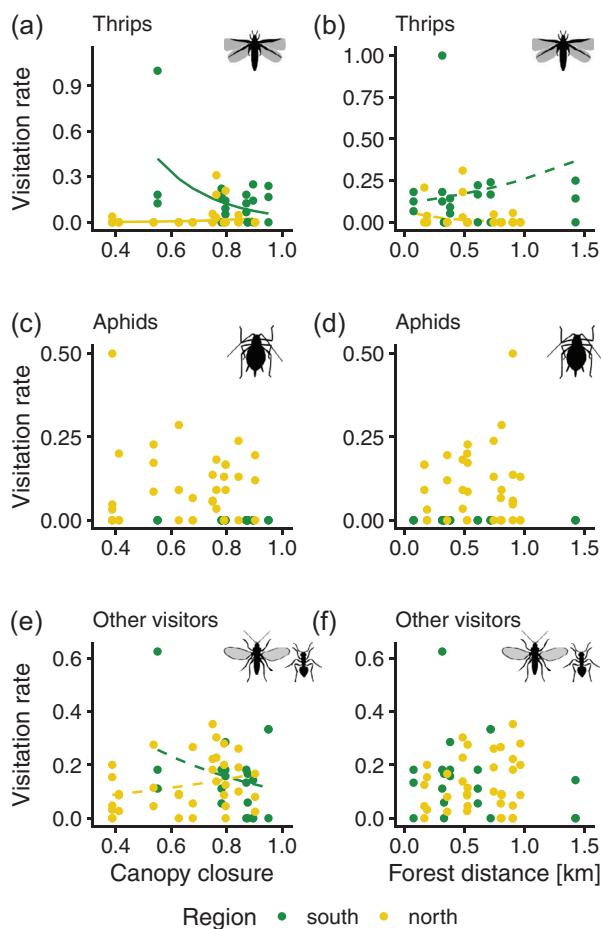
FIGURE 3 Visitation rates of thrips (a and b), aphids (c and d) and all other visitors, excluding aphids and thrips (e and f), in function of canopy closure (left column) and forest distance (right column). Visitations rates per round are calculated by dividing total visits by number of collected flowers per round in the 19 agroforests and are shown with dots (green for the south, yellow in the north). Full lines are simulations of significant interactions from generalized linear mixed models; dashed lines represent simulations of marginally significant interactions (Table S4)

3.2 | Pollen quantity

We found an average of 31 ± 1.2 (mean ± SE) pollen grains deposited per flower (*n* = 517), and only four flowers (0.8%) set fruit (Figure S4). On these four flowers, an average of 111 ± 19.2 pollen grains were deposited, while an average of 30.7 ± 1.2 pollen grains were deposited on styles of flowers that did not set fruit (*n* = 513).

3.3 | Hand pollination

Fruit set was remarkably low in both pollination treatments, but significantly higher for hand-pollinated flowers (GLMM: *z* = −6.76, *P* < 0.001; Figure 4; Table S5). Under natural pollination, 2% of the observed open flowers set fruit in total (39 out of 1952), whereas manual pollination resulted in a total fruit set rate of 7% (70 out of 968).
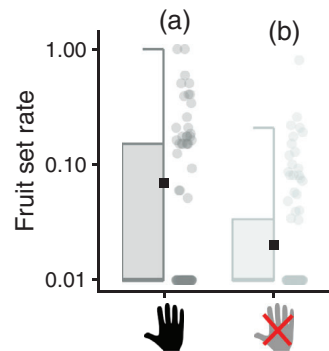
FIGURE 4 Fruit set rates of manually (dark grey circles) and naturally pollinated (light grey circles) cacao flower and total rates per treatment (black squares). Fruit set rate is the number of healthy cherelles divided by open flowers counted and/or pollinated 6 days earlier, pooled across seven weekly visits. For plotting purposes, we added 0.01 to the original values and used a logarithmic scale. Letters indicate significant differences (binomial GLMM, Table S5)

4 | DISCUSSION

In this study, we aimed to reveal key drivers of cacao pollination services (Figure 1) by sampling flower visitors, quantifying pollen deposition and hand pollinating flowers in Peru, part of the native region of the crop. Our results show (i) regional variation in the most abundant flower visitors and visitation rates throughout different seasons, as well as limited changes in visitation rates along a canopy closure gradient; (ii) low fruit set and pollen deposition in a native cacao variety of Peru; and (iii) beneficial but restricted effects of hand pollination on fruit set of native cacao.

