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Diversity of natural pollinators in cocoa agroforests
in the Peruvian Amazon

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Declaration

“I hereby declare that this thesis entitled Diversity of natural pollinators in cocoa agroforests in the Peruvian Amazon is my own work and all the sources have been quoted and acknowledged by means of complete references.”

In Prague,

.....

Martina Snášelová

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Abstract

Despite the importance of cocoa (*Theobroma cacao*, L.), the world's yields remain relatively low. A very low fruit set of cocoa relative to the numerous produced flowers has been connected to low pollination intensity. Abundance of cocoa pollinators (mostly Diptera: Ceratopogonidae) is influenced mainly by the availability of breeding material. One of the best breeding opportunities for pollinators represent ecosystems with heterogeneous shade cover and those where substrates of decomposing cocoa leaf litter and fruit husks are available in sufficient amount. Pollinator-friendly practices would lead to higher number of pollinators and consequently to a higher yield. The objective of this study was the evaluation of cocoa agroforests as a suitable habitat for pollinators in general and cocoa pollinators in particular and its subsequent potential to yield enhancement. The experiment was conducted in three different cocoa agroforestry systems in the Peruvian Amazon with different characteristics, including vegetation structure, canopy cover and soil cover. Insect trapping took place in parallel to monitoring the phenological patterns of the flowering and fruit set. Afterwards evaluation of captured insects was conducted. Chosen families of Hymenoptera and Diptera order were determined to morphospecies and their abundance and diversity was calculated. The abundance of pollinators in general was the highest in the system with the highest number of species of shade trees and with the highest shade cover. There were no differences in insect species diversity and richness among systems. The abundance of Ceratopogonidae insects was very low in all systems and that is why they were excluded from our study. Other potential cocoa pollinators are small individuals from the Diptera order (namely the families Cecidomyiidae, Drosophilidae and Phoridae). Results indicated that agroforestry systems with adequate canopy shade cover and enough leaf litter could be considered as the best habitat for cocoa pollinator enhancement. However, an additional long-term survey needs to be done.

Key words: Diptera order, flowering, *Theobroma cacao*, L., tree species diversity, yield

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List of abbreviations

ACATPA Association of Technified Cocoa Growers of Padre Abad

AFS agroforestry system

AFSs agroforestry systems

BCCH Biologische Bundesantalt, Bundessortenamt and Chemische Industrie

FF flower and fruit

1 Introduction

Cocoa (*Theobroma cacao*, L.) belongs to the world's most important tropical cash crops. It has been traditionally grown in shaded agroforestry systems (AFSs). Despite the importance of this crop, the world average yields remain relatively low, which is limited by various factors including natural resources, climate and the type and intensity of production system. While traditional cocoa agroforests usually have lower cocoa production, intensive cocoa full-sun monoculture plantations exhibit higher yields in the short-term at the expense of extensive application of fertilizers and pesticides, increased pest outbreaks and causing more environmental damages compared to AFSs (Claus et al. 2018). Nevertheless, AFSs provide many benefits, such as stress reduction, cocoa plant protection, longer economic life, biodiversity value and others. They might even be sufficient for sustainable cocoa production with satisfactory yields, if the so-called pollination gap, was resolved.

One of the major limiting factors in the crop production is the lack of successful pollination (Groeneveld et al. 2010). Pollinators play an essential role in plant reproduction in most terrestrial ecosystems and represent an important ecosystem service to maintain agricultural production. Very low fruit set of cocoa relative to the numerous produced flowers has been reported by several studies and has been connected to low pollination intensity (Falque et al. 1995; Bos et al. 2007; Frimpong et al. 2011). Cocoa is strictly entomophilous. The main natural successful pollinators of cocoa are ceratopogonid midges (Ceratopogonidae) of the Dipteran order and particularly those from *Forcipomyia* genera (Glendinning 1972; Kaufman, 1975; Young 1982; Adajaloo & Oduro 2013). However, according to various authors (Young 1968b; Winder 1978; De Schawe et al. 2016; Toledo-Hernández et al. 2017; Claus et al. 2018) some other insect species visiting cocoa flowers may also contribute to cocoa pollination, especially when midges are scarce. Abundance of pollinators is influenced mainly by the availability of sufficient amount of material where they breed, which is generally found in moist, shaded and rather cooler habitats. One of the best breeding

opportunities for pollinators are ecosystems with heterogeneous shade cover, those in which bananas or plantains are present and also those where midge-specific substrates of decomposing cocoa leaf litter, fruit husks and slices of banana pseudostem are available in sufficient amount (Young 1986; Adajaloo et al. 2013).

The determination of suitable habitat with good breeding substrate for the pollinators could be used as a model for the change of management in cocoa cultivation. Pollinator-friendly practices could lead to higher number of pollinators and consequently to a higher yield. Good harvests from already existing cocoa plantations could decrease the pressure on agricultural land expansion, help to reduce deforestation and contribute to biodiversity conservation.

The aim of this study was the evaluation of cocoa agroforests as a suitable habitat for pollinators with a focus on the major cocoa pollinators, and its subsequent potential to yield enhancement.

2 Literature Review

2.1 Cocoa (*Theobroma cacao*, L.)

Theobroma is a small genus of Malvaceae family, which comprises over 20 species. Although there are several cultivated species such as *T. grandiflorum*, *T. angustifolium* and *T. bicolor*, the only commercially cultivated one is *Theobroma cacao*, L. It was cultivated by the Mayas over 1,500 years ago, and was considered to be of divine origin. The name of the genus, *Theobroma*, means food of the gods (Kalousová 2013; Motamayor et al. 2002).

It is native to humid lowland tropical rainforest in Amazon region in South America (Cuatrecas 1964). There are three main genetic groups traditionally described as Criollo, Forastero and Trinitario. Criollo is nowadays highly prized and rare, less bitter and more aromatic than other varieties, but with a low vigour and yield. Forastero trees are significantly hardier than Criollo trees and produce cheaper cocoa beans, they are used for 80 % of world chocolate production. Forasteros can be further subdivided into very diverse populations with different geographic origins: Upper Amazon, Lower Amazon, Orinoco and the Guianas. Trinitario is a hybrid group originating from crosses between Criollo and Forastero and is more disease resistant and productive (Motamayor et al. 2002). The cultivation of cocoa is concentrated in a belt between 15°N and 15°S of the Equator. Currently the majority of cocoa is produced in Africa (around 66 % of world production), with Côte d'Ivoire and Ghana as leader production countries, followed by Asia (18 %) and Americas (15 %) (FAOSTAT 2016).

2.1.1 Botanical description

T. cacao (Figure 1) is a small evergreen tree growing up to 4-8 m in height. It has a woody, straight trunk with a smooth bark of pale brownish colour.

The root system is formed by well-defined main (orthotropic) taproot reaching depth up to 2 m and lateral (plagiotropic) roots which can be found in the upper 20 cm of the soil.

Leaves are alternate, entire, simple, oblong and pointed with a firm texture. The average leaf size is 20 cm long (exceptionally reaching length up to 50 cm) and 8-10 cm wide. The colour of young leaves is pale green to pink to purple, later becoming dark green. New leaves develop in a series of flushes occurring 4 to 5 times a year (Kalousová 2013).

The fruit, sometimes called pod, is an ovoid indehiscent drupe with fleshy pericarp, 10-30 cm long and 7-9 cm wide. The young cocoa fruit smaller than 5 cm is called cherelle (Figure 2). The weight of a ripe fruit is about 500 g. It usually contains 30-40 seeds covered in white mucilaginous pulp. The white to dark purple seed (cocoa bean) is recalcitrant with two cotyledons. It contains 45 – 65 % of fat (of total weight of dried seeds), proteins and starch. The pigment cells are composed of polyphenols and purines (theobromine and caffeine). The first production is at age of 3–4 years (starting from seedling stage), full production is reached at 10 years (Garcia 2000).

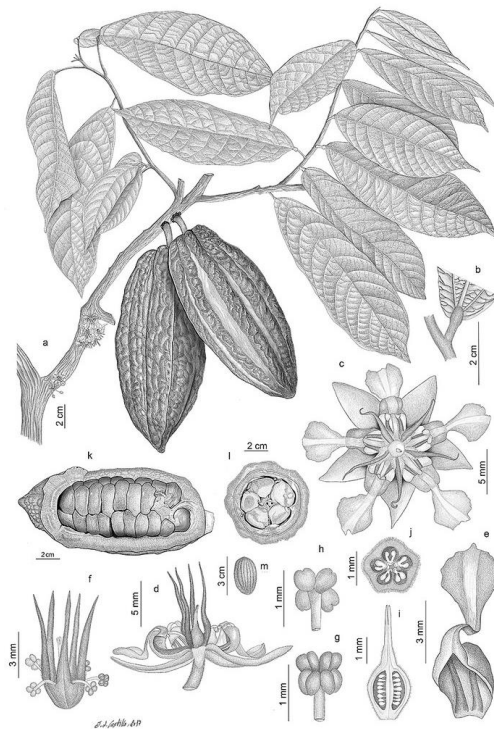


Figure 1 *Theobroma cacao*, L. (Royal Botanic Garden Sydney, source: flickr.com)



Figure 2 Young cherelle of cocoa and flower buds

2.1.2 Cocoa flowering and pollination

The tree is cauliflorous - cocoa inflorescences are grouped in clusters on the trunk and older branches. These clusters called flower cushions i.e. thickened lower-producing leaf axils may have between 14-48 flowers. These very small flowers (0.5 - 1 cm across) are white to pink. They are bisexual, regular and pentamerous consisting of five petals alternate with five sepals, hood and ligule, ten stamens (five fertile and five non fertile staminodes) and one pistil with superior ovary, tubular branched style and five stigmata. The tree produces up to 125,000 flowers per tree each year (Falque et al. 1995). The flowering is simultaneous within a single plantation/region. The flowering season of Upper Amazon genotype is year-round with the 2 slight peaks occurring during wet season (in Peru starting in November/December) after which gradually decline. Flowering occurs approximately 6 months before the harvest of cocoa beans (the main harvest season in Ucayali region is between May and August).

T. cacao prefers allogamous mating system and its self-incompatibility in genetics is considered unique among the identified systems in flowering plants. Wild populations of Forastero originating in South America contain predominantly self-incompatible individuals whereas some traditional varieties such as Amelonado (Lower Amazon type), Criollo and others are self-compatible. Several Upper Amazon genotypes segregate both for the self-compatibility and the self-incompatibility. ‘Modern’ Criollo are often self-incompatibles (Efombagn et al. 2009).

While the flower production can be very high, the pollination rate stays low. In general, only 10 % of the flowers are naturally pollinated and the proportion of flowers giving rise to mature fruit is even lower, usually below 5 % only (Stephenson 1981; Falque 1995; Bos et al. 2007; Groeneweld et al. 2010). This low pollination rate is caused by several following factors. Since the stigma of flowers is receptive to pollination only from sunrise to sunset on the day that the flower opens, the released pollen remains viable for a maximum of 48 h and the unpollinated flowers abscise within about two days, the opportunity for pollination is very small (Madell 2005). Because of the special flower structure, where the floral parts are intricately arranged, cocoa is strictly entomophilous and generally excludes pollination by other insect than by ceratopogonid midges thanks to their body size and morphology (Klein et al. 2008). As midges live and breed in wet and cool conditions, lack of pollinators, especially during the dry season, can occur (Young 1982). At least 35 suitable pollen grains have to be deposited on the receptive parts of the flower to achieve successful pollination (Frimpong et al. 2014). However, the fruit production is likely to depend on resources like nutrients, light and water available to the plant, Groeneveld et al. (2010) proved stronger cocoa yield limitation by pollination than by plant resources.

The growth and maturation process of the cocoa fruit lasts approximately 150 days and is divided into two phases. During the first phase many young pods are lost by the fruit abortion (so called cherelle wilt), even after successful pollination, which is another reason for low fruit set. It is a self-thinning mechanism and serves to balance nutrient allocation in the tree. Up to 80 % of cherelles do not reach their maturity due to

this phenomenon. During the cherelle wilt, the young pod will shrivel, turn black, and become rapidly colonized by pathogens, while remains on the tree. Cherelles can wilt up to day 100 after fruit set. The second phase starts about 85 after the pollination. Pericarp and ovule growth decrease and the embryo start to develop from about 0.2 to 3 cm. On the day 140 the embryo has completed its development and pod starts ripening (McKelvie 1956).

2.1.3 Cocoa pollinators

Cocoa is generally considered to be pollinated by so called cocoa midges, small biting Diptera flies of the family Ceratopogonidae. Most prominently the pollination is performed by adult ceratopogonid midges females of *Forcipomyia* genera (Figure 3) (Young 1983). Males also pollinate, but to a lesser extent which is probably determined by the need of females to feed on the protein-rich pollen grains, necessary for egg maturation (Claus et al. 2018). Within that genus, the most frequently reported pollinators belong to the subgenera *Euprojoannisia* (before: *Proforcipomyia* and *Euforcipomyia*) (Winder 1977). According to Winder (1975) there are several other genera, which can serve as successful pollinators such as *Dasyheleas* spp., *Atrichopogons* spp., *Stylobezzia* spp. and *Culicoides* spp.

The body length of ceratopogonid midge is about 3 mm. According to Kaufmann (1975) the normal flight range is about 5–6 m, meaning they mostly deposit pollen from a certain cocoa tree on the neighbouring tree. However, distance travelled during which pollination is performed, can reach up to 50 m. They occur both above and below the canopy. Biggest pollination intensity is in the morning between 8:30 and 11 am and again in the afternoon after 3:00 pm (Saunders 1959; Frimpong et al. 2009).

