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Study of the soil macrofauna in tropical cacao-based agroforestry systems in the Dominican Republic

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Preface

The present document does not include all the data collected during the training period. Several data were collected in view of treating additional research questions that will be addressed in the frame of a scientific publication to be submitted by the end of 2021.

These data are presented in Appendix 1.

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1 Introduction

Agroecology is “the application of ecological concepts and principles to the design and management of sustainable agroecosystems” (Gliessman et al. 1998). Francis et al. (2003) defined agroecology as “the integrative study of the ecology of the entire food systems, encompassing ecological, economic and social dimensions”. It goes beyond the study of the immediate impacts of agriculture on the environment and requires a complete understanding of the agroecosystems as part of the food systems, and the consideration of their complex and countless interactions. Agroforestry is a practice coherent with the current agroecological knowledge. It is an agricultural land use combining at least one perennial ligneous, with crops and/or cattle, in a spatial and temporal arrangement that often aims at optimizing ecological and economical interactions between the components (Young 1989, Somarriba 1992). It encompasses highly contrasted agroecosystems, from input-intensive and mechanized plantations intercropping only two species to family grown, highly diverse and ecologically intensive agroecosystems. Agroforestry takes many forms around the world. Each agroforestry system is the fruit of a different history and culture, and answers specific needs by using the natural resources and possibilities of the place where it emerges. For instance, it goes from the association of oak trees, grassland and pigs in Spain for a specific high-quality meat market (Smith 2010), to the family gardens of Maya population in México compound of multistrata annuals and perennial crops for the family’s consumption (Diaz-Santana 2012). It takes place from cold areas like Scandinavia, where silvo pastoralism is practiced with fodder pollards inside meadows (Smith 2010), to tropical areas like the Caribbean region where cacao trees are grown in association with a number of cultivated plants (Sánchez 2019).

Agroforestry is generally acknowledged a sustainable agricultural practice, in term of productivity and environmental conservation (Young 1989, Nair 1993, Scroth and Harvey 2007). Agroforestry practices bring economical resilience by diversifying the production, and reduce erosion compared to annual monocrops (Estrada 2000). Plant and root litter enhanced in agroforestry systems compared to annual monocrop systems may increase the activity of soil fauna (Akinnifesi et al. 1999). Cacao-based AFS have a better environmental impact than monospecific systems (Vaast and Somarriba 2014).

The soil macrofauna provides crucial ecosystem services, it fosters the functioning of the overall ecosystems through the soil ecosystems. “Soils are a key reservoir of global biodiversity, which ranges from microorganisms to flora and fauna. This biodiversity has a fundamental role in supporting soil functions and therefore ecosystem goods and services associated with soils” (FAO et al. 2020). Similarly, Wagg et al. (2014) showed that the soil biodiversity is a key factor in regulating ecosystem services. More specifically, Marichal et al. (2014) found positive correlations between ecosystem services and soil macrofauna density.

As expressed earlier, the soil macrofauna provides ecosystem services that allow food production, but inversely, some food production systems such as organic cacao-based AFS play a huge role in preserving soil macrofauna, by creating ecological corridors for many species.

Cacao-based AFS are known for preserving both wild and cultivated biodiversity (Suárez et al. 2021). Organic cacao-based AFS play a huge role in preserving soil macrofauna and create ecological corridors for many species. They allow their soil macrofauna to provide ecosystem services that ensure the sustainability of food production. Several studies reveal that agroforestry systems have higher abundance and diversity of soil invertebrates than annual crop systems (Mujeeb Rahman et al. 2012, Pauli et al. 2011, Marsden et al. 2020). Indeed, agroforestry systems are generally constructed on a higher density and diversity of plants than annual crop systems, so they offer more ecological niches for macrofauna, as well as for microorganisms and fauna, which in turn foster the development of macrofauna.

The cacao-based agroforestry systems of this study are managed without agrochemicals, without tillage and with more cultivated and wild diversity than monocrop systems (Deheuvelds et al. 2020).

In order to understand the effects of agroforestry practices on macrofauna, it is important to investigate the relationships between soils' chemicals and physical properties, and below-ground biodiversity, which for the moment has not been much studied (Marsden et al. 2020, FAO et al. 2020). Several studies focus on specific groups of soil macrofauna, especially earthworms, beetles, ants and termites (De Bruyn and Conacher 1990, Diamé et al. 2018, Conceição et al. 2019, Gongalsky et al. 2021), comparing their diversity in different land uses (Lal 1988, Marasas et al. 2001, Delabie et al. 2007, Mujeeb Rahman et al. 2011, Marsden et al. 2020). AFS, in terms of influences on biodiversity, are often compared with forests, pastures or monocrop systems, and even other contrasted AFS (Mujeeb Rahman et al. 2012, De Beenhouwer et al. 2013, Suárez et al. 2021). However, few studies compare agricultural practices inside the same type of AFS. The influences of crop residues application on the soil macrofauna has been studied in annual cultures (Lavelle et al. 2001, Ayuke et al. 2004, De Aquino et al. 2008) but seldom in agroforestry systems. García-Tejero and Taboada (2016) studied the effect of different microhabitat, including pruning residues, on the soil macrofauna. Few studies have tested the influence of cacao husks on the soil macrofauna. Prastowo (2020) tested the influence of cacao husks and cacao leaf on the earthworms populations, but not on the whole soil macrofauna. Other studies focused on ants population living in cacao pods, without link to the soil (Fowler 1993, Castaño-Meneses et al. 2015). The influence of the ageing of the plantation has been studied by Kamau et al. (2017) in Kenya. They assessed the spatial influence of trees in maize-based AFS at different ages of cultivation. They showed that the content of lignin in the litter varied with the age of the plantation. The influence of trees density and diversity on the soil macrofauna is more documented (Pauli et al. 2010, Suárez et al. 2021).

