Title: How habitat heterogeneity affects pollinator's communities in cocoa-based agroforestry systems?

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Abstract: In the humid tropics, a significant amount of the agricultural landscape where cocoa (*Theobroma cacao*) is grown is managed as agroforestry systems. As pressure to intensify cocoa production is increasing, the current worldwide trend for the intensification of cocoa production aims at significant reductions of the shade canopy. However, this trend implies losing the potential to produce valuable ecosystem services. Among those services, pollination is a limiting factor of cocoa production that has been investigated in the 70's and 80's essentially by trapping methods. The genus *Forcypomia* sp. is acknowledged to be the main insect responsible for cocoa pollination, when other insects such as ants and Trips sp. would play a secondary role. Regulation and habitat provision for cocoa pollinating insect communities are poorly documented in the literature. Most pollinating species are known from trapping methods in the immediate surroundings of the tiny cocoa flowers and no study has succeeded so far in observing and describing the insects actually visiting the inside of the cocoa flowers. In this investigation, we characterized habitats for pollinating insects at plot scale, in a 1.5 ha cocoa-based agroforestry system located in the Peruvian Amazon. Local climate, topography, leaf litter's biomass, composition and water content, cocoa trees and associated plant diversity and structure, were described, as well as farmer's practices. Based on habitats characterization, we compared the pollinating insects' communities of two contrasted habitats within the same plot. We used a digital video recording system that allowed us to monitor and record all insects visiting cocoa from 6:30 am to 05:30 pm during the main flowering season. Each habitat was sampled in 2 or 3 different locations when possible and 20 to 30 flowers were monitored in each repetition, resulting in a total amount of 180 monitored flowers. Our results showed that the diversity and the frequency of insects visiting cocoa flowers are influenced by habitat quality. The heterogeneity of habitat often found in cocoa-based agroforestry system is mostly due to farmer's practices relying on opportunistic shade management. Pollinating insects' communities do not always rely specifically on the Forcypomia genus but rather depend on habitat quality. These results open good perspectives for the ecological intensification of cocoa production in Agroforestry Systems.

Introduction

During the past two centuries, the impact of human activities on landscapes and biodiversity at global scale has considerably increased (DeFries *et al*., 2004). More food production is needed to feed the growing human population, and terrestrial natural habitats are being massively converted into agro-ecosystems (August *et al*., 2002; Forman, 1995; Sala *et al*., 2000; Laurance, 1999). In tropical areas, perennial crops such as oil palm, tea, rubber, coffee and cocoa represent a significant amount of the cultivated land (Neufeldt *et al*., 2012), especially at the forest margin of the Amazon (Laurance *et al*., 2001). Some of these crops may have strong negative impacts on ecosystem services, affecting local biodiversity, soils and waters. Others, such as cocoa-based agroforestry systems in the humid tropics, have more limited impacts and provide interesting synergies between socio-economic and environmental challenges. (Schroth *et al*., 2004 ; Vandermeer *et al*., 1998). These cropping systems, where the main crop is associated with a number of other cultivated plants on the same plot, often offer higher plant biodiversity levels and improved ecosystem services than in mono-cropping systems (Deheuvels *et al*., 2014, 2012; Malézieux *et al*., 2009; Chen *et al*., 1999). In particular, the presence of different strata, among the cultivated plant species, creates micro-climate conditions that can be favorable for wild plant and animal species (Martin-Chave *et al*., 2016; Burgess, 1999). Because they provide a wide variety of habitats and food, agroforestry systems are known to be of importance for the populations of pollinating animals (Varah *et al*., 2013; Jha and Vandermeer, 2010; Klein *et al*., 2007). Most of the area (95%) where cocoa is grown is cultivated by small farmers (Rice and Greenberg, 2000) on farms smaller than 10 hectares (Nolte, 2014; MINAGRI, 2003) with low investment capacity, family workforce and low risk strategies often including agroforestry. There, the design of the cocoa plantation is rarely conventional and both the cocoa trees and the associated plants often present a heterogeneous distribution on the cocoa plot (Gidoin *et al*., 2014; Matey *et al*., 2013; Ngo Bieng *et al*., 2013; Deheuvels *et al*., 2012). Since before the early 70's, the cocoa tree was known to be a species pollinated by insects (Glendinning, 1972), with only 5% of its flowers receiving enough pollen to get fecundated. A number of insects families are hold responsible for the pollination of the cocoa tree, such as Cecidomyiidae (Garibaldi et al., 2011), Ceratopogonidae (Young, 1982; Winder, 1978), but also ants, mealybugs, Thrips and Cicadella (Orwa et al., 2009). Following Frimpong et al. (2011) and Young (1982), we make the hypothesis that the heterogeneity of plant distribution in cocoa-based agroforestry systems reflects farmer's practices and has an influence on the composition and abundance of cocoa-pollinating insects at the micro-habitat level. In the Peruvian Amazon, we studied micro-habitats at plot level in one single cocoa-based agroforestry system, including topography, plant composition and structure, and local climate. We then monitored and compared pollinating insect populations in two major micro-habitats during the dry season. In this communication we present very preliminary results of our study to be published in 2018.