Adults live only between 1 to 12 days (under laboratory conditions) and a complete life cycle covers about 28 days: 3 days after deposition eggs hatch, 12 days later larvae transform into pupae and 3 day later an adult develops (Claus et al. 2018).



Figure 3 *Forcipomyia* spp. on a slide (Adjaloo 2012)

Ceratopogonid midges are relatively demanding regarding their breeding habitat. They seek rather humid, cooler and dark environment which can be represented by rotten organic substrate such as tree rot holes, between buttresses of shade trees, decaying cocoa pod husks, cocoa leaf litter, decomposing banana pseudostems and epiphytic bromeliads (Winder 1978). Especially availability of remains of plantains/bananas have been found to be a good breeding substrate for midges. There have been found positive relationships also between the abundance of pollinators and the leaf litter cover and remains of decomposing fruit on the ground (Córdoba et al. 2013). Oppositely cleaning of the ground-litter, the common practice in numerous AFSs is believed to depress the pollinator midge populations by destroying their breeding microhabitats, so that cocoa pollinators may suffer when organic waste is removed from plantations (Winder 1977). Sufficient amount of organic matter (decomposing fruit), leaf litter and other suitable breeding material could increase the population of midges and subsequently the pod-set in cocoa farms (Young 1982; Frimpong et al. 2011).

Also level of shade is an important factor influencing midge populations and Young (1986b) has observed a negative correlation between number of mature pods per cocoa tree and distance from large canopy (shade) trees suggesting increased pollination rate beneath shade-trees canopies.

Shade tree species composition may be possibly another feature with the impact on midges. Cocoa is often grown in simple polyculture with just one or two shade trees. A more heterogeneous ground litter resulted from more diverse agroforestry system (AFS) could probably promotes colonization by a greater number of pollinating midge species than homogeneous ground litter of cocoa farms with the simple floristic structure (Young 1982).

Population of *Forcipomyia* spp. also depends on climatic conditions of the region and the season in a particular area (Adjaloo & Oduro 2013).

Despite the evidence for their role as pollinators of cocoa, pollinator surveys have often found low abundances of ceratopogonid midges and literature suggests that other Dipteran families and even other insect orders visiting cocoa flowers may also contribute to cocoa pollination. According to Toledo-Hernández et al. (2017) ceratopogonid species represent only about 40 % of flower visitors. Among the most important Dipterans with a potential to pollinate cocoa are following.

Some members of Drosophilidae (2-3 mm) are confirmed as possible pollinators of cocoa (Winder 1978) and have been captured by Young (1986) on cocoa flowers.

Cecidomyiidae with their body length from 0.5-2 mm have been mentioned in various articles as cocoa flower visitors (Winder 1977; Winder 1978; Young 1983; Young 1985) and Lucas (1981) even found them carrying large quantities of pollen grains.

The adults of Phoridae with size between 1.5-3 mm feed on flower nectar and therefore they could carry cocoa pollen. They were captured by De Schawe et al. (2016) on cocoa flowers. Furthermore, some Phorids such as *Megaselia* pollinate *Herrania*

species of Malvaceae family which is closely related to *Theobroma* genus (Young 1986).

There are various other Dipteran families, namely Sciaridae and Mycetophilidae, Sphaeroceridae, Chironomidae, Tachinidae, Chloropidae and Psychodidae captured on cocoa flowers, however with no evidence for their pollinator activity. But due to their adequate morphology and feeding behaviour they might contribute to pollination too (Young 1986; Winder 1978; De Schawe et al. 2016; Toledo-Hernández et al. 2017).

Although Lauxaniidae and Stratiomyiidae have not been captured directly on cocoa flowers but only collected from leaf litter and rotten pods (Winder 1972), small individuals of these two orders could be included in potential cocoa pollinators because they might visit flowers to feed on nectar.

Apart from Diptera insects some members of other orders such as Hemiptera (aphids, coccids and cicadellids), Thysanoptera (thrips) and small Hymenoptera (Eulophidae and Platygasteridae) have been documented to visit cocoa flowers but their contribution to pollination is considered to be probably low (Claus et al. 2018; De Schawe et al. 2016).

Ants could potentially have indirect influence on cocoa pollination. The ant *Azteca chartifex spiriti* (in Brazil) has been observed to attract ceratopogonid midges (Claus et al. 2018). On the other hand, Toledo-Hernández et al. (2017) reported that ant communities disturb pollinators and so enhance their movement, leading to more frequent flower visitation and enhanced pollination success.

Wild bees *Lasioglossum* sp. and *Hypotrigona* have been documented to visit cocoa flowers in Africa and *Lasioglossum* in particular was identified as effective cocoa pollinator (Raju 1975; Frimpong et al. 2015). Also Young (1985) offers that bees, specifically stingless bees (*Trigona jaty*) in Costa Rica have an impact on fruit set in cocoa trees in direct sunlight, however with only minimal impact in areas of cocoa with shade where natural pollinator activity is high. Further there is some evidence of the

pollination potential of *Trigona testaceicornis*, *T. coryina*, *T. pallida*, *Nannotrigona testaceicornis punctata*, *Paratrigona lineata subnuda*, and *Plebeia mosquito* but cocoa pollination by those species is probably only coincidence (Raju 1975; Soria 1975).

2.1.4 Ecological requirements of cocoa

Climatic factors are essential in encouraging optimum growth and particularly precipitation is important yield determining factor. Cocoa plants respond well to relatively high temperatures between 23°C to 32°C with the optimum of 25°C. At average less than 23°C, the flowering is reduced. It requires an annual rainfall level of between 1,500 mm and 2,500 mm well-distributed over the year (Durán Ramírez 2010).

Cocoa grows best in very humid conditions. At night and early morning it usually fluctuating around 100 % and falling to 70 – 80 % during the day.

For proper development shading should be reduced, letting through 70 % of the light (Kalousová 2013).

Cocoa has the capacity to adapt to a wide variety of soil types. It can develop well in soils with a pH in the range of 5-8, with optimal value of 6.5. The soil should be at least 1.5 m deep, with the minimum of 3.5 % of organic matter in the most upper layer (Durán Ramírez 2010).

2.2 Cocoa agroforests

Cocoa is naturally an understory plant that requires a canopy of shade, which would protect it against direct sun and wind. This can be provided by other tree species planted in the cocoa plantation. Although shade trees may sometimes compete with cocoa, they play several roles in cocoa production and provide along with canopy cover and transmitted sunlight many other multiple economic, social and environmental benefits. Regarding the environment, the main advantages are regulation of air humidity, nutrient availability and increase in the efficiency of nutrient use, increase in amount of soil organic matter that may affect physiological processes of flowering,

protection of the soil and reduction of erosion, and protection against pests and weed growth. As for social and economic services it is for example diversification of production and subsequent income from their sale. But one of the most important things is they also enable increased pollination services by providing habitat for insect pollinators associated with the formation of the fruits (David 2005; Franzen and Mulder 2007; Zakariyya et al. 2016). It is however very important to balance the level of shade. In general, shade increases humidity of the plantation. If there is not enough shade, the plantation can get too dry in the dry season and contrary too much shade implicate too humid environment during the rainy season, which can increase the incidence of pest and diseases.

There are various limiting factors, both intrinsic (genetic, physiological) and extrinsic (environmental), and also agronomic resources such as water stress, deficiency of light and nutrients and others, which regulate fruit set in cocoa.

One extrinsic limiting factor is supposed to be pollination by insect, already described in 2.1.3. Low pollinator abundance and the subsequent inadequate pollination of cocoa has been attributed to the removal of suitable pollinator habitat (i.e. moist decomposing organic matter for egg oviposition and larval development) (Kaufmann 1975; Winder 1978) that is usually associated with the intensification of cocoa cultivation: full-sun cultivation without shade trees. Cocoa-shade tree agroforestry systems thus may present an appropriate option for cocoa sustainable cultivation. Recent research suggests that providing additional, midge specific substrates of decomposing cocoa leaf litter, slices of banana pseudostem and cocoa fruit husks can increase fruit set (Adjaloo et al. 2013). Additionally to that, some management practices that generally promote pollinator communities (i.e. habitat manipulation and the reduction of pesticide use) may also provide benefits to other animals in agroecosystems (Forbes & Northfield 2017).

2.2.1 Cocoa agroforests in Peru

Cocoa production in Peru (Figure 4) is continuously increasing and in the year 2017 121,825 tons of cocoa beans were produced. The average yield is 839.2 kg ha⁻¹ (FAOSTAT 2016). Between 80 to 90 % of world's cocoa production is produced in smallholder subsistence systems (World Cocoa Foundation 2014) but almost all of the cocoa production is used for export and only a small portion is consumed locally (Kalousová 2013).

Agroforestry may be introduced at various stages in agricultural development and a common case is introducing tree crops into shifting cultivation systems. Most traditional cocoa farms were established by removing the forest understorey and thinning the forest canopy. The fertility of the soil is temporarily increased by burning the previous forest or fallow vegetation.

The majority of the trees in cocoa agroforests are native tree species, and also occur in surrounding forest fragments, however exotic leguminous trees and/or marketable timber or fruit trees are also introduced.

One of the most popular trees in Peruvian cocoa agroforests are often leguminous shade trees such as guaba (*Inga Edulis* C. Martius), timber tree species like bolaina (*Guazuma crinita* Martius) and capirona (*Calycophyllum spruceanum* (Bentham) Hooker f. ex Schumann), tree crops such as mango (*Mangifera indica* L.), avocado (*Persea americana* Mill.), citruses (*Citrus* spp.) and various palm species (e.g. *Bactris gasipaes* Kunth, *Cocos nucifera* L., *Mauritia flexuosa* L.f.).

Smallholder farmers usually associate their young cocoa trees with annual and semi-perennial food crops, until either the shading by the trees becomes too intensive or the fertility of the soil has been exhausted. Plantain and banana (*Musa* spp.) are used for temporal shade during first years of establishment.

However, recent agricultural science focus on maximizing cocoa production and has developed new hybrid cocoa varieties that require full sun, so that there is a gradual

shift towards the elimination of shade trees in the cocoa landscape and some farmers even switch to full-sun production because of lower labour costs and higher short-term yields. Although it results in increased yields, to maintain them it requires the use of chemical fertilizers and greater use of herbicides, due to that full-sun conditions also encourage the growth of weeds.



Figure 4 Cocoa production in Peru (Ministry of Foreign Trade and Tourism 2018)

The important organization in Peruvian Amazon which deals with cocoa production is ACATPA (Association of Technified Cocoa Growers of Padre Abad). This association consists of 300 rural families in the Padre Abad province, dedicated to the management of AFSs based on cocoa and the commercialization of high-quality cocoa grains and production of certified and organic cocoa. According to ACATPA majority of the varieties of cocoa grown by the farmers from their organization is CCN 51 (75%). CCN 51((ICS-95 x IMC-67) x CCN-1) is highly productive, disease-resistant and precocious cultivar from Ecuador, that produces large pods and beans after only two years of transplanting to the field. However the flavour is much worse than flavour of traditional

fine varieties and it is also cheaper, therefore, cocoa farmers get less money for their cocoa beans (Amores et al. 2011). The rest of varieties (15%) are composed of ICS 39 (a local clone of Trinitario Trinidad and Tobago), IMC 36 and EET 12 (local clones).

3 Objective of the Thesis

The objective of the thesis was the evaluation of cocoa agroforests as a suitable habitat for pollinators in general (order Hymenoptera and Diptera), with a special focus on the potential cocoa pollinators (selected orders of Diptera) and its subsequent potential to yield enhancement.

The specific objectives were following:

- To assess the pollinator diversity and abundance in various cocoa agroforests in Peruvian Amazon;
- To evaluate the influence of tree species composition, shade cover and soil cover on abundance and diversity of pollinators;
- And to assess the phenological patterns of flowering and fruit set (cocoa production) and its relation with pollinators.

Based on the objectives we have set following hypothesis:

H1: The highest pollinator diversity and abundance is in the agroforests with highest tree diversity and more complex vegetation structure;

H2: The high canopy shade cover, and soil coverage with organic material has a positive influence on abundance of pollinators;

H3: There is a positive relationship between the abundance of pollinators and subsequent cocoa production.

4 Materials and Methods

4.1 Study area

The Peruvian Amazon region, one of the most species rich forested area in the world, is located in the lowlands on the eastern side of Andes. The research was conducted around the town of Alexander von Humboldt situated at 86 Km of the Federico Basadre road which connects Lima to Pucallpa (Figure 5). It lies in the Ucayali region, Padre Abad province, Irazola district, the zone of Lowland Forest, which dominates the Peruvian Amazon.

