Soil Macroinvertebrate Functional Groups composition and structure in High, Medium, and Low-Input Cacao Farms of Upper-Amazonian Ecuador

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Abstract

On the margins of the western Amazon Rainforest, cacao agroforestry represents a type of semi-disturbed ecosystem that covers most of the arable land on earth. Surrounded by shade trees and shedding leaves of their own, cacao plantations can create a rich leaf litter habitat for detritivores and other animals that help cycle nutrients to the O-layer below. This study examined soil and leaf litter macrofauna (insects, myriapoda, molluscs, arachnids, worms, etc) from two distinct cacao Chakras in Tena (.28 and .18 ha) and from two cacao farms (3.48 and 4.49ha) in Chontapunta, all sites situated in the Ecuadorian Oriente. Each site can be categorized into high, medium and low-input farming when framing agricultural strategies in terms of the amount of machinery and bought chemicals used. We predicted that if herbivore presence was high, then diversity of other species would be lower. We also predicted that sap-sucking insect biodiversity would be high, when in fact the most abundant guild was saprophagous, or organisms that break down dead organic matter. In Chakra 1, herbivore presence was disproportionately high and other guilds much lower in number. Using aspirators and Berlése traps, we found that the most jumping spiders were found in the monoculture, and that ant diversity was highest in a shaded cacao plantation. Hymenoptera made up the greatest proportion of macrofauna orders in the medium input farm. The biggest contributor to macrofauna biodiversity was Chakra 2, which was situated across a river away from chickens and most unintentional human disturbances. The second chakra and both higher-input plots shared similar proportions of saprophagous organisms. In Chakra 1, many more herbivores were found. Chakra 1 was done at night, while the other sites were done during the day. All of this considered, the proportion of guilds and the proportions of orders were similar across three cultivation styles. Although the amount of social insects and herbivores was variable across three different sites, the amount of saprophagous organisms remained somewhat constant at about 35 percent.

Resumen

Al límite de la Amazonía occidental, la agroforestería del cacao representa el paisaje seminatural que cubra la mayoría del terreno arable del mundo. Rodeado por árboles de sombra que arrojen hojas y arrojando sus propias hojas también, plantaciones de cacao pueden crear un medioambiente rico para detritívoros y otros animales que ayudan a reciclar nutrientes en la capa O por abajo. Este estudio examinó macrofauna del suelo y hojarasca (como insectos myriapoda, mollusca, araña, buzones, y más) de dos Chakras de cacao distintas en Tena (que miden .28 ha y .18 ha) y de dos granjas (3.48ha y 4.49ha) en Chontapunta. Todos los sitios ubicados en el Oriente del Ecuador. Cada sitio puede ser categorizado como alto, medio, o bajo aporte de cultivo en términos de máquinas y químicos comprados. Predecimos que si la presencia de herbívoros fuera alta, la diversidad de otras especias sería más baja. También predecimos que la cantidad de insectos que chupan savia sería alta, pero en realidad el más común insecto era el sapófago, o los que descomponen material orgánico muerto. En Chakra 1, la cantidad de herbívoros era desproporcionadamente alta y otros gremios estaban mucho más bajo en número. Usando aspiradores y trampas Berlése, encontramos que las arañas saltando eran encontradas en el monocultivo y que la diversidad de hormigas era más alta en la granja de nivel de aporte medio. El más grande contribuidor a diversidad de macroinvertebrados era Chakra 2, la cual estaba situada a través de un río lejos de gallinas y la mayoría de alteraciones humanas no intencionales. La segunda chakra y los dos sitios de aporte grande compartían proporciones semejantes de organismos saprofágos. En Chakra 1, muchos más herbívoros han sido encontrados. Chakra 1 se recolectó en la noche, en cambio los otros se hicieron en el día. Considerando eso, la proporción de gremios y las proporciones de órdenes eran semejantes a través de los tres estilos de cultivo. Aunque la cantidad de insectos sociales y herbívoros

era variable a través de los tres sitios, la cantidad de organismos saprofagos permanece constante a aproximadamente un 35 por ciento.

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Introduction

More than 5000 years ago, cacao was spread across the Americas by the Valdivia coastal culture on boats from Ecuador to other lands in south and central America (Valderrama et al. 2020). Cacao is an important cash crop for farmers in the upper Napo basin in Ecuador and many other tropical regions around the world, so it is crucial to understand the ecology of its cultivation.