Applied research aims at answering problems with practical solutions. Understanding the effects of agricultural practices on the plants, the soil and its macrofauna in cacao-based agroforestry systems will enable to design and manage agroecosystems that are environmentally, socially, culturally and economically sustainable.

These are the reasons why the objective of this study is to assess **the influence of temporal heterogeneity and agricultural practices on the soil and its macrofauna in tropical cacao-based agroforestry systems**. This objective raises four hypotheses:

- 1- The temporal heterogeneity caused by the ageing of the cacao plantation influences the soil quality and the abundance and diversity of the soil macrofauna.
- 2- The heterogeneity of plant composition and distribution at plot scale evolves during the production cycle of the cacao plantation, and influences the abundance and diversity of the soil macrofauna.
- 3- Crops residues, and in particular cacao pod husks, influence the soil quality and the diversity and abundance of the soil macrofauna
- 4- The interactions between the temporal heterogeneity and the presence of crop residues influence the soil quality and the abundance and diversity of the soil macrofauna.

We offer here to test these 4 hypotheses in the manner described below.

2 Literature review

Microorganisms, mesofauna and macrofauna all depend on each other through complex food webs interactions (Sofa et al. 2020). Each population of a community has a different functionality in the ecosystem, and some have been more studied than others: the roles of microorganisms have been extensively highlighted, conversely to the roles of macrofauna (Sofa et al, 2020). Among macrofauna, the role of earthworms, ants and termites have been more studied than other taxa (De Bruyn and Conacher 1990, Jouquet et al. 2011, Lang and Russell 2020). These three taxa are recognized as ecosystem engineers (Jones et al. 1994) that modify the soil structure and increase the circulation of nutrients, gases, water and energy, influencing their availability for other organisms (Jouquet et al. 2006, FAO et al. 2020). Earthworms have been particularly studied. Their bioturbation services depend on their functional traits (Blouin et al. 2013, Tsiafouli et al. 2015, Pelosi et al. 2015). Their abundance and functional groups composition are positively correlated with water infiltration rates (Spurgeon et al. 2013). In the tropics, earthworms increase the growth of cultivated plants, especially the shoot growth of perennial plants (Brown et al. 2004, Van Groenigen et al. 2014). However, the influence of agroforestry systems on soil fauna has been more studied for macrofauna than for micro and mesofauna. Among macrofauna, most studies have focused on earthworms, beetles, ants, termites and spiders (Marsden et al. 2020)

The ant's family is often recognized as the best bioindicator to evaluate the degree of health and degradation of an ecosystem (Brown, 1991), because they are very sensitive to ecological changes. It is the group that most impacts its habitat and community (Delabie et al. 2007). Sanabria et al. (2014) identified ant species that can be used as bioindicators of different soil-based ecosystem services. They also regulate crop-damaging insects, including some cacao pests (Diamé et al. 2018, Delabie et al. 2007). Termites, as social insects, also greatly contribute to ecosystem services (Elizalde et al. 2020), especially in the tropics. They create biostructures that impact the soil physical properties and they influence the distribution of natural resources on which the whole biota depends (Jouquet et al. 2011). Among other taxa that have been studied in connection with ecosystem services are the predators *Araneae*, which highly contribute to pest control (Rousseau et al. 2013)

Macrofauna abundance and diversity first depend on geography. Specific richness decreases with latitude: soil fauna density and diversity are higher in tropical areas, and when getting closer to the equator (Gaston 2000, Decaëns 2010). Species richness is generally lower on islands, where it decreases when the distance from the continent increases (Marcon 2015). This is especially true for the ants (Decaëns 2010). Diversity and abundance also depend on habitat fragmentation (Decaëns 2010), topography (Liu and Li 2008), climate and microclimate, season (Rozen et al. 2013) humidity and distance to the sea (Zheng et al. 2020). Flora density and diversity are other important biological factors affecting soil macrofauna (Gholami et al. 2016, González and Zou 1999).

It is as well affected by physical factors like soil's texture, especially percentage of silt (Gholami et al. 2016), density and compaction (Radford et al. 2001). For example, a significant negative correlation between bulk density and abundance of ants was proven (Nanganoa et al. 2019).

It has been demonstrated that the percentage of nitrogen in the soil, modified by fertilizers application (Edwards and Lofty 1982), as well as the percentage of organic matter and nutrients (Lavelle et al. 2001, Decaëns 2010), alter the diversity and abundance of soil organisms. Influences of the content of potassium, calcium and magnesium (Rousseau et al. 2012), pH (Decaëns 2010) and cations exchange capacity (Bradham et al. 2006) on the density and diversity of soil macrofauna has also been proven. Pesticides drastically decrease the abundance and diversity of soil macrofauna (Nare et al. 2017). Insecticides have a large effect on soil organisms, but earthworms are the most sensitive (Pelosi et al. 2014). They are also sensitive to fungicides, especially copper-based fungicides that causes long-term reduction of their populations, and to herbicides, as some products directly kill them (FAO and ITPS 2015).

