Pollination and pest control along gradients of shade cover and forest distance in Peruvian cacao agroforestry landscapes

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SUMMARY

Chapter I – Introduction

Global trade of beans of the cacao tree (*Theobroma cacao*), of which chocolate is produced, contributes to the livelihoods of millions of smallholder farmers. The understorey tree is native to South America but is nowadays cultivated in many tropical regions. In Peru, a South American country with a particularly high cacao diversity, it is common to find the tree cultivated alongside non-crop trees that provide shade, in so-called agroforestry systems. Because of the small scale and low management intensity of such systems, agroforestry is one of the most wildlife-friendly land-use types, harbouring the potential for species conservation. Studying wildlife-friendly land-use is of special importance for species conservation in biodiversity-rich tropical regions such as Peru, where agricultural expansion and intensification are threatening biodiversity. Moreover, there is a growing body of evidence that shows co-occurrence of high biodiversity levels and high yield in wildlife-friendly cacao farming. Yet studies are restricted to non-native cacao countries, and since patterns might be different among continents, it is important to improve knowledge on wildlife-friendly agroforestry in native countries.

Because studies of wildlife-friendly cultivation processes are still largely lacking for South America, we set out to study multiple aspects of cacao productivity in agroforests in Peru, part of cacao´s region of origin. The natural pollination process of cacao, which is critically understudied, was investigated by trapping flower visitors and studying pollen deposition from macrophotographs (Chapter II). Next, we excluded birds, bats, ants and flying insects and squirrels from cacao trees in a full-factorial field experiment

and quantified these animals´ contribution to cacao fruit set, fruit loss and yield (Chapter III). Lastly, we aimed to assess whether fruit quantity and quality of native cacao increases through manually supplementing pollen (Chapter II and IV), and whether microclimatic conditions and the genetic background of the studied varieties limit fruit set (Chapter IV).

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

Given the importance of cacao pollination for the global chocolate production, it is remarkable that fruit set limitations are still understudied. Knowledge on flower visitation and the effect of landscape context and local management are lacking, especially in the crop's region of origin. Moreover, the role of pollen deposition in limiting fruit set as well as the benefits of hand pollination in native cacao are unknown. In this chapter, we aimed to close the current knowledge gaps on cacao pollination biology and sampled flower visitors in 20 Peruvian agroforests with native cacao, along gradients of shade cover and forest distance. We also assessed pollen quantities and compared fruit set between manually and naturally pollinated flowers. We found that herbivores were the most abundant flower visitors in both northern and southern Peru, but we could not conclude which insects are effective cacao pollinators. Fruit set was remarkably low (2%) but improved to 7% due to pollen supplementation. Other factors such as a lack of effective pollinators, genetic pollen incompatibility or resource unavailability could be causing fruit set limitations. We conclude that revealing those causes and the effective pollinators of cacao will be key to improve pollination services in cacao.

Chapter III – Quantifying services and disservices provided by insects and vertebrates in cacao agroforestry landscapes

Pollination and pest control, two ecosystem services that support cacao yield, are provided by insects and vertebrates. However, animals also generate disservices, and their combined contribution is still unclear. Therefore, we excluded flying insects, ants, birds and bats, and as a side effect also squirrels from cacao trees and we assessed fruit set, fruit loss and final yield. Local management and landscape context can influence animal occurrence in cacao agroforestry landscapes; therefore, shade cover and forest distance were included in the analyses. Flying insects benefitted cacao fruit set, with largest gains in agroforests with intermediate shade cover. Birds and bats were also associated with improved fruit set rates and with a 114% increase in yield, potentially due to pest control services provided by these animals. The role of ants was complicated: these insects had a positive effect on yield, but only close to forest. We also evidenced disservices generated by ants and squirrels, causing 7% and 10% of harvest loss, respectively. Even though the benefits provided by animals outweighed the disservices, trade-offs between services and disservices still should be integrated in cacao agroforestry management.

Chapter IV – Cross-pollination improves fruit set and yield quality of Peruvian native cacao

Because yields of the cacao tree are restricted by pollination, hand pollination has been proposed to improve yield quantity and potentially, also quality. However, low self- and cross-compatibility of native cacao, and abiotic conditions could cancel out hand pollination benefits. Yet, the impact of genetic constraints and abiotic conditions

on fruit set have not been assessed in native cacao so far. To increase our understanding of the factors that limit fruit set in native cacao, we compared manual self- and cross-pollination with five native genotypes selected for their sensorial quality and simultaneously tested for effects of soil water content, temperature, and relative air humidity. We also compared quality traits between manually and naturally pollinated fruits. Success rates of self-pollination were low (0.5%), but increased three- to eightfold due to cross-pollination, depending on the genotype of the pollen donor. Fruit set was also affected by the interaction between relative air humidity and temperature, and we found heavier and more premium seeds in fruits resulting from manual than natural pollination. Together, these findings show that reproductive traits of native cacao are constrained by genetic compatibility and abiotic conditions. We argue that because of the high costs of hand pollination, natural cross-pollination with native pollen donors should be promoted so that quality improvements can result in optimal economic gains for smallholder farmers.

Chapter V – Discussion

In this thesis, we demonstrated that the presence of flying insects, ants and vertebrates, local and landscape management practices, and pollen supplementation interactively affected cacao yield, at different stages of the development from flower to fruit. First, we showed that fruit set improved by intermediate shade levels and flower visitation by flying insects. Because the effective cacao pollinators remain unknown, we recommend shade cover management to safeguard fruit set rates. The importance of integrating trade-offs in wildlife-friendly management was highlighted by lower harvest losses due to ants and squirrels than the yield benefits provided by

birds and bats. The maintenance of forest in the landscape might further promote occurrence of beneficial animals, because in proximity to forest, ants were positively associated with cacao yields. Therefore, an integrated wildlife-friendly farming approach in which shade cover is managed and forest is maintained or restored to optimize ecosystem service provision, while minimizing fruit loss, might benefit yields of native cacao. Finally, manual cross-pollination with native genotypes could be recommended, due to improved yield quantity and quality. However, large costs associated with hand pollination might cancel out these benefits. Instead, we argue that in an integrated management, natural cross-pollination should be promoted by employing compatible genotypes in order to improve yield quantity and quality of native cacao.

ZUSAMMENFASSUNG

Kapitel I – Einleitung

Der weltweite Handel mit den Bohnen des Kakaobaums (*Theobroma cacao*) trägt zum Lebensunterhalt von Millionen von Kleinbauern bei. Der Unterholzbaum, aus dessen Bohnen Schokolade hergestellt wird, ist in Südamerika beheimatet, wird aber heute in vielen tropischen Regionen angebaut. In Peru, einem der Länder mit einer besonders hohen Kakaovielfalt, wird der Baum häufig zusammen mit schattenspendenden Bäumen in so genannten Agroforstsystemen angebaut. Aufgrund der Kleinräumigkeit und der geringen Bewirtschaftungsintensität solcher Systeme ist die Agroforstwirtschaft eine der wildtierfreundlichsten Landnutzungsformen, die ein großes Potenzial für den Artenschutz bietet. Die Erforschung wildtierfreundlicher Landnutzungsformen ist besonders wichtig für den Artenschutz in artenreichen tropischen Regionen wie Peru, in denen die Ausweitung und Intensivierung der Landwirtschaft die biologische Vielfalt bedroht. Darüber hinaus gibt es immer mehr Belege dafür, dass eine hohe Artenvielfalt mit hohen Erträgen im wildtierfreundlichen Kakaoanbau einhergeht. Die Studien beschränken sich jedoch auf nicht ursprüngliche Kakaoländer, und da die Muster auf den verschiedenen Kontinenten unterschiedlich sein könnten, ist es wichtig, das Wissen über wildtierfreundliche Agroforstwirtschaft in den Ursprungsländern zu verbessern.

Da Studien über wildtierfreundliche Anbauprozesse in Südamerika noch weitgehend fehlen, haben wir uns vorgenommen, verschiedene Aspekte der Kakaoproduktivität in Agroforstbetrieben in Peru, einem Teil der Ursprungsregion des Kakaos, zu untersuchen. Der natürliche Bestäubungsprozess von Kakao, der wenig erforscht ist, wurde durch das Einfangen von Blütenbesuchern und die Untersuchung der Pollenablage anhand von Makrofotografien untersucht (Kapitel II). Als Nächstes haben wir gemeinsam Vögel, Fledermäuse, Ameisen und Fluginsekten und Eichhörnchen vom Zugang zu Kakaobäumen ausgeschlossen und den Beitrag dieser Tiere zum Fruchtansatz, Fruchtverlust und Ertrag von Kakao quantifiziert (Kapitel III). Schließlich wollten wir feststellen, ob sich die Fruchtmenge und -qualität des heimischen Kakaos durch die händische Zugabe von Pollen erhöht (Kapitel II und IV) und ob der genetische Hintergrund der untersuchten Sorten und die mikroklimatischen Bedingungen den Fruchtansatz limitieren (Kapitel IV).

Kapitel II – Besuch der Kakaoblüten: Geringer Polleneintrag, geringer Fruchtansatz und Dominanz von Pflanzenfressern

Angesichts der Bedeutung der Kakaobestäubung für die weltweite Schokoladenproduktion ist es bemerkenswert, dass der Fruchtansatz noch immer nicht ausreichend erforscht ist. Insbesondere in der Herkunftsregion der Pflanze fehlt es an Wissen über die Blütenbesucher und die Auswirkungen von Landschaft und Bewirtschaftung. Darüber hinaus sind die Rolle des Polleneintrags bei der Limitierung des Fruchtansatzes sowie die Vorteile der Handbestäubung bei einheimischem Kakao unbekannt. In diesem Kapitel wollten wir die derzeitigen Wissenslücken über die Bestäubungsbiologie von Kakao schließen und haben in 20 peruanischen Agroforsten mit einheimischem Kakao bei unterschiedlicher Beschattung und Waldentfernung Proben von Blütenbesuchern genommen. Wir untersuchten auch die Pollenmenge und verglichen den Fruchtansatz zwischen händisch und natürlich bestäubten Blüten. Wir stellten fest, dass Pflanzenfresser sowohl im Norden als auch im Süden Perus die häufigsten Blütenbesucher waren, konnten aber nicht feststellen, welche Insekten effektive Kakaobestäuber sind. Der Fruchtansatz war bemerkenswert niedrig (2 %), verbesserte sich aber durch die Pollenergänzung auf 7 %. Andere Faktoren wie ein Mangel an wirksamen Bestäubern, genetische Polleninkompatibilität oder die Nichtverfügbarkeit von Ressourcen könnten die Ursache für den geringen Fruchtansatz sein. Wir kommen zu dem Schluss, dass die Aufdeckung dieser Ursachen und der effektiven Bestäuber des Kakaos der Schlüssel zur Verbesserung der Bestäubungsleistungen im Kakao sein wird.

Kapitel III – Quantifizierung der von Insekten und Wirbeltieren in agroforstwirtschaftlichen Kakaolandschaften erbrachten Ökosystemdienstleistungen und Gegenleistungen

Bestäubung und Schädlingsbekämpfung, zwei Ökosystemleistungen, die den Kakaoertrag unterstützen, werden von Insekten und Wirbeltieren erbracht. Allerdings erbringen die Tiere auch andere Leistungen und ihr kombinierter Beitrag ist noch unklar. Daher haben wir Fluginsekten, Ameisen, Vögel und Fledermäuse und als Nebeneffekt auch Eichhörnchen vom Zugang zu den Kakaobäumen ausgeschlossen und den Fruchtansatz, den Fruchtverlust und den endgültigen Ertrag bewertet. Die Bewirtschaftung auf lokaler und Landschaftsebene kann das Vorkommen von Tieren in Kakao-Agroforstlandschaften erhöht werden; daher wurden auch die Beschattung und die Entfernung zum nächsten Wald in die Analysen einbezogen. Fluginsekten begünstigten den Fruchtansatz von Kakao, wobei die größten Zugewinne in Agroforsten mit mittlerer Beschattung zu verzeichnen waren. Vögel und Fledermäuse wurden ebenfalls mit verbesserten Fruchtansatzraten und einer 114%igen Ertragssteigerung in

Verbindung gebracht, was möglicherweise auf die Schädlingsbekämpfung durch diese Tiere zurückzuführen ist. Die Rolle der Ameisen war kompliziert: Diese Insekten wirkten sich positiv auf den Ertrag aus, aber nur in Waldnähe. Wir haben auch negative Auswirkungen von Ameisen und Eichhörnchen festgestellt, die 7% bzw. 10 % der Ernteverluste verursachten. Auch wenn die Vorteile der Tiere die Nachteile überwiegen, sollte ein Ausgleich zwischen den Vor- und Nachteilen in die agroforstliche Bewirtschaftung von Kakao integriert werden.

Kapitel IV – Kreuzbestäubung verbessert den Fruchtansatz und die Ertragsqualität von einheimischem peruanischem Kakao

Da die Erträge des Kakaobaums durch die Bestäubung eingeschränkt werden, wurde die Handbestäubung vorgeschlagen, um die Ertragsmenge und möglicherweise auch die Qualität zu verbessern. Die geringe Selbst- und Kreuzkompatibilität der einheimischen Kakaosorten und die abiotischen Bedingungen könnten jedoch die Vorteile der Handbestäubung einschränken. Die Auswirkungen genetischer Limitierungen und abiotischer Bedingungen auf den Fruchtansatz wurden bei einheimischem Kakao bisher noch nicht untersucht. Um die Faktoren besser zu verstehen, die den Fruchtansatz bei einheimischem Kakao einschränken, verglichen wir die händische Selbst- und Kreuzbestäubung mit fünf einheimischen Genotypen, die aufgrund ihrer aromatischen Qualität ausgewählt wurden, und untersuchten gleichzeitig die Auswirkungen von Bodenwassergehalt, Temperatur und relativer Luftfeuchtigkeit. Außerdem verglichen wir die Qualitätsmerkmale zwischen händisch und natürlich bestäubten Früchten. Die Erfolgsrate der Selbstbestäubung war gering (0,5 %), stieg jedoch durch Kreuzbestäubung um das Drei- bis Achtfache, je nach

Genotyp des Pollenspenders. Der Fruchtansatz wurde auch durch die Wechselwirkung zwischen relativer Luftfeuchtigkeit und Temperatur beeinflusst, und wir fanden schwerere und hochwertigere Samen in Früchten, die durch manuelle Bestäubung entstanden waren, als in den natürlich bestäubten. Diese Ergebnisse zeigen, dass die Fortpflanzungseigenschaften des einheimischen Kakaos durch genetische Kompatibilität und abiotische Bedingungen eingeschränkt werden. Wir argumentieren, dass aufgrund der hohen Kosten der Handbestäubung die natürliche Kreuzbestäubung mit heimischen Pollenspendern gefördert werden sollte, damit Qualitätsverbesserungen zu optimalen wirtschaftlichen Gewinnen für die Kleinbauern führen können.

Kapitel V – Diskussion

In dieser Arbeit haben wir gezeigt, dass die Anwesenheit von Fluginsekten, Ameisen und Wirbeltieren, die Bewirtschaftungspraktiken auf lokaler und Landschaftsebene sowie die Pollenergänzung den Kakaoertrag in verschiedenen Entwicklungsstadien von der Blüte bis zur Frucht interaktiv beeinflussen. Zunächst haben wir gezeigt, dass sich der Fruchtansatz durch eine mittlere Beschattung und den Blütenbesuch durch Fluginsekten verbessert. Da die effektiven Bestäuber des Kakaos noch nicht bekannt sind, empfehlen wir, die Beschattung so zu gestalten, dass der Fruchtansatz gesichert ist. Wie wichtig es ist, bei einer wildtierfreundlichen Bewirtschaftung Kompromisse einzugehen, zeigt sich daran, dass die Ernteverluste durch Ameisen und Eichhörnchen geringer sind als die Ertragsvorteile durch Vögel und Fledermäuse. Die Erhaltung des Waldes in der Landschaft könnte das Vorkommen von Nützlingen weiter fördern, da Ameisen in der Nähe von Wäldern positiv mit den Kakaoerträgen verbunden waren.

Daher könnte ein integrativer, wildtierfreundlicher Anbauplan, bei dem die Beschattung und der Waldabstand so gesteuert werden, dass die Bereitstellung von Ökosystemleistungen optimiert und gleichzeitig der Verlust von Früchten minimiert wird, den Erträgen des heimischen Kakaos zugutekommen. Schließlich könnte die händische Kreuzbestäubung mit einheimischen Genotypen aufgrund der verbesserten Ertragsmenge und -qualität empfohlen werden. Die hohen Kosten der händischen Bestäubung könnten diese Vorteile jedoch zunichtemachen. Stattdessen sollte im Rahmen einer integrativen Bewirtschaftung die natürliche Kreuzbestäubung durch den Einsatz kompatibler Genotypen gefördert werden, um die Quantität und Qualität der Erträge von einheimischem Kakao zu verbessern.

CHAPTER I

INTRODUCTION

CHAPTER I INTRODUCTION

Agricultural expansion and intensification are one of the largest threats to global biodiversity. Wildlife-friendly farming, a strategy that combines agricultural productivity with biodiversity conservation, is therefore proposed to avoid further threats to biodiversity. Agroforestry is such a wildlife-friendly farming strategy, in which understorey crop trees are cultivated below non-crop trees that provide shade. Benefits of such systems include, but are not limited to, income diversification, improved abiotic conditions, and ecosystem service provision by high levels of biodiversity that are associated with this type of land use. Even though the economic and ecological benefits of agroforestry are manyfold, it is also assumed that yields are lower than in conventional system. An example of a crop tree grown in agroforestry systems that could benefit from improved ecosystem services to increase yields, is the cacao tree (*Theobroma cacao*). Many smallholders' livelihoods depend on the global trade of the crop tree´s beans, especially in South American countries that are within the crops native range. Win-win scenarios of high diversity levels and improved ecosystem service provision in cacao agroforestry landscapes, as well as relationships with shade cover and forest distance are relatively well-studied, but evidence is mainly restricted to non-native countries. This work fills that knowledge gap by investigating animal-provided ecosystem services, such as pollination and pest control, within cacaos native range.

I.1 Agricultural expansion and its impact on biodiversity loss

As a result of concurrent population and consumption growth, food demand has been on a sharp rise (Tilman, Balzer, Hill, & Befort, 2011). To meet the rising food demand, the proportion of land under cultivation has been expanding, too (Godfray et al., 2010). In 2011, almost 40% of the worlds land was cultivated and models forecasted continuous increases in population growth until at least 2050 (Foley et al., 2011), with likely consequences for agricultural expansion. Before the onset of the 21st century, scientists already warned for the multiple consequences for the integrity of natural ecosystems due to agricultural expansion (Tilman, 1999). Multiple adverse effects of agricultural expansion include, but are not limited to, nutrient pollution, greenhouse gas emissions and habitat destruction (Tilman et al., 2001), endangering global biodiversity (Kehoe et al., 2017).

Indeed, our global biodiversity is at risk. The impact of anthropogenic activities is associated with severe species declines: populations of vertebrates and invertebrates reduced 25 and 45% since the 16th century, respectively (Dirzo et al., 2014). Among all causing agents of global species declines, agricultural activities are threatening the global biodiversity the most, after overexploitation. Agricultural development is putting more species at risk than urban development, pollution, introduction of invasive species, and even climate change (Maxwell, Fuller, Brooks, & Watson, 2016). The current large-scale biodiversity loss due to agricultural activities is of large concern, because of the implied loss of ecosystem functions and services (Newbold et al., 2015). It is clear that the large-scale loss of biodiversity and its associated regulating, provisional, and cultural ecosystem services is problematic for multiple aspects of human well-being (Cardinale, 2012). For example, lower biodiversity levels can have a negative impact on agricultural yields through reduced essential provisioning ecosystem services, such as pollination and pest control (Cardinale, 2012; Cardinale et al., 2012).

I.2 Reconciliation of agriculture and biodiversity

Increasing food production to achieve food security for a steadily growing population, is a major challenge of this century. Harmonizing this major challenge with the loss of biodiversity has been heavily debated in scientific circles and conservation planning (Fischer et al., 2014; Laurance, Sayer, & Cassman, 2014; Shackelford, Steward, German, Sait, & Benton, 2015). One approach is *land sparing*, a solution in which agricultural production is intensified with the aim to increase yield such that more land can be spared and dedicated to biodiversity conservation instead (Green, Cornell, Scharlemann, & Balmford, 2005). In the areas dedicated to conservation, biodiversity is isolated from human activities (Swallow et al., 2009), and in agricultural areas, production is intensified. But, intensification might be associated with unsustainable activities such as use of agrochemicals and depletion of groundwater due to irrigation (Baudron & Giller, 2014; Phalan, Green, & Balmford, 2014). Even though intensification is projected to spare an area the size of the Indian subcontinent (Phalan et al., 2014), crop yield increases do not always guarantee that land is effectively set aside for conservation (Phalan, Onial, Balmford, & Green, 2011; Ramankutty & Rhemtulla, 2012).

Land sharing, a contrasting approach to land sparing, is proposed by conservationists to reduce the impact of agriculture on biodiversity by combining both objectives on the same land (Baudron & Giller, 2014). Both biodiversity and agricultural objectives could be combined by wildlife-friendly farming, a strategy that allows biodiversity to co-occur in less-intensively managed farmland (Phalan et al., 2011).

Some have warned however that this approach might result in lower yields on the one hand (Green et al., 2005), and limited advantages for animals on the other hand (Phalan et al., 2011). At the same time, increasing awareness of the beneficial effects of biodiversity on agricultural production, has opened the way for win-win scenarios, in which biodiversity and yield are jointly optimized (Macfadyen, Cunningham, Costamagna, & Schellhorn, 2012). Moreover, it was increasingly recognized that socio-economical complexity should be incorporated into this framework, as subsidence smallholder farming is the way of life for many in the tropics (Fischer et al., 2014; Scariot, 2013). More recently, Grass et al. (2019) posed that *land sharing* and *land sparing* must not be mutually exclusive strategies. Instead, *land sparing* could be combined with biodiversity-friendly farming in the landscape. *Land sharing* can promote biodiversity and associated ecosystem services in agricultural land, while biodiversity still relies on connectivity between set-aside conservation areas (Grass et al., 2019).