This study was conducted in the upper Amazon basin, where Cacao is a native species. In 2018, Ecuador was producing 65% of fine-flavored dry beans, which sell at a premium price (Montenegro et al (2018). Today, although it produces only a fraction of global cocoa supply, cacao in Ecuador is renowned for its quality that comes from traditional, usually non mechanized production methods, in which farmers sell their crop for a higher price than they could for conventional farming (Montoya et al 2020). Ninety percent of global cacao production comes from smallholder farmers, which is the primary form of cultivation around the upper Napo River near Tena. (Adeniyi, 2022) Although the greatest amount of production in Ecuador comes from the Andean Chocó and the coast of Manabi (Direct communication, 2023), the cacao farms of Napo and other Amazonian provinces are valuable contributors to Ecuador's well-known aromatic quality, with distributors selling to local chocolate companies such as Kallari and Pacari.

Cacao is often cited as more integrated with the surrounding ecosystem than other cash crops because of the various planted species of shade trees required for a quality yield. Being among native trees in many cases, cacao plants and their surrounding vegetation have a variety of insect herbivores such as members of Formicidae, order Hymenoptera. Cacao needs to be pollinated by midge flies (*Nematocera*) to produce fruit, which comes from only about 5% of flowers (Groeneveld et al 2010). This type of fly has been found to breed in cacao husks, which are left on the ground close to cacao trees to encourage pollination. Leaf litter created from cacao trees themselves as well as shade trees can create a type of forest floor with other diverse macroinvertebrate life. The leaf litter layer is an interface between life in aboveground vegetation and soil dwellers, and the principal natural provider of nutrients in tropical agroforestry systems. Although there are macroinvertebrates(>2mm), mesoinvertebrates (.2-2mm), and microinvertebrates (<.2mm) (Oliveira, 2018), this study will be focusing on macroinvertebrates because they are much easier to count without technical identification skills. Depending on the type of macrofauna, soil decomposition can either be sped up or broken down in their presence. Frouz (2018) stated that earthworms coating soil with a clay layer after digesting it makes the soil accessible to microorganisms. Easily digestible polysaccharides and changes in pH can make it harder for decomposing microbes to take hold. Still, other macrofauna masticate leaves and benefit microbes which increase in their feces. In Cacao Agroforests, 41 percent of species composition of insects can be attributed to coleoptera, higher than other places (Perry, J et al 2016)

- *Chakras* produce more food than most subsistence farms. (Armengot et al, 2016). However, with low crop prices, farmers have little money and must look for alternative income sources. As a farming system, Chakras represent more than production; they have an altruistic meaning. Chakras are not just a food provider, but also a host to many culturally important plants and animals. Occupying less space than conventional farms, they provide more subsistence and conserve soil better. Chakras do not produce much revenue, 50 cents per pound and each chakra plot could produce about 150-300 pounds per year (personal communication, 2023, Coc-Huelva et al 2017) In the surveyed Chakra, there were many cultivars of cacao, including nacional, alongside subsistence plants such as plantains. The owners used Glyphosate sometimes for weeds but do not have extra money to buy fertilizer. They weeded their land with machete and weedwacker, planting with machete only.
- In the *High Input* or *Conventional* system at Chontapunta, mainly the Nacional variety (McCarthy and Silva, 2023). Cacao pods were already ready in late November, earlier than around Tena. The pods prefer a humid but dry environment to yield fruit. The rainy season is from May to July. Uses pesticides sprayed with automated sprayers around the tree, organic biol, which is a mix of ash, whey, water, molasses, and milk. and inorganic fertilizers equally.
- In the *Medium input* system in Chontapunta, part of the site is abandoned and the cacao trees are being covered by jungle. Due to higher tree presence and patches of grass, this system resembled a chakra. Litter varied, perhaps the most diverse substrate with noticeably more woody material, cacao husks in some areas and barely other coverage in others. It was also a smaller area being cultivated than the map would suggest because of some flooding and overgrowth, which also might have contributed to the development of some diseases.

Avadi (2023) said that carbon loss impacts of cacao production in Ecuador are comparatively lower in than in other cacao-producing countries. Clonal varieties such as CCN-51 were more common in monocrop systems. Monoculture cacao is more commonly produced than aromatic types. The majority of cacao produced on Ecuadorian coast Impacts per t are much higher in intensive producers than in extensive ones, although intensive producers produce slightly more. Most cacao produced in Ecuador comes from smallholders. Therefore, agronomists and ecologists should focus on these types of cultivation. Amazonian smallholders fertilize less but also use more pesticides such as herbicide, fungicide, and other chemical pesticides .

Several pests can cause extensive damage to cacao including Rusty grain beetle (Bharathi, 2023), Ceratocystis wilt, frosty pod rot, and witch's broom (Valderrama 2020) which are being made more intense by climate change (Cilas et al 2020). Some insects are vectors for disease. This study will provide valuable monitoring data that can be used to compare to past studies in which insect communities may have been different due to a cooler climate.

The goals of this project are to see how cacao farm production relates to availability of macroinvertebrates in the soil, as they influence soil quality which directly affects the ability of cacao plants to get nutrients for growth.