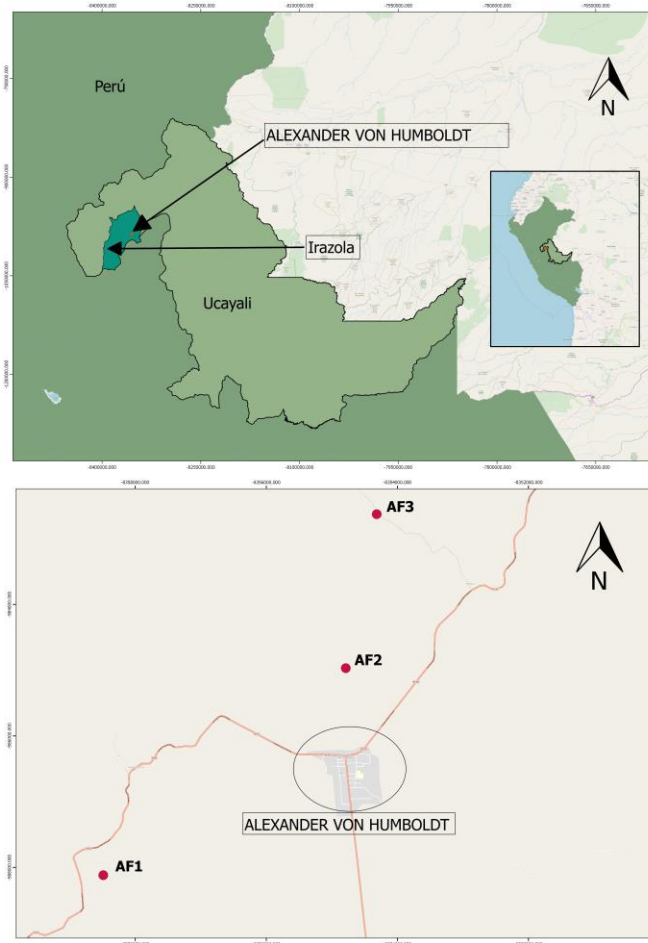


Figure 5 Map of study site (source: QGIS)

The climate is hot and humid (during both days and nights) with only slight fluctuation of temperatures throughout the year. The area is characterized by changing of wet and dry season. Wet season lasts from November/December till March/April during which the heavy rains are concentrated and it is a bit colder. The rest of the year is dry and these months are also the warmest. The rainfall ranges from 2,500 to 3,500 mm, the mean annual temperature is 26.1 °C and mean annual relative humidity reaches 85 % (Figure 6).

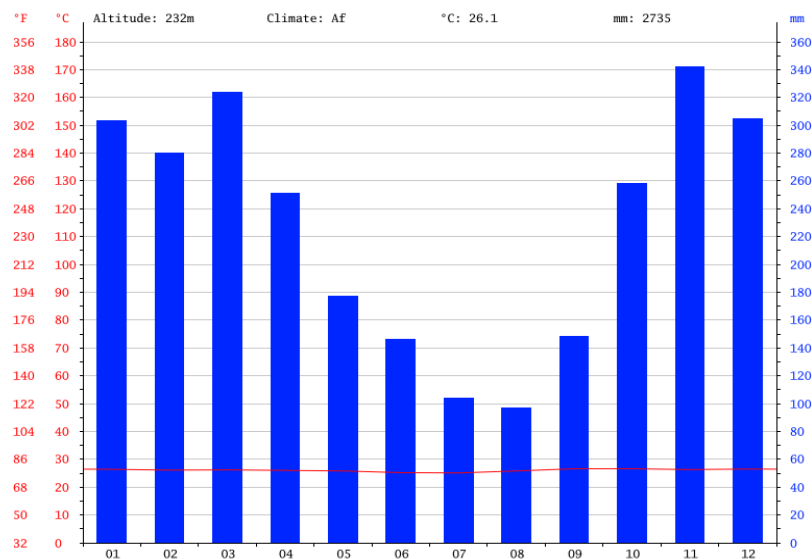


Figure 6 Climate diagram for Alexander von Humboldt (source: climate-data.org)

The original vegetation was lowland humid tropical forest, at an altitude of between 250 and 350 m asl, but large areas have been deforested and nowadays it consists mainly of agricultural land, pastures, secondary forests and the residues of the forest. Due to this inadequate forest management resulting in mass deforestation, there are many environmental problems connected to this part of the country such as torrential rains and floods, soil degradation, and loss of biodiversity.

The rural residents are dedicated mainly to agriculture (slash-and-burn), livestock breeding, forestry and other land-based production (Gonzales 2008). It is one of a major

cocoa growing areas in Peruvian Amazon. Beside the cocoa production, farmers usually cultivate several annual staple crops like rice (*Oryza sativa* L.) and maize (*Zea mays* L.) but also many perennial crops such as bananas and plantains (*Musa* spp.), cassava (*Manihot esculenta* Crantz), sugar cane (*Saccharum officinarum* L.), citruses (*Citrus* spp.), pineapple (*Ananans comosus* L. Merr), papaya (*Carica papaya* L.), coconut (*Cocos nucifera* L.) and camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh) (Vebrová, 2012). Some of these plants are commonly cultivated in AFSs with various local multipurpose tree species.

4.2 The selected agroforestry systems

Data were collected in three cocoa AFSs. Cocoa farms were selected according to their various vegetation structure and other plot characteristics with the help of ACATPA from their list of the cocoa growing farmers. During the selection of the three appropriate systems we focused on differences in their characteristics, but also on good accessibility of the farm and willingness of the owners to cooperate. We particularly tried to choose farms with similar age of cocoa trees and size of the cocoa plot, but with different level of canopy shade cover and tree diversity, various slope, different intensity of management and consequent various coverage of soil and amount of organic matter as potential breeding site for midges. This selection resulted into three types of cocoa agroforests.



Figure 7 Traditional AFS

Traditional AFS

The first one was farm with high canopy shade cover, rich in species of shade trees, used mainly for subsistence and diversification of production, in a hilly landscape, with traditional type of management, which refers to habitual management carried out by indigenous producers, which could be considered as minimal management. This means no use of pesticides and chemicals and rare or no use of fertilizers (manure of livestock or other farms animals such as guinea pig). Organic material was not manipulated at all and was left on the ground. The pruning, control of weeds and other cultivation practices were very limited. The majority of the cocoa trees was hybrid CCN 51 variety but also variety UF 13 on small proportion (20 % of area) (Figure 7).



Figure 8 Organic AFS

Organic AFS

The second farm was characterized by medium level of shade and medium level of tree diversity compared to other two farms. The majority of shade trees was used for wood production. The terrain was rolling and type of management was classified as organic certified. Only natural products authorized by certification program were used for fertilization and control of pests. Compared to the first farm the management practices were more intensive e.g. more frequent pruning and a certain level of manipulation of organic matter, meaning for example adding of decomposing fruits to some trees, covering bare soil surface with leaves etc. The only cocoa variety grown was CCN 51 (Figure 8).



Figure 9 Conventional AFS

Conventional AFS

The third farm was almost full-sun cocoa AFS with just a few species of shade trees, the relief was flat and type of management was considered as conventional. This was the only farm where pesticides and chemicals (except for insecticides) were used and also the management practices were more frequent than in the case of first and second farm. There was installed a system of irrigation used two times per week during dry season and one time per week during wet season. This AFS was regularly cleaned and organic matter was removed and subsequently added to only few selected trees. The variety of cocoa trees was CCN 51 as in the previous cases (Figure 9).

The inter-tree spacing between cocoa trees was on all of the AFSs 3x3 m.

To gain additional relevant information to completely document and characterize the cocoa growing systems and management, farmers were interviewed using a simple

semi-structured questionnaires. Questions included history (previous crops, beginning of cultivation of cocoa), size of the plot, age and variety of cocoa trees, inter-tree spacing, yield, selling price, management (thinning, fertilization, harvest...) and tree diversity in the whole system (Table 1).

Table 1 General characteristics of selected cocoa AFSs

Farm samples	Shade cover	Tree diversity	Size (ha)	Slope	Age of cocoa trees (years)	GPS data	Soil cover
Traditional	high	high	2.5	hilly	10	8°50'33"S 75°5'4"W	rich in organic material
Organic	medium	medium	1.25	undulating	6	8°48'58.4"S 75°03'12.3"W	medium
Conventional	low	low	3	flat	9	8°47'19"S 75°3'6"W	bare soil

4.3 Data collection

Data were collected from June to September 2017 in selected AFSs which corresponds to culmination of dry season. In each AFS three cocoa trees, so called "central" trees were chosen which represented average state of the vegetation on the farm, meaning that its appearance, height, number of flowers and fruits was found out to be an ordinary cocoa tree in the particular AFS. The distance between the central trees on the farm was at least 15 m. Approximately 5-7 meters from each of the central tree, three other cocoa trees have been selected randomly, which means 12 trees in total in each cocoa AFS (36 in total) (Figure 10). All trees were similar in variety, size, and overall health and properly marked.

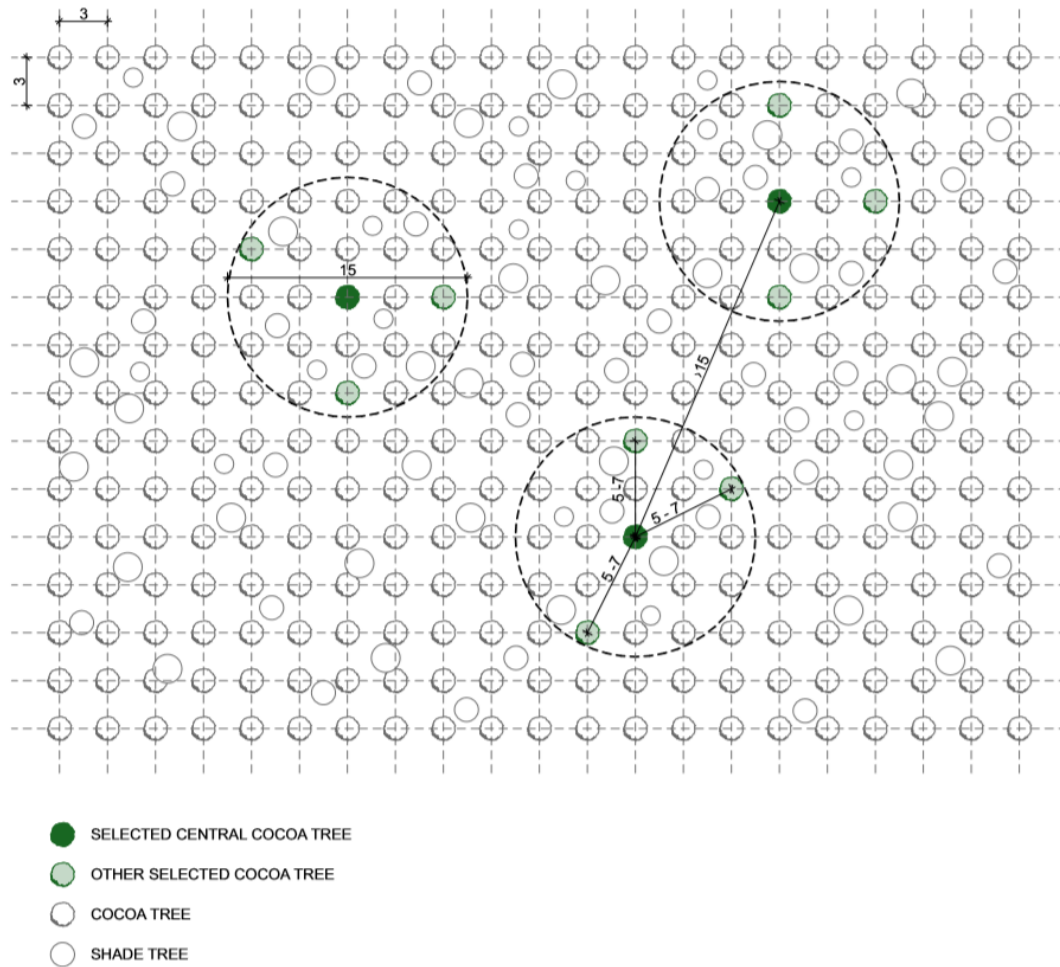


Figure 10 Schematic drawing of a segment of one AFS (ArchiCAD)

4.3.1 Vegetation survey

Following vegetation and other characteristics of the AFSs were measured for the further analysis of its structure and diversity.

Shade cover

Spherical densitometer with use of a standardized methodology by Lemmon (1956) was utilized for measuring the shade cover. Shade cover was measured on each of the selected cocoa trees.

Soil cover

A quadrat (1 m x 1 m) was set around each of selected cocoa trees (the cocoa tree was placed in the centre of the cell) and spatial percentage cover of grass, bare soil, leaf litter, organic material (decomposing fruit) was estimated in each of these cells.

Floral and fruit phenology of cocoa

The observation of floral and fruit phenology of cocoa trees was done at the beginning of each trapping session, thus every two to three weeks which was enough time for a new distinguishable pod (cherelle) to develop. The observations of new pods, flower buds and open flowers were done only on central trees (3 in each of the AFS, 9 in total).

We used the modified BCCH (Bleiholder et al. 1991) to determine the stages of flowers and fruits for assessment of flower and fruit production. Collected data regarding floral phenology included number of mature flower buds and newly opened flowers. Data related to fruit phenology consists of new pods only. During every measurement of cherelles we marked them with a permanent marker to be distinguished by the next measuring session when they were remarked. Flowers and fruits observed in the tree canopy above 2 m in height were inaccessible and thus excluded from the counts. Same for those found lower than 30 cm on the trunk.

Shade trees species diversity, richness and density

All shade trees (other species than cocoa) within a circle (plot) with $r = 7.5$ m around each central cocoa tree were counted and identified to species level in order to determine the tree density (trees ha^{-1}) and tree species richness and diversity. Despite the herbaceous character of *Musa* spp. they were included into shade trees counts, due to their important role in providing shade and breeding substrate in form of decomposing pseudostems.

4.3.2 Insect collection and identification

Insect trapping was done using the same methods in all samples. On each of the selected cocoa tree, four insect traps have been installed near the flower cushions (one of the traps attached to the trunk near the ground, the rest attached to the branches). The traps were white plastic buckets with shiny surface and with volume of 1 liter (Figure 11). The colour was chosen based on the similarity to cocoa flowers to attract the pollinators. They were refilled with fixative solution consisting of water, salt and liquid detergent. Exposure of traps ranged from 3 to 5 days (depending on the possibility of insect collection related to weather conditions). Collection of trapped insects was repeated three times in each agroforest, which means one trapping session every two to three weeks. The caught insect was sluiced with fresh water on the sieve and stored in a special plastic bag properly marked by the locality label. All the samples were conserved in ethanol (96 %) for the long-lasting fixation and stored for further taxonomical classification in laboratory. Additionally, to these traps we tried to use aspirator to collect the insect directly from the cocoa flowers but due to their low abundance it turned out to be non-functional. The insect was standardly preserved on entomological pin or glued on triangular card. Afterwards it was classified to order and family levels and subsequently into morphospecies by experienced entomologists.