I.3 Tropical agroforestry: a wildlife-friendly farming strategy

In the tropics, population growth and agricultural expansion is more rapid than elsewhere (Foley et al., 2011; Laurance et al., 2014). The tropics are also home to a huge amount of the world's biodiversity (Barlow et al., 2018), which is also disproportionally threatened by multiple anthropogenic pressures (Barlow et al., 2016; Laurance et al., 2014). Biodiversity losses from highly diverse tropical regions can have severe impacts on global diversity levels, hence the importance in the general debate (Newbold et al., 2015). Because of the large proportions of the tropics that are covered by untouched rainforest, it was suggested that *land sparing* was the strategy best suited for the tropical realm (Ramankutty & Rhemtulla, 2012). Yet, it was also recognized that species existence can rely on agricultural fields (Wright, Lake, & Dolman, 2012). This evidence points to *land sharing* as beneficial strategy. A wildlife-friendly lang sharing approach typically found in the tropics is agroforestry, the combined management of shade trees with agricultural crops (Perfecto & Vandermeer, 2008; Tscharntke et al., 2015). These systems can provide habitat for species outside of conservation areas, while minimizing pressure for exploitation of protected land (Bhagwat, Willis, Birks, & Whittaker, 2008). Because of the relatively high value for species maintenance, agroforestry has potential to reconcile the food security challenge with biodiversity conservation goals (Bhagwat et al., 2008; Perfecto & Vandermeer, 2008).

The maintenance of shade trees in tropical agroforestry has multiple short and long term benefits such as carbon sequestration (Rajab, Leuschner, Barus, Tjoa, & Hertel, 2016), optimization of microclimatic conditions (Lin, 2007; Niether, Armengot, Andres, Schneider, & Gerold, 2018), and can support high biodiversity levels, associated to pest and weed control and pollination services (Tscharntke et al., 2011, 2012). Social and economic benefits of agroforestry with shade trees include profitability and cost-efficiency for smallholder farmers (Jezeer, Verweij, Santos, & Boot, 2017). Moreover, the high levels of agroforestry-related species diversity that provide crucial ecosystem services such as pollination and pest control, are an important argument for land sharing approaches with wildlife-friendly farming strategies (Tscharntke et al., 2012). Meanwhile, the body of evidence that wildlife-friendly farming can increase crop yield, is growing (Pywell et al., 2015). An example of a crop that holds potential for employing benefits of high biodiversity levels, is cacao (*Theobroma cacao* L.). Cacao is cultivated in agroforestry settings throughout the tropics, and its capacity of supporting high levels of biodiversity (Clough, Faust, & Tscharntke, 2009) can even be combined with high yields (Clough et al., 2011).

I.4 Ecosystem services in cacao agroforestry landscapes

The conversion of forest to cacao agroforest does not have a strong effect on biodiversity levels (Steffan-Dewenter et al., 2007). Consequently, the relatively high biodiversity levels that are maintained in cacao agroforestry can have positive effects on the delivery of multiple ecosystem services (De Beenhouwer, Aerts, & Honnay, 2013). This can be of special importance for cacao, considering that current yields are often below of what could be achieved under ideal circumstances (Abdulai et al., 2020; Vanhove et al., 2020). One of the reasons for low cacao yields, is pest and disease occurrence. Pest pressure in cacao agroforests can be so high that fruit loss due to insect pests can blur pollination benefits (Bos, Steffan-Dewenter, & Tscharntke, 2007a). However, insectivorous birds and bats can successfully control some of the most economically important cacao pests (Maas, Clough, & Tscharntke, 2013), highlighting the importance of vertebrate conservation in and around cacao agroforests.

Pollination is another main factor constraining current yields (Groeneveld, Tscharntke, Moser, & Clough, 2010). Cacao is a predominantly self-incompatible crop and requires cross-pollination by insects for successful fruit set (Toledo-Hernández, Wanger, & Tscharntke, 2017). Hence, improving pollination by ecological intensification, i.e. optimizing ecosystem services while reducing agrochemical inputs, might lead to yield gains. Remarkably, there are major knowledge gaps limiting our ability to do so (Toledo-Hernández et al., 2017). Because the crops´ pollinating agents remain largely unidentified (Chumacero de Schawe, Kessler, Hensen, & Tscharntke, 2016; Toledo-Hernández et al., 2017), it remains challenging to improve natural populations of beneficial insects (Toledo-Hernández et al., 2020). Since pollination and pest control are mediated by biodiversity (Dainese et al., 2019), managing on-site biodiversity levels could be a promising way to improve cacao yields in agroforestry landscapes (Clough et al., 2011).

Concretely, investigating management strategies that could improve on-site biodiversity levels is a crucial step to improve our knowledge of ecosystem service delivery in cacao. Management factors that influence biodiversity and the ecosystem services they provide are (i) shade cover and (ii) forest proximity (Gras et al., 2016). Shade management of non-cacao trees can improve flower visitation rates and the diversity and occurrence of birds and bats (Faria, Paciencia, Dixo, Laps, & Baumgarten, 2007; Hanf-Dressler, 2020; Toledo-Hernández et al., 2021). Similarly, in agroforests closer to forest, we can find more birds, bats, and certain insects (Ocampo-Ariza et al., 2022; Schroth et al., 2011). Drought and temperature stress can be co-responsible for suboptimal yields (Abdulai et al., 2018; Lahive, Hadley, & Daymond, 2019), but these abiotic constraints could be alleviated by managing agroforest characteristics such as shade cover and forest proximity. Shade cover can improve growing conditions for cacao by decreasing temperature and increasing humidity (Blaser et al., 2018; Lahive et al., 2019) and forest patches within the landscape can affect microclimatic conditions in agricultural fields, close to the forest edge (Schmidt, Jochheim, Kersebaum, Lischeid, & Nendel, 2017). Forest proximity and shade cover might thus be tools to optimize biodiversity levels and ecosystem service delivery in cacao agroforestry, improving overall value of wildlife-friendly cacao farming.

To develop sound wildlife-friendly farming practices it is crucial to improve our holistic understanding of ecosystem delivery in cacao agroforests. This can help reveal potential interactive effects and trade-offs between ecosystem services that affect cacao yield. In coffee for example, there is evidence of pollination and pest control interactively affecting fruit quality and quantity (Classen et al., 2014; Martínez-Salinas et al., 2022), but potential interactions in cacao remain to be quantified. Understanding how insect pollination and pest control by vertebrates jointly constrain fruit set and yield is an important piece of information which is currently still lacking in cacao.

I.5 Study design and experimental set-up

I.5.1 Study area

Peru is a megadiverse tropical country with a tremendously large biodiversity of ecosystems and associated to its countless ecosystems (Fajardo, Lessmann, Bonaccorso, Devenish, & Muñoz, 2014). As an illustration of high biodiversity levels: About 20% of the worlds bird and bat diversity, is found in Peru (Rodríguez & Young, 2000). This megadiverse country faces considerable biodiversity loss risk because high species loss coincides with low investments in conservation and rapid agricultural expansion (Kehoe et al., 2017). Biodiversity loss and its consequences can be particularly severe in biodiversity rich countries in which poverty is high, such as Peru (Newbold et al., 2015). The country also harbours a large diversity of cacao varieties, since it is one of the Amazonian home countries of the crop tree (Thomas et al., 2012). This might result in low cross compatibility levels, especially in cacao clones and varieties that were selected for sensory traits rather than compatibility, as is often the case in Latin America (Zhang & Motilal, 2016).

In Peru, planted varieties are often selected for their aromatic properties, as the country is specialized in cultivation and export of highly aromatic *fine or flavour cocoa* (ICCO, 2022; César Armando Romero & Vargas, 2016). This is of large economic importance, because fine or flavour cocoa, as opposed to bulk cacao, can be sold at premium market prices (ICCO, 2022). Even though Peru is among the world's top 10 producer countries, most of the cacao production is in smallholder agroforestry systems where organic cacao is cultivated (FAO, 2022; César Armando Romero & Vargas, 2016). Smallholder agroforestry in Peru is diverse: Forest patches are interspersed in the landscape and a variety of shade trees grow in different densities among the cacao trees. Such diverse management strategies offer an excellent opportunity for comparative research on ecosystem services in differently shaded cacao agroforests in a landscape with remaining forest patches.

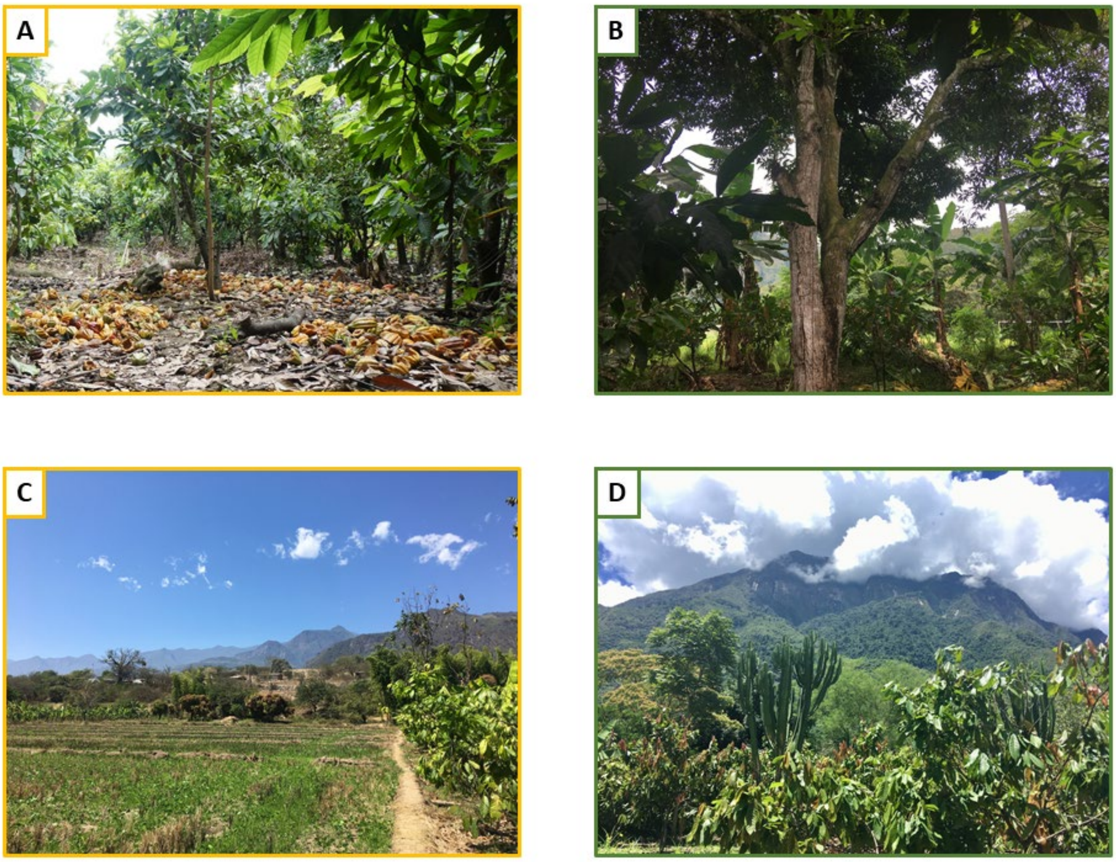

Figure I.1: Typical cacao agroforests in A) northern and B) southern Peru, and landscapes with nearby forest and shade trees over cacao plants in the agroforests in C) northern and D) southern Peru.

I.5.2 Study regions and sites

a) Southern Peru: wet and humid montane ecozone

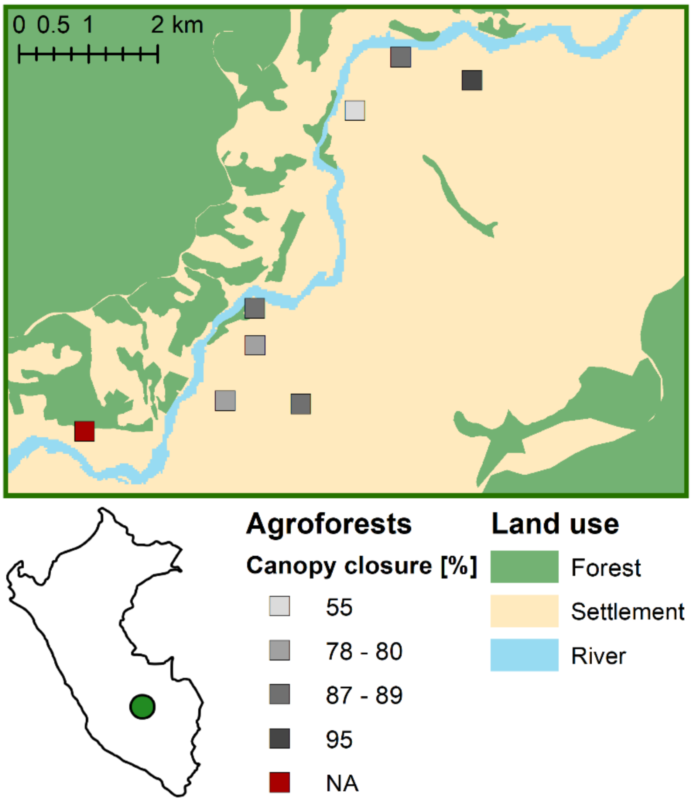

Figure I.2: Southern study region in Peru (black outline), around the town of Echarati (green circle). The seven agroforests included in visitation rate models (grey squares) and one agroforest without canopy closure data (red square) were surrounded by wet and humid forest (green patches), a river (blue) and anthropogenic and-use (beige).

Two biogeographically contrasting cacao-growing areas in Peru with a distinct climate, vegetation type and biogeography were included in the research: the dry northern lowlands, west of the Andes (Figure I.1A and I.1C), and the humid south-eastern Andean slopes (Figure I.1B and I.1D). The southern study area, in the foothills east of the Andes, was situated near Echarati in the Cusco department (S12.768999°, W72.578451°, 987 m.a.s.l.; Figure I.2). 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 to 200 mm per month and during the dry season, about 50mm per month (Merma & Julca, 2012; SENAMHI, 2020b). In the southern agroforests, introduced hybrid clones are cultivated alongside the local native variety, called *Chuncho cacao*. To conduct our experiments, we selected eight organic agroforests, smaller than 3 ha and with trees ranging between 5 and 65 years old. Gravity-fed flood canals and aspersion are used for irrigation, but only when needed during the dry season.

b) Northern Peru: tropical dry forest ecozone

The study area in the north was located around the farmer community of La Quemazón, in the department of Piura which is situated in the coastal northwest of Peru (S5.312249°, W79.718996°, 240 m.a.s.l.; Figure I.3). In this area the local variety, *Piura white cacao*, is cultivated. The area is characterized by the dominance of seasonally dry tropical forest vegetation and the climate is hot and semi-arid (SENAMHI, 2020a). Most of the annual rainfall (235 mm) occurs during the short, wet season from December until March (Ocampo-Ariza et al., 2022). In the dry months, rainfall is close or equal to 0 mm. Twelve 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 to 20 days by means of gravity-fed flood canals.

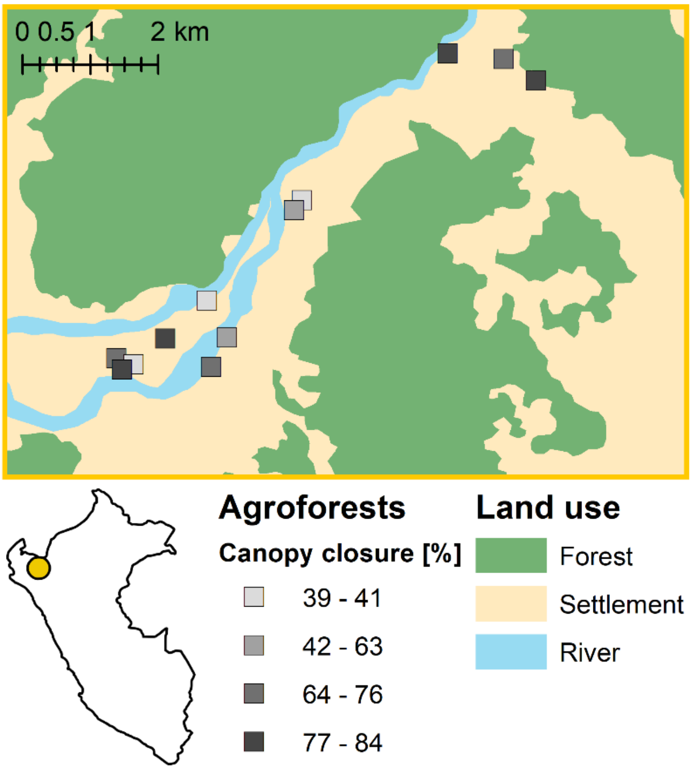

Figure I.3: Northern study region in Peru (black outline), close to the village of La Quemazón (yellow circle). 13 agroforests (grey squares) are surrounded by a river (blue), anthropogenic land-use or settlement (beige) and tropical dry forest (green). One of the farms was used for one part of the experiments only (flower visitation), another additional farm was used in all other experiments, excepting flower visitation studies. Therefore, 13 different study sites are displayed.

c) Site characterisation

All agroforests included in this thesis were selected along gradients of shade cover, measured as canopy closure, and forest distance, the shortest distance from each study site to the nearest forest. 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. Minimum canopy closure was 39% in northern and 55% in southern Peru, while the maximum in the north was 90%, and 95% in the south. We calculated forest proximity, i.e., using ArcMap 10.5.1 by 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). In the south, distances ranged from 0.075 to 1.42 km, in the north from 0.046 to 0.965 km. Cacao tree density and abundance were comparable throughout all study plots: in most of the agroforests, trees were planted following a 3 x 3 m grid, with few exceptions of 3.5 m grids.

I.5.3 Experiments and research questions

*Table I.1: Description of the study sites included in the different experiments that are part of this thesis. In total, 13 agroforests were studied in the north, but only 12 sites were included per experiment. The two sets of agroforests indicated with * and ° differ by one site that was substituted.*

	Experiment	***Chapter***	***Sites***
1A	Flower visitor surveys	Chapter II	12 northern agroforests* 8 southern agroforests
1B	Pollen surveys	Chapter II	4 northern agroforests
2A	Hand pollination: overall	Chapter II	12 northern agroforests°
2B	Hand pollination: genotypes	Chapter IV	12 northern agroforests°
3	Vertebrate and insect exclusion	Chapter III	12 northern agroforests°

For this doctoral thesis, we conducted multiple experiments[1], mostly concentrated in the northern agroforests, in the surroundings of tropical dry forest (Table I.1, Figure I.4). To assess the state of natural pollination, we trapped visitors from cacao flowers in both northern and southern Peru, and we counted the amount of pollen grains deposited on flowers in some of the northern study sites (Chapter II). We also conducted hand pollination experiments in which we supplemented flowers from

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[1] Throughout this thesis, the plural pronouns were preferred over the singular, as most of the work for this thesis was conducted within a collaborative research network, and many of the experiments were conducted together with Carolina Ocampo-Ariza, and many other people without which this work would have not been possible.

northern agroforests with additional pollen and analysed how this affected fruit set rates (Chapter II). In the same experiment, we alternated between self-pollination and cross-pollination with different native genotypes from the cacao variety native to the northern region. The results, showing genetic limitations of fruit set, are complemented with microclimatic variables (Chapter IV). Lastly, to assess the contributions of insects and vertebrates to yield of cacao, we conducted exclusion experiments in 12 northern agroforests. We excluded birds, bats, squirrels, ants and flying insects from cacao trees, and assessed the impact of excluding these animals on fruit set, fruit loss and yield (Chapter III). Additionally, we studied whether flower visitors, hand pollination and animals´ contributions to yield depended on local shade and landscape management (Figure I.4).

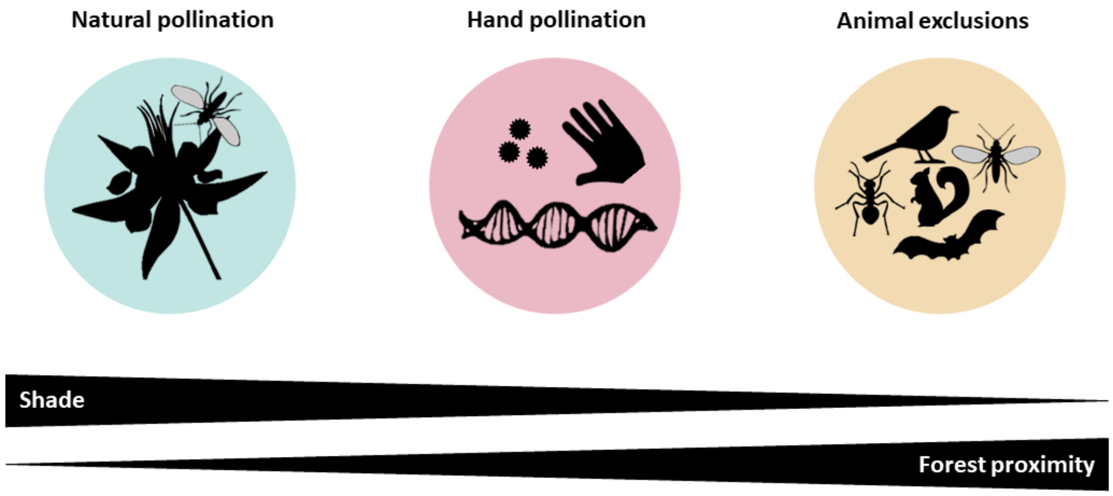


Figure I.4: Schematic experimental set-up of this doctoral thesis. Three different experiments were conducted in cacao agroforests in Peru, among gradients of shade cover and forest proximity: (i) Flower visitors and pollen deposition were surveyed to assess natural pollination, (ii) hand pollination experiments with genetically different pollen donors were conducted to examine whether manual pollination could improve fruit set, and (iii) birds, bats, squirrels, ants, flying insects were excluded from trees to assess these animals´ contributions to fruit set, fruit loss and yield.

a) Flower visitor and pollen surveys: What is the state of natural pollination?

Fruit set and pollination are necessary for cacao fruit production and the pollination biology of the plant limits fruit set severely (Groeneveld et al., 2010). Even though the crop and its pollination biology have been under investigation for decades, there are still gaps in our understanding of the pollination process. Fruit set depends on minuscule insects that visit flowers and deposit sufficient viable and compatible pollen grains on the stigmas (Toledo-Hernández et al., 2017). However, it is not certain which insects are the effective pollinators of the crop. For a long time, it has been assumed that midges are the main pollinating agent (Entwistle, 1972), but recently, it was acknowledged that the dominant insect visitors of the flowers differ among continents (Chumacero de Schawe et al., 2016; Toledo-Hernández et al., 2017) and along farm and landscape management (Toledo-Hernández et al., 2021). Fruit set can be linked to the amount of pollen deposited on the stigma (Falque, Lesdalons, & Eskes, 1996; Mena-Montoya et al., 2020), but the amount of pollen deposited in natural conditions, as well as the effect of pollen deposition on fruit set remain to be quantified. The origin of the pollen influences fruit set, too. Unsuccessful fruit set occurs when incompatible pollen from a flower with the same genetic background is deposited and will likely not result in fruit set (de Almeida & Valle, 2009; Ford & Wilkinson, 2012).