In summary, soil organisms and the soil chemical and physical parameters influence each other through complex interactions (Devine et al. 2014)

Agroforestry as a practice is an alternative to the agricultural intensification based on agrochemicals and mechanization (Tubenchlak et al. 2021). This last type of agriculture reduces abundance and functional

diversity of soil biota, especially of larger-sized organisms such as earthworms and macroinvertebrates (Postma-Blaauw 2010). It also reduces the diversity of soil fauna and the complexity of soil food webs. Especially, drastic losses of native earthworm species have been observed in highly disturbed agricultural systems (Feijoo et al 2011). Agricultural intensification also reduces the mean body mass of soil invertebrates, and shrinks the functional and taxonomical diversity of earthworms (Tsiafouli et al. 2015). The mean body mass (corresponding to the mean individual weight) is an important functional traits of earthworms, as their effect on some soil physical parameters, like the bulk density, depends on their body mass (Lang and Russel 2020). Conversely, Agroforestry improves the soil physical qualities on which macrofauna relies (Cherubin et al. 2019). The complementarity in root structure between the different species constituting an agroforestry system may prevent soil compaction and then preserve macrofauna habitats. As an example, cacao AFS have lower bulk density than pastures (Suárez et al. 2021) and their contribution to ant's species conservation has been proven by Delabie et al. (2007)

Cacao-based agroforestry systems in the DR show similarities with cacao producing countries of Central America and Africa in terms of associated plant's diversity and density. In one parcel around 10 associated species are usually grown, the cacao density ranges from 658 to 880 trees per hectare and the associated plant density from 100 to 281 trees per hectare (Deheuvels et al. 2020). The cacao tree is native from the Amazon rainforest, where it has adapted to the shade of higher trees. It does not require full sunlight to reach its maximum photosynthetic capacity. When not fertilized, cacao reaches its maximum yield at 50% full daylight transmitted (Wood and Lass 2008). Young cocoa trees under 3 years old even do better under shade, which impacts the light intensity reaching the leaves, but also creates a microclimate that prevents moisture stress (Wood and Lass 2008).

3 Material and methods

3.1 Choice of the study site

3.1.1 The Dominican Republic

The Dominican Republic was chosen as a study site because an opportunity was offered by the Cacao Forest Project for accessing agroforestry cacao-based plantations. Its location makes it adequate to study **tropical macrofauna**. The country is the first **organic cacao** producer worldwide and 48% of the national production comes from organic **agroforestry systems** (Notaro 2019).

The Cacao Forest Project is working in the Dominican Republic since 2016. It has been conceptualizing and implementing four innovative cacao-based agroforestry systems (AFS) for improving farmer's revenue. Cacao Forest also wants to complete its assessment of the system's sustainability by evaluating their ecological impacts.

The Dominican Republic (Fig.1) occupies the eastern two-thirds of the Hispaniola island, in the Caribbean area. It is bounded to the north and east by the Atlantic Ocean, to the south by the Caribbean Sea, and to the west by the land border with Haiti. It is located 70.16 West and 18.74 North.



Figure 1 - Location of the Dominican Republic in the Caribbean. Adapted from Google maps, 2021. Caribbean, License Map data, available through <<https://www.google.fr/maps/place/Caribbean/@18.4335985,-81.3317603,5z/data=!3m1!4b1!4m5!3m4!1s0x8eb9e309d5a>>

In the Dominican Republic, agrochemicals use and mechanization are mostly limited to landed estates or *latifundia*, for rice and exportations crops like sugarcane. Smallholders, who represent the majority of the agricultural land in the country, generally do not rely on such techniques inducing large investments (Republica Dominicana 2021). Cacao represents a significant part of the agricultural land with 25% of the cultivated area, where only rice cultivation (30%) occupies a larger area (Gobierno de la República Dominicana, 2020). In the Dominican Republic, cacao is almost exclusively grown under agroforestry systems (AFS), often in environmentally sensitive regions (Siegel and Alwang 2004). The regions where cacao is grown have a relative dry winter that reduces the incidence of pests and diseases, making them adapted for organic cacao production (Siegel and Alwang 2004). Plus, the country has been preserved from common cacao diseases in tropical areas like *Moniliophthora*, thanks to its insular position (Notaro 2019). Cacao-based agroforestry systems often present lower densities of cacao trees than in cacao monocrops. They also present several interactions with a number of associated crops. As a consequence, the cocoa yield is often lower but is also compensated by a wide range of products either sold or self-consumed. In the Dominican Republic, more than 60 plant species have been found cultivated in association with cocoa trees (Deheuvels et al. 2020).

The diversity of associated crops, their densities and the cacao trees' density vary among plots and also inside the plots. This heterogeneity reflects farmer's strategies. Moreover, cacao farmers adapt their practices during the cacao trees' growing cycle and production dynamics. During the first 3 to 4

unproductive years after the plantation of the cacao trees, farmers usually intercrop annual or short-cycle crops such as plantain, pineapple or yam, to feed their families and/or to generate revenue. Those crops tend to be eliminated as the cacao trees close their canopies and other perennial associated crops grow taller and enter productive phase.

In the smallholders' cacao-based AFS of the Dominican Republic, the harvest of cocoa and associated crops are often performed by the farmer himself or by a family member. The cacao pods are tumbled down from the trees. They are gathered on the ground at an opening site almost always located inside the plantation and beans are often extracted on the same site, for commodity reasons. The remaining cacao husks are often left decomposing in heaps where the extraction of the beans was realized.

3.1.2 San Cristóbal province

Cacao Forest established experimental plantations in two areas of the Dominican Republic. Among them, we chose *San Cristóbal*, situated 70°10' West and 18°42' North (Fig. 2)