Figure 11 Insect trap

Even though we detected presence of Ceratopogonidae insects in our samples their abundance was very low. Because of their small size, fragility and damages caused during the capture, we were not able to identify them properly. Due to those facts they were excluded from our study and we conducted the data evaluation and analysis only for chosen insect families from our samples, which were appropriate for the purpose of our study. The suitability was evaluated according to their functional role assigned, adult feeding habit, the size and other characteristics found in scientific literature (see 2.1.3. Cocoa pollinators). We decided to include in our study evaluation of chosen families of Hymenoptera and Diptera order, despite the fact, that they are not considered as primary pollinators of cocoa. The abundant presence of members of those orders could be explicated by the fact that they feed on and pollinate other tree species in the agroforestry systems. In addition, small individuals could serve as potential additional cocoa pollinators.

4.5 Data evaluation and analysis

We compared the vegetation structure and pollinator characteristics on two levels: in total, corresponding to all values calculated from all collected species from each AFS (sample), and for each cocoa tree circle (plot) separately. Data from cocoa circles were used for statistical analysis. Nevertheless, the abundance, diversity index (Shannon) and species richness obtained by pooling all three circles could give better approximation to the reality due to larger scale (Vebrová 2012).

Vegetation survey

For shade and soil cover characteristics, we evaluated all (12) selected cocoa trees/each AFS. All evaluated trees within one circle around the central cocoa tree included (four cocoa trees) were pooled together and mean value of each plot was used for the statistical analysis. Data from all sample plots were also pooled for whole system and evaluated separately for each AFS.

The counts of flowers and fruits were performed only on central cocoa trees (3) in each AFS. Data from three consecutive counts were pooled together for each tree, giving the value for each plot. These values were used for the statistical analysis. Data from all sample plots were also pooled for whole system and evaluated separately for each AFS.

For evaluation of vegetation structure: shade trees species diversity, species richness and density of non-cocoa trees were calculated within each of the plots separately. To evaluate shade trees species diversity, Shannon index was calculated by equation [2] described below. Shade trees species richness was expressed as the number of non-cocoa tree species. Shade trees density was calculated for each plot too. These values were used for the statistical analysis. Data from all sample plots were also pooled for whole system and evaluated separately for shade tree abundance, species richness and diversity for each AFS.

Insect evaluation

Only particular families of Hymenoptera and Diptera order were used for the study. The evaluation was divided into two sections. At first, pollinators in agroforestry systems that are considered to be pollinators in general were evaluated. There we focus on members of both Hymenoptera and Diptera order (including some species that could serve as potential cocoa pollinators). For evaluation of potential cocoa pollinators, we focused only on specific Diptera families which were selected based on the literature review as described in chapter 2.1.3 (Cocoa pollinators).

The insect captured during the entire sampling period were pooled together for analysis of chosen orders and evaluated separately for selected AFSs. For evaluation of insect abundance, we calculated the number of individuals per plot. These values were used for the statistical analysis. It was also evaluated within each AFS by pooling all the individuals in sample plots. For evaluation of insect species richness, we calculated the number of species per plot. These values were used for the statistical analysis. It was also evaluated within each AFS by combining all the species recorded in sample plots. For estimation of species richness was used Jackknife species estimator. To evaluate insect diversity, Shannon index was calculated per plot. These values were used for the statistical analysis. It was also evaluated within each AFS by combining all the species and their abundances recorded in sample plots. Via the Sørensen similarity coefficient we compared the percentage of insect species similarity across the study sites. Unique and shared species within and among AFSs were visualised in a Venn diagram using an online database hosted by Bioinformatics and Evolutionary Genomics (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

The calculation of the indices is based on following equations:

$$\text{Jackknife species estimator } S = s + \left(\frac{n-1}{n}\right)k \quad [1]$$

Where s represents total number of observed species in n quadrats, n is total number of quadrats samples and k is the number of unique species.

This estimate is based on the observed frequency of rare species in the community (Krebs 1994).

$$\text{Shannon index } H = - \sum_{i=1}^S p_i \ln(p_i) \quad [2]$$

where p_i is proportion of the species relative to the total number of individuals, S is the number of species.

Shannon index relates to the number of species in the community to the relative abundance of each species, so it accounts for both abundance and evenness of the species present. This index expresses the uncertainty of predicting the species of a random sample. The uncertainty decreases along with decrease of evenness and with the number of species, i.e. the value of the Shannon index increases as diversity increases. The average value ranges from 1.5 - 3.5 (Magurran 1988).

$$\text{Sørensen similarity coefficient } S_S = \frac{2a}{2a+b+c} \quad [3]$$

Sørensen similarity coefficient uses presence/absence data for two samples (in this case, AFSs), and weight matches and mismatches in species composition between two samples. It gives greater "weight" to species common to the quadrats than to those found in only one quadrat. It is frequently multiplied by 100 % and may be represented in terms of dissimilarity (i.e. $D_S = 1.0 - S_S$) (Krebs 1994).

Statistical analysis

Statistical analysis of both vegetation structure and pollinator characteristics was performed using SPSS 22.0 program (IBM SPSS, Inc. Chicago, USA). The measured variables were checked for two basic ANOVA assumptions: normality of distribution (Shapiro-Wilk's test) and homogeneity of variance (Levene's test). Variables which fulfilled both assumptions were statistically analysed using parametric analysis of variance (ANOVA). When appropriate (ANOVA $p < 0.05$), Post-hoc Tukey HSD (honest significant difference) was used to determine the differences among the three

studied AFSs. For variables which did not fulfil ANOVA assumptions, non-parametric Kruskal-Wallis test was performed.

Relationship among variables

For identification of the key drivers influencing the abundance and diversity of potential cocoa pollinators, multiple stepwise regressions were performed. The stepping criteria employed for the entry and removal of the variables were based on the significance level of the F-value and were set to 0.05.

The analysis of principle components (PCA) was used to evaluate the relationships among variables. Two principle components (PC1 and PC2) were extracted through Varimax orthogonal rotation. The treatments were further plotted in the orthogonal space defined by PC1 and PC2 to detect the similarity within the agroforestry systems and the differences among them.

5 Results

5.1 Vegetation survey

In total, we found 53 trees belonging to 15 species in traditional AFS, 70 trees belonging to 8 species in organic AFS and 24 trees belonging to 10 species in conventional AFS (Table 2).

While there were no differences in shade trees density among AFSs, the Shannon index of tree species diversity was shown to be the highest in traditional AFS (1.66), intermediate for conventional (1.32), and the lowest in organic (1.09). The statistical analysis revealed significant differences in shade cover and shade trees species richness among the AFSs. Both variables were significantly lower in organic and conventional AFS than in traditional. All types of soil cover and number of new pods, new buds and open flowers were comparable among study sites. There was high variability among trees within an AFS in FF phenology characteristics. In the traditional AFS the range of number of new pods, new buds and open flowers were 0-12, 0-173 and 4-48, respectively. In the organic AFS it varied between 0-4, 0-84 and 0-100 and in conventional the range was 0-14, 0-229 and 0-45. However, we did not find any significant differences among various systems.

Table 2 Vegetation characteristic in traditional, organic and conventional AFSs; means per plot \pm standard error; (n=3)

Variable	Unit	Traditional AFS	Organic AFS	Conventional AFS	ANOVA	KW
Total AFS						
Shade tree abundance	No. of trees per AFS	53	70	24		
Shade tree species richness	No. of species per AFS	15	8	10		
Shannon index of shade trees species diversity		2.39	1.4	1.83		
Means per plot						
Shade trees density	No. of trees per ha	999.73 \pm 303.57	1320.40 \pm 245.22	452.71 \pm 117.80	n.s.	
Shade trees species richness	No. of species per plot	7 \pm 0.58a	4.67 \pm 0.33b	4.33 \pm 0.33b	s.	
Shannon index of shade trees species diversity		1.66 \pm 0.13a	1.09 \pm 0.14b	1.32 \pm 0.08ab	s.	
Canopy shade cover	%	79.96 \pm 2.99a	50.80 \pm 4.76b	36.04 \pm 5.19b	s.	
Soil cover						
Grass	%	15.25 \pm 3.14	5 \pm 0.90	20 \pm 4.13		n.s.
Bare soil	%	19.83 \pm 6.5	10.83 \pm 5.90	39.17 \pm 5.77	n.s.	
Organic matter	%	8.75 \pm 4.53	65 \pm 7.84	19.17 \pm 4.48		n.s.
Leaf litter	%	53.67 \pm 5.47	19.17 \pm 4.47	37.92 \pm 6.81	n.s.	
New pods	No. per central tree	9.33 \pm 3.84	2.67 \pm 1.33	7.67 \pm 3.71		n.s.
New buds	No. per central tree	256.67 \pm 52.26	50.33 \pm 47.35	162 \pm 99.33	n.s.	
Open flowers	No. per central tree	89.33 \pm 15.68	51.67 \pm 44.73	64 \pm 28.16	n.s.	

Values in rows followed by the same letter are not significantly different; s. statistically significant, n.s. statistically non-significant ($p < 0.05$, Tukey's SD test) using analysis of variance (ANOVA) for parametric and Kruskal-Wallis test (KW) for non-parametric distribution

5.2 Pollinators

5.2.1 Pollinators in agroforestry systems

We captured and identified in total 323 insect individuals. We found 71 morphospecies of Hymenoptera belonging to 15 families and 97 morphospecies of Diptera insects belonging to 20 families (Table 3). The families with the highest variety of species and number of individuals were: Vespidae (17 morphospecies, 55 individuals) and Apidae (13 morphospecies, 43 individuals) of Hymenoptera order and Phoridae (29 morphospecies, 44 individuals) of Diptera order. The order Phoridae was the most abundant group found in traditional AFS (37 individuals). In case of organic the most abundant order was Vespidae (20 individuals) and in conventional it was Apidae (19 individuals). In total, we found 163 insects belonging to 95 morphospecies in traditional AFS, 81 insects belonging to 52 morphospecies in organic AFS and 79 insects belonging to 50 morphospecies in conventional AFS.

The abundance of insect individuals differed significantly among the systems, being the highest in the traditional AFS and comparable in organic and conventional AFS (Table 4). Statistically there were no differences in species richness among AFSs, however the highest estimated species richness according to Jackknife was in traditional AFS (145.67), followed by organic (75.33), and the lowest in conventional (73.33). The statistical analysis did not reveal differences in insect species diversity.

Table 3 Families of selected pollinators (Diptera and Hymenoptera order) found in traditional, organic and conventional AFSs; their abundance (detailed table including morphospecies see Appendix)

Order	Family	Traditional	Organic	Conventional
Diptera				
	Phoridae	37	2	5
	Drosophilidae	12	7	3
	Mycetophilidae	19	0	0
	Cecidomyiidae	6	3	9
	Chloropidae	4	1	3
	Stratiomyiidae	5	2	0
	Sciaridae	3	1	2
	Sarcophagidae	1	1	3
	Dolichopodidae	1	1	1
	Richardiidae	3	0	0
	Tachinidae	1	1	1
	Clusiidae	0	2	0
	Lauxaniidae	2	0	0
	Ulidiidae	2	0	0
	Asilidae	1	0	0
	Culicidae	0	0	1
	Chironomidae	0	0	1
	Neriidae	1	0	0
	Platystomatidae	1	0	0
	Psychodidae	1	0	0
Hymenoptera				
	Vespidae	18	20	17
	Apidae	10	14	19
	Halictidae	2	10	6
	Scelionidae	7	6	2
	Bethylidae	10	4	0
	Pteromalidae	9	2	2
	Ichneumonidae	2	1	1
	Braconidae	1	1	0
	Mutillidae	1	0	1
	Pompilidae	2	0	0
	Ampulicidae	0	0	1
	Dryinidae	0	1	0
	Evanidae	1	0	0
	Megachilidae	0	1	0
	Proctotrupidae	0	0	1
Total individuals		163	81	79
Total families		28	20	19

Table 4 Pollinator characteristic in traditional, organic and conventional AFSs; means per plot \pm standard error; (n=3)

Variable	Unit	Traditional AFS	Organic AFS	Conventional AFS	ANOVA	KW
Total AFS						
Abundance	No. of insects per AFS	163	81	79		
Species richness	No. of species per AFS	95	52	50		
Jackknife estimate of species richness		145.67	75.33	73.33		
Shannon index of species diversity		4.27	3.75	3.61		
Means per plot						
Abundance	No. of insects per plot	54.33 \pm 8.76a	27 \pm 5b	26.33 \pm 3.71b		s.
Species richness	No. of species per plot	38.67 \pm 7.06	19.33 \pm 2.91	19.33 \pm 1.86		n.s.
Shannon index of species diversity		3.41 \pm 0.18	2.79 \pm 0.20	2.84 \pm 0.10		n.s.

Values in rows followed by the same letter are not significantly different; s. statistically significant, n.s. statistically non-significant ($p < 0.05$, Tukey's SD test) using analysis of variance (ANOVA) for parametric and Kruskal-Wallis test (KW) for non-parametric distribution

Using Sørensen similarity coefficients, the highest similarity was observed between organic and conventional system and the lowest between traditional and conventional system (Table 5). All three AFSs shared only 7 species (Figure 12).