Because of the existing knowledge gap on major aspects of natural cacao pollination and the necessity of pollination for fruit development, we addressed some of the uncertainties concerning pollination of cacao by conducting flower visitor and pollen deposition surveys in northern and southern Peru (Chapter II). Visitors were trapped from flowers by applying insect sticky glue to flowers for 24 hours, in 12 agroforests that differed in proximity to forest and shade cover (Figure I.5A-B). Moreover, pollen was counted from macro-photographs of cacao flower stigma (Figure I.5C), and the fruit set rates of photographed flowers was monitored. Specifically, we asked:

Q1. Which insects are the main flower visitors of cacao?

Q2. How do flower visitation rates differ across gradients of forest proximity and shade cover?

Q3. How much pollen is deposited on flowers and how does this affect fruit set rates?

b) Hand pollination: Can pollination limitation be mitigated?

Hand pollination is a strategy to improve fruit set quantity of tropical crops (Wurz, Grass, & Tscharntke, 2021), such as coffee or passion fruit (Bos, Veddeler, et al., 2007; Klein, Cunningham, Bos, & Steffan-Dewenter, 2008). It can also be a tool to control the quality of seeds, which can be of special importance in cash crops (Wurz et al., 2021). In other crops, manual cross-pollination improved seed content and fruit weight, whereas self-pollination resulted in increased formation of defective seeds (Stein et al., 2017). Manual pollination has also been proposed as a solution to enhance cacao fruit set, because fruit set is especially constrained by pollination (Groeneveld et al., 2010). Through hand pollination, typically, many more pollen grains are deposited than in natural pollination, resulting in improved quality and yield gains up to 69% (Falque et al., 1996; Toledo-Hernández et al., 2020). Gains, however, depend on abiotic limitations and the genetic make-up and cross-compatibility of the considered varieties (de Almeida & Valle, 2009; Samantha Jay Forbes et al., 2019; Valle, De Almeida, & De O.

Leite, 1990). This might be especially true in native cacao, which has lower self-compatibility levels than clonal varieties selected for their compatibility traits. Nevertheless, the effect of hand pollination and the role of the pollen donor genotype remain to be tested in native cacao.

Next to compatibility, stress factors such as drought and high temperatures can limit cacao reproduction as well (Abdulai et al., 2020), although some varieties might have larger tolerance ranges (Ceccarelli et al., 2021). Because microclimatic variables can, on the one hand limit cacao fruit set, and can be affected by canopy closure (Lahive et al., 2019) and forest distance (Schmidt et al., 2017) on the other hand, we monitored soil water content, relative air humidity and temperature throughout the hand pollination experiment (Chapter IV). During this experiment, flowers of eight trees of 12 northern agroforests were pollinated manually by rubbing the free anthers of the pollen donor flower to the stigma of the receptor, using either self- or cross-pollen (Figure I.5D). For self-pollination, we used pollen of other flowers from the receptor trees and for cross-pollination, we collected flowers from five different genotypes of the native cacao variety *Piura white cacao.* Seven days after hand-pollinating all flowers encountered on a 35-cm-long branch section, fruit set of pollinated flowers was recorded (Chapter II and IV). Of fruits that were harvested from both hand pollinated and natural flowers, we also measured fruit quality traits: we weighted fruits and beans and counted the number of normal and premium white beans per pod (Chapter IV). With this dataset, we set out to answer the following questions:

> Q4. Are fruit set rates of manually pollinated flowers higher than those of naturally pollinated flowers?

Q5. Are fruits from manually pollinated flowers of higher quality than those naturally pollinated flowers?

Q6. How do fruit set rates of cross-pollinated flowers differ from self-pollinated flowers and among pollen donor genotypes?

Q7. How do soil water content, relative humidity and temperature affect fruit set rate in native cacao?

Q8. How do forest distance and canopy closure affect soil water content, relative humidity, and temperature in cacao agroforests?

Figure I.5: Set-up and methodology of the three experiments described throughout this thesis. In the first surveys, flower visitors were trapped from flowers with insect sticky glue (A-B), and pollen was counted from macro photographs (C). In the hand pollination experiment, various native cacao genotypes were used to manually deposit pollen on flowers (D). In the third experiment, birds and bats were excluded from cacao trees with large cages built around them (E), flower visitors were excluded with cages covered with fine mesh (F-G) and ants were excluded by covering plastic collars with insect sticky glue so the insects could not crawl up the trees (H).

c) Exclusion experiments: How much do animal groups contribute to yield?

High biodiversity levels can coincide with high yields in cacao agroforests (Clough et al., 2011). Biodiversity can provide ecosystem services that are crucial for crop yields, such as pollination services by flying insects (Garibaldi et al., 2018; Knight et al., 2018; Tscharntke et al., 2012). Insectivorous birds and bats as well as certain arthropods may provide pest control services, either by preying on insect pests directly, or by preying on mesopredators that control herbivore populations (Cassano, Silva, Mariano-Neto, Schroth, & Faria, 2016; Samantha J. Forbes & Northfield, 2017; Maas et al., 2016). Yet, high biodiversity levels might also be associated to disservices: Rodents and other mammals are notorious fruit predators in agroecosystems (Cassano, Rios, & Gaiotto, 2021; Linden et al., 2019). The quantification of animals' contributions to services and disservices is important for increasing our knowledge on biodiversity-friendly cacao agroforestry. Although the single contributions of flying insects, birds, bats and ants are relatively well-studied in Asian cacao (Bos, Veddeler, et al., 2007; Maas et al., 2013; Wielgoss et al., 2014), the combined contributions of animals still remain to be investigated in native cacao.

Management of agroforests, such as forest proximity and shade cover, can affect the abundance and diversity of animals that provide ecosystem (dis-)services in cacao (Gras et al., 2016; Hanf-Dressler, 2020; Toledo-Hernández et al., 2021). To inform management more adequately, it is important to integrate such local and landscape management factors in analyses of biodiversity-friendly farming. To this end, we set-up a field experiment in which we jointly manipulated access of birds, bats, squirrels, ants and flying insect to cacao trees, in eight trees in each of the 12 northern agroforests (Figure I.5E-H). We quantified the contribution of each animal group to fruit set, fruit loss and yield, and assessed whether contributions varied across forest proximity and shade cover (Chapter III). The specific research questions were:

Q9. How do birds, bats, ants, and flying insects affect fruit set, fruit loss and yield of native cacao in northern Peru?

Q10. How is the contribution of these animal groups affected by management of nearby forest and shade cover?

CHAPTER II

CACAO FLOWER VISITATION: LOW POLLEN DEPOSITION, LOW FRUIT SET AND DOMINANCE OF HERBIVORES

CHAPTER II CACAO FLOWER VISITATION: LOW POLLEN DEPOSITION, LOW FRUIT SET AND DOMINANCE OF HERBIVORES

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. 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. 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. 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. 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.

II.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 (C.A. Romero & Urrego-Vargas, 2016). Therefore, identifying limitations of pollination success (Figure II.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, e.g. 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, particularly so in self-incompatible cacao varieties (Toledo-Hernández et al., 2020). 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; Samantha Jay 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 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.

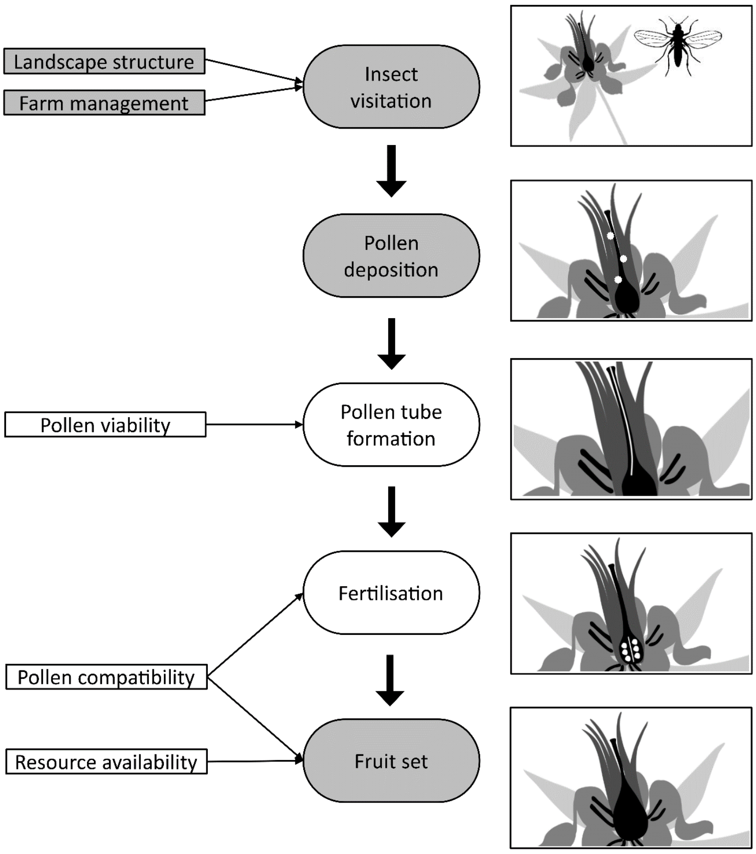


Figure II.1: Conceptual overview of the cacao pollination process, depicting several steps preceding fruit set (ovals), including relevant drivers and limitations (rectangles). Variables addressed in this study are highlighted in grey. Insect visitation is necessary for pollen deposition and may depend 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 fertilisation (Claus, Vanhove, Van Damme, & Smagghe, 2018; Falque, Vincent, Vaissiere, & Eskes, 1995)*. Finally, pollen compatibility and resource availability can affect setting of fruits even until after fertilisation* (de Almeida & Valle, 2009; Ford & Wilkinson, 2012).

II.2 Material and methods

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

II.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 northern 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 II.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 II.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 to 200 mm per month and during the dry season, about 50mm per month (Merma & Julca, 2012; SENAMHI, 2020b). In the southern agroforests, introduced hybrid clones are cultivated alongside the local native variety, called chuncho cacao.

II.2.2 Site selection and characterisation

In the northern study region, twelve 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 to 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, i.e., 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 x 3 m grid, with few exceptions of 3.5 m grids.

II.2.3 Surveys

a) 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 hours 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 hours after glue application, most of the flowers had abscised, a process that is normal in cacao (24-36 hours, Toledo-Hernández et al., 2017). Therefore, not all flowers could be recollected and numbers of retrieved flowers differed among trees and farms (Table II.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 categorised as other Diptera, Hymenopterans were either classified as parasitoid wasps, ants, or other Hymenoptera.

b) 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 & Forrest, 2017). Pollen deposition is usually quantified destructively, i.e., 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 II.S2).

We took 7704 macro photographs of 518 flowers, spread over 5 agroforests and different shooting days. Data of two consecutive years was included (Table II.S2). Normal cacao flower lifetime is about 24-36 hours (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 to 28 hours 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).

c) Hand pollination

To compare natural pollination with manual pollination, we hand-pollinated flowers of eight receptor trees in each of the twelve 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 35cm 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 35cm branch section on each tree and followed the development of all open flowers on the other branch section over a period of seven 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

(*Cooperativa Agraria Norandino Ltda.*). 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 II.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 six days after pollination, divided by the number of open flowers recorded six 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 seven weeks of the experiment.

II.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.

a) Flower visitors

We used generalized linear mixed effect models (GLMM) with the package lme4 (Bates, Mächler, Bolker, & Walker, 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 II.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 was excluded from all models, because of incomplete canopy closure assessments (Q14, Table II.S1, Figure II.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 DHARMa package (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.

b) *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, Fuentes-Ramirez, Gregoire, Altamirano, & Yaitul, 2018), the success events were too rare to perform any meaningful statistical analysis.

c) *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 (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 II.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 II.S3).

II.3 Results

a) *Flower visitors*

In total, 304 flower visitors were collected from 1,179 flowers (1 visitor per 3.88 flowers), 7% of the entire visitor community were midges (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 II.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 II.2B), the dominant visitors were thrips (65%), followed by midges (14%), parasitoid wasps (10%), other Diptera (9%), ants (7%), and immature arthropods (6%).

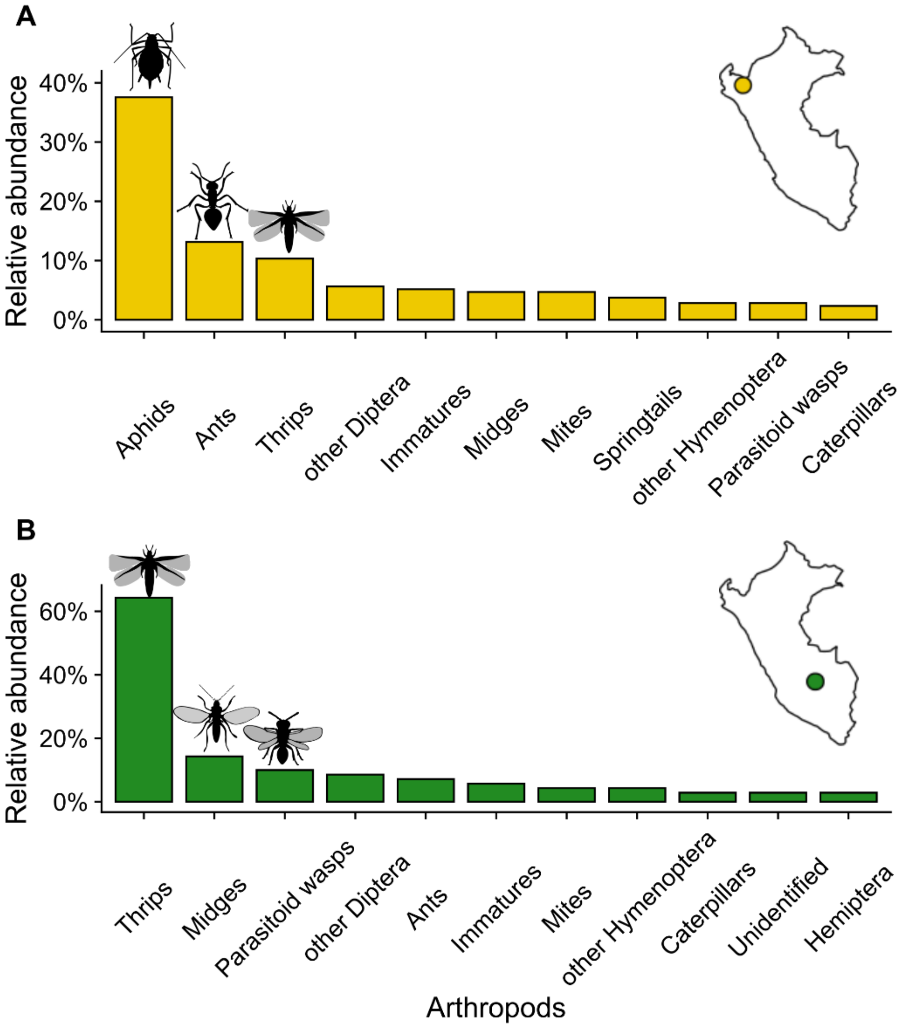


Figure II.2: Relative abundances of top 11 arthropod groups per region (maps with circles) trapped with insect sticky glue from flowers in northern (A) and southern (B) Peru. Only groups with relative abundances >2% are shown.

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 II.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 II.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 II.3B). Neither canopy closure nor forest distance influenced visitation rates of aphids, which was the most abundant visitor in the north (Figure II.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 II.3E). Finally, visits by other arthropods did not seem to be affected by increasing forest distance (Figure II.3F).

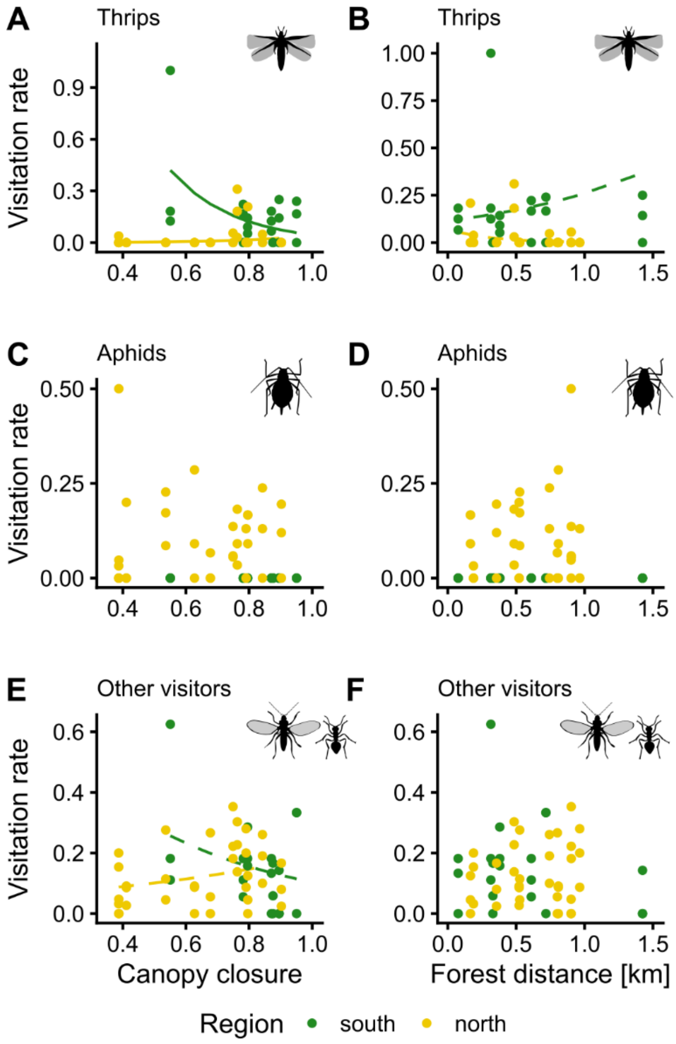


Figure II.3: Visitation rates of thrips (A – B), aphids (C – D) and all other visitors, excluding aphids and thrips (E – F), in function of canopy closure (left column) and forest distance (right column). Visitations rates per round are calculated by dividing total visits by number of collected flowers 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 II.S4).

b) 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 II.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).

c) 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 II.4, Table II.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).

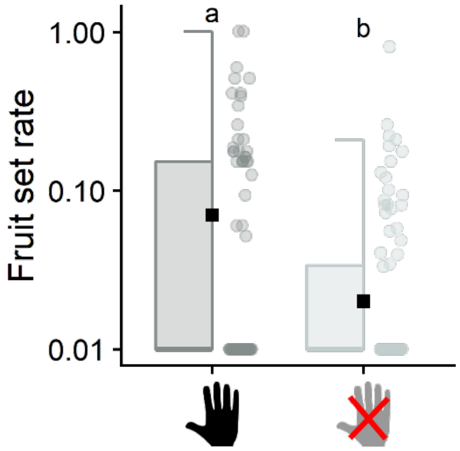


Figure II.4: Fruit set rates of manually (dark grey circles) and naturally pollinated (light grey circles) cacao flower and total rates per treatment (black squares). Fruit set rate is the number of healthy cherelles divided by open flowers counted and/or pollinated six days earlier, pooled across seven 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 II.S5).

II.4 Discussion

In this study, we aimed to reveal key drivers of cacao pollination services (Figure II.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, Veddeler, 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 is 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, Veddeler, 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.

II.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.

II.6 Supplementary information

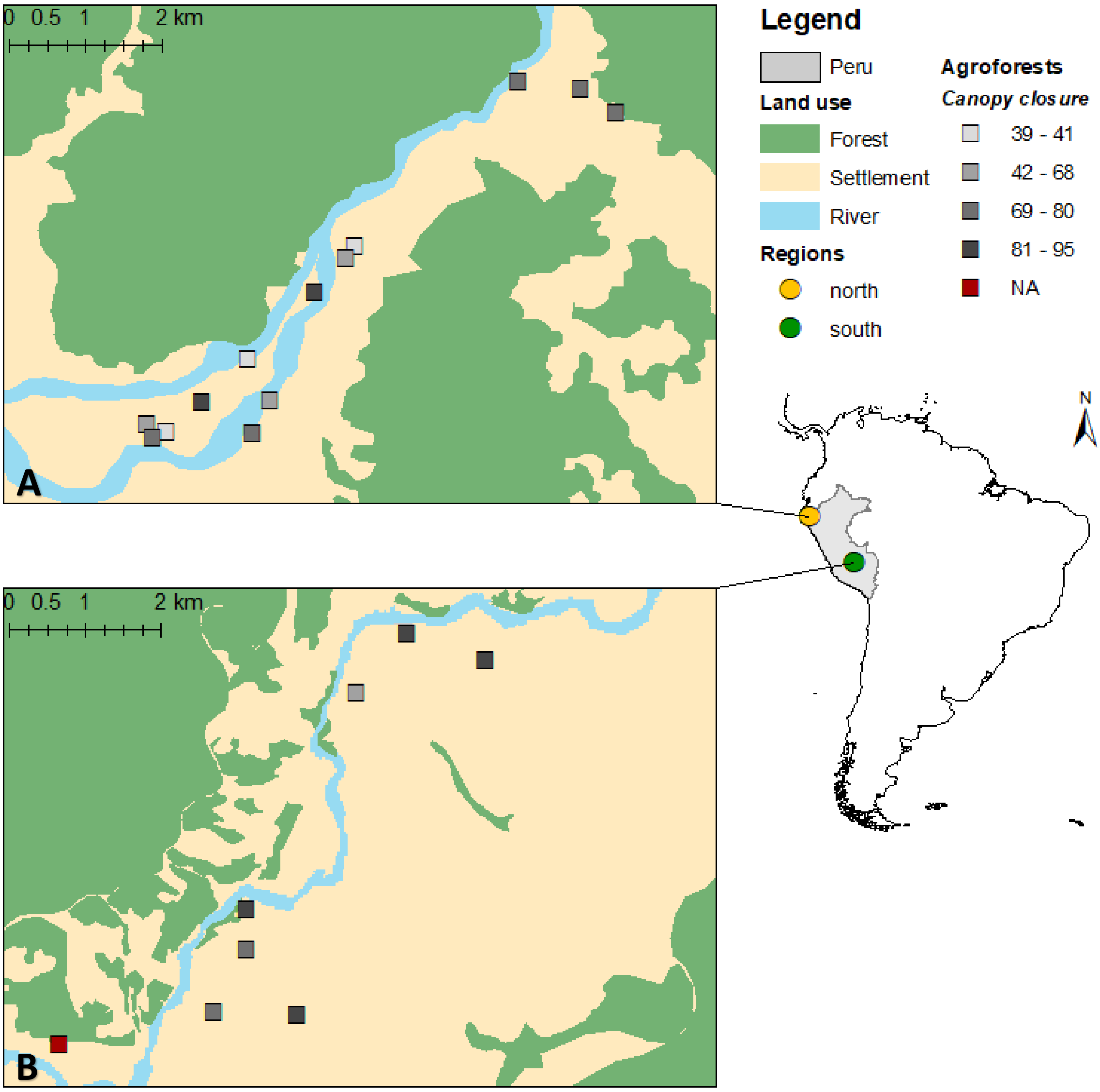


Figure II.S1: Northern and southern study regions (yellow and green circles, respectively) in Peru (grey fill). In the north (A), 12 agroforests (grey squares) in which flower visitors were surveyed, and one additional agroforest in which hand pollination was studied are surrounded by a river (blue) and dry forest (green), close to the village La Quemazón. Around the town of Echarati in the south of Peru (B), the seven agroforests included in visitation rate models (grey square) and Q14, the one agroforest without canopy closure data (red square) were surrounded by wet and humid forest (green patches).

