



*“Cocoa fruit has improved greatly”  
“In one tree, around three hundred fruits”  
“A lot of broken branches because of too many fruits”  
“Pollination is indeed very tiring, but the yields are very satisfying”  
“I keep pollinating and my family is always good”*

(Cocoa farmers of Napu Valley)

# Cocoa pollination as a potential yield driver under changing management and climate



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**D7**

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## General Introduction





## General Introduction

### *Ecological Intensification and Cocoa Production in a Changing World*

Agricultural intensification has become the main strategy to counteract the increasing demand for food commodities of a rapidly growing population (Godfray et al., 2010; Bommarco et al., 2013). The resulting simplification, high dependency on chemical inputs, and areal expansion of agricultural systems into pristine habitats can indeed increase yields in the short term. In cocoa (*Theobroma cacao* L.), the rapid expansion of monocultures with high agrochemical input, at the cost of primary forest, results in substantial yield improvements in the main producing regions of West Africa, South East Asia, and Latin America (Donald, 2004). In Ghana, for example, the adoption of new cocoa varieties and fertilizers increased yields by 42% between 1980 and 2000 (Edwin and Masters, 2005). However, the cocoa expansion to natural forest often leads to deleterious effects on biodiversity, ecosystems and human well-being on the long term (Tscharntke et al., 2005; Ruf and Schroth, 2004; Deguines et al., 2014) (Fig. 1A). In Central Sulawesi, Indonesia, cocoa farming is conducted using former natural forestland instead of other land types such as grassland, because farmers prefer to plant cocoa in thinned or cleared pristine forest as it reduces labor costs and soils are perceived as more fertile (Ruf and Schroth, 2004). Such practices in Central Sulawesi cause an accelerated forest degradation, and a drastic change in species composition of key functional groups such as amphibians (Wanger et al., 2011), birds (Maas et al., 2009), and invertebrates (Bos et al., 2007).

In contrast to agricultural intensification, ecological intensification is an alternative approach to achieve higher yields by enhancing ecosystem services such as pollination with known beneficial effects on crop yields (Fig. 1B) (Bommarco et al., 2013; Kleijn et al., 2018). There is scientific evidence highlighting the yield and income benefits of pollination services in many agricultural systems across the world (Garibaldi et al., 2016). In the case of cocoa, there are three main reasons supporting its great potential for ecological intensification by enhancing pollination services. First, cocoa is the third largest traded commodity in the world, just after coffee and sugar cane (Donald, 2004). Second, its production is strongly dependent on small-scale agriculture (Tscharntke et al., 2011). And third, yields are highly threatened by pest and diseases, and prolonged droughts associated to climate change (Läderach et al., 2013; Wanger et al., 2014). Worldwide, cocoa yields have declined at an average rate of 2.5% since 2013 (ICCO, 2019). In Indonesia, the third largest producer, yield losses are even more alarming, as they fell by 36% from 2014 to 2018 (ICCO, 2019). These values show the necessity for sustainable alternatives to mitigate yield losses,

to meet the by 2.5% increasing global demand for chocolate production, and secure the livelihood of small-scale farmers, who produce 90% of global cocoa (ICCO, 2018; Clough et al., 2009). Little understood cocoa pollination services, as I will argue in the present work, can increase cocoa production and provide benefits for the environment and farmer livelihoods.



**Figure 1.** The conventional (A) and ecological (B) intensification approaches. In Central Sulawesi, Indonesia, cocoa production is strongly dependent on the expansion of monocultures into pristine forest and on a high use of agrochemicals (A). On the other hand, in Bahia, Brazil, cocoa mainly grows under the shade of diverse native trees from the Atlantic Forest, presumably enhancing biodiversity and ecosystem services (B).

### *Understanding Cocoa Pollination for Effective Ecological Intensification*

The pollination success of cocoa flowers is critical to improve yields, because yield deficits are linked to pollen rather than other plant resource limitations, such as

fertilizer and water availability. A study in Ghana showed that enhancing pollen grain deposition has a direct effect on the number of seeds produced per cocoa pod (Falque et al., 1995) Furthermore, a small-scale hand pollination study in Central Sulawesi found that enhancing flower pollination from 10% to 40% increases yields by up to 350kg/ha (Groeneveld et al., 2010). While mainly ceratopogonid flies (Diptera) (Kaufmann, 1973; Winder, 1978a; Young and Severson, 1994), but also other insect groups (Entwistle, 1972; Winder, 1978b; Young, 1981; Adjaloo et al., 2012), have been suggested to be pollinators of cocoa, we know surprisingly little about the identity of cocoa flower visitors, the landscape, farm management and plant-performance effect on their communities, and their importance to increase yields. The high dependence of cocoa flowers on potentially only a few species of ceratopogonid flies for pollination and a high general susceptibility of pollinators to climate change in the tropics (Kjøhl et al., 2011) suggests further impacts on crop yields (Garibaldi et al., 2013).

Effective landscape and farm management strategies can substantially enhance cocoa flower visitors (Fig. 2) (Young, 1986; Tschardt et al., 2011; Frimpong et al., 2011), fruit set (Forbes and Northfield, 2017), and potentially improve yields (Groeneveld et al., 2010). For enhancing cocoa flower visitors and pollination success, it is necessary to facilitate flower visitor movement from adjacent habitats and increase suitable nesting sites inside the farm. For example, managing surrounding habitats and farms for multi-strata vegetation and their soil organic litter could have a positive effect on flower visitor communities, because they prefer cool and moist habitats that are dark and rich in litter debris (Kaufmann, 1975; Young, 1986; Klein et al., 2008; Tschardt et al., 2011). In general, cocoa flower visitors breed in rotting fruits and similar substrates in the litter layer, and are expected to have smaller dispersal ranges than bees. For example, ceratopogonid flies can only fly up to 6m, although their foraging range can be enlarged by wind even up to 3km (Chumacero de Schawe et al., 2013). If farmers remove shade and leaf litter, flower visitor populations can be expected to depend on adjacent habitats, such as forest patches, where suitable breeding sites are available. However, little is known about the links between cocoa pollination, the management of the entire production landscape, and yield response (Young, 1986; Frimpong et al., 2011). Therefore, it is important to understand the pollination ecology of the cocoa and develop effective strategies for flower visitor management not only to sustainably increase yields, but also to buffer the effects of climate change (Wanger et al., 2014).

## Research Aims and Questions

In my PhD research I aim to understand the ecological intensification potential of pollination services for increasing yields and improving farmer income. I argue that disentangling the role of pollination for improving yields, and the effect of landscape, farm management, and plant-performance on flower visitors will help in providing management recommendations of how to enhance pollination service in cocoa. Here below, I present my main research questions, which I address in four thesis chapters:

1. What is the state of the art of cocoa pollination and where lie the major research gaps?
2. What are the main cocoa flower visitors and their pollinator potential?
3. What are the landscape, farm, and plant-performance effects on cocoa flower visitors?
4. What is the contribution of ecological intensification to improve cocoa yields?
5. What ecological intensification recommendations can be provided to farmers for improving yields?

In *Chapter I*, I develop the state of the art of cocoa pollination research. Here, I compile all available scientific literature from the past 65 years to elaborate an exhaustive review on the neglected role of cocoa pollination for improving yields. In particular, I discuss topics such as cocoa pollination and production cycle, and elaborate a comprehensive species list of the cocoa flower visitors and discuss their pollinator potential. In the last chapter sections, I provide hypotheses, sustained by scientific evidence, on how landscape and farm management may enhance pollinators and pollination services.

In *Chapter II*, I discuss the identity of cocoa flower visitors and their response to landscape and farm management practices as well as to plant-performance factors. Here, I conducted the first large scale field study on cocoa flower visitors following a correlative and experimental approach. In the correlative approach, I investigated the effect of landscape and farm management practices and plant performance on cocoa flower visitor species' richness and abundance. In the experimental approach, I manipulated soil organic litter amounts to understand its effect on pollinator communities.

In *Chapter III*, I focus on contrasting pollination and agrochemical intensification for improving cocoa yields. Here, I conducted the first large scale hand pollination

experiment in Indonesia encompassing 128 cocoa trees across eight farms. In this experiment, I contrasted pesticide and fertilizer use with hand pollination to determine the major driver for fruit set, cherelle wilt, pest and diseases, and final yield. Furthermore, I developed three hand pollination scenarios and calculated the economic benefit to farmers at the local, regional and national level.

Finally, in *Chapter IV*, I develop an analytical framework to integrate innovative genetic editing tools with ecological intensification principles. Here, I expanded my scope to perennial cocoa, citrus and coffees crops, and identified the major constraints for the brand new CRISPR/cas technology to be implemented in farm conditions. In the final section of this chapter, I provide agroecological-based solutions (e.g. pollination, grafting, and biostimulants) that may help bridging this implementation gap.

## Research Approach

The field studies described in *Chapter II and III* took place between October 2016 and November 2017 as part of a collaboration agreement within the University of Göttingen and the University of Tadulako, in Palu, Central Sulawesi. During my stay in Palu, I was kindly hosted by Prof. Dr. Basir Cyio, Prof. Dr. Alam Anshary, and Dr. Aiyen Tjoa, who provided valuable support for establishing my research at their University. In the University of Tadulako, I worked in close collaboration with Dr. Nur Edy's and Dr. Shahabuddin Saleh's research groups, and integrated 10 of their BSc. students to conduct their thesis within my research projects. Furthermore, 28 cocoa farmers, 10 local workers, and two international volunteers supported in the set-up of the field sites, the data collection and laboratory analysis (Fig. 2).

### *Study Site*

I conducted my field studies in the region of Napu Valley (S1° 27' 48", E120° 21' 6") in Central Sulawesi, Indonesia (Fig. 3A) located 100km southeast of Palu. This area has been part of scientific activities conducted by the Collaborative Research Center (STORMA) since 2000 (Tscharntke et al., 2007, 2010). The Napu Valley is part of the Wallacea biodiversity hotspot, situated in a lower mountain zone between 1,100 to 1,200m a.s.l., and with a mean annual precipitation ranging from around 1,500 to 3,000mm per year (Maas et al., 2009; Smiley and Kroschel, 2010). The Lore National Park extends up to the mountains that surround the Napu Valley (Fig. 3B). This natural area is known for hosting a great number of endemic and endangered species (Weber et al., 2007).

The population in the Napu Valley predominantly consists of local Napu, Javanese and Makassar small-scale farmers. The population has rapidly grown in the past decades because of the national transmigration program supporting human migration from overpopulated to less populated islands for conducting agricultural activities. Cocoa farming is the major activity of farmers in this region and in all Central Sulawesi, which alone contributes to 70% of cocoa produced in Indonesia (Wijaksono, 2016). Other predominant activities are rice cultivation and vegetable gardening (Fig. 3C). The accelerated population growth has caused conversion of pristine forest for new human settlements and farmlands. These drastic land use changes are having devastating consequences for biodiversity conservation (Weber et al., 2007).

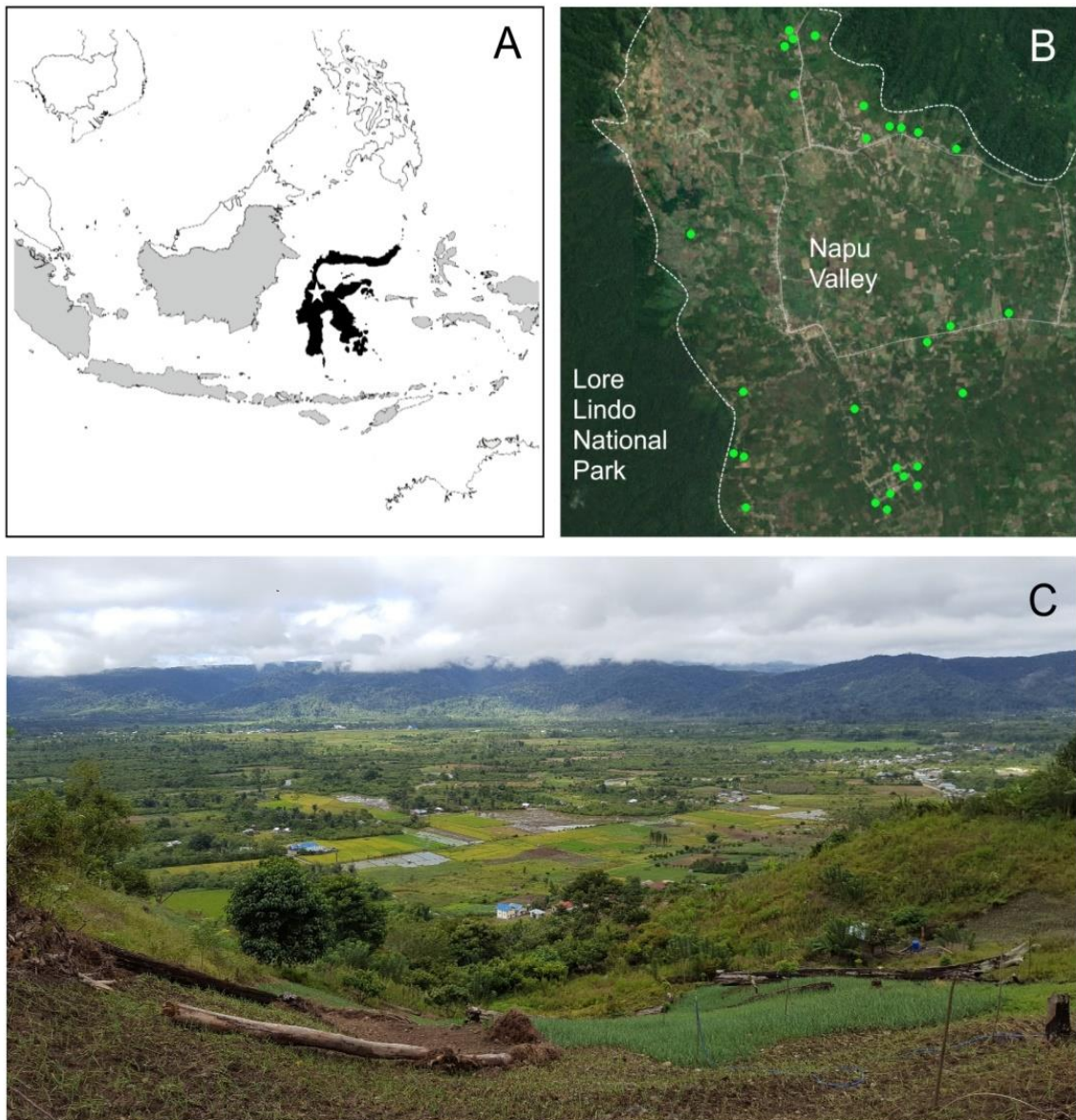


**Figure 2.** The field work team for my research in Central Sulawesi, Indonesia. All the research activities were conducted with the support of professors, lectures and 10 BSc. students of the University Tadulako, 28 cocoa farmers, 10 local workers, and two international volunteers.

### *Landscape and Farm Characterization*

In my research studies, I argue that cocoa flower visitor species' richness and abundance (*Chapter II*), and fruit set and yields (*Chapter III*) are driven by a landscape and farm management context. At the landscape level, I hypothesized that i) habitats with multi-strata vegetation (e.g. forest and cocoa agroforestry) in contrast to habitats with a single-strata (i.e. vegetable gardens, settlements, and open areas) surrounding the cocoa farms provide suitable habitats for flower visitors and ii) farms proximate to natural forest promote flower visitor movement to the farms. At the farm level, I hypothesized that i) an increased canopy cover provided by shade trees, and

ii) soil litter amounts provide optimal microclimatic conditions for flower visitors to develop.



**Figure 3.** The area of my studies was located in the region of Napu Valley, Central Sulawesi, Indonesia (A). Here, I visited and characterized 28 farms (green dots) situated along a forest distance gradient (dotted white-line) (B). Cocoa, rice, and vegetable farming are the main income activities in this region (C).

To answer my hypotheses, at the initial stage of my research, I visited and characterized 28 cocoa farms located along a forest distance gradient (Fig. 3B). The main idea of the visits was to have a general overview of the cacao systems in the Napu Valley. During the visits, I interviewed the farmers to record household socioeconomics (i.e. number of household members, farmer age, gender and level of education, main income activities) and farm management (i.e. farm organic and agrochemical inputs used, weeding, litter management, cocoa tree pruning, shade



management). Furthermore, I visited each cocoa farm together with the farmer to characterize the agricultural system (e.g. cocoa tree density, age, height and DBH, shade tree species' richness, density and percentage of canopy cover, and soil litter amount) and the habitat identity surrounding the farms. Finally, I conducted my studies in a subset of farms from the 28 farms characterized. I selected the farms across a gradient of i) forest distance, ii) percentage of multi-strata vegetation surrounding the farm, iii) percentage of canopy cover provided by shade trees, and iv) amount soil litter. I provide detailed information of the study design for each research study in *Chapter II – Field Study Design* pp. 70, and *Chapter III – Experimental Design* pp. 97.

### *Manipulation of Litter Amounts and Monitoring of Flower Visitors*

The research studies described in *Chapter II* encompass a correlative and experimental approach. In the correlative approach, I recorded flower visitors under current farm management practices (gradient of soil organic litter) in 18 cocoa farms with the help of two field assistants. In the experimental approach, I manipulated soil organic litter amounts in 24 cocoa farms with the help of 10 BSc. students and two field assistants. Each approach was carried out for four months (see *Chapter II - Field Study Design* pp. 70).



**Figure 4.** The preparation of the field sites for the experimental approach. Here I increased (high, A), maintained (control, B), and lowered (low, C) soil organic litter in 24 cocoa farms. First, I calculated the average soil litter amount in the 28 farms surveyed ( $2.3\text{kg/m}^2$ ) (D). Then I removed the litter to its lowest levels possible ( $0.6\text{kg/m}^2$ ) in the low litter treatment (E) and doubled the average litter in the high litter treatment (F).

The litter manipulation in the experimental approach consisted in lowering (low litter treatment), maintaining (control litter treatment) and increasing (high litter treatment) soil litter amounts in each of the eight farms per treatment (Fig. 4A-C). First, I calculated the average soil litter amounts from the selected 28 cocoa farms, giving  $2.3\text{kg/m}^2$  (Fig. 4D). Then, I removed the soil organic litter from the eight selected low-litter-treatment farms to its lowest level possible under the field conditions ( $<0.6\text{kg/m}^2$ ) (Fig. 4E). The litter from these farms was transported to the field house in Napu and mixed together. Then, I doubled the average recorded litter amounts ( $4.6\text{kg/m}^2$ ) in the eight high-litter-treatment farms (Fig. 4F). For the control-litter treatment, I adjusted it to  $2.3\text{kg/m}^2$ . Finally, a group of four students did a monthly litter monitoring and maintained the litter levels in each litter-treatment farm.

The minute size ( $\sim 5\text{mm}$ ) of the main cocoa flower visitors and its low visitation frequencies, make direct observations of flower visitation a difficult task. A solution to solve this problem was proposed by de Schawe et al. (2018), who used “Insect Tangle Glue Trap” (Tanglefoot©), hereafter referred as “insect glue” (Fig. 5A). The method consists in carefully applying a small quantity of glue on the flower, so that the visitor is trapped by the sticky glue when landing in the flower (Fig. 5B). Later on, the arthropod sample is carefully rinsed with a solvent solution, such as ethyl acetate, to remove the glue from its body. Using de Schawe et al. (2018) method, I monitored 11,664 flowers in the correlative ( $n=6,480$ ) and experimental approach ( $n=5,184$ ) with the help of four BSc. students. Before starting my research, I conducted a five days training in which the students monitored four cocoa trees adjacent to our field house in Napu. Each student applied the insect glue to 10 newly open flowers. The second part of the training consisted in providing guidance on effective identification of the major arthropod groups (Fig. 5C).

Finally, at the end of my research, I transported all identified hymenopteran and dipteran samples to the University of Jambi in Sumatra, where the specialist Rico Fardiansah sorted them to family and genus (Fig. 5D, E).

### *Hand Pollination Study*

In *Chapter III*, I present the results of my two large-scale pollination experiments (i.e. partial hand pollination and full hand pollination) conducted in Indonesia. The aim of my study was to understand the role of hand pollination and agrochemical intensification for improving yields. I trained two students and six field assistants who helped me to run the full experiment that lasted around eight months.

First, I selected 12 cocoa farms from the 28 farms. In each farm, I established four agrochemical treatments (i.e. fertilizer, insecticide, fertilizer+insecticide, control), where I doubled the average amounts of fertilizer (Nitrogen, Phosphorus, Potassium or NPK=186.5kg/ha/year) and insecticide (concentrate=1.7l/ha/year) recorded in the 28 farms surveyed. In the partial hand pollination experiment I daily hand-pollinated easily accessible flowers <2m height (or the 13% of flowers/tree) in four cocoa trees per treatment in rates ranging from 0 (control) to 13% during 60 consecutive days. In our full hand pollination experiment I hand-pollinated all flowers (100% of flowers/tree) in eight cocoa trees in an additional farm also for 60 consecutive days. I provide a detailed description of the study design for both experiments in *Chapter III – Experimental Design* pp. 97).



**Figure 5.** The flower visitor monitoring method as described by de Schawe et al. (2018). Here, I applied Insect Tangle Trap Glue (Tanglefoot©) (A) in 11,664 cocoa flowers to record cocoa flower visitors (B). First, I trained four BSc. students to conduct the flower monitoring and further arthropod identification to order level (C). At the end of the field season, I transported all hymenopteran and dipterans samples to the University of Jambi, Sumatra, for further identification to family and genus level (D-E).

For comprehensive purposes, I divided the hand pollination study in five parts that cover the cocoa production cycle (from the flower to the harvest):

- 1. Flower monitoring.** In the early morning, I counted all open flowers in each selected tree (hereafter “pollen-receptor tree”) (Fig. 6A). Then, I calculated the number of flowers to be pollinated according to the assigned pollination rate. For example, if flower counts of a given tree was 100, and hand pollination



**Figure 6.** The complete hand pollination approach from flowering to the harvest. First, we quantified the flowers (A) and marked the flowers selected for hand pollination (B). Then, we collected flowers from three trees in an additional farm (C), and hand pollinated the marked flowers (D). After hand pollination, we quantified fruit set (E), cherrille wilt (F), and pest and diseases (G). The harvesting of mature fruits took place around six months after hand pollination (H). Fermentation and drying was conducted following local practices (I). Finally, we recorded fresh and dry weigh (kg/tree) (J).

rate assigned was 10%, then the number of flowers to be pollinated was 10. Then, I randomly picked and marked with pins and labels (with the pollination day and date) the flowers to be pollinated (Fig. 6B).

- 2. Hand pollination.** I followed the methods described by Falque et al. (1995) and Groeneveld et al. (2010) that consisted in collecting open flowers coming from three new trees (hereafter “pollen-source trees”) in an additional farm not included in the study (Fig. 6C). This approach increases the genetic pool and avoids fruit abortion due to tree self-incompatibility. I randomly hand-picked one flower per pollen-source tree (tree flowers in total) and carefully, one by one, rubbed their anthers in the marked flower styles of the pollen-receptor tree (Fig. 6D). I removed flowers not pollinated to prevent open pollination.
- 3. Fruit set.** I recorded successful pollination, or fruit set, two days after hand pollination (Fig. 6E). This is because only successfully pollinated flowers remain on the tree 48h after pollination, while the unsuccessfully pollinated fall down (Wood and Lass, 2008). For example, fruit set of hand pollination in days one and 60 were recorded in days three and 62, respectively.
- 4. Fruit losses.** A large proportion of young fruits rotten and shrank in the first months of development. This phenomenon also known as cherelle wilt, or fruit abortion, is a plant regulating process associated with the limited plant energy resources available for fruit development (Fig. 7F) (Wood and Lass, 2008), which eventually causes an early fruit abortion. I daily quantified the fruit abortion for two weeks and later weekly until the harvest. Additional fruit losses caused by the cocoa mosquito (*Helopeltis* sp.) pest and black pod disease (*Phytophthora* sp.) (Fig. 6G) were quantified weekly until the harvest.
- 5. Harvest.** The harvest took place around six months after the hand pollination started. Here, we collected all harvestable fruits and quantified the proportion of healthy and unhealthy (attacked by pest and diseases) fruits. We opened the harvested fruits to extract the fresh beans, and weighted them (fresh bean weight kg/tree) (Fig. 6H). The fermenting and drying of beans was carried out following the local practices consisting in a seven-days fermentation in rice sacks, and a two to three days open-sun drying (Fig. 6I). The final yields quantified as dry been weight (kg/tree) (Fig. 6J).

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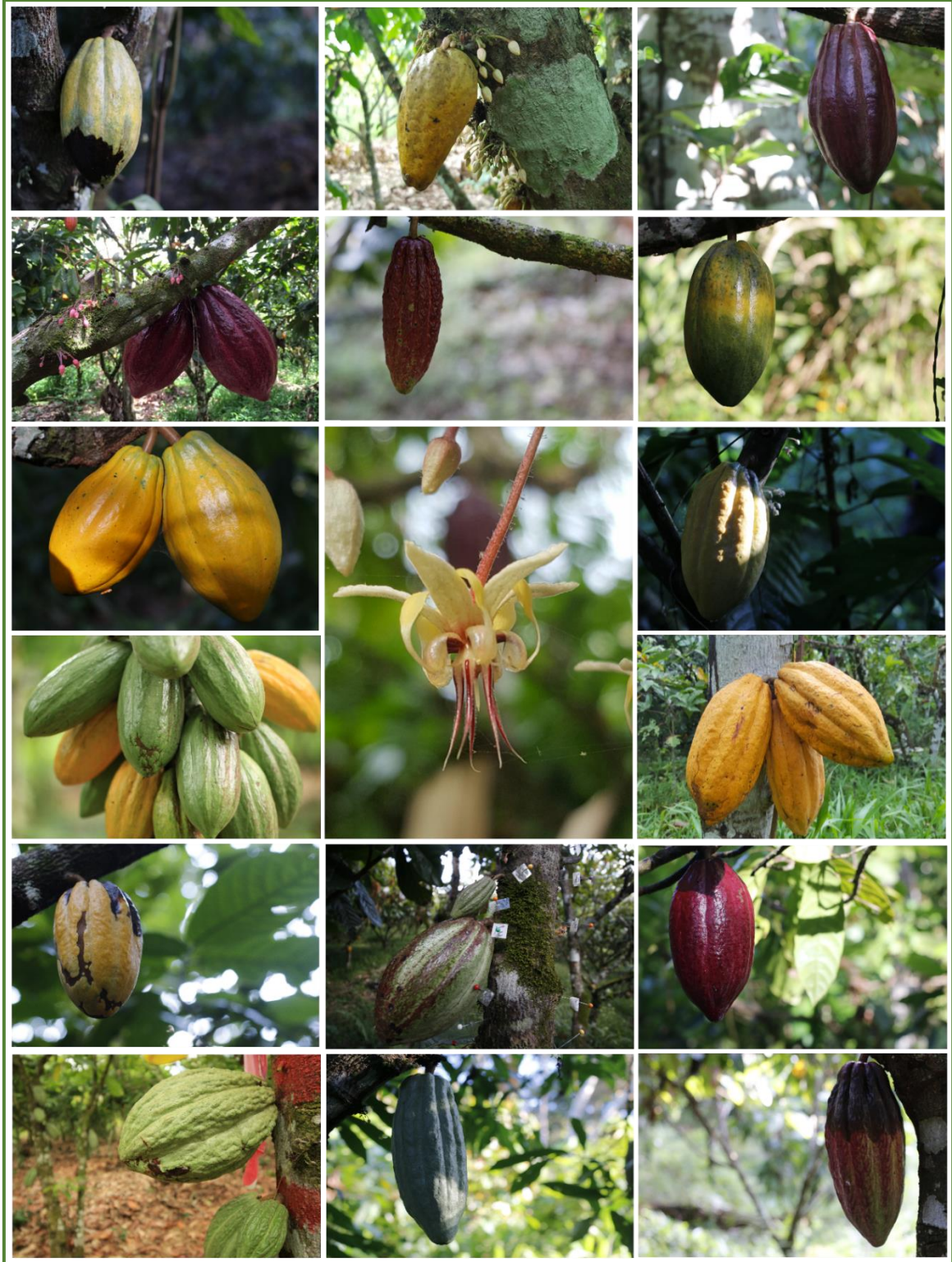
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# Chapter I

## Neglected Pollinators: Can Enhanced Pollination Services Improve Cocoa Yields? A Review



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## Abstract

The negative effects of climate change on cocoa production are often enhanced through agricultural intensification, while research institutions and enterprises try to minimize yield gaps with production strategies mitigating climate risk. Ecological intensification is such a production strategy, whereby yield increase is promoted through reduced agrochemical inputs and increased regulating ecosystem services such as pollination. However, we still know little about cocoa pollination ecology and services, although they appear to be key to understanding yield functions. Here, we provide an extensive literature review on cocoa pollination focusing on three main aspects: non-plant (external) and plant regulated (internal) factors affecting pollination, pollinator agents and ecological intensification management for enhancing pollination success and yield. Pollination services by many arthropod groups such as ants, bees, and parasitic wasps, and not only ceratopogonids, may be a way to increase cocoa productivity and secure smallholders' income, but their role is unknown. Several environmental and socioeconomic factors can blur potential pollination benefits. Surprisingly, knowledge gaps preclude our understanding of how to (i) identify the major pollinator species, (ii) disentangle the direct or indirect role of ants in pollination, (iii) design effective habitat improvements for pollination (by litter and shade management), and (iv) quantify the yield gaps due to pollination limitation. Optimizing cocoa pollination alone appears to be a powerful ecological tool to increase the yield of smallholders, but experimental research is required to validate these results in a realistic setting. In general, industry, governments and smallholders need to develop more joined efforts to ecological production strategies. In particular, farm-based management innovations building on robust scientific evidence must be designed to meet the increasing demand for chocolate on the global market and mitigate cocoa yield gaps. This review suggests that diversified systems and associated ecosystem services, such as pollination, can help to achieve such goals.

**Keywords:** Agroforestry system, climate change, cocoa, ecological intensification, pollination service, pollinator, shade, yield

## Introduction

Climate change is predicted to have severe impacts on the environment and the crop production (Challinor et al., 2014) through prolonged droughts, pest and disease

outbreaks and variations in climate extremes (Rosenzweig et al., 2001; Lobell et al., 2011). In the past 30 years, yields of wheat and maize, two of the most important staple crops, declined considerably due to irregular weather events (Lobell et al., 2011). Similarly, to wheat and maize, cocoa (*Theobroma cacao* L.) as the third largest legal crop commodity worldwide (Donald, 2004) has seen significant climate-related yield deficits over the past three years (ICCO, 2016a). In the major producer countries Ghana and Ivory Coast, climate change threatens the current suitability of land used for cocoa, and will likely force farmers to adopt strategies enhancing agroecosystem resilience through management improvements (Clay, 2004; Franzen and Borgerhoff Mulder, 2007; Clough et al., 2009; Läderach et al., 2013; Schroth et al., 2016). International enterprises and research institutions are aware of these constraints (ICCO, 2015; MARS, 2017), but current cocoa production strategies lack effective ways of securing long-term yield and climate adaptation. For example, farmers are advised to intensify their production through replacement of old and heterogeneous plant material with genetically engineered varieties, and trained to efficiently apply pesticides and fertilizer to reduce yield gaps (WCF, 2016a; MARS, 2017). This approach increases short-term yields and farmer benefits but it can have long-term disadvantages such as biodiversity loss, disruption of essential ecosystem services and the dependence of farmers on external inputs (Tschardt et al., 2011). Sustainable cocoa production strategies need to buffer current yield deficits while assuring long-term ecological and economic benefits for all cocoa stakeholders.

Compared to conventional agriculture, highly diversified systems and their provision of ecosystem services through ecological intensification may be key for long-term solutions (Ponisio et al., 2015). Ecological intensification balances external inputs and advocates for the enhancement of ecosystems services through farm-based adaptations to reduce yield gaps and improve farmer livelihoods (Bommarco et al., 2013). For example, yield gaps in small-scale agricultural systems can be reduced by enhancing pollination services (Garibaldi et al., 2016). Simple management improvements such as the addition of shade trees can have positive effects on both pollination and yield while also likely reducing climate risks (Wanger et al., 2014). Indeed, pollination success rather than nutrient limitation determines cocoa fruit set and yield in Indonesia (Groeneveld et al., 2010). However, major knowledge gaps include pollination ecology, pollinator agents, and their potential for ecological intensification. Addressing these pressing research issues will help to overcome the climate-related yield crises in cocoa production (Deheuvels et al., 2014a, 2014b; COCOAPOP, 2016).

Here, we review the limited knowledge on cocoa pollination to discuss the ecological and economic potential of pollination services to buffer current yield deficits. We present three important topics that address the main aspects and constraints on cocoa pollination ecology: (i) external and internal factors affecting pollination success from flowering to harvesting, (ii) the role of pollinator agents on pollination intensification, and (iii) current ecological intensification methods for increasing yields. In the final section, we discuss where future research efforts are needed.

## Literature Review

We reviewed published literature from the last 60 years (from 1939-2016) in the Web of Science and Google Scholar using the search string “TS= ((cocoa OR cacao) AND (pollinat\*))”, with additional searches of relevant studies on cocoa and climate change, hand pollination, arthropod diversity and ceratopogonids ecology. We found 108 books, articles, PhD dissertations and manuals, of which 67 were available for revision (see Electronic Supplementary Material, Table S1). We also extracted information from the title, abstracts of unavailable literature where possible, and merged it together with the available literature in a common database. Overall, there were two main peaks in the number of publications: 1971-1985 and 2001-2016 with 36 and 37 published materials, respectively. The majority of study sites of the published material were in Ghana (23), Costa Rica (11) and Brazil (8). We found only two published reviews and two book chapters specifically focusing on pollination and pollinator ecology (Glendinning, 1972; Winder, 1978a; Entwistle, 1972; Wood and Lass, 2008) published in the 1970s and 1980s with studies dating back to 1910. In recent years three review papers on agroforestry systems highlighted the importance of pollination ecology for cocoa yields (Donald, 2004; Klein et al., 2008; Tscharrntke et al., 2011, Wielgoss et al., 2014).