Table 5 Species similarity Sørensen index (%) of pollinators in traditional, organic and conventional AFSs

	Traditional	Organic	Conventional
Traditional			
Organic	20.14		
Conventional	17.78	22.22	

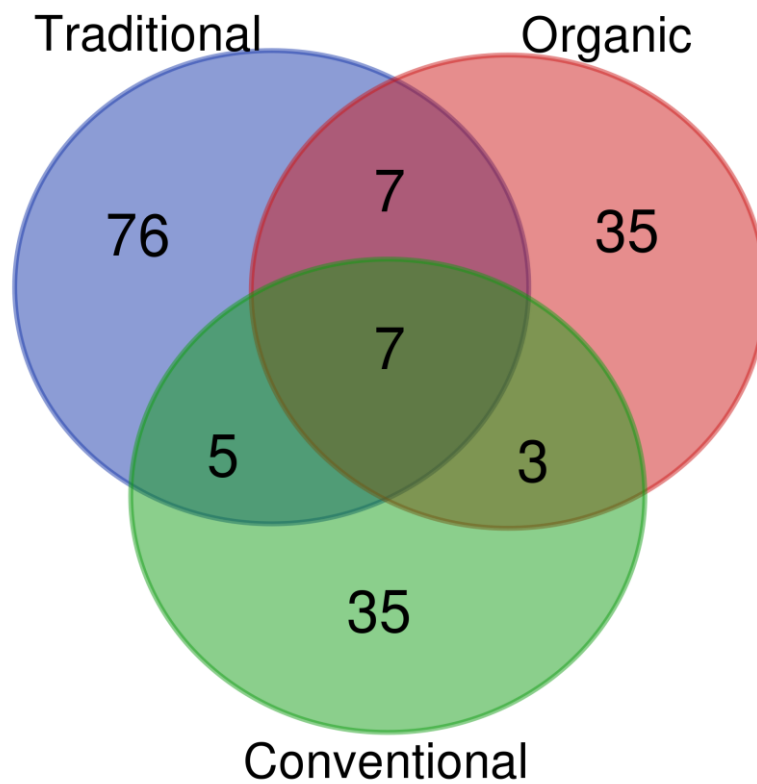


Figure 12 Venn diagram of similarity of pollinators species found in selected AFSs; in total identified 168 morphospecies

5.2.2 Potential cocoa pollinators

Total of 131 insect individuals of Diptera order belonging to 11 families and 82 morphospecies were collected (Table 6). The family with the highest variety of species

and number of individuals was Phoridae (29 morphospecies, 44 individuals), followed by Drosophilidae (10 morphospecies, 22 individuals) and Cecidomyiidae (14 morphospecies, 18 individuals). The order Phoridae was the most abundant order in Traditional system (37 individuals). In the Organic the most abundant were insects of Drosophilidae (7 individuals) and in Conventional it was the order Cecidomyiidae (9 individuals). In total, we found 90 insects belonging to 52 morphospecies in traditional AFS, 17 insects belonging to 16 morphospecies in organic AFS and 24 insects belonging to 24 morphospecies in conventional AFS.

The total abundance of insects did not differ significantly between the systems (Table 7), however evaluating the relative abundance by families, significant difference among the systems was found in case of Phoridae family (Figure 13), being the lowest in organic AFS when compared to traditional and conventional AFSs. Traditional and conventional AFSs had a comparable abundance of Phoridae insects. Statistically there were no differences in species richness among systems, nevertheless same as in the case of pollinators in AFS, the highest estimated species richness according to Jackknife was in traditional AFS (82). But contrary to evaluation of pollinators in AFSs, the second was conventional AFS (36.67), and the lowest organic (23.33). The statistical analysis revealed differences in insect species diversity. The Shannon index of species diversity was shown to be lowest in organic AFS (1.66) and comparable in conventional (1.99), and traditional AFS (2.74).

Table 6 Families of potential cocoa pollinators (Diptera order) found in traditional, organic and conventional AFSs; their abundance (detailed table including morphospecies see Appendix)

Family	Traditional	Organic	Conventional
Phoridae	37	2	5
Drosophilidae	12	7	3
Mycetophilidae	19	0	0
Cecidomyiidae	6	3	9
Chloropidae	4	1	3
Stratiomyiidae	5	2	0
Sciaridae	3	1	2
Tachinidae	1	1	1
Lauxaniidae	2	0	0
Chironomidae	0	0	1
Psychodidae	1	0	0
Total individuals	90	17	24

Figure 13 Relative abundance of potential cocoa pollinators of selected families of Diptera orders in traditional, organic and conventional AFSs; values in one column followed by the same letter are not significantly different at $p < 0.05$; (n=3)

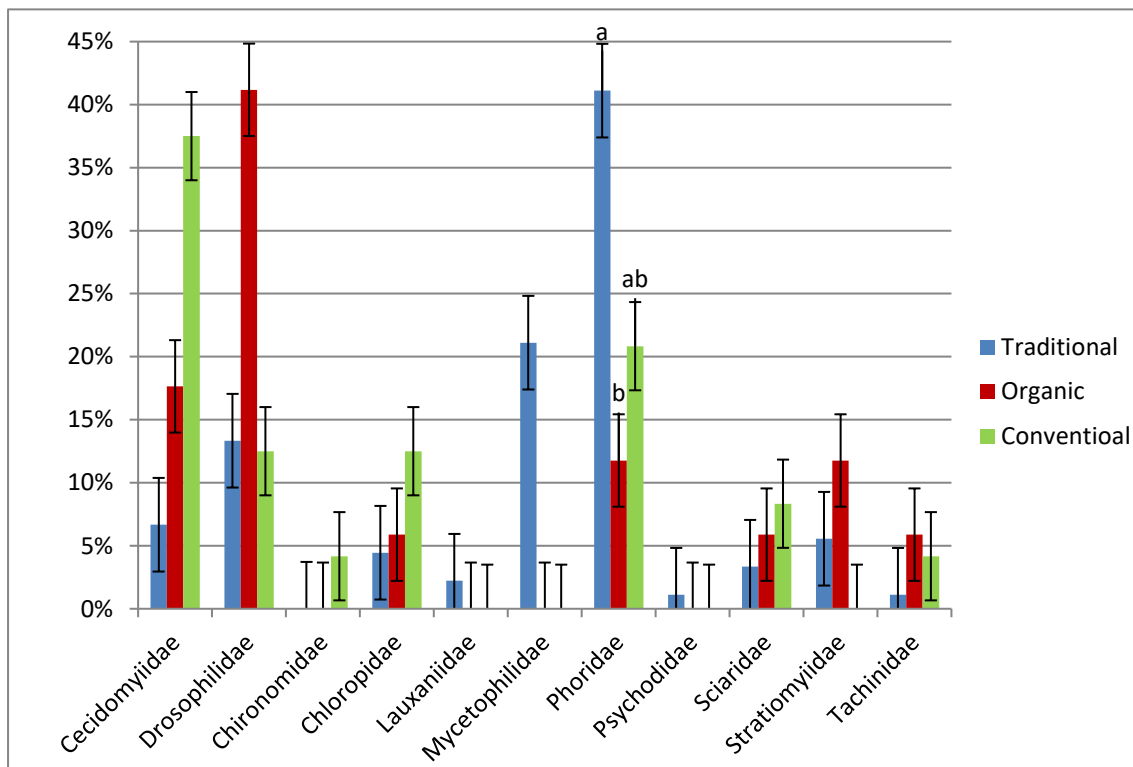


Table 7 Potential cocoa pollinator characteristic in traditional, organic and conventional AFSs; means per plot \pm standard error; (n=3)

Variable	Unit	Traditional AFS	Organic AFS	Conventional AFS	ANOVA	KW
Total AFS						
Abundance	No. of insects per AFS	90	17	24		
Species richness	No. of species per AFS	52	16	24		
Jackknife estimate of species richness		82	23.33	36.67		
Shannon index of species diversity		3.64	2.75	3.18		
Means per plot						
Abundance	No. of insects per plot	30 \pm 8	5.67 \pm 0.67	8 \pm 2.08		n.s.
Species richness	No. of species per plot	20.67 \pm 5.49	5.33 \pm 0.33	8 \pm 2.08		n.s.
Shannon index of species diversity		2.74 \pm 0.27a	1.66 \pm 0.05b	1.99 \pm 0.30ab	s.	

Values in rows followed by the same letter are not significantly different; s. statistically significant, n.s. statistically non-significant ($p < 0.05$, Tukey's SD test) using analysis of variance (ANOVA) for parametric and Kruskal-Wallis test (KW) for non-parametric distribution

Respect to species composition, the most resembling were traditional and conventional system and the most different traditional and organic system according to Sørensen similarity coefficients (Table 8). All three localities shared only 3 species (Figure 14).

Table 8 Species similarity Sørensen index (%) of potential cocoa pollinators in traditional, organic and conventional AFSs

	Traditional	Organic	Conventional
Traditional			
Organic	15.15		
Conventional	25.00	16.67	

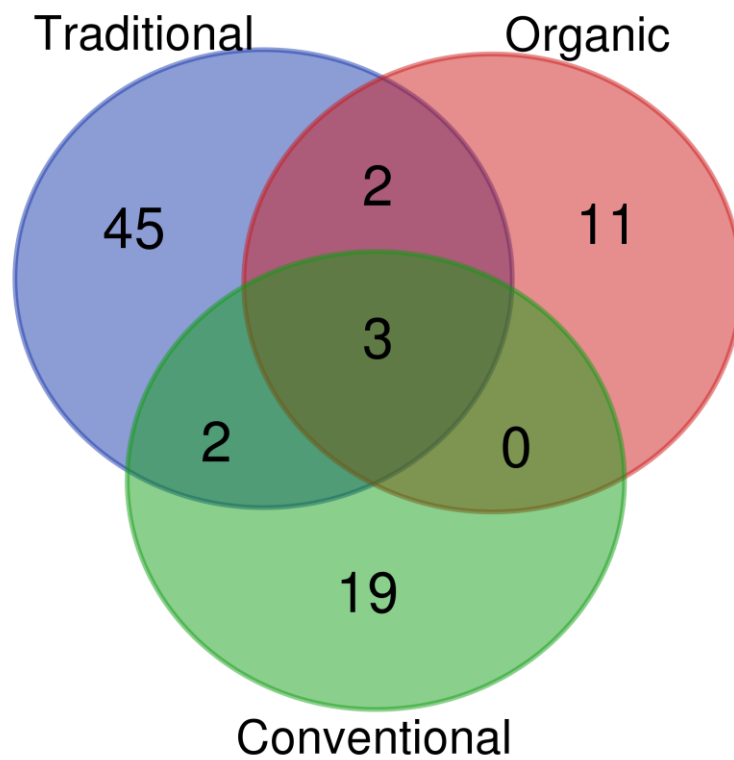


Figure 14 Venn diagram of similarity of potential cocoa pollinators species found in selected AFSs; in total identified 82 morphospecies

5.3 Relationship among variables

5.3.1 Regression analysis

Both potential cocoa pollinator abundance and diversity (Shannon index) were explained by vegetation survey data (Table 2). Canopy shade cover and leaf litter explained 61.7 % and 66.2 % of total variation, respectively (Table 9).

Table 9 The vegetation characteristics explaining the variability of potential cocoa pollinator abundance and diversity detected in stepwise regression analysis

Dependent variable	Constant	Correlation coefficient	Independent variable	R ²	p-value
Potential cocoa pollinator abundance	-12.93	+ 0.494	Canopy shade cover	0.617	0.012
Potential cocoa pollinator diversity (Shannon index)	1.246	+ 0.024	Leaf litter	0.662	0.008

The regression analysis between cocoa production (number of new pods) and its possible influencing factors (potential cocoa pollinator abundance and diversity, shade cover, number of new buds, open flowers and shade tree density) was also conducted but none of the variables was detected to have statistically significant influence.

5.3.2 Principal Components Analysis (PCA)

In total, 67.88 % of data variation were explained in PCA analysis. The majority of variables were grouped within PC1, with pollinator species richness and cocoa pollinator species richness having the highest scores. New buds were related to variables in both PC1 and PC2. The PC2 included the grass cover, bare soil, new pods (positively) and organic matter (negatively). The highest PC2 scores were found for grass and bare soil (Table 10). When the variables were plotted in the orthogonal space defined by PC1 and PC2 (Figure 15), we could observe that both pollinator and cocoa pollinator characteristics were positively influenced by the variables presented in PC1 with shade cover and leaf litter being the most important predictor. PC2 has a very little

influence on pollinator characteristics. The PCA revealed that some vegetation (shade tree diversity, shade tree species richness and shade cover), leaf litter and pollinator variables (pollinator species richness, diversity and abundance; cocoa pollinator species richness, diversity and abundance) were on the positive side of the PC1 axis, which indicated their mutual dependence. On the other hand, PC2 grouped grass cover, bare soil cover and number of new pods (positive side of PC2 axis) and shade tree density (negatively).