Figure II.S2: Macro photographs of two opposite sides of the style of a cacao flower, on which several bright-white pollen clumps can be seen. We estimated that 170 pollen grains were found on the style, but fruiting was not successful in this flower.

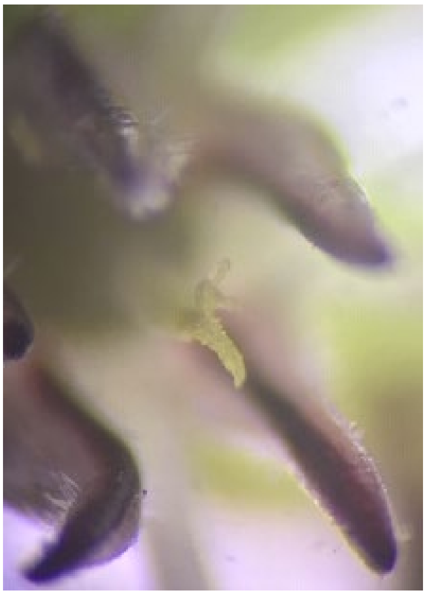


Figure II.S3: The amount of pollen deposited on the style after manual pollination, showing that the style is full of pollen grains (rough surface; the style surface is smooth in flowers where no pollen was deposited). View through stereomicroscope.

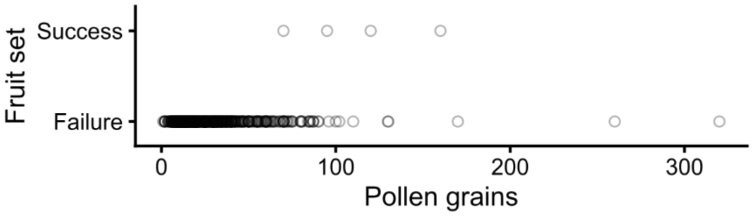


Figure II.S4: Number of pollen grains deposited on styles of monitored cacao flowers exposed to natural pollination. The Y axis indicates whether pollination resulted in successful fruit set (Success) or failure within seven days after photographing.

Table II.S1: Canopy closure, forest distance and totals of collected flowers and arthropod visits for the three visitor groups analysed, in northern and southern agroforests.

Agroforest ID	Canopy closure	Forest distance [km]	Collected flowers	Thrips	Aphids	Other visitors
south						
Q02	0.55	0.315	28	12	0	8
Q10	0.78	0.610	38	5	0	4
Q18	0.90	1.424	13	2	0	1
Q23	0.95	0.717	52	8	0	9
Q25	0.88	0.330	42	0	0	3
Q33	0.87	0.076	34	4	0	4
Q37	0.80	0.380	37	3	0	7
north						
P01	0.39	0.188	82	1	1	10
P08	0.68	0.801	55	0	1	5
P09	0.39	0.902	56	0	9	1
P10	0.84	0.743	74	1	8	13
P11	0.79	0.965	68	0	3	13
P13	0.90	0.356	96	0	11	8
P20	0.63	0.808	52	0	4	4
P25	0.75	0.903	57	1	5	14
P30	0.80	0.166	70	5	10	4
P32	0.76	0.485	84	14	8	19
P40	0.41	0.522	105	0	7	7
P41	0.54	0.526	86	0	13	13

Table II.S2: Number of successes and failures of fruit set per agroforest.

	Fruit set	
Agroforest ID	Success	Failure
P01	1	1
P10	0	4
P13	1	5
P09	2	496
P11	0	8

Table II.S3: Number of open flowers and cherelles and fruit set rates per agroforest, after manual and natural pollination. Fruit set rates are the number of cherelles divided by open/ pollinated flowers.

	Natural pollination			Manual pollination		
Agroforest ID	Flowers	Cherelles	Fruit set rate	Flowers	Cherelles	Fruit set rate
P01	101	8	0.079	31	4	0.129
P08	195	2	0.010	140	10	0.071
P09	198	1	0.005	69	7	0.101
P10	141	0	0.000	61	5	0.082
P11	107	2	0.019	65	1	0.015
P20	150	2	0.013	101	11	0.109
P25	216	10	0.046	108	16	0.148
P30	202	0	0.000	135	6	0.044
P32	267	0	0.000	82	1	0.012
P40	111	5	0.045	37	4	0.108
P41	83	3	0.036	38	0	0.000
P42	181	5	0.028	101	5	0.050

Table II.S4: Regression summary of generalized mixed models for the most abundant flower visitor groups, i.e., thrips, aphids and other visitors.

	thrips			aphids			other flower visitors		
Predictors	*Incidence Rate Ratios*	*Statistic*	*p*	*Incidence Rate Ratios*	*Statistic*	*p*	*Incidence Rate Ratios*	*Statistic*	*p*
(Intercept)	0.19	-3.11	**0.002**	0.06	-3.99	**<0.001**	0.18	-6.26	**<0.001**
Canopy closure	0.41	-1.44	0.149	1.75	0.66	0.512	0.7	-1.27	0.203
Region [Piura]	0.06	-3.77	**<0.001**				0.72	-1.13	0.259
Forest distance	1.31	0.7	0.484	0.92	-0.14	0.891	1.02	0.08	0.938
Canopy closure * Region [Piura]	5.74	2.2	**0.028**				1.79	1.87	0.062
Forest distance * Region [Piura]	0.26	-1.91	0.056				1.12	0.4	0.69
Random Effects									
σ^2	4.03			2.55			2.2		
τ_{00}	0.67_{Farm}			0.12_{Farm}			0.01_{Farm}		
ICC	0.14			0.05			0		
N	19_{Farm}			12_{Farm}			19_{Farm}		
Observations	57			36			57		
Marginal R^2 / Conditional R^2	0.369 / 0.459			0.004 / 0.049			0.030 / 0.033		

Table II.S5: Summary of the binomial GLMM used to analyse differences in fruit set rates between natural and manual pollination (Pollination treatment).

	Fruit set rates		
Predictors	*Risk Ratios*	*Statistic*	*p*
(Intercept)	0.17	-10.43	**<0.001**
Pollination treatment [NAT]	0.46	-6.76	**<0.001**
Random Effects			
σ^2	1		
τ_{00} Plant:Farm	0.59		
τ_{00} Farm	0.11		
ICC	0.04		
N Plant	93		
N Farm	12		
Observations	181		
Marginal R^2 / Conditional R^2	0.124 / 0.160		

CHAPTER III

QUANTIFYING SERVICES AND DISSERVICES PROVIDED BY INSECTS AND VERTEBRATES IN CACAO AGROFORESTRY LANDSCAPES

CHAPTER III QUANTIFYING SERVICES AND DISSERVICES PROVIDED BY INSECTS AND VERTEBRATES IN CACAO AGROFORESTRY LANDSCAPES

Animals provide services such as pollination and pest control in cacao agroforestry systems, but also disservices. Yet, their combined contributions to crop yield and fruit loss are mostly unclear. In a full-factorial field experiment in north-western Peru, we excluded flying insects, ants, birds, and bats from cacao trees and assessed several productivity indicators. We quantified the contribution of each group to fruit set, fruit loss and marketable yield and evaluated how forest distance and canopy closure affected productivity. Fruit set dropped (from 1.7% to 0.3%) when flying insects were excluded and tripled at intermediate (40%) compared to high (>80%) canopy cover in the non-exclusion treatment. Fruit set also dropped with bird and bat exclusion, potentially due to increased abundances of arthropods preying on pollinators, or of flower herbivores. Overall, cacao yields more than doubled when birds and bats had access to trees. Ants were generally associated with fruit loss, but also with yield increases in agroforests close to forest. We also evidenced disservices generated by squirrels, leading to significant fruit losses. Our findings show that several functional groups contribute to high cacao yield, while trade-offs between services and disservices need to be integrated in local and landscape-scale sustainable management of cacao agroforestry.

III.1 Introduction

Ecosystem services such as pollination and pest control support yields of globally important crops, thus ensuring a considerable part of the world's food supply (Garibaldi et al., 2018; Tscharntke et al., 2012). These nature-based services are biodiversity-driven (Dainese et al., 2019) and provided by multiple animal groups. Vertebrates such as birds and bats, as well as arthropods, may control pest populations (Samantha J. Forbes & Northfield, 2017; Gras et al., 2016), while bees and many other animals are important crop pollinators (Knight et al., 2018). But animals can also cause substantial disservices: arthropod species are typical pests threatening yields of many crops. Aside from arthropods pests, rodents or other mammals can damage or raid fruits (Cassano et al., 2021; Linden et al., 2019). Some animal taxa can be involved in more than one ecosystem service (Lundin, Rundlöf, Jonsson, Bommarco, & Williams, 2021), while other taxa, are known to provide both services and disservices in the same crop system (Wielgoss et al., 2014), which can result in management trade-offs. Interactions among services exist as well. For example, beneficial effects of pollination on yields can depend on the level of pest control, e.g. by herbivores lowering attractiveness to pollinators (Lundin, Smith, Rundlöf, & Bommarco, 2013). Therefore, assessing both ecosystem services and disservices is essential to account for potential trade-offs and interactions in biodiversity-friendly and sustainable crop management (Gagic, Marcora, & Howie, 2019). Yet, only a handful of studies have addressed multiple services and disservices simultaneously (Classen et al., 2014; Segre, Segoli, Carmel, & Shwartz, 2020).

In cacao, a tropical crop grown in agroforestry systems that can be wildlife-friendly (Clough et al., 2011), multiple animal groups mediate yields. Animal pollination is strictly limiting productivity: the exclusion of flower visitors can result in fruit set values equal or close to zero (Bos, Veddeler, et al., 2007), even though the identity of pollinator species remains unclear (Chumacero de Schawe et al., 2016). Pollination gains can be undermined by insect pest infestations causing fruit loss (Bos, Steffan-Dewenter, et al., 2007a), but these pests can be successfully controlled by birds and bats. Yield gains have been attributed to arthropod control by flying vertebrates (Cassano et al., 2016; Maas et al., 2013). Other vertebrates, such as squirrels and other rodents, prey on mature cacao fruits and can cause severe harvest losses (Cassano et al., 2021). Harvest loss can also be due to fungal infections, and by propagating fungal spores, ants can enhance fruit loss (Bos, Steffan-Dewenter, et al., 2007a; Gras et al., 2016; Wielgoss et al., 2014). However, ants can also support yield gains, through reduction in flower and leaf herbivory (Gras et al., 2016). Knowledge on combined effects of animal groups is key to improve our understanding of services and disservices, which in turn might allow developing more efficient management recommendations for profitable and sustainable biodiversity-friendly cacao agroforestry.

The abundance and diversity of services and disservices provided by animals in cacao agroforests are also affected by agroforest and landscape characteristics, such as shade cover and forest distance (Gras et al., 2016). Shade cover provided by the canopy of non-cacao trees in agroforests, can improve growing conditions for cacao (Blaser et al., 2018), the occurrence of birds and bats (Ocampo-Ariza et al., 2022), and cacao flower visitation rates (Toledo-Hernández et al., 2021). On the other hand, high shade cover can promote the occurrence of pest species and counteract natural pest control (Bos, Steffan-Dewenter, et al., 2007a). Forest proximity can also influence pest control and pollination, as forest remnants in the landscape provide habitat to many animals, including flying vertebrates and arthropods (Klein et al., 2008), potential natural enemies of cacao pests. For example, typically, more birds and bats can be found foraging in cacao agroforests closer to the forest than at further distances (Ocampo-Ariza et al., 2022). As for arthropods, there is evidence of certain cacao flower visitors (Vansynghel et al., 2022) and ant species (Schroth et al., 2011) being impacted by increasing distance to forest, though this is not consistent across studies (Toledo-Hernández et al., 2021). Forest distance and shade cover thus have important implications for biodiversity and its ecosystem functions they provide.

Understanding the complex interactions between animals, the services and disservices they provide, and their dependence on local and landscape characteristics is crucial for aiding decision-making in sustainable cacao agroforestry management., We quantified multiple ecosystem services and disservices in cacao agroforests established in a Peruvian tropical dry forest environment, using exclusion cages and barriers to prevent access of certain animal groups to cacao trees. We excluded flying insects, ants, birds and bats and measured four productivity parameters: fruit set, marketable yield and fruit loss. We analysed fruit loss due to squirrels separately from other fruit loss causes, as these rodents are an important pest species in the study region. Additionally, we assessed how forest distance and canopy cover affected productivity to identify key animal-driven services and disservices.

III.2 Methods

III.2.1 Study area

We performed the study in twelve organic cacao agroforests located around the farmer community of La Quemazón, in northwestern Peru (S5.31°, W79.72°, 240 m.a.s.l.; Supplementary Figure III.S1). The region is characterized by a hot and semi-arid climate, with mean annual rainfall of 235 mm, mostly concentrated between December and March, and a native vegetation cover of submontane, seasonally dry tropical forests (Ocampo-Ariza et al., 2022; SENAMHI, 2020a). To compensate for low water availability in the dry season, agroforests are irrigated by means of gravity-fed flood canals every four weeks on average, depending on water availability

The cacao agroforests ranged in size between 0.3 and 1.1 ha, had comparable cacao planting densities (3 x 3 m or 3.5 x 3.5 m planting grids) and age (5- to 10-year-old) but differed in shade cover (39-84%) and distance to forest (0.1-1.2km). Shade cover was assessed using a Forest Suppliers® spherical densiometer with convex mirror, by averaging the readings of canopy closure [%] in 20 points spread over an area of about 0.15 ha, to obtain a mean value per agroforest. Distance from each agroforest to the nearest forest [km] was calculated with ArcMap 10.5.1, using a land-use map of Piura (Otivo Barreto, 2010) updated through ground-truthing (Hanf-Dressler, 2020; Ocampo-Ariza et al., 2022).

III.2.2 Exclusion experiments

We established three vertebrate exclusion treatments in September 2019 (Figure III.1) with exclusion of birds and bats, only birds or only bats, and one open control treatment in each of the twelve selected cacao agroforests and maintained them functional for approximately one year, until October/November 2020. Vertebrate exclusions consisted of cages with a size of 2 m wide, 5 m long and 3 m high, each containing two adult cacao trees. Pairs of experimental trees were spaced by 6 to 9 meters, in an area of approx. 0.15 Ha. The scaffolds of the structure were made of bamboo poles, and fishing mesh with 2.5 cm openings was used to cover all sides and roof of the cage, preventing the access of birds and/or bats. Selectivity was ensured by differential opening times of each treatment: (1) control treatments consisted of two cacao trees per agroforest left permanently accessible to vertebrates and without a cage constructed around them; (2) bird exclusion cages were kept closed during the day (6:00 – 18:00) and open during the night (18:00-6:00), to allow the access of nocturnal vertebrates; (3) bat exclusion cages were kept open during the day and closed during the night; (4) full exclusions were permanently closed. By excluding flying vertebrates, we also excluded squirrels, notorious fruit predators in the region (Ledesma, 2022).

One of the two trees per vertebrate exclusion treatment was subject to an ant exclusion treatment consisting of a vinyl cone located at the base of the trunk, covered with Schacht® insect sticky glue, to prevent ants from crawling up the plant from the ground (Figure III.1). The vinyl cones were tied with rubber tires to the cacao bark at around 30 cm height and isolation foam was stuffed between the cone and the bark (Supplementary Figure III.S2D). Further, we used cotton wool to stuff cracks, to avoid the smallest ants from crawling up the cacao trees. To also eliminate tree-nesting ants, we applied small doses of a plant-based insecticide Atoxin 15 EC (10ml/l) with a pipette inside existing ant nests, and when necessary, the application was repeated every two weeks for the entire duration of the experiment. Glue layers were refreshed every two weeks, to prevent the glue from drying out. Experimental trees were pruned regularly, so that the crowns and branches of trees within each cage did not touch each other or the nets, to avoid ant recolonization.

We excluded flying flower visitors on each of the 96 experimental trees by covering a 35 cm long branch section with UV-stabilized polypropylene gauze (0.5 mm mesh size), supported by an aluminium framework, and sealed with plant wire (Supplementary Figure III.S2B). To permit the access of ants, we inserted little twigs between the nets and the cacao branches, but only in the trees without ant exclusions (Supplementary Figure III.S2C). Although we aimed to selectively exclude ants only, other crawling insects, such as beetles or bugs could have also entered the exclusion cages through the twig, and likewise, could have been excluded by the ant-barriers.

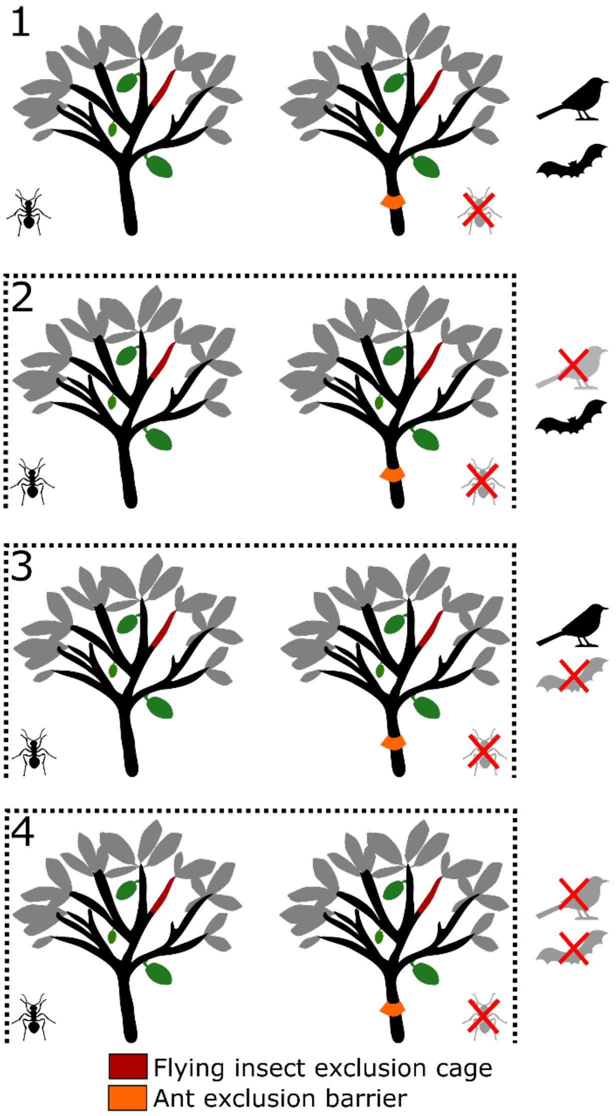


Figure III.1: Set-up of experiments in each of the 12 cacao agroforests. Each vertebrate exclusion treatment (1 – control, full bird and bat access; 2 – closed during the day, no bird access; 3 – closed during the night, no bat access; 4 – permanently closed, no bird nor bat access) included two cacao trees, one of which was subject to exclusion barriers covered with insect sticky glue (orange) to prevent ants' access (ant exclusion). On all experimental trees, flying insect exclusion cages were installed to prevent access of flying insects. To permit ants´ entrance, twigs were inserted, but only in trees without ant exclusion.

III.2.3 Productivity indicators

From November 2019 until October/November 2020, we conducted biweekly counts of all recently fertilized fruits (measuring between 1 and 3 cm) and open flowers on each tree. Flower counts started two weeks earlier than the fruit counts and were repeated every 14 days. Small fruits were summed per tree, over the year. We multiplied the sum of daily flower counts by 7 to obtain the total number of flowers that could have given rise to the observed fruits. These counts only include fruits over 1 cm that are older than 1 week, as in other studies (Vansynghel et al., 2022), and fruits smaller than 3 cm, that are approximately 3 weeks old. Subsequently, small fruits were divided by the total number of flowers, to obtain an estimate of yearly fruit set [%] per tree.

Additionally, we performed biweekly counts of harvested and lost fruits. Squirrel-related fruit loss [%] per tree was established as the proportion of non-harvested mature fruits, i.e., fruits that were large and almost harvestable but were not marketable due to seed predation by squirrels (Supplementary Figure III.S5). We pooled all other, non-squirrel-related causes of fruit loss (Supplementary Figure III.S3), i.e., insect damage, germinated seeds, or malformed seeds to calculate non-squirrel fruit loss [%]. Cacao beans from harvested fruits were dried in the sun and then weighed with a 0.01 g pocket scale to obtain a final measurement of dry weight. The dry weight per tree [kg] was summed per tree over all counts (over a period of one year) and then multiplied by the number of trees/ha typical for our study area (1100 cacao trees, at a 3 x 3 m planting grid) to obtain a total yield value [kg/ha].

III.2.4 Data analysis

We constructed generalized linear mixed effect models (GLMM) using R Statistical Software (RCoreTeam, 2021) in R Studio 4.1.2 (RStudio Team, 2021) to evaluate the effects of our exclusion treatments on productivity indicators. All models were assembled in the “glmmTMB” package (Brooks et al., 2017). Diagnostic plots and tests for overdispersion and zero-inflation were done with the “DHARMa” package (Hartig, 2018), adapting the probability distribution when necessary. Model performance indicators were extracted with package "performance" (Lüdecke, Ben-Shachar, Patil, Waggoner, & Makowski, 2021) and Wald χ^2-tests (Anova type II) reported were conducted with package "car" (Fox & Weisberg, 2019). Predictions were obtained with package "ggeffects" (Lüdecke, 2018).