Overall, we found a large diversity of flower visitors, but very distinct visitation patterns in the northern and southern study regions, which could have been (partly) due to different climatic circumstances during sampling. The low percentage of midge visitors (7%) found on cacao flowers in our study coincides with findings from studies in Asia and South America (Chumacero de Schawe et al., 2016; Toledo-Hernández et al., 2021). Herbivores—aphids in the north and thrips in the south—were the most abundant flower visitors. Although both groups have been reported to transport cacao pollen grains, it is more likely that their net effect on fruit set is neutral or adverse (Entwistle, 1972). Aphids are likely to negatively affect fruit set, because of their sap-sucking diets and association with honeydew-collecting ants (Maas et al., 2013). Thrips might contribute to pollination mainly through their high relative abundances which may compensate for the minimal amount of pollen they typically carry with their hairy-fringed wings, although a substantial part of pollen transported by thrips might be self-pollen (Entwistle, 1972; Mound, 2005). In our study, the functional role of midges, aphids and thrips remains unconfirmed. In the light of these uncertainties, methodologies that allow to demonstrate transport of outcross-pollen should be developed to confirm functional roles of flower visitors in future investigations.

The lack of a strong relationship between forest distance and visitation rates was contrary to our expectations of finding higher

visitation rates in forest vicinity, as was the case in studies carried out in Asia (Klein et al., 2008; Toledo-Hernández et al., 2021). Possibly, other management variables, such as canopy closure and habitat management, play a bigger role in insect visitation to flowers of native cacao. In the north, visitation rates tended to be associated with increasing canopy closure, while in the south, during the wet season, an opposite trend prevailed. Shade trees decrease transmitted radiation, lower air temperatures and increase relative humidity (Niether et al., 2018; Tscharntke et al., 2011). Especially under intensely dry circumstances as in the north, buffering of extreme environmental conditions in the agroforests could have benefited flower visitation. In the south, the high cloud cover during the wet season might have limited transmitted radiation. Under denser canopies, the radiation could have been below the threshold necessary for insects to visit flowers (Liporoni et al., 2020).

We were not able to relate fruit set with pollen quantities measured directly on cacao trees in the northern study region, because fruit set rates were extremely low (0.8%) compared to the 10% reported from Indonesia (Groeneveld et al., 2010). This could be problematic for final yields, because in cacao, the majority of pollinated flowers do not develop into harvestable fruits (Bos et al., 2007). Considering that we observed several cases of pollination failure in spite of high amounts of pollen deposited, other factors such as pollen viability, pollen compatibility and resource availability may be limiting fruit set even more than previously thought. Pollination failures are also commonly caused by low pollen viability (Wilcock & Neiland, 2002) and viability in turn can be affected by high temperatures and drought. Potentially, extraordinarily high temperatures in our northern study region have induced more pollination failures than expected. Alternatively, and more likely, the narrow genetic basis of the native variety used for our experiments (Thomas et al., 2012) resulted in limited compatibility (Rodger & Ellis, 2016), while climatic conditions could have aggravated fruit set failures. It is critical that future studies aim to understand the relative contributions of pollen quantity, resource availability and compatibility to pollination failure to allow designing locally adapted (hand-)pollination strategies that improve fruit set.

The average pollen deposition on freely pollinated flowers (30 grains) was much lower than the threshold for pollination success (115 grains) established from experimental evidence (Falque et al., 1995), indicating there might be a severe pollination deficit in Peruvian cacao agroforestry systems. Low relative abundance of pollinating flower visitors, lack of pollen deposition by the most frequent visitors and regular incompatibility might have contributed to this deficit. To be able to identify the pollination dynamics of this crop, it is necessary to determine whether and how much pollen different flower visitors carry during a visit. For example, female ceratopogonids can carry over 700 pollen grains (Entwistle, 1972), but data of pollen loads of other frequent cacao flower visitors are lacking, potentially because the appropriate methods still need to be developed. We did not detect pollen in the glue (with stereoscopes), and previously, only one insect was found to be carrying pollen by visual inspection (Chumacero de Schawe et al., 2016). Combining pollen estimation from macro photography with controlled insect visitation would be ideal for confirming pollen loads,

visitation frequencies and ultimately, pollinator identity of flower visitors.