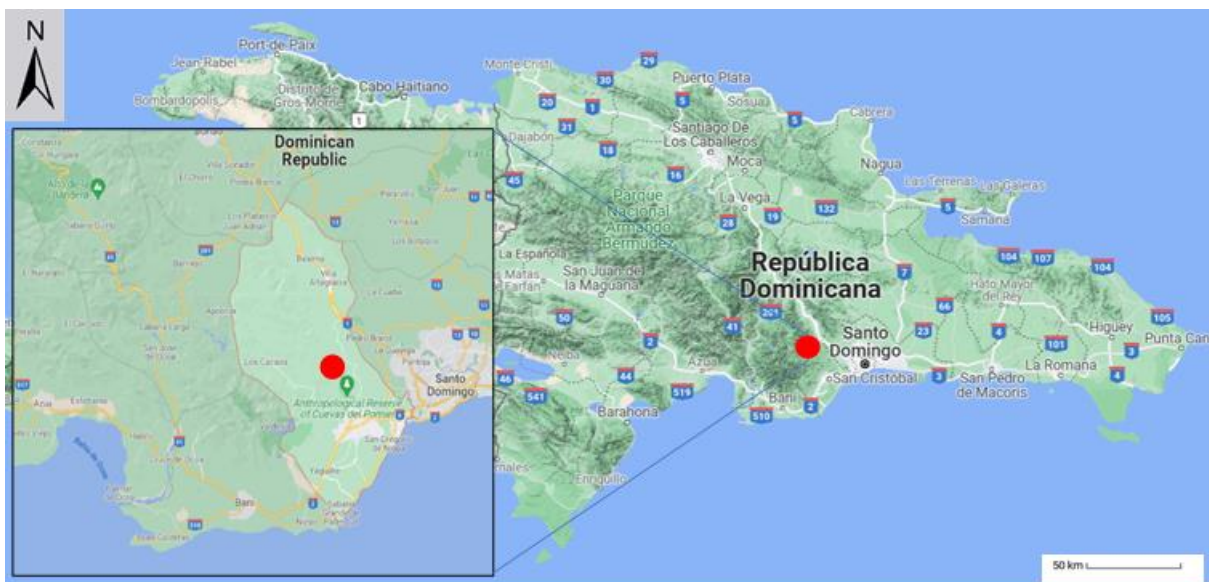


Figure 2 - Location (red dots) of San Cristóbal province and study area in the Dominican Republic. Adapted from Google maps, 2021. Dominican Republic, License Map data, available through <https://www.google.fr/maps/place/Dominican+Republic/@18.6691753,-71.251424,8z/data=!3m1!4b1!4m5!3m4!1s0x8eaf8>

This province has a winter dry equatorial climate (Aw in the Köppen classification) (Kottek et al. 2006). Average annual temperatures and precipitations range from 19°C (minimum monthly average) to 32 °C (maximum monthly average), and from 1600 mm (minimum annual average) to 1800 mm (maximum annual average), respectively . The average soil conditions are 3.2% organic matter, pH 6.9, 0.3 meq K/100 mL, 18 ppm Ca, 4.3 ppm Mg and 10 ppm P (Dehevels et al. 2020).

This province accounts for 1.7% of the national cacao production (Notaro 2019). The cacao-based AFS of San Cristóbal have a lower density of cacao trees and a higher density of associated plants than in

other main cacao producing areas of the Dominican Republic (Deheuvels and Notaro 2017). It presents concentrated cacao plantations areas, and the cacao-based AFS are similar enough to form a same agroecosystem, but still present internal variations. This study was carried out in a restricted area around the village *Loma Verde* in San Cristóbal (Fig.2).

3.1.3 Criteria for selecting cacao plantations

Our experimental design was an adaptation of a split-plot (Jones and Nachtsheim 2009), using purposive sampling (Ritchie et al. 2003). The final experimental plan is presented in appendix 3 and relies on a total sample of 24 cacao-based agroforestry plantations managed organically and without tillage, and accessible by road in a 1 hour radius from *Loma Verde*.

The criteria used to select our 24 cacao plantations were:

- The age of the plantation
- An area greater than 1000m²
- The presence of cacao pods heaps

The selection of the cacao plantations, delineation of the plots, selection of the sampling points and macrofauna sampling were realized from April, the 19th to June, the 2nd.

The agricultural practices evolve during the production cycle of cocoa plantations, especially in term of plant composition and distribution. In order to test the hypotheses 1, 2 and 4, cacao plantations were sampled among four age classes shown in Table 1.

Table 1 - Distribution of the sample of 24 cacao-based agroforestry plantations into four age-classes.

Age class	Description	Number of parcels
[0-3]	Initial stage, the trees are young and unproductive.	6
[4-10]	Transition stage, the trees are growing and some, including cacao trees, start their production.	6
[11-25]	Stabilized stage, the fruit trees are productive.	6
>70	Senescent stage, the production is reduced.	6

The age of each plantation is the number of years since its creation and was given by the owner.

We chose to leave a wide gap of 45 years between age classes 3 and 4, in order to maximize our chances to register any effect of aging on soil macrofauna and physico-chemical properties

3.1.4 Sampling plots in cocoa plantations

In each cocoa plantation, a 1000 m² plot was materialized following Rousseau et al. (2012), with stakes and strings and located in the best possible manner to avoid contact with the borders of the plantation. For this purpose, a transect walk was performed on each cocoa plantation, following Francis et al. (2012) and as shown in appendix 4.

San Cristobal cacao AFS are small and irregular in shape (Notaro 2019). For this reason, we had to adapt the shape of our sampling plots. Plots were square shaped, their dimensions were 20m x 50m or 25m x 40m, accordingly to the shape of the plantation and always in order to avoid contact with the borders.

The sampling plot delimitation was performed with stakes and strings, the day before macrofauna sampling for minimizing disturbance. Strings were removed just after macrofauna samplings in order not to interfere with farmer's work. The stakes were left standing until soil sampling

3.1.5 Sampling points in the sampling plots

The microtopography, low canopies and high tree densities made a reliable delineation method necessary to ensure a similar distance among sampling points in each plot. The first corner of the sampling plot was established following the Pythagorean theorem. Then, a stake was placed each 10 meters by a person visually guided by a second person to ensure linearity. If the last side of the plot did not have the expected length, the distance between each facing stake was measured for rectification (appendix 5).

On each plot, five sampling points were established at least five meters apart following the TSBF macrofauna sampling method (Anderson and Ingram 1989) (See paragraph 3.1.5.3) and using a purposive sampling method (Ritchie et al. 2003). This method allowed us to split plots between two alternate situations: (i) "soil covered with leaf litter" and (ii) "soil covered with cacao pod husks". The experimental plan was unbalanced (Table 2) because there were no pods husks in unproductive cacao plantations aged [0-3] and because we found less cacao pods heaps than expected in young and very old cacao plantations.