We expect to find that herbivorous guilds will be present in areas with higher leaf and grass coverage. This can in turn increase predator population and make the overall count of insects higher. The proportion of herbivores may diminish biodiversity because they could be dominant. One possibility for which guilds we expect is that there will be more abundance and richness of sap-sucking and xylophagous insects, as was found in Novais et al (2016).

Objectives:

1. Identify macroinvertebrate orders and feeding groups present
2. Determine difference in composition of feeding groups between higher and lower levels of input
3. Determine macroinvertebrate species richness and abundance in high and low input farms
4. Examine how leaf litter and organic soil layers differ between cultivation methods
5. Infer how the unique strategies of cacao cultivation may contribute to macroinvertebrate diversity

Figure 1: Tena Chakra Sites at 530 masl, showing Chicken present (bottom left) and chicken absent (top right) chakras.(Google satellite images, 2023)	**Figure 2:** High input agriculture sites at 380 masl (Google satellite images, 2023). The lower right had few chickens, while the upper right had many.

Figure 3 (above): the two yellow dots by Tena and Chontapunta signify the two locations of cacao cultivations in the study. (Google Earth satellite images, 2023)

Figure 4 a Cacao monoculture in Chontapunta, Ecuador, M1. Author: Joshua Puyear

Figure 5 Typical chakra setup with shade trees. In this case, the trees are the native balsa, which is a profitable lumber tree. Author: Joshua Puyear

Figure 6: Berlése funnel setup. Author: Josh Puyear

Materials and Methods

Study Area

Four different sites were surveyed, two near Tena , Ecuador, (coordinates) and two in Chontapunta, Ecuador (coordinates). The sites C1 and C2 in Tena were Chakras, the traditional Kichwa polyculture lifeway. Sites M1 and M2 in Chontapunta were not considered chakras. Chontapunta is 53 kilometers further from the Andes and 180 meters lower than the Tena site. For each site, the perimeter was walked and photos were taken of the site. Then, plots were selected using the Random Walk method, as outlined in University of Minnesota’s Great Lakes Worm Watch (2023). For larger sites, a variety of landscape features of the site were chosen to avoid remaining in the same area.

Site	Size (ha)	Location	Chickens?
C1	.28	1°00'42"S 77°50'58"W	Y
C2	.18	1°00'34"S 77°50'56"W	N
M1	3.48	0°56'59"S 77°22'24"W	N
M2	4.49	0°56'44"S 77°22'40"W	Y

Collection of samples

One square meter of substrate, which includes the leaf litter and the organic layer, was taken from each of the 5 surveyed trees per plot. Under each tree, the square meter was divided into one 50 cm^2 half on one side of the tree and the other 50cm^2 half on the other side. The rectangles, which were made with string and wooden stakes, were oriented so that the narrow side was within .3 meters of the base of the tree. Using an aspirator before doing any digging, the plot was visually scanned in a back-and-forth pattern for approximately 5 minutes to look for animals crawling on the surface of the leaf litter. The purpose of this initial scan was to find animals that could hide in the leaf litter upon disturbance but could easily be taken as samples using the aspirator. Macroinvertebrates that had been taken were placed in a bag. Leaf litter was collected from the surface with a rake to avoid being bitten by bullet ants or other feisty critters and placed into a rectangular tub, where the leaves were sifted through a few small handfuls at a time to find more macroinvertebrates that could easily be obtained by hand. The same process was repeated with the O layer of soil, which usually only constituted less than a centimeter of the soil's depth. Using a shovel, the soil was scraped with a gardening shovel. Litter and soil samples were placed in separate plastic bags for later processing.

In the field, percent canopy cover (25, 50, 75, or 100 percent) as well as rough estimates of the amount of leaf litter and O-layer in later plots (to the nearest centimeter) were estimated. In the first Chakra plot with chickens, O leaf litter layers were not separated; rather both were collected at once. With the lack of macroinvertebrate presence, It was obvious where the O layer transitioned to an A/B layer because decomposed organic matter from leaf litter had a distinct grainy texture in all plots that differed from the clayey, smooth soil characteristic of tropical areas with high amounts of leaching and rainfall. Only digging a few more centimeters from the O layer, there was a slight orange tint to the soil that suggested oxidation. When the leaf litter and insect data was taken on one side, I moved to the opposite side of the tree. After a tree was done, the location of the tree was saved using Google Maps and the process was repeated.