Our results show a limited dependency of cacao on pollen deposition: hand-pollinating flowers alleviated observed fruiting limitations, though fruit set remained low (increase from 2% to 7%). Larger gains were observed in Indonesia, where fruit set increased from 10% to 51%, though only 6.3% of the initially formed fruits was eventually harvested (Toledo-Hernández et al., 2020), which is a common observation in cacao (Bos et al., 2007). Properties of cacao varieties might influence contrasts between continents: outside of the Americas, plantations consist mainly of hybrid varieties bred in clonal design for steady production and auto-compatibility (Zhang & Motilal, 2016), whereas productivity of the native variety we studied is more variable, and potentially more reliant on cross-pollination than hybrid varieties. Conducting inter- and cross-compatibility trials with planted varieties to maximize gains is therefore strongly recommended. In the light of pollinator uncertainty, hand pollination could be applied to mitigate pollen limitations in the field and improve fruit set rates, though thorough assessments would be needed to calculate yield gains in the longer term.

5 | CONCLUSION

Despite years of intensive research on the pollination services in cacao, multiple knowledge gaps remain, underpinning the difficulty of related research. Based on the dominance of herbivore visitors and the low pollen deposition and fruit set rates we found, we urge the confirmation of the main cacao pollinator in regions of origin of cacao, and the cause of low fruit set rates. Our results demonstrate that with hand pollination, it is possible to alleviate fruit set limitations, although only partly. The limited hand pollination gains in native cacao might be due to pollen incompatibility—and it will be crucial to determine the relative importance of limitations other than pollen quantity (i.e. pollen compatibility and resource availability) to increase fruit set rates. Confirming pollinator identity will also be key to make recommendations on farm and landscape management to maximize visitation rates. To this end, we recommend combining new and existing techniques to study pollen deposition quantities of different arthropod visitors, permitting the development of management interventions to maximize the visitation rates of the groups that deposit sufficient viable and compatible pollen.

INCLUSION STATEMENT
Several authors from different countries collaborated on the work presented in this study, conducted within a larger collaborative framework in Peru. Hypotheses and research questions were developed after stakeholder meetings with local universities, farmers' organizations and governmental institutions ensuring applied relevance of the research. Peruvian students and field assistants made a significant contribution to the implementation of field experiments and data collection. Further, preliminary results have been presented to regional farmer communities and printed results were distributed to farmers

participating in the project. Local literature was consulted whenever relevant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

J.V., C.O.-A., B.M., E.T., T.T. and I.S.-D. conceived the research and planned fieldwork. J.V. and C.O.A. selected field sites and J.V. led and C.O.-A. supported collection of field data. N.-C.S. and C.U.-S. collected and transcribed hand pollination data. T.H.-D. collected and transcribed canopy and forest proximity data. J.V. and E.M.-P. performed the data analysis. J.V. led manuscript writing, supported by C.O-A., E.M.-P, B.M., E.T., T.T. and I.S.-D. All authors commented on and approved the final version.

DATA AVAILABILITY STATEMENT

The original data are archived and available at the open science framework data repository: https://doi.org/10.17605/OSF.IO/PUV4K (Vansynghel, 2022).

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/2688-8319.12140

ORCID

Justine Vansynghel https://orcid.org/0000-0002-4250-7016
Carolina Ocampo-Ariza https://orcid.org/0000-0002-4106-5586
Bea Maas https://orcid.org/0000-0001-9461-3243

REFERENCES

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Blaser, W. J., Oppong, J., Hart, S. P., Landolt, J., Yeboah, E., & Six, J. (2018). Climate-smart sustainable agriculture in low-to-intermediate shade agroforests. *Nature Sustainability, 1*(5), 234–239. https://doi.org/10.1038/s41893-018-0062-8

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

Brown, B. V., Borkent, A., Cumming, J. M., Wood, D. M., Woodley, N. E., & Zumbado, M. A. (2009). *Manual of Central American Diptera: Volume 1 & 2.* NRC Research Press.