## The Cocoa Cycle from Flower to Harvest

The cocoa pollination cycle from flowering to pollination, fruit set, fruit development and pod harvest is regulated by external and internal mechanisms, which can be divided into four phases: flowering, flower opening and pollination, flower fecundation and fruit set, and harvesting (Fig. 1) (Table 1).

*Flowering (Phase I)*

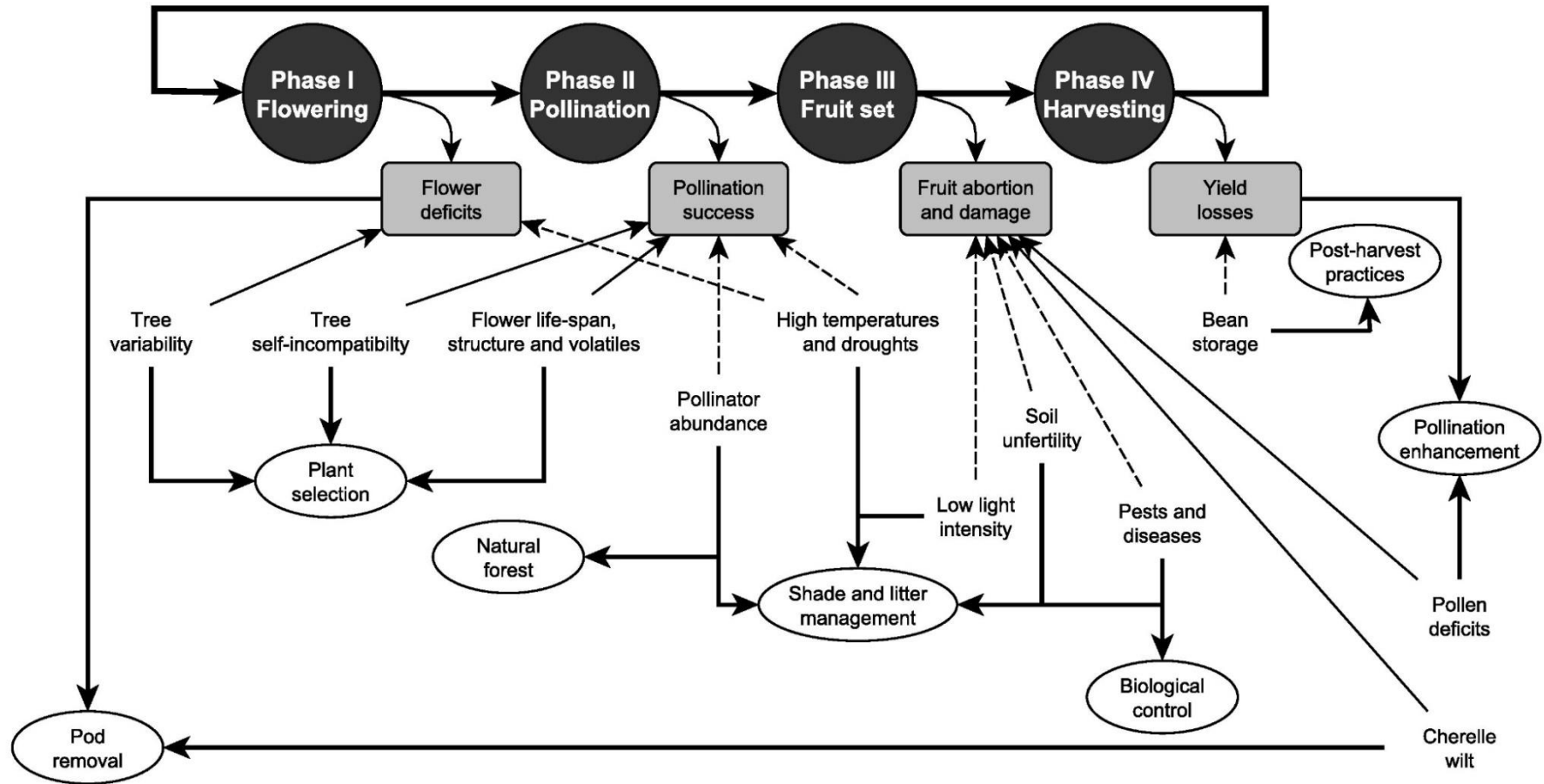
The cocoa tree (*Theobroma cacao* L.) produces up to 125,000 miniature white and pink-purple nectar-guide flowers of 10-15 mm diameter in clusters of 14 to 48 flower cushions, also referred as cauliflowers, along its main branches (Glendinning, 1971; Wood and Lass, 2008; Falque et al., 1995; Somarriba et al., 2010). Cushions are formed in old leaf-axis of young healthy wood, with flower buds requiring in general 30 days to emerge and mature (Wood and Lass, 2008). The floral structure comprises five sepals, five petals, 10 stamens (male structure), one ovary (female structure) of five chambers containing the ovules, and five unfertile elongated staminodes (Glendinning, 1971; Wood and Lass, 2008).

*Flower Mechanisms to Enhance Pollinator Visitation*

Flower color, structure and volatiles are highly important for inducing pollinator visitation. For example, staminodes are highly attractive to pollinators due to their color and odor molecules produced (Young et al., 1984; Young and Severson, 1994). Young and Severson (1994) studied the effect of steam distilled oil properties and attractiveness to pollinators in nine genetically contrasting cocoa cultivars in Costa Rica. They classified the cultivars in three differentiated clusters, of which one ancestral-type of cocoa formed one cluster alone with the highest molecular weight compound and attractiveness. These results indicate that cultivars from artificial selection methods can be less attractive to pollinators than wild types of cocoa cultivars, highlighting the potential of native cocoa varieties for enhancing pollinators and pollination success.

*Driving Factors for Flower Production*

Precipitation is known as the main driver initiating cocoa flowering (Glendinning, 1972; Wood and Lass, 2008). Glendinning (1972) observed that flower frequency in plantations in Ghana was low during the dry season (January-March), but increased throughout the rainy season (April). Similar flowering patterns are described in the Americas, West Africa and South East Asia (Young, 1983; Omolaja et al., 2009; Chumacero de Schawe et al., 2013; Bos et al., 2007a). Beside precipitation, factors such as cultivar genetics and management practices can affect flower production. In Ghana, for example, flowering pauses in Lower-Amelonado between June and November, while Upper-Amelonado produces flowers throughout the year (Falque et al., 1995). Glendinning (1972) observed that flower abundance increased during



**Figure 1.** The four phases (black boxes) of the cocoa pollination cycle and the main external (break arrows) and internal (continuous arrows) drivers for flower and pollination deficits, fruit mortality and overall yield losses (gray boxes). The adoption of landscape and farm level practices (white boxes) can significantly reduce the effect of these external and internal pressures on cocoa yields.

**Table 1.** Description of the cocoa pollination cycle (external and internal) and the management strategies to overcome the trade-offs in each phase.

Phase	External (Non-plant regulated)		Internal (Plant-regulated)		Reference
	Mechanism (Trade-offs)	Management	Mechanism (Trade-offs)	Management	
I Flowering	<b>Precipitation</b> - Rainfall promotes mobilization of soil nutrients.	<b>Farm level</b> - Enhance soil water retention, and reduce evaporation through shade canopy and litter cover.	<b>Cultivar genetics</b> - All year round flowering cultivars (e.g. Upper Amelonado).  <b>Staminodes</b> - Color and odor molecules.	<b>Farm level</b> - Cultivar selection to reduce tree genetic variability. Avoid flower damage during harvest. Pod removal.	Bos et al., 2007a; de Schawe et al., 2013; Falque et al., 1995; Frimpong-Anin et al., 2014; Glenndining, 1972; Omolaja et al., 2009; Somarriba et al., 2010; Wood and Lass, 2008.
	<b>Pollinator agents</b> - Low pollination visitation (pollinator abundance) and inefficacy to carry pollen grains (pollinator identity).	<b>Landscape level</b> - Pollinator habitat conservation through forest preservation.  <b>Farm level</b> - Secure microclimatic conditions for pollinators development (moisture, light intensity, organic matter) through plant biodiversity, shade canopy and litter cover.	<b>Flower lifespan</b> - Limited to 48 hours.  <b>Pollen deposition</b> - Minimum 35-40 pollen grains.  <b>Self-incompatibility</b> - Fusion failure between male and female gametes to for the zygote.  <b>Tree-incompatibility</b> - Poor plant material promotes High tree variability generate.  <b>Staminodes</b> - Staminode-style distance.	<b>Self-incompatibility and tree-incompatibility</b> - Incompatibility is proportional, rather than absolute. Control self-incompatibility and tree genetic variability by an appropriate selection of cultivars.	
III Flower fecundation and fruit set	<b>Fruit mortality</b> - Pest and pathogens outbreaks.	<b>Farm level</b> - Improve phytosanitary practices (e.g. removal of diseased fruits) to minimize disease widespread.	<b>Fruit mortality</b> - <i>Cherelle wilt</i> induced by low photosynthetic rates, and water and soil nutrient limitations.	<b>Farm level</b> - Enhance soil water retention and soil nutrients through litter cover. Enhance light intensity through appropriate shade tree selection (e.g. canopy structure) and management (e.g. pruning).	Bos et al., 2007a, 2007b; Entwistle, 1972; Falque et al., 1995; Groeneveld et al., 2010; Müntzing, 1947; Wood and Lass, 2008.
IV. Harvesting (beans/pod)	<b>Pollen deficits</b> - Associated to pollinator visitation rates and pollen carrying capacity. Maximum beans/pod with 200 pollen grain, maximum pollinator carrying capacity 16-23 grains per flight.  <b>Post-harvest</b> - Pest and pathogens outbreaks.	<b>Landscape level</b> - Pollinator habitat conservation through forest preservation.  <b>Farm level</b> - Enhance pollinator visitation rates by maintaining high pollinator densities (habitat conservation). Separate diseased and healthy pods during the harvest. Appropriate bean storage.	<b>Cultivar genetics</b> - Less pollen grains needed to reach maximum beans/pod production (e.g. Upper Amazon).	<b>Farm level</b> - Cultivar selection of high beans/pod varieties with less pollen grains.	Falque et al., 1995; Young, 1982, 1986.



harvesting, suggesting that pod removal can also trigger flowering, as the tree allocates energy in flower production rather than fruit development (Entwistle, 1972; Valle et al., 1990; Bos et al., 2007a).

### *Flower Opening and Pollination (Phase II)*

Flower opening, or anthesis, starts in the afternoon when a fully mature flower bud splits out, and it continues opening during the night. Early in the morning, the anthers release the pollen grains when the flower is fully open, and later in the afternoon the style matures. This is the stage when the flower is more receptive for pollination (Wood and Lass, 2008; Chumacero de Schawe et al., 2013). The average flower lifespan is two days after opening, and un-pollinated flowers abscise after 36 hours (Glendinning, 1972; Entwistle, 1972; Groeneveld et al., 2010).

Cacao pollination is the process of pollen transportation and deposition on the flower style performed by a pollinator agent (Falque et al., 1995; Wood and Lass, 2008). Pollination success occurs when a minimum of 35 to 40 pollen grains (Entwistle, 1972; Kaufmann, 1975), are deposited on the style (Falque et al., 1995). The general consensus is that small ceratopogonids (Diptera) are the main cocoa pollinator agents (Entwistle, 1972; Winder, 1978a; Young, 1982, 1986; Tscharnke et al., 2011). Some authors have reported other insects as casual flower visitors, but the majority failed in recording fruit set to corroborate their pollination efficacy (Winder, 1978b; Adjaloo and Oduro, 2013; Deheuvels, et al., 2014a, 2014b; de Schawe et al., 2018). Pollination via non-animal agents, such as wind, is unlikely due to the plant self-incompatibility in most cocoa trees and the flower structure (Posnette, 1940; Chapman, 1964; Leston, 1970), but successful wind pollination has been reported in Costa Rica (Glendinning, 1972). Wind can play a major role by transporting the pollinators along larger distances, as directed ceratopogonids flights can only cover few meters (Bos et al., 2007b; Klein et al., 2008).

### *Internal and External Drivers of Pollination Success*

Pollination self-incompatibility is one of the main drivers of pollination success. This plant-regulated mechanism was first described by Pound and Marshall in 1932-1933 (Adu-Ampoman et al., 1990), and widely addressed by other researchers (Posnette, 1940; Entwistle, 1972; Wood and Lass, 2008). Self-incompatibility in cocoa is contrasting with other self-incompatible plants, as pollen tubes develop normally, but the male gamete fails fusing the female gamete to form a zygote in 25 to 100% of

cases. This prevents ovary development, and the flower abscises four days after pollination (Wood and Lass, 2008). High proportions of the cultivar Amazon and Trinitario are presumably self-incompatible (Wood and Lass, 2008; Falque et al., 1995). However, self-compatible cocoa populations are also common. For example, self-pollination was found to be highly prevalent in the cultivars Criollo and Forastero in Bolivia, while 7 to 14% of the wild cocoa populations were self-compatible (Chumacero de Schawe et al., 2013).

Tree-incompatibility can considerably affect pollination success (Entwistle, 1972; Kaufmann, 1975; Wood and Lass, 2008; Somarriba et al., 2010). Tree-incompatibility occurs when pollen fails pollinating other flowers of the same tree individual (Somarriba et al., 2010). Inappropriate practices of poor genetic plant material selection can mean that 70-80% of the yield is produced by only 20-30% of the trees (Somarriba et al., 2010; Royaert et al., 2011). Other factors driving pollination success are flower staminodes arrangement, and pollinator species identity, ecology and behavior. A study in Ghana that examined three types of staminodes-style distance categories (i.e. converging, parallel and splay), concluded that splay staminodes resulted in the lowest pollination rates (Frimpong-Anin et al., 2014), as pollinators landing in splay staminodes fail in rubbing their pollen-covered body with the style. Although ceratopogonid population fluctuations synchronize with flowering peaks (Kaufmann, 1975; Young, 1983), and flower surplus may increase pollinator visitation and pollination rate, only about 5 to 10% of flowers are naturally pollinated (Falque et al., 1995; Bos et al., 2007b). One reason for the low cocoa pollination rate is low pollinator visitation and high inefficiency to carry pollen grains. In Ghana, for example, Kaufmann (1975) reported that mainly ceratopogonid males of eight species (out of 70 species present) are attracted to cocoa flowers, and only few of them succeeded in carrying pollen grains to enable pollination.

### *Flower Fecundation and Fruit Set (Phase III)*

Phase III starts after successful pollination, indicated by the flowers remaining on the tree for more than two days after opening. A total of five to seven months are required for fruit development (Wood and Lass, 2008). During the first 40-50 days (first growth period) the zygote divides to form the embryo, then in the coming 35-45 days (second growth period) the ovule and pod growth slows down, and as a consequence the embryo growth stagnates. In the last growing period, characterized by the accumulation of fat content, the embryo consumes the endosperm, which is accumulated in the ovule (Wood and Lass, 2008). Pathogen and insect outbreaks

(external) and plant regulated (internal) factors prevent 62 to 79% of pollinated flowers developing into fruits (Bos et al., 2007a).

#### *External and Internal Drivers of Fruit Mortality*

Cherelle wilt or active fruit abortion is a plant-induced mechanism controlling fruit mortality, which commonly occurs at two early stages (50 and 70 days after pollination) of fruit development (Falque et al., 1995; Bos et al., 2007a; Wood and Lass, 2008). A cherelle wilted pod stops growing, turns black, shrinks, and becomes rapidly colonized by pathogens, while the pod remains on the tree (Wood and Lass, 2008). The main drivers of cherelle wilt are low photosynthetic rates inducing pollen incompatibility, as well as water and nutrient limitations in poor soils (Bos et al., 2007a). Bos et al. (2007a) investigated the principal causes of fruit losses in two distinct cocoa agroforestry types (planted and natural forest shade) in Central Sulawesi, Indonesia. They found cherelle wilt as the main driver of fruit mortality in plantations with forest shade compared to planted shade systems, but later pest outbreaks in planted shade systems prevented significant yield differences. They argue that planted shade by leguminous trees significantly contributed to enhancing soil nutrients, compared to forest systems where leguminous trees were not abundant. Furthermore, pathogen attack was the second most important limiting factor controlling 19% of fruit mortality, mainly occurring in homogeneous shade systems. Although insect attacks were of less importance than cherelle wilt and pathogens, early fruit damage by insects promoted subsequent pathogen colonization.

The results from Bos et al. (2007a) highlighted the significant impact of external and internal drivers of fruit losses, causing over 72% pod losses in Indonesia. However, small management adjustments, such as removal of cherelle wilted and diseased pods, can reduce yield gaps associated to fruit abortion. For example, a study conducted in Nigeria proved that phytosanitary practices (pod removal of diseased pods) reduced the incidence of black pod disease by 30%, and increased yield (Ndoumbe-Nkeng et al., 2004).

#### *Fruit Development and Harvest (Phase IV)*

The last part of the cocoa production cycle is the yield phase (IV). It comprises harvesting the healthy pods, containing the seeds, called beans. External (non-plant regulated) and internal (plant regulated) factors, such as (i) cultivar genetics, (ii)

flower physiology, structure and abundance, (iii) tree self-incompatibility, (iv) pollinator ecology, (v) fruit abortion, (vi) pest/pathogen attacks, and (vii) management practices (shade systems, removal of diseased pods) will strongly affect the overall cocoa tree yield, which is on average 10 to 32 healthy pods per tree (Young, 1982, 1986).

Maximizing seed content per pod is of major importance, as beans are the primary raw material for the chocolate industry. For example, Falque et al. (1995) observed that seed production in healthy pods is regulated by the number of pollen grains deposited on the style, reaching a maximum number of seeds at >200 pollen grains. This number is far beyond the 16-23 grains that the main cocoa pollinators can transport per flight. In another study, Falque et al. (1996) investigated the effect of pollination intensity on seed production in the self-compatible Forastero Lower-Amazon Amelonado and in the self-incompatible Forastero Upper-Amazon clones. He found that the self-compatible trees require less pollen grains to reach maximum seed production compared to the self-incompatible trees. These findings suggest that pollen deficits and associated yield gaps are contingent on cultivar genetics and on the limited pollen carrying capacity of the pollinators.

#### *Harvest practices contribute to yield deficits*

Yields can be further affected by post-harvest practices, as beans are susceptible to mould and pest/pathogen outbreaks (Entwistle, 1972; Dharmaputra, 1999; ICCO, 2016b). The post-harvest procedure involves several farm-level processing steps before beans are dried and stored for commercialization. The steps include (i) pulp pre-condition for microorganism colonization, (ii) partial pulp removal to reduce beans acidity, and (iii) bean fermentation (Kongor et al., 2016). The majority of raw cocoa produced worldwide (70%) comes from small-scale farms of <10 ha (Donald, 2007) that generally lack of technical and infrastructure capacity to conduct appropriate post-harvest practices. Despite the strict international quality standards ensuring the commercialization of safe and wholesome chocolate products (CAOBISCO/ECA/FCC, 2015), it is likely that small producers sometimes compromise the production quality for an increased volume of raw cocoa, as quality is rarely controlled by local intermediaries (Levai et al., 2015). For example, many cocoa producers in Peru, Ghana and Indonesia harvest, ferment and dry both diseased/damaged and healthy fruits together (see Electronic Supplementary Material, Fig. S1) (pers. obs.). These practices contribute to high pest and disease levels and are detrimental to the raw cocoa quality (Levai et al., 2015; Kongor et al., 2016).

## The Cocoa Pollinators

### *The Ceratopogonids*

Over the past four decades, cocoa pollination has been associated with few insect pollinator species (Glendinning, 1971; Entwistle, 1972; Young, 1981). Our literature review found that at least 56 species and morphospecies of ceratopogonids (predominantly *Forcipomyia* sp.) and cecidomyiids (both Diptera) provide 45.6% of all cocoa flower visitors in the Americas, West Africa and South East Asia (Fig. 2). These results are in line with the most common perception that 3 mm sized ceratopogonids are the main pollinators of cocoa (Entwistle, 1972; Winder, 1978a; Young, 1982, 1983, 1985, 1986; Tscharrntke et al., 2011).

### *Ceratopogonid Natural Habitats*

To date, we know little about the ceratopogonids' ecology and behavior, as only a limited number of species are extensively described from a few countries including Ghana, Brazil and Costa Rica (Kaufmann, 1973, 1975; Winder, 1977; Young, 1982, 1983, 1986). Ceratopogonids are widely distributed in highly diverse tropical forests, where multi-strata vegetation provides cool and dark habitats as well as rich organic substrates for ceratopogonids development (Kaufmann, 1975; Young, 1986).

Ceratopogonids are unselective pollinators, meaning that they actively forage and may pollinate different plant species (Young, 1986), but very little is understood about the ceratopogonids' natural habitats and foraging behavior. Many authors agree that highly plant diverse shade agroforests are attractive habitats for ceratopogonid development, as they resemble their natural habitats (Glendinning, 1972; Kaufmann, 1975; Tscharrntke et al., 2011).

### *Ceratopogonid Life Cycle*

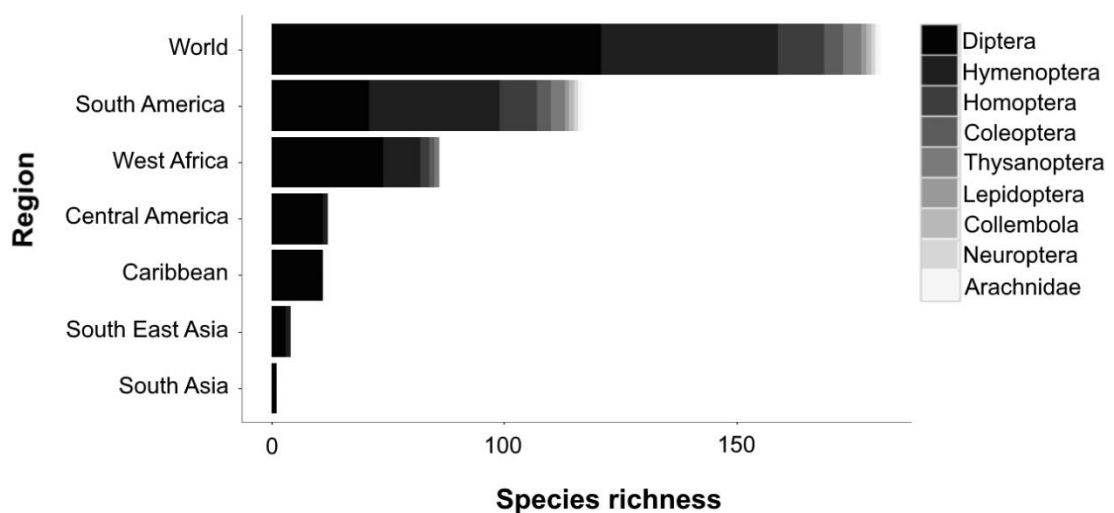
Ceratopogonids have a complex life cycle, with populations fluctuating over the year. Ceratopogonids are abundant during the rainy season, and decline over the dry season, irrespective of flower resources other than cocoa (Kaufmann, 1975).

Ceratopogonids are generally active during the initial hours of the day and a few hours before sunset. During this time, ceratopogonids swarm and forage flowers in a 5-6m radius, although a maximum distance of 3km has been reported (Entwistle, 1972; Chumacero de Schawe et al., 2013). Ceratopogonids are gregarious insects living in groups throughout their life cycle. The females lay about 200 eggs during

rainy days in nutrient rich substrates. It is during the first three life stages (egg, larvae, and pupae) that ceratopogonids are more vulnerable to predator attacks from other invertebrates such as mites, ants, millipedes, and pseudoscorpions (Kaufmann, 1974, 1975). The Ceratopogonids life cycle ends after 22 to 24 days. Larvae and pupae stages last 10-13 and 2-3 days respectively, and adults can live in average 12 days depending on food and water resources available. (Kaufmann, 1974; Somarriba et al., 2010).

### Management Practices Affecting Ceratopogonid Populations

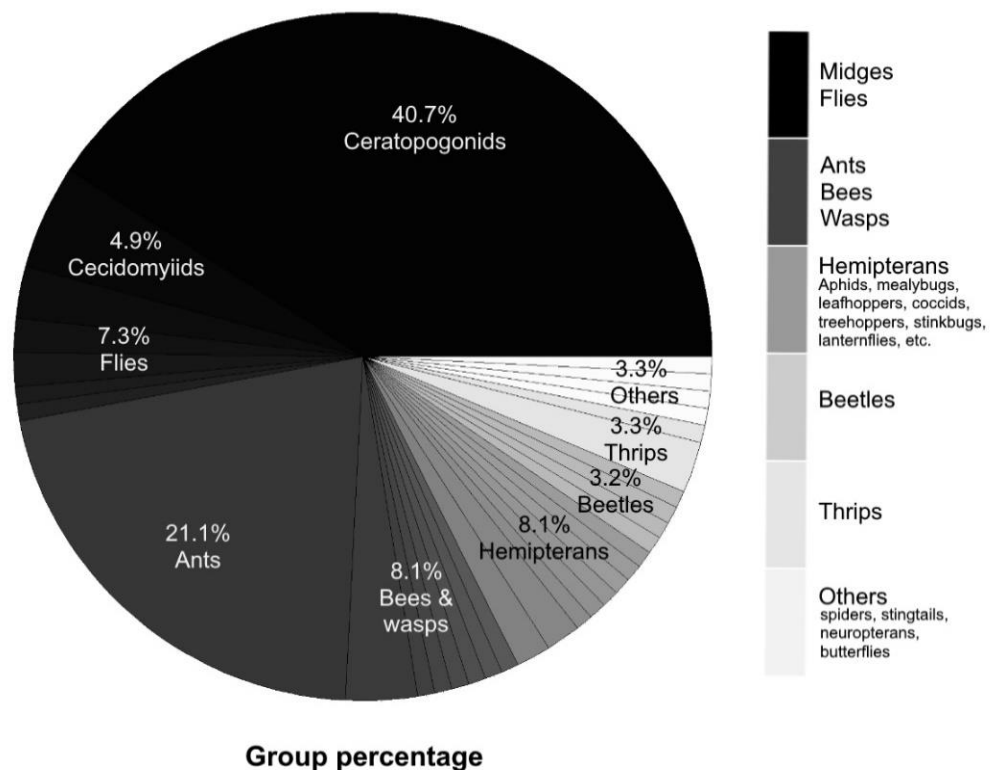
Farming practices can considerably affect ceratopogonid populations (Entwistle, 1972; Kaufmann, 1975; Young, 1982; Tschardt et al., 2011; Kwapong and Frimpong-Anin, 2013). For example, maintaining high tree shade diversity, enriching plantation soil with nutrient-rich litter substrates (such as banana peels, cocoa husks, and dead trunks), and preserving nearby forest patches considerably increases pollinator abundance (Winder and Silva, 1972; Young, 1982), pollination success and fruit set (Soria, 1978; Yong, 1982, 1983). On the other hand, use of insecticides has a deleterious effect on ceratopogonids (Entwistle, 1972), but the type, amount and periodicity of the application can alter the extent of the pollinator population damage (Ibrahim, 1990; Kwapong and Frimpong-Anin, 2013).



**Figure 2.** The cocoa flower visitors sorted by world region from the literature review. The main arthropod visitors are dipterans, followed by hymenopterans and homopterans. For more details, see Electronic Supplementary Material, Table S2.

Ceratopogonids Monitoring Methods

The observation in real time of ceratopogonids visiting cocoa flowers is particularly difficult. However, there are at least six direct and indirect observation methods that have been used to monitor ceratopogonid populations. The direct methods are (i) handpicking flower with pollinators and collection in small sealed glasses (Kaufmann, 1973, 1975; Winder, 1977; Adjaloo and Oduro, 2013), and (ii) motorized suction pump use (Frimpong et al., 2011; Kwapong and Frimpong-Anin, 2013). The indirect methods are (iii) UV-color pan, (iv) sticky traps (Frimpong et al., 2011; Tarmadja, 2015), (v) Mc Phail distilled flower traps (Frimpong et al., 2011), and (vi) flower sticky-glue traps (de Schawe et al., 2018).



**Figure 3.** The worldwide percentage of cocoa flower visitors sorted by families from the reviewed literature. Ceratopogonids represent only 40.7% of species visiting flowers, followed by ants (21.1%), bees and wasps (8.1%), and hemipterans (8.1%). Data of organisms not identified to species were not included. The flies group represents five families of Diptera (Chironomidae, Drosophilidae, Psychodidae, Sciaridae and Sphaeroceridae). For more details, see Electronic Supplementary Material, Table S2.

Direct methods are more time consuming, require full time human attention, in contrast to indirect survey methods covering on average 24 hours. However, indirect methods (besides flower sticky-glue traps) are not specific to cacao pollinator identity, fail to prove flower visitation, and can be only recommended for overall community

surveys that may or may not include cocoa pollinator species. Among the indirect methods, Frimpong et al. (2009) concluded that UV-color traps, particularly yellow, are the most efficient in terms of sampling effort, in contrast to McPhail distilled flower traps and motorized aspirators (direct method).

A more innovative direct recording device was implemented in Costa Rica consisting of a video-recording camera activated once an insect landed on a flower (Deheuvels et al., 2014). However, its bulky dimensions and the fact that only one flower is recorded at a time (pers. comm.), makes it substantially difficult for an extensive usage in large experimental trials.

### *Non-Ceratopogonid Species as Pollinator Agents*

Other invertebrates can also be important for cocoa pollination. In Bolivia, de Schawe et al. (2018) surveyed 1160 flowers and captured only 6 ceratopogonid specimens, representing 2% of all visitors. In contrast, hymenopterans (mainly small parasitic wasps) were the most abundant visitors with 118 individuals. In Ghana, Adjaloo and Oduro (2013) collected from direct (hand picking flowers) and indirect (pan and McPhail trap) monitoring methods 578 ceratopogonids representing 25% of the total insect abundance in the cocoa plantations. In Brazil, Winder (1977) collected only 81 ceratopogonids in 12,000 flowers surveyed. In Costa Rica, Bravo et al. (2011) reported 7.8% of ceratopogonid specimens collected from litter emerging traps. Therefore, it seems likely that ceratopogonids are not the only cocoa pollinators and that other insect groups can act as pollinators as well (Winder, 1978b; Deheuvels et al., 2014a, 2014b).

Our literature review shows that non-ceratopogonid species represent almost half of the flower visitors with groups such as ants and bees being the most representative (Fig. 3). Ant communities have been suggested to indirectly facilitate pollination by disturbing pollinators and enhancing their movement, leading to more frequent flower visitation and enhanced pollination success. Fruit set of cacao flowers was positively related to ant abundance and was significantly lower under experimental ant exclusion in Sulawesi, Indonesia (Wielgoss et al., 2014). This “pollinator-disturbance” mechanism enhancing pollination has also been observed in other studies (Altshuler, 1999; Greenleaf and Kremen, 2006; Philpott et al., 2006).



## Ecological Intensification, Yield Deficits, and Pollination Services

### *Climate Risk on Cocoa Production – Ecological Intensification as a Solution*

Current cocoa yield deficits are amplified by short-term production strategies focused on yield, but with long-term devastating environmental, social, and economic impacts for the industry, farmers, and national governments. In an attempt to close yield gaps and overcome climate risks, major cocoa enterprises and research institutions invest a great deal of resources in technological advances (WCF, 2016b; MARS, 2017). For example, plant-breeding programs for high-yielding varieties may close yield gaps and can also benefit regional economies and farmers through stable incomes (Clay, 2013; Franzen and Borgerhoff Mulder, 2007). This has been observed in the Ghanaian cocoa sector, where the distribution and adoption of new cocoa varieties and increased fertilizer use is associated with 42% of yield increments during 1980-2000. (Edwin and Masters, 2005). However, these programs may fail in the long term if the necessary practices, such as shade tree management, to buffer climate impacts are not implemented, or if the costs of adopting such strategies exceed the overall farm profit (Leakey, 2014). Thus, cost effective farm-based management adaptations to counteract climatic risks, to reduce yield gaps, and to secure farm income are urgently needed (Wanger et al., 2014; Schroth et al., 2016).

Ecological intensification to increase economic and ecological benefits through the enhancement of biodiversity and ecosystem services, such as pollination, may reduce yield gaps and enhance food security for small-scale farmers (Bommarco et al., 2013; Garibaldi et al., 2016). Even though cocoa agroforests are less biodiverse than pristine forests, they are much more diverse than intensified full-sun systems (Franzen and Borgerhoff Mulder, 2007). The high multi-strata arrangement due to shade trees and rich plant diversity, the pollinator dependency, and the small-scale farm management make cocoa agroforests a suitable candidate for ecological intensification (Donald, 2004; Clough et al., 2009; Deheuvels et al., 2012, 2014; Córdoba et al., 2013; Matey et al., 2013).

Surprisingly, our understanding is limited of how to (i) identify the major pollinator species, (ii) disentangle the direct or indirect role of ants in pollination and other abiotic agents (e.g. wind), (iii) design effective habitat improvements for pollinators, and (iv) quantify the yield gaps due to pollination limitation. Optimizing cocoa pollination alone appears to be a powerful ecological tool to increase potentially

double yield, but needs comprehensive experimental work (Falque et al., 1995; Bos et al., 2007a, 2007b; Groeneveld et al., 2010).

### *Management Strategies to Enhance Pollination Services*

Cocoa yield gaps are driven not only by nutrient limitation but also by pollination limitations (Bos et al., 2007a; Groeneveld et al., 2010). According to a small-scale study on hand pollination in Sulawesi, Indonesia, increasing pollination rates by 10% to 40% translates into benefits to farmers by increasing their attainable yields by up to 350kg/ha (Groeneveld et al., 2010). Hand pollination trials in Ghana also revealed that enhancing pollen deposition (pollination intensity) up to 238 grains increased the number of seeds per pods (Falque et al., 1995). These results suggest that pollination rate and pollinator limitations are major factors affecting cocoa yields. Pollination limitation could be minimized by the adoption of the following landscape and farm level management strategies.