Table 10 Scores of PC1 and PC2 in the PCA

Rotated component Matrix		
Variables	PC1 47.93 %	PC2 19.96 %
Pollinator species richness	0.96*	
Cocoa pollinator species richness	0.96	
Cocoa pollinator abundance	0.95	
Pollinator diversity (Shannon index)	0.94	
Pollinator abundance	0.93	
Cocoa pollinator diversity (Shannon index)	0.92	
Shade cover	0.76	
Leaf litter	0.75	
Shade tree species richness	0.71	
Shade tree diversity (Shannon index)	0.70	
Open flowers		
Grass		0.91
Bare soil		0.89
New pods		0.71
Organic matter		-0.69
New buds	0.53	0.59
Shade tree density		

*The scores lower than 0.5 not shown

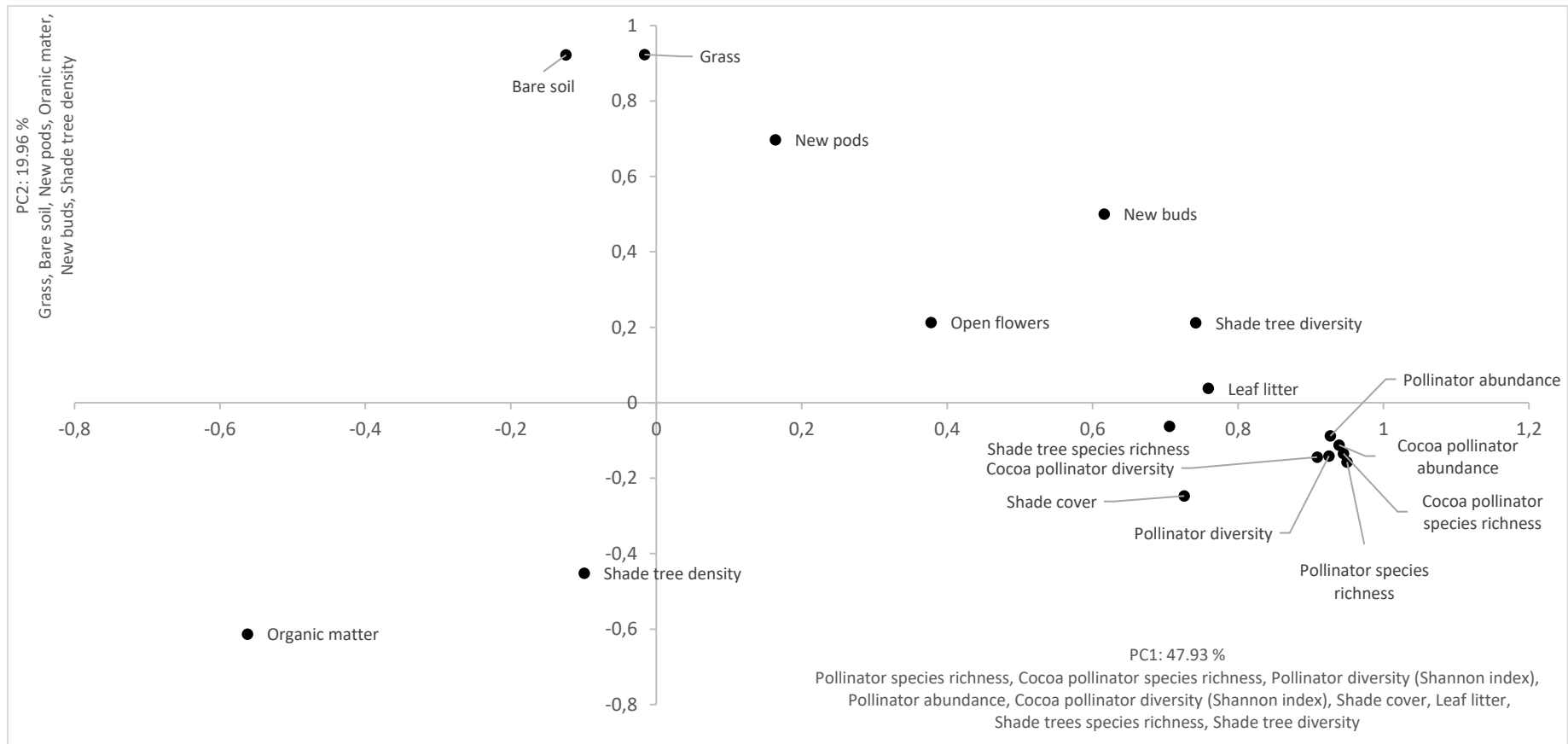


Figure 15 Loading plots for studied variables on Principle Components

Loading plots of variables spaced in the orthogonal space defined by PC1 and PC2 revealed that the AFSs differed considerably in their traits (Figure 16). We can see that plots in each of the AFSs are clustered (they are relatively close to each other and clearly separated from the others). The plots of traditional AFS are all on the positive side of the X axes, which is defined by PC1 variables with positive components. The difference between organic and conventional is more defined by Y axis, so that the differences between these two samples are better described by PC2 variables.

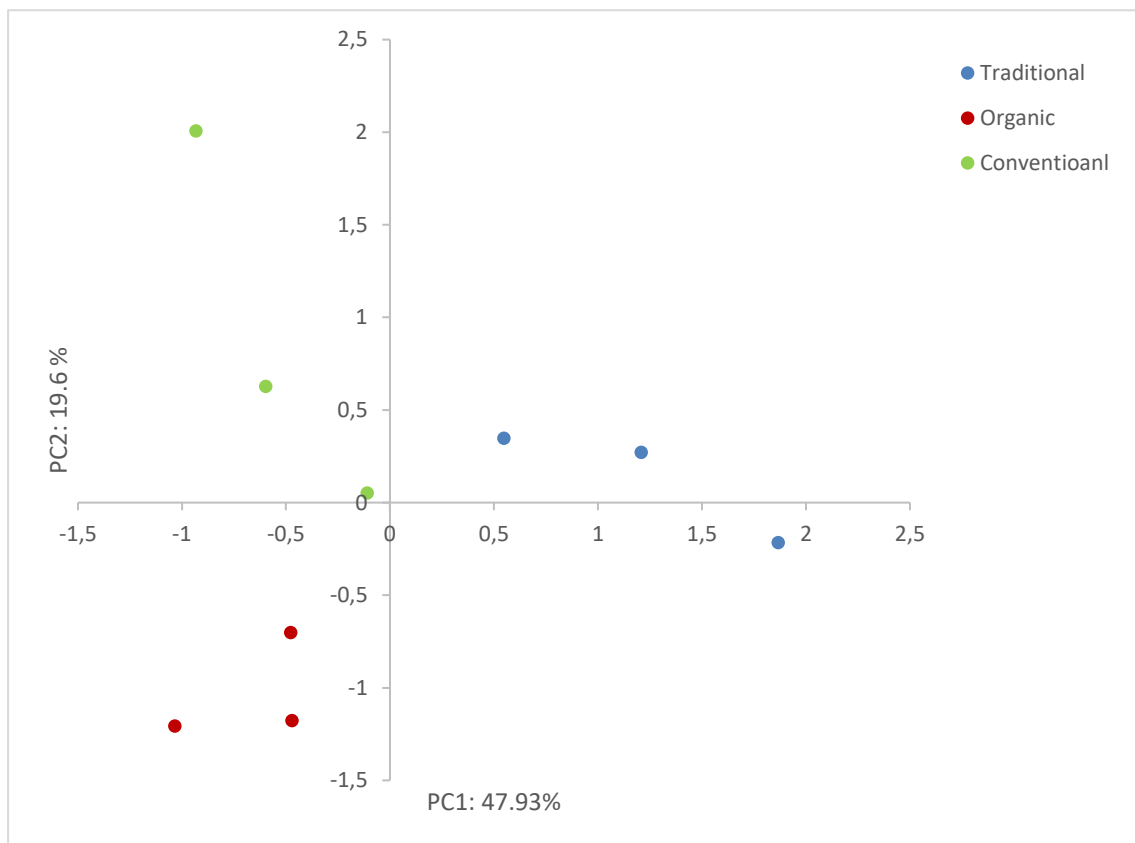


Fig. 16. PCA loadings plots of sampled AFSs

6 Discussion

6.1 Pollinator abundance and diversity

The low abundances of Ceratopogonidae found and identified in the present thesis confirmed previous studies that detected relatively low natural occurrence of these insects (Winder 1977; Bravo et al. 2011; Ramos Serrano 2011; Chumacero de Schawe et al. 2016; Salazar-Díaz & Torres-Coto 2017). This low occurrence of pollinators is even more pronounced in dry season, which was also our case. We collected the data in summer months when the dry season culminates and these climate conditions were affecting the insect occurrence. Despite the fact that Ceratopogonids were for years considered as the only natural successful pollinators of cocoa, it seems likely that other insect groups can act as pollinators as well. This statement confirmed Toledo-Hernández et al. (2017) with his recent pollinator literature review, where suggests that cocoa is unlikely to rely on a single group of insects for pollination. For this reason, we decided to include in our study other Dipteran families and even order (Hymenoptera). Although these groups of insects are for cocoa pollination marginal, they can contribute, above all in the season when the main pollinators are not so abundant.

Many of evaluated Hymenopteran families are well-known pollinators, which explain their presence as they are probably attracted by other tree species in the AFS. The most abundant family in our samples was Vespidae. Even though majority of members of this family are predators, many species visit flowers, where they feed on nectar and so serve as pollen vectors. Although they are not considered as possible cocoa pollinators, they contribute to pollination of other plant species, which are important in diversity of AFSs. Members of Apidae, the second most abundant group in our AFSs, are also important plant pollinators, namely e.g. *Apis mellifera* or *Bombus* spp. Raju (1975) and Soria (1975) even suggest some of the *Trigona* species as effective cocoa pollinators. Unfortunately, we could not have classified this genus to species level, but their presence was detected in all our samples suggesting their possible role in cocoa pollination. Also, one individual of *Lasioglossum* sp., the member of Halictidae

(wild bees), that were identified as effective cocoa pollinator (Raju 1975; Frimpong et al. 2015), was found in conventional AFS. According to Toledo-Hernández et al. (2017) bees and wasps represent up to 8.1 % of species visiting cocoa flowers.

The members of the rest of the Hymenopteran families were not very abundant in our samples, moreover they probably could not contribute to cocoa pollination, since most of them are parasitoids, meaning their primary source of food are not flowers. However, they occasionally feed on flower nectar and so could serve as successful pollinators of shade tree and other plant species in the AFSs. The same conclusion could be applied to Diptera species Asilidae, Clusiidae, Culicidae, Dolichopodidae, Neriidae, Platystomatidae, Richardiidae, Sarcophagidae, Ulidiidae). Their abundances were not very high and they are for pollination marginal or does not provide pollination services at all.

Among the Diptera species, that could serve as potential cocoa pollinators, the highest number of both individuals and variety of species was found for the family Phoridae, Drosophilidae and Cecidomyiidae. The species of Phoridae family were also captured by De Schawe et al. (2016) feeding on cocoa flower tissue, which implicates that they might carry cocoa pollen. Besides they were already suggested by Young (1984) playing a role in cocoa pollination. The lowest relative abundance of this order was found in organic AFS (only 2 individuals). Despite their numbers were statistically comparable in traditional and conventional AFSs, we can observe a certain trend of being markedly bigger in traditional AFS. This would support our hypothesis, that higher shade tree diversity and species richness (which was found the highest in traditional AFS) could improve cocoa pollinator diversity and abundance. The statistical analysis probably did not reveal the differences because of their overall low abundances. Nevertheless, this deficiency could be overcome in further studies by longer examination period or by various repetitions of insect capture. Cecidomyiidae, were not so abundant and their numbers were similarly low among our study sites but they were present in variety of species. They are known to be pollinating agents since they were observed to pick-up pollen grains in Ivory Coast (Lucas 1981). Adajloo

(2012) in Ghana confirmed, that they deposit pollen on the stigmas of the cocoa flowers and even discovered, that their visitation frequency of cocoa flowers was even higher than that of *Forcipomyia* midge, generally believed to be a main cocoa pollinator. *Drosophilidae* was another Dipteran family confirmed as possible pollinators of cocoa (Winder 1978). Their numbers of individuals in our samples were quite equal, however in traditional AFS, they were again the most abundant.

Other members of Dipteran families (*Mycetophilidae*, *Chloropidae*, *Stratiomyiidae*, *Sciaridae*, *Tachinidae*, *Lauxaniidae*, *Chironomidae* and *Psychodidae*) were captured but they have not been stated in scientific literature as possible pollinators. There are however various studies dealing with cocoa pollination, where they have been mentioned as flower visitors. For example, De Schawe et al. (2016) found individuals of *Chloropidae*, *Sciaridae*, *Tachinidae* and *Mycetophilidae* in Bolivia. These orders could be found also in our samples. Another similar record can be found in study of Young (1986) in Costa Rica where he monitored presence of *Sciaridae*, *Chironomidae*, *Psychodidae* and *Mycetophilidae* family. Those findings are also confirmed in literature review of Toledo-Hernández et al. (2017) where he presents members of *Chironomidae*, *Drosophilidae*, *Psychodidae*, *Sciaridae* and *Sphaeroceridae* as species visiting cocoa flowers. In view of that fact, they could be appropriate targets for further studies.

Although it is true that with the sampling method we used, we cannot assure that the sampled insects were responsible for the pollination service. The former plan was to use aspirator that would make us sure that the insects were at least inside the flower but due to low occurrence of flying insects this technique could not be used. Nevertheless, considering the placing of traps near flower cushions and character of evaluated insects consulted with scientific literature, we can suppose, that these selected individuals mentioned above could be potential cocoa pollinators.

Even though we can observe the highest numbers of individuals and number of insect species in traditional system as expected, the overall abundance and species

richness of potential cocoa pollinators was found to be comparable between the systems according to statistical analysis. Our hypothesis (H1) about positive effect of complex vegetation structure on abundance and diversity of cocoa pollinators was not confirmed. Our study however provides interesting suggestions and so represent a good scientific background for further research, on which base the methodology could be optimize.