Figure 1.6, a typical plot, with stems and vegetation as well as old cacao husks and leaf litter. In the Amazon, there is constant decomposition happening

Analysis of Data

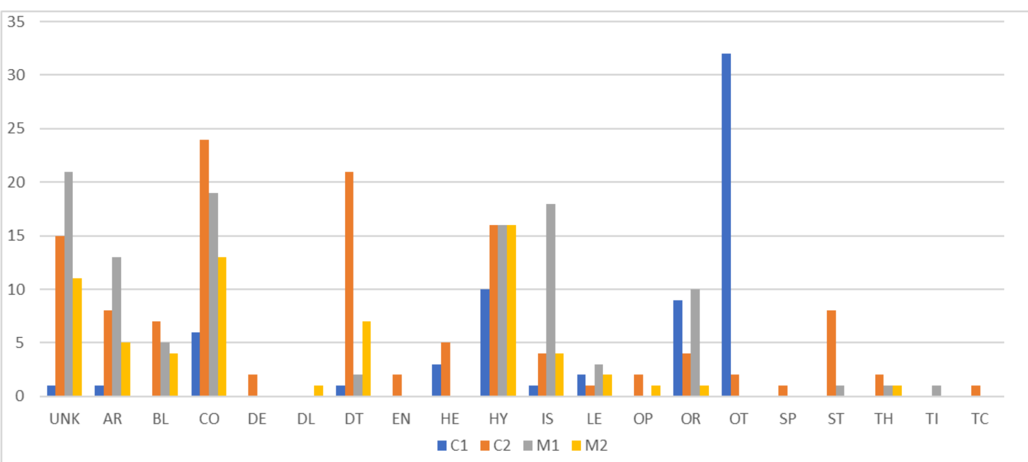
When possible, species were identified to the level of family and order, following the example of Deheuvels et al (2014) . Insects that were too small to separate manually were passively extracted using the Berlése funnel, in which soil or leaf litter is placed on a mesh at the top of a funnel and the insects fall into a jar of alcohol at the bottom of the funnel as they move down to avoid the hot, dry environment of the soil. (Berkeley, C 2023). In the lab, A Plugable digital viewer (Plugable Technologies, 2023) microscope to differentiate between morphospecies. I used the soil insect book and a few different spider electronic keys for insects and spiders. Photos of all taxa were uploaded to iNaturalist.

For analyzing Beta Diversity, we used the ade4 package in R to calculate the Jaccard index. This index is used to compare how the species in each site influenced replacement and richness. The Jaccard index uses two types of diversity measures, replacement and richness. It compares two or more sets of species, looking at different places and/or across time.

$$\text{richness} \quad \frac{|b-c|}{a+b+c} \qquad\qquad \text{replacement} \quad \frac{2 * \text{minimum}(b,c)}{a+b+c}$$

(Legendre, P 2014)

Results



Code	Order
UNK	unknown
AR	Araneae
BL	Blattodea
CO	Coleoptera
DE	Dermaptera
DL	Diplura
DT	Diptera
EN	Entomobryomorpha
HE	Hemiptera
HY	Hymenoptera
IS	Isopoda
LE	Lepidoptera
OP	Opisthopora
OR	Oribatida
OT	Orthoptera
SP	Spirobolidea
ST	Stylommatophora
TH	Thysanoptera
TI	Trichoniscidae
TC	Tricladida

Table 1*:* Codes for major orders of insects and other macroinvertebrates

Figure 2: Relative perceived abundance of major orders of insects and other macroinvertebrates. Refer to table 1 for a guide to each code on the X-axis.

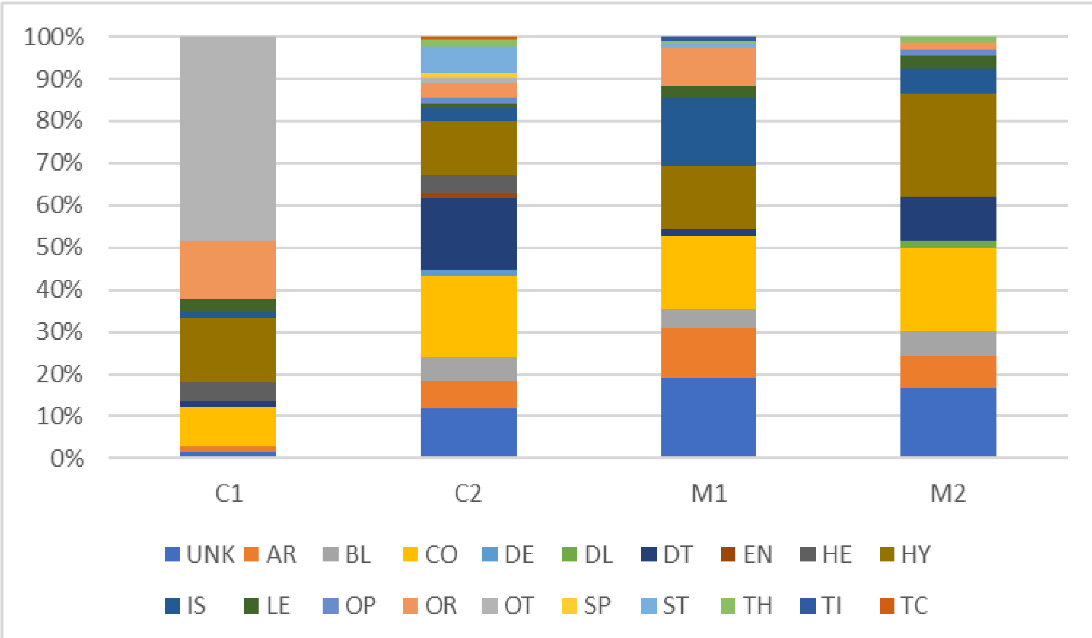
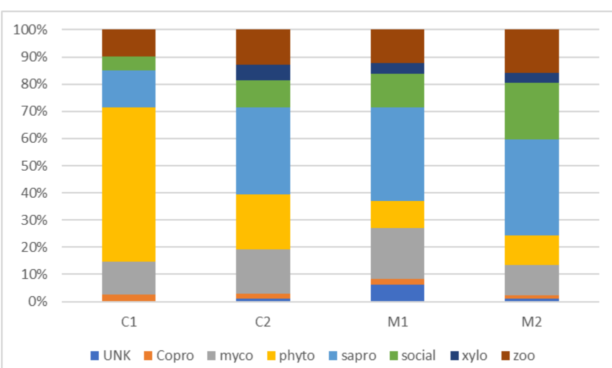