#### *Landscape Level*

Natural forest ecosystems are highly important habitats for ceratopogonid populations (Kaufmann, 1975; Young, 1986). Studies on crop pollination services agree that beneficial organisms migrate from forest to adjacent agroforestry systems, and insect diversity and abundance at the farm level decrease when nearby forest patches are missing (Perfecto and Vandermeer, 2002; Klein et al., 2003, 2003b). A study on coffee pollinators in Indonesia found that pollinator diversity and abundance was significantly affected by forest distance, and predicted 18% yield losses in 20 years due to a continuous forest fragmentation and pollinator decline (Priess et al., 2007). In Costa Rica, Young et al., (1982) observed that pollinators rapidly colonize cocoa agroforests adjacent to forest remnants, as they provide necessary foraging resources and breeding places for their optimal development. Therefore, forest conservation is necessary for enhancing pollinator abundance, otherwise serious economic losses due to insufficient pollination services occur. However, a study in Ghana, where no significant relation between forest distance and pollinator abundance was observed (Frimpong et al., 2011), indicates the importance of further research to fully understand the effect of natural forest fragmentation on cocoa pollinator communities and their pollination services.

*Farm- level*

Management practices at the farm level include the enhancement of ceratopogonid-foraging resources and breeding habitats and the reduction of insecticide use by enhancing beneficial organisms through the conservation of diversified agroforestry systems (Clough et al., 2011; Tschardt et al., 2011). This is supported by Young (1982, 1983) who investigated the effect of shade tree species composition and rotting material on ceratopogonid diversity, abundance, and fruit set. His results suggest that fruit set increases in mixed-shade agroforests dominated by rotten banana plant residues due to the higher ceratopogonid populations than in systems with homogenous shade and full sun systems. Cocoa epiphytes communities may also provide substrates to pollinators, as suggested by Sporn et al. (2007), who found slightly lower fruit set in plantations where epiphytes were removed. The authors argue that these results could be due to pollinator decline through loss of breeding habitats.

Insecticide applications have negative effects on pollinators, although some chemical compounds are more harmful than others (Kwapong and Frimpong-Anin, 2013). Further investigation is required to provide farmers with appropriate recommendations about the best types of insecticides and adequate doses and timing of application. Shade agroforestry systems typically host a great diversity of herbivore predators, which allows minimal insecticide use (Klein et al., 2002; Tschardt et al., 2008; Wielgoss et al., 2013). Therefore, agroforestry system adoption is encouraged as a wildlife-friendly alternative providing pollination, and other important functional services, as well as competitive yields (Clough et al., 2011).

*Trade-off between Pollination and other Yield-affecting Factors*

The cocoa industry is strongly encouraged to find scientific solutions preventing yield losses associated with nutrient resource limitations (Edwin and Masters, 2002; Souza et al., 2009). However, plant-pollination limitation is a major fact that is still largely ignored (ICCO, 2017; MARS, 2017). Cocoa-pollination limitation as an important cause of yield deficits was suggested by only a few early researchers (McKelvie, 1960; Glendinning, 1972), but has recently gained more traction (Bos et al., 2007a, 2007b; Groeneveld et al. 2010; COCOAPOP, 2016). There is debate however, over whether cocoa fruit-set and seed production deficits are controlled by pollen limitation or only by resource limitation, and whether including pollination into the conventional water/nutrient resource management scheme will result in higher yields.

Empirical evidence from nutrient resource versus pollen resource limitation experiments in Sulawesi, Indonesia, leads to the conclusion that fruit losses are mainly driven by pollination deficits, rather than water and nutrient limitations, at least in this region (Groeneveld et al., 2010). However, fruit mortality can blur the potential of pollination services and strongly influence yields, as observed by Bos et al. (2007a) who reported 71% fruit losses caused mainly by early fruit abortion, followed by pest outbreaks and insect attacks, respectively. Moreover, Bos et al. (2007a) observed that fruit mortality incidence was strongly correlated with plant shade diversity. For instance, he found that fruit abortion was higher in natural- as opposed to planted-shade systems, while the opposite was observed for pathogen and pest attacks that were more common in planted-shade systems, but overall yields did not differ between systems. The authors argue that plant homogeneity in planted-, compared to natural-shade systems, would lead to reduced resilience within the plantation to cope with pest outbreaks. A surprising result, however, was that canopy cover had no effect on yields, suggesting that micro-climatic conditions, such as light intensity or humidity, were not limiting factors.

Although results of Bos et al. (2007a) discard light intensity as a limiting factor for fruit mortality, this must be taken with caution as other studies reveal that higher canopy cover affects yields, at least when canopy cover is >40% (Clough et al., 2011; Steffan-Dewenter et al., 2007). Clough et al. (2011) found a negative correlation between canopy cover and yield in the same study region, which may be due to differences in the number and identity of study sites. Therefore, maintaining a 40% shade cover of trees which promotes N-fixation, water retention and soil organic matter (Bos et al., 2007a; Isaac et al., 2007; Clough et al., 2011; Somarriba et al., 2013), as well as appropriate selection of cocoa cultivars (Somarriba et al., 2010; Schneider et al., 2016) are critical to optimize plantations for long-term yield stability and to balance trade-offs between pollination services and other yield-affecting factors.

## Future Research

Our literature review showed that pollination services by many and not just one invertebrate group is likely and may be enhanced through the preservation of natural forest habitats and the maintenance of highly diverse agroforestry systems with 40% canopy cover, which are more resilient to climate change than other agricultural systems. Many external and internal factors previously described, affect fruit set and yield along the cocoa production cycle, which need to be taken into account through

adequate management practices. These include intermediate canopy cover, a well-developed litter layer, landscape management with nearby forest patches, and the appropriate plant varieties to enhance beneficial organisms such as pollinators and soil nutrients, while minimizing climatic risks. In the following section, we highlight the emerging knowledge gaps and required research needs.

### *Knowledge Gaps and Research Needs*

#### *Pollinator Agents*

Our literature review suggests that cocoa is unlikely to rely on a single group of insects for pollination (Fig. 2, 3). While many recent studies on flower visitors recorded a large abundance of ants, and parasitic wasps, contrasting the very low proportion of ceratopogonids, no study has recorded pollination success dependent on the species' identity (Winder, 1978b; Adjaloo and Oduro, 2013; Deheuvels et al., 2014a, 2014b; de Schawe et al., 2018). This is one of the main challenges for disentangling the role of non-ceratopogonids in cocoa pollination, and more innovative study designs and sampling methods are necessary. Deheuvels et al. (2014a) developed an interesting monitoring solution, by video-recording real-time flower visitation, and similar monitoring methods are emerging in other research fields (Steen, 2017). Video recording has the potential to reveal insect visitation rate and duration, as well as behavior on the flower. However, further development adjustments are needed to make such monitoring methods logistically and economically viable for large-scale experiments.

#### *Self-incompatibility*

Self-incompatibility is one of the major determinants for pollination success (Lloyd and Schoen, 1992). While self-incompatibility is inherited in cocoa, studies have found that self-compatibility can also occur (Posnette, 1940; Chumacero de Schawe et al., 2013). Cocoa self-compatibility appears to occur only in a small proportion of analyzed cases (Glendinning, 1972). Researchers in Costa Rica successfully mapped self-compatible populations (Brown et al., 2008), and further genetic studies determined the genomic regions inducing mechanisms (Royaert et al., 2011). Future studies in other regions are needed to improve our understanding of self-compatibility in cocoa and will also help breeding self-compatible and high yielding cultivars (Phillips et al., 2009; Yamada et al., 2013). Furthermore, self-compatibility opens a wide spectrum for new potential pollinator agents, such as wind and the so far

neglected role of ants. The lack of evidence highlights the need for future investigations addressing pollinator agents and the pollination-resource limitation mechanism in self-compatible trees.

### *Pollination and Resource Limitation Affecting Yields*

Disentangling the role of cocoa pollination services and resource limitation on yields is crucial for coping with the current cocoa crisis associated to environmental and social pressures. While published evidence shows that nutrients and water availability are two important limiting factors for yield (Edwin and Masters, 2002), gaps of knowledge still exist on how enhancing pollination services can help minimizing these yield gaps. Despite important efforts to increase productivity by tackling resource limitation, less attention is given to the role of pollinators and increasing pollination success (ICCO, 2017; MARS, 2017). At least in specific geographic regions such as Sulawesi (Indonesia), low productivity of cocoa is mediated by pollination rather than soil nutrient limitation (Bos et al., 2007a, 2007b; Groeneveld et al., 2010). Small-scale hand pollination experiments show that increasing pollination rate by 10 to 40% can double yield, translating to direct economic benefits for the smallholders (Groeneveld et al., 2010), but larger experiments in other producing regions are necessary to support these results.

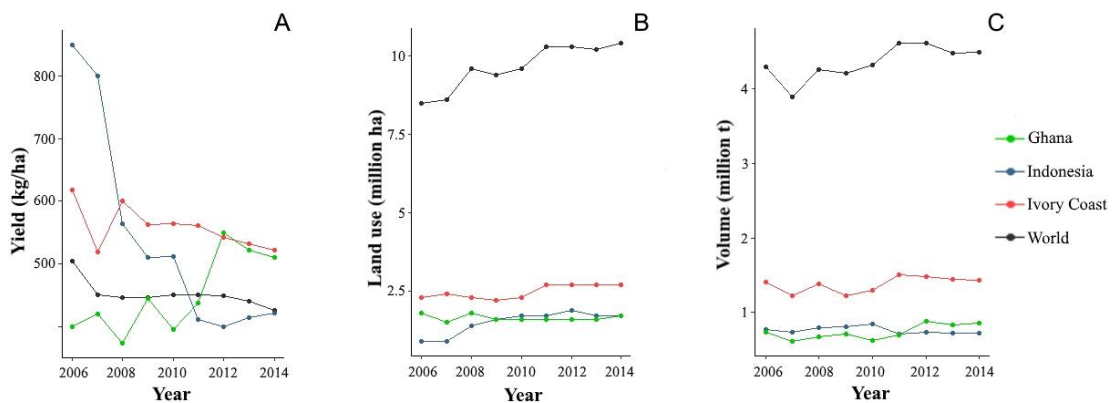
Furthermore, we know that only less than <10% of total flowers in a tree are successfully pollinated in natural conditions (Falque et al., 1995), which suggests inefficient transport and deposition of pollen grains to secure fruit set (Ashman et al., 2004). The factors driving pollinators' inefficiency can be diverse, including environmental changes, inadequate management or traits of the pollinator species (Young, 1982, 1983; Ashman et al., 2004). Knowledge gaps limit our capability for a comprehensive conclusion and management recommendation. Further studies are needed to better understand the still neglected role of pollinators and the driving forces of pollination services in cocoa production to develop successful management strategies mitigating cocoa yield deficits.

### *Environmental Drivers and Management of Pollination and Cocoa Yields*

Natural forest conservation is crucial for maintaining pollination services of many pollinator-dependent crops, such as cocoa (Kaufmann, 1975; Young, 1986; Ricketts, et al., 2004). Worldwide, the 35% of the food production comes from crops directly affected by pollination (Klein et al., 2007), and losing this important functional service

will have tremendous economic impacts (Losey and Vaughan, 2006; Gallai et al., 2009). In addition, forest conversion to agriculture increases vulnerability to climate change (Tilman et al., 2001; Bonan et al., 2008). Recent evidence emphasizes the negative effects of prolonged droughts and temperature increases on cocoa yield, land use suitability, and production variability in West Africa (Läderach et al., 2013; Schroth et al., 2016).

Despite worldwide rise in cocoa prices, farmers are failing to maintain or increase farm productivity to reduce their poverty. Seeking for livelihood improvements, smallholders tend to either switching to more profitable production systems, or leave the dwelling and migrate to bigger cities (Rice and Greenberg, 2000; Franzen and Borgerhoff Mulder, 2007; Tschardt et al., 2012).



**Figure 4.** The cocoa bean production worldwide (black), in Ivory Coast (red), Ghana (green), and Indonesia (blue) over the period 2006-2014 (FAOSTATS, 2017). Considerable yield losses at the farm level are observed worldwide with Indonesia and Ivory Coast being most affected (A). Despite a regular land use conversion (B), the overall bean volume is stagnant (C), while data from 2014-2016 show that volumes continued declining (see Electronic Supplementary Material, Table S2).

All these environmental, social and economic constraints only put cocoa production at a higher risk. To increase economic profits, farmers maintaining cocoa production tend to reduce shade in their plantations at the cost of the traditionally highly diverse and resilient shade systems, although this conversion should negatively affect pollinator diversity and abundance (Young, 1982, 1983; Tschardt et al., 2011).

Surprisingly, we ignore how climatic constraints affect insect pollinators communities and pollination services on cocoa, despite the evidence that rising temperatures impact survival, development, and distribution of many beneficial functional groups, such as parasitoids and predators (Bale et al., 2002; Hence et al., 2007), as well as pollinators (Potts et al., 2010). Further studies need to address how landscape, environmental conditions, and agroforestry management impact on cocoa

pollinators. For instance, further investigation of the effect of plantation distance to forest and the microclimatic variations within different shade canopy covers on ceratopogonid population dynamics will help improving agroforestry management for enhancing pollinator and securing pollination services.

## Conclusions

In our review we found that external (non-plant regulated) and internal (plant regulated) mechanisms strongly affect (i) flowering, (ii) pollination success, (iii) fruit development and (iv) cocoa yield. These mechanisms are driven by environmental, economic and social factors that all together put cocoa productivity at a risk. The available empirical evidence suggests that the adoption of management practices at the landscape and farm level can enhance pollinators and pollination services. However, further research is necessary to cover knowledge gaps on the pollination role played by non-ceratopogonid insects, the effect of management intensification (e.g. shade cover, litter management, inputs used) on pollinator population and how other trade-off may blur the potential benefits of pollination services. For instance, the effect of rising temperature and increasing droughts, associated with climate change, on cocoa pollinators have not been addressed so far, despite the clear evidence showing negative effects of climate change on multiple insect functional groups. Improving our knowledge on cocoa pollination based on scientific research will help us elucidating the until to the date neglected role of pollinators on securing cocoa yields under climate change.

The current global picture shows an increasing chocolate consumption in countries such as China, Indonesia and Brazil that will likely enhance the pressure on cocoa yields further, regardless of exacerbating climate risks. Cocoa stakeholders therefore face the challenge to act quickly in favor of increasing productivity. The industry sector is in need to close yield gaps induced by considerable yield losses associated with climate change and inappropriate strategies for crop adaptation (see Electronic Supplementary Material, Table S2) (Läderach et al., 2013; Schroth et al., 2016). The FAO reported continuous farm-level yield declines since 2006, when yields decreased from 505.1kg/ha to 426.5kg/ha in 2014, a 15.6% loss (FAOSTATS, 2017) (Fig. 4). Worldwide, production has dropped by 3.3% from 2013 to 2015, and estimates for 2016 predict a further 1.8% decline from 2015 (ICCO, 2016a). In West Africa, which contributes 70% of the global cocoa production, yields are predicted to decline by 2% annually (Kongor et al., 2016). The three main producer countries are



the most affected with losses of over 50 thousand tonnes of cocoa beans for each, the Ivory Coast and Ghana, and 75 thousand tonnes for Indonesia from 2013 to 2016 (see Electronic Supplementary Material, Table S2) (ICCO, 2016a).

It is likely that over the next years, cocoa production will continue to increase only at the cost of forest and traditional shade system, reducing the resilience to climatic risks. Climate change effects in Ivory Coast and Ghana are likely to have considerable negative impacts on cocoa production. This suggests that farmers have to encroach forested areas to maintain production, switch to more profitable crops, or migrate to metropolitan areas (Rice and Greenberg, 2000; Franzen and Borgerhoff Mulder, 2007; Tschardt et al., 2012; Läderach et al., 2013; Schroth et al., 2016). In general, governments, industry and farmers must engage in partnerships to master the challenges ahead based on robust scientific evidence. A step forward is The World Cocoa Conference in 2016 announcing public-private collaborations to tackle the effects of climate change on cocoa production and farmer livelihoods (Clough et al., 2009; WCF, 2016b). Other additional strategies are the creation of incentives for farmers and industry to adopt ecological intensification practices. These incentives can be promoted by a premium price for ecologically certificated cocoa (Rice and Greenberg, 2000; Franzen and Burgorhoff, 2007; Clough et al., 2011; Tschardt et al., 2015), as the consumption of sustainable chocolate products is increasing by 2 to 3% annually (Sustainable Cocoa Initiative, 2017). Furthermore, diversified agricultural systems may be the way forward for the adoption of ecological intensification practices. Therein, pollination of cocoa trees is likely to play a major or even key role, but understanding of cocoa pollinators, of the high flower-fruit ratio in cocoa and the associated drivers of cocoa yield requires further investigation. Given the great importance of cocoa as a commodity, it is surprising how little is known of how pollination can be maintained and enhanced under global change.

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Electronic Supplementary Material



**Figure S1.** Since quality produced is rarely controlled by local buyers, small-scale farmers in Napu, Sulawesi generally harvest healthy and diseased fruits together as a strategy to increase raw cocoa volume.

**Table S1.** List of the total papers reviewed. Abbreviations: A = available for review; NA = not available for review

No.	Author	Year	Country/Region	Study type	Title	Availability
1	Adjaloo and Oduro	2013	Ghana	Field	Insect assemblage and the pollination system of cocoa ( <i>Theobroma cacao</i> L.)	A
2	Adjaloo et al.	2012	Ghana	Field	Spatial distribution of insect assemblage in cocoa farms in relation to natural forest	A
3	Adu-Ampomah et al.	1991	Ghana	Lab	Use of irradiated pollen as mentor pollen to induce self-fertilization of two self-incompatible Upper Amazon cacao clones	A
4	Ameyaw et al.	2013	Ghana	Lab	Investigation on cacao swollen shoot virus (cssv) pollen transmission through cross-pollination	A
5	Anikwe et al.	2007	Nigeria	Field	Studies of the ant-plant mutualism in the Nigerian cocoa agroecology	A
6	Baker and Hasenstein	1997	Puerto Rico	Lab	Hormonal changes after compatible and incompatible pollination in <i>Theobroma cacao</i> L.	A
7	Bastide et al.	1993	Ivory Coast	NA	Production of cocoa hybrid seeds in the cote-divoire – various hand pollination trials	NA
8	Beek et al.	1977	Costa Rica	NA	Some factors affecting fat-content in cacao beans ( <i>Theobroma cacao</i> L.), with emphasis on effect of pollinator parent	NA
9	Bisseleua et al.	2009	Cameroon	Field	Biodiversity conservation, ecosystem functioning, and economic incentives under cocoa agroforestry intensification	A
10	Bouharmont	1960	Congo	Lab	Cytological investigations on fruiting and incompatibility in cacao	NA
11	Boussard	1981	NA	Review	Pollinisation. Arbres fruitierset cacaoyers	NA
12	Bos et al.	2007a	Indonesia	Field	Shade tree management affects fruit abortion, insect pests and pathogens of cacao	A
13	Bos et al.	2007b	Indonesia	Field	Caveats to quantifying ecosystem services: fruit abortion blurs benefits from crop pollination	A
14	Bravo et al.	2011	Costa Rica	Field	Factores que afectan la abundancia de insectos polinizadores del cacao en sistemas agroforestales	A
15	Brew et al.	1993	Ghana	NA	Preliminary-observations on the classification of <i>Forcipomyia</i> midges (Diptera, Ceratopogonidae) of Ghana with special reference to species involved in the pollination of cocoa ( <i>Theobroma cacao</i> L.)	NA
16	Bystrak and Wirth	1978	North America	Review	The North American species of <i>Forcipomyia</i> , subgenus <i>Euprojoannisia</i> (Diptera: Ceratopogonidae)	A

Table S1- Continued

No.	Author	Year	Country/Region	Study type	Title	Availability
17	Chumacero de Schawe	2013	Bolivia	Doctoral thesis	Pollination ecology and spatial genetic population structure of wild and cultivated species of cacao ( <i>Theobroma</i> ) in Bolivia	A
18	Chumacero de Schawe	2016	Bolivia	Field	Abundance and diversity of flower visitors on wild and cultivated cacao ( <i>Theobroma cacao</i> L.) in Bolivia	A
19	Cilas et al.	1987	Togo	NA	Study of the variations in natural pollination of cocoa tree in Togo – influence on the pod seed filling rate	NA
20	Cilas et al.	2010	Latin America	Lab	Relations between several traits linked to sexual plant reproduction in <i>Theobroma cacao</i> L.: number of ovules per ovary, number of seeds per pod, and seed weight	A
21	Cope	1939	NA	NA	Compatibility and fruit-setting in cacao. Eight Annual Report on Cacao Research for 1938 ICTA	NA
22	Cope	1940	NA	NA	Agents of pollination in cacao. 9 <sup>th</sup> Annual Report of Cacao Research for 1939	NA
23	Cope	1940	NA	NA	Studies in the mechanism of self-incompatibility in cacao I and II. 9 <sup>th</sup> annual report	NA
24	Córdoba et al.	2013	Panama	NA	Pollinators, pollination and cocoa production potential in agroforestry systems of Boca del Toro, Panama	NA
25	Cortez	2009	Ghana	Field	Pollination efficiency in the major trunk and branch axis of the cauliflorous <i>Theobroma Cacao</i> L.	A
26	de Schawe et al.	2013	Bolivia	Field	Gene flow and genetic diversity in cultivated and wild cacao ( <i>Theobroma cacao</i> ) in Bolivia	A
27	de Souza and Venturieri	2010	Brazil	Field	Floral biology of Cacauhy ( <i>Theobroma speciosum</i> – Malvaceae)	A
28	Decazy et al.	1985	Ivory Coast	NA	Long-term effects of insecticide treatment on pollination conditions and cocoa tree production in the ivory-coast	NA
29	Dereffeye et al.	1980	NA	NA	Study on the pollination of the cocoa tree on the basis of insect traffic – mathematical-model and simulation	NA
30	Donald	2004	World	Review	Biodiversity impacts of some agricultural commodity production systems	A
31	Edwards	1973	Ghana	NA	Pollination studies on Upper Amazon cocoa clones in Ghana in relation to production of hybrid seed	NA
32	Efron et al.	2005	Papua New Guinea	Field	Analysis of the factors affecting yield and yield variability in the SG2 cocoa hybrid variety in Papua New Guinea	A
33	Entwistle	1972	World	Review	Pests of cocoa	A

Table S1- Continued

No.	Author	Year	Country/Region	Study type	Title	Availability
34	Falque et al.	1992	Ghana	Lab	Gamma-irradiation of cacao ( <i>Theobroma cacao</i> L.) pollen: effect on pollen grain viability, germination and mitosis and on fruit set	A
35	Falque et al.	1994	Ghana	Lab	Pod and seed development and phenotype of the M1 plants after pollination and fertilization with irradiated pollen in cacao ( <i>Theobroma cacao</i> L.)	A
36	Falque et al.	1995	Ivory Coast	Lab	Effect of pollination intensity on fruit and seed set in cacao ( <i>Theobroma cacao</i> L.)	A
37	Falque et al.	1996	Ivory Coast	Lab	Comparison of two cacao ( <i>Theobroma cacao</i> L.) Clones for the effect of pollination intensity on fruit set and seed content	A
38	Forbes and Northfield	2016	Australia	Field	Increased pollinator habitat enhances cacao fruit set and predator conservation	A
39	Ford and Wilkinson	2012	UK	Field	Confocal observations of late-acting self-incompatibility in <i>Theobroma cacao</i> L.	A
40	Frimpong et al.	2009	Ghana	Field	Dynamics of cocoa pollination: tools and applications for surveying and monitoring cocoa pollinators	A
41	Frimpong et al.	2011	Ghana	Field	Dynamics of insect pollinators as influenced by cocoa production systems in Ghana	A
42	Frimpong-Anin et al.	2014	Ghana	Field	Structure and stability of cocoa flowers and their response to pollination	A
43	Glendinning	1971	World	Review	Natural pollination of cocoa	A
44	Groeneveld et al.	2010	Indonesia	Field	Experimental evidence for stronger cacao yield limitation by pollination than by plant resources	A
45	Hurtado	1960	Peru	Field	The effects of field pollination on cocoa production	NA
46	Irizarry and Rivera	1998	Puerto Rico	NA	Early yield of five cacao families at three locations in Puerto Rico.	NA
47	Kaufmann	1973	Ghana	Field	Preliminary observations on Cecidomyiid midge and its role as a cocoa pollinator in Ghana	A
48	Kaufmann	1974	NA	NA	Cocoa pollination by males of <i>Forcipomyia-squamipennis</i> (Diptera-Ceratopogonidae) in Ghana	NA
49	Kaufmann	1974	Ghana	Field	Behavioral biology of a cocoa pollinator, <i>Forcipomyia inornatipennis</i> (Diptera: Ceratopogonidae) in Ghana	A
50	Kaufmann	1975	Ghana	Field	Ecology and behavior of cocoa pollinating Ceratopogonidae in Ghana, W. Africa	A
51	Kaufmann	1975	Ghana	Field	Studies on the ecology and biology of a cocoa pollinator, <i>Forcipomyia squamipennis</i> I. & M. (Diptera, Ceratopogonidae), in Ghana	A
52	Kaufmann	1975a	Ghana	NA	Ecology and behavior of cocoa pollinating Ceratopogonidae in Ghana, West Africa	NA

Table S1- Continued

No.	Author	Year	Country/Region	Study type	Title	Availability
53	Kaufmann	1975b	Ghana	NA	Efficient, new cocoa pollinator, <i>Lasioglossum sp</i> (Hymenoptera – Halictidae) in Ghana, West-Africa	NA
54	Klein	2008	World	Review	Advances in pollination ecology from tropical plantation crops	A
55	Kwapong and Frimpong-Anin	2013	Ghana	Field	Pollinator management and insecticide usage within cocoa agroecosystem in Ghana	A
56	Kwapong et al.	2014	Ghana	Field	Pollination and yield dynamics of the cocoa tree	A
57	Lachenaud	1994	Ivory Coast	NA	Variations in the number of beans per pod in <i>Theobroma cacao</i> L. in the Ivory-Coast. 1. The role of pollen	NA
58	Lachenaud	1995	Ivory Coast	NA	Variations in the number of beans per pod in <i>Theobroma cacao</i> L. in the Ivory-Coast. 2. Pollen germination, fruit setting and ovule development	NA
59	Lucas	1981a	Togo	NA	Study of cocoa tree pollination conditions in Togo	NA
60	Lucas	1981b	NA	NA	Influence of insecticide treatments against cocoa tree mirids on pollination conditions	NA
61	LLano	1947	NA	NA	Cacao growing	NA
62	McKelvie	1955	Ghana	NA	Cherelle wilt. Annual Report West African Research Institute	NA
63	McKelvie	1956	Ghana	Field	Cherelle wilt of cacao I. Pod development and its relation to wilt	A
64	McKelvie	1960	Ghana	Field	Cherelle wilt of cacao II. Wilt in relation to yield	A
65	Mossu and Paulin	1981	NA	NA	Influence of flowering and pollination on cocoa tree yields – mathematical relationships between experimental-data – yield equation	NA
66	Müntzing	1947	Ecuador	Field	Some observations on pollination and fruit setting in Ecuadorian cacao	A
67	Omolaja	2009	Nigeria	Field	Rainfall and temperature effects on flowering and pollen productions in cocoa	A
68	Paravais et al.	1977	NA	NA	Observations on free pollination in <i>Theobroma-cacao</i> – mathematical-analysis of data and modeling	NA
69	Paulin	1981	NA	NA	Contribution to the study of the flower biology of the cocoa tree – result of artificial pollinations	NA
70	Paulin et al.	1983	NA	NA	Study on seasonal-variations of pollination and fructification conditions in a cocoa tree plantation	NA
71	Pineda	1953	Colombia	Field	Problemas de incompatibilidad en el cacao	A
72	Posnette	1942	West Africa	NA	Natural pollination of cocoa <i>Theobroma leicocarpa</i> Bern. On the Golden Coast. I	NA



Table S1- Continued

No.	Author	Year	Country/Region	Study type	Title	Availability
73	Posnette	1942	West Africa	NA	Natural pollination of cocoa <i>Theobroma leiocarpa</i> Bern. On the Golden Coast. II	NA
74	Posnette	1950	West Africa	NA	The pollination of cacao in the Gold Coast	NA
75	Reffye et al.	1978	NA	NA	Influence of pollination hazards on the yields of the cocoa tree – mathematical-model and simulation.	NA
76	Royaert et al.	2011	Costa Rica	Lab	Identification of marker-trait associations for self-compatibility in a segregating mapping population of <i>Theobroma cacao</i> L.	A
77	Sabatier	2013	Indonesia	Modelling	Production and robustness of a cacao agroecosystem: effects of two contrasting types of management strategies	A
78	Sari and Susilo	2015	Indonesia	Field	Phenology of flowering and pod maturity on Some cocoa ( <i>Theobroma cacao</i> L.) clones	A
79	Saunders	1959	NA	Methodology	Methods for studying Forcipomyia midges, with special reference to cacao-pollinating species (Diptera, Ceratopogonidae)	NA
80	Sereno et al.	2006	Brazil	Primary	Genetic diversity and natural population structure of cacao ( <i>Theobroma cacao</i> L.) From the Brazilian Amazon evaluated by microsatellite markers	A
81	Soetardi	1950	NA	NA	The importance of insects in the pollination of <i>Theobroma cacao</i> L.	NA
82	Somarriba et al.	2010	Costa Rica	Manual	Sexual reproduction of cacao	A
83	Soria et al.	1978	Brazil	Field	Breeding places and sites of collection of adults of <i>Forcipomyia</i> spp, midges (Diptera, Ceratopogonidae) in cacao plantations in Bahia, Brazil: a progress report	A
84	Soria and Wirth	1977	NA	NA	Life-cycles of cacao pollinating midges ( <i>Forcipomyia</i> spp) and some notes on larval behavior in laboratory	NA
85	Sporn et al.	2007	Indonesia	Field	Is productivity of cacao impeded by epiphytes? An experimental approach	A
86	Tarmadja	2015	Indonesia	Field	The cacao flower visitor insects diversity and their potential as pollinators	A
87	Tscharntke et al.	2011	World	Review	Multifunctional shade-tree management in tropical agroforestry landscapes – a review	A
88	Valle et al.	1990	Brazil	Primary	Energy costs of flowering, fruiting, and cherelle wilt in cacao	A
89	Voelcker	1940	Nigeria	NA	The degree of cross pollination in cacao in Nigeria	NA
90	Wanger	2014	World	Opinion	Pollination curbs climate risk to cocoa	A
91	Wielgoss	2015	Indonesia	Field	Interaction complexity matters: disentangling services and disservices of ant communities driving yield in tropical agroecosystems	A
92	Winder	1972	NA	NA	Cacao pollination – microdiptera of cacao plantations and some of their breeding places	NA

Table S1- Continued

No.	Author	Year	Country/region	Study type	Title	Availability
93	Winder	1977	Brazil	Field	Field observations on Ceratopogonidae and other Diptera: Nematocera associated with cocoa flowers in Brazil	A
94	Winder	1977	NA	NA	Biology, activity, and behavior of cocoa pollinators	NA
95	Winder	1978	Brazil	Field	The role of non-dipterous insects in the pollination of cocoa in Brazil	A
96	Winder	1978	World	Review	Cocoa flower Diptera; their identity, pollinating activity and breeding sites	A
97	Winder and Silva	1972	Brazil	Field	Cacao pollination: microdiptera of cacao plantations and some of their breeding places	A
98	Wirth	1991	Brazil	NA	New and little-known species of <i>Forcipomyia</i> (Diptera, Ceratopogonidae) associated with cocoa pollination in Brazil	NA
99	Wood and Lass	1985	World	Review	Cocoa	A
100	Young	1981	Costa Rica	Field	The ineffectiveness of the stingless bee <i>Trigona jaty</i> (Hymenoptera: Apidae: Meliponinae) as apollinator of cocoa ( <i>Theobroma cacao</i> L.)	A
101	Young	1982	Costa Rica	Field	Effects of shade cover and availability of midge breeding sites on pollinating midge populations and fruit set in two cocoa farms	A
102	Young	1982	Costa Rica	Field	Pollen-collecting by stingless bees on cacao flowers	A
103	Young	1983	Costa Rica	Field	Seasonal differences in abundance and distribution of cocoa-pollinating midges in relation to flowering and fruit set between shaded and sunny habitats of the ILa Lola cocoa farm in Costa Rica	A
104	Young	1985	Central America	NA	Studies of Cecidomyiid midges (Diptera,C) as cocoa pollinators ( <i>Theobroma cacao</i> L.) in Central-America	NA
105	Young	1986	Costa Rica	Field	Habitat differences in cocoa tree flowering, fruit-set, and pollinator availability in Costa Rica	A
106	Young et al.	1989	Costa Rica	Field	Pollination biology of <i>Theobroma</i> and <i>Herrania</i> (Sterculiaceae) — 3. Steam-distilled floral oils of <i>Theobroma</i> species as attractants to flying insects in a Costa Rican cocoa plantation	A
107	Young	1989	Costa Rica	NA	Pollination biology of <i>Theobroma</i> and <i>Herrania</i> (Sterculiaceae). 4. Major volatile constituents of steam-distilled floral oils as field attractants to cacao-associated midges (Diptera, Cecidomyiidae and Ceratopogonidae) in Costa-Rica	NA
108	Young and Severson	1994	Mexico	Field	Comparative analysis of steam distilled floral oils of cacao cultivars ( <i>Theobroma cacao</i> L., Sterculiaceae) and attraction of flying insects: implications for a <i>Theobroma</i> pollination syndrome	A

**Table S2.** List of species per arthropod group (Class or Order) found on cocoa flowers. NA is for samples not identified to species.