We used a traditional null hypothesis testing approach in which we only included ecologically relevant fixed effect variables and interactions. We restricted ourselves to a priori hypotheses to avoid overparameterizing our models. In all models, shade cover and forest distance were scaled, i.e. the values were subtracted by the mean and divided by standard deviation. In the first model, we assessed the effect of exclusion treatments and farm characteristics (shade cover and forest distance) on cacao fruit set rates. We used a betabinomial distribution with logit link function, using flowers as weights and site as random effect variable. Flying insects, ants, and vertebrate exclusions, as well as canopy closure and forest distance were included as fixed effects. We also included two-way interactions of flying insect exclusion with canopy closure, forest distance, ant exclusion and vertebrate exclusion, as each of these parameters could affect the way insect exclusion altered fruit set. E.g., canopy closure and forest distance can affect insect abundances directly, and since ants and vertebrates might be involved in predator-prey relations with flying insects, we considered those the interactions of interest for the fruit set model.

Second, we evaluated changes in cacao fruit loss due to squirrels (squirrel fruit loss/mature fruits), using a model with binomial distribution and logit link, using numbers of mature fruits per tree as weights. Fixed effect variables included were ant exclusion, vertebrate exclusion, canopy closure and forest distance, as well as the two-way interactions between the exclusion treatments and forest distance and canopy closure, respectively. We considered the interaction of ant and vertebrate exclusion not meaningful, because other, non-squirrel related fruit loss cannot be detected when pods are attacked by squirrels. Therefore, this interaction was left out of the analysis. Third, cacao fruit loss due to other causes (non-squirrel fruit loss) was analysed with a similar model as for squirrel-related fruit loss, the only difference being the inclusion of the vertebrate and ant exclusion interaction in this model. We assumed the interaction could be meaningful, for example when birds and bats have different ant predation rates. Fourth, we modelled cacao yield with a hurdle-gamma model (ziGamma), a distribution used to model continuous data with non-constant error that allows zero as a response, overcoming the restriction of a classical gamma distribution to strictly positive observations (Magnusson et al., 2021). We included site as random effect variable; all other fixed effect variables and their interactions were included as in the non-squirrel fruit loss model.

III.3 Results

In total 3,337 young cacao fruits developed in total (mean per tree: 35.5 ± 3.0). Only 702 fruits fully matured, 596 of which were harvested, 52 were lost due to squirrel seed predation and 54 were lost due to other, non-squirrel related causes. Average yield was 220.0 ± 23.9 kg/ha (Supplementary Table III.S1). Mean fruit set rates were 1.7 ± 0.2% for natural pollination and 0.3 ± 0.1% for the flying insect exclusion treatment. Mean natural fruit set rates doubled from 1.3 ± 0.3% under full vertebrate exclusion to 2.6 ± 0.5 % when both birds and bats had access to the cacao trees, irrespective of ant exclusion (Figure III.2A, Table III.1). In open controls, predicted fruit set decreased exponentially with increasing canopy closure, from 3% under intermediate (39%) canopy closure to 1% under high canopy closure (84%, Figure III.2B, Table III.1).

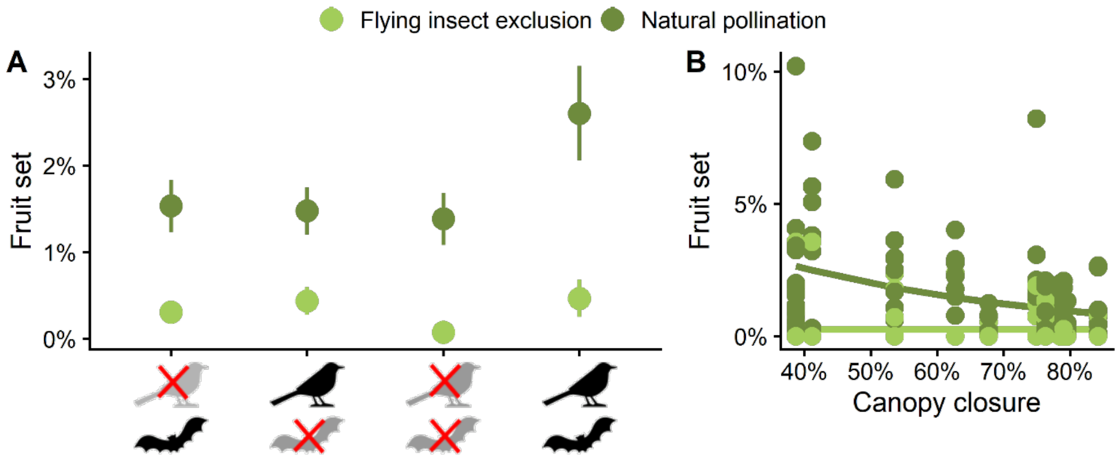


Figure III.2: Yearly fruit set rates per tree (mean ± SE, dots and whiskers) as a function of flying insect and vertebrate exclusion (A) and flying insect exclusion and canopy closure (B). Fruit set rates under flying insect exclusion (light green) were measured at the branch level; fruit set of open controls were measured at the tree level (dark green). For statistics see Table III.1.

Squirrel fruit loss was highest in the treatments in which all vertebrates, including squirrels, had access to the trees (10.2 ± 3.8%), and was lower when partial and full exclusion treatments prevented squirrel access to cacao trees (Figure III.3A, Table III.1). Ant access was related to an increase in non-squirrel related fruit loss, from 4.2 ± 1.3%

to 6.9 ± 2%, independent of shade cover and forest distance (Figure III.3B, Table III.1). Yields more than doubled (114% higher) when both birds and bats had access to trees (331.2 ± 62.9 kg/ha, Figure III.4A, Table III.1), than under full vertebrate exclusion (153.6 ± 27.7 kg/ha). In the presence of birds and bats, yield decreased 28% when ants had access (291.9 ± 79.8 kg/ha), compared to ants being absent (374.1 ± 101.0 kg/ha, Supplementary Figure III.S4). However, in the presence of only birds, ants seemed to benefit yields: their access improved yields by 43%, from 168.2 ± 52.2 kg/ha to 240.8 ± 83.7 kg/ha. Yield also decreased with distance to forest, in the presence of ants but not in their absence. Predicted values ranged from 612 kg/ha next to the forest to 98 kg/ha at distances further than 1 km from the forest. No such effect was observed on trees from which ants were excluded (Figure III.4B, Table III.1).

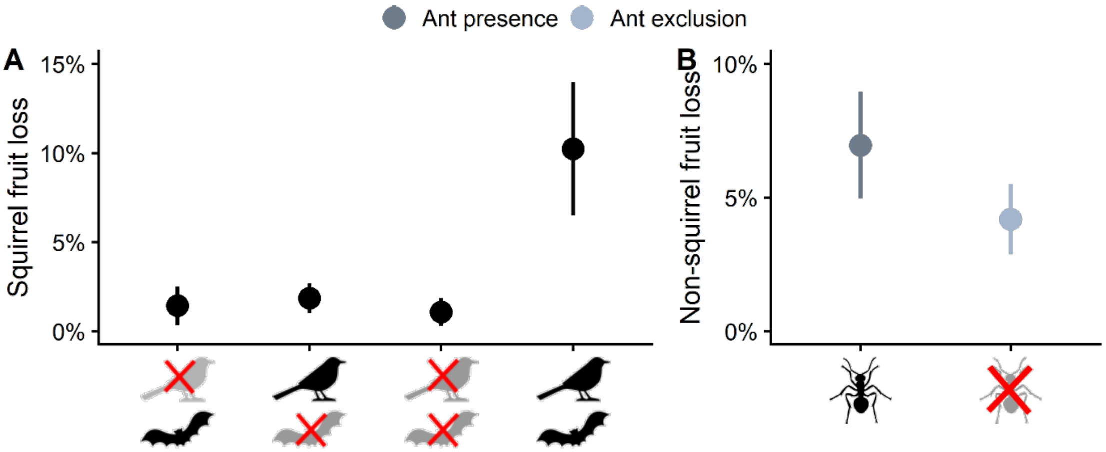


Figure III.3: Fruit loss per tree due to squirrels (squirrel fruit loss) as a function of vertebrate exclusion treatments (A) and non-squirrel fruit loss as a function of ant exclusion treatments (B). Dots and whiskers (means +- SE, totalled per tree): black, all data; light blue, trees without ants; and dark blue, trees with ants. For statistics see Table III.1.

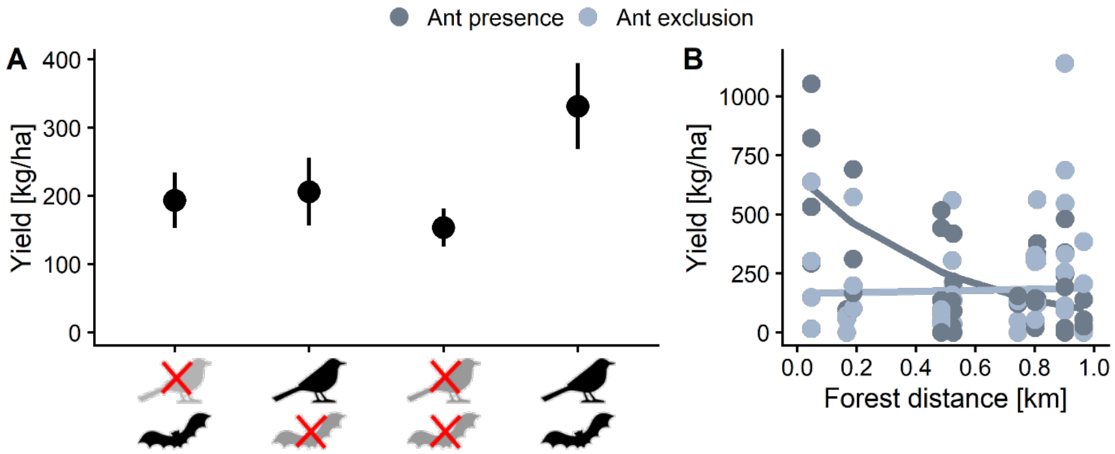


Figure III.4: Yield per tree as a function of vertebrate exclusion treatments (A) and ant exclusion treatments (B). Dots and whiskers (means +- SE, totalled per tree): black, all data; light blue, trees without ants; and dark blue, trees with ants. For statistics see Table III.1.

*Table III.1: Results of type II analysis of variance with generalized linear mixed effects models relating model parameters to fruit set [%], fruit loss [%] and yield [kg/ha]. Parameters include exclusion treatments (excl.) of flying insects, vertebrates and ants, and site characteristics (canopy closure and forest distance, both scaled) and two-way interactions. In all models, site is included as random factor. df, degrees of freedom; excl. = exclusion. Significance codes: *** P < 0.001, ** P < 0.01, * P < 0.05, ° P < 0.1.*

Model parameters	χ^2	df	P
Fruit set [%]			
Flying insect excl.	51.472	1	<0.001***
Vert excl.	22.126	3	<0.001***
Ant excl.	0.854	1	0.355
Canopy closure	5.935	1	0.015*
Forest distance	0.163	1	0.687
Flying insect excl.*Ant excl.	0.233	1	0.629
Flying insect excl.*Vert excl.	4.732	3	0.192
Flying insect excl.*Canopy closure	3.657	1	0.056°
Flying insect excl.*Forest distance	0.067	1	0.795
Squirrel fruit loss [%]			
Vert excl.	24.265	3	<0.001***
Ant excl.	1.978	1	0.160
Canopy closure	0.531	1	0.466
Forest distance	0.319	1	0.572
Vert excl.*Forest distance	0.365	3	0.947
Vert excl.*Canopy closure	1.334	3	0.721
Ant excl.*Forest distance	2.336	1	0.126
Ant excl.*Canopy closure	0.558	1	0.455
Non-squirrel fruit loss [%]			
Vert excl.	3.573	3	0.311
Ant excl.	7.785	1	0.005**
Canopy closure	0.380	1	0.538
Forest distance	0.380	1	0.537
Vert excl.*Ant excl.	1.752	3	0.626
Vert excl.*Forest distance	3.225	3	0.358
Vert excl.*Canopy closure	3.813	3	0.282
Ant excl.*Forest distance	2.157	1	0.142
Ant excl.*Canopy closure	0.191	1	0.662
Yield [kg/ha]			
Vert excl.	12.192	3	0.007**
Ant excl.	0.144	1	0.704
Canopy closure	0.407	1	0.524
Forest distance	0.002	1	0.962
Vert excl.*Ant excl.	6.486	3	0.090°
Ant excl.*Canopy closure	3.086	1	0.079°
Ant excl.*Forest distance	16.854	1	<0.001***
Vert excl.*Canopy closure	2.493	3	0.477
Vert excl.*Forest distance	3.470	3	0.325

III.4 Discussion

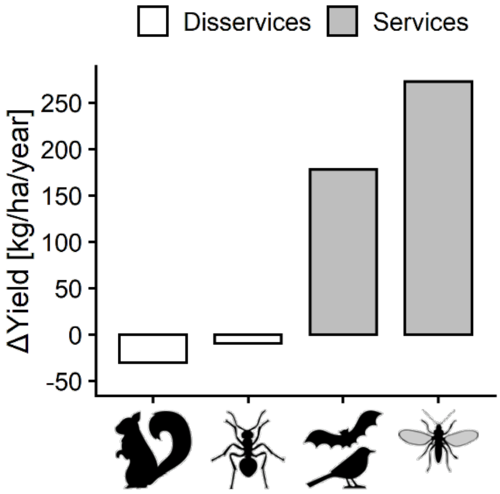


Figure III.5: Summary of changes in yield [kg/ha/year] associated with the presence of squirrels, ants, birds and bats and flying insects. For clarity, services interacting with local and landscape factors, i.e., forest distance and canopy closure have been left out of the figure. Ecosystem services (positive yield change) are grey, disservices (negative yield change) white. For detailed calculations, see supplementary methods; for standard errors, see Figures III.2-4.

Understanding interactions and trade-offs between ecosystem services and disservices of animals is crucial for establishing biodiversity-friendly and sustainable management strategies, to achieve higher-yielding cacao agroforests. Here, we provided a first quantification of the complex interactions between services and disservices in cacao agroforestry. Through our full-factorial experiment, including the year-round assessment of fruit set, fruit loss and yield, we identified insects and vertebrates' impact on cacao productivity. Fruit set increased with flying insects as well as birds and bats occurrence. Yield increased with bird and bat occurrence. With ant occurrence, yield increased too, but only in agroforests close to forest. Yet, ants also caused minor fruit loss (annually: - 9.2 kg/ha), though fruit loss due to squirrels was of larger importance (annually: -30.1 kg/ha, Figure III.5). Yield gains due to birds and bats and flying insects were larger: 177.6 kg/ha and 272.8 kg/ha, respectively. Our simultaneous assessment of services and support the design of local and landscape-scale sustainable management strategies that maintain functional biodiversity and maximize benefits for smallholder farming.

III.4.1 Flying insect services: fruit set increase

Mean fruit set dropped from 1.7% to 0.3% under flying insect exclusion, underpinning the importance of flying insects as pollinators of cacao that ensure fruit set and yield (Bos, Veddeler, et al., 2007; Groeneveld et al., 2010). Therefore, farm management in favour of flying arthropods can likely enhance yield, despite current knowledge gaps about the precise identity of cacao’s pollinators (Vansynghel et al., 2022). Here, pollination services were better supported by intermediate than high shade cover. This observation is concordant with previous evidence of high yield values in cacao with intermediate shading (Blaser et al., 2018; Gras et al., 2016) while conserving biodiversity (Clough et al., 2011). It is also in line with previously evidenced correlations of shade tree density and abundances with flying insects that are pollinator candidates (Toledo-Hernández et al., 2021). It is thus likely that shade management can contribute to creating microclimatic conditions that favour flying insect visitors (Young, 1982), and can serve as a tool to enhance cacao yields.

III.4.2 Bird and bat services: fruit set increase

Flying vertebrate access enhanced fruit set. However, in the absence of data on arthropod abundances, we can only speculate about the underlying processes. A direct effect through birds and bats pollinating the crop seems unlikely: these vertebrates are much larger than the tiny cacao flowers (1-2 cm intersection). Indirect effects such as increased pollination and/or reduced herbivory (Maas et al., 2016; Schmitt et al., 2021), are more likely to explain our observations. The absence of birds and bats may have resulted in an increased density of mesopredators, which may have reduced the abundance of cacao pollinators. Indeed, exclusion of flying vertebrates has been linked to higher abundances of spiders and ants (Cassano et al., 2016), which in turn may prey on cacao pollinators, causing lower fruit set rates. Further, access of birds and bats to cacao trees is expected to negatively impact the densities of aphids and other herbivores (Maas et al., 2013), preventing flower damage and potentially fruit abortion, hence increasing fruit set. However, detailed data on arthropod densities and food webs is required to test the hypotheses of potential pollination increase and/or herbivory reduction due to the joint occurrence of birds and bats.

III.4.3 Bird and bat synergistic services: yield increase

Our study showed that birds and bats have a large contribution to cacao yields: their presence increased yield by 114%. The contribution we found, is larger than reported before (Gras et al., 2016; Maas et al., 2013), maybe due to the involvement of birds and bats in fruit set rates, and presumably, also in pest control, as in other studies. Both in previous and current studies, the cacao yield increase found in the presence of both birds and bats, was higher than the single benefits provided by birds or bats alone (Maas et al., 2013). Such synergistic effects are common when different groups provide complementary ecosystem services (Garibaldi et al., 2018), also in current study. It is probable that birds and bats have complementary diets, by consuming insects with different ecological functions. For example, one group could be consuming mostly leaf-consuming insects, while the second one consumes mostly flower herbivores or potential cacao pests (Straub, Finke, & Snyder, 2008). Moreover, the differences in day and night-time activity peaks of the two taxa might guarantee no enemy-free time for potential cacao pests (Ferrante, Barone, Kiss, Bozóné-Borbáth, & Lövei, 2017), which might be critical for arthropods whose activity peaks change during their lifetime (e.g Lepidoptera with palatable larvae) (Heinrich, 1979). In order to safeguard and improve birds' and bats´ synergistic contributions to yield, strategies such as creating artificial nesting and roosting spaces for birds and bats could be considered (García, Miñarro, & Martínez-Sastre, 2021; Kelm, Wiesner, & Von Helversen, 2007). However, benefits of such strategies should be locally assessed because the successes vary across regions (Maas, Ocampo-Ariza, & Whelan, 2021).

III.4.4 Ant-related services and disservices

The contribution of ants to cacao fruit production is complex (Bisseleua, Begoude, Tonnang, & Vidal, 2017; Gras et al., 2016; Wielgoss, Clough, Fiala, Rumede, & Tscharntke, 2012), probably because their contributions depend on species identity and community properties (Maas et al., 2016; Wielgoss et al., 2014). On one hand, we found higher levels of fruit loss related to ant presence, but at the same time, close to forest cacao yield tended to be higher in trees to which ants had access. By forming symbioses with sap-sucking herbivores, and by propagating fungal infections, ants can provide disservices in cacao (Gras et al., 2016; Wielgoss et al., 2012). Detailed mapping of food webs in cacao agroforests would be required to unravel which of these mechanisms was causing ant-related fruit loss. Despite the ant-related fruit loss, yield benefited from ant occurrence in proximity to forest patches. Perhaps, some ant species that provide beneficial services to cacao agroforestry systems are dependent on the forest as a refuge or for reproduction. Therefore, maintaining existing forest patches in agricultural landscapes might be beneficial to enhance yield. Known ways in which ants contribute to cacao fruit development are through pest control or aiding pollination by enhancing visitation of small insect visitors of flowers (Samantha J. Forbes & Northfield, 2017; Wielgoss et al., 2014), but the functional ecology of ants largely depends on the species (Philpott & Armbrecht, 2006). Because of the varied functional ecologies of ants, identifying the role of different ant species will be crucial to confirm the positive combined effect of forest maintenance, ant presence and increased yields (Bos, Steffan-Dewenter, & Tscharntke, 2007b; Delabie et al., 2007).

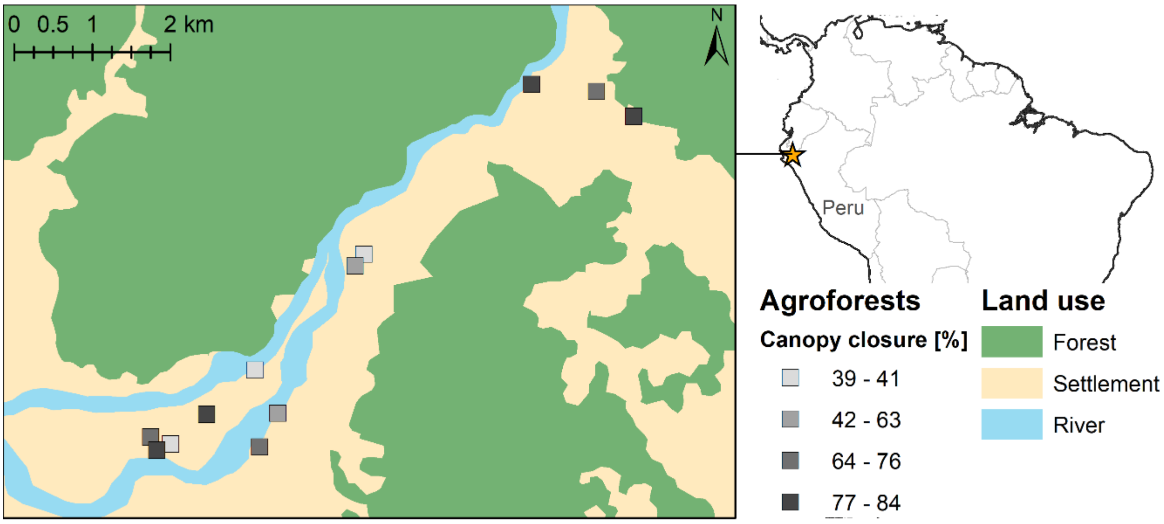
III.4.5 Squirrel-related disservices: fruit predation

We quantified an important disservice of vertebrates in cacao: fruit predation by squirrels caused an average loss of 10% of mature fruits from unmanipulated trees, totalling to 30 kg/ha annual yield loss. The lower squirrel-related fruit loss in the partial vertebrate exclusion than in the control trees which did not have cages built around them, might indicate that exclusion cages deter squirrels, even when the nets are open. An alternative explanation is that by opening of the nets during dusk and dawn, when squirrels are most active, they avoided the caged trees more than the free-standing ones. Fruit predation by squirrels and other rodents is not an isolated case. For example in Brazil (Cassano et al., 2021), other rodent species prey on cacao pods and in Ecuador, farmers hunt native squirrels because they cause fruit losses of up to 30% (Palate Mazo, 2019). Proposed control methods for squirrels are nest destruction, biocontrol by introduction of natural enemies and immunocontraception to eradicate populations (Dunn, Marzano, Forster, & Gill, 2018). Given the large harvest losses due to squirrel predation, research is needed on realistic management alternatives as to minimize squirrel disservices in cacao.