Chumacero de Schawe, C., Kessler, M., Hensen, I., & Tscharntke, T. (2016). 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

Claus, G., Vanhove, W., Van Damme, P., & Smagghe, G. (2018). *Challenges in Cocoa Pollination: The Case of Cote dt'Ivoire* (Issue June). https://doi.org/10.5772/intechopen.75361

Clay, K. (2004). Fungi and the food of the gods. *Nature, 427*(6973), 401–402. https://doi.org/10.1038/427401a

de Almeida, A.-A., & Valle, R. R. (2009). Cacao: Ecophysiology of growth and production. In F. DaMetta (Ed.), *Ecophysiology of tropical tree crops* (pp. 1–34). Nova Science Publishers.

Entwistle, P. F. (1972). *Pests of cocoa.* Longman.

Falque, M., Lesdalons, C., & Eskes, A. B. (1996). Comparison of two cacao (*Theobroma cacao* L.) clones for the effect of pollination intensity on fruit set and seed content. *Sexual Plant Reproduction, 9*(4), 221–227. https://doi.org/10.1007/BF02173102

Falque, M., Vincent, A., Vaissiere, B. E., & Eskes, A. B. (1995). Effect of pollination intensity on fruit and seed set in cacao (*Theobroma cacao* L.). *Sexual Plant Reproduction, 8*(6), 354–360. https://doi.org/10.1007/BF00243203

FAO. (2020). Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/

Forbes, S. J., Mustiga, G., Romero, A., Northfield, T. D., Lambert, S., & Motamayor, J. C. (2019). Supplemental and synchronized pollination may increase yield in Cacao. *Hortscience, 54*(10), 1718–1727. https://doi.org/10.21273/HORTSCI12852-18

Ford, C. S., & Wilkinson, M. J. (2012). Confocal observations of late-acting self-incompatibility in *Theobroma cacao* L. *Sexual Plant Reproduction, 25*(3), 169–183. https://doi.org/10.1007/s00497-012-0188-1

Gibb, T. J., & Oseto, C. Y. (2006). *Arthropod collection and identification: Field and laboratory techniques.* Elsevier/Academic Press.

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

Hartig, F. (2018). *DHARMa: Residual diagnostics for hierarchical (multi-level /mixed) regression models.* R package version 0.2.0. https://CRAN.R-Project.Org/Package=DHARMa

Jezeer, R. E., Verweij, P. A., Santos, M. J., & Boot, R. G. A. (2017). Shaded coffee and cocoa – Double dividend for biodiversity and small-scale farmers. *Ecological Economics, 140*, 136–145. https://doi.org/10.1016/j.ecolecon.2017.04.019

Klein, A.-M., Cunningham, S. A., Bos, M. M., & Steffan-Dewenter, I. (2008). Advances in pollination ecology from tropical plantation crops. *Ecology, 89*(4), 935–943. https://doi.org/10.1890/07-0088.1

Liporoni, R., Cordeiro, G. D., Prado, P. I., Schlindwein, C., Warrant, E. J., & Alves-dos-Santos, I. (2020). Light intensity regulates flower visitation in Neotropical nocturnal bees. *Scientific Reports, 10*(1), 1–11. https://doi.org/10.1038/s41598-020-72047-x

Maas, B., Clough, Y., & Tscharntke, T. (2013). Bats and birds increase crop yield in tropical agroforestry landscapes. *Ecology Letters, 16*(12), 1480–1487. https://doi.org/10.1111/ele.12194

Macinnis, G., & Forrest, J. R. K. (2017). Quantifying pollen deposition with macro photography and 'stigmagraphs.' *Journal of Pollination Ecology, 20*(2), 13–21. https://doi.org/10.26786/1920-7603(2017)six

Mena-Montoya, M., García-Cruzatty, L. C., Cuenca-Cuenca, E., Pinargote, L. D. V., Villamar-Torres, R., & Jazayeri, S. M. (2020). Pollen flow of Theobroma cacao and its relationship with climatic factors in the central zone of the Ecuadorian Littoral. *Bioagro*, *32*(1), 39–48.