Surprisingly on the second place in insect diversity and abundance was conventional system. The same findings were made in case of Jackknife estimate of species richness. The statistical analysis also revealed statistically significant differences in insect species diversity (Shannon index of species diversity), being the highest for traditional AFS, followed by conventional, and the lowest in organic AFS. These facts were confirmed by Sørensen similarity coefficient, which showed, that as for species composition, the most similar were traditional and conventional system. We assume that the overall low abundance of insects in organic AFS could be caused by the age of the farm. As traditional and conventional system had more or less the same age being both older than the organic system, their microclimate was likely more stable and suitable for insects. It is probable that, older trees had more developed canopies and thus there were not such fluctuations of humidity and temperature as in younger AFS. Young (1986b) also found more insects on abandoned, meaning heavily shaded cocoa farm reverted to secondary tropical forest, however contrary to our results, observed lower flowering, which ascribes to intense shade.

6.2 Relationship among variables

Shade cover was according to conducted linear regression the best descriptor of potential cocoa pollinator abundance, or in other words, it suggested that more potential cocoa pollinators can be found in shaded habitats. The highest abundance was indeed in the traditional AFS, which was the one with the highest shade cover. This founding supports the first part of our hypothesis (H2). Therefore, it could be used as the most suitable indicator of cocoa pollination. Furthermore, the maintenance of high vegetation coverage in order to increase pollinator (and other beneficial insects) abundance can

have important positive impacts on cocoa productivity as a result of higher litter input, maintenance of soil moistures and regulation of microclimate within AFSs.

The increased shade cover could be directly linked with the amount of litter on the soil surface. As pollinator diversity can be affected by the amount of breeding material (leaf litter), the indirect effect of shading through leaf litter accumulation on pollinator abundance and diversity could be expected. Adjaloo et al. (2013) in his study suggested, that leaf litter should be considered as the most appropriate substrate for midges, however he only focused on the abundance and did not evaluate the litter impact on pollinator diversity. Winder and Saliva (1972) on the other hand indicated, that rotten cacao pods provide the best substrate with regard to both species diversity and total numbers of insects collected. Surprisingly, the amount of organic matter (rotten fruits) did not have an influence on abundance nor diversity of potential cocoa pollinators in our study, meaning that the second part of our hypothesis (H2) was disproved. It was probably caused by random character of farm management in organic AFS and fertilization and irrigation in conventional AFS. However, it plausible, that different species of midges may have different substrate preferences, as Winder and Saliva (1972) suggested in their study: leaf litter was found to be an important breeding place specifically for the subgenus *Forcipomyia*. In evaluation of the best breeding substrate should be then considered local specifics.

We were able to explain relatively high percentage of data variation in PCA analysis. As we can see in Figure 15, all pollinator characteristics were the most influenced by shade cover and leaf litter (both positively), which confirms the results of linear regression. The traditional system was characterized by pollinator and potential cocoa pollinator variables, shade cover, leaf litter and shade trees species diversity and species richness, which support our hypothesis, that shade and complex vegetation structure could be an important factor influencing cocoa pollinator populations. Moreover, all these variables showed a mutual dependence. That is why regression revealed dependency between potential cocoa pollinator diversity and leaf litter, resulting from heterogenous vegetation of the traditional system. Organic system is on

the other side defined the most by organic matter and shade tree density and conventional by bare soil and grass cover.

6.3 Floral and fruit phenology

As already mentioned before, traditional AFS recorded the highest number of insects, while organic system was the one that registered the least. The same pattern we can observe regarding the numbers of open flowers and fruits recorded. The same findings were observed by Salazar-Díaz and Torres-Coto (2017) in Costa Rica. This fact was probably caused by the particularities of farm management.

Nevertheless, it is true, that Groeneveld et al. (2010) proved that the yield is more determined by the number of flowers pollinated than by plant resources. That is to say, low abundances of cocoa pollinators could have important implications for the productivity, despite our results, that did not reveal this connection (caused also by unsuitability of collection of FF phenology data, see below).

For precision of the observation, flower buds, open flowers and cherelles should be counted at 30-day intervals based on the 28 days that a flower bud takes to fully develop and open (Swanson et al. 2005) and the approximately 2 days survival span of open flowers (McKelvie 1962). Due to lack of time we could not conduct our study within this time frame, which could have impact on our results. Another problem with the short-term character of this study (and limited technical and personnel support) is, that it did not allow us to collect sufficient data for FF phenology for exploration of factors explicating the fruit set, that implies cocoa productivity. Our hypothesis (H3) therefore could not be confirmed. A larger sample size would be necessary.

We can observe that all counts of flower buds, open flowers and cherelles have a tendency to decrease from the first to the last measurement of counts, which is probably caused by climatic conditions. For complexity of the study, both dry and wet season should be examined. Since our study was conducted during the dry season only it does not take into account the weather and climate changes.

Although we used the same sampling methods in all plots, we tried to control the hours of the day in which the samples were taken, the same clones of trees were selected and others conditions with possible influence on our results were tried to kept the same in all plots, there were other variables that could not be controlled, such as the different microclimates and available nutrient resources in each of the plots evaluated. These factors which could not be measured in this kind of study could have an influence on our results.

7 Conclusion

Considering the scarcity of midges in our study region, we suggest that Ceratopogonidae alone were probably too rare to be main cocoa pollinators, at least during the dry season. Potential additional pollinators would be mainly small individuals of Diptera (Cecidomyiidae, Drosophilidae and Phoridae).

The abundance of pollinators in general was the highest in traditional agroforestry system, meaning the one with the highest number of species of shade trees and with the highest canopy shade cover. Regarding evaluation of potential cocoa pollinators, their abundance and species richness were comparable in all the systems. The conservation of trees, which provide adequate shade cover could be the key factor for cocoa pollinator enhancement and better cocoa yields. Clearly, the removal of shade trees, as often occurs in the intensive cocoa production systems, could be detrimental to cocoa pollinators. Leaf litter was found to have a positive influence on the diversity of potential cocoa pollinators. It could be considered the best breeding substrate for increasing diversity of cocoa pollinators in this particular zone. Therefore, it should be left on the ground and if need added around the trees. We did not find a link between fruit set and abundance of cocoa pollinators.

Our study contains a summary of recent information about cocoa pollinators in the Peruvian Amazon, which has not yet been done in this area. The collected insects have enriched entomological collection and contributed to deepen the knowledge in this field of study. Our results could be used as recommendations for correct management in cocoa cultivation favoring the pollination services.

Since cocoa is worldwide highly demanded commodity of a big importance for both market and growers, the further research should be focused on optimizing and increasing yields from sustainable agriculture. Additional long-term survey is needed to study the relations between cocoa pollinators and fruit set. It would be interesting to focus the further research on non-ceratopogonid species as pollinator agents.

8 References

- Adjaloo MK. 2012. Pollination ecology of Upper Amazon cocoa and breeding substrates of cocoa pollinators in the Ejisujuabeng district of the Ashanti region. Ghana. [Ph.D. Thesis]. Kwame Nkrumah University of Science and Technology. Ghana.
- Adjaloo MK, Banful B, Oduro W. 2013. Evaluation of breeding substrates for cocoa pollinator. *Forcipomyia* spp. and subsequent implications for yield in a tropical cocoa production system. *American Journal of Plant Sciences* **4**: 204–211.
- Adjaloo MK, Oduro W. 2013. Insect assemblage and the pollination system in cocoa ecosystems. *Journal of Applied Biosciences* **62**: 4582-4594.
- Amores FM, Vasco SA, Eskes AB, Suarez C, Quiroz JG, Loor RG, Teran MM. 2011. On-farm and on-station selection of new cocoa varieties in Ecuador. Final report of the CFC/ICCO/Bioversity international project on “cocoa productivity and quality improvement: a participatory approach **59**: 59-72.
- Bos MM, Veddeler D, Bogdanski AK, Klein AM, Tschardt T, Steffan-Dewenter I, Tylianakis JM. 2007. Caveats to Quantifying Ecosystem Services: Fruit Abortion Blurs Benefits from Crop Pollination. *Ecological Applications* **17**: 1841-1849.
- Canfield RH. 1941. Application of the line interception method in sampling range vegetation. *Journal of forestry* **39**: 388-394.
- Claus G, Vanhove W, Van Damme P, Smagghe G. 2018 Challenges in Cocoa Pollination: The Case of Côte d’Ivoire. Pages 39-58 in Mokwala PW. editor. *Pollination in Plants*. InTechOpen. London.
- Córdoba C, Cerda Bustillos R, Deheuvels O, Hidalgo E, DeClerck F. 2013. Polinizadores. polinización y producción potencial de cocoa en sistemas agroforestales de Bocas del Toro. Panamá. *Agroforestería en las Américas* **49**: 26-32.
- Cruz-Neto O, Machado IC, Duarte JA, Lopes AV. (2011). Synchronous phenology of hawkmoths (Sphingidae) and Inga species (Fabaceae–Mimosoideae): implications for the restoration of the Atlantic forest of northeastern Brazil. *Biodiversity and conservation* **20**: 751-765.
- Cuatrecasas. J. 1964. Cocoa and its allies. A taxonomic revision of the genus *Theobroma*. *Smithsonian Institution* **36**: 379–614.
- David S. 2005. Learning about sustainable cocoa production: a guide for participatory farmer training 1. Integrated crop and pest management. Sustainable Tree Crops Program. International Institute of Tropical Agriculture. Yaoundé.
- De Schawe CC, Kessler M, Hensen I, Tschardt T. 2018. Abundance and diversity of flower visitors on wild and cultivated cocoa (*Theobroma cocoa* L.) in Bolivia. *Agroforestry Systems* **92**: 117-125.

- Durán Ramírez F. 2010. Cultivo y explotación del cocoa. Grupo Latino Editores. Colombia.
- Efombagn MIB, Sounigo O, Eskes AB, Motamayor JC, Manzanares-Dauleux MJ, Schnell R, Nyassé S. 2009. Parentage analysis and outcrossing patterns in cocoa (*Theobroma cocoa* L.) farms in Cameroon. *Heredity* **103**: 46-53.
- Falque M., Vincent A, Vaissiere BE, Eskes AB. 1995. Effect of pollination intensity on fruit and seed set in cocoa (*Theobroma cocoa* L.). *Sexual Plant Reproduction* **8**: 354-360.
- FAO. 2018. FAOSTAT: online database. FAO. Rome. Available from <http://www.fao.org/faostat/en/#data> (accessed March 2019).
- Forbes S, Northfield TD. 2017. Increased pollinator habitat enhances cocoa fruit set and predator conservation. *Ecological Applications* **27**: 887-899.
- Franzen M, Mulder MB. 2007. Ecological, economic and social perspectives on cocoa production worldwide. *Biodiversity and Conservation* **16**: 3835-3849.
- Frimpong EA, Gordon I, Kwapong PK, Gemmill-Herren B. 2009. Dynamics of cocoa pollination: tools and applications for surveying and monitoring cocoa pollinators. *International Journal of Tropical Insect Science* **29**: 62-69.
- Frimpong EA, Gemmill-Herren B, Gordon I, Kwapong PK. 2011. Dynamics of insect pollinators as influenced by cocoa production systems in Ghana. *Journal of Pollination Ecology* **5**: 74-80.
- Frimpong EA, Adjaloo MK, Kwapong PK, Oduro W. 2014. Structure and stability of cocoa flowers and their response to pollination. *Journal of Botany* **2014**: 6.
- Frimpong EA, Bosu PP, Adjaloo MK, Braimah H, Kwapong PK, Oduro W. 2015. Some fact about cocoa pollination. University of Cape Coast Printing Press. Ghana.
- Gamito S. 2010. Caution is needed when applying Margalef diversity index. *Ecological Indicators* **10**: 550-551.
- Garcia CL. 2000. Cultivo del cocoa en la amazonía peruana. Ministerio de Agricultura. Lima.
- Glendinning DR. 1972. Natural pollination of cocoa. *New Phytologist* **71**: 719-729.
- Groeneveld JH, Tschardt T, Moser G, Clough Y. 2010. Experimental evidence for stronger cocoa yield limitation by pollination than by plant resources. *Perspectives in Plant Ecology Evolution and Systematics* **12**: 183-191.
- Kabir M, Webb E 2009. Household and homegarden characteristics in southwestern Bangladesh. *Agroforestry systems* **75**: 129-145.
- Kalousová M. 2013. Morphological and genetic diversity of cocoa (*Theobroma cocoa* L.) in San Alejandro. Peruvian Amazon [MSc. Thesis]. Czech University of Life Sciences Prague. Prague.