Figure 3*:* Orders as a percentage of total individuals found in each plot.

Many of the herbivores in figure 4 are the orthoptera in figure 3 for site C1. In this site, there were 49 herbivores, compared to the rest of the sites that had 40 or less, However, even among the other plots for figure 4, no two graphs look alike. However, all sites had at least 12% Hymenoptera, illustrating how foundational and ubiquitous of an order it is. Most of the hymenoptera seen in the study are ants. Another common order across all sites was Coleoptera, which makes up about 20 percent of the latter 3 sites. The most prevalent feeding group for each site is phytophagous for C1 and saprophagous for the rest of the groups. Isoptera were more common in M1 and M2 than in C1 or C2.



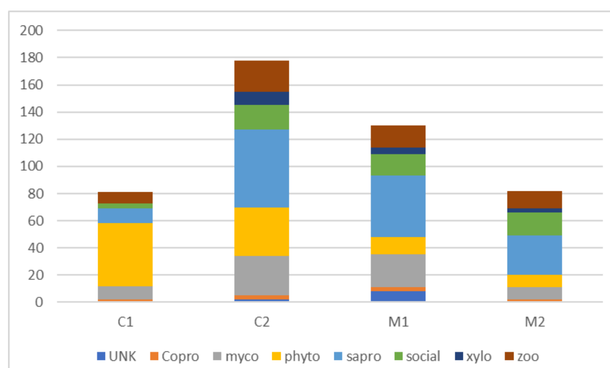


Figure 4 (left) Percentages of each guild found in the chakras.

Figure 5 (right) Total number of each organism from its respective guild found in each plot.

With a more refined methodology in the latter three sites, they ended up looking much more similar to each other than to C1, as illustrated in Figure 3.1. M1 has a greater proportion of unknown orders than C2 or M2, which could have included larvae. M2 has the greatest proportion of zoophagous organisms and social insects, the latter of which for this study was almost exclusively ants. According to Figure 3.2, the greatest number of samples collected came from C2, the chakra across the river and away from chickens. C1 and M2 saw fewer overall numbers of insects.

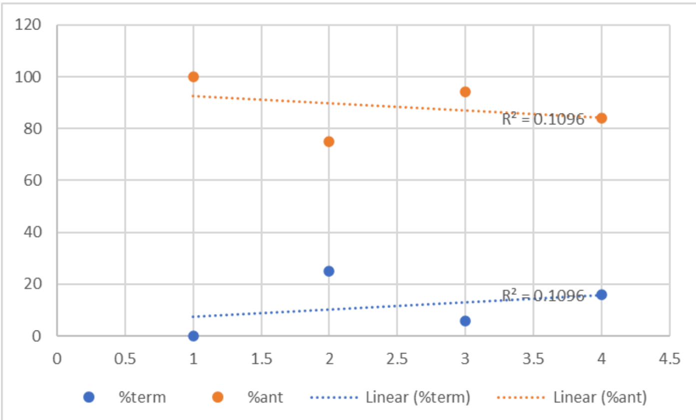
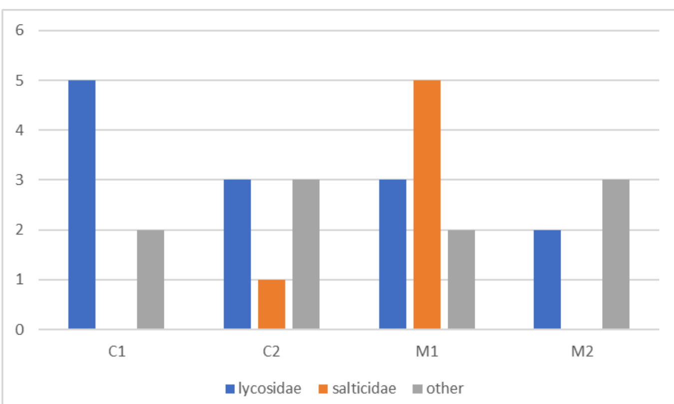