Materials and Methods

Study site: the study was conducted between April and July 2017, during one of the two annual flowering periods of the cocoa trees, on a 5 years old cocoa plantation. This plantation was cultivated as an agroforestry system by a local farmer and located in the Peruvian Amazon, Ucayali region, Irazola District. There, the cocoa trees were 95% CCN-51 cocoa clone and 5% Peruvian undescribed native varieties, in association with fruit and timber trees, as well as other service trees. There, the humid warm tropical climate is appropriate for cocoa cultivation, with an 80% average annual relative humidity, a 2500 mm average annual rainfall concentrated from November to March, and a 26°C average annual temperature. The surface of the cocoa plot was 1.5 ha and was measured with a GPS. The last treatments on this plot were applied in 2015: 1 herbicide application and 1 insecticide application.

Sampling units and habitat biological and topographic descriptors: a grid has been built that covered the whole plot to serve as a reference for coordinates of each plant and sampling sites. Starting from the origin point (South: 8°50.958'; West: 75°06.898'), the grid was based on 10 m x 10 m geo-referenced square sampling units which elevation was registered at the center. On each 100 m² sampling unit, we identified each living plant (cocoa and non-cocoa) and reported their coordinates on the grid. Total canopy cover was assessed with a densitometer at 1m above the ground at the center of each sampling unit. Total height, canopy shape and height, as well as dbh at 1.3 m above the ground were reported for each living plant. In the case of cocoa trees and plants lower than 1.3 m, dbh was measured at 0.3 m above the ground. Each 100 m² sampling unit was divided into four 5 m x 5 m sub-sampling units at the center of which leaf litter was sampled and weighted fresh and after drying 1 hour at 105°C.

Local climate monitoring: daily rainfall, temperatures (min/max), and relative humidity (min/max) were registered at the plot level. Maximum wind speed was registered every 30 minutes from 6:30 am to 5:30 pm during the pollinating insect monitoring periods.

Monitoring of the pollinating insects: three sampling units were selected per micro-habitat. They were chosen for presenting the highest distance from the borders of the habitat. On each sampling unit, 10 cocoa flowers were monitored each one from 6:30 am to 5:30 pm, using a video camera system described by Steen *et al*. (2011). Each flower was selected for monitoring 24h before opening and isolated. Each insect visiting a given flower was registered and identified at Family level. Insects were then classified according to their activity in relation with the reproductive parts of the flower, i.e. touching or not-touching male or female reproductive parts.