Region	Group	Family	Species				
Central America	Diptera	Cecidomyiidae	NA				
		Ceratopogonidae	<i>Atrichopogon fusculus</i> <i>Ceratopogon spp.</i> <i>Culicoides diabolicus</i> <i>Dasyhelea spp.</i> <i>Forcipomyia nodosa</i> <i>Stilobezzia spp.</i>				
		Chironomidae	NA				
		Drosophilidae	NA				
		Psychodidae	NA				
		Sphaeroceridae	NA				
		Hymenoptera	Apidae	<i>Trigona jati</i>			
		Caribbean	Diptera	Ceratopogonidae	<i>Culicoides imicola</i> <i>Dasyhelea theobranatis</i> <i>Forcipomyia falcifera</i> <i>Forcipomyia mortuifolii</i> <i>Forcipomyia nana</i> <i>Forcipomyia quasiingrami</i> <i>Forcipomyia setigera</i> <i>Forcipomyia venusta</i> <i>Stilobezzia limai</i> <i>Stilobezzia tomensis</i> <i>Forcipomyia stylifera</i>		
				Arachnida	NA		
				Coleoptera	Staphylinidae Anthribidae Chrysomelidae	NA NA <i>Colaspis ornata</i>	
Collembola	NA			NA			
Diptera	Cecidomyiidae			<i>Coquillettiomyia spp.</i> <i>Clinodiplosis spp.</i> <i>Lestodiplosis spp.</i>			
	Ceratopogonidae			<i>Atrichopogon spp.</i> <i>Culicoides glabellus</i> <i>Culicoides paraensis</i> <i>Dasyhelea borgmeieri</i> <i>Dasyhelea williamsi</i> <i>Forcipomyia brachyrhynchus</i> <i>Forcipomyia bromeliae</i> <i>Forcipomyia fuliginosa</i>			
	South America			Diptera	Ceratopogonidae	<i>Forcipomyia harpegonata</i> <i>Forcipomyia genualis</i> <i>Forcipomyia pictoni</i> <i>Forcipomyia quasiingrami</i> <i>Forcipomyia jipajapae</i> <i>Forcipomyia lesliei</i> <i>Stilobezzia spp.</i> <i>Smittia sp.</i>	
					Chironomidae	NA	
					Psychodidae	NA	
					Sciaridae	NA	
					Homoptera	Aphididae	<i>Toxoptera aurantii</i>
						Cicadellidae	<i>Xestocephalus ancorifer</i>
Fulgoridae						NA	
Margarodidae		<i>Iceryni spp.</i>					
Membracidae		<i>Bolbonota pictipennis</i>					
Pentatomidae		NA					
Pseudococcidae	<i>Planococcus citri</i>						
Hymenoptera	Apidae	<i>Eulaema meriana</i>					
	Cynipidae	<i>Trybliographa sp.</i>					
	Bethylidae	<i>Dissomphalus sp.</i>					
	Eulophidae	<i>Tetrastichus sp.</i>					

Table S2- Continued

Region	Group/Order	Family	Species
South America	Hymenoptera	Formicidae	<i>Azteca</i> sp.
			<i>Brachymyrmex heeri</i>
			<i>Brachymyrmex pictus</i>
			<i>Camponotus abdominalis</i>
			<i>Camponotus senex</i>
			<i>Crematogaster curvispinosa</i>
			<i>Brachymyrmex heeri</i>
			<i>Crematogaster evallans</i>
			<i>Crematogaster limata</i>
			<i>Crematogaster nigropilosa</i>
			<i>Crematogaster parabiatica</i>
			<i>Crematogaster sumichrasti</i>
			<i>Brachymyrmex heeri</i>
			<i>Iridomyrmex</i> sp.
			<i>Labidus praedator</i>
			<i>Monomorium floricola</i>
			<i>Nylanderia</i> sp.
			<i>Pheidole</i> sp.
			<i>Rogeria subarmata</i>
			<i>Solenopsis</i> sp.
<i>Tapinoma melanocephalum</i>			
<i>Wasmannia rochai</i>			
		Platygasteridae	<i>Synopeas</i> sp.
		Scelionidae	<i>Telenomus</i> sp.
	Neuroptera	NA	NA
	Lepidoptera	Geometridae	NA
	Thysanoptera	Thripidae	<i>Frankliniella parvula</i>
			<i>Frankliniella fulvipennis</i>
			<i>Selenothrips rubrocinctus</i>
South East Asia	Diptera	Cecidomyiidae	NA
		Ceratopogonidae	<i>Dasyhelea</i> spp.
			<i>Forcipomyia theobroma</i>
	Hymenoptera	Formicidae	NA
South Asia	Diptera	Ceratopogonidae	<i>Forcipomyia theobroma</i>
West Africa	Coleoptera	NA	NA
		Diptera	Cecidomyiidae
		Ceratopogonidae	<i>Cecidomyiids</i> spp.
			<i>Atrichopogon</i> spp.
			<i>Culicoides citroneus</i>
			<i>Forcipomyia ashantii</i>
			<i>Forcipomyia castanea</i>
			<i>Forcipomyia basendjiorum</i>
			<i>Forcipomyia biannulata</i>
			<i>Forcipomyia brincki</i>
			<i>Forcipomyia cacaoi</i>
			<i>Forcipomyia inornatipennis</i>
			<i>Forcipomyia clastrieri</i>
			<i>Forcipomyia indecora</i>
			<i>Forcipomyia fuliginosa</i>
			<i>Forcipomyia hirsuta</i>
			<i>Forcipomyia litoraurea</i>
			<i>Forcipomyia lepidota</i>
			<i>Forcipomyia macronyx</i>
			<i>Forcipomyia randensoides</i>
			<i>Forcipomyia squamipennis</i>
			<i>Stilobezzia spirogyrae</i>
			<i>Stilobezzia africana</i>
		Drosophilidae	<i>Scaptodrosophila triangulifer</i>
	Homoptera	Aphididae	<i>Toxoptera</i> spp.
	Hymenoptera	Apidae	<i>Hypotrigona aurajoi</i>
			<i>Liotrigona parvula</i>
		Formicidae	<i>Camponotus acvapimensis</i>
			<i>Crematogaster depressa</i>
			<i>Crematogaster calriventris</i>
			<i>Crematogaster africana</i>
		Vespidae	<i>Pheidole megacephala</i>
			NA

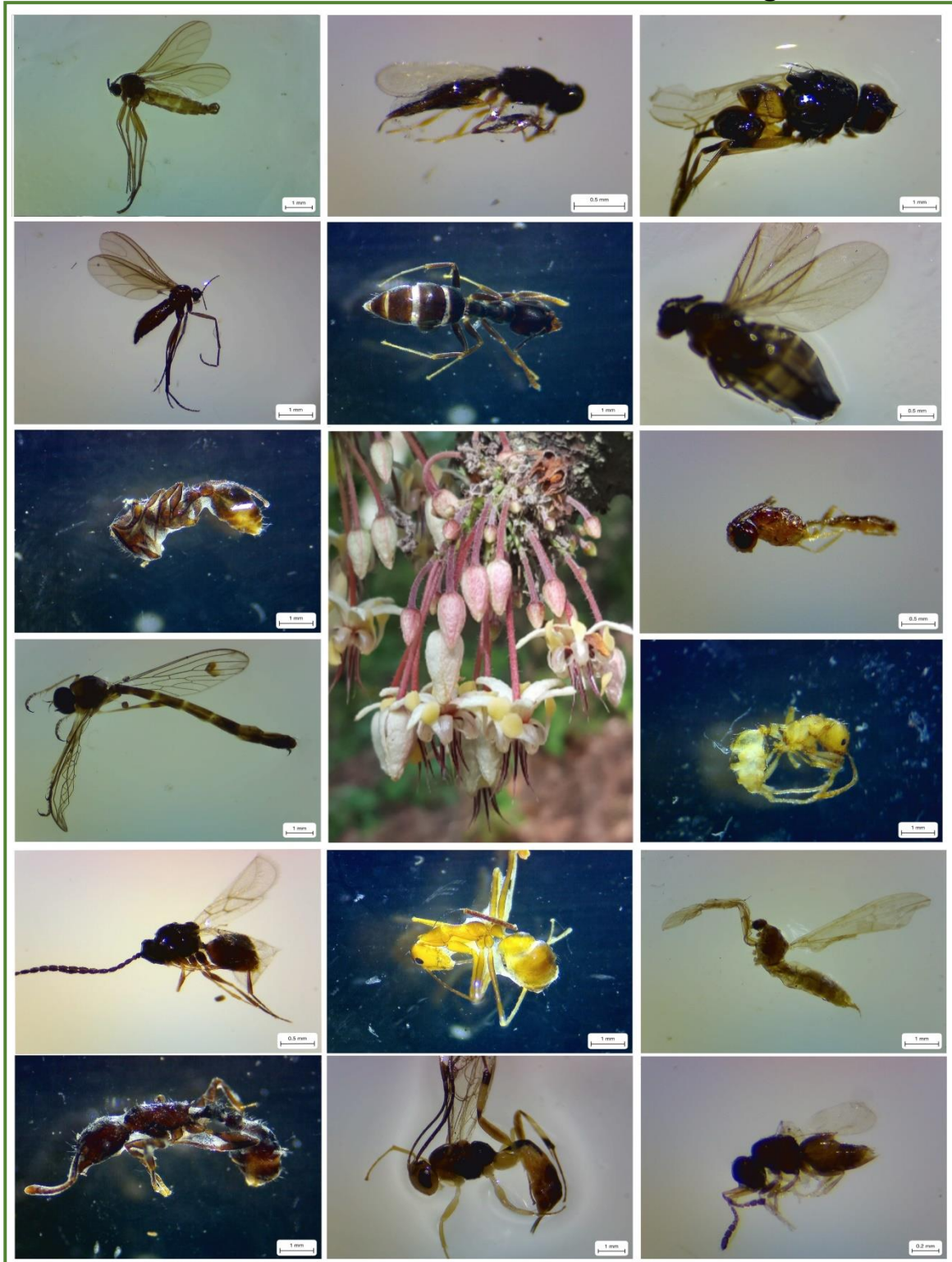
**Table S3.** The current yields (in thousands of tons) of cocoa beans in the main producing countries from 2014-2016. The balance is the percentage of deficits in 2016 compared with 2014 yields<sup>1</sup>.

	Estimates		Forecasts	Balance (%)
	2014	2015	2016	2014-2016
Ivory Coast	1746	1796	1690	-3.2
Ghana	897	740	840	-6.4
Ecuador	234	250	230	-1.7
Brazil	228	230	210	-7.9
Indonesia	375	325	300	-20.0
World	4372	4230	4154	-5.0

<sup>1</sup>Table modified from ICCO Quarterly Bulletin of Cocoa Statistics, Vol. XLII, No. 1; Cocoa

## Chapter II

### Landscape Complexity and Farm Management Moderate Cocoa Flower Visitors in Indonesian Agroforests



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## Abstract

Cocoa (*Theobroma cacao* L.) is a self-incompatible and pollinator-dependent tree crop. Despite cocoa is one of the major trade commodities globally, there are important knowledge gaps about flower visitor and pollinator identity, farm and landscape management effects on their populations, and yield improvements through pollination services. Here we investigate how farm and landscape-level management affects cocoa flower visitors in a correlative and an experimental approach. We analyzed flower visitation of 11,664 cocoa flowers in 18 (correlative approach) and 24 (experimental approach) farms of Central Sulawesi, Indonesia. In the correlative approach, we focused on the relation of distance to natural forest, complex habitats surrounding the farms, canopy cover, amount of soil leaf-litter, and the ratio of flower abundance to the number and richness of cocoa flower visitors. In the experimental approach, we focused on yields and a leaf litter manipulation, because increase in leaf-litter management has been suggested to enhance flower visitor populations. We found that ants and dipterans are the main flower visitors, and although ceratopogonids are presumed to be the main cocoa pollinators, we did not capture any. In the correlative approach, complex habitats surrounding the farms (forests and shaded cocoa agroforests) and a canopy cover above 40% enhanced ant and dipteran richness and abundance, whereas forest distance had no effect. Again in the experimental approach, complex habitats surrounding the farm rather than farm-level leaf-litter manipulation increased dipteran and ant species richness and abundance. Yield increase was related to higher dipteran abundance.

*Synthesis and Application:* Maintaining landscapes with multi-strata vegetation surrounding the farm, a 40% canopy cover, and minimum leaf-litter of  $>0.6\text{kg/m}^2$  can enhance species richness and abundance of cocoa flower-visiting dipterans and ants, which appear to be important in sustaining cocoa yields. Future research needs to better understand the contribution of different species groups to pollination. The role of the different dipteran species as well as the direct or indirect contribution of ants is still unknown. Only a better understanding of flower visitor identity and their ecology will help to give reliable management recommendations for farmers to improve their yields through cocoa pollination services.

**Keywords:** Cocoa, flower visitor, forest, Indonesia, landscape, pollination services, canopy cover, soil leaf-litter

## Introduction

Cocoa (*Theobroma cacao* L.) is the third largest export crop commodity worldwide (Donald, 2004), but faces yield declines associated with limited resilience to climate change. Increasing temperatures, prolonged drought, and increased pest and disease outbreaks are affecting cocoa yields, jeopardizing the livelihood of small-scale farmers in the main producing regions of West-Africa (Läderach et al., 2013; Schroth et al., 2016), South East Asia (Bunn et al., 2017) and South America (Gateau-Rey et al., 2018). While conventional intensification strategies, highly dependent on agrochemical inputs, can help boosting cocoa productivity, it will not be sufficient for stabilizing high yields. Thus, the role of ecological intensification, through the enhancement of ecosystem services such as pollination (Bommarco et al., 2013; Motzke et al., 2015), is seen as a sustainable strategy for securing cocoa yields in the long term (Wanger et al., 2014; Toledo-Hernández et al., 2017). For example, a study in Central Sulawesi, Indonesia, found that increasing amount of hand-pollinated flowers from 10 to 40%, can enhance cocoa yields by at least 30% at the farm level (Groeneveld et al., 2010).

Cocoa is a self-incompatible plant that depends on insects for pollination success (Falque et al., 1995; Wood & Lass, 2008), but we know surprisingly little about the cocoa pollinator identity and ecology. The majority of studies acknowledge tiny ceratopogonids (Diptera) as the most common and main cocoa pollinators (Entwistle, 1972; Young, 1982; Tschardt et al., 2011; Arnold et al., 2018). The pollination role of non-ceratopogonids is regarded as secondary, although they represent an even larger proportion of flower visitors (Deheuvels, 2017; Toledo-Hernández et al., 2017; Claus et al., 2018; de Schawe et al., 2018).

While there is little evidence on the habitat suitability for non-ceratopogonid flower visitors, ceratopogonids require dark and humid conditions with rich soil organic matter, as found in ecosystems with high tree canopy cover, to develop and thrive (Kaufmann, 1975; Young, 1982, 1986; Tschardt et al., 2011; Toledo-Hernández et al., 2017). As ceratopogonids can fly only short distances of a few meters (Chumacero de Schawe et al., 2013), complex habitats with multistrata vegetation such as forest patches and agroforestry systems (Kaufmann, 1975; Young, 1986; Tschardt et al., 2011; Toledo-Hernández et al., 2017) that are adjacent to the farm can enhance flower visitation. Enriching soil organic litter substrates increased ceratopogonid abundance in Trinidad & Tobago (Bridgemohan et al., 2017) and fruit set in Australia (Forbes and Northfield, 2017), while there is no evidence that



increasing the amount of leaf-litter generally increases populations of cocoa flower visitors.

Despite the potential of pollination services for sustainably increasing cocoa yields, there are major research gaps of understanding 1) the identity of the cocoa flower visitors and 2) the landscape and farm-management practices driving habitat suitability and their populations. Here we conducted the first large-scale study to test landscape and farm-level effects on cocoa flower visitors and yield. We used flower visitor data of a correlative and an experimental approach to understand the effect of landscape (forest distance to farm, identity and percentage of complex habitats surrounding the farm), farm-management (canopy cover, soil leaf-litter amount), and cocoa tree performance (flower abundance) on the species richness and abundance of cocoa flower visitors. We hypothesized that 1) complex landscapes (farms closer to forest, farms with multistrata-vegetation in the surrounding) and diverse agroforests (farms with high canopy cover and high levels of soil leaf-litter), and 2) farm leaf-litter management (strongly modified amounts of leaf-litter) affects flower visitor diversity and abundance, thereby improving cocoa yields.

## Materials and Methods

### *Study Site*

The study was conducted in the region of Napu Valley (S1° 27' 48", E120° 21' 6") located at the boundaries of Lore Lindu National Park, in Central Sulawesi, Indonesia (Electronic Supplementary Material, Fig. S1A) (Wanger et al., 2011). The Napu Valley is situated in a lower montane zone between 1,100 to 1,200 m a.s.l., with a mean annual precipitation of ~1,500mm year<sup>-1</sup> (Smiley & Kroschel, 2010). Cocoa farming and vegetable gardening are the two main income activities in the study region (Motzke et al., 2016).

### *Farm Characterization*

We randomly selected and characterized 28 cocoa farms distributed across the Napu Valley (Electronic Supplementary Material, Fig. S2A-C). Farms were characterized based on semi-structured interviews with the farmers, and farm visits to record household socioeconomics and management practices. In the interviews we recorded farmer gender and age, number of household members, farm income and use of chemical inputs. During the farm visits we collected data on cocoa tree

density, height and age, as well as shade tree species richness, density and canopy cover, soil leaf-litter amount, and yields (dry bean weight g/tree).

### *Landscape Characterization*

We quantified the farm distance to forest, and the identity and percentage of habitats surrounding the farm. Habitats surrounding the farm included secondary forest, high and low shaded cocoa agroforests, vegetable gardens, settlements and open areas. We based our classification of suitable (complex habitats with 1-2 vegetation layers, such as forests and agroforests) and unsuitable habitats (simple or no vegetation strata, such as vegetable gardens, settlements, and open areas) assuming that small cocoa flower visitors (3-7mm large) require moist and dark microclimatic conditions to proliferate (Kaufmann, 1975; Toledo-Hernández et al., 2017). We classify habitat-suitability for flower visitors as percentage of suitable habitats (forest, high and low shaded cocoa agroforests) in a 100m radius from the plot center (Electronic Supplementary Material, Table S1).

### *Field Study Design*

We used a combined correlative and experimental approach to determine farm management effects on cocoa flower visitors. In the “correlative approach”, our aim was to determine the landscape- (forest distance, flower visitor habitat-suitability), farm (canopy cover, soil leaf-litter amount), and tree-level (flower production) variables driving the richness and abundance of the main cocoa flower visitors. In the “experimental approach”, we tested the effect of farm leaf-litter management (low and high amounts of leaf-litter) on flower visitor species richness and abundance. We conducted the correlative approach between January-April 2017 on a total of 18 farms (Electronic Supplementary Material, Fig. S1B, Table S2). In each farm, we established a 40m<sup>2</sup> plot along a gradient of forest distance (100-3400m), habitats suitable for flower visitors in the farm surroundings (30-100%), canopy cover (0-80%), and soil leaf-litter amount (0-5kg/m<sup>2</sup>). We conducted the experimental approach between May-August 2017 on 24 farm plots of the same area as in the correlative approach (40m<sup>2</sup>) (Electronic Supplementary Material, Fig. S1C, Table S3). We grouped the 24 plots in three soil leaf-litter treatment categories (low, high, and untreated [control] amounts of soil leaf-litter) hereafter “leaf-litter treatment” with eight plot replicates each. Leaf-litter treatments were established within a forest

distance (100-3,400m) and canopy cover (0-80%) gradient, and with a 100m minimum distance between farms.

To establish the leaf-litter treatments, we first calculated the average soil leaf-litter amount in the 28 farms, which was 2.3 (SE $\pm$  0.2) kg/m<sup>2</sup>. Soil leaf-litter was composed mainly by small tree branches (<1m long), rotten trunks, stems, leaves and cocoa pods. We doubled (>4.6kg/m<sup>2</sup>), removed (<0.6kg/m<sup>2</sup>) and maintained (~2.3kg/m<sup>2</sup>) soil leaf-litter amounts for the high, low, and control treatments, respectively. Fallen un-rotten trunks (>1m) and weeds did not provide decayed organic matter that may serve as flower visitor habitats, thus we did not remove them from the low leaf-litter treatments. We balanced and recorded leaf-litter amounts every two weeks to maintain the treatments' leaf-litter levels (Electronic Supplementary Material, Fig. S2D-F).

We monitored six cocoa trees per plot throughout the experiment to link flower visitors and yields. One day before starting the flower visitor monitoring we removed all open flowers and fruits at any development stage from the whole tree. This was done to ensure that we only monitor the successfully pollinated flowers and the resulting fruits in the experimental approach. Furthermore, we marked all fruits occurring during the experiment, and monitored them every two weeks to record their development. Finally, we harvested all matured fruits around six months after the experiment started, and quantified fresh and dry bean weight (g/tree).

### *Flower Visitors Monitoring*

We randomly selected a total of 12 cocoa trees of the same age for each of the plots in the correlative and experimental approaches, and hence, a total of 504 trees (correlative=216 trees, experimental=288 trees; Electronic Supplementary Material, Fig. S1D). The selected trees showed no evidence of pest and/or diseases and were distributed at a minimum distance of 10m from other selected trees and from the plot margins. Furthermore, we grouped the selected 12 cocoa trees in four subsets of three trees each, and numbered each subset as S1, S2, S3 and S4. We monitored flower visitors weekly starting with S1 and ending with S4 for 12 consecutive weeks. This means that we sampled three times each tree-subset (Electronic Supplementary Material, Fig. S1D).

We applied "Insect Tangle Glue Trap" (TangleFoot<sup>®</sup>) as described by de Schawe et al. (2018) in 10 (correlative approach) and six (experimental approach) easily accessible flowers (<2m tree/height) per tree and per sampling round (Electronic

Supplementary Material, Fig. S1E). We monitored less flowers in the experimental approach, because of higher labor requirements (72 trees more than in the correlative study) across the 24 leaf-litter treatments. We collected the flowers with glued visitors 24 hours after the glue was applied, and identified flower visitors in the laboratory (Electronic Supplementary Material, Fig. S3A-C). We identified all flower visitors to taxonomic class and order, dipterans and hymenopterans to family and morpho-species level (Electronic Supplementary Material, Fig. S3D-F), and ants (Formicidae) to genus level (Electronic Supplementary Material, Fig. S3F).

### *Data Analysis*

We conducted all the data analyses in R statistic software (R Core Team, 2019). First, we analyzed all flower visitor data of both approaches at the plot level using the Shannon and Simpson diversity indexes in the “vegan” package (Oksanen et al., 2007). In a second step, we conducted generalized linear mixed effect models (GLMMs) (Burnham & Anderson, 2003) with the dataset sorted by flower using the “lme4” package (Bates et al., 2015). The full model of the correlative approach included the 1) dipteran and ant (the two main flower visitors in our study) species richness and abundance as dependent variables, and 2) landscape (forest distance [m], flower visitor habitat-suitability [%]), 3) farm (shade canopy cover[%], leaf-litter amount [kg/m<sup>2</sup>]), and 4) tree performance (cocoa flower abundance) as predictor (fixed effects) variables. Furthermore, we included trees nested in farms as random effect to account for nestedness of the design and unexplained variation in the data. Many of the monitored flowers were not visited and, hence, we used the glmmTMB in the “glmmTMB” package to account for zero-inflation and ran models with a negative-binomial distribution to correct for overdispersion (Brooks et al., 2017). We then followed a model selection approach to identify the most parsimonious models using the “MuMIn” work-packages ( $\Delta AICc \leq 2$ ; Barton, 2013). As several models satisfied the  $\Delta AICc \leq 2$  cut-off criterion for best fit models (Anderson, 2007), we averaged coefficients of these models (Tab. 2). For the experimental approach, we also used generalized linear mixed effect models of dipteran and ant species richness and abundance as response variable, and forest distance (m), pollinator-habitat-suitability (%), shade canopy cover (%), and leaf-litter (high >4.6kg/m<sup>2</sup>, low <0.6kg/m<sup>2</sup>, control ~2.3kg/m<sup>2</sup>) treatment (categorical), and yields (dry bean weight g/tree) as predictor variables. We also nested trees in farms as random effect to account for the nestedness of the design. Finally, we carried out a least-square-means analysis for a multiple comparison to quantify the effects of the leaf-litter

treatments on cocoa flower visitor species richness and abundance using the “lsmmeans” package (Lenth, 2018).

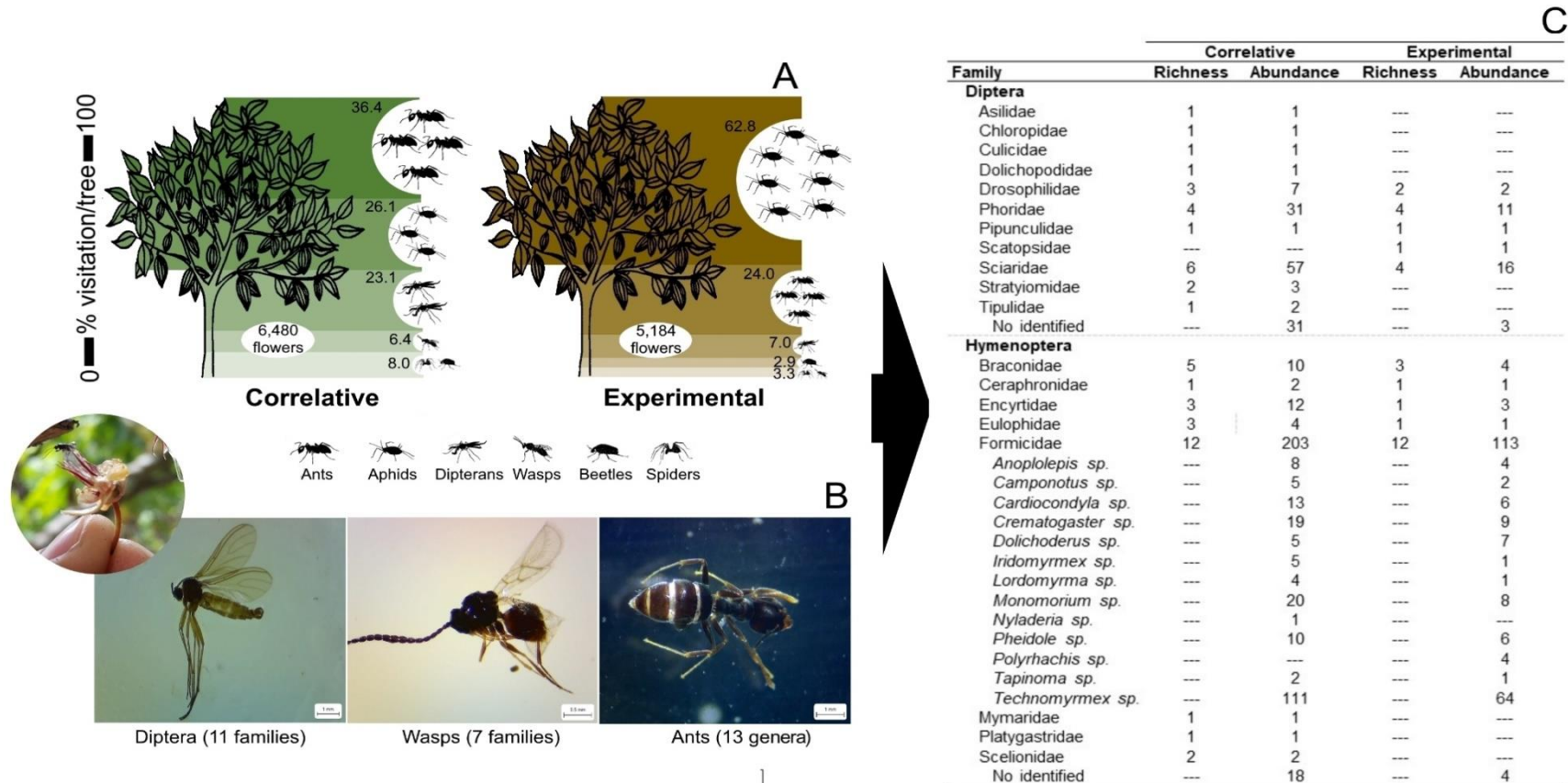
## Results

### *Overall Flower Visitors*

In total, we monitored 11,664 cocoa flowers (correlative=6,480 flowers, experimental=5,184 flowers) throughout January-August 2017 (Fig. 1A, Electronic Supplementary Material, Table S2, S3) and recorded a total of 1,491 flower visitors ( $n=770$  in the correlative and  $n=721$  in the experimental study). This corresponds to a 14.9% flowers visited in the correlative and 11.1% in the experimental approach. A total of 417 individuals captured ( $n=180$  in the correlative and  $n=237$  in the experimental) were at early development stages (larvae) or bodies were incomplete, making identification impossible.

### *Flower Visitor Species Richness and Abundance*

We found that the most predominant groups in the correlative study ( $n=590$ ) were ants (Hymenoptera-Formicidae; 36.4%;  $n=215$ ), aphids (Hemiptera-Aphididae; 26.1%;  $n=154$ ), dipterans (Diptera; 23.1%;  $n=136$ ) and parasitic wasps (Hymenoptera-Parasitica; 6.4%;  $n=38$ ), altogether providing 92% of total captures. In the experimental study ( $n=484$  individuals identified), the most abundant groups were aphids (62.8%;  $n=304$ ), ants (24%;  $n=116$ ), dipterans (7%;  $n=34$ ) and beetles (Coleoptera; 2.9%;  $n=14$ ) representing 96.7% of all flower visitors (Fig. 1A, Electronic Supplementary Material, Table S2, S3). At the family level, we recorded 10 dipteran and 7 hymenopteran families in the correlative study, with Sciaridae (Diptera;  $n=57$ ) and Formicidae (Hymenoptera;  $n=203$ ) being the most abundant families (Fig. 1B, C). In the experimental study, we recorded a total of five dipteran and four hymenopteran families, and similar to the findings in the correlative approach, Sciaridae ( $n=12$ ) and Formicidae ( $n=113$ ) were the two most common families (Fig. 1B, C). At the species level (excluding ants), we found 37 (Diptera=21, Hymenoptera=16) morphospecies in the correlative, and 18 (Diptera=12, Hymenoptera=6) morphospecies in the experimental approach (Fig. 1C). Furthermore, we recorded the highest species richness in Sciaridae (correlative=6, experimental=4), and Braconidae (correlative=5 experimental=3) families for both studies (Fig. 1C). Furthermore, we identified 13 ant genera, being *Technomyrmex*



**Figure 1.** The cocoa flower visitors from the correlative and experimental approaches in Central Sulawesi, Indonesia. From the 11,664 flowers monitored in total, the most dominant groups were ants (36.4%), aphids (26.1%) and dipterans (23.1%) in the correlative, and aphids (62.8%), ants (24%) and dipterans (7%) in the experimental (A) approach. These groups made the 92 and 96.7% of all cocoa flower visitors in the correlative and experimental approach, respectively. In both experiments combined, we recorded a total of 11 dipteran and 7 parasitic wasp families, and 13 ant genera (B). The families Sciaridae (Diptera) and Formicidae (Hymenoptera) showed the highest species richness and abundance, while *Technomyrmex sp.* (Formicidae) was the most abundant ant genus (C).

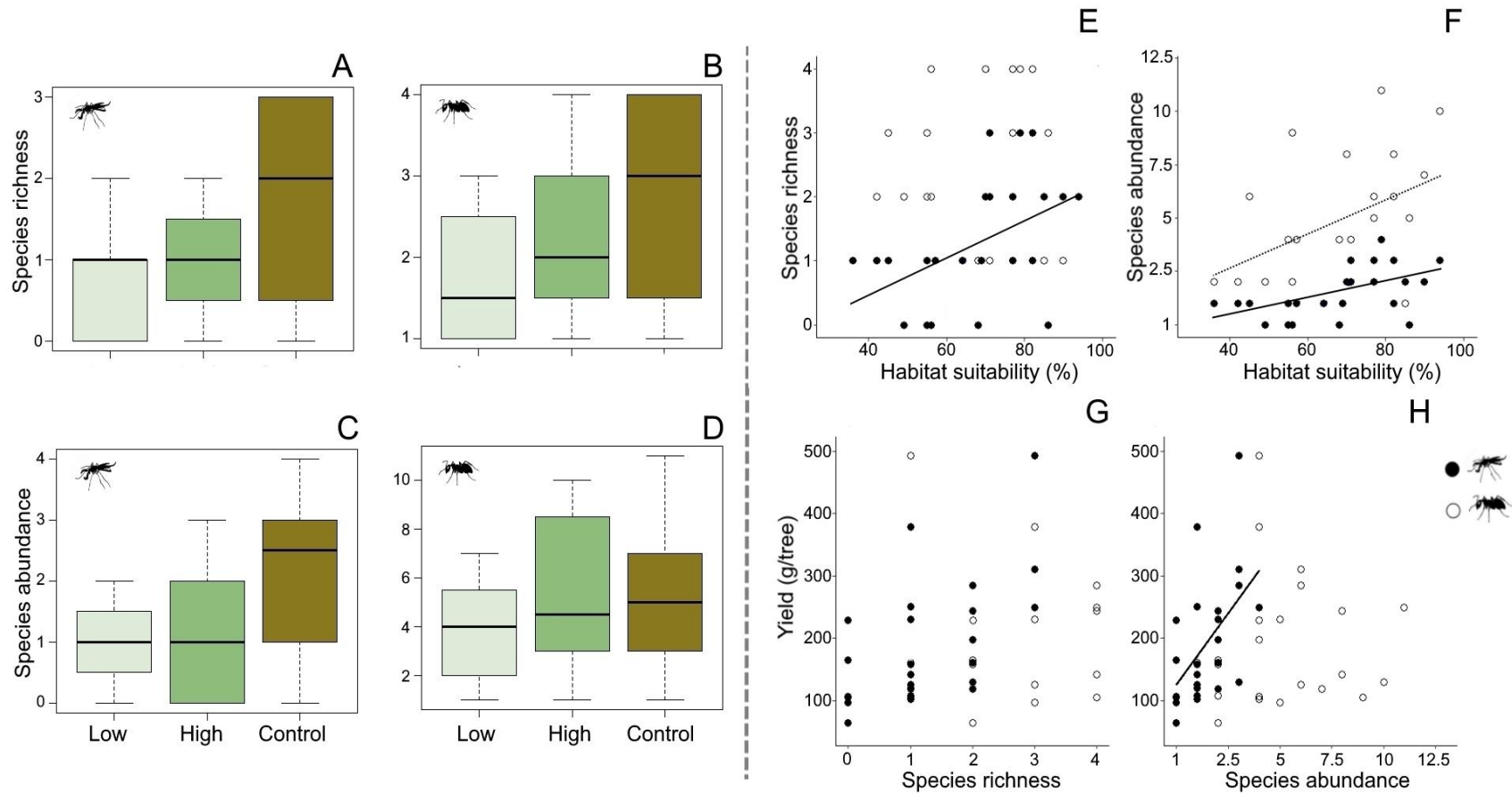
sp. (correlative=111, experimental=64) and *Tapinoma* sp. (correlative=2, experimental=1) the most and least dominant. The genus *Nyladeria* sp. ( $n=1$ ) and *Polyrhachis* sp. ( $n=4$ ) were only present in the correlative and experimental study, respectively (Fig. 1C).