Figure 6 Comparing ants and termites, the two main social insects present in the plots

The R squared coefficient for each line of best fit is very weak, at .1096. However, there does appear to be a trend that as termites increase, ants go down.

Plot	Canopy Avg
C1	25
C2	50
M1	0
M2	25

Table 2.: Table of average canopy cover for each site, which was estimated to the nearest interval of 25 percent.



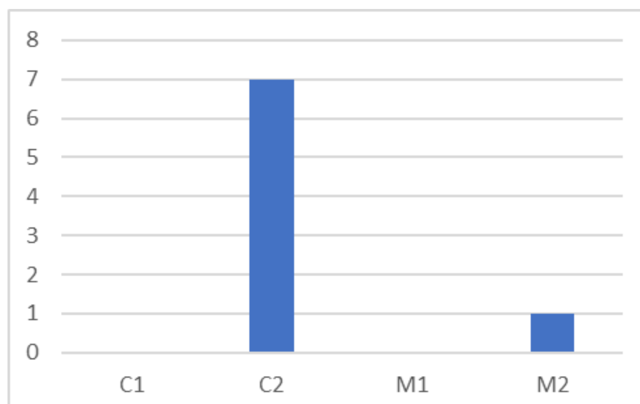


Figure 7 Two main families of spiders found, Lycosidae and Salticidae, across the plots.

Figure 8 Count of individual snails. There were none in C1 or M1.

Richness		Replacement	
Sstotal	0.270367	Sstotal	1.112191
Bdtotal	0.090122	Bdtotal	0.37073
LCBD richness		LCBD Replacement	
C1	0.183595	C1	0.283404
C2	0.411363	C2	0.209892
M1	0.19994	M1	0.253197
M2	0.205102	M2	0.253506
RichDif/Bdtotal	0.195556	Repl/Bdtotal	0.804444
		Bdtotal	0.460853

Table 3 Richness and replacement beta diversity using the Jaccard calculation.

For species richness, the highest local contribution to richness (number of species) is C2, a traditional chakra without chickens. Since we only saw a lot of orthoptera species in chakra 1, which had more grass that was not removed, the highest local contribution to replacement biodiversity is from C1.

L Depth index	O Depth index	Moisture index	%canopy cover index
1.45	1.65	2.4	20
2.325	1.65	2	37.5
0.825	0.3	1.9	0
0.6	0.4	2.8	25

Table 4.1 Comparing the environmental conditions of each plot. From top to bottom, C1, C2, M1, M2

The highest L depth index was in Chakra 2 at an L depth index of 2.32. Chakra 2 shared the deepest O depth index at 1.65. The chakra sites have greater depth of leaf litter and both have over 20 canopy cover index.

Analysis

Plots that get mowed with machetes require more effort to remove weeds to the same standard as a weedwacker, which should permit more grass and herb-associated insects to remain. This could provide food for larger herbivores such as chickens. Adding nutrient deposition in the form of manure and scavenging, they can restructure macroinvertebrate communities in farms. Considering this, we didn't see a lot of change between guild distribution between plots C2, M1, and M2 according to Figure 2.2. The chickenless plot across the river experienced frequent overgrowth because when it rains, the river can't be safely crossed. As a result, an entire ecosystem of open area insects can move into the chakra during these somewhat frequent periods of overgrowth. This could have influenced insect diversity, but it is a consistent enough reality of chakra farming that I was able to include it as a part of the study. Although an overgrown area was never surveyed for this project, we had to remove the grass from a place that had freshly been cut a few days earlier.

Considering that there should have been more spiders in areas with chickens according to Garcia (2023), it was surprising that the plot with the greatest proportion of arachnids (Figure 3.1, Figure 4.2) was the highest input approach to cacao farming. The addition of organic fertilizer might have encouraged many of the same insects that colonize areas with chicken manure. Chickens as predators are nondiscriminatory and catch what they can. However, we would expect to see fewer phytophagous insects like orthopter because they live closer to the surface and are easier for the chickens to spot.