Statistical analysis: a multivariate analysis followed by a cluster analysis, using the R- Vegan package (Oksanen et al., 2017). allowed identifying micro-habitats. Kuskall-Wallis tests were performed for quantitative variables and Chi² tests for qualitative ones, in order to identify the habitat discriminating variables. Non parametrical tests (Kruskall-Wallis) were conducted to compare the number of potentially effective pollination activity between the two habitats. The effect of the wind on the composition of pollinating insects has been tested by Spearman non-parametrical correlation test.

Preliminary results

The Figure 1 presents the results of the cluster analysis and the repartition of micro-habitats 1 and 2 within the cocoa plot. A transition area between the two habitats is clearly visible.

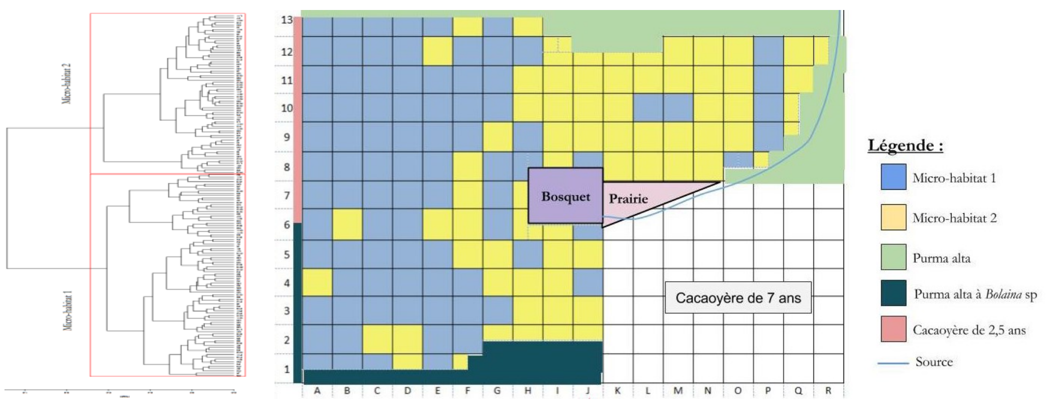


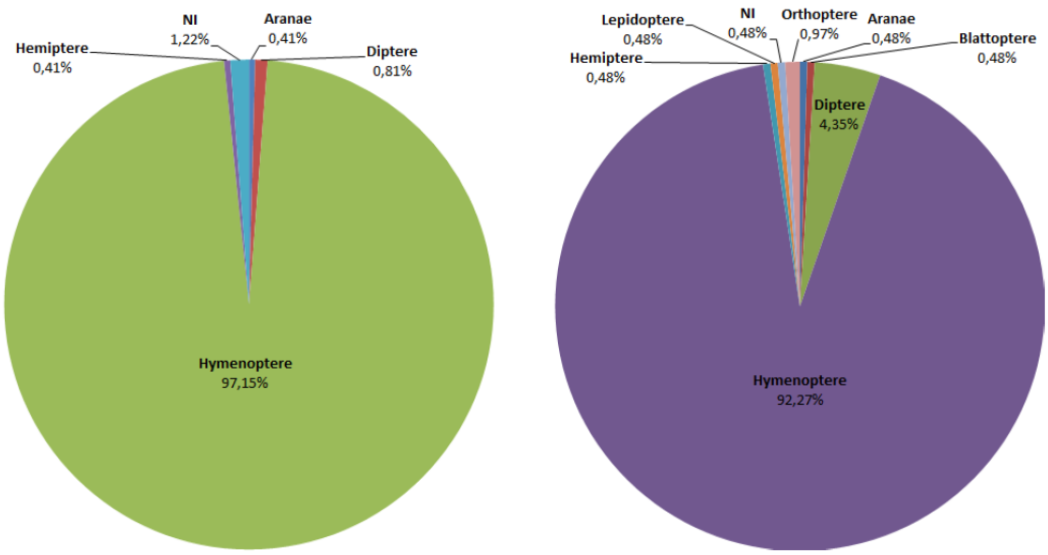
Figure 1. *Cluster analysis and its translation on the geo-referenced grid and on each 100 m² sampling unit of the cocoa-based agroforestry plantation.*

Among the 55 variables tested, these two micro-habitat significantly differed according to 12 variables described in **Table 1**.