### *Correlative Approach*

The results from the generalized linear mixed-effect models in the correlative approach (Table 1A, B, Electronic Supplementary Material, Table S4) showed that at the landscape level, the percentage of habitat-suitability for dipteran and ant flower visitors showed a positive effect on species richness (Diptera  $p=0.012$ , Formicidae  $p=0.046$ ) and abundance (Diptera  $p=0.024$ , Formicidae  $p=0.048$ ) of both insect groups. Forest distance did not have a significant effect on dipteran and ant species richness (Diptera  $p=0.313$ , Formicidae  $p=0.466$ ) or abundance (Diptera  $p=0.130$ , Formicidae  $p=0.333$ ). At the farm level, we found a positive effect of canopy cover on dipteran species richness ( $p=0.019$ ) and abundance ( $p=0.006$ ), but no effect on ant species richness ( $p=0.567$ ) and abundance ( $p=0.930$ ). Furthermore, leaf-litter amount significantly affected ant species richness ( $p=0.034$ ) and abundance ( $p=0.088$ ), but not dipteran species richness ( $p=0.796$ ) and abundance ( $p=0.907$ ). At the tree level, we found that cocoa flower abundance had a positive effect on dipteran species richness ( $p=0.012$ ) and abundance ( $p=0.032$ ), but not on ant species richness ( $p=0.794$ ) and abundance ( $p=0.785$ ).

### *Experimental Approach*

The results of the experimental approach showed that the flower visitor habitat-suitability in the landscapes surrounding the farms, but not the leaf-litter treatment (Fig. 2A-D; Table 1C, D, 2), significantly affects dipteran species richness ( $p=0.025$ ) and dipteran and ant abundance (Diptera  $p=0.012$ , Ant  $p=0.022$ ) (Fig. 2E, F; Table 1C, D). We also found that cocoa yields were correlated to dipteran abundance ( $p=0.022$ ), but only marginally to dipteran species richness ( $p=0.085$ ), while we found no relation of yields to ant species richness ( $p=0.902$ ) and abundance ( $p=0.968$ ) (Fig. 2G-H; Table 1C, D). Other variables did not affect any group or metric (Table 1C, D).



**Figure 2.** Results of the experimental approach on the effect of soil leaf-litter manipulation (high >4.6kg/m<sup>2</sup>, low <0-6kg/m<sup>2</sup>, control ~2.3kg/m<sup>2</sup>), flower visitor habitat-suitability (%) on the main cocoa flower visitor dipterans and ants, and their correlation with yield (dry bean weight g/tree) in Central Sulawesi, Indonesia. We did not find a significant effect of increasing (high) soil leaf-litter, maintaining (control) or decreasing (low) soil leaf-litter (A-D), an increased pollinator habitat-suitability showed a significant effect on dipteran species richness (E) and dipteran and ant abundance (F). Furthermore, dipterans marginally (G) and significantly (H) increased with an increasing yield. Linear regression (dipterans=solid, ants=dotted) lines (E-H) show statistically significant effects ( $p \leq 0.05$ ; Table 1C, D).



CHAPTER II - COCOA FLOWER VISITORS IN INDONESIAN AGROFORESTS

**Table 1.** Results of the correlative and experimental approach on the effect of landscape, farm management, plant performance and yield variables on the main cocoa flower visitor in Central Sulawesi, Indonesia. In the correlative approach we investigated the response of species richness and abundance of dipterans (A), and ants (B) to forest distance (m), flower visitor habitat-suitability (%), shade canopy cover (%), leaf-litter amount (kg/m<sup>2</sup>), and flower abundance. In the experimental approach we manipulated soil leaf-litter (high >4.6kg/m<sup>2</sup>, low <0.6kg/m<sup>2</sup>) amounts, and recorded yields (dry bean weight g/tree) to understand leaf-litter effect on dipterans (C) and ants (D). Significant *p-values* are highlighted in bold.

	Species richness					Abundance				
	Coefficient	SE	Adjusted SE	<i>z-value</i>	<i>p-value</i>	Coefficient	SE	Adjusted SE	<i>z-value</i>	<i>p-value</i>
<b>Correlative</b>										
<b>A. Dipteran</b>										
Intercept	-4.341	0.315	0.315	13.770	< 0001	-4.093	0.144	0.144	28.494	< 0001
Forest distance	0.167	0.165	0.165	1.009	0.313	0.235	0.156	0.156	1.510	0.130
Habitat-suitability	0.526	0.208	0.208	2.525	<b>0.012</b>	0.446	0.198	0.198	2.250	<b>0.024</b>
Canopy cover	0.337	0.144	0.144	2.343	<b>0.019</b>	0.384	0.140	0.140	2.737	<b>0.006</b>
Leaf-litter amount	-0.055	0.215	0.215	0.258	0.796	0.024	0.210	0.210	0.117	0.907
Flower abundance	0.535	0.213	0.213	2.517	<b>0.012</b>	0.420	0.196	0.196	2.142	<b>0.032</b>
<b>B. Ant</b>										
Intercept	-4.006	0.349	0.349	11.458	< 0001	-3.472	0.087	0.087	39.636	< 0001
Forest distance	-0.077	0.105	0.105	0.730	0.466	-0.098	0.101	0.101	0.968	0.333
Habitat-suitability	0.201	0.101	0.101	1.994	<b>0.046</b>	0.201	0.101	0.101	1.979	<b>0.048</b>
Canopy cover	0.057	0.100	0.100	0.572	0.567	0.008	0.096	0.096	0.087	0.930
Leaf-litter amount	0.247	0.117	0.117	2.119	<b>0.034</b>	0.181	0.106	0.106	1.706	<b>0.088</b>
Flower abundance	-0.030	0.116	0.116	0.261	0.794	0.032	0.116	0.116	0.273	0.785
<b>Experimental</b>										
<b>C. Dipteran</b>										
Intercept	-5.271	0.312	0.312	16.884	< 0001	-5.129	0.293	0.293	17.490	< 0001
Forest distance	-0.212	0.214	0.214	0.988	0.323	-0.124	0.190	0.190	0.656	0.512
Habitat-suitability	0.552	0.246	0.246	2.248	<b>0.025</b>	0.561	0.223	0.223	2.519	<b>0.012</b>
Canopy cover	-0.047	0.205	0.205	0.231	0.817	0.040	0.184	0.184	0.215	0.830
Low leaf-litter	-0.397	0.547	0.547	0.725	0.468	-0.395	0.532	0.533	0.742	0.458
High leaf-litter	-0.756	0.564	0.564	1.340	0.1804	-0.645	0.545	0.545	1.184	0.236
Yield	0.309	0.180	0.180	1.718	0.0858	0.344	0.162	0.162	2.127	<b>0.033</b>
<b>D. Ant</b>										
Intercept	-4.532	0.176	0.176	25.767	< 0001	-3.854	0.140	0.140	27.596	< 0001
Forest distance	0.046	0.141	0.141	0.329	0.742	0.083	0.130	0.130	0.635	0.525
Habitat-suitability	0.079	0.147	0.147	0.538	0.590	0.266	0.116	0.116	2.295	<b>0.022</b>
Canopy cover	0.086	0.136	0.136	0.634	0.526	-0.015	0.112	0.112	0.134	0.893
Low leaf-litter	-0.413	0.371	0.371	1.113	0.266	-0.230	0.303	0.303	0.757	0.449
High leaf-litter	-0.125	0.346	0.346	0.359	0.719	-0.041	0.287	0.287	0.143	0.887
Yield	0.019	0.150	0.150	0.123	0.902	0.005	0.120	0.120	0.040	0.968

**Table S2.** Results of the multiple comparison analysis of leaf-litter manipulation (high >4.6kg/m<sup>2</sup>, low <0-6kg/m<sup>2</sup>, control ~2.3kg/m<sup>2</sup>) on dipteran and ant species richness (A), and abundance (B) in the experimental approach.

	Coefficient	SE	df	t-ratio	p-value
<b>A. Species richness</b>					
<b>Diptera</b>					
Control–High	0.865	0.454	5177	1.905	0.137
Control–Low	0.696	0.491	5177	1.418	0.332
High–Low	0.169	0.557	5177	0.304	0.950
<b>Ant</b>					
Control–High	-0.0335	0.254	5177	-0.132	0.990
Control–Low	0.1522	0.279	5177	0.545	0.849
High–Low	-0.1857	0.282	5177	-0.658	0.788
<b>B. Species abundance</b>					
<b>Diptera</b>					
Control–High	0.855	0.427	5177	2.003	0.112
Control–Low	0.541	0.434	5177	1.246	0.426
High–Low	0.314	0.506	5177	0.621	0.808
<b>Ant</b>					
Control–High	0.041	0.250	5177	0.163	0.985
Control–Low	0.199	0.275	5177	0.724	0.749
High–Low	-0.158	0.281	5177	-0.563	0.840

## Discussion

In this first study combining correlative and experimental evidence of landscape and farm management effect on cocoa flower visitors, we found that ants and dipterans are the major visitors of cocoa flowers in Indonesia. Even when manipulating leaf-litter as a farm-level variable, we find that landscape complexity surrounding the farm is most important to maintain cocoa flower visitors, compared to farm or tree level management.

### *Flower Visitation and Flower Visitors*

We recorded a flower visitation ranging from 11.1% flowers visited in the experimental approach, to 14.9% in the correlative approach. These values are higher than those known from Brazil (2.1%; Winder, 1977) and Ghana (7.5%; Frimpong et al., 2009), but lower than 30% visitation rate reported in Bolivia (de Schawe et al., 2018). Such variations in flower visitation rates can be associated with monitoring method (e.g. suction sampler vs Insect Tangle Glue), weather (e.g. temperature and rainfall) and farm management (e.g. canopy cover, soil organic matter), but they also highlight the need to better understand factors changing between cocoa producing regions (Winder, 1978).

Similar results from our correlative and experimental approach that ants (correlative=36.4%, experimental=24%) and to a lesser extent dipterans (correlative=23.1%, experimental=7%) are the most important flower visitors were reported using direct flower observations in cocoa farms of Peru (94.7% ants, 2.6% dipterans with camera recordings; Deheuvels et al., 2017), and Bolivia (48.5% ants, 23.5% dipterans with Insect Tangle Glue; de Schawe et al., 2018). We observed a strong decrease in dipteran and ant species richness and abundance in the experimental approach in contrast to the correlative approach, which can be due to the seasons when each study was conducted (correlative=rainy, experimental=dry; Gunawan, 2006).

The strong presence of aphids in flowers in the correlative (26.1%) and experimental (62.8%) approach may indicate a high pest incidence, rather than flower visitation for pollination, as aphids are known to feed on flowers and young fruits (Maas et al., 2013). The remaining groups (e.g. parasitic wasps, coleopterans, orthopterans, thysanopterans, collembolans, spiders, etc.) visited cocoa flowers only in a few cases, which is consistent with a global review of cocoa pollinators (Toledo-Hernández et al., 2017).

#### *Pollinator Potential of the Main Flower Visitors*

In our correlative and experimental approach, we did not capture a single ceratopogonid, which is in contrast to previous assumptions and findings (Entwistle, 1972; Young, 1982; Tschardt et al., 2011; Toledo-Hernández et al., 2017), and suggests that the role of ceratopogonids as main cocoa pollinators has been largely overestimated. Very few or even no observations of ceratopogonid flower visits are in line with reports from Brazil (Winder, 1997), Ghana (Frimpong et al., 2009), and Bolivia (de Schawe et al., 2018) where this family made only up to 2% of the total visitors on cocoa flowers. In our study, the relatively high altitude of the Napu Valley (~1,100-1,200m a.s.l.) and related low mean annual temperature and precipitation may adversely affect climate-sensitive ceratopogonids (Arnold et al., 2018).

The high abundance of ants in the correlative (34%) and experimental (24%) approach confirm the strong likelihood of their role as pollinators, both directly and indirectly. As direct pollinators, ants of the size of ceratopogonids (~5mm), such as *Monomorium* sp., could easily access anthers and thus actively transport pollen grains between flowers. However, direct pollination may be more likely to occur in a self-compatible tree in contrast to self-incompatible trees such as cocoa, as they

require less pollen to fertilize its own flowers (Falque et al., 1995). As indirect pollinators, ants disturb pollinators after landing on cocoa flowers, thereby promoting pollinator movement to other trees (Wielgoss et al., 2014, Toledo-Hernández et al., 2017). This ant-pollinator disturbance hypothesis was widely recorded in tropical ecology studies (Altshuler, 1999; Philpott et al., 2006).

### *Landscape Effect on Flower Visitors*

In our correlative and experimental approach, we found that the habitat-suitability for flower visitors surrounding the farm, not forest distance, is the major landscape driver of the species richness and abundance of dipteran and ants. This is different to the widespread pattern in tropical habitats, where pollinator diversity and flower visitation decreases with increasing forest distance (Ricketts et al., 2008; Klein et al., 2003; Klein, 2009). However, these results are mainly based on bees, suggesting that our results may be due to the specific plant-pollinator interaction in cocoa. Social and solitary bees (Apidae) can cover large flying distances (Klein, 2009), but they rarely forage or effectively deposit pollen on cocoa flowers (Young, 1981). In contrast, the tiny cocoa pollinators may cover only a few meters within each flight (Toledo-Hernández et al., 2017), and can only travel larger distances if supported by wind (Chumacero de Schawe et al., 2013). Thus, even if forest provides suitable habitats, flower visitors may only travel to farms in the immediate surroundings.

We also found that farms surrounded by higher percentage of suitable habitats for flower visitors (complex surroundings with at least one vegetation stratum of forest and cocoa agroforests) have higher dipteran and ant species richness and abundance than simple habitats with no vegetation strata (simple surroundings with no vegetation strata of open areas, settlements, vegetable gardens). This is because higher stratification provides better microclimatic conditions (low sunlight intensity, moisture, rich soil organic nutrients and foraging resources) for pollinator reproduction (Kaufmann, 1975; Young 1986; Tschardt et al., 2011; Toledo-Hernández et al., 2017), and, hence, the ability to promote pollinator movement to the farm, increasing flower visitation.

### *Farm Management Effect on Flower Visitors*

In the correlative and to a lesser extent in the experimental approach we observed that a higher canopy cover positively affected the main cocoa flower visitors, dipterans and ants. This can be associated to the microclimate given by shade trees

(Young, 1986; Tschardtke et al., 2011), as their canopy reduces light intensity, increases humidity, and buffers farm temperatures (Wanger et al., 2018). Dead leaves and branches of shade trees contribute to enrich the soil organic substrates which are presumably important for securing flower visitor habitats and enhancing their populations (Kaufmann, 1975; Young, 1986; Tschardtke et al., 2011; Toledo-Hernández et al., 2017; Arnold et al., 2018). Furthermore, dipterans primarily visit cocoa trees to collect nectar from flowers (Kaufmann, 1973), while ants play other roles in complex plant-insect interactions such as pest-predator and pest-facilitator, plant-herbivore, and pollinator-facilitator (Wielgoss et al., 2014). This may explain why dipterans but not ants were driven by flower abundance in the correlative approach.

Contrary to our hypothesis and in contrast to our correlative approach, where we found an effect of landscape configuration on leaf-litter amounts on dipterans, accumulating or removing leaf-litter did not lead to significant effects on dipterans and ants in our experimental approach. This suggests that even smaller amounts of highly nutrient-rich leaf-litter cover (e.g.  $<0.6\text{kg/m}^2$  in our low treatment) could be sufficient for securing habitats and stabilizing pollinator populations, as observed for dipterans in the correlative approach. Another reason could be that we need longer sampling periods ( $>12$  weeks in our approach) for observing a turnover on cocoa pollinator populations.

## Conclusion and Outlook

In this first large scale study of cocoa flower visitors in Indonesia, we show that flower visits were dominated by ants and dipterans. The fact that we did not capture a single ceratopogonid, the presumed main cocoa pollinator, shows that so far little acknowledged ants may play a major role in cocoa pollination than previously argued. In both, the correlative and experimental approach, complex habitats surrounding the farms and canopy cover in the farms played a role as habitat for cocoa flower visitors. Therefore, we suggest that for promoting flower visitors in cocoa farm it is necessary to 1) preserve habitats with a multistrata-vegetation surrounding the cocoa farms (e.g. forest, agroforests), 2) maintain a recommended 40% canopy cover (Clough et al., 2011), and 3) minimum levels of nutrient-rich litter ( $>0.6\text{kg/m}^2$ ).

In general, we need long term cross-continental studies to target the major cocoa pollination knowledge gaps. For instance, the so far hidden identity and biology of the flower visitors and potential pollinators need to be revealed. Studies should focus on

disentangling the role of ants as direct or indirect cocoa pollinators, as well as the pollination services and disservices. For this, we need new sophisticated pollinator monitoring methods, such as automatic camera recording (Steen et al., 2017) that enable a precise quantification of flower visitation frequencies, visit durations, and fruit set and yield. Furthermore, we need landscape and farm management studies with detailed quantification of habitats surrounding the farm (e.g. plant identity and abundance, flower resources, canopy cover etc.) and soil leaf-litter composition and quality (e.g. leaf litter identity, water and nutrient content, decomposition rate, etc.) and their effect on cocoa flower visitors. Addressing these important issues will provide a better understanding of the complex cocoa-pollinator ecology, and will help to give farmer management recommendations for building climate change-resilient and sustainable cocoa production systems and facilitate biodiversity conservation.

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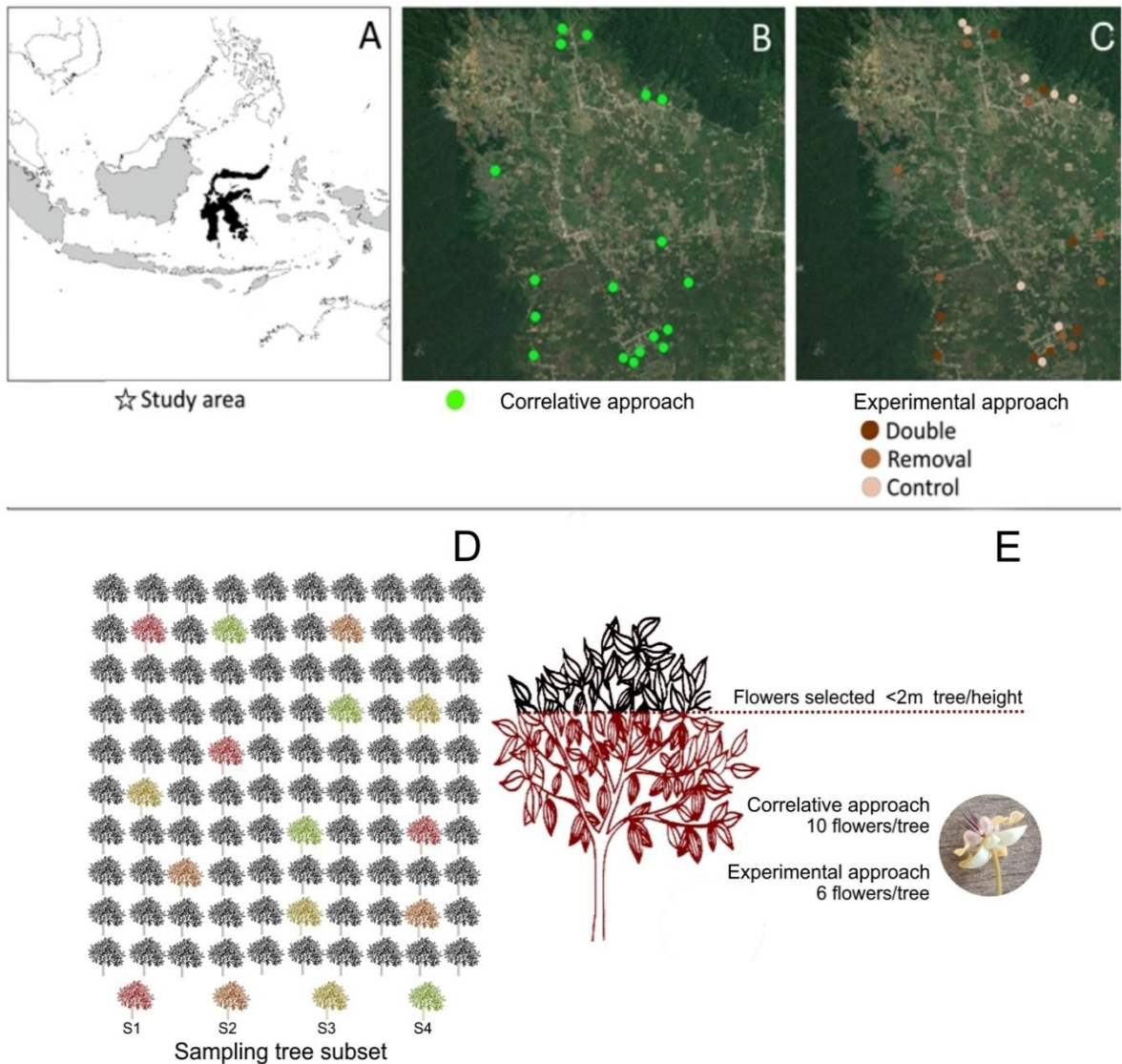


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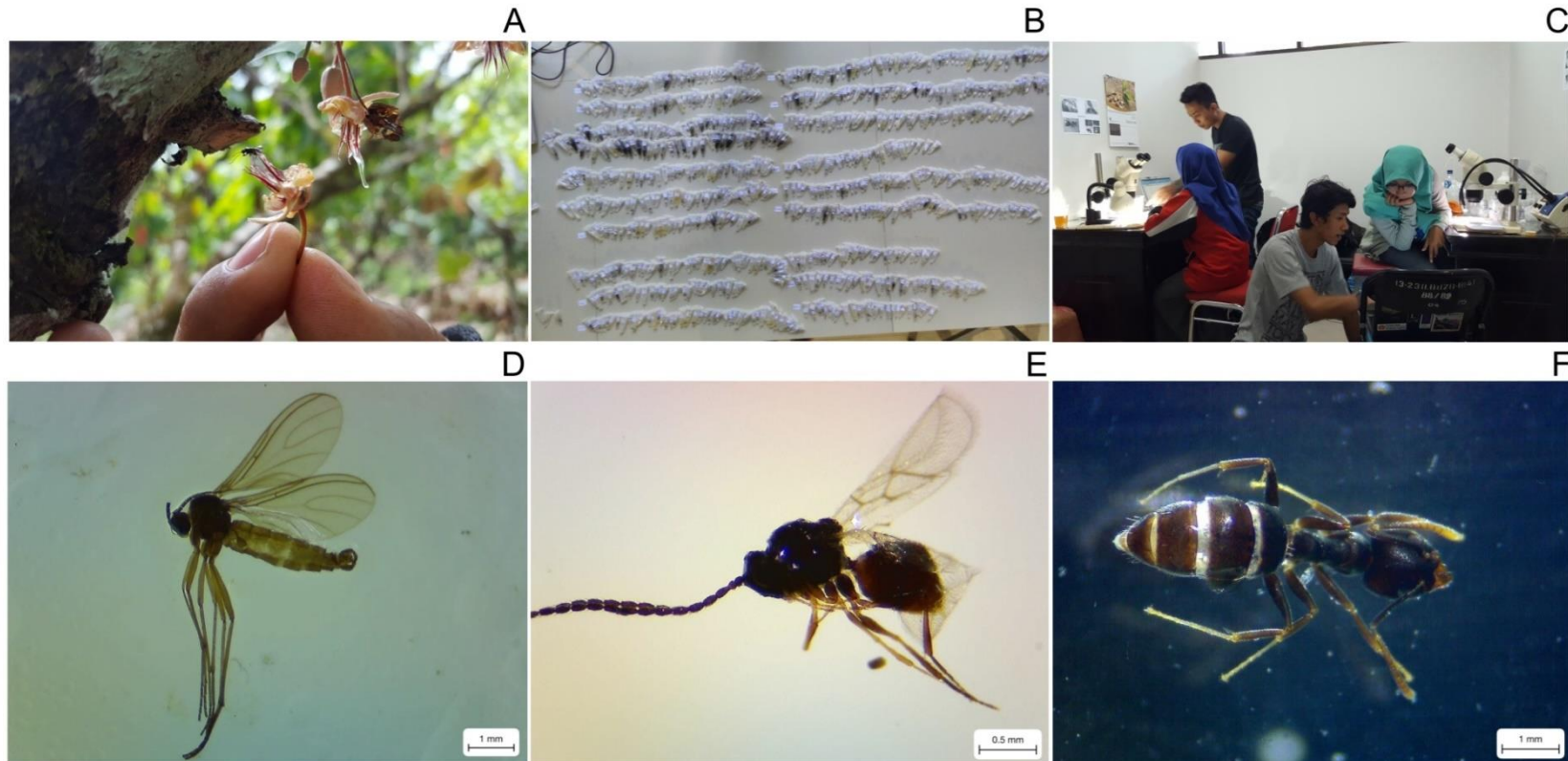
## Electronic Supplementary Material



**Figure S1.** The study area in the region of Napu Valley, Central Sulawesi, Indonesia (A) where we conducted a correlative (B) and an experimental (C) approach, as well as the schematic overview on tree selection (D) and flower visitor monitoring (E). First, we sorted the 12 selected cocoa trees in four subsets of three trees each (i.e. S1, S2, S3 and S4) (D). We monitored subset S1 (red trees) in week 1, while S2 (brown trees), S3 (yellow trees) and S4 (green trees) in week 2, 3 and 4, respectively. We repeated the monitoring cycle in weeks 5 and 9 starting always with subset S1 and continuing with S2 (weeks 6 and 10), S3 (weeks 7 and 11), and S4 (weeks 8 and 12). In each sampling week we applied “Insect Tangle Glue” in 10 (correlative) and 6 (experimental) cocoa flowers below 2m height) (E).



**Figure S2.** The characterization of farms in the Napu Valley, Central Sulawesi (A-C) and the soil litter treatments of the experimental approach (D-F). The farm characterization included farmer interviews (A) and recording cocoa tree density, height and age, shade tree species richness, density and percentage of canopy cover (B), and soil litter amount (C). In the soil treatments we doubled (high  $>4.6\text{kg/m}^2$ ) (D), removed (low  $<0.6\text{kg/m}^2$ ) (E) and maintained (control  $\sim 2.3\text{kg/m}^2$ ) (F) soil litter amounts in the 24 cocoa farms throughout the length of the study (May-August 2017).



**Figure S3.** We monitored 11,664 flowers during the field sampling season (January-August 2017) in the correlative and experimental studies. We applied "Insect Tangle Glue" to the staminodes of each flower selected (A, flower in the background). After 24 hours, we collected flowers with glued flower visitors (A, hand-picked flower), stored them in eppendorf tubes with ethanol 70% (B) and transported to the laboratory for identification (C). We identified all arthropods first classified to class and order. Later on, we classified dipterans (D) and hymenopterans (E, F) to family and morphospecie level, while ants (Formicidae) to genus (F).

CHAPTER II - COCOA FLOWER VISITORS IN INDONESIAN AGROFORESTS

**Table S1.** The farm-surrounding landscapes and the percentage of flower visitor habitat-suitability for the 18 cocoa farms in the correlative approach. We divided farm-surrounding landscapes in complex (i.e. 1-2 vegetation strata) and simple (i.e. 0 strata). Complex landscapes are habitats with low (0-20%) and high (40-80%) shade cocoa agroforests, and secondary forest; while simple landscapes are open area, vegetable garden, and settlement. We then calculated the percentage cover for each habitat in a 100 m radius from the plot center. The percentage of habitat-suitability is the sum of complex land use systems.

Farm	Farm-surrounding (%)																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
<b>Simple</b>																		
<i>Open area</i>	17	51	15	35	14	11	24	17	8	0	32	6	27	7	23	8	17	20
<i>Vegetable garden</i>	47	0	4	20	16	10	3	11	7	6	0	5	7	7	16	15	21	9
<i>Settlement</i>	0	0	10	0	0	0	4	16	0	0	0	7	2	0	6	0	0	14
<b>Complex</b>																		
<i>Low-shade cocoa</i>	10	49	71	24	0	0	0	18	25	94	62	72	13	14	3	25	17	27
<i>High-shade cocoa</i>	16	0	0	21	70	79	63	38	60	0	0	0	51	72	52	52	34	30
<i>Secondary forest</i>	10	0	0	0	0	0	6	0	0	0	6	10	0	0	0	0	11	0
<b>% Habitat-suitability</b>	36	49	71	45	70	79	69	56	85	94	68	82	64	86	55	77	62	57

CHAPTER II - COCOA FLOWER VISITORS IN INDONESIAN AGROFORESTS

**Table S2.** Results of the correlative approach on the response (flower visitation, species diversity indexes (Shannon, Simpson), and flower visitor abundance, and the predictor (forest distance [m], pollinator-habitat-suitability [%], canopy cover [%], soil litter amount [kg/m<sup>2</sup>], and flower abundance). In total we monitored 6,480 flowers in 12 weeks.

Plot	Forest distance (m)	Habitat-suitability (%)	Canopy cover (%)	Mean litter amount (kg/m <sup>2</sup> )	Mean cocoa flowers	Flower visitation (%)	Diptera and hymenoptera species richness	Shannon index	Simpson index	Total abundance	Diptera	Hymenoptera (Ant)	Hymenoptera (Wasp)	Hemiptera (Aphid)	Coleoptera	Collembola	Orthoptera	Thysanoptera	Neuroptera	Isopoda	Aranae	Larvae	No identified
1	108	36	4.3	4.5	89	16.4	2	0.601924	0.320312	44	0	15	0	15	1	0	1	0	0	0	1	0	11
2	130	49	0.7	2.3	81	19.2	5	1.695743	0.800000	47	4	7	0	19	0	1	0	0	0	0	0	0	16
3	306	71	0.0	3.0	53	10.8	8	1.853327	0.780992	35	6	11	3	4	1	0	0	0	0	0	1	0	9
4	426	45	36.1	3.7	96	15.0	13	2.186306	0.820302	41	9	12	5	8	0	0	0	0	0	0	0	0	7
5	192	70	28.9	2.2	47	13.6	10	2.128265	0.860000	47	4	17	6	8	2	0	0	0	0	0	0	0	10
6	479	79	37.8	3.0	17	16.1	9	2.039184	0.847034	44	10	20	0	5	0	0	1	1	0	0	0	0	7
7	387	69	48.9	2.6	30	14.4	9	2.053945	0.841600	46	5	15	3	7	3	2	1	0	0	0	0	0	10
8	168	56	50.2	1.8	13	15.3	7	1.748067	0.770000	38	2	11	1	11	2	0	1	1	0	0	0	1	8
9	170	85	60.0	2.2	27	11.7	11	2.132880	0.842222	40	9	13	1	8	0	0	0	1	0	0	0	0	8
10	2949	94	11.0	2.2	55	13.9	13	1.987041	0.791115	54	14	20	3	6	0	0	0	0	1	0	0	1	9
11	3094	68	3.5	3.1	44	11.4	7	1.514904	0.709677	39	7	11	3	3	1	0	1	0	0	0	0	4	9
12	3169	82	9.3	0.6	43	15.6	11	1.965963	0.780024	41	9	10	3	8	1	0	0	0	0	0	0	0	10
13	1773	64	30.4	2.5	32	11.1	5	1.163723	0.545000	34	7	3	0	9	1	1	0	0	0	0	1	1	11
14	2140	86	31.9	1.1	20	11.9	6	1.560487	0.720000	39	4	11	2	10	1	1	0	0	0	0	1	0	9
15	2398	55	35.2	1.8	33	14.7	4	1.123889	0.595336	41	2	10	0	11	1	2	1	0	0	0	0	0	14
16	2842	77	59.2	1.5	42	13.1	11	1.824350	0.727273	39	8	9	3	9	1	1	0	0	0	1	0	1	6
17	2216	62	48.5	1.4	66	12.2	10	1.815266	0.745069	43	8	12	2	9	1	0	0	1	0	0	2	0	8
18	2396	57	75.0	1.4	58	16.7	13	1.904627	0.765052	58	28	8	3	4	1	4	0	0	0	0	0	0	10



CHAPTER II - COCOA FLOWER VISITORS IN INDONESIAN AGROFORESTS

**Table S3.** Results of the experimental approach on the response of cocoa flower visitors to litter management (high >4.6kg/m<sup>2</sup>, low <0.6kg/m<sup>2</sup>, and control ~2.3kg/m<sup>2</sup>). In total we monitored 5,184 flowers in 12 weeks.