- Kaufmann T. 1975. Studies on the ecology and biology of a cocoa pollinator. *Forcipomyia squamipennis* I. & M. (Diptera. Ceratopogonidae). in Ghana. *Bulletin of Entomological Research* **65**: 263-268.
- Krebs CJ. 1994. *Ecological methodology*. University of British Columbia.
- Klein AM, Cunningham SA, Bos M, Steffan-Dewenter I. 2008. Advances in pollination ecology from tropical plantation crops *Ecology* **89**: 935-943.
- Lemmon PE. 1956. A spherical densiometer for estimating forest overstory density. *Forest science* **2**: 314-320.
- Lojka B. 2012. *Agroforestry for the Peruvian Amazon*. LAMBERT academic publishing. Prague.
- Lucas P. 1981. Etude des conditions de pollinisation du cocoyer au Togo. *Café. Cocoa Thé* **25**:113–120.
- Madell S. 2005. *Theobroma cocoa: from bud to bean*. World Agroforestry. Tava. Available from http://www.worldagroforestry.org/treesandmarkets/inforesta/documents/agrof_cons_biodiv/cocoa%20frm%20bud%20to%20bean.htm (accessed May 2017).
- Magurran AE. 1988. *Ecological diversity and its measurement*. Princeton University Press. Princeton.
- McKelvie AD. 1956. Cherelle wilt of cocoa I. Pod development and its relation to wilt. *Journal of Experimental Botany* **7**: 252-263.
- Ministry of Foreign Trade and Tourism. 2018. *Cocoa in Peru a Rising Star*. Ministry of Foreign Trade and Tourism. Peru. Available from <http://www.peru.org.tw/web/data/file/userfiles/files/Cocoa%20Peru%20Promperu.pdf> (accessed November 2018).
- Motamayor JC, Risterucci AM, Lopez PA, Ortiz CF, Moreno A, Lanaud C. 2002. Cocoa domestication I: the origin of the cocoa cultivated by the Mayas. *Heredit* **89**: 380.
- Nair PR. 1993. *An introduction to agroforestry*. Kluwer Academic Publishers. Dordrecht.
- Popic TJ, Davila YC, Wardle GM. 2013. Evaluation of common methods for sampling invertebrate pollinator assemblages: net sampling out-perform pan traps. *PLoS one* **8**: e66665.
- Rice R, Greenberg R. 2000. Cocoa cultivation and the conservation of biological diversity. *Ambio* **29**: 167-173.
- Raju KS. 1975. An efficient. new cocoa pollinator. *Lasioglossum* sp. (Hymenoptera: Halictidae) in Ghana. West Africa. *Turrialba (IICA)* **25**: 90-91.
- Ramos Serrano RM. 2011. Estudio de la diversidad de insectos polinizadores en sistemas agroforestales de cocoa y su relacion con la productivad y diversidad de

- especies del Dosel [MSc. Thesis]. Universidad de San Pedro Sula. San Pedro Sula.
- Saunders LG. 1959. Methods for studying *Forcipomyia* midges. with special reference to cocoa-pollinating species (Diptera. Ceratopogonidae). *Canadian Journal of Zoology* **37**: 33-51.
- Salazar-Díaz R, Torres-Coto V. 2017. Estudio de la dinámica de polinizadores del cultivo de cacao (*Theobroma cacao*) en tres sistemas de producción. *Revista Tecnología en Marcha* **30**: 90-100.
- Simpson EH. 1949. Measurement of diversity. *Nature* **163**: 688p.
- Soria SDJ. 1975. O papel das abelhas sem ferrão (*Meliponinae*) na polinização do cacauzeiro na América tropical. *Revista Theobroma (Brasil)* **5**: 12-20.
- Sporn SG, Bos MM, Gradstein SR. 2007. Is productivity of cocoa impeded by epiphytes? An experimental approach. *Agriculture, ecosystems & environment* **122**: 490-493.
- Stephenson AG. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annual review of ecology and systematics* **12**: 253-279.
- Toledo-Hernández M, Wanger TC, Tschardt T. 2017. Neglected pollinators: Can enhanced pollination services improve cocoa yields? A review. *Agriculture, ecosystems & environment* **247**: 137-148.
- Vebrová H. 2012. Tree diversity in cocoa agroforestry in San Alejandro. Peruvian Amazon [MSc. Thesis]. Czech University of Life Sciences Prague. Prague.
- Young AM. 1982. Effects of shade cover and availability of midge breeding sites on pollinating midge populations and fruit set in two cocoa farms. *Journal of Applied Ecology* **1**: 47-63.
- Young AM 1983. Seasonal differences in abundance and distribution of cocoa pollinating midges in relation to flowering. fruit-set and pollinator availability in Costa Rica. *Journal of Tropical Ecology* **2**: 163-186.
- Young AM, Schaller M, Strand M. 1984. Floral nectaries and trichomes in relation to pollination in some species of *Theobroma* and *Herrania* (Sterculiaceae). *American Journal of Botany* **71**: 466-480.
- Young AM. 1986. Distribution and abundance of Diptera in flypaper traps at *Theobroma cacao* L. (Sterculiaceae) flowers in Costa Rican cacao plantations. *Journal of the Kansas Entomological Society* 580-587.
- Young AM. 1986b. Habitat differences in cocoa tree flowering. fruit-set. and pollinator availability in Costa Rica. *Journal of Tropical Ecology* **2**: 163-186.
- Zakariyya F, Sulistyowati E, Rahayu DS. 2016. Abundance of Pollinator Insect (*Forcipomyia* spp.) of Cocoa Under Some Shade Trees. *Pelita Perkebunan (a Coffee and Cocoa Research Journal)* **32**: 91-100.

- Winder JA, Silva P. 1972. Cacao pollination: Microdiptera of cacao plantations and some of their breeding places. *Bulletin of Entomological Research* **6**: 651-655.
- Winder JA. 1978. Cocoa flower Diptera; their identity, pollinating activity and breeding sites. *Pans* **24**: 5-18.
- Winder JA. 1977. Recent research on insect pollination of cocoa. *Cocoa Growers Bulletin* **25**: 11-19.
- World cocoa foundation. 2014. Cocoa Market Update. World cocoa foundation. Available from <http://www.worldcocoafoundation.org/wp-content/uploads/Cocoa-Market-Update-as-of-4-1-2014.pdf> (accessed May 2017).
- World agroforestry centre. 2017. Growing Cocoa Beans. World agroforestry centre. Available from http://www.worldagroforestry.org/treesandmarkets/inaforesta/documents/agrof_co ns_biodiv/Ch.3-Growing-Cocoa-Beans.pdf (accessed May 2017).

Appendix A

Table A Insect morphospecies of selected pollinators (Hymenoptera and Diptera order) found in traditional, organic and conventional AFSs; in grey potential cocoa pollinators; their abundance

Order	Family	Morphospecie	Traditional	Organic	Conventional
Hymenoptera					
	Ampulicidae				
		Ampulex sp.1	0	0	1
	Apidae				
		Apis melifera	1	1	2
		Apoica sp.20	0	1	0
		Bombus sp.33	0	0	1
		Ceratina sp.35	0	2	1
		Eucerini sp.29	0	0	7
		Euglosa sp.34	0	1	0
		Leiopodus lacertinus	0	1	0
		Melitoma sp.27	0	1	0
		Monoeca sp.26	4	0	0
		Paratetrapedia sp.25	1	3	0
		Trigona sp.30	1	4	0
		Trigona sp.31	3	1	5
		Xylocopa sp.36	0	0	3
	Bethylidae				
		Bethylidae sp.1	1	0	0
		Bethylidae sp.2	5	0	0
		Bethylidae sp.3	1	3	0
		Bethylidae sp.4	1	0	0
		Bethylidae sp.5	0	1	0
		Bethylidae sp.6	1	0	0
		Bethylidae sp.7	1	0	0
	Braconidae				
		Braconidae sp.1	0	1	0
		Braconidae sp.2	1	0	0
	Drynida				
		Dryinidae sp.53	0	1	0
	Evaniidae				

	Evaniidae sp.1	1	0	0
Halictidae				
	Augochlora sp.39	0	2	0
	Augochlora sp.40	0	1	1
	Augochlora sp.42	1	0	0
	Augochlora sp.43	0	1	0
	Augochlora sp.44	0	3	0
	Augochloropsis sp.38	0	2	0
	Corynura sp.45	1	1	0
	Lasioglossum sp.47	0	0	1
	Pereirapis semiaurata	0	0	4
Ichneumonidae				
	Ichneumonidae sp.1	0	1	0
	Ichneumonidae sp.2	1	0	1
	Ichneumonidae sp.3	1	0	0
Megachilidae				
	Hypanthidioides sp.37	0	1	0
Mutilidae				
	Mutilidae sp.51	1	0	0
	Mutilidae sp.52	0	0	1
Pompilidae				
	Pompilidae sp.49	1	0	0
	Pompilidae sp.50	1	0	0
Proctotrupidae				
	Proctotrupidae sp.1	0	0	1
Pteromalidae				
	Pteromalidae sp.54	1	0	0
	Pteromalidae sp.55	3	0	0
	Pteromalidae sp.56	0	0	1
	Pteromalidae sp.57	0	1	0
	Pteromalidae sp.58	0	1	0
	Pteromalidae sp.59	0	0	1
	Pteromalidae sp.60	1	0	0
	Pteromalidae sp.61	4	0	0
Scelionidae				
	Scelionidae sp.1	3	6	2
	Scelionidae sp.2	2	0	0
	Scelionidae sp.3	1	0	0
	Scelionidae sp.4	1	0	0

b

Vespidae				
	Chartergus sp.23	1	0	0
	Chartergus sp.24	2	0	0
	Metapolybia tatua	0	1	0
	Parachartergus apicalis	0	1	0
	Polybia dimidiata	2	2	0
	Polybia liliacea	1	0	4
	Polybia rejecta	9	6	9
	Polybia rufitarsis	0	3	0
	Polybia sp.11	2	0	0
	Polybia sp.12	0	3	0
	Polybia sp.13	1	0	0
	Polybia sp.14	0	2	0
	Polybia sp.15	0	0	1
	Polybia sp.17	0	0	2
	Polybia sp.18	0	1	0
	Synoeca surinama	0	0	1
Diptera				
Asilidae				
	Asilidae sp.1	1	0	0
Cecidomyiidae				
	Cecidomyiidae sp.1	1	0	1
	Cecidomyiidae sp.10	0	0	1
	Cecidomyiidae sp.11	0	0	1
	Cecidomyiidae sp.12	1	0	0
	Cecidomyiidae sp.13	0	0	1
	Cecidomyiidae sp.14	0	0	1
	Cecidomyiidae sp.15	0	0	1
	Cecidomyiidae sp.2	0	1	0
	Cecidomyiidae sp.3	1	0	0
	Cecidomyiidae sp.4	1	0	0
	Cecidomyiidae sp.5	0	0	1
	Cecidomyiidae sp.6	0	1	0
	Cecidomyiidae sp.7	2	1	0
	Cecidomyiidae sp.8	0	0	1
	Cecidomyiidae sp.9	0	0	1
Chironomidae				
	Chironomidae sp.1	0	0	1
Chloropidae				

	Chloropidae sp.1	1	0	0
	Chloropidae sp.2	1	0	0
	Chloropidae sp.3	0	1	0
	Chloropidae sp.4	0	0	1
	Chloropidae sp.5	1	0	0
	Chloropidae sp.6	1	0	0
	Chloropidae sp.7	0	0	1
	Chloropidae sp.8	0	0	1
Clusiidae				
	Clusiidae sp.1	0	1	0
	Clusiidae sp.2	0	1	0
Culicidae				
	Culicidae sp.1	0	0	1
Dolichopodidae				
	Dolichopodidae sp.1	1	0	0
	Dolichopodidae sp.2	0	1	0
	Dolichopodidae sp.3	0	0	1
Drosophilidae				
	Drosophilidae sp.1	1	0	0
	Drosophilidae sp.10	7	1	1
	Drosophilidae sp.2	1	0	1
	Drosophilidae sp.3	0	1	0
	Drosophilidae sp.4	0	1	0
	Drosophilidae sp.5	0	0	1
	Drosophilidae sp.6	1	0	0
	Drosophilidae sp.7	0	1	0
	Drosophilidae sp.8	2	1	0
	Drosophilidae sp.9	0	2	0
Lauxanidae				
	Lauxaniidae sp.1	1	0	0
	Lauxaniidae sp.2	1	0	0
Mycetophilidae				
	Mycetophilidae sp.1	1	0	0
	Mycetophilidae sp.2	10	0	0
	Mycetophilidae sp.3	2	0	0
	Mycetophilidae sp.4	1	0	0
	Mycetophilidae sp.5	5	0	0
Neriidae				
	Neriidae sp.1	1	0	0
Phoridae				

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Phoridae sp.1	3	0	0
Phoridae sp.2	1	0	0
Phoridae sp.3	6	0	0
Phoridae sp.4	1	0	0
Phoridae sp.5	1	0	0
Phoridae sp.6	0	0	1
Phoridae sp.7	1	0	0
Phoridae sp.8	1	0	0
Phoridae sp.9	4	1	1
Phoridae sp.10	1	0	0
Phoridae sp.11	0	0	1
Phoridae sp.12	0	0	1
Phoridae sp.13	1	0	0
Phoridae sp.14	1	0	0
Phoridae sp.15	1	0	0
Phoridae sp.16	1	0	0
Phoridae sp.17	1	0	0
Phoridae sp.18	0	0	1
Phoridae sp.19	1	0	0
Phoridae sp.20	1	0	0
Phoridae sp.21	1	0	0
Phoridae sp.22	1	0	0
Phoridae sp.23	1	0	0
Phoridae sp.24	2	0	0
Phoridae sp.25	0	1	0
Phoridae sp.26	1	0	0
Phoridae sp.27	1	0	0
Phoridae sp.28	2	0	0
Phoridae sp.29	2	0	0
Platystomatidae			
Platystomatidae sp.1	1	0	0
Psychodidae			
Psychodidae sp.1	1	0	0
Richardidae			
Richardidae sp.1	3	0	0
Sarcophagidae			
Sarcophagidae sp.1	0	1	1
Sarcophagidae sp.2	0	0	1
Sarcophagidae sp.3	1	0	1
Sciaridae			

	Sciaridae sp.1	3	1	1
	Sciaridae sp.2	0	0	1
Stratiomyiidae	Stratiomyiidae sp.1	0	1	0
	Stratiomyiidae sp.2	0	1	0
	Stratiomyiidae sp.3	2	0	0
	Stratiomyiidae sp.4	1	0	0
	Stratiomyiidae sp.5	1	0	0
	Stratiomyiidae sp.6	1	0	0
Tachinidae	Tachinidae sp.1	1	0	0
	Tachinidae sp.2	0	1	0
	Tachinidae sp.3	0	0	1
Ulidiidae	Ulidiidae sp.1	1	0	0
	Ulidiidae sp.2	1	0	0
Total individuals		163	81	79
Total morphospecies		95	52	50
Total individuals of potential cocoa pollinators		90	17	24
Total morphospecies of potential cocoa pollinators		52	16	24