Merma, I., & Julca, A. (2012). Descripción del medio y bases de zonificacion del Alto Urubamba, Peru. *Ecologia Aplicada*, *11*(1), 13–21. https://doi.org/10.21704/rea.v11i1-2.421

MINAM. (2015). *Mapa Nacional de Cobertura Vegetal*. Memoria Descriptiva.

Mound, L. A. (2005). Thysanoptera: Diversity and interactions. *Annual Review of Entomology*, *50*(6), 247–269. https://doi.org/10.1146/annurev.ento.49.061802.123318

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), 38. https://doi.org/10.1007/s13595-018-0723-9

Otivo Barreto, J. A. (2010). Mapa ocupación del territorio y uso actual del suelo. Memoria Descriptiva.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing.

Reitan, T., & Nielsen, A. (2016). Do not divide count data with count data; a story from pollination ecology with implications beyond. *PLoS ONE*, *11*(2), e0149129. https://doi.org/10.1371/journal.pone.0149129

Rodger, J. G., & Ellis, A. G. (2016). Distinct effects of pollinator dependence and self-incompatibility on pollen limitation in South African biodiversity hotspots. *Biology Letters*, *12*(6), 10–13. https://doi.org/10.1098/rsbl.2016.0253

Rodríguez, L. O., & Young, K. R. (2000). Biological diversity of Peru: Determining priority areas for conservation. *AMBIO: A Journal of the Human Environment*, *29*(6), 329–337. https://doi.org/10.1579/0044-7447-29.6.329

Romero, C. A., & Vargas, E. U. (2016). *Estudio del Cacao en el Perú y en el Mundo*.

Salas-Eljatib, C., Fuentes-Ramirez, A., Gregoire, T. G., Altamirano, A., & Yaitul, V. (2018). A study on the effects of unbalanced data when fitting logistic regression models in ecology. *Ecological Indicators*, *85*(November), 502–508. https://doi.org/10.1016/j.ecolind.2017.10.030

SENAMHI. (2020a). *Accumulated rainfall per month in Morropon, Piura*. https://www.senamhi.gob.pe

SENAMHI. (2020b). *Climas del Perú - Mapa de Clasificicación Climática Nacional*. Author.

Thomas, E., van Zonneveld, M., Loo, J., Hodgkin, T., Galluzzi, G., & van Etten, J. (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

Toledo-Hernández, M., Tscharntke, T., Tjoa, A., Anshary, A., Cyio, B., & Wanger, T. C. (2020). Hand pollination, not pesticides or fertilizers, increases cocoa yields and farmer income. *Agriculture, Ecosystems and Environment*, *304*(18), 107160. https://doi.org/10.1016/j.agee.2020.107160

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), 1–8. https://doi.org/10.1016/j.biocon.2021.109106

Toledo-Hernández, M., Wanger, T. C., & Tscharntke, T. (2017). Neglected pollinators: Can enhanced pollination services improve cocoa yields? A review. *Agriculture, Ecosystems and Environment*, *247*(February), 137–148. https://doi.org/10.1016/j.agee.2017.05.021

Tscharntke, T., Clough, Y., Bhagwat, S. A., Buchori, D., Faust, H., Hertel, D., Ho, D., Juhrbandt, J., Kessler, M., Perfecto, I., & Scherber, C. (2011). Multifunctional shade-tree management in tropical agroforestry landscapes – A review. *Journal of Applied Ecology*, *48*, 619–629. https://doi.org/10.1111/j.1365-2664.2010.01939.x

Vansynghel, J. (2022). *Data*. Open Science Framework. https://doi.org/10.17605/OSF.IO/PUV4K

Wickham, H. (2016). *ggplot2– Elegant graphics for data analysis* (2nd ed.). Springer-Verlag.

Wilcock, C., & Neiland, R. (2002). Pollination failure in plants: Why it happens and when it matters. *Trends in Plant Science*, *7*(6), 270–277. https://doi.org/10.1016/S1360-1385(02)02258-6

Zhang, D., & Motilal, L. (2016). Origin, dispersal, and current global distribution of cacao genetic diversity. In B. Bailey & L. Meinhardt (Eds.), *Cacao diseases: A history of old enemies and new encounters* (pp. 3–31). Springer International Publishing. https://doi.org/10.1007/978-3-319-24789-2_1

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.