Habitat descriptor	Variable	Statistic value	df	P
Topography inside the plot	Slope inclination and orientation (*)	106,6	2	< 0,001
	Relative elevation coefficient (*)	84,7	8	< 0,001
	Micro topography (*)	56,9	1	< 0,001
Soil cover including dead biomass and vegetation under the cocoa trees	% of opened pods on the ground	7,6	1	0,006
	% of leaf litter on the ground	12,0	1	0,001
	% of medium sized herbaceous plants	16,0	1	< 0,001
Associated plants	% of service plants	8,2	1	0,004
Shade plant structure	% of shade trees with spherical shaped canopy	8,7	1	0,003
	% of shade trees with inverted pyramid shaped canopy	9,5	1	0,002
	% of short plants [0 - 0,65 m] in total height	9,1	1	0,003
	% of plants with short trunk [0 - 0,50 m]	13,2	1	< 0,001
	% of plants with high dbh [0.67 – 4.71 m]	8,6	1	0,003

Table 1. *Variable showing significant differences between Habitat 1 and Habitat 2, based on the X^2-test for qualitative variables (*) and the Kruskal-Wallis chi-squared test for other variables.*

Figure 2 presents the insect orders observed in the two micro-habitats and show that in both cases hymenoptera (mostly ants) represent more than 92% of the visiting insects.



Micro habitat 1 **Micro habitat 2**

Figure 2. *Composition of insects visiting cocoa flowers in micro habitats 1 and 2 at the Order level. NI = unidentified insects.*

The only significant correlation between wind speed and the visiting frequency of pollinating insects has been observed for the Hymenoptera order (**Table 2**).

Order	Rho	S Statistic	P
Aranae	< 0.001	1519000	0.9812
Blattoptera	-0.11	1687600	0.1156
Diptera	-0.09	1657600	0.1978
Hymenoptera	0.21	1198500	**0.00203****
Heteroptera	0.002	1524600	0.9765
Lepidoptera	-0.11	1687600	0.1156
Orthoptera	-0.04	1575700	0.6087

Table 2. *Spearman correlation test between wind and cocoa flower visiting frequency for each taxa registered by video camera.*

Discussion

Cocoa based agroforestry systems are often cultivated without any identifiable design for shade plants distribution. In addition, the annual mortality of the cocoa trees and the associated plants results in a high heterogeneity of plant population structure in these systems. Finally, all dead plants are not always replaced by the same species if replaced at all. This process is well described in Jagoret (2011). It results in a complex spatial heterogeneity of plant distribution at plot level, which offers a variety of micro habitats for small animals, including pests and pollinators (Ngo Bieng et al., 2013; Gidoin et al., 2014).
This plant distribution heterogeneity combines with the topographic condition of the plot. Small intermittent rivers often cross cocoa plantations and it is not rare to find steep slopes of different inclinations and orientations, some parts of the plantation receiving more sunlight than others.
We evidenced this spatial heterogeneity due to both topographic and plant distribution on one single cocoa plot in the Peruvian Amazon.
We also evidenced that the famous Forcypomia cocoa midge (Diptera, Ceratopogonidae) is not always the main pollinating agent for cocoa. A huge variety of insects have been described in the literature as cocoa pollinating agents in Africa, Latin America and Asia, including Ceratopogonidae, but also Cecidomyiidae, Thysanoptera, Aphidae, Psyllidae, Formidae, Hemiptera and Apidae (De Reffye *et al*., 1980; Lucas, 1981; Boussard, 1981; Paulin *et al*., 1983; Young, 1985; Mavisoy, 2009). Plot management, especially topography interacting with plant distribution, is certainly playing an important role to regulate the populations of pollinating insects at plot level.
The data collected in this study are still under analysis and our approach will also include the possible effects of land uses surrounding the cocoa plantation.

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