3.1.5.1 Presence/Absence of cacao husk heaps

In order to test the hypotheses 3 and 4, we selected cacao plantations where at least one heap of decomposing cacao husk was found, except for the parcels of the age class [0-3] because no cacao was produced at this stage. The cacao husk too recent (less than two weeks after harvest) were excluded and no cacao husk heaps was older than two month. The information was provided by the owner or, in few cases, it was estimated visually.

Table 2 – Contingency table of the experimental plan

Age class	Type of soil cover		Total
	Soil covered with heaps od cacao pod husks (repetitions)	Soil covered with leaf litter (repetitions)	
[0-3]	0	30	30
[4-10]	6	24	30
[11-25]	14	16	30
>70	10	20	30
Total	30	90	120

3.1.5.2 Sampling points for plant inventory

In order to test the hypotheses 2 and 4, the cultivated vegetation's density and diversity were measured. From the stake marking the sampling point, a disc of 4 meter radius (50.27m²) was established with measuring tape. Each plant higher than 1 meter inside this disc was inventoried following Deheuvels and Notaro (2017). However, in the age class [0-3], this heigh limit was reduced to 40cm, because most of the cultivated vegetation in this initial stage was smaller than 1m, and keeping this limit would have led to underestimate the actual vegetation density and diversity. There was few vegetation between 40cm and 1m in the other age classes.

3.1.5.3 Sampling points for macrofauna

The components of soil biodiversity are classified in three groups following their size: microorganisms, mesofauna and macrofauna. Microorganisms are from 20 to 200 µm long. It is the most abundant and diverse group, reasons why its study is complex. Besides, due to their size, the sampling and identification methods require advanced equipment. Mesofauna includes all organisms from 200 µm to 10 mm long. They have limited burrowing capacity (Ruiz and Lavelle 2008). Conversely, macrofauna includes animals from 2mm to 2cm long (DeLuca et al. 2019) which generally have an important burrowing activity. The study focuses on soil macrofauna.

The macrofauna sampling was carried out following an adaptation of the TSBF method (Anderson and Ingram 1989) : a 25cm x 25cm x 10 cm (6.25 dm³) metal frame was used to extract separately the litter, the 0 to 10 cm soil layer and the 10 to 20 cm soil layer. The litter and the soil layers were separated to reduce the time required for soil extraction. It also reduced the time laps between soil extraction and macrofauna extraction. The adaptation from the original method was also made to limit the escape of macrofauna during the extraction. We sampled the 20 to 30 cm subsoil horizon in the first six plots, and then discarded these samples because (i) this horizon was the most time consuming and (ii) these first samples represented less than 5% of the total macrofauna found.

First, the metal frame was systematically placed on each sampling point, and the litter found inside the metal frame was rapidly placed in a closed plastic box. Then, the metal frame was driven 10 cm down into the soil with a heavy hammer until its top reached the surface. The entire soil inside the frame was extracted with a shovel and placed into a different closed plastic box. The same process was executed for the second and last soil horizon.

After each monolith extraction, the macrofauna was hand-sorted with tweezers from each plastic box and placed inside referenced flasks filled with 70% alcohol. Each earthworm was photographed on site for color and pigmentation information.

A team of 5 young local volunteers was trained and hired to help with the sampling. They were trained to the adapted TSBF extraction method and received thorough and repeated explanations and instructions. Two of them helped extracting the monoliths and the whole team participated to the hand-sorting of invertebrates. The sampling was always realized during the morning

3.1.5.4 Sampling points for soil quality assessment

In order to test the hypothesis 1, 2, 3 and 4, soil was sampled following Mahler and Tindall (1994). In the plots without cacao husk heaps, a composite soil sample was produced out of 3 points randomly selected among the 5 macrofauna sampling points. In the plots with cacao husk heaps, one composite sample was made out of the sampling points covered with leaf litter, and a second one was made out of the sampling points covered with cacao husks. At each point, after removing the leaf litter layer, the first 20 cm of the soil were extracted with a spade. Subsamples were mixed together in a box and an approximative weight of 400g was extracted. A total of 38 composite soil samples was obtained.

In addition, bulk density was determined using the small cylinders method described in Baize (2018): The first 20 cm of the soil were extracted with a sampling cylinder of known volume. In the plots without cacao husks, the sampling was realized at a point randomly selected among the 5 sampling points. In the plots with cacao husk heaps, one was randomly selected among the sampling points under cacao pod husks, and a second one was randomly selected among the sampling points covered with leaf litter.

3.2 Processing the macrofauna samples

3.2.1 Identification

The macrofauna identification was carried out at the biological control laboratory of the UASD (*Universidad Autónoma de Santo Domingo*), under binocular loupes and by using several books and identification keys (Chu 1949, Fernández and Sharkey 2006, McGavin et al. 2002, Triplehorn and Johnson 2018). Macrofauna originally refers to individuals sized from 2mm to 20mm (DeLuca et al. 2019). In our study the invertebrates bigger than 20mm, belonging to taxonomical groups considered as macrofauna, were included. The taxon *Entognatha*, *Collembola*, *Diplura* and *Acari* which are

sometimes considered as mesofauna (Gongalsky 2021) were included in the study because collected individuals entered the chosen size range of 2 to 20mm.