III.5 Summary and conclusions

In summary, we quantified the benefits that insects, birds and bats provide to cacao yield by improving fruit set rates and marketable yield, but we also showed that squirrels and ant species can provide important disservices by enhancing fruit loss (Figure III.5). Because the yield losses by ants and squirrels represent significant income losses for farmers (9.2 and 30.1 kg/ha annually, respectively), management should aim at minimizing these disservices. Nevertheless, the positive yield contributions by biodiversity surmount the yield losses. Yield gains due to flying insects´ could mount to 272.8 kg/ha annually, whereas birds and bats provide benefits of 177.6 kg/ha per year. Our results also show variations in contributions of ants and flying insects, due to forest distance and shade cover (not shown in Figure III.5). Based on our findings, we propose that biodiversity-friendly and sustainable management should (1) comprise intermediate levels of shade cover of around 40%, to foster populations of flying insects that are indispensable for fruit set success; (2) maintain or restore forest patches at distances of only a few hundred meters to maintain beneficial effects on marketable yields; and (3) implement management strategies that account for interactions among services and disservices.

III.6 Supplementary information



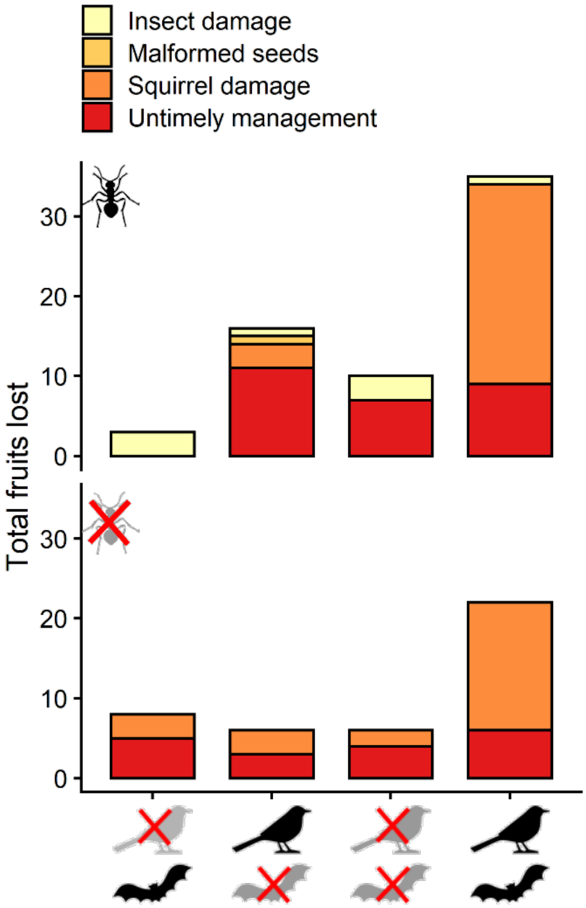
Supplementary Figure III.S1: Land-use map of the study area, showing all 12 agroforests used in the experiments, including their level of canopy closure [%] (grey-scale). Forest patches are green, the river is coloured blue, and all anthropogenic land-use was classified as settlement, are coloured beige.

Supplementary Figure III.S2: Experimental exclusion cages for vertebrates, constructed with nylon nets covering the pruned cacao trees (A). Cages to exclude flying flower visitors, tightly sealed with plant wire to ensure no ants had access, on the trees on which an ant exclusion barrier was installed (B), and the adapted version that ensured ant access by adding two small branches, to be used by crawling insects (C).

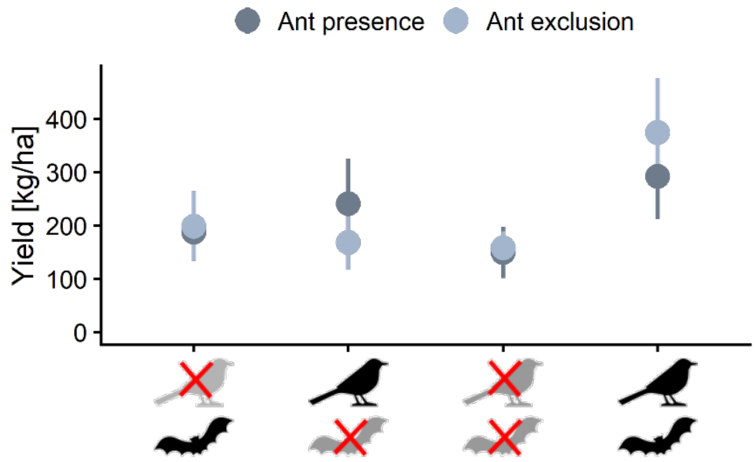
Cone-shaped ant exclusion barriers were covered with fresh glue every two weeks, to prevent the glue from getting to dry (D).

Supplementary Table III.S1: Yearly totals, means and standard errors (per tree) of flowers, young fruits, harvested and lost fruits and yield during the experiment. The raw data per tree was used for modelling purposes. Here, only naturally pollinated treatments are shown.

	Total	Mean ± SE
Young fruits [n]	3,337	35.5 ± 3.0
Harvested [n]	596	6.3 ± 0.6
Squirrel fruit loss [n]	52	0.5 ± 0.2
Non-squirrel fruit loss [n]	54	0.6 ± 0.1
Yield [kg/ha]	20,684.3	220.0 ± 23.9



Supplementary Figure III.S3: Fruit losses per category per ant exclusion treatment (upper panel: with ants, lower panel: without ants) for all vertebrate exclusions.



Supplementary Figure III.S4: Yield [kga/ha] as a function of vertebrate and ant exclusion treatments.

Supplementary Figure III.S5: Cacao pod that has been damaged by squirrels: the peel is gnawed away, and seeds are removed from the open pod. Picture taken by Lisanne Abts.

Supplementary methods

To obtain the changes in yield related to the occurrence of different animal groups, we converted fruit set and fruit loss rates to kg/ha. Yield increase due to the joint occurrence of birds and bats was measured in kg/ha already, so no data was converted. We calculated this value by subtracting the mean yield without birds and bat from the mean yield with bird and bat occurrence (values plotted in Figure III.4). We used the mean percentages, as plotted in Figure III.3A to convert squirrel-related fruit losses to yield losses. Yield change was defined as the difference between squirrel-related fruit loss with squirrel access and fruit loss under complete vertebrate exclusion (without squirrel access), multiplied by the mean yield achieved in the control treatments (without vertebrate exclusion). To convert ant-related fruit losses to yield changes, we used the mean percentages as plotted in Figure III.3B. We calculated yield change by multiplying the mean yield achieved in the control treatment (without vertebrate exclusion) with the difference between fruit loss in ants' presence and absence. We converted the fruit set percentages from open pollination (A; 1.7%) and under flying insect exclusion (B; 0.3%), to yield differences as well. We assumed that the fruit set rate from open pollination (i.e. flying insects present) gave rise to the mean yield of the control treatment, without vertebrate exclusion. We then calculated the yield loss under flying insect exclusion by multiplying the yield under natural pollination with the proportion (B/A). The change in yield related to flying insects was defined as the difference between yield of the control treatment and the yield loss under flying insect exclusion.

CHAPTER IV

CROSS-POLLINATION IMPROVES FRUIT SET AND YIELD QUALITY OF PERUVIAN NATIVE CACAO

Picture taken by Evert Thomas

CHAPTER IV CROSS-POLLINATION IMPROVES FRUIT SET AND YIELD QUALITY OF PERUVIAN NATIVE CACAO

Even though the cacao tree (*Theobroma cacao*) is a billion-dollar crop species, yields are greatly restricted by pollination limitation. Hand pollination has been proposed to counter pollination deficits, thus improving yield quantity and potentially also quality. However, low rates of self- and cross-compatibility in native cacao, may limit hand pollination benefits severely. Genetic constraints and abiotic conditions can thus limit fruit set, yet their effects have not been assessed in native cacao. To increase our understanding of fruit set limitations of native cacao, we compared manual self-pollination with cross-pollination using five native genotypes selected for their sensorial quality as pollen donors. Simultaneously, we assessed how soil water content, temperature, and relative humidity influenced fruit set and evaluated qualitative differences between manually and naturally pollinated fruits. Self-pollination success was very low (0.5%) but increased 3 to 8-fold in cross-pollination experiments, with success rates depending on the genotype of the pollen donor. Further, seed weight and the proportion of premium seeds were higher in fruits resulting from manual than from natural pollination. Additionally, there was a trade-off between flower counts and fruit set rates on manually pollinated branches, and fruit set rates were influenced by the interaction of temperature and relative humidity. Together, our findings suggest that reproductive traits of native cacao systems are constrained by both genetic compatibility, and abiotic factors. Natural cross-pollination with native pollen donors

should be promoted because quality improvements and when avoiding costs of hand pollination costs, can result in net economic gains for smallholders.

IV.1 Introduction

Global crop production is increasingly limited, owing to unreliable or absent insect and pollinator populations (Bennett et al., 2020; Gallai, Salles, Settele, & Vaissière, 2009). Hand pollination, in which pollen is supplemented to flowers by hand or with tools such as brushes, is increasingly being explored as a strategy to overcome pollination limitations and to ensure crop production (Wurz et al., 2021). Hand pollination in tropical cash crops such as passion fruit and coffee has been shown to improve fruit quantity (Bos, Veddeler, et al., 2007; Klein et al., 2008) and seed quality through control of the origin of the pollen donors (Wurz et al., 2021). For example, in partially self-compatible cash crops, manual cross-pollination improved seed content and fruit weight, whereas self-pollination resulted in more defective seeds (Stein et al., 2017). Pollen supplementation can result in increased fruit filling, i.e. a higher number of seeds per fruit, due to the more pollen being deposited when pollinating flowers manually (Falque et al., 1996).

Hand pollination has also been proposed to enhance fruit set in cacao, a tropical tree crop, as a potential solution for natural pollination limitations (Groeneveld et al., 2010; Toledo-Hernández et al., 2020). Insects, presumably midges, are needed for cross-pollination of cacao flowers (Bos, Steffan-Dewenter, et al., 2007a; Chumacero de Schawe et al., 2016; Toledo-Hernández et al., 2017). However, recent work in native cacao agroforests in Peru found low rates of insects visitation and pollen deposition, resulting in very low fruit set rates (Vansynghel et al., 2022). Fruit set rates can be increased substantially through hand pollination (69%, Toledo-Hernández et al., 2020), but yield gains depend on resource availability on the one hand, and genetic makeup and compatibility of the considered cacao genotypes on the other hand (Samantha Jay Forbes et al., 2019). Especially in native cacao cultivars and varieties, compatibility might play an important role. Cacao is native to the Amazon basin and within its native range, predominantly outcrossing, i.e. pollen of a genetically distant donor plant is needed for successful fruit set (Ford & Wilkinson, 2012; Sicard & Lenhard, 2011; Thomas et al., 2012). Up to date, most selection of native cacao has been oriented towards elite sensorial traits (ICCO, 2022), as opposed to the partially self-compatible varieties that have been introduced into Asia and Africa (Zhang & Motilal, 2016). It is therefore likely that limited self- and cross-compatibility partially explains the lower fruit set gains related to hand pollination in cacao's native distribution range (Vansynghel et al., 2022), but this remains to be experimentally tested.

Cacao fruit set is not only limited by the amount and origin of the pollen that is deposited, but also by growth conditions such as water availability, temperature regimes and reproductive traits (de Almeida & Valle, 2009). Notably, flowering density, i.e. the amount of flowers available for pollination might influence fruit set. When very few or no flowers are available, chances of successful pollination might be low. By contrast, when too many flowers are pollinated, net gains might become restrained owing to fruit abortion (Bos, Veddeler, et al., 2007). Due to the abortion of young fruits, a common phenomenon in cacao that presumably helps to balance plant resources (Valle et al., 1990), only a small fraction of pollinated flowers will eventually develop into harvestable fruits (Groeneveld et al., 2010). Native to the humid Amazon basin, the water demand of cacao is relatively high. Low soil moisture content has an adverse effect on cacao flowering, fruit set and yield (Moser et al., 2010; Schwendenmann et al., 2010), while relative air humidity effects are still largely unknown (Lahive et al., 2019). Cacao is often grown in places with high temperatures which can enhance cacao flowering density (Lahive et al., 2019), whereas low temperatures have been found to inhibit pollen germination (Aneja, Gianfagna, Ng, & Badilla, 1992). Moreover, native cacao varieties may be adapted to local microclimatic conditions, as is the case for landraces of other crops (Ficiciyan, Loos, Sievers-Glotzbach, & Tscharntke, 2018), and show different patterns related to microclimatic factors. Yet, the interactive effects of temperature and relative humidity on fruit set in native cacao have not been measured so far.

Cacao agroforestry management is highly diverse: Canopy closure and proximity to forest, respectively, vary within the landscape. These management factors can affect abiotic growth and reproduction conditions in multiple ways. The common practice of intercropping shade trees with cacao has multiple economic and ecological benefits (Blaser et al., 2018; Jezeer et al., 2017). On the one hand, shade trees provide habitat to a diversity of wildlife, including potential cacao pollinators, and on the other hand create a physiological buffer against extreme temperatures (Lin, 2007; Toledo-Hernández et al., 2021; Tscharntke et al., 2011). Furthermore, by intercepting irradiation, shade trees can increase relative air humidity in agroforests (Niether et al., 2018). Also, forest patches within the landscape mosaic can affect microclimate conditions in adjacent agricultural plots. Within a 150m distance interval from the forest edge, temperatures have been found to be lower while relative air humidity and soil humidity tend to be higher (Schmidt et al., 2017). Buffering extreme climatic conditions through shade and forest management might be of particular importance in regions where relative humidity is low, and temperatures are high.

In this study, we compared cacao productivity resulting from self- and cross-pollination and analysed potential interactions with microclimatic conditions. We considered five different genotypes of a Peruvian native cacao cultivar, selected for superior productivity and sensorial quality, as pollen donors for carrying out cross-pollinations. We also compared pod filling, seed and fruit weight and percentage of premium beans, between naturally and manually pollinated fruits. Because our study area was situated in the tropical dry forest ecozone, we expected low soil water content, elevated temperatures, and low relative humidity to be limiting cacao flowering and fruit production, either directly or indirectly. To identify management opportunities that guarantee optimal microclimatic conditions, we further assessed how local agroforestry and landscape context (canopy closure and forest distance, respectively) affect soil water content, temperature, and relative humidity.

IV.2 Methods

IV.2.1 Study system

This research was carried out in eleven organic cacao agroforests, located around the farmer community of La Quemazón, in north-western Peru (S5.31°, W79.72°, 240 m.a.s.l.). The north-western region of Peru is characterized by submontane seasonally dry tropical forests (Fremout, Thomas, & Elena Gutierrez Miranda, 2021) and a hot and semi-arid climate, with mean annual rainfall of 235 mm, mostly concentrated between December and March (SENAMHI, 2020a). During the dry months rainfall is close to zero and water availability is limited. To compensate for the resulting water shortage, agroforests are irrigated by means of gravity-fed flood canals every two to three weeks, or more depending on water availability. Agroforests in the study region mainly consisted of seed-grown cacao trees pertaining to the native cultivar called Piura white cacao (Spanish: *Cacao blanco de Piura*; Figure IV.S1), which is part of the internationally known Nacional genetic group (Arevalo-Gardini et al., 2019). Piura white cacao is classified as a high-quality “fine or flavour aroma” cultivar with white beans that is generally valued well above the global stock market price of cacao (ICCO, 2022). The local cooperative, *Cooperativa Agraria Norandino Ltda.* established a working collection or clonal garden of about 50 clones of several high-yielding and high-quality genotypes of Piura white cacao.

The cacao agroforests we considered belonged to smallholder famers, all cooperative members that manage their farm organically, and ranged between 0.3 and 1.1 ha in size. The agroforests had comparable cacao densities (3 x 3 m or 3.5 x 3.5 m planting grids) and were of similar age (5- to 10-year-old) but differed in shade cover (gradient from medium to high; 39% to 85%) and distance to the nearest forest patch (0.17 to 0.96 km). Shade cover was assessed using a Forest Suppliers® spherical densiometer with convex mirror, by averaging the readings of canopy closure [%] at 20 points spread over an area of about 0.15 ha, to obtain a mean value per agroforest. Distance to forest [km] was calculated with ArcMap 10.5.1, using a land-use map of Piura (Otivo Barreto, 2010) updated through ground-truthing (Hanf-Dressler, 2020).

IV.2.2 Data collection

On all eight experimental trees of the eleven farms, we selected a 35-cm long branch segment and between 11/10/2019 and 26/11/2019, we manually pollinated all freshly opened flowers encountered on these eight branch segments. Every other day, farms were visited, and flowers were pollinated with either self-pollen or cross-pollen. To do so, we rubbed anthers of pollen donors to stigmas of receptor flowers, and did not isolate flowers from visitors before or after supplementing pollen (Groeneveld et al., 2010; Toledo-Hernández et al., 2020; Vansynghel et al., 2022). For manual self-pollination, we used pollen from other flowers available on the same experimental trees. For cross-pollination, we used pollen from freshly opened flowers collected from five superior genotypes available in the clonal garden or working collection established by *Cooperativa Agraria Norandino Ltda.* The five genotypes included in this experiment were chosen based on the constant availability of flowers.

On each of the 8 experimental trees per farm, we pollinated all freshly opened flowers encountered on the 35-cm branch segment, every second day. On a given day, we pollinated all receptor branches with the same pollen donor, which was either self-pollen or one of the five genotypes. Upon the next repetition, two days later, another pollen donor was used, until we had repeated the whole series four times. Therefore the branch sections of experimental trees were pollinated a total of 24 times: four times with self-pollen and four times with each of the five genotypes (hereafter referred to as GT1 – GT5). Pollinated flowers were carefully labelled to track the pollen donor and monitor fruit set posteriorly. Six days after hand pollination, we assessed fruit set of the labelled flowers. Fruit set was defined as the number of small fruits, divided by the number of pollinated flowers six days earlier. Five to six months later, we harvested all marked mature fruits of the hand pollination treatment but could not retrieve the pollen donor anymore upon harvesting, as the information from the labels was not noted by harvesting farmers. To compare fruit quality traits of manually pollinated fruits, with fruits resulting from natural pollination, we harvested mature fruits from a second 35-cm branch section of the same 96 experimental trees. For both naturally and manually pollinated fruits, we sun-dried the beans per fruit, and weighted them using an electric pocket scale with 0.01 g accuracy to obtain fruit weight values [g]. We also counted the number of seeds per fruit (pod filling) and assessed how many seeds were white, to obtain a proportion of premium beans [%]. The total weight of all beans per pod was divided by the number of beans per pod to obtain average seed weight values [g].

To assess relative air humidity [%] and temperature [°C], we placed iButton hygrochron DS1923 dataloggers in one experimental tree in each farm (iButton, https://i-button.co.uk). Every hour, temperature and relative humidity were measured. We extracted daily maximums of air humidity and temperature, because mean and minimum values were too correlated with other variables. Mean daily soil water content of all experimental trees was obtained with a Delta-T SM150T soil moisture sensor. We averaged four readings, one in each of the wind directions, on the same day of pollination at a 1.5-m circular distance around the cacao tree base, and transformed these from Volts to volumetric percentage with the formula for organic soils ("User Manual for the SM150T Soil Moisture Sensor," 2016). Because of lower correlation values with other variables, instead of using maximum or minimum values, we averaged the values per tree and day, to obtain a mean volumetric soil water content [vol%] per tree.

IV.2.3 Statistical analysis

All analyses were performed with R v. 4.1.2 (R Core Team, 2020) in RStudio 2021.09.1+372 for Windows (RStudio Team, 2021). Plots were assembled with the packages “cowplot” and “ggplot2” (Wickham, 2016; Wilke, 2020). Generalized linear mixed effect models were constructed with the package “glmmTMB” (Brooks et al., 2017) unless indicated otherwise. Continuous predictor variables were scaled in all models, i.e., population mean subtracted and divided by standard error. Root Mean Square Error or RMSE and Sigma were the performance indicators used for all models, extracted with “performance” package (Lüdecke et al., 2021). Model fits were inspected using the same package. No strong violations were detected.

First, we explored the effect of the genotype of the pollen donor and abiotic factors on cacao pollination success, fruit set rate and flowering density. We conducted a two-part hurdle analysis in which the response is split into a count variable and a binary variable, and two models with different distribution are run (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Partitioning the data allows to model the non-zero counts separately from the binary, zero-generating process. Moreover, the effects of predictors can be tested separately for the zero-generating process and the count process (Zuur et al., 2009), which allows for more realistic estimations (Radzevičiūtė, Theodorou, Schlegel, & Paxton, 2021). Factors that determine whether a flower is pollinated at all, could be of less importance to the count process, i.e. how many of the pollinated flowers on one branch will set fruit (Radzevičiūtė et al., 2021). Therefore, our original response variable, the number of successes divided by pollinated flowers, was split into a binary variable, including ones and zeroes, and a count variable from which the zeroes were left out. The binary variable (pollination success), in which successful fruit set cases were converted to ones and the failures to zero, was modelled with a binomial distribution to assess the likelihood of successful pollination. The random effect variable was trees nested within agroforests, fixed effect variables were pollen donor genotype (self-pollination and cross-pollination with GT1- 5) , soil water content, temperature, and relative humidity. Two-way interactions between abiotic variables were included, as well as their quadratic terms. Fruit set rate, the count variable, was analysed with a binomial distribution with a probit link function. The random effect variable included was trees nested within agroforests; weights were counts of pollinated flowers. Fixed effect variables were counts of pollinated flowers and two-way interactions between abiotic variables, as well as their main first and second order effects, and counts of pollinated flowers.

Second, we assessed how management and landscape affected abiotic factors, with three models including abiotic factors as response variable and canopy closure [%] and forest distance [km] as fixed effect variables. In the models with temperature and humidity as response variable, a gaussian distribution was used. Because data was collected at the farm level, agroforest identity and the measuring date were included as random effect variables. The model with soil water content [vol%] as response variable included both measuring date and tree identity nested within farm as random effect variables, since water content was measured at the tree level.