Plot	Litter treatment	Yield (g/tree)	Flower visitation (%)	Diptera and hymenoptera species richness	Shannon index	Simpson index	Total abundance	Diptera	Hymenoptera (Ant)	Hymenoptera (Wasp)	Hemiptera (Aphid)	Coleoptera	Collembola	Orthoptera	Thysanoptera	Aranae	Larvae	No identified
1	High	108.1	5.09	2	0.600166	0.512397	15	0	2	0	12	1	0	0	0	0	0	0
2		158.7	8.33	3	0.953271	0.506667	30	2	4	0	22	0	0	0	0	0	0	2
3		119.7	8.33	4	1.209254	0.609418	32	0	9	0	13	0	0	0	0	0	1	9
4		125.3	5.56	3	0.720125	0.346667	13	2	1	0	7	0	0	0	0	0	0	3
5		106.3	18.52	4	1.161725	0.621302	91	3	10	0	24	0	0	0	0	0	1	53
6		118.9	8.33	5	1.179234	0.574669	40	1	8	0	22	0	0	0	0	0	0	9
7		228.4	9.26	2	0.687436	0.395000	25	1	4	0	12	0	0	0	0	0	1	7
8		229.9	5.56	3	0.775129	0.394558	21	0	5	0	14	0	0	0	0	0	2	0
9	Low	165.6	4.63	2	1.039721	0.625000	13	1	2	0	5	2	0	0	0	0	0	3
10		197.9	6.94	4	1.549826	0.775510	31	1	2	2	17	0	0	0	0	0	0	9
11		104.4	3.70	2	0.950270	0.560000	10	1	1	0	1	2	0	0	0	0	2	3
12		161.1	9.72	4	1.389681	0.710744	28	1	6	0	9	2	0	0	0	0	0	10
13		130.0	6.02	1	0.686962	0.493827	35	0	4	0	17	1	0	0	0	0	3	10
14		141.4	10.65	3	1.083255	0.613333	38	2	7	0	21	0	0	1	0	0	1	6
15		102.3	7.41	2	0.859967	0.512397	24	0	4	0	10	1	0	0	0	0	1	8
16		97.3	8.33	4	1.215388	0.512397	31	2	5	0	13	0	0	0	0	0	1	10
17	Control	493.4	12.04	6	1.192335	0.541420	27	3	4	2	7	1	0	0	0	0	0	10
18		243.6	13.43	8	1.464693	0.615556	31	2	8	3	10	1	0	0	0	0	0	7
19		249.9	13.43	7	1.442426	0.643599	44	4	11	0	20	0	0	0	1	0	0	8
20		64.8	11.11	2	0.367649	0.169421	28	0	2	0	12	1	0	0	0	0	1	12
21		310.4	12.04	7	1.114838	0.463734	26	3	6	1	9	1	0	0	0	0	0	6
22		251.1	12.04	2	0.344598	0.156250	29	1	1	0	6	0	1	0	0	1	1	18
23		379.3	14.81	3	0.573516	0.266118	33	1	4	0	10	0	2	0	0	0	0	16
24		284.2	12.04	8	1.201482	0.489619	26	3	6	2	11	1	0	0	0	0	1	2

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## Chapter III Hand Pollination, not Pesticides or Fertilizers, Increases Cocoa Yields and Farmer Income



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## Abstract

Increasing demand for cocoa and climate-related yield declines have sparked a multi-stakeholder debate on cocoa production strategies. Agrochemical inputs and pollination enhancement through hand pollination are two strategies to increase yields. Here, we test both strategies with field experiments in Indonesia. We show that even partial hand pollination (13% of easily accessible flowers/tree), and not fertilizers or insecticides, increases yield/tree by 51%. The more laborious 100% hand pollination of the entire tree increases yield/tree by 161%, and farmer's annual net income from 994 USD/ha up to 2,754 USD/ha, or 69% in the study area, after accounting for farm operational, hand pollination labor, and opportunity costs. Thus, intensifying cocoa pollination appears to be a potential solution for closing cocoa yield gaps and should be considered in the current industry-led discussion of designing farms for mitigation of climate change.

**Keywords:** Agrochemical intensification, climate change, cocoa, hand pollination, farmer income, sustainability, yield

## Introduction

The International Cocoa Organization (ICCO) forecasts that climate-related production challenges led to yield declines of 2.3% in the world cocoa production from 2017 to 2018 (ICCO, 2018a). Climate predictions suggest that by 2050, a 2°C temperature increase would intensify current drought events and pest outbreaks, leading to further cocoa yield declines in major producer countries (Läderach et al., 2013; Schroth et al., 2017). In Indonesia, the third largest cocoa producer globally, shade tree removal tends to increase yields and immediate monetary benefits. However, this negatively affects the functional diversity of predators that can enhance farm resilience to pest outbreaks (Donald, 2004; Steffan-Dewenter et al., 2007; Clough et al., 2009). Yields and income of small scale farmers owning less than 2 ha of land continue to decline due to dwindling yields of cocoa trees beyond 25-30 years old. The volatile world market price for cocoa, abandonment of old unshaded cocoa farms, and migration to urban areas further add to the economic insecurity of smallholders (Donald, 2004, Hettig et al., 2017).

This so-called cocoa “boom-and-bust cycle” (Tschardt et al., 2011) is addressed in the “Cocoa Action” efforts of the World Cocoa Foundation (WCF) trade group and

other multi-stakeholder sustainability initiatives. Such initiatives primarily aim to both mitigate climate change impacts by preserving tropical rainforest, and to improve the livelihood of small-scale cocoa farmers, which contribute 90% of the global cocoa production (Donald, 2004; Gockowsky and Sonwa, 2011; Läderach et al., 2013; Schroth et al., 2017; WCF, 2018). While these sustainability initiatives promote sophisticated breeding technologies for climate-resistant varieties (MARS, 2018a) and improvements in fertilizer use (MARS, 2018b), they largely neglect alternative approaches such as enhancing pollination to improve cocoa yields sustainably (Young, 1982; Falque et al., 1995; Groeneveld et al., 2010; Wanger et al., 2014; Forbes and Northfield, 2017; Toledo-Hernández et al., 2017).

Cocoa is a cross-pollinated plant that highly depends on specialized insects for successful pollination (Entwistle, 1972; Young, 1986; Toledo-Hernández et al., 2017). Pollen limitation, as less than 10% of flowers in a tree are successfully pollinated in natural conditions, appears to be a major factor to improve yields in Indonesia (Groeneveld et al., 2010; Wanger et al., 2014; Toledo-Hernández et al., 2017). In experiments in Ivory Coast, increased pollen deposition rates on the style enhanced the number of seeds per fruit (Falque et al., 1995). Intensifying pollinator-flower visitation through landscape (e.g. forest conservation) and farm management (e.g. shade canopy cover) practices for improving pollinator habitats can increase pollination rates, pollen deposition, and improve fruit set (Young, 1982; Wanger et al., 2014; Forbes and Northfield, 2017; Toledo-Hernández et al., 2017).

However, no study so far has compared the performance of agrochemical intensification with pollination enhancement strategies in a realistic field experiment including landscape and farm management context, to identify the best approach for increasing cocoa yields. Further, studies linking hand pollination and economic performance of cocoa production strategies are lacking. Here, we use hand pollination and commonly applied fertilizers and insecticides to contrast the respective effects of pollination and chemical intensification on cocoa yield related variables (fruit set, harvested fruits and yields), and the pollination contribution for improving farmer income in Central Sulawesi, the major cocoa producing region of Indonesia (Witjaksono, 2016).

## Materials and Methods

### *Study Site*

The study area is located in the region of Napu Valley (S1° 27' 48", E120° 21' 6"), at the forest margins of Lore Lindu National Park, in Central Sulawesi, Indonesia (Fig. 1A) (Tscharntke et al., 2007, 2010). Lore Lindu National Park is one of the most important and well-preserved tropical forest remnants in Sulawesi and lies within the Wallacea bio-geographic region, a biodiversity hot spot. The Lore Lindu's more than 2,000 km<sup>2</sup> area is habitat to a large number of endangered flora and fauna, which are highly threatened by human action (Weber et al., 2007).

Cocoa farming is one of the major income-generating activities in the study region and Central Sulawesi, with 70% of Indonesia's cocoa production coming from this region (Witjaksono, 2016). This has led to immigration of people from other Indonesian islands seeking livelihood improvements through the cocoa cultivation sector. For instance, the cocoa expansion in the study area strongly contributed to increased household income, closing the poverty gap by over 18% between 2006 to 2013 (Hettig et al., 2017). However, increasing population density, cocoa demand, and subsequent land conversion to farmland has put high pressure on the remaining pristine tropical forest areas (Weber et al., 2007).

### *Farm Survey*

We conducted semi-structured surveys in 28 cocoa farms to characterize our study area and management practices. A summary of data collected in the farm survey is available in Electronic Appendix Table S1. Questionnaires included the cocoa tree varieties and age; amount and periodicity of fertilizer and insecticides use; and yields as dry cocoa beans weight. Further, we estimated the number of cocoa and shade trees/ha, and canopy cover as mean percentage in one ha farm using digital photographs and processing software Image J (Wanger et al. 2009; Maas et al., 2013). We calculated the farm distance to nearest forest margin in meters using satellite images and GPS coordinates.

### *Experimental Design*

We conducted two separate hand pollination experiments: 1) hand pollination of flowers below 2m in height (13% of all flowers/tree; hereafter "partial hand

pollination"), and 2) hand pollination of all flowers on a tree (100% of the flowers/tree; hereafter "full hand pollination").

**1) Partial hand pollination.** This experiment aimed to calculate the effect of increased hand pollination of flowers, and landscape (forest distance) and farm management factors (agrochemical inputs, canopy cover) on yields (Electronic Appendix Figure S1). First, we selected 12 of the 28 surveyed farms (hereafter "plots") with a minimum distance of 200 m between each plot. The areas surrounding the plots consisted in average of 67% secondary forest combined with low and high shaded cocoa, and 33% vegetable gardens, settlements and open areas. We sorted the selected plots into four combined forest distance (close=0-800 m, far=1,500-3,400 m) and canopy cover (low= 0-20%, high= 40-80%) categories to evaluate the potential effect of forest distance and canopy cover on pollination and yields. Studies suggests that forest habitats and canopy cover provide suitable microclimatic conditions for pollinators, thus farms adjacent to forest and with high canopy cover may benefit from additional pollination (Clough et al., 2011; Tschardt et al., 2011; Toledo-Hernández et al., 2017). Each category had three plot replicates: 1) close to forest and low canopy cover, 2) close to forest and high canopy cover, 3) far to forest and low canopy cover, and 4) far to forest and high canopy cover categories (Fig.1B).

In each plot, we established four subplots of 10 m<sup>2</sup> area, separated by a minimum distance of 10 m for our agrochemical intensification treatments (Fig. 1C). In these subplots, we doubled the amounts of agrochemical inputs commonly used in the 28 surveyed farms (Electronic Appendix Table S1). Our four treatments were: 1) double fertilizer (Nitrogen, Phosphorus and Potassium or granule NPK= 373 [SE± 82.8] kg/ha), 2) double insecticide (Capture® Concentrate= 3.4 [SE± 1.1] l/ha), 3) double fertilizer (granule NPK= 373 [SE± 82.8] kg/ha) and double insecticide (Capture® Concentrate= 3.4 [SE± 1.1] l/ha), and 4) control where agrochemicals and fertilizer were not used (granule NPK= 0 kg/ha, Capture® Concentrate= 0 l/ha) (Fig. 1C). We followed the farmer periodicity of agrochemicals used for one harvesting season (six months), in which fertilizer is applied once (at the initial stage), and insecticide twice (at the initial stage and three months later). One month before starting the experiments farmers avoided application of any agrochemicals in the plots until the experiment ended.

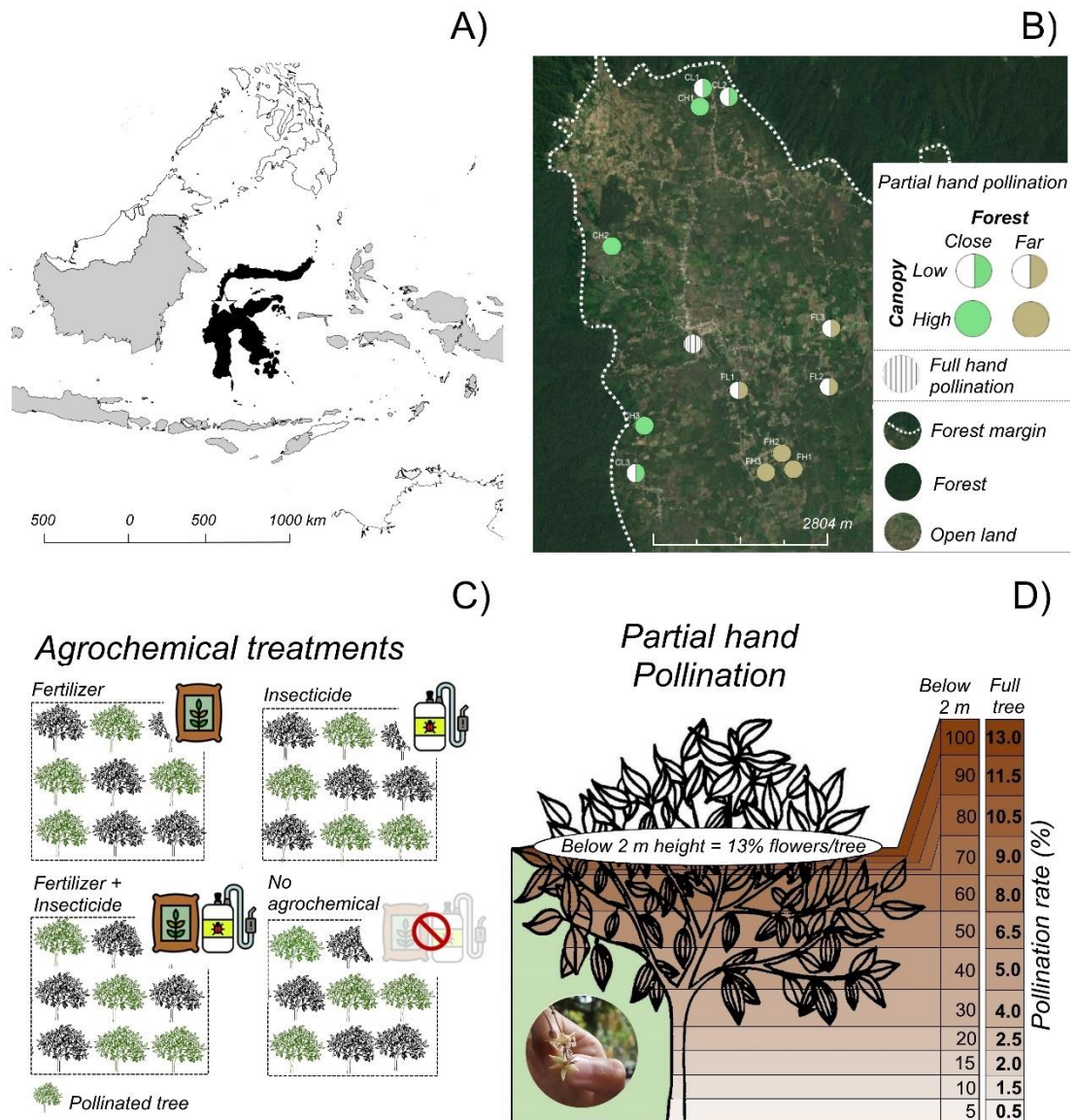
In each subplot, we randomly selected four cocoa trees of the most common variety in the study area, which is a hybridization between the Forastero and Trinitario types (Bos et al., 2007a; Groeneveld et al., 2010) with no evidence of pest and disease

attacks and of ages ranging from 5 to 19 years, thus, a total of 192 cocoa trees for the whole experiment. Following Groeneveld et al. (2010), we hand pollinated accessible flowers below 2 m height (or 13% of the flowers/tree) in 12 different rates, ranging from 0.5 ( $\bar{X}$ = 14.4 flowers/tree SE $\pm$ 3.0) to 13% ( $\bar{X}$ = 231.3 flowers/tree SE $\pm$ 28.0), thus comprising 16 cocoa trees for each rate category (Fig. 1D; Electronic Appendix Table S2). We additionally selected 12 cocoa trees as control (0% hand pollination; one for each plot) and monitored pollination success below 2 m height to contrast fruit development in our treatment trees with that under natural pollination.

**2) Full hand pollination.** To verify effects of the partial hand pollination experiment on the full-tree hand pollination on yields, we used a separate available plot of the close-to-forest distance category and with <10% canopy cover (Fig. 2A, Electronic Appendix Figure S1). In this plot, we randomly selected eight healthy cocoa trees. We clustered the selected trees in two age categories: four young (ca. 6 years old), and four old (ca. 36 years old). An average of 2,009 (SE $\pm$ 202.5) open flowers/tree were hand pollinated. We used the average tree yield recorded of all 28 farms to quantify the contribution of 100% hand pollination on yields in contrast to natural pollination in Napu Valley (Electronic Appendix Table S2).

#### *Hand Pollination Method*

We conducted the hand pollination of flowers daily for 60 days (from April to May 2017) following the labor effort of Groeneveld et al. (2010) and the method described by Falque et al. 1995). A detailed description on the hand pollination method is available in Electronic Appendix Figure S3. First, we removed all open flowers and fruits at any development stage from the tree one day before starting the experiments to promote tree resource allocation on flowering (Valle et al., 1990; Toledo-Hernández et al., 2017). On the next day, we pollinated each newly opened flower using three collected flowers from three different trees in farms not included in the experiments. This procedure aims to minimize potential tree self-incompatibility, and pollen grain deficits affecting pollination success. From the flowers collected, we carefully removed the flower petals to access the anthers containing the pollen grains. To perform the pollination, we rubbed the five anthers from each of the three flowers in the flower style of the flower pollinated. We removed flowers not hand pollinated below 2 m height to avoid natural pollination from unpollinated flowers, and to minimize the variability of resources between trees allocated to flower production.



**Figure 1.** The study area of the partial (13% flowers/tree) and full (100% flowers/tree) hand pollination experiment in the Napu Valley of Central Sulawesi, Indonesia (A). We selected 12 cocoa farms (plots) and sorted them into two landscape (close to forest= 0-800 m, far to forest= 1,500-3,400 m) and two farm management (low canopy cover=0-15%, high canopy cover= 40-80%) categories, with three replicates for each category (B). In each plot, we established four agrochemical intensification treatments where we doubled the average amount of chemicals applied (double fertilizer, double insecticide, double fertilizer and insecticide=, and no agrochemical application at all) (C). We randomly selected four cocoa trees from each treatment to be hand pollinated (green trees) plus one control tree/plot (natural pollination= 0% hand pollination rate) (C). Finally, we hand pollinated easily accessible flowers below 2 m height (corresponding to 13% flowers/tree) at 12 different rates ranging from 0.5 to 13% flowers/tree, with 16 tree replicates per hand pollination rate category (Electronic Appendix Figure S1) (D). In a separate farm (B, white dot), we conducted the full hand pollination experiment where we hand pollinated all flowers of eight cocoa trees (Electronic Appendix Figure S2).

### Fruit Monitoring and Harvesting

We conducted the fruit monitoring (absolute number of fruit set, aborted, and fruit losses) and harvesting (absolute number of harvested fruits, and dry bean weight) of



the hand pollinated flowers at a tree base (Electronic Appendix Figure S3). We monitored all hand-pollinated flowers three days after hand pollination to record number of fruit sets and number of early aborted fruits. Published evidence suggests that successful pollination, or fruit set, occurs within the first 48h, while unpollinated flowers wilt and fall down from the tree after this time frame (Toledo-Hernández et al., 2017). We monitored un-aborted fruits daily for two weeks to estimate fruit development and number of cherelle wilted fruits. Cherelle wilt is a plant-regulated process, generally occurring within the first weeks of fruit development that causes fruits shrinking, darkening, and drying due to lack of nutrient resources to better sustain the remaining, non-wilted fruits (Wood and Lass, 2008). Further, we monitored fruits weekly until harvest to record incidences of the two most common cocoa pest and diseases in the study area: the mirid bug (*Helopeltis* sp.) pest and black pod (*Phytophthora* sp.) disease (Bos et al., 2007a). The harvest took place approximately six months after hand pollination started and lasted around three months (October-December 2017). We harvested and counted all fruits reaching maturity per tree from hand pollinated flowers. We fermented and dried the pooled seeds from healthy and diseased (fruits with signs of mirid bugs and black pod disease) fruits per tree following the regular practice by farmers to minimize income losses (Electronic Appendix Figure S4), and quantified dry bean weight per tree.

### *Statistical Analysis*

We analyzed only the data of the hand pollinated mature trees (age 10 to 19 years) from the partial hand pollination, because very young trees (age five to eight years) turned out to produce only very few fruits (Electronic Appendix Figure S5). This gave a total sample size of two (instead of three) plot replicates per landscape and per farm management categories and 128 (instead of 192) trees with eight to 12 (instead of 16) trees per each hand pollination category (Electronic Appendix Figure S1). We used model selection with a generalized linear mixed effects modelling approach (Burnham and Anderson, 2003) of the data with Poisson distribution pooled by tree. We quantified the effect (fixed effect variables) of (1) hand pollination (%), (2) forest distance (m), (3) canopy cover (%), and (4) agrochemical intensification treatments on the response variables (1) number of fruit set, (2) number of cherelle wilts, (3) number of pest and diseased, and (4) number harvested fruits, and (5) yield (kg dry bean weight). We included agrochemical intensification subplots nested in farm plots as random effect variables to estimate trees specific variations. All predictor and random effect variables were continuous, besides chemical intensification, which we treated

as a categorical variable. We ran the models with negative-binomial distribution to correct for overdispersion (Brooks et al., 2017), and scaled continuous predictors (i.e. count data= fruit set, cherrille wilt, pest & diseases, harvested fruits; proportion data= yield) to account for variables with different measuring scales (Bolker et al., 2009, Bruce et al., 2020). Then, as several models satisfied the  $\Delta AICc \leq 2$  cut-off criterion for best-fit models (Anderson, 2007), we averaged the coefficient of the best fitting models (Electronic Appendix Table S3). Finally, we conducted a least square means analysis for a multiple comparison to quantify the effect of our agrochemical intensification treatments on each of the response variables (Electronic Appendix Table S4) (Lenth, 2018).

### *Hand Pollination and Farmer Income*

To understand the economic implications of pollination for cocoa farmers and household livelihood, we calculated the overall effect of pollination intensification on income, or pollination-related net income ( $I_{Pollnet}$ ), by extrapolating yield increase from our full hand pollination experiment across farms in Napu Valley (Electronic Appendix Table S5). We calculated the  $I_{Pollnet}$  per hectare in four steps: (1)  $I_{Gross}$ = Gross income, (2)  $OC$ = Operational costs, (3)  $HP$ = Hand pollination effort, and (4)  $OP$ = Opportunity costs using data from our 28 farm surveys in 2017.

**1) Gross income ( $I_{Gross}$ ).** We defined the  $I_{Gross}$  as the monetary value of the farmer's harvested cocoa dry beans (yield). This was calculated as the product of yield increase compared to the control trees ( $Y_{\Delta}$ = kg dry beans/ha) and the marketable price of cocoa or  $CP$  (Electronic Appendix Table S6).

$$I_{Gross} = Y_{\Delta} * CP$$

**2) Operational costs (OC).** The  $OC$  are the economic investments of a farmer to run operation of the farm and include purchase of agrochemical and organic inputs (fertilizers, herbicides, insecticides) and labor for farm management (e.g. tree pruning, weeding, agrochemical/organic input application, harvesting, processing).

$$OC = \sum \text{inputs} + \text{labor}$$

Based on information of inputs (69.9 USD/ha,  $SE \pm 19.3$ ) and labor (26.3 USD/ha  $SE \pm 5.5$ ) costs from our farm survey, we know that in 2017 the  $OC = 96.2$  ( $SE \pm 24.8$ )

USD/ha (Electronic Appendix Table S1). The calculated labor value is the annual mean expense recorded in the farm surveys. It accounts for a seven to eight-day worker salary supporting in farm management activities at 3.7 USD/day (8-9 hours) rate.

**3) Hand Pollination Effort (HP).** The *HP* is the product of the pollinated days multiplied by the investment for hand pollination one ha farm area (*PI*).

$$HP = N \text{ day(s) pollination} * PI$$

We recorded from the hand pollination trainings prior our study (Electronic Appendix Figure S3) that a worker can fully (100% flower/tree) hand pollinate 77 trees (one tree in ~7 minutes, without collecting any flower and fruit development data) in one working day. This means a *PI* of 39 (SE±3.7) USD/day at a worker salary of 3.7 USD/day in 2017. Thus, we assumed that 10-11 workers will be required to full hand pollinate 813.5 (SE±389) trees/ha (from farmer surveys) in one day (eight to nine-hour work). As we pollinated flowers daily for 60 days in the hand pollination experiment, the *HP* costs were 2,342.4 (SE±219.2) USD/ha. However, successful pollination can be assumed to occur every second day (30 working days), because flowers are receptive to pollen for even more than 48 hours after opening (Toledo-Hernández et al., 2017). This reduces *HP* cost to 1,171.2 (SE±109.6) USD/ha.

**4) Opportunity Costs (OP).** The *OP* accounts for income that a household member may receive if working in other activities outside the farm (hand pollination) (Buchanan, 1991). To account for *OP*, we assumed a 100% hired labor for pollination, thus the *OP* was one (100%= 1). As in our study area, the salaries are equal regardless the local labor activity carried out, this makes *HP* and *OP* identical.

$$OP = 1$$

**5) The Pollination Related Net-Income ( $I_{Pollnet}$ )** Finally,  $I_{Pollnet}$  is the earnings a farmer obtains after subtracting the *OC*, *HP* and *OP* from the  $I_{Gross}$ .

$$I_{Pollnet} = (I_{Gross} - OC) - (HP * OP)$$

*The  $I_{Pollnet}$  scenarios justification*

To understand the possible variations on the potential benefits of hand pollination on farmers' income in Napu Valley, we developed three pollination-related net income increase scenarios accounting for two *HP* (daily and every second day for 60 days) and three *OC* (high, average and low *OC*). We summarized the results of the income scenarios in Electronic Appendix Table S5.

**1) Conservative.** We assume the same hand pollination labor as in our experimental study and the highest (maximum value) farm operation costs reported in our survey (*HP* daily for 60 days and high *OC*).

$$I_{Pollnet}=I_{Gross}-378.8 \text{ USD/ha (2,342.4 USD/ha*1)}$$

**2) Realistic.** We assume half of the pollination labor as in our experimental study and the average farm operation costs reported in our surveys (*HP* every second day for 60 days and high *OC*).

$$I_{Pollnet}=I_{Gross}-96.2 \text{ USD/ha (1,171.2 USD/ha*1)}$$

**3) Optimistic.** We assume half of the hand pollination labor as in our experimental study and the lowest (minimum value) farm operational costs (*HP* every second day for 60 days and no *OC*).

$$I_{Pollnet}=I_{Gross}-1.8 \text{ USD/ha (1,171.2 USD/ha*1)}$$

## Results and Discussion

### *Partial Hand Pollination Effects on Yield Related Variables*

In our partial hand pollination experiment, we analyzed 128 cocoa trees pollinated up to 2 m above the ground (13% of all flowers/tree) for 60 days and found that pollination and not agrochemical intensification increased all cocoa yield related variables (Table 1, Fig. 2). Similar to Groeneveld et al. (2010), partial hand pollination of 13% flowers/tree increased dry bean weight of the hand pollinated flowers by 51.3% per tree ( $p < 0.0001$ ; Table 1E, Fig. 2F) compared to natural pollinated (0% hand pollination, i.e. control) trees.

Our multiple comparison analysis shows that hand pollination in our control treatments provides similar fruit set, cherelle wilt, pest and disease load, harvested fruits, and yields compared to both, fertilizer and insecticide addition, as well as their combination (Electronic Appendix Table S4). The results of the fertilizer treatment on yield can be associated to the nature of Central Sulawesi soils, which are considered very fertile with soil nitrogen stock of 9,900 kg/ha (Dechert et al., 2005; Groeneveld et al., 2010), suggesting no nutrient limitations in our study area. Furthermore, the evolution of resistance of pests to insecticides (Entwistle, 1972; Asogwa and Dongo, 2009), and its little targeted applications, affecting also beneficial pest predators (Croft and Brown, 1975, Syarief et al., 2017), may be the major reason for the neutral or even negative effects of spraying insecticides on pest and diseases.

In contrast to naturally pollinated trees, fruit set of the partial (13% flowers/tree) hand pollinated trees increased more than seven times ( $p < 0.0001$ ; Table 1A, Fig. 2B) and the amount of harvested cocoa fruits increased by 85.1% ( $p < 0.0001$ ; Table 1D, Fig. 2E), despite a 30 times increase in early fruit abortion or cherelle wilt ( $p < 0.0001$ ; Table 1B, Fig. 2C), and 10 times higher fruit losses to pest and diseases ( $p < 0.0001$ ; Table 1C, Fig. 2D). The premature fruit abortion, i.e. the cherelle wilt (Falque et al., 1995), allows the cocoa tree to allocate energy towards the development of remaining fruits and, hence, may explain the increase in fruit set and fruits.

At the landscape level we found that forest distance did not affect any yield related variables (Table 1). This may be due to the ecology and behavior of tiny cocoa pollinators (Ceratopegonidae and other small insects); single flights cover only a few meters (Chumacero de Schawe et al., 2013) and, hence, forest to farm movement for flower foraging may be limited. At the farm level, canopy cover had a negative effect on fruit set ( $p = 0.028$ ; Table 1A) and led to lower pest and diseases infestation ( $p < 0.028$ ; Table 1C). This highlights the trade-offs of agroforestry systems (Clough et al., 2011; Tschardt et al., 2011; Blaser et al., 2018). For instance, shade trees may compete with cocoa for nutrients (Isaac et al., 2007), but may also enhance predators and predation of herbivores (Maas et al., 2013).

**Table 1.** The generalized linear mixed effect model results of the agrochemical intensification and partial hand pollination (13% flowers/tree) experiment in cocoa trees of Central Sulawesi, Indonesia. We investigated the response of amount of (A) fruit set, (B) amount of cherelle wilt, (C) pest and disease (number infested fruits), (D) number harvested fruits, and (E) yield (dry bean weight g/tree) to agrochemical intensification treatments (double fertilizer [Fertilizer], double fertilizer and double insecticide [Fertilizer+Insecticide], and double insecticide [Insecticide]) and farm parameters Forest distance (m) and Canopy cover (%). Significant *p-values* highlighted in bold.

	Coefficient	SE	Adjusted SE	z-value	p-value
<b>A Fruit set</b>					
Intercept	3.904	0.103	0.104	37.421	<b>&lt;0.0001</b>
Hand pollination	0.494	0.030	0.031	16.009	<b>&lt;0.0001</b>
Forest distance	0.071	0.090	0.091	0.779	0.436
Canopy cover	-0.184	0.083	0.084	2.191	<b>0.028</b>
Fertilizer	0.087	0.175	0.177	0.490	0.624
Insecticide	0.259	0.175	0.177	1.465	0.143
Fertilizer+Insecticide	0.173	0.175	0.177	0.979	0.328
<b>B Cherelle wilt</b>					
Intercept	2.685	0.257	0.260	10.327	<b>&lt;0.0001</b>
Hand pollination	0.488	0.047	0.047	10.331	<b>&lt;0.0001</b>
Forest distance	0.167	0.250	0.252	0.662	0.508
Canopy cover	-0.263	0.242	0.245	1.073	0.283
Fertilizer	0.075	0.303	0.306	0.246	0.805
Insecticide	0.294	0.303	0.306	0.963	0.335
Fertilizer+Insecticide	-0.074	0.304	0.307	0.242	0.809
<b>C Pest and diseases</b>					
Intercept	3.718	0.112	0.113	32.907	<b>&lt;0.0001</b>
Hand pollination	0.553	0.033	0.033	16.703	<b>&lt;0.0001</b>
Forest distance	0.096	0.100	0.101	0.951	0.342
Canopy cover	-0.208	0.093	0.094	2.200	<b>0.028</b>
Fertilizer	0.093	0.213	0.215	0.430	0.667
Insecticide	0.271	0.213	0.215	1.261	0.207
Fertilizer+Insecticide	0.157	0.213	0.215	0.731	0.465
<b>D Harvested fruits</b>					
Intercept	2.057	0.036	0.037	55.732	<b>&lt;0.0001</b>
Hand pollination	0.144	0.031	0.032	4.539	<b>&lt;0.0001</b>
Forest distance	-0.059	0.033	0.033	1.767	0.077
Canopy cover	-0.010	0.031	0.032	0.316	0.752
Fertilizer	-0.012	0.091	0.091	0.135	0.893
Insecticide	0.082	0.088	0.089	0.919	0.358
Fertilizer+Insecticide	0.067	0.089	0.090	0.747	0.455
<b>E Yield</b>					
Intercept	5.607	0.052	0.053	105.668	<b>&lt;0.0001</b>
Hand pollination	0.095	0.015	0.015	6.338	<b>&lt;0.0001</b>
Forest distance	-0.064	0.044	0.044	1.463	0.143
Canopy cover	-0.023	0.046	0.047	0.487	0.626
Fertilizer	-0.008	0.073	0.074	0.108	0.914
Insecticide	0.027	0.073	0.074	0.361	0.718
Fertilizer+Insecticide	0.127	0.073	0.074	1.708	0.088

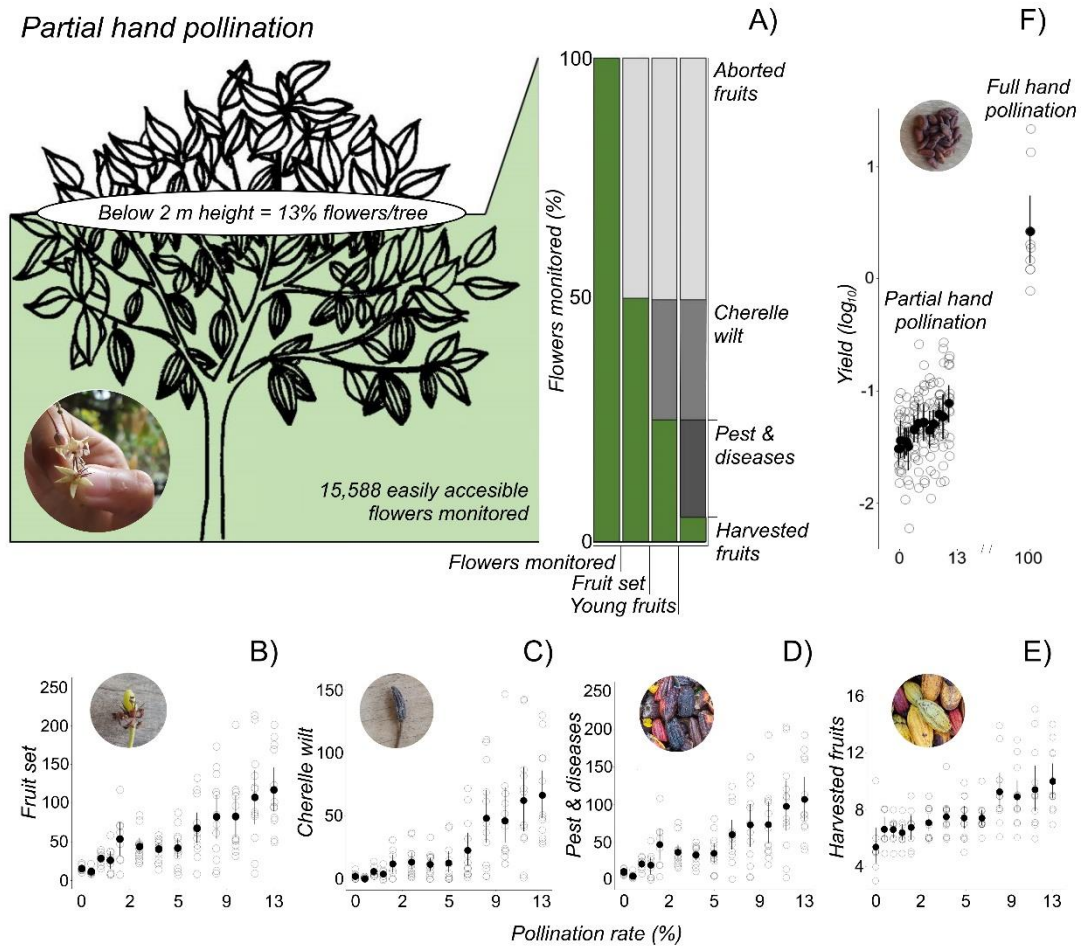
### *The Cocoa Fruit Development*

In the partial hand pollination experiment, we recorded a fruit set of 50.8% ( $N= 7,920$  flowers) of all 15,588 hand pollinated flowers examined, while only 6.3% ( $N= 1,015$  flowers) developed harvestable fruits (Fig. 2A, Electronic Appendix Table S2). Fruit set in the control trees, where flowers were naturally pollinated ( $N= 1,177$  flowers examined), was of 10.3% ( $N= 121$  flowers), and only 3.6% ( $N= 43$  flowers) reached

maturity and were harvested. In the full hand pollination experiment, fruit set was of 47.5% ( $N= 7,635$  flowers) of the 16,072 flowers examined, while only 2.7% ( $N= 428$  flowers) developed to harvestable fruits (Electronic Appendix Table S2). The increased fruit set after hand pollination, and the general low levels of harvestable fruits are in accordance with previous studies in Central Sulawesi (Bos et al., 2007b; Groeneveld et al., 2010).