3.2.1.1 Taxonomic approach

Each invertebrate was identified at taxon level, and classified by morphotype (usually, one morphotype corresponds to one species), except from the earthworms. The first morphotype identified in a taxon was registered as the morphotype 1 of this taxon and so forth for each new morphotype. Each morphotype was photographed with the binocular loupe, and conserved in 70% alcohol in an individual 1.5mL flask. Taxa were generally Orders, with some exceptions: earthworms formed one taxon, *Hemiptera* was considered as an Order, even if some taxonomists consider *Hemiptera* as a superorder that includes *Homoptera* and *Heteroptera*. This choice was made for the sake of consistency, because it was the systematic used in the books and determination keys that were used for identification. Moreover, *Hemiptera* is a monophyletic group clearly identifiable based on its mouthparts and this classification is used by many entomologists (Forero 2008, Entomological Society of America 2021). The termites were classified following the old systematic as *Isoptera*. Indeed, they became *Blattodea* in 2018 for phylogenetic evidence (Inward et al. 2007, Milius 2018) but it was important to separate them from the other morphotypes of the order *Blattodea* as they have different functions, principally due to their social organization and bioturbation services.

3.2.1.2 Functional approach

An exclusively taxonomic approach of the diversity would not provide a full understanding of how soil macrofauna diversity can be influenced. We chose to include in our study an assessment of the functional diversity (Postma-Blaauw et al. 2010). Some species have similar functions and can replace one another in their ecosystem, they form the same functional group (Brussaard 1998). The balance between functional groups is a condition to sustainable agroecosystems (Marasas et al. 2001). Macrofauna species influence each other especially through competition for food and habitat (Decaëns 2010).

The taxa were organized in four functional groups according to their trophic and rheagocic function: predators, phytophagous, detritivores and ecosystem engineers (Arditi et al. 2005). The rheagocic group “ecosystem engineers” gathered the earthworms, *Isoptera* and *Hymenoptera* (Table 3). They could have been grouped according to the three trophic categories (predator, phytophagous or detritivore), but this classification was chosen because the ecosystem engineers have strong impacts on their environment, that are not directly linked to their trophic role. It seemed important to adopt this second approach because “The functional importance of invertebrate activities is often disproportionate to their actual abundance” (Anderson 1988, cited in Lavelle 1996).

The principal functional traits of earthworms were measured. The earthworms of each sample were individually weighted with a balance of a 0.1 mg accuracy to obtain their body mass (mg) and calculate

their total biomass (mg.m^{-2}). We considered that the fresh body weight was measured, as the alcohol used for conservation approximately replace the body water. They were measured with a ruler of 1 mm accuracy to get their average body length (mm). The body mass and the body length are commonly used functional traits for soil invertebrates (BETSI 2021). The adult specimens were photographed with the binocular loupe for further identification.

Table 3 - Functional classification used for soil macrofauna

Predators	Phytophagous	Detritivore	Engineer
<i>Acari</i>	<i>Coleoptera</i> (larvae)	<i>Blattodea</i>	Earthworms
<i>Araneae</i>	<i>Dermoptera</i>	<i>Collembola</i>	<i>Hymenoptera</i>
<i>Coleoptera</i> (adult)	<i>Diptera</i> (adult)	<i>Diplopoda</i>	<i>Isoptera</i>
<i>Diplura</i>	<i>Stylommatophora</i>	<i>Diptera</i> (larvae)	
<i>Pseudoscorpionida</i>	<i>Pulmonata</i>	<i>Embiopoda</i>	
<i>Schizomida</i>	<i>Hemiptera</i>	<i>Isopoda</i>	
<i>Scolopendrida</i>	<i>Lepidoptera</i>	<i>Psocoptera</i>	
<i>Scorpiones</i>	<i>Neuroptera</i>		
<i>Uropygi</i>	<i>Orthoptera</i>		
	<i>Thysanoptera</i>		

3.2.2 Density and diversity determination.

3.2.2.1 Density

The number of individuals of each morphotype in each one of the three layers (litter, 0-10 cm layer, 10-20 cm layer) was recorded during the identification.

However, litter and both horizons were gathered for the statistical analysis because we considered them as a whole system, in which macrofauna is constantly moving. It is especially true for anecic earthworms that feed on the litter but live in vertical galleries (Blouin et al. 2013), or ants that forage in the litter and nest underground (Fowler 1993).

For each sampling point, the density of each taxon and the density of each functional group was calculated, using the software Excel and its interface for VBA programming. The abundance by sampling point was multiplied by 16 to get the density (ind.m^{-2}).

3.2.2.2 Diversity

The soil macrofauna diversity was also calculated by sampling point. Three classical diversity indices were calculated (Marcon et al. 2015) with Excel's interface for VBA programming and with the package *vegan* (Oksanen et al. 2020) in R:

1. The **specific richness (S)**, which is the number of species (here morphotypes) that were found at a given sampling point.

2. The **Shannon index (H)**, which combines species richness and species evenness (the relative proportions of each species encountered), is expressed as:

$$H = - \sum_{s=1}^S p_s \ln p_s.$$

Where S is the number of species or morphotypes encountered, and p_s is the proportion of species s.

3. The **Simpson index (E)**, which is an evenness index, that slightly varies with S. It goes from 0 to 1 and can be interpreted as the probability that two individuals randomly selected belong to the same species. It decreases with the regularity of the distribution of the taxa and is expressed as:

$$E = 1 - \sum_{s=1}^S p_s^2.$$

Where S is the number of species or morphotypes encountered, and p_s is the proportion of species s.

3.3 Plant inventories

3.3.1 Density

The number of plants inventoried in the 4 meters radius disc around the sampling point was divided by the area of this disc (50.27m^2), to get a density measure comparable with other studies (ind.m^{-2})

3.3.2 Diversity

The same 3 classical diversity indices presented in paragraph 2.8.2.2 were calculated for the vegetation.

3.4 Soil analysis

In order to test the hypotheses 1, 2, 3 and 4, the 38 composite soil samples were sent to the laboratory less than 24h after sampling, for chemical and physical analysis.