According to figure 3.2, there were more herbivores in C1 (which was surveyed at 5 in the morning, before a lot of the chickens came out) and C2 (which didn't have chickens). According to Garcia (2023), While rove beetle, dung beetle, and spider density increased with the presence of chickens in an agricultural plot, hymenoptera, coleoptera, and hemiptera presence decreased.

It was less common to hear about insects than non-insect pests like fungi when discussing the occurrence of pests with landowners.However, there are insects that aid in the dispersion of other pathogens that can cause harm to cacao. For this reason, the benefit of insects should be addressed on a case-by-case basis. Stink Bugs (Hemiptera, Pentatonidae) may facilitate the movement of fungus into cacao Coleoptera and diptera feed on diseased pods and can spread BPR since they fly. Ceratocystis has a symbiotic relationship with insect disperser C. fimbriata may be attracted by fruity aromas in cacao, which are more common (Bailey and Meinhardt, 2016) Shade reduces insect pest occurrence which is more common under full sun. Further study is needed to have a more precise estimation of shade cover on the cacao in the different chakras and to test if shade cover is a significant influence on soil insect presence. At least indirectly, more shade usually means that there are more overstory trees to provide leaf litter, which is habitat for soil dwelling macrofauna.

One variable that will always be present in cacao farms is dry cacao pods in the substrate. Mariano and Melo (2015) discuss how pods are a microhabitat that are hosts to ants and a diverse array of other organisms when they compared cacao pods still on the tree to those on the ground. Class Symphyla, class Gastropoda, and class Diplopoda were among orders found in pods on the ground, which are saprophagous and zoophagous. According to our results, the saprophagous feeding group made up at least 30 percent of C2, M1, and M2, while still making up 12 percent of C1 even though the soil wasn't taken out. Creation of rough woody habitat replicated by the cacao pods can be found in places with dense canopy cover, such as M2, or primarily mechanical cleaning, such as for C2.

Earthworms, ants, and termites are ecosystem engineers that are present throughout all of our plots and should be expected almost everywhere due to their wide diet range and niches. They create diverse organic mineral structures, the kind that works as a natural fertilizer for crops like cacao (Oliviera, 2018).

In Maraun et al (2008) Orb mites increased with decreasing elevation despite losses in the organic layer. However, diversity of larger macroinvertebrates was comparatively low because of the acidic soil in the area. Between the chakras and monoculture sites of this study, there was a drop in elevation by about 150 meters. Orbitada were found in all three sites where soil was collected. In this study, orbitada is included in table 1. Which shows that C1 and M1 have markedly higher numbers of this mite. This type of mite is mycophagous and coprophagous, so it could be benefiting from increased chicken manure or additions of fertilizer.

Presence of grass insects in samples was due to the cut grass especially in the chakras, which were mechanically thinned most of the time. There was always enough grass to host herbivores, even if it was cut close to the substrate.

Discussion

Even at the highest intensity plot surveyed, the owners were implementing more ecological farming methods such as biol, an organic fertilizer made from biological ingredients. The owner had been through training from Yachana foundation, which is a conservation NGO in the area. T came to his farm to give training, they sprayed pesticides around the farm. Organic fertilizer is more expensive than the ready made chemical fertilizers, but they try to use it. One example is cocoa butter and whey that they spray over the plants with mechanical sprayers. Although they went through ecological farming training, they were not pursuing an organic certification. It was not clear which pesticides he used. Higher shade inputs hurt production, but can also lead to higher quality. Higher shade systems also reduce the risk of insect pests. Most chakra owners are more concerned with diseases like pod rot, so trying to understand insect predation was veering from conventional concerns about this cash crop. However, in both the monoculture plot and in the chakra, a stype of ant called "Hormiga Arriera" (*Atta cephalotes*), was indicated that eats the flowers, preventing the pods from developing. (Mosquera, 1976, *Croplife*, 2020). If cacao trees are weakened due to full sun conditions, complications from herbivores such as Hormiga Arriera could be more pronounced.

Although the second site in Chontapunta was named M2, it was not a monoculture because there were more shade trees than required, at a canopy cover index of 25. The forest was encroaching towards the back of the site and the trees had not been trimmed at the time of data collection, which further lent its appearance to that of a Chakra. Each of the three growers in this study had slightly different strategies, and the dynamic nature of cacao vegetative makeup opens opportunities for high insect diversity.

There was green plant debris in Chakra 2 due to the use of mechanical weeding for all cultivation to varying degrees. In cacao agroforests, due to this randomness of microhabitats, there is unplanned biodiversity: weeding only during certain periods leaves a host of insects that can provide unknown ecosystem services (greenberg, 2020). Seasons with more harvesting might see higher insect div since some are still in tree & others fallen (Mariano and Melo, 2015) Higher input agriculture (Monoculture 1) sold at 40 cents per pound versus the 51.6 cents per pound received for cacao cultivated in Chakras. However, seeing both Chakras and the monoculture, it was clear that under the same conditions, the M plots were producing much more cacao. However, the costs of losing some insect pests could be greater.