Lastly, to compare quality of harvested cacao fruits between natural and manual pollination, we constructed four simple models with quality indicators as response variable, and hand pollination versus natural pollination as the only predictor. Fruits of all pollen donor genotypes, and self-pollination were pooled, because the genotype could not be distinguished during the harvesting of pods. Pod filling, i.e. the number of seeds per pod, was analysed using a generalized linear model with a negative binomial distribution, implemented in "MASS" package (Ripley et al., 2018). Seed and fruit weight were fitted with a simple linear model with gaussian distribution, and proportion premium beans with a generalized linear model with binomial distribution, including total number of beans as weights.

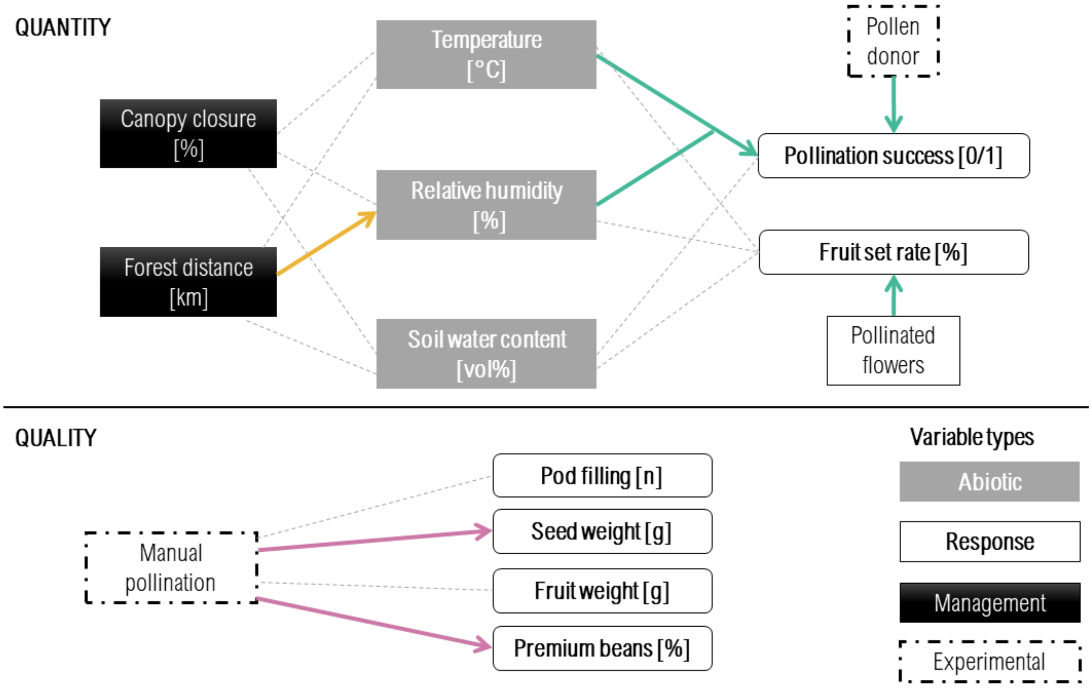


Figure IV.1: Overview of tested (grey dashed lines) and significant (arrows colored according to Figure IV.2-4; full line) associations between variables, to investigate quantity and quality traits of cacao fruits after manual pollen supplementation. Arrows that join indicate interactive effects. Genotype of the pollen donor and manual pollination were the experimentally manipulated variables (boxes; dot dashed line), whose effect on several response variables (rounded boxes; full line) was tested in various models, including abiotic variables (grey boxes) and management variables (black boxes).

IV.3 Results

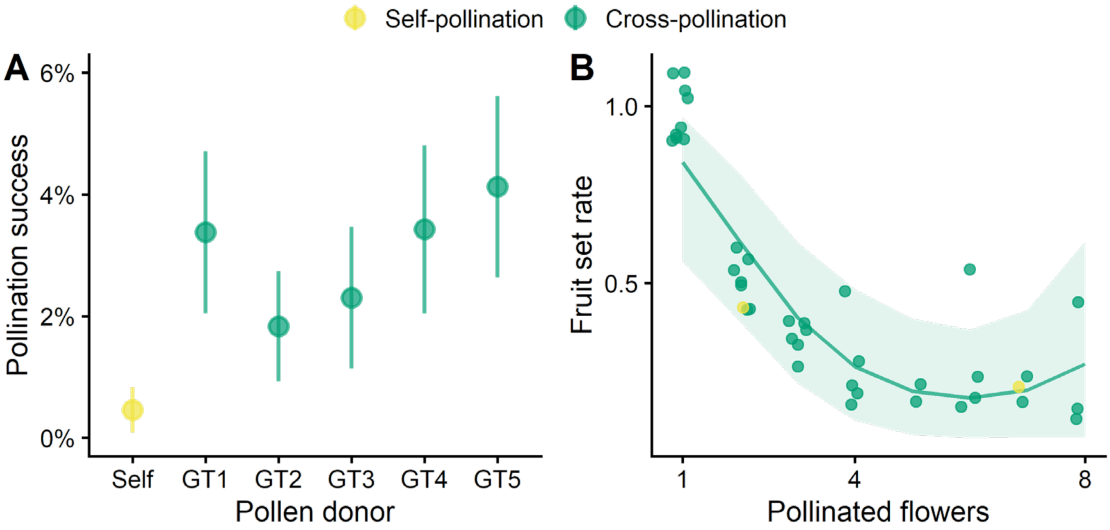


Figure IV.2: A) Mean ± SE hand pollination success (fruit set/pollinated flowers) of manually pollinated flowers (cross-pollination; green circles) with different genotypes (GT1-5) of the Piura white cacao cultivar as pollen donors or pollen from the receptor tree itself (self-pollination; yellow circles). B) Predictions of the association of counts of pollinated flowers on branch sections with the zero-truncated count variable fruit set rate. Yellow dots are fruit set rates of self-pollinated flowers, green dots of cross-pollinated flowers.

Out of 1761 hand-pollinated flowers included in the pollination success analyses, 45 set fruit which corresponds to 2.6% of the total. Manual self-pollination resulted in the lowest average success rate (0.5 ± 0.4%, Figure IV.2A). Cross-pollination with some but not all genotypes, resulted in higher pollination successes than self-pollination (Table IV.S1, Figure IV.2A). Cross-pollination success rates ranged from 1.8 ± 0.9% (pollen donor GT2; not statistically different from self-pollination success) to 4.1 ± 1.5% (pollen donor GT5; Figure IV.2A). Pollination success also improved at higher air temperatures, but only when relative humidity remained under 90% (Table IV.S1). When pollination was successful, the number of fruits set were negatively associated with the number of pollinated flowers (Table IV.S1, Figure IV.2B), but not with any of the other abiotic variables considered.

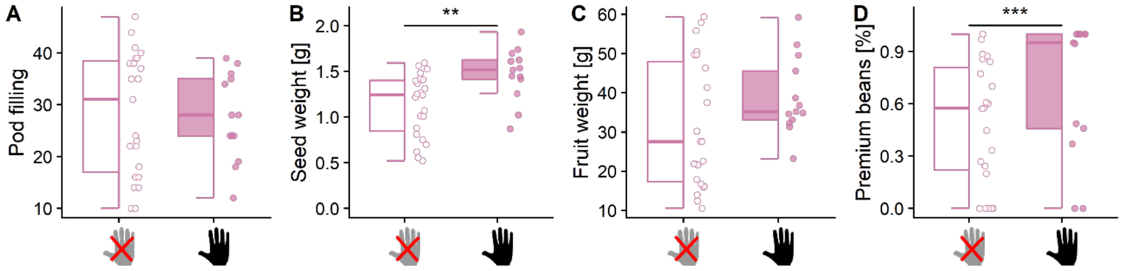


*Figure IV.3: Comparison of quality parameters between naturally (white) and manually pollinated fruits (pink): pod filling (A), seed weight (B), fruit weight (C) and premium bean content (D). Significance codes: *** $p < 0.001$, ** $p < 0.01$. For statistics, see Table IV.S3.*

Of all 45 flowers that set fruit in the manual pollination treatment, 22 fruits were lost due to pest infestation, fungal infections, untimely management, or fruit abortion. Of the 13 fruits that reached maturity and were harvested, seed weight (Figure IV.3B) and proportion of premium beans (Figure IV.3D) of manually pollinated fruits were higher than in the natural pollination treatment (Table IV.S2). No such differences were detected in pod filling nor fruit weight (Figure IV.3A and IV. 3C; Table IV.S2).

Contrary to expectations, temperature did not decrease with canopy closure increasing from 0.39 to 0.85 (Figure IV.4A, Table IV.S3). Relative humidity decreased, from 94% to 88%, across a gradient of 0.17-1.20 km away from forest (Figure IV.4B, Table IV.S3). Soil water content remained unaffected by either forest distance, or canopy closure.

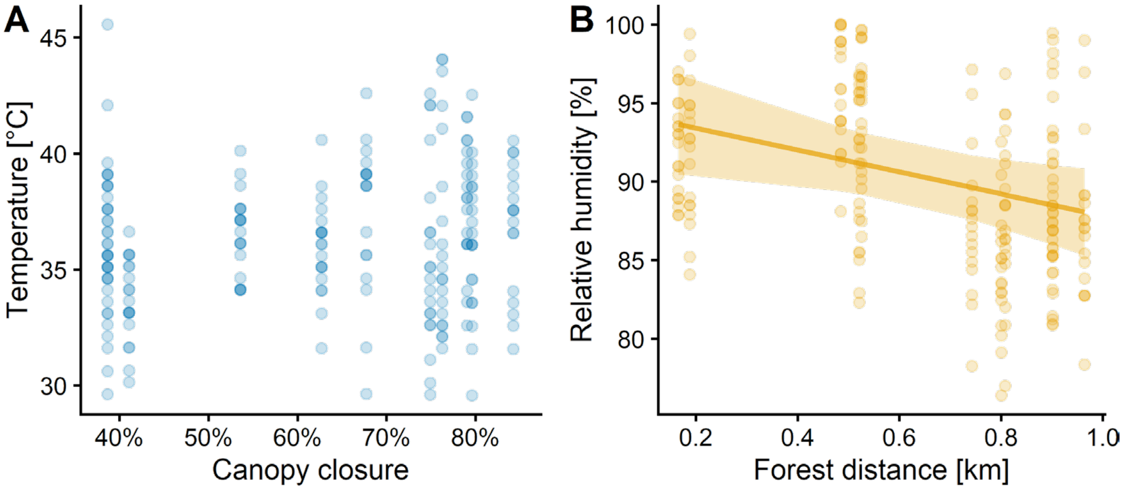


Figure IV.4: Associations between local farm management (canopy closure), landscape context (forest distance) and abiotic variables (temperature and relative humidity). For statistics, see Table IV.S2.

IV.4 Discussion

In this study, we examined productivity differences in native cacao through hand pollination using different genotypes as pollen donors, in interaction with both abiotic factors and management variables. We showed (i) higher fruit set when manually supplementing cross-pollen to cacao flowers, compared to self-pollination, with fruit set gains strongly depending on the genotype of the pollen donor and (ii) larger seeds of higher quality in fruits resulting from manual pollen supplementation. We also evidenced that (iii) abiotic factors such as relative humidity and temperature play a regulating role in reproductive processes in cacao, and that (iv) in agroforests closer to forest, average relative humidity is higher.

The three- to eightfold increase in pollination success due to manually supplementing cross-pollen, suggests this could be a valuable tool to reduce fruit set limitations of native cacao. However, pollination success also depended strongly on the genotype of the pollen donor. Interestingly, patterns remained consistent among 96 receptor trees that differed in genetic background. This emphasizes the importance of selecting suitable pollen donors, regardless of pollen receptor, to ensure that investments in hand pollination return maximal fruit set gains.

Hand pollination also improved some aspects of fruit quality: Seed weight and proportion of premium seeds were positively impacted by hand pollination, which is of economic relevance for smallholders' incomes. We used a native fine flavour cacao cultivar that is overall being sold at higher market prices than bulk cacao (ICCO, 2022). High proportions of white, premium beans are particularly sought after and are the subject of exclusivity contracts with chocolate makers who pay up to the double of stock market prices. As such, the improved bean quality and enhanced seed weight might be translated in even larger farmer benefits. We did not find more beans per fruit due to manual pollen supplementation, opposing to what has been previously reported (Falque et al., 1996), potentially indicating that these traits depend on the pollen receptor. Pollen donor and receptor should thus be matched both in terms of compatibility properties and desired quality traits to make sure investments in hand pollination, are returned.

Fruit set after hand pollination was also influenced by the number of pollinated flowers, and by abiotic factors, such as temperature and relative humidity. The cacao tree naturally regulates the number of fruits that simultaneously develop, by abortion of fruits less than three months old, presumably due to resource allocation (Valle et al., 1990). It is thus expected that not all hand-pollinated flowers can develop at the same time. We also observed abortion of set fruits in our study: only 45 out of 1761 pollinated flowers set fruit, and of those 45, only 13 developed in mature fruits. Other hand pollination studies also found that large proportions of the pollinated fruits did not develop into harvestable fruits (Bos, Veddeler, et al., 2007; Toledo-Hernández et al., 2020).

The interactive effects of relative air humidity and temperature on fruit set indicate that abiotic effects also play a role in limiting native cacao yields. Higher temperature improved fruit set rates only when relative humidity was below 90%, which could indicate a that fruit set of the native cacao cultivar we studied might be limited by a combination of high relative air humidity and high temperature. We did not identify any short-term effect of measured soil water content on fruit set, corroborating previous findings (Groeneveld et al., 2010). Though this was beyond the scope of the study, it would be interesting to understand the long-term effects of soil water content on fruit set or even abortion rates. Especially in the face of climate change, it could be of particular importance to identify temperature and drought tolerant cacao (Ceccarelli et al., 2021).

Because landscape management affected abiotic effects, forest conservation and restoration could be a tool to improve abiotic conditions for cacao fruit set. We found that forest distance affected relative air humidity on a larger scale than has been previously reviewed (Schmidt et al., 2017). However, it is possible that the dry tropical forest in our study region has specific dynamics, that go beyond the patterns reviewed by Schmidt and colleagues. Potentially, drought adaptation mechanisms of plants could favour water retention and avoid water loss through evaporation. Contrary to expectations, we did not find that increased canopy closure increased humidity or lowered temperatures. The lack of observed effects of canopy closure on temperature, does not mean shade cover is not of importance for cacao pollination: Intermediate shade cover of about 40% has been found to promote flower visitation by certain insects (Toledo-Hernández et al., 2021), pollination success (Vansynghel et al., in preparation) and cacao yields (Blaser et al., 2018).

Overall, before adopting hand pollination as a supplementation or replacement of natural pollination, economic and genetic aspects should be considered. Especially in native cacao varieties that might be limited by low compatibility levels, pilot compatibility trials should be conducted. Even though managing the pollen source can provide considerable economic benefits, hand pollination is related to high costs, also in cacao (Toledo-Hernández et al., 2020). Therefore, combining cross-compatible genotypes in grid-like field arrangements (e.g. López et al., 2021; N'Zi et al., 2017) that facilitate natural cross-pollination, might be a more cost-effective measure than manual pollination. Provided that abiotic conditions such as temperature and relative air humidity are not limiting fruit set, the enhanced quality and improved fruit set of naturally cross-pollinated native cacao could eventually translate in higher benefits for farmers.

IV.5 Supplementary Information

Figure IV.S1: Cacao pod of the Peruvian native cultivar Piura white cacao, which is named after its premium quality white seeds. The cultivar is famous for its high-quality seeds and fine flavour properties and can be sold at higher prices than bulk cacao. Picture taken by Evert Thomas.

Table IV.S1: Generalized linear mixed effects models relating model parameters to flowering density, pollination success and fruit set rate. Parameters include maximum daily temperature (Tem), Hum = maximum daily relative humidity (Hum), mean soil water content per tree (Wat), pollen donor genotypes 1-5 compared with self-pollination (PollenGT1-5) and counts of pollinated flowers. Quadraatic terms are indicated with (^{2}). Given are Odds Ratios (OR), Risk Ratios (RR), and Confidence Intervals (CI), P values (p, RMSE = Root Mean Square Error and sigma.

	Pollination success [1/0]			Fruit set ratio [%]		
	OR	CI	p	RR	CI	p
(Intercept)	0.01	0.00 – 0.05	**<0.001*****	0.93	0.67 – 1.28	0.647
PollenGT1	5.29	0.99 – 28.26	**0.051°**			
PollenGT2	2.75	0.45 – 16.78	0.273			
PollenGT3	4.64	0.77 – 27.78	0.093°			
PollenGT4	6.59	1.11 – 39.16	**0.038***			
PollenGT5	9.73	1.75 – 54.12	**0.009****			
Wat	0.67	0.22 – 2.04	0.476	0.86	0.28 – 2.66	0.794
Tem	0.04	0.00 – 16.83	0.290	1.38	0.01 – 218.01	0.900
Hum	161.2	0.00 – $1.41x10^6$	0.467	0.9	0.00 – 19718.94	0.983
Wat^2	1.78	0.66 – 4.82	0.259	1.07	0.34 – 3.38	0.910
Tem^2	38.39	0.09 – 16456.20	0.238	0.71	0.00 – 122.57	0.896
Hum^2	0.01	0.00 – 5716.10	0.472	1.03	0.00 – 24320.89	0.995
*Wat * Tem*	0.94	0.65 – 1.35	0.725	0.98	0.70 – 1.38	0.928
*Wat * Hum*	1.03	0.72 – 1.46	0.873	1.01	0.74 – 1.39	0.930
*Tem * Hum*	0.63	0.39 – 1.00	**0.052°**	1.11	0.75 – 1.63	0.613
Flowers				0.12	0.03 – 0.53	**0.005****
Flowers^2				4.71	1.16 – 19.17	**0.030***
RMSE	0.221			0.114		
Sigma	1			1		

Table IV.S2: Model summaries of the effect of hand pollination respective to natural pollination (predictor variable) on pod filling (negative binomial), seed and fruit weight (gaussian) and light beans (binomial with probit link function) as response variables. Given are estimates (Est.), Incidence Rate Ratios (IRR), Risk Ratios (RR), Confidence Intervals (CI), P values (p) and sigma and Root Mean Square Error (RMSE) as error estimates.

Pod filling [n]				Seed weight [g]			
	IRR	*CI*	*p*		*Est.*	*CI*	*p*
(Intercept)	28.39	24.22 – 33.43	**<0.001*****	*(Intercept)*	1.13	0.99 – 1.27	**<0.001*****
Hand poll.	0.97	0.74 – 1.27	0.840	*Hand poll.*	0.34	0.11 – 0.57	**0.004****
RMSE	10.476			*RMSE*	0.314		
Sigma	1.045			*Sigma*	0.323		
Fruit weight [g]				**Premium beans [%]**			
	Est.	*CI*	*p*		*RR*	*CI*	*p*
(Intercept)	31.49	25.41 – 37.57	**<0.001*****	*(Intercept)*	0.96	0.87 – 1.05	0.350
Hand poll.	7.46	-2.65 – 17.58	0.143	*Hand poll.*	1.45	1.24 – 1.70	**<0.001*****
RMSE	13.945			*RMSE*	0.357		
Sigma	14.349			*Sigma*	4.524		

Table IV.S3: Summaries of associations between local management (canopy closure) and landscape (forest distance) on farm microclimate (maximum temperature and humidity) and soil properties around the trees (mean soil water content). Given are estimates (Est.), Confidence Intervals (CI) and P values (p) and sigma and Root Mean Square Error (RMSE).

	Temperature [°C]		
	Est.	CI	p
(Intercept)	36.2	35.35 – 37.04	**<0.001*****
Canopy closure	0.46	-0.06 – 0.98	0.085°
Forest distance	0.21	-0.39 – 0.80	0.495
RMSE	1.846		
Sigma	2.05		
	Relative humidity [%]		
	Est.	CI	p
(Intercept)	0.9	0.88 – 0.92	**<0.001*****
Canopy closure	0	-0.01 – 0.02	0.804
Forest distance	-0.02	-0.03 – -0.00	**0.017***
RMSE	0.017		
Sigma	0.019		
	Soil water content [vol%]		
	Est.	CI	p
(Intercept)	0.32	0.28 – 0.35	**<0.001*****
Canopy closure	0.02	-0.01 – 0.04	0.262
Forest distance	-0.01	-0.04 – 0.02	0.646
RMSE	0.071		
Sigma	0.076		

CHAPTER V

DISCUSSION

CHAPTER V DISCUSSION

The extent of land under agricultural production has been increasing due to both population and consumption growth, at the expense of biodiversity conservation. In agroforestry and other types of wildlife-friendly farming strategies, high biodiversity levels can be combined with high yields through improved ecosystem service provision. In cacao, optimized provision of pollination and pest control services has the potential to improve yields, but patterns have not been quantified in the crops native range. Because of the importance of the crop for smallholders' livelihoods, we aimed to fill this knowledge gap by studying animal-provided ecosystem services.

We found that the dominant visitors of flowers were herbivores and not midges, the main pollinator candidates, and that natural pollen deposition was low. We also demonstrated that management interventions such as manual pollen supplementation can affect the first stage of fruit formation and depending on the genotype of the pollen donor, can increase fruit set three- to eightfold. The interaction between temperature and relative humidity was of importance for fruit set as well. Flying insects, intermediate shade cover and birds and bats provided a large benefit to annual cacao production by increasing fruit set. Weeks after initial fruit set, ants and squirrels generated fruit loss, but the disadvantages due to these groups did not outweigh the benefits provided by other animals in cacao agroforests.