Chemical analyses included:

- The pH, measured by a potentiometric pH-meter. It determines the voltage difference between two electrodes disposed in the soil solution.
- The exchangeable cations Ca^{2+} , Mg^{2+} , Na^+ (meq/L) were measured by the ammonium acetate method. It consists in mixing the soil solution with an excess of ammonium solution, which exchanges its cations with the soil exchangeable cations, and then measuring the difference between the initial and the remaining ammonium content.
- The electrical conductivity (mS/cm), was determined by measuring the conductance of the soil solution with a conductivity meter.

- The calcium carbonate CaCO_3 content (%) was measured by volumetric calcimetry: in this method, the CO_3^{2-} is converted in CO_2 by adding hydrochloric acid to the soil solution, and the volume of the CO_2 released is measured to determine the initial CaCO_3 content.
- The organic matter (%), organic carbon (%) and nitrogen (%) were measured by the Walkley-Black method where the oxidizable matter is oxidized by a potassium dichromate solution. The remaining dichromate is then titrated with ferrous sulphate, and the amount of C in the initial soil solution is deducted. The organic matter and the Nitrogen content were determined from the organic content.
- The phosphorus content P (mg/kg) that was measured by near infrared reflectance spectroscopy.

Physical analyses included:

- The texture, that was measured by the Bouyoucos method where the density of the soil solution is measured by a hydrometer after two hours of sedimentation, and the particles size is determined from this density. It is expressed in percentages of sand, silt and clay;
- The bulk density where the dry weight of the bulk density samples was divided by the volume extracted.

All the analysis were performed by the soil laboratory of the INDRHI (*Instituto Nacional de Recursos Hidráulicos*) in Santo Domingo.

3.5 Statistical analysis

All the statistical analysis were realized using R (R core team 2021).

3.5.1 Data exploration by descriptive analysis

3.5.1.1 PCA on macrofauna densities

In order to test the hypotheses 1 and 3, a PCA was performed on the macrofauna densities.

Principal Component Analysis was used to resume the database into synthetic variables and compare the samples points according to these synthetic variables, using the packages *ade4* (Thioulouse et al. 2018), *adegraphics* (Siberchicot et al. 2017), *FactoMineR* (Le et al. 2008) and *Factoshiny* (Vaissie et al. 2021). These first analysis aimed at revealing if the sampling points were correlated according to the **macrofauna** variables, and if these variables were correlated together.

Once the correlations between samples according to the minimum best explicative principal components was revealed, it was attempted to explain these correlation by the variables **age of the plantation** and **type of soil cover**, using clustering and between-class analysis (BCA) with the package *ade4* (Thioulouse et al. 2018). With this same package, a Monte-Carlo test based on 999 replicates was used to test the significance of the differences between clusters.

3.5.1.2 PCA on soil parameters

In order to test the hypotheses 1 and 3, the same analysis as presented in the paragraph 3.5.1.1 were performed with the database of soil chemical and physical parameters.

3.5.1.3 Complementary descriptive analysis

The hypotheses 1 and 3 question the responses of the soil quality and its macrofauna to two independent variables. As it is known that soil organisms and soil quality influence each other, it is interesting to explore the correlations between them in the frame of this study, in order to get a better understanding of the results. For this purpose, a co-inertia analysis was performed between the PCA on macrofauna densities and the PCA on soil parameters, to reveal covariations between both datasets. A Monte-Carlo test based on 999 replicates on the sum of eigenvalues of this co-inertia analysis was performed to test the significance of the covariation. The package *ade4* was used for both analysis. A correlation test based on Pearson coefficient was performed to reveal correlations between the variables of each dataset.

3.5.2 Explicative analysis

3.5.2.1 Univariate analysis

The hypothesis 2 was tested in two stages.

The first stage aimed at testing the evolution of the agricultural practices, in terms of plants density and diversity, during the different phases of the production cycle of the cacao plantation. This was part of the hypothesis 2. For this purpose, two one-way ANOVAs were performed to compare the means of Simpson index and density of the vegetation between the 4 age classes. Linear models were established with, as response variables (i) the density of plants, and (ii) the Simpson index of plants, and as explanatory variable the age of the plantation. The assumption of normality and homoscedasticity were graphically verified. The model was tested by calculating the F-test and its p-value with the function *aov* of the package *stats* (R core team 2021). The significance of the differences between the means of the levels of the independent variable was tested with the Tukey test, with the function *TukeyHSD* of the package *stats*.

The second stage aimed at testing the influence of the heterogeneity of plant composition and distribution on the soil macrofauna abundancy and diversity. For this purpose, four two-by-two Pearson's correlation tests were performed between (i) the total density of macrofauna and the density of vegetation (ii) the total density of macrofauna and the diversity (Simpson index) of vegetation (iii) the diversity of macrofauna and the density of vegetation (iv) the diversity of macrofauna and the diversity of vegetation.

3.5.2.2 Multivariate analysis

In order to test the hypotheses 1, 3 and 4, several models were created and compared to test the effect of the age class and the cacao pods on the soil parameters and on the macrofauna density and diversity,

using the packages lmerTest (Kuznetsova et al 2017) and emmeans (Russel 2021). The assumption of normality of the residuals was verified graphically, and with the Shapiro-Wilk test. When the residuals did not follow a normal distribution, the response variable was transformed by the function logarithm. When the normal distribution could not be reached, we realized *generalized linear models* (GLMs). GLMs allow the residuals of the response variable to follow a different distribution than the normal distribution (Poisson, binomial, binomial negative, Gamma). The law to which the residuals best fitted was searched visually and with the Chi-squared test. The homoscedasticity was checked visually.

For each response variable, the 6 models presented in table 4 were compared.