Methods were improved during the study. For example, at first the funnels for Berlése were placed on a table, making them very top-heavy. A few samples were lost due to this. Later, still without a lamp, the funnels were suspended like garden baskets from string close to a steel ceiling to maximize the head absorption effects during the day, acting like its own heat lamp. A more precise method for extracting insects can be employed, wherein each berlése funnel is held under a heat lamp for 15 days to extract the maximum number of insects possible. (Palacios-Vargas et al., 2007; Cassagne et al., 2003, 2006; Chagon et al., 2001) With only 1 day in the funnel for this project, there was comparatively little time to extract stubborn macroinvertebrates. One idea to improve predictions about macroinvertebrate occupancy would be to analyze abiotic soil quality factors that interest users of conventional agriculture to have more uniform and concrete metrics that can help to predict production differences between traditional and high-input farming methods (Rousseau et al 2012). One more concrete measure would be to determine soil layers by doing a profile test, so we can have a better idea of where to start counting the O layer and to know where the A layer starts. Having more tailored mesh to the size and texture would aid in more precisely extracting insects using the Berlése method. It is important to store specimens in a durable container because ants ate through the plastic storage bags.

Novais et al (2016) looked at native versus nonnative tree species' effects on insect pests. What they found was that xylophagous insects may have been lower . We have to know if planted alien vs native trees have an effect. Another nonnative species with an unclear impact is chickens. It would be a good idea to study chickens' effects on soil macroinvertebrates being such a common form of livestock that is ubiquitous in tropical rural settings. Chicken meat and eggs are among the most popular forms of protein for Ecuadorians and every landowner encountered had an area with free-range or semi-free range chickens. With their omnivorous habits, Chickens can select slower macroinvertebrates that might play a role in nutrient cycling. Since chickens are normally released twice a day and literally physically take away insects, they could be playing a bigger role than any human impact, which are not as physical or direct. The effects of free-roaming chickens has recently attracted attention because of a growing global demand for meat.

Another variable that wasn't examined in this study was how leaf litter from different types of trees, both native and nonnative affectl macrofauna, particularly in the substrate. For aquatic macroinvertebrates, there was shown to be little effect in Firmino et al (2021). However, Novais et al (2016) bring up that for cacao herbivores, surrounding vegetation naturally affects from the bottom up which insects live in an agroforest from a foundational standpoint. All plots were part of a checkerboard of agricultural fields and fallow land or secondary forest, which could serve as a source for more insects.

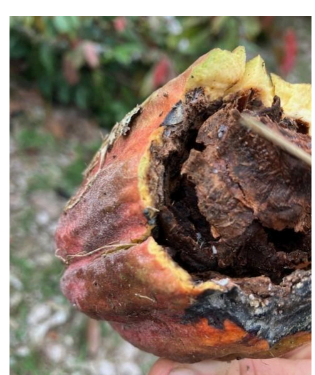
Conclusion

Soil macroinvertebrates can both speed and slow litter and organic matter decomposition and thus nutrient cycling, through predation on other detritivores or digestion of their sources of energy on one hand, or through mechanical breakdown on the other. Substrate macroinvertebrates include cacao's pollinating midge species during their larval stages, and they can also contain insects that are vectors for disease. In a freezer bag full of soil, one can find four or more trophic levels present as well as many orders of macroinvertebrates. C2 contributed the most to biodiversity in terms of numbers (see Figure 5, where C2 is the highest at 180 individuals out of 375 total) and in species richness (according to the Jaccard index, C2 makes up 42 percent of replacement diversity. However, each site contributed several unique species and the total diversity couldn't be encountered with this project alone. In the chakras overall, there were more herbivores due to the manual method of trimming the chakra.

Macroinvertebrate guild and order proportions being very similar across cultivation strategies shows that certain methods such as fertilizing or use of pesticides maybe hasn't tipped the trophic balance in the realm of macroinvertebrates. A joint study of plant and insect diversity could help shed light on how the ecosystem foundation interacts with farming inputs. Another opportunity for future study are substrate macroinvertebrates for other cash crops like vanilla. This experiment should be repeated with more precise identification of smaller soil fauna and standardized collection methods. The species diversity of soil insects in chakras should be further examined because their habitat is dynamic. What was a meadow full of beetles yesterday becomes a lawn the next.

Appendix A: Some examples of species found in all three plots. Left to right, Coleoptera larva, Lycosidae, and Camponotus.

Appendix B: A rotting cacao pod is a host to many insects



Appendix C: The road bordering M2 shows that there were many native palms and other trees that could be a source for insect diversity

Appendix D: A chakra owner seeds cassava root, known in South America as yuca

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