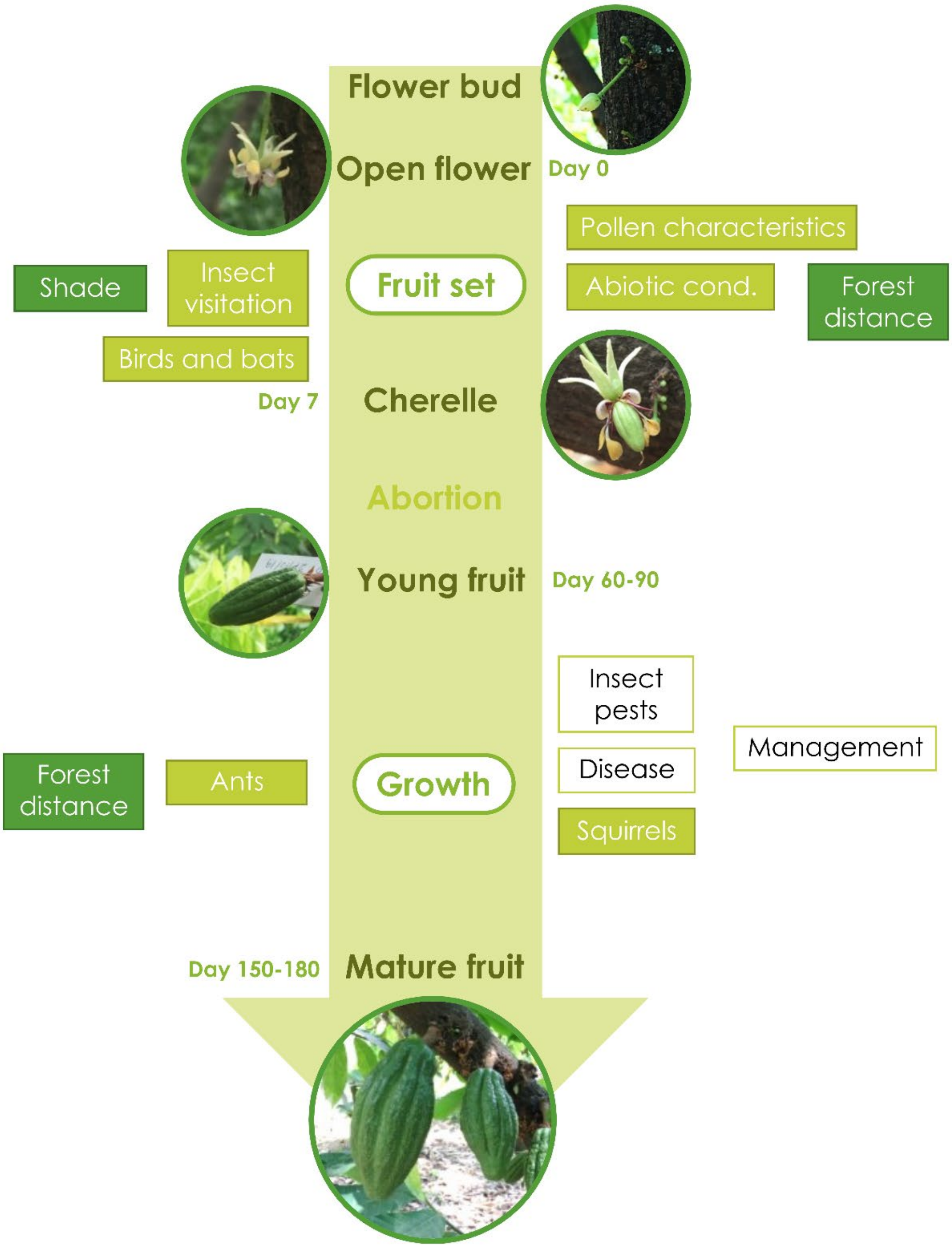


Figure V.1: Schematic summary of the factors that affect cacao yield (light green boxes), along the developmental timeline from flower to fruit (green arrow). Pollen characteristics, insect visitation, birds and bats and abiotic conditions (cond.) can affect the early fruit set process, whereas ants and squirrels alter yields in a later stage, during growth. Forest distance and shade (dark green boxes) indirectly affected fruit set and growth, by regulating insect visitation, abiotic conditions (cond.), and ant communities. Insect pests, diseases, and timely management (green outlined boxes) also affect the growth process, but these were not the main topic of study. Abbreviations: cond. = conditions.

V.1 Factors affecting cacao yield at different developmental stages

V.1.1 The first prerequisite of fruit set: **flowering**

Flower buds form continuously on the flower cushions, and although activity peaks before and during the wet season have been observed, new buds can form and open throughout the year (Claus et al., 2018). Flower buds usually open early in the morning and remain receptive to flower visitation and pollen deposition for about 36 to 48 hours (Toledo-Hernández et al., 2017). Because flowering is a first requirement for flower visitation by insects, pollen deposition, and subsequent fruit set, flower availability could be another factor limiting fruit set (day 0 to day 2, Figure V.1). In Chapter IV, we saw that pollinating more flowers on a branch does not necessarily result in a higher total number of fruits, at least not when flowers are manually pollinated on the same day. The branch-level trade-off between the number of pollinated flowers and fruit set might have implications for maximal fruit set rates.

Moreover, in Chapter II, the naturally pollinated branches we found more flowers than in the manually pollinated branches, where fruit set was generally higher. This suggests that the cacao tree invests less energy in flower production when fruit set is higher. This energy-saving phenomenon has been well documented, though it is the first time that trade-offs are studied at the branch level (Groeneveld et al., 2010; Toledo-Hernández et al., 2020; Valle et al., 1990). Because of the non-linear return of investments, it is important to take branch-level trade-offs between flowering and fruit set into account when designing hand-pollination strategies.

V.1.2 Flower visitation and pollen deposition

During their two-day lifespan (day 0 to day 2, Figure V.1), cacao flowers get visited by insects from diverse taxonomical groups, which transport and deposit pollen on the reproductive parts of the flowers (Chumacero de Schawe et al., 2016). Such insect-mediated pollen transfer from a genetically different pollen donor is essential for cacao, as the crop is predominantly outcrossing (Chumacero de Schawe, Durka, Tscharntke, Hensen, & Kessler, 2013). Although dipteran midges are frequently shortlisted candidates, dominant visitors differ among regions of the world (Toledo-Hernández et al., 2017). In Chapter II, it was discussed that herbivores such as thrips and aphids are the most abundant flower visitors of native cacao in Peru, but also that it remains unknown which insects are the main cacao pollinators. Due to the lack of evidence of pollen transfer and deposition, we could only speculate about the functional role of flower visiting insect. It is important to identify the main pollinators, as they are indispensable for transfer of cross-pollen and fruit set.

The urgency to identify cacao´s main pollinators is underlined in Chapter III, in which we demonstrated a fruit set rate increase due to flying insects. The contribution of flying insects was even higher in farms with intermediate shade cover. Thus, flying insects make an important contribution to fruit set, and their conservation is crucial to underpin cacao productivity. Maintaining an intermediate shade cover could be an appropriate strategy to conserve pollination services provided by flying insects. Without identifying the pollinating agents, however, it is difficult to design more specific management strategies. As long as it remains unclear which insects are pollinating the crop, conservation measures that address flying insects in general are needed, which might lack efficiency. Therefore, new techniques, for example macro-photography as described here, should be applied to study the different pollen loads that get deposited by different insects. Knowledge on which insects transfer how much pollen, and from which pollen donor to receptor cacao flowers, would increase our overall understanding of cacao pollination tremendously. This improved understanding could help to target conservation measures directly at cacao´s effective pollinators.

V.1.3 Pollen characteristics affect the **early fruit set process**

Several characteristics of the pollen that is deposited on flowers will determine the likelihood of pollination success. Pollination failures can be due to many reasons; they are particularly worth identifying when pollination success is remarkably low (Li, Tscharntke, Saintes, Buchori, & Grass, 2019; Wilcock & Neiland, 2002). This is the case in native cacao from northern Peru: Fewer than 1% of the flowers set fruit, which is contrasting to the success rates up until 10% that are reported from other parts of the world (Chapter II). Our findings of an extremely low pollination success rate highlight the importance of a better understanding of the interplay of factors that explain pollination failures.

Higher pollen loads improve cacao fruit set and also pod filling (Falque et al., 1996, 1995), and more than 115 pollen grains should be deposited for higher odds of pollination success (Falque et al., 1995). A pollen quantity threshold alone does not explain unsuccessful fruit set despite high pollen deposition. Pollen viability for example can affect germination of pollen grains and subsequent fruit set. Deposition of pollen older than 6 to 24 hours, will likely result in unsuccessful fruit set (García Talledo, Bazurto Zambrano, García Cruzatty, & Zambrano Gavilanes, 2019). Pollen viability can also be affected by abiotic factors such as drought and temperature (Mena-Montoya et al., 2020), but it remains to be confirmed whether these factors also limit fruit set in the field.

Based on the patterns discussed in Chapter IV, it is more likely that genetic incompatibility plays a crucial role in the determination of pollination success. Cross-pollinated flowers set fruit three to eight times more often than self-pollinated flowers, with success rates depending on the genotype of the pollen donor. Cross-compatibility likely explains a large proportion of the pollination failures that were observed in Chapter II. Long-term breeding programmes may have eliminated compatibility limitations in clonal cacao, as opposed to native cacao that is rather selected for its sensorial traits. It is thus possible that compatibility is a stronger limitation of fruit set rates of native cacao and for this reason, it is of utmost importance to conduct compatibility trials to obtain optimized fruit set rates.

V.1.4 Vertebrates' indirect contribution to **fruit set**

Even though the crop is pollinated by insects, vertebrates can also have a positive impact on cacao yield (Gras et al., 2016). In Chapter III, we showed that the benefits of birds and bats were reflected in a yield increase of 114%, which is higher than bird and bat benefits quantified in other parts of the world (Maas et al., 2013). Unexpectedly, we also documented a fruit set rate increase due to the presence of birds and bats, but since they are considerably larger than cacao flowers, their direct contribution is very unlikely.

Birds and bats known benefits to cacao yield are through pest control (Maas et al., 2013), in which the flying vertebrates either supress pest population directly, or through cascading effects on intermediate predatory insects. In Chapter III, it was shown that the benefits of birds and bats were reflected in a yield increase of 114%, which is higher than what has been quantified in other parts of the world (Maas et al., 2013). Surprisingly, a fruit set rate increase due to presence of birds and bats was also documented, but since birds and bats are considerably larger than cacao flowers, it is very unlikely that flying vertebrates pollinated cacao flowers.

A more probable explanation for the increase in fruit set rates is that flying vertebrates controlled the abundance or activity of other functional insect groups, through indirect cascading effects (Cassano et al., 2016). Potentially, flying vertebrates reduced mesopredator abundance or activity such as that of spiders and ants (Maas et al., 2013). In turn, reduced mesopredator abundances could have had negative effects on herbivores, or even positive effects on pollinators. However, birds and bats´ access to cacao trees could also have had a direct effect, reducing the abundance or activity of flower herbivores, such as aphids, or of pollinating insects by preying on them. Arthropod data would be needed to reveal direct and indirect ecological interactions in exclusion experiments. Even though the mechanisms through which these flying vertebrates improved cacao fruit set could not be unravelled here, these animals clearly contribute positively to native cacao yield in Peru. For optimal fruit set and yield benefits in cacao agroforests, their contributions should be safeguarded by appropriate conservation measures.

V.1.5 Abiotic interactions affect **fruit set**

Fruit set is also affected by abiotic variables such as relative air humidity, temperature and soil water content (Lahive et al., 2019). Management characteristics such as shade tree maintenance and irrigation could regulate cacao yields, through their impact on abiotic variables (Carr & Lockwood, 2011; Lahive et al., 2019). Opposed to our expectations of the dry climate in northern Peru to be limiting cacao yields (Carr & Lockwood, 2011; SENAMHI, 2020a), no direct effect of soil water content on fruit set was detected. Irrigation might have played a role in overcoming any potential drought effects, or drought could have affected yield in the long term, as in other studies (Moser et al., 2010; Schwendenmann et al., 2010), but neither hypothesis was tested explicitly in this thesis. However, an interaction between relative air humidity and temperature on fruit set was apparent from the models described in Chapter IV. Higher temperature improved fruit setting rates, but only when humidity was below 90%. Fruit set of the native cacao cultivar we considered here, may be limited by a combination of high relative air humidity and high temperature, which could have implications for climate change resilience of native cacao agroforestry systems.

V.1.6 After fruit set: **physiological abortion**

Seven days after the opening of a cacao flower (day 0-7, Figure V.1), incompatible pollinations will have manifested (Ford & Wilkinson, 2012) and the flowers that got successfully pollinated will continue to develop (Toledo-Hernández et al., 2017). After the formation of cherelles, and before harvesting the mature fruits, the fruit will continue to grow and ripen on the tree for another 5 to 6 months (de Almeida & Valle, 2009). During these months, a large proportion of the formed cherelles and fruits will not reach maturity, for example due to abortion of immature fruits for example (Bos, Steffan-Dewenter, et al., 2007a).

Abortion of cherelles has been well documented in cacao: 58% of pollinated fruits do not reach maturity within the first three months after fertilization (Bos, Veddeler, et al., 2007). Even though we did not specifically address abortion in our study design, some indirect data on abortion was presented in Chapter IV. Of the 45 fruits formed after hand pollinating 1761 flowers, only 13 fruits could be harvested – the other 22 fruits did not reach maturity and could not get harvested. However, it remains unclear which percentage of fruit loss was due to abortion. Many factors that were studied in this thesis, such as resource availability and shade management (Bos, Steffan-Dewenter, et al., 2007a; de Almeida & Valle, 2009), can affect abortion, and therefore, understanding their relation would complete our understanding of yield limitations. Quantification of the strongest limitations along the cacao developmental process could help to identify management priorities.

V.1.7 Reasons for fruit loss

There are multiple reasons why young fruits eventually do not get harvested, such as pest and disease occurrence. Both within and outside of cacao´s native range, hectares of cacao have been wiped out due to fungal infestations and insect pests such as herbivorous bugs can compromise cacao yields severely (Díaz-Valderrama, Leiva-Espinoza, & Catherine Aime, 2020; Hebbar, 2007; Maas et al., 2013; Wielgoss et al., 2012). The data presented in Chapter III demonstrated that even though pest- and disease-related fruit loss is not negligible, it is not the most frequent cause of fruit loss.

Instead, squirrel seed predation and untimely management were the main reasons for fruit loss in northern Peru.

Untimely management could be relatively easy to address and was therefore of particular interest. We observed that many of the pods that were harvested untimely, had germinated seeds inside their pods. This means that either the fruit was left too long on the tree and when management would have been on time, the fruit could have been harvested. According to regional cacao growers, premature germination can also be a symptom of fungal infection. Interestingly, fungal infestation can also be partly mitigated by timely management, since the removal of infected fruits helps to prevent further spread (Krauss & Soberanis, 2001). As such, it is of large importance to schedule regular phytosanitary control, removal of infested fruits and harvesting times.

As a side effect of installing cages to prevent birds and bats access to cacao trees, squirrels were excluded. Even though our observation of 11% of harvest loss due to squirrel seed predation, is less than in other regions (Ledesma, 2022; Palate Mazo, 2019), the loss is still of considerable economic importance to farmers. Squirrel fruit predation was not related to forest distance or shade cover, so no concrete management actions to address squirrel fruit loss could be recommended in Chapter III. Still, the considerable yield losses generated by squirrels and other rodents (Cassano et al., 2021) cancel out the benefits of other animals such as birds, bats and flying insects, and therefore, should be minimized. Thus, to safeguard the pollination and pest control benefits provided by fauna in cacao agroforests, strategies to avoid vertebrate fruit loss should be prioritized.

V.1.8 The complicated role of ants: more fruit loss, but also higher yield

The contribution to cacao yield of some animals is straightforward. They either cause a net loss, as is the case for squirrels, or a net gain, as is the case for flying insects and birds and bats. The net effect of ants on cacao fruit loss and yield, however, is not so straightforward (Wielgoss et al., 2014), which was also apparent in our study systems. Ants are known to form symbioses with pests and can promote fungal infections in cacao agroforests (Gras et al., 2016; Wielgoss et al., 2012). Potentially, the 7% ant-related fruit loss that was found in Chapter III had similar origins, but we do not know whether ants promoted fungal infections or pest infestations in our study system.

In our study system, ants´ contributions were not solely negative: Yield increases due to ants were also recorded, though their positive effect was restricted to sites near the forest (Chapter III). It is possible that forest-associated ant species could have improved cacao yields in those agroforests by enhancing visitation of small insect visitors (Wielgoss et al., 2014) or supporting pest control (Samantha J. Forbes & Northfield, 2017). Nevertheless, conclusions on the net effect of ants in Peruvian cacao agroforests are limited by knowledge gaps on ant species in agroforests near to and far from forest. Because the role of ants depends on community properties (Wielgoss et al., 2014), knowing which ants fulfil functional roles is urgently necessary. Combining this knowledge can serve as a base for identifying which ant species fulfil the most important functional roles and will help to increase overall systemic understanding of ants´ multiple contributions to cacao yield.

V.1.9 Improved **quality of harvested fruits** due to manual pollination

After a 150 to 180-day maturing period (day 150 – 180, Figure IV.1), cacao fruits are ready to be harvested. The fruits are large and swollen, and after cutting the fruit open, seeds can be extracted, fermented, and dried in the sun. Seed quality is of special importance in the native cacao that was studied here, because of the unique aromatic properties and the price-premium associated with it (Arevalo-Gardini et al., 2019; ICCO, 2022; Kadow, Bohlmann, Phillips, & Lieberei, 2013). Seed quality is largely influenced by postharvest processes (Levai et al., 2015), and as we illustrated in Chapter IV, also by manual pollination. Because cacao is usually sold to buyers or cooperatives per dry weight, the increased bean weight due to manual pollination is of economic importance for farmers. Moreover, the proportion of premium beans was higher in manually pollinated fruits, underlining that overall fruit quality can be optimized by pollination with native pollen donors. Promoting manual cross-pollination among native genotypes might translate in meaningful farmer benefits, but, manual pollination might also be associated to large labour costs (Wurz et al., 2021). To avoid these costs, optimized plantation designs with cross-compatible genotypes in rows might be a more cost-effective solution.

V.2 Limitations and perspectives

Throughout section V.1, some limitations of the studies presented in this dissertation are mentioned, for example, the lack of data on arthropod abundances that limit our understanding of the ecological interactions driving ecosystem services and disservices. Nevertheless, even though insect data would increase our integrative understanding of the system, even without arthropod abundance data, the effects of the animal exclusions were strong enough for reliable quantification, comparison and discussion.

Another, yet undiscussed but important challenge of cacao research, is the sample size difficulty due to the high flower to fruit ratio. Only a very small fraction of all thousands of produced cacao flowers sets fruit. Even though producing more flowers than fruits, is a long-known phenomenon in hermaphroditic plants (Sutherland, 1987), it complicates research advances in pollination ecology. Advanced statistical methods to deal with unbalanced data in logistical regression exist (Salas-Eljatib et al., 2018), yet many more samples are needed to achieve sufficient replicates for sound statistical analyses. This limitation became especially apparent in Chapter II, in which we discuss the pollination dynamics of cacao. We observed fruit set rates of less than 1%, which is extremely low compared with the 10% that was previously reported (Groeneveld et al., 2010) and concluded that our ability to relate pollen quantities with fruit set success was limited by these low success rates.

The low fruit set rates observed in our study, could mean that natural fruit set rates of up until 10% (Groeneveld et al., 2010) might be a methodological overestimation. This overestimation might be due to the difficulty of counting all flowers formed by cacao trees, as new flowers open and drop within a time span of 24-48 hours, and hundreds of new flowers appear on branches every day. Nevertheless, it is also possible that our data was taken in an exceptionally dry year, during which pollination onset was later than usual and fruit set was exceptionally low. Another explanation for low fruit set rates could be that native cacao has different compatibility properties, as discussed extensively in Chapter IV. Likewise, when sampling visitors from cacao flowers, care should be taken to obtain large enough sample sizes to draw conclusions on the effective pollinators of cacao.

Due to the challenges involved in monitoring flower visitors, pollen deposition, and subsequent fruit set, there are still major research gaps that limit our understanding of the main pollinating agents of cacao. This is remarkable, given the global importance of the crop for the chocolate industry. Identifying cacaos most effective pollinators will rely on combining knowledge of flower visitors, visitation frequencies, pollen loads, and the genetic origin of the deposited pollen. Sampling intensity will need to be increased so that enough replicates for sound statistical analyses are available. Because more successful fruit set cases are registered in hand pollination experiments, it is a commonly used technique to study cacao pollination ecology. Hand pollination also has known benefits for crop productivity, at least in the short term. Due to the high costs related to manual pollen supplementation, however, improving natural pollination might be a more cost-effective alternative. Because of the strict dependency on insects for natural cross-pollination, that was demonstrated in Chapter III, it is urgent to target conservation of insect that visit cacao flowers.

V.3 Summary and conclusions

Pollination and pest control are ecosystem services that are provided by multiple animal groups and support yields of the cacao tree in wildlife-friendly agroforestry systems. Because knowledge of provision of these ecosystem services is restricted to non-native cacao regions, the aim of this thesis was to fill a knowledge gap on the relationships between ecosystem service provision and local management in native cacao agroforestry landscapes. We found that the occurrence of flying insects and vertebrates, local and landscape management practices, and pollen supplementation interactively affected cacao yield, at different stages of the development from flower to fruit (Figure V.1).

In cacao, most of the flowers do not develop into a harvestable fruit, due to, among other reasons, low fruit set rates. Improving fruit set might thus contribute to yield improvements and could translate into larger farmers benefits. Here, we demonstrated that fruit set benefitted from flying insects that visit flowers, although there is no certainty about which visitors are pollinating flowers. As long as the main effective cacao pollinators remain unknown, general conservation measures that target flying insects are needed to safeguard fruit set. Shade management could aid to improve cacao fruit set further, as demonstrated by the highest fruit set gains under intermediate shade cover.

Not all flowers that set fruit will mature into harvestable fruits. Even after the critical phase of fruit set, fruits are being lost due multiple causes, among which squirrel and ant activity. The harvest loss due to squirrels and ants is of economic concern to farmers, therefore, strategies to minimize animal-caused fruit loss should be developed. Nevertheless, because the animal-caused losses were smaller than the 114% yield increase due to joint occurrence of birds and bats, for example, it is recommended that conservation of beneficial fauna within and around agroforests is included in conservation and landscape planning. As exemplified by the large beneficial effects of ants on cacao yield in agroforests near to forest, forest maintenance and restoration might be an appropriate way to conserve communities that benefit cacao yields maximally.

Lastly, because both quality and quantity of yield improved due to deposition of native pollen, manual cross-pollination with native genotypes could be another way to improve farmers´ incomes, although large costs associated with hand pollination might cancel out these benefits. Therefore, we propose an integrated wildlife-friendly farming approach in which shade cover is managed and nearby forests are conserved and restored to maximize pest control and pollination services provided by ants, flying insects and birds and bats, while minimizing fruit loss. In such integrated management, combining compatible genotypes as such that natural cross-pollination is promoted, could further improve yield benefits of native Peruvian cacao.

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AUTHOR CONTRIBUTIONS

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Data analysis	JV	EAM	COA	
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