We found overall fruit losses before the harvest of 87.5% ( $N= 6,929$ ) in the partial hand pollination experiment, and of 67.8% ( $N= 82$ ) in the control trees. In the full hand pollination experiment, we recorded fruits losses of 94.4% ( $N= 7,202$ ) before the harvest. The overall fruit losses in our hand pollination experiments are similar to published results of hand pollination studies in Indonesia (72% to 92%) (Bos et al. 2007a; Groeneveld et al., 2010) and Brazil (79%) (Hasenstein and Zavada, 2001), but higher than in the Ivory Coast (29%) (Falque et al., 1995). The main reasons for cocoa fruit losses before the harvest are internal factors such as early fruit abortion or cherelle wilt within the first two weeks after pollination, while external factors such as pest and diseases occur throughout the fruit development (Falque et al., 1995; Bos et al., 2007a; Toledo-Hernández et al., 2017).

In the partial hand pollination experiment, we observed an early fruit abortion (cherelle wilt) of 45.1% ( $N= 3,584$ ) of the successfully hand pollinated flowers, and of 14.9% ( $N= 18$ ) in the control trees. In the full hand pollination experiment, the 70% ( $N= 5,347$ ) of all flowers developing into fruits were early aborted. The cocoa mirid bug (*Helopeltis* sp.) and the black pod disease (*Phytophthora* sp.) caused fruit losses before the harvest of 77.1% ( $N= 3,345$ ) in the remaining 4,336 un-aborted fruits in the partial hand pollination, while pest and diseases caused 62.1% ( $N= 64$ ) fruit losses of the remaining 103 un-aborted fruits in the control trees. In the 2,288 un-aborted fruits of the full hand pollination experiment, the 81.3% ( $N= 1,860$ ) of fruits were lost by the mirid bugs and black pod disease. Our high incidence of pest and diseases and their contribution to fruit losses was higher than the 23% previously reported in hand pollination experiments in Central Sulawesi (Bos et al., 2007a). Signs of cocoa mirid bugs were found on 74.5% ( $N= 756$ ) and 55.8% ( $N= 24$ ) of the 1,015, and 43 harvested fruits of the partial hand-pollinated and control trees, respectively. We found black pod disease on 23.3% ( $N= 236$ ) and 28% ( $N= 12$ ) of the harvested fruits, and only 2.3% ( $N= 23$ ) and 16.3% ( $N= 7$ ) were not affected by any pest or disease. The 56.8% ( $N= 243$ ) and 22.4% ( $N= 12$ ) of the 428 harvested fruits in the full hand pollination experiment showed incidence of pest and disease, while 20.8% ( $N= 89$ ) presented no signs of mirid bugs and black pod.



**Figure 2.** The cocoa pollination-production cycle (A) and the positive effect of pollination intensification on fruit set (B), cherrille wilt (C), pest and diseases(D), harvested fruits (E), and the combined yield results from the partial (13% flowers/tree, solid line) and the full hand (100% flowers/tree, dotted line) pollination experiment (F). The cocoa pollination-production cycle starts with the fruit set of young flowers recorded 48 hours after pollen deposition on the flower style (A). In the partial hand pollination experiment, more than half of flowers dropped (A, Aborted flowers). Over one quarter of young fruits from non- aborted flowers naturally aborted after two weeks (A, Cherelle wilt). Pests and diseases occurring before the harvest contributed in further fruit losses (A, Pest & diseases). Finally, only 6.3% of pollinated flowers matured to harvestable fruits (A, Harvested fruits). The black dots in figures B to F indicate the means and vertical bars the standard error of the response variable.

### The Economic Benefit of Pollination in Napu Valley

Our partial (13% flowers/tree) and full (100% flowers/tree) hand pollination experiment led to a yield increase per tree of 51.3% or 0.117 (SE±0.008) kg/tree and of 161.5% or 1.066 (SE±0.298) kg/tree, respectively (Table 2A). Extrapolating our yield results in both hand pollination experiments to farm level using data from 28 farms across our study site, considering an average of 813.5 trees/ha (SE±76.1) from our 28 farms surveyed, translates to a farm yield increase from 185.5 (SE±18.7) kg/ha



to 280.6 (SE±25.2) kg/ha, and from 536.9 (SE±49.6) kg/ha to 1,404.4 (SE±292) kg/ha in the partial hand and full hand pollination experiment, respectively.

The extrapolation of a 161.5% yield increase from the full (100% flowers/tree) hand pollination experiment and calculation of the three different pollination-related net income increase ( $I_{Pollnet}$ ) scenarios (Table 2B, Electronic Appendix Table S5), shows that the realistic ( $I_{Pollnet}$  = 1,582.1 USD/ha, 59.3% change) and optimistic ( $I_{Pollnet}$  = 1,677.3 USD/ha, 68.8% change) scenarios provide favorable income returns. In contrast, the conservative scenario shows negative income returns ( $I_{Pollnet}$  = 129.1 USD/ha, -87.3% change) because of the assumption of an overly high labor investment. Overall, these results suggest that a realistic cost calculation (including current farmer operational, and opportunity costs, and hand pollination labor) of hand pollinating all flowers on a cocoa tree can be highly profitable for farmers in Napu Valley.

#### *Hand Pollination and Cocoa Sustainability*

In general, our results are in line with previous studies in our study area suggesting that pollen rather than resource limitation is a major factor determining yields (Bos et al., 2007b; Groeneveld et al., 2010). These findings may provide guidance to develop diversified farming strategies that include pollination to improve yields and farmer's income.

However, we consider three main aspects requiring attention in order to elucidate the full potential of cocoa hand pollination for promoting sustainability in cocoa. First, our study was carried out for one harvesting season, limiting our understanding on the effect of hand pollination on tree resources and long term yield stability. For instance, cocoa trees under intensive hand pollination may allocate resources to sustain highly energy demanding fruits (Bos et al., 2007b), leading to a resource depletion and abnormal flowering and fruit abortion (Valle et al., 1990). Furthermore, tree aging, a major issue in most of the growing regions in the world where trees are over 25 years, may reduce the effectiveness of hand pollination for improving yields (Wessel and Quist-Wessel, 2015). Hence, we suggest that future research should focus on long-term yield stability over several harvesting seasons under hand pollination and on tree physiology parameters that can influence hand pollination effectiveness.

Second, the high labor costs of hand pollination, as we observe in the conservative scenario, may blur the economic benefit to farmers. Future studies should determine

the best labor cost-benefit hand pollination rate (e.g. from 20% to 40% of all flowers). We need also more detailed analyses of the pollination labor efforts (to identify hand pollination minimum time interval and time-saving, skilled applications of hand pollination) to reach maximum attainable yields. In addition, the quantification of hand pollination effects on global cocoa yields and farmer income in major producer countries will help to understand the true potential of hand pollination strategies for sustainable production.

Lastly, Natural pollination is a sustainable and inexpensive alternative to hand pollination, but the effects of different farm management strategies on pollinators at the farm (e.g. shade tree and soil litter management) and landscape level (e.g. forest conservation), as well as cocoa pollinator identity and their potential breeding sites are little understood (Young, 1982; Frimpong et al., 2011; Forbes and Northfield, 2017; Toledo-Hernández et al., 2017). Multi-stakeholder initiatives such as 'Deforestation Free Cocoa' (UNFCCC, 2018) and 'International Cocoa Initiative' (Cocoa Initiative, 2018), which focus on ecological farming approaches, will help to leverage the full pollination potential in practice.

## Conclusions and the Way Forward

In this large-scale study we show that hand pollination can increase cocoa yields by 51.3% with partial hand pollination of 13% of flowers, and by 161.5% with full hand pollination of all flowers, resulting in an annual farmer income by up to 68.8%, independent of fertilizer and insecticide inputs. In the near future, research studies should give priority to understand the long term sustainability of hand pollination, and to develop alternative methods and tools to optimize procedures and to reduce pollination labor, thereby increasing income benefits. Our results suggest that cocoa pollination deserves a more prominent position in cocoa research and stakeholder discussions to sustainably meet the global cocoa demand increases of 2.5 to 3% annually (ICCO, 2018b), secure farmers' livelihoods, and to end deforestation in a time of global climate change.

**Table 2.** The summary results of the partial (13% flowers/tree), and full (100% flowers/tree) hand pollination experiments on cocoa yields at the tree level ( $Y_{\Delta/tree}$ ), and yield extrapolations at the farm level ( $Y_{\Delta/ha}$ ) (A), and the results of the pollination-related income increase ( $I_{Pollnet}$ ) scenarios (B). The hand pollination of cocoa increases yields by 51.3% in the partial, and 161.5% in the full hand pollination experiment (A). The result extrapolations of a 161.5% yield increase at the farm level ( $Y_{\Delta/ha}$ ) as recorded in our full hand pollination experiment (A) translates into farmer income benefits after accounting for hand pollination costs in the realistic and optimistic scenarios, but not in the conservative scenario (detailed calculations of the  $I_{Pollnet}$  are available in Electronic Appendix Table S5).

<b>A. Hand pollination experiments</b>						
	<b>Partial hand pollination</b>			<b>Full hand pollination</b>		
	<i>Value</i>	<i>SE</i>	<i>% Change</i>	<i>Value</i>	<i>SE</i>	<i>% Change</i>
$Y_{\Delta/tree}$ (kg/tree) <sup>1</sup>						
No pollination	0.228	0.023	0.0	0.660	0.061	0.0
Pollination	0.345	0.031	51.3	1.726	0.359	161.5
$Y_{\Delta/ha}$ extrapolations (kg/ha) <sup>2</sup>						
No pollination	185.5	18.7	0.0	536.9	49.6	0.0
Pollination	280.6	25.2	51.3	1,404.1	292.0	161.5
<b>B. Pollination-related income increase (<math>I_{Pollnet}</math>) (USD/ha)</b>						
<i>Scenario</i> <sup>3</sup>	<i>Description</i>			<i>Value</i>	<i>SE</i>	<i>% Change</i>
No pollination	Natural pollination			993.7	100.7	0.0
Conservative	HP daily for 60 days + high OC			129.1	592.8	-87.0
Realistic	HP every second day for 60 days + medium OC			1,582.9	592.8	59.3
Optimistic	HP every second day for 60 days + low OC			1,677.3	592.8	68.8

<sup>1</sup> Cocoa yield results of the partial and full hand pollination experiments at the tree level (kg/tree).

<sup>2</sup> Yield increase extrapolation of the partial and full hand pollination experiments to the farm level (kg/ha) considering 813.5 (SE±389) tree/ha from the 28 farm surveys (Electronic Appendix Table S1).

<sup>3</sup> The  $I_{Pollnet}$  scenarios assume the costs of two hand pollination effort (HP), and maximum (high), average (average), and minimum (low) operational costs (OC) recorded in the 28 farms surveys (Electronic Appendix Table S1).

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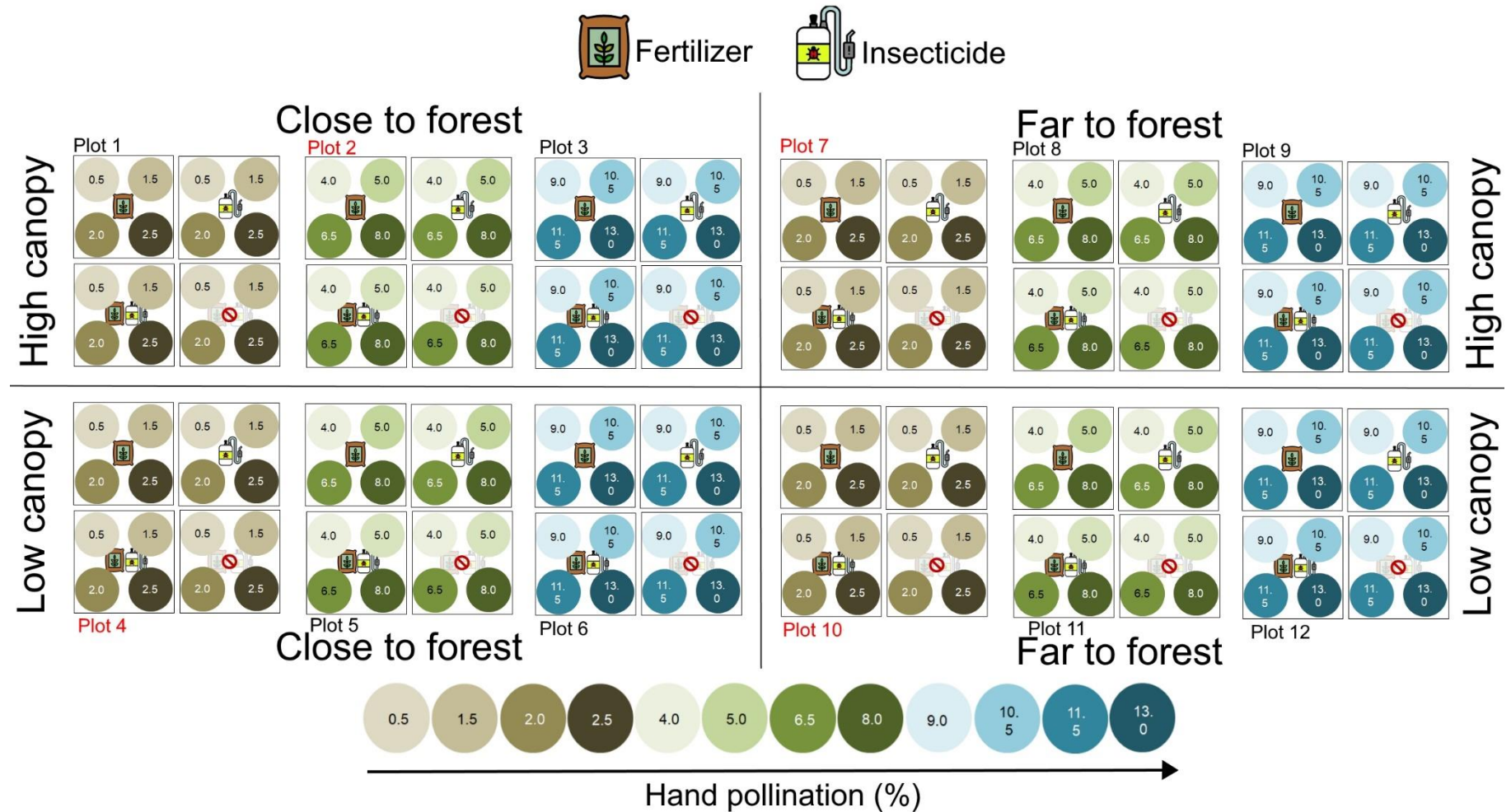
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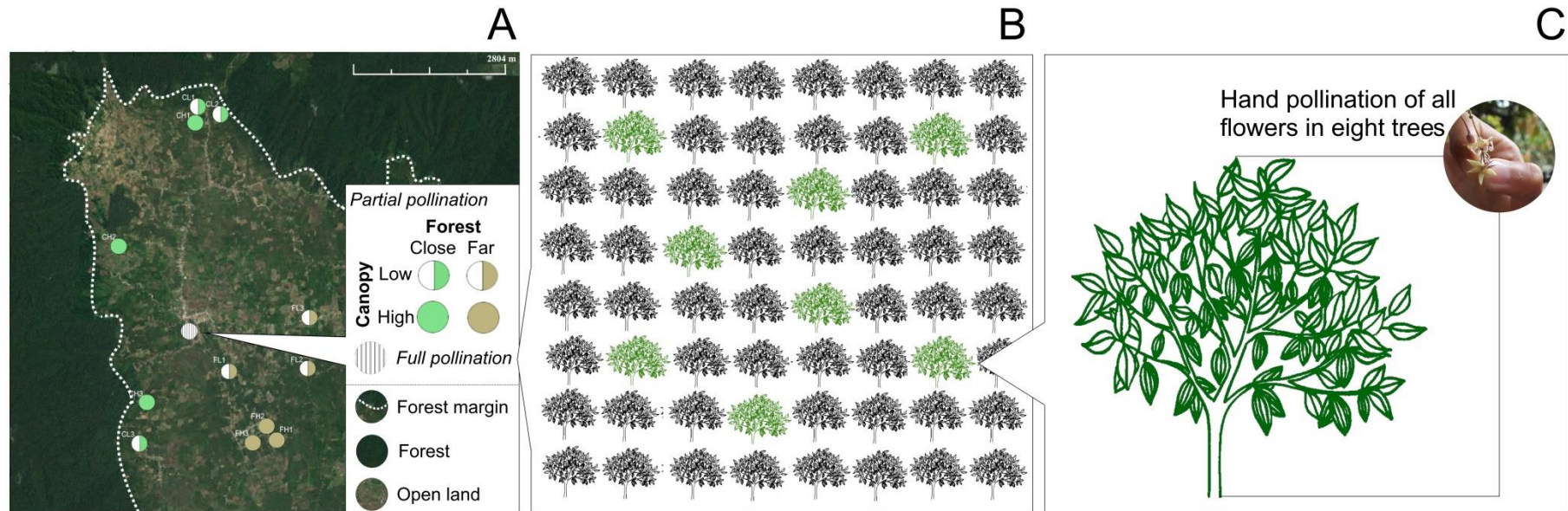
Electronic Supplementary Material



**Figure S1.** Schematic figure of our experimental design in the partial (13% flowers/tree) hand pollination explained in five steps. 1) Farm selection – First, we selected 12 cocoa farms (plots) from our 28 surveyed. 2) Landscape and farm management - Then, we sorted our 12 plots in four combined forest distance (close: 0-800 m, far= 1,500, 3,400 m) and canopy cover (low= 0-20%, high= 40-60%) categories, with three plot replicates for each category: (1) close to forest and high canopy cover (Plot 1,2,3), (2) close to forest and low canopy cover (Plot 4,5,6); (3) far to forest and high canopy cover (Plot 7,8,9), and (4) far to forest and low canopy cover (Plot 10,11,12).

### CHAPTER III – HAND POLLINATION AND YIELDS

3) Agrochemical treatments – In each plot we established four agrochemical treatment subplots (black-contoured squares) and doubled the amount of fertilizer and insecticides used in the study area: (1) fertilizer, (2) insecticide, (3) combined fertilizer and insecticide, (4) no agrochemical use or control. We then selected four healthy cocoa trees per subplot (represented here as circles) for hand pollination. 4) Hand pollination - We hand pollinated easily accessible flowers below 2 m height (or 13% flowers/tree) at 12 different rates: 0.5, 1.5, 2.0, 2.5 (transition-brown circles), 4.0, 5.0, 6.5, 8.0 (transition-green circles), 9.0, 10.5, 11.5 and 13.0 (transition-blue circles) %. The 12 pollination rates were represented in each of the forest distance and canopy cover categories. 5) Statistical analysis - We discarded from the statistical analysis 64 younger cocoa trees (ages 5-8 years) from the plots 2,4,7,10 (in red letters) because they produced few fruits (see Appendix Figure S5), reducing the sample size from 192 to 128 trees.).



**Figure S2.** The study area (A) and schematic plot design (B) of the full (100% flowers) hand pollination experiment (C). We selected four old (ca. 36 years old) and four young (ca. 6 years old) trees in a low canopy cover (<10%) plantation near to a forest margin (B). As in the partial hand pollination, we daily hand pollinated all flowers during 60 day (C) and monitored pollination success, fruit set, cherelle wilt, pest and diseases and yields.

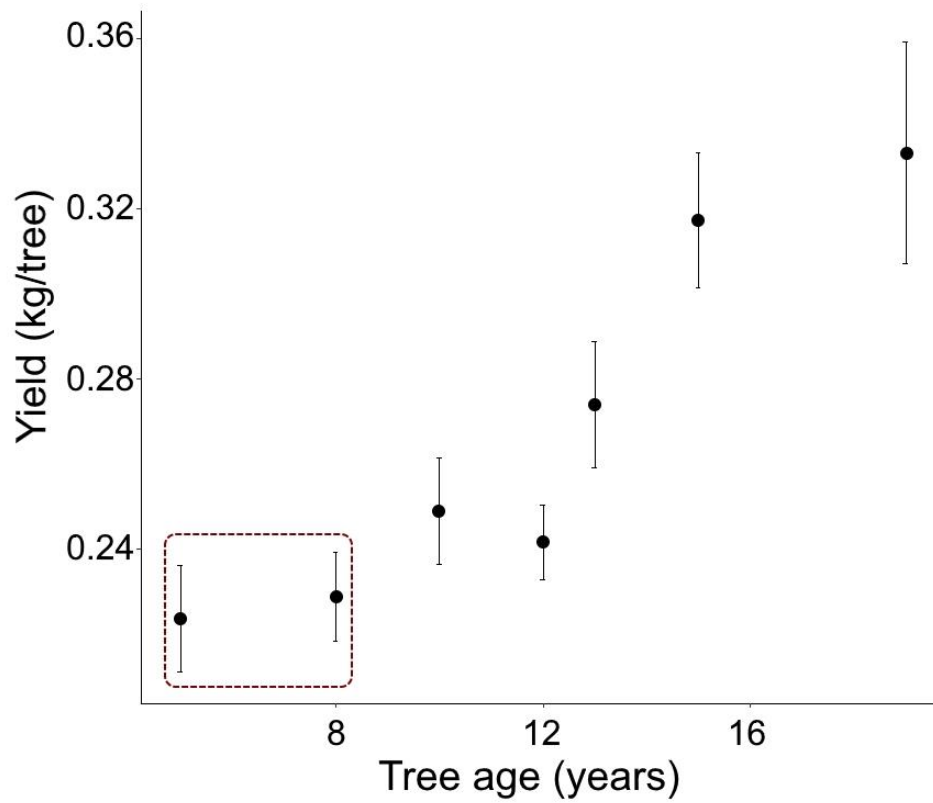


**Figure S3.** The hand pollination of cocoa flowers was carried out by a team of eight local helpers and students, who worked together in our experimental sites. Before the hand pollination experiments started, the team received a one week theoretical and practical training. Once the training concluded, we started the hand pollination of cocoa flowers in our experiments, which involved five steps: **1) Flower monitoring** - In the early morning, we counted all open flowers of each selected tree (hereafter “pollen-receptor tree”) (A). Then, we calculated the number of flowers to be pollinated according to the assigned pollination rate. For example, if flower counts of a given tree was 100, and hand pollination rate assigned was 10%, then the number of flowers to be pollinated was 10. Then, we

randomly picked and marked (with pins labeled with pollination day and date) the flowers to be pollinated (B). **2) Hand pollination** - We hand pollinated cocoa flowers for 60 consecutive days following the methods described by Falque et al. (1995) and Groeneveld et al. (2010). First we collected open flowers from three new trees (hereafter “pollen-source trees”) in an additional farm not included in the study (C). This approach increases the genetic pool and avoids fruit abortion due to tree self-incompatibility. We randomly hand-picked one flower per pollen-source tree and carefully rubbed their anthers in the marked flower styles of the pollen-receptor tree (D). Finally, we removed flowers not pollinated to prevent open pollination. In average, hand pollination (D) of all flowers in a tree (3-4 m height) required ~7 minutes. **3) - Fruit set** - We recorded successful pollination, or fruit set, three days after hand pollination (E). This is because only successfully pollinated flowers remain on the tree 48h after pollination, while the unsuccessfully pollinated fall down (Wood and Lass, 2008; Toledo Hernandez et al., 2017). For example, we recorded fruit set in day tree for hand pollination carried out in day one, or fruit set in day 62 for hand pollination carried out in day 60. **4) Fruit losses** - A large proportion of young fruits rotten and shrank in the first months of development. This phenomenon also known as cherelle wilt, or fruit abortion, is a plant regulating process associated with the limited plant energy resources available for fruit development (F) (Wood and Lass, 2008), which eventually causes an early fruit abortion. We daily quantified fruit abortion for two weeks and later weekly until the harvest. We also weekly quantified fruit losses caused by the mirid bug (*Helopeltis* sp.) pest and black pod disease (*Phytophthora* sp.) (G) until the harvest. **5) Harvest** - The harvest took place around six months after the hand pollination started. Here, we collected all harvestable fruits and quantified the proportion of healthy and diseased fruits. We opened the harvested fruits to extract the fresh beans, and weighted them (fresh bean weight kg/tree) (H). We fermented and dried cocoa beans following the local practices consisting in a seven-days fermentation in rice sacks, and a two to three days open-sun drying (I). We quantified final yields as dry bean weight (kg/tree) (J).



**Figure S4.** An example of the harvested fruits in the study sites. We recorded a high incidence of pest and diseases, mainly black pod disease (*Phytophthora* sp.) and cocoa mosquito (*Helopeltis* sp.) Farmers generally combine seeds from healthy and diseased fruits to reduce yield losses.



**Figure S5.** We observed that 64 very young trees (five to eight years) in our partial (13% flowers/tree) hand pollination experiment produced few fruits, and thus average yields were lower (red-dotted square) in contrast to older (10-19 years) trees. In our statistical analysis we only used the data of trees older than 10 years ( $n=128$ ) to avoid any previously unforeseen effect of age and yields.



**Table S1.** The summary data collected from the farm surveys. We interviewed 28 farmers across the Napu Valley, Indonesia in 2017.

<b>Variable</b>	<b>Unit</b>	<b>Mean</b>	<b>Min</b>	<b>Max</b>	<b>SE</b>	<b>N</b>
<b>Dependent variable</b>						
<i>Cocoa yield</i>	kg/ha	537.0	257.0	660.0	50.000	28
<b>Agricultural parameters</b>						
<i>Cocoa trees</i>	Trees/ha	813.5	389.0	2,060.0	76.136	28
<i>Cocoa tree age</i>	Year	12.3	5	25	5.143	28
<i>Farm size</i>	ha	0.9	0.2	2.0	0.098	28
<b>Management practices</b>						
<i>Farm surrounding</i>						
Forest and cocoa	%	67.4	36	94	3.245	28
<i>Canopy cover</i>	%	28.6	0	59.2	4.743	28
<i>Agrochemical use</i>						
Fertilizer	kg/ha/year	186.5	0.0	600.0	42.176	28
Insecticide	l/ha/year	1.7	0.0	13.4	0.567	28
Herbicide		2.5	0.0	20.0	0.844	28
<i>(OC) Operational cost</i>						
Fertilizer	USD/ha/year	36.1	0.0	116.4	8.182	28
Insecticide	USD/ha/year	21.3	0.0	174.7	7.317	28
Herbicide	USD/ha/year	12.5	0.0	90.0	3.796	28
Labor	USD/ha/year	26.3	0.0	66.0	5.471	28
Total	USD/ha/year	96.2	1.8	378.8	24.767	28

**Table S2.** The fruit development results of the partial (13% flowers/tree) (A) and full (100%flowers/tree) (B) hand pollination experiment. Here we present the average and standard errors (SE±) at the tree level. In the full hand pollination experiment, we used the data on yields at a tree level from the 28 farm surveys to compare the yield increases. *NA* accounts for not available data.

Experiment	% Pollination (<2m height)	% Pollination (full tree)	N trees	Open flowers	Hand pollinated flowers	Fruit set	Cherelle wilt	Pest and diseases	Harvested fruits	Yield
<b>(A) Partial</b>	Control	0.0	8	147.1 (39.0)	0	15.1 (1.4)	2.3 (1.0)	10.3 (1.3)	5.4 (0.7)	0.228 (0.023)
	5	0.5	8	330.5 (48.5)	14.4 (3.0)	11.3 (1.4)	0.6 (0.3)	5.4 (1.2)	6.6 (0.4)	0.244 (0.024)
	10	1.5	8	431.1 (51.7)	46.3 (6.1)	27.9 (2.9)	5.5 (1.5)	21.3 (2.8)	6.6 (0.3)	0.239 (0.016)
	15	2.0	8	342.8 (81.6)	52.1 (13.8)	25.8 (6.3)	4.0 (1.5)	19.4 (6.3)	6.4 (0.3)	0.238 (0.017)
	20	2.5	8	506.6 (144.8)	100.5 (29.3)	53.4 (12.2)	12.1 (4.0)	46.9 (11.7)	6.8 (0.5)	0.234 (0.022)
	30	4.0	12	276.9 (40.5)	87.3 (11.9)	43.8 (7.0)	13.5 (3.6)	36.4 (5.4)	7.1 (0.3)	0.267 (0.017)
	40	5.0	12	218.5 (22.8)	85.7 (9.5)	40.3 (3.9)	11.6 (2.8)	33.1 (3.5)	7.5 (0.4)	0.290 (0.030)
	50	6.5	12	165.7 (30.5)	95.1 (15.6)	41.7 (12.8)	12.8 (4.4)	35.3 (7.1)	7.4 (0.4)	0.291 (0.025)
	60	8.0	12	214.0 (28.1)	128.0 (16.2)	67.3 (10.7)	22.7 (7.1)	59.8 (10.6)	7.4 (0.3)	0.270 (0.023)
	70	9.0	12	250.3 (54.7)	176.4 (38.3)	82.1 (15.8)	48.3 (11.8)	72.8 (15.4)	9.3 (0.7)	0.281 (0.019)
	80	10.5	12	194.0 (38.0)	158.2 (29.5)	82.4 (14.0)	46.2 (11.1)	73.5 (14.1)	8.9 (0.6)	0.306 (0.019)
	90	11.5	12	207.3 (35.6)	194.7 (32.6)	107.1 (18.6)	62.3 (13.4)	97.7 (18.3)	9.4 (0.8)	0.311 (0.034)
	100	13.0	12	231.3 (28.0)	231.3 (28.0)	116.9 (15.3)	66.6 (10.6)	106.9 (15.1)	10.0 (0.6)	0.345 (0.031)
<b>(B) Full</b>	Control	0.0	28	NA	0.0 (0.0)	NA	NA	NA	NA	0.660 (0.061)
	---	100	8	2009 (202.5)	2009 (202.5)	954.4 (97.8)	1723 (167.0)	232.5 (29.5)	53.5 (10.8)	1.726 (0.359)

**Table S3.** The average best fitting models ( $\Delta AICc \leq 2$ ) from the model selection analysis. We investigated the effect of the fixed effect variables 1) pollination rate (*Rate*), 2) forest distance (*Forest*), 3) canopy cover (*Canopy*) and agrochemical intensification treatments (*Treat*) on fruit set (A), cherelle wilt (B), pest and diseases (C), harvested fruits (D), and yield (E). *Int* represents the intercept term.

	<i>Int</i>	<i>Rate</i>	<i>Forest</i>	<i>Canopy</i>	<i>Treat</i>	df	LogLik	AICc	Delta	Weight
<b>A Fruit set</b>										
Rate + Canopy	3.917	0.494	---	-0.188	---	5	-983.615	1977.7	0.00	0.542
Rate + Forest + Canopy	3.917	0.494	0.060	-0.176	---	6	-983.360	1979.4	1.69	0.232
Rate	3.917	0.494	---	---	---	4	-985.574	1979.5	1.75	0.226
Null	---	---	---	---	---	3	-1108.810	2223.8	246.09	0.000
<b>B Cherelle wilt</b>										
Rate	2.690	0.486	---	---	---	4	-772.336	1553.0	0.00	0.490
Rate + Canopy	2.691	0.489	---	-0.271	---	5	-771.758	1554.0	1.01	0.296
Rate + Forest	2.691	0.487	0.184	---	---	5	-772.080	1554.7	1.65	0.214
Null	---	---	---	---	---	3	-826.860	1659.9	106.91	0.000
<b>C Pest and diseases</b>										
Rate + Canopy	3.728	0.553	---	-0.2134	---	5	-1078.259	2167.0	0.00	0.525
Rate + Forest + Canopy	3.727	0.552	0.084	-0.1969	---	6	-1077.859	2168.4	1.4	0.260
Rate	3.728	0.553	---	---	---	4	-1080.235	2168.8	1.79	0.215
Null	---	---	---	---	---	3	-1215.310	2436.8	269.8	0.000
<b>D Harvested fruits</b>										
Rate + Forest	2.059	0.144	-0.058	---	---	5	-269.229	548.9	0.00	0.508
Rate	2.06	0.145	---	---	---	4	-270.830	550.0	1.03	0.303
Rate + Forest + Canopy	2.059	0.145	-0.061	-0.0144	---	6	-269.121	550.9	1.99	0.188
Null	---	---	---	---	---	3	-276.990	560.2	11.22	0.000
<b>E Yield</b>										
Rate	5.614	0.095	---	---	---	4	-1202.672	2413.7	0.00	0.541
Rate + Forest	5.614	0.096	-0.063	---	---	5	-1201.753	2414.0	0.33	0.459
Null	---	---	---	---	---	3	-1222.490	2451.2	37.50	0.000

**Table S4.** The results of the multiple comparison analysis of agrochemical intensification treatments (double fertilizer [Fertilizer], double fertilizer and double insecticide [Fertilizer+Insecticide], double insecticide [Fertilizer]) and no agrochemical application [control]) on cocoa fruit set (A,F), cherelle wilt (B,G), pest and diseases (C,H), harvested fruits (D,I), and yield (E,J) in the partial (13% hand pollination) experiment.

	Ismean	SE	df	Lower CL	Upper CL
<b>A Fruit set</b>					
<i>Control</i>	3.79	0.134	119	3.52	4.05
<i>Fertilizer</i>	3.87	0.134	119	3.61	4.14
<i>Fertilizer+Insecticide</i>	3.96	0.134	119	3.69	4.23
<i>Insecticide</i>	4.05	0.134	119	3.78	4.31
<b>B Cherelle wilt</b>					
<i>Control</i>	2.62	0.300	119	2.02	3.21
<i>Fertilizer</i>	2.69	0.301	119	2.10	3.29
<i>Fertilizer+Insecticide</i>	2.55	0.302	119	1.95	3.14
<i>Insecticide</i>	2.91	0.300	119	2.32	3.51
<b>C Pest and diseases</b>					
<i>Control</i>	3.60	0.158	119	3.28	3.91
<i>Fertilizer</i>	3.69	0.158	119	3.38	4.00
<i>Fertilizer+Insecticide</i>	3.76	0.159	119	3.44	4.07
<i>Insecticide</i>	3.87	0.158	119	3.56	4.18
<b>D Harvested fruits</b>					
<i>Control</i>	2.02	0.064	119	1.90	2.15
<i>Fertilizer</i>	2.01	0.064	119	1.88	2.14
<i>Fertilizer+Insecticide</i>	2.09	0.062	119	1.97	2.21
<i>Insecticide</i>	2.11	0.061	119	1.98	2.23
<b>E Yield</b>					
<i>Control</i>	5.58	0.062	119	5.46	5.70
<i>Fertilizer</i>	5.57	0.062	119	5.45	5.69
<i>Fertilizer+Insecticide</i>	5.70	0.062	119	5.58	5.83
<i>Insecticide</i>	5.60	0.062	119	5.48	5.73

Table S4 - Continued

		Coefficient	SE	df	t-ratio	p-value
<b>F</b>	<b>Fruit set</b>					
	<i>Control–Fertilizer</i>	-0.087	0.176	119	-0.497	0.960
	<i>Control–Fertilizer+Insecticide</i>	-0.174	0.175	119	-0.990	0.755
	<i>Control–Insecticide</i>	-0.259	0.175	119	-1.478	0.454
	<i>Fertilizer–Fertilizer+Insecticide</i>	-0.086	0.175	119	-0.493	0.960
	<i>Fertilizer–Insecticide</i>	-0.172	0.175	119	-0.980	0.761
	<i>Fertilizer+Insecticide–Insecticide</i>	-0.085	0.175	119	-0.487	0.962
<b>G</b>	<b>Cherelle wilt</b>					
	<i>Control–Fertilizer</i>	-0.077	0.303	119	-0.253	0.994
	<i>Control–Fertilizer+Insecticide</i>	0.073	0.304	119	0.240	0.995
	<i>Control–Insecticide</i>	-0.295	0.303	119	-0.973	0.765
	<i>Fertilizer–Fertilizer+Insecticide</i>	0.150	0.305	119	0.491	0.961
	<i>Fertilizer–Insecticide</i>	-0.218	0.303	119	-0.719	0.889
	<i>Fertilizer+Insecticide–Insecticide</i>	-0.367	0.304	119	-1.209	0.622
<b>H</b>	<b>Pest and diseases</b>					
	<i>Control–Fertilizer</i>	-0.093	0.214	119	-0.436	0.972
	<i>Control–Fertilizer+Insecticide</i>	-0.158	0.214	119	-0.740	0.881
	<i>Control–Insecticide</i>	-0.271	0.213	119	-1.270	0.583
	<i>Fertilizer–Fertilizer+Insecticide</i>	-0.065	0.214	119	-0.304	0.990
	<i>Fertilizer–Insecticide</i>	-0.178	0.213	119	-0.834	0.838
	<i>Fertilizer+Insecticide–Insecticide</i>	-0.113	0.213	119	-0.529	0.952
<b>I</b>	<b>Harvested fruits</b>					
	<i>Control–Fertilizer</i>	0.012	0.091	119	0.136	0.999
	<i>Control–Fertilizer+Insecticide</i>	-0.067	0.089	119	-0.755	0.874
	<i>Control–Insecticide</i>	-0.082	0.088	119	-0.929	0.789
	<i>Fertilizer–Fertilizer+Insecticide</i>	-0.079	0.089	119	-0.891	0.810
	<i>Fertilizer–Insecticide</i>	-0.095	0.089	119	-1.064	0.712
	<i>Fertilizer+Insecticide–Insecticide</i>	-0.015	0.087	119	-0.174	0.998
<b>J</b>	<b>Yield</b>					
	<i>Control–Fertilizer</i>	0.008	0.073	119	0.109	0.999
	<i>Control–Fertilizer+Insecticide</i>	-0.127	0.073	119	-1.725	0.316
	<i>Control–Insecticide</i>	-0.027	0.073	119	-0.364	0.983
	<i>Fertilizer–Fertilizer+Insecticide</i>	-0.135	0.073	119	-1.834	0.263
	<i>Fertilizer–Insecticide</i>	-0.035	0.073	119	-0.473	0.965
	<i>Fertilizer+Insecticide–Insecticide</i>	0.100	0.073	119	1.360	0.527

**Table S5.** Summary results of the extrapolated yields at the farm level ( $Y_{\Delta/ha}$ ) using the 161.5% yield increase of the full (100% flowers/tree) hand pollination experiment. We calculated the gross income ( $I_{Gross}$ ) and pollination-related net income ( $I_{Pollnet}$ ) scenarios (conservative, realistic, optimistic) consider the average cocoa prices ( $CP$ ), opportunity ( $OP$ ) and operational costs ( $OC$ ), and hand pollination effort ( $HP$ ) by the time the data was collected (2017) in Napu Valley.

	Unit	Napu Valley (Local)		
		Value	SE	% Change
<b>Fixed values</b>				
<i>Yield increase</i> <sup>1</sup>	---	161.5		---
<i>(CP) Cocoa prices</i> <sup>2</sup>	USD/kg dry	2.03	0.023	---
<i>(PI) Pollination investment</i> <sup>3</sup>	USD/ha/day	39.0	3.7	---
<i>(OP) Opportunity cost</i> <sup>4</sup>	---	1.0	---	---
<b>Extrapolations</b>				
<i>(Y<sub>Δ/ha</sub>) Yield/ha</i>				
No pollination <sup>5</sup>	kg/ha	536.9	49.6	0.0
Pollination		1,404.1	292.0	161.5
<i>(I<sub>Gross</sub>) Gross-income</i>				
No pollination	USD/ha	1,089.9	100.7	0.0
Pollination		2850.3	592.8	161.5
<i>(OC) Operational costs</i> <sup>6</sup>				
Low OC	USD/ha	1.8	---	---
Average OC		96.2	24.8	---
High OC		378.8	---	---
<i>(HP) Hand pollination effort</i>				
<i>HP 30 days</i>	USD/ha	1,171.2	109.6	---
<i>HP 60 days</i>		2,342.4	219.2	---
<i>(I<sub>Pollnet</sub>) Pollination related net income increase</i>				
No pollination	USD/ha	993.7	100.7	0.0
Pollination conservative		129.1	592.8	-87.0
Pollination realistic		1,582.9	592.8	59.3
Pollination optimistic		1,677.3	592.8	68.8

<sup>1</sup>The *Yield increase* is the 161.5% yield increases from the full hand pollination experiment (Table 2A).

<sup>2</sup>The average global cocoa prices ( $CP$ ) in 2017.

<sup>3</sup>The pollination investment ( $PI$ ) is the labor invested for one-day hand pollination of 1ha farm.

<sup>4</sup>The opportunity cost ( $OP$ ) assuming a 100% ( $OP=1$ ) hired labor for pollination.

<sup>5</sup>The yields extracted from the 28 farm surveys in Napu Valley (Electronic Appendix Table S1).

<sup>6</sup>The maximum (high), average (average), and minimum (low) operational costs ( $OC$ ) reported in the 28 farm surveys in 2017 (Electronic Appendix Table S1).

**Table S6.** Summary of global dry cocoa prices (*CP*) in USD/kg from 2001 to 2017 (ICCO, 2018, INDEXMUNDI, 2018). We used the *CP* from 2017 (the year of our study) to calculate the pollination-related net income increase ( $I_{PollNet}$ ) scenarios in Napu Valley (see Electronic Appendix Table S7).

Cocoa Prices (USD/kg)		
Year	Mean	SE
2001	1.07	0.023
2002	1.54	0.026
2003	1.78	0.043
2004	1.55	0.043
2005	1.54	0.026
2006	1.59	0.018
2007	2.00	0.025
2008	2.58	0.084
2009	2.89	0.107
2010	3.13	0.053
2011	2.98	0.105
2012	2.39	0.031
2013	2.44	0.068
2014	3.06	0.039
2015	3.13	0.054
2016	2.89	0.076
2017	2.03	0.023

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## Chapter IV CRISPR/cas: Minding the Implementation Gap for Gene-Edited Crops



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## Abstract

CRISPR/cas shows promising results to improve pest-resistance in major agricultural commodities. In tree crops such as citrus, coffee, or cocoa, a major challenge is the implementation gap when transferring gene-edited varieties from the laboratory to the farm. The speed and complexity of ecological and evolutive crop-pest interactions is beyond what can currently be managed through gene-editing alone. This threatens successful farm adoption, stable producer income, and food security. We propose an integrated grafting and CRISPR nursery approach that is broadly applicable to gene-edited tree crop varieties. Interdisciplinary research efforts are critical to developing sustainable implementation plans that integrate gene-editing technologies.

**Keywords:** Biostimulants, climate change, crspr/cas, cocoa, gene editing, grafting, hand pollination, pest and diseases, yield

## Introduction

CRISPR/cas is a key technique for gene-editing and a valuable new tool to improve global human welfare (Barrangou et al., 2007; Doudna et al., 2014). This innovative technique makes it possible to delete or replace sections of an organism's genome to generate a desired phenotype. CRISPR technology has the potential to underpin the development of cures for cancer (Xia et al., 2019) and HIV (Ophinni et al., 2018), and even bring back extinct fauna (Schultz-Bergin, 2018). In agriculture, CRISPR/cas shows promise for improving crop quality and pest-resistance (Ma et al., 2016), reducing undesirable compounds such as acrylamide in potatoes (Kim et al., 2015), and prolonging food product shelf life (Walz, 2016). Successful examples of CRISPR implementation mainly come from annual plants, where new gene-edited varieties can be planted and harvested within a growing season. However, the technology has also been applied to perennial tree crops, including cocoa, coffee, and citrus.

Currently, tropical cocoa and coffee trees require three to five years from planting to the first harvest, and drastic yield losses are linked to droughts as well as pest and disease outbreaks, such as black pod disease in cocoa and berry borer in coffee (Tscharncke et al., 2011). CRISPR/cas research in cocoa largely focuses on developing resistance to black pod disease *Phytophthora tropicalis* (Fister et al., 2019), and in coffee the breeding time could potentially be reduced from 30 to six years (Breitler et al., 2018). Coffee modification with CRISPR has also targeted the

development of caffeine-free beans to reduce the costs of producing decaffeinated coffee (Tropical Bioscience, 2018). However, CRISPR approaches face major challenges; coffee gene-editing is limited by the low genetic diversity available in *Coffea arabica*, the most cultivated coffee variety (Campos et al., 2017), and for both, coffee and cocoa, there are challenges in conferring gene-edited traits to the whole tree (Fister et al., 2018). In recent years, the citrus industry has seen losses worth USD 7 billion in the United States of America due to ‘Huanglongbing’ or Citrus Greening virus (CGV) (Spreen et al., 2014). CRISPR/cas-related research has focused on designing CGV resistance (Jia et al., 2014, 2017; Chen et al., 2018; Clark et al., 2019), but developing multi-loci encoding resistance genes remains a challenge.

Apart from the technical hurdles that remain in the creation of gene-edited varieties (GEV) in the laboratory, CRISPR/cas experts agree that an additional challenge is to bridge the implementation gap: moving gene-edited varieties from the laboratory to the farm (Doudna, 2017; IGI, 2018). Here, we suggest a general solution to bridge the implementation gap for tree crops by exposing GEV early to the ecological complexity and co-evolutive crop-pest and disease processes on working farms (Tabashnik et al., 2013, 2017). The proposed approach not only reduces the requirements for second-stage bio-technical input (‘up-dating’, Fig. 1) by avoiding resistant pests and diseases, it also increases the likelihood of socio-economic benefits accruing to small-scale producers (Schroth et al., 2016). We first illustrate the problems of gene-edited tree crops associated with the implementation gap, and then suggest a grafting and CRISPR/cas nursery solution to incorporate GEV quickly at the farm level (Fig. 1).

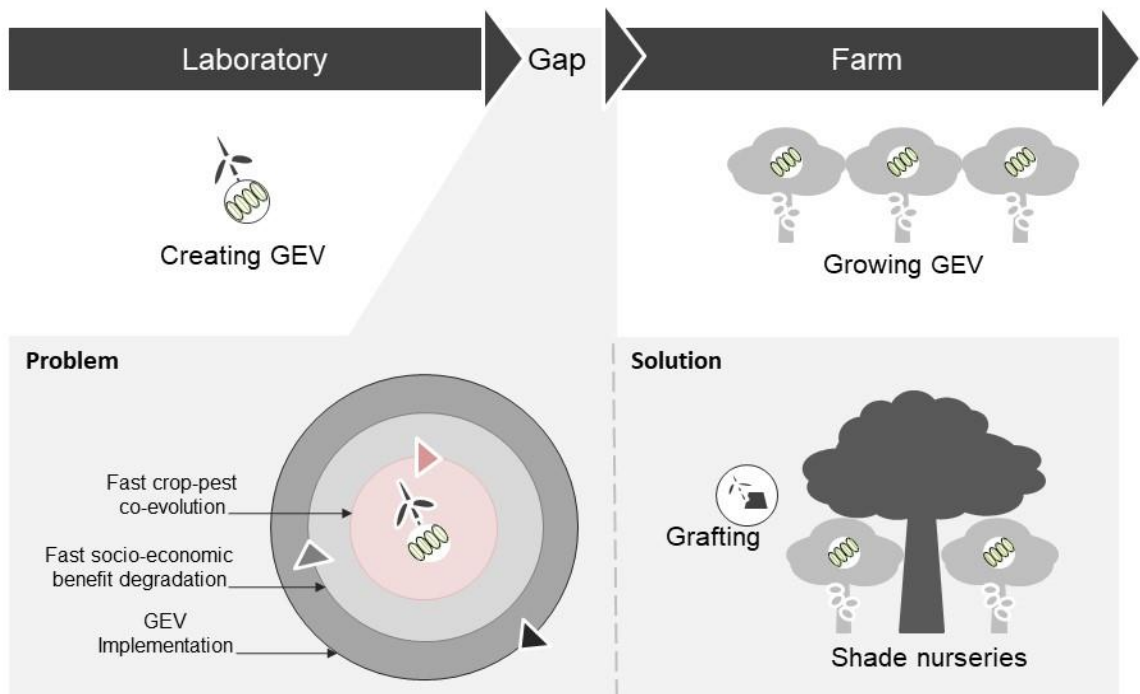
## The Tree Crop Implementation Gap – Two Problems

There are two major but tractable problems that together constitute the tree crop implementation gap. The first is the ecological complexity and the co-evolutive crop-pest/disease processes in farms that can lead to the rapid rise of resistant pests and diseases (Tabashnik et al., 2013, 2016, 2017). The second is the risk of implementation failure if ecological and economic pressures on small-scale producers are disregarded.

When a tree genome is CRISPR-edited for pest or disease resistance, the editing is based on the pest or disease reference genome at time point T1 (PDG<sub>T1</sub>) (Fig. 2). When the gene-edited variety (GEV) is introduced at the farm at time point T2 or T3, following an approximate five to 10-years implementation period (Jaganathan et al., 2018; Walz, 2018), co-evolutive crop-pest processes will have modified PDG<sub>T1</sub> to

PDG<sub>T2</sub> or PDG<sub>T3</sub>. Hence, GEV efficacy against the pest or disease is likely to be reduced. When PDG<sub>T3</sub> evolves to resistant PDG<sub>T4</sub>, gene-editing updates to the GEV may be needed to target the genome of the new resistant pest. This plant/pest arms race already presents a challenge for pest management, but plant-pest/disease co-evolution may be faster, or simply different, under climate change, potentially exacerbating this implementation challenge (Johnson et al., 2018).

## Implementation gap in gene-editing tree crops



**Figure 1.** An overview of the implementation gap problem and solution. A gene-edited variety (GEV) is created in the laboratory, but there is a time gap before implementation on the farm. Rapid co-evolutionary crop-pest/disease processes, in which pests evolve resistance to the GEV likely occur before large-scale implementation of the GEV. This leads to a vicious cycle that requires an ‘updating’ of the GEV (modification of the gene-editing to accommodate newly arisen resistance to the trait as it was first expressed in the GEV) to respond to new pest resistance, and declines of socioeconomic benefits for farmers. We propose a solution to this problem based on a gradual, partial replacement of old trees with new GEVs by grafting and planting of GEV seedlings. This solution is suitable for a wide variety of tree crop species.

Because yield declines due to pests and diseases cannot be counteracted by a new GEV at T1, farmer income is reduced (McCook and Vandermeer, 2015; WCF, 2018), and farmers may consider growing alternative crops or seeking other employment opportunities (Tschardt et al., 2012). If the GEV has low efficacy against the PDG at T3 or T4, this can lead to yield declines or a complete production collapse. Declining economic returns from decreasing yields may encourage small-scale farmers to expand their area of crop production, abandon target crop production, or find

alternative income sources (Clough et al., 2009). Neither of these outcomes is desirable, because when farmers increase their area of production, it can put pressure on forested and biodiverse habitats (Clough et al., 2009), and when farmers abandon production, it can exacerbate supply deficits. Thus, implementation of new CRISPR/cas-modified tree crop varieties must aim to keep up with the evolutionary processes that shape pest and disease genomes, provide climate adaptation potential, and maintain a stable income for farmers to secure the 'willingness to produce' the crop.

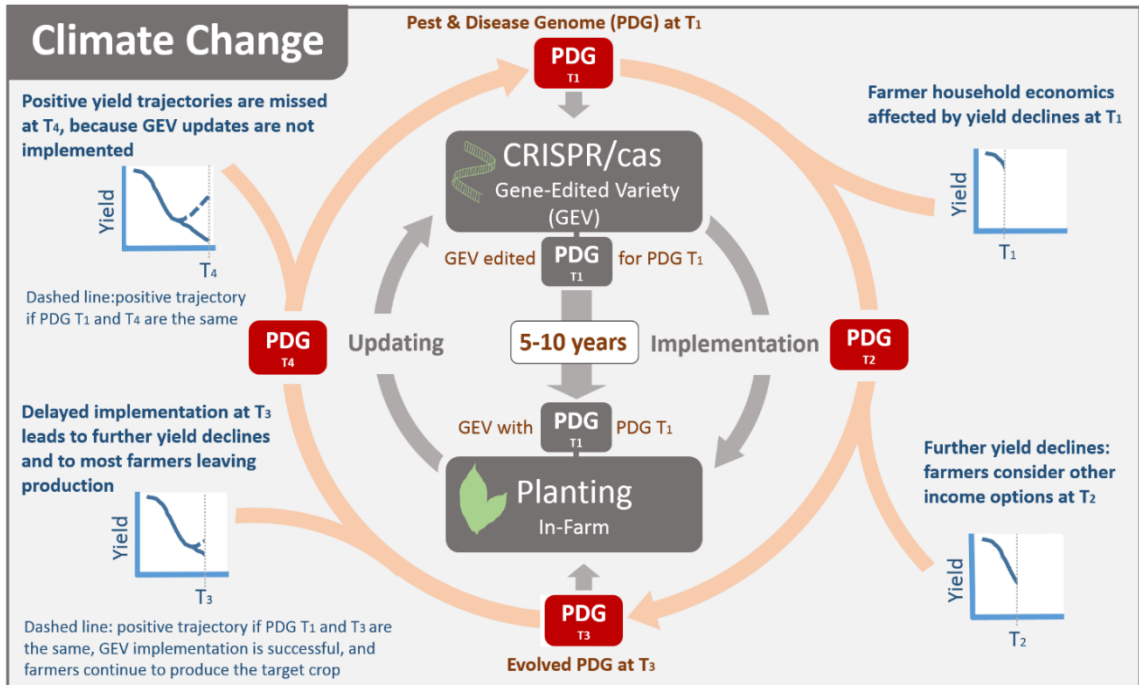
## A Solution - Grafting and CRISPR/cas Nurseries

We propose a combined grafting-CRISPR/cas nursery solution to bridge the implementation gap, and to avoid GEV-pest/disease Red Queen (Bell, 1982) cycles and dependency on GEV-updates. Grafting is a technique whereby two plant individuals are joined to produce a new specimen carrying the desired traits (Mudge et al., 2009). When a GEV is grafted at the base of an old tree (OT) trunk (side-grafting) or onto a shoot growing directly from the OT trunk (chupon-grafting), the new variety grows on the old rooting system (Somarriba et al., 2011). Conveniently, grafting can be performed on many tree crops including cocoa, coffee, and citrus.

Following traditional plant breeder examples, a CRISPR/cas nursery could hold numerous GEV types with different optimized traits, such as pest or drought resistances. Producers could harvest scions (newly emerging shoots) from the CRISPR/cas nursery and graft them onto OT in existing plantations (Fig. 3A). GEV seedlings could also be produced in the nursery and be used to replace a proportion of OTs in the plantation, where they can grow under the shade of the remaining OTs (Fig. 3B). Planting GEV seedlings would avoid potential negative interactions between GEV and OT genomes when grafting.

While the GEV material is immature, during the first two to five years of the GEV grafting or replanting in the plantation, crop yields may be low compared to yields achieved in the mature plantation. In accordance with ecological intensification principles (Bommarco et al., 2013), these losses may be mitigated by the sensible use of chemical inputs and benefit from pollination services as well as biostimulants in the plantations (Fig. 3A2 & B2). Artificially increasing pollination success by hand pollination has been associated with yield increases in various tree crops (Groeneveld et al., 2010; Toledo-Hernández et al., 2017), and mitigation of climate impacts (Wanger et al., 2014) in plantations. Biostimulants are non-fertilizer, non-pesticide

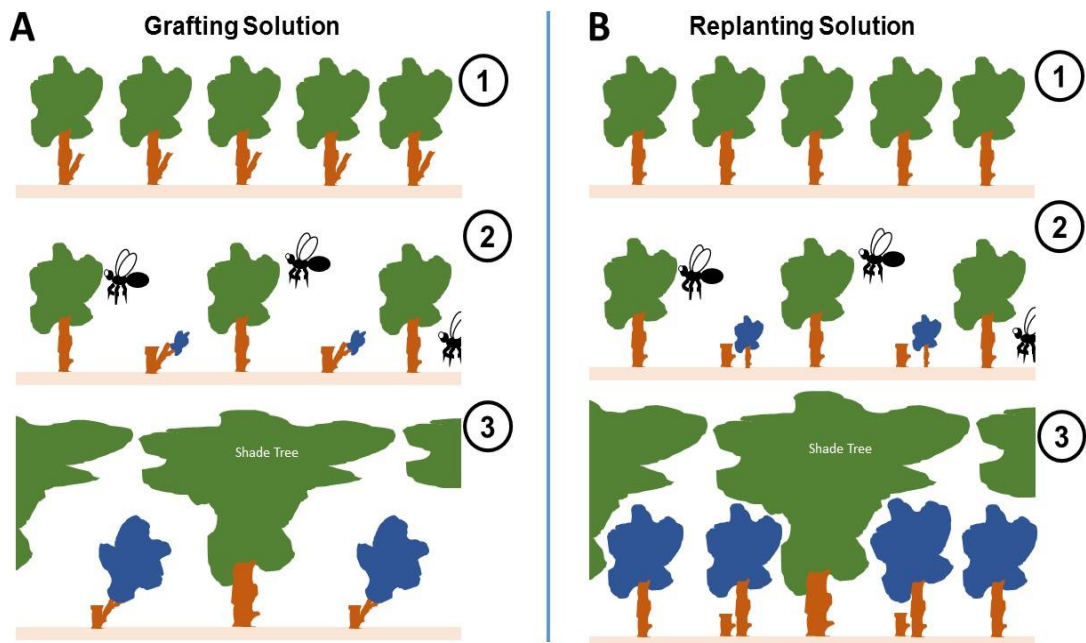
agricultural amendments that improve plant productivity, stress tolerance, and disease resistance (Russo and Berlyn, 1991; Richardson et al., 2004, 1991; Yakhin et al., 2017; Saa et al., 2015). The benefit of a management approach based on ecological intensification, including biostimulants, is that in general the cost for chemical inputs and the environmental impact are reduced.



**Figure 2.** The problems of the implementation gap. It can take five to ten years to develop a new gene-edited tree crop Variety (GEV) in the laboratory that is resistant to a given pest/disease, fulfil all regulatory requirements, distribute it to farmers, and adopt the GEV into large scale production. During this time, evolutionary pressures will have altered the pest or disease genome (PDG) from  $PDG_{T1}$  (top of the orange circle) to  $PDG_{T2}$  and  $PDG_{T3}$  (right and bottom of the orange circle). When a GEV's genome engineered to have resistance to  $PDG_{T1}$  is presented with pests or diseases with  $PDG_{T3}$  or  $T4$  at time  $T3$  or  $T4$ , updates are needed to make the GEV effective against the new pest and disease adaptations (left of the orange circle). If the process of GEV implementation and updating is slow, this can lead to a Red Queen-like cycle wherein the GEV is constantly being updated, but is never quite sufficiently ahead to win against the pest or disease. The resulting dependency on GEV-updates is likely to increase economic pressure on producers, already financially strained by decreasing yields, causing farmland expansion or farm abandonment.

When the introduced GEVs reach peak production, the remaining OT in the plantation should be replaced with GEVs and shade tree species. Pollen transfer between the various grafted or replanted CRISPR/cas varieties from the CRISPR/cas nurseries will ensure high genetic diversity within the plantation and long-term in-situ conservation of genetic diversity of the crop. Shade and fruit trees provide additional income for small-scale producers. In addition to shade, these trees provide pollinator habitats, and minimize competition for water, light and soil nutrient resources between the GEV trees (Tschardt et al., 2011; Wanger et al., 2018) (Fig. 3). Shade trees in

the plantation may also help slow the development of resistant pests, as, according to the ‘refuge strategy’ (Jin et al., 2015), these trees allow survival of non-resistant pests, which can mate with pests that may be developing resistance, slowing or stemming the development of resistance. Plantations with high genetic diversity and numerous GEVs adapted to different yield threats, such as drought and pests, will provide within-farm resilience, stabilize farmers’ income, protect ecosystem services, such as biological pest control and pollination, and create climate-smart farms (Tscharncke et al., 2011; Citrus Industry, 2018).



**Figure 3.** A solution to the implementation gap for gene-edited tree crops. In an existing plantation, a proportion of existing old trees (green trees) would be replaced with gene-edited varieties (GEVs; blue trees) using grafting techniques (A1) or replanting (B1). The grafts and GEV seedlings would come from CRISPR/cas nurseries (for details see text). To minimize yield losses in the first few years, hand pollination or active enhancement of natural pollinator visitation, as well as considered use of fertilizers and biostimulants could be implemented. Old trees would provide shade for the GEVs (A2, B2). When the GEVs reach peak production, additional old trees could be replaced with other tree species, including other fruit crops, to provide shade and pollinator habitat, and minimize competition for resources (A3, B3). This system would capture the benefits of GEVs and highly resilient agroforestry systems, preserve existing crop genetic diversity, and increase the stability of farmers’ incomes.

## Interdisciplinary Research and the Way Forward

In these exciting times of new technologies applied to major agricultural commodities, it is critical to acknowledge the interdisciplinary nature of the implementation gap problem. Our suggested solution is part of the larger CRISPR/cas research ecosystem, which includes research to design crops to meet a range of production needs and overcome production challenges, develop faster and more effective genetic

engineering techniques (Citrus Industry, 2018), understand physiological trade-offs between desired traits, and improve techniques to handle target traits encoded by multiple genes. In addition to these areas of research directly related to gene-editing technology, geneticists need to work with agro-ecologists to ensure the design of ecologically sensitive, climate-smart agricultural production systems that protect and enhance ecosystem services

Here, we propose a first and tangible solution to bridge the implementation gap for CRISPR/cas-modified tree crops, to move them from the laboratory to the farm. With solutions such as this, CRISPR/cas technology can contribute to the creation of sustainable, climate-smart agricultural production systems. New genetic varieties may also play a vital role in facilitating ecological intensification of agricultural production (Bommarco et al., 2013; Kleijn et al., 2019). With this toolbox for 'next-generation agriculture', and by blending modern breeding with agroecological techniques, there is the possibility to increase global and local food security and secure farmers' livelihoods in an uncertain future (Ramankutty et al., 2018). However, failing to address additional research requirements and the interdisciplinary nature of the implementation challenges may have devastating environmental and socioeconomic consequences, in particular for small-scale tree crop producers.

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## Summary

In my doctoral research, I combined a literature review with field experiments to investigate the pollination ecology of cocoa, the third largest export commodity worldwide. The aim of my work was to elucidate the potential of ecological intensification, through pollination services, for increasing yields and improving farmer income in the region of Napu Valley, in Central Sulawesi, Indonesia. I divided my doctoral dissertation into four chapters, formatted as peer-reviewed scientific manuscripts. In *Chapter I*, I compiled all available scientific literature from the past 65 years to elaborate an exhaustive review on the neglected role of cocoa pollination. In *Chapter II*, I report the results of my field studies to quantify the relative importance of landscape, farm, and plant performance, as well as soil organic litter for species richness and abundance of cocoa flower visitors. In *Chapter III*, I present results of a large-scale hand pollination experiment and contrast the contribution of pesticide and fertilizer uses with that of pollination on fruit set and yield for improving farmer income. Finally, in *Chapter IV*, I present recommendations on how to translate gene-editing technologies from the laboratory to the farm, expanding the scope to three perennial crops: cocoa, citrus, and coffee.

My findings in *Chapter I* and *II* provide evidence that mainly ants and to a lower extend dipterans are the main flower visitors of cocoa. This suggests that neglected ants may play a larger direct and indirect role in cocoa pollination than previously acknowledged. Multi-strata vegetation surrounding the farms (e.g. forests, agroforests), the shade provided by the tree canopy cover in the farm, and the amount of tree flowers are the main factors explaining flower-visitor species richness and abundance. Contrary to my initial hypothesis, increased amounts of soil litter in my experimental approach did not affect flower visitors. In *Chapter III*, the hand pollination experiments show that pollination, and not pesticides or fertilizers, is the main driver of fruit set and yields. Moreover, I found that hand pollinating 13% of cocoa flowers in a tree increases yields by 51%, and hand pollination of all flowers (100% of flowers/tree) by 260%. These yield increases have a direct effect on farmer livelihoods as their income increases by 69%, 50% and 85% at the local (study region), regional (Central Sulawesi) and national (Indonesia) level. Finally, in *Chapter IV*, I give comprehensive recommendations on how to integrate innovative gene editing technologies with traditional ecological intensification strategies, such as pollination services and grafting. These recommendations aim to bridge the gap between lab and farm implementation.

In conclusion, I found that pollination services play a major role for increasing cocoa yields. While I prove that hand pollination can increase yield notably, it is still highly labor intensive. Thus, a well-structured working plan and the creation of innovative pollination tools are needed to reduce the implementation costs. On the other hand, natural pollination by enhancing flower visitation through management adjustments at the landscape and farm level should become an ecological alternative to hand pollination, but future studies need to detect the still hidden identity of pollinators and their ecology. The results from my work suggest that the preservation of multi-strata vegetation in the surroundings and high canopy cover enhances flower visitor populations and flower visitation, and this presumably improves yields. As litter amount did not appear to affect pollinator species' richness and abundance, further studies should focus on soil litter quality rather than quantity. Finally, I argue that in the future cocoa stakeholders need to find alternative management practices to agrochemical intensification. These practices should successfully integrate ecological, such as pollination services, and promising gene editing approaches for increasing cocoa production sustainably, improve farmer livelihoods, and preserve biodiversity in times of climate change.

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## Declarations

1. I, hereby, declare that this Ph.D. dissertation has not been presented to any other examining body either in its present or a similar form.

Furthermore, I also affirm that I have not applied for a Ph.D. at any other higher school of education.

Göttingen, .....

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Manuel Toledo-Hernández

2. I, hereby, solemnly declare that this dissertation was undertaken independently and without any unauthorised aid.

Göttingen, .....

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