Table 4 - Predictive variables included in the different models

		Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Fixed parameters	Type of soil cover	x	x		x		
	Age class	x	x	x			
	Interaction of the type of soil cover and the age class	x					
Random parameter	Plot	x	x	x	x	x	

Models were compared with the Akaike Information Criterion (AIC). Its expression is $AIC = -2 * \log(L) + 2*k$. L is the likelihood of the model and measures its goodness of fit to the data. K is the number of parameters in the model (Burnham et al. 2011). For each response variable, a complete model with the fixed effects of the age class, the type of soil cover, their interaction and the random effect of the plot (Model 1) was created. For this model, the distribution law best fitting to the residuals was searched. Then, all nested models, including the null model, were established (Models 2 to 6). The AIC of the models were calculated and compared together to select the best fitting model. For this model, the marginal means of the predictive factors were calculated. The significance of the difference between the marginal means was determined by calculation of the standard error. If the standard errors of the marginal means of two modalities did not intercept, they were considered as significantly different. The packages that were used allow for analysis on unbalanced dataset. However, when the full model with interaction was the best fitting model, the analysis was performed again with a different dataset from which all the plots of the age class [0-3] were excluded. It improved the understanding and interpretation of the results.

4 Results

12 413 individuals of 608 morphotypes and 27 taxa were identified. The most abundant taxa were *Hymenoptera* (43.2%), *Isoptera* (22.7%) and earthworms (11.8%). The most diverse taxa were *Coleoptera* (123 morphotypes), *Araneae* (114 morphotypes), *Hemiptera* (53 morphotypes) and *Hymenoptera* (44 morphotypes). A table summarizing all the data collected is presented in appendix 2

4.1 Data exploration by descriptive analysis

The mean abundances and diversity of soil macrofauna in the four age classes and two types of soil cover are presented in appendix 6.

4.1.1 Exploration of the macrofauna dataset

4.1.1.1 Taxonomic approach

4.1.1.1.1 Plot level

In this first PCA, the statistical units were the plots and the variables used to create the hyperspace were the taxa (Fig.3).

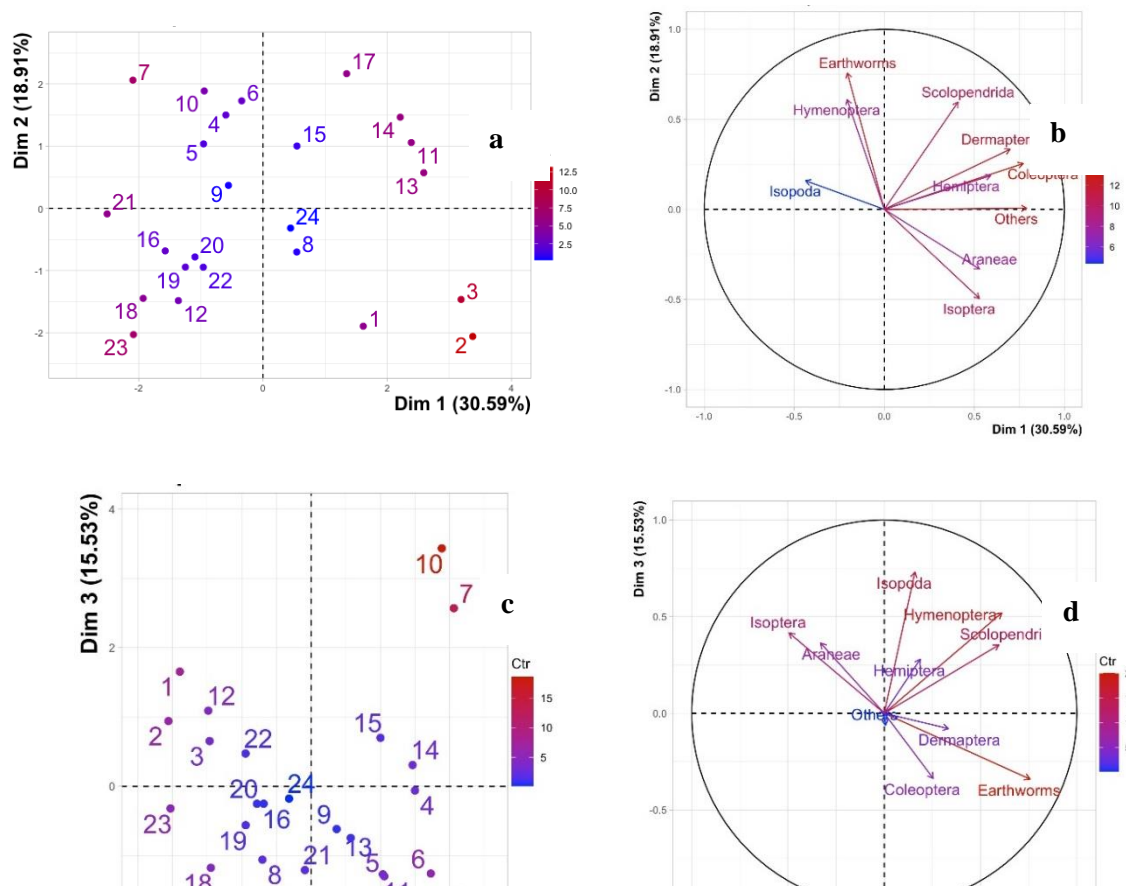


Figure 3 - PCA of the taxa abundances(per plots). (a) Graph of the plots according to the axes 1 and 2 (b) Correlation circle according to the axes 1 and 2 (c) Graph of the plots according to the axes 2 and 3 (d) Correlation circle according to the axes 2 and 3

The first three axes of the PCA accounted for 65.02 % of the variance. The first axis (eigenvalue = 30.59%) represented a positive gradient of Others, *Coleoptera* and *Dermaptera* abundances. The second axis (eigenvalue = 18.91%) represented a positive gradient of earthworms, *Hymenoptera* and *Scolopendrida* abundances. The third axis (eigenvalue = 15.53%) represented a positive gradient for *Isopoda* and *Hymenoptera* abundances.

Plots were separated into 5 clusters along the first two axis.

PC 1 was negatively correlated with the cluster 1 and positively correlated to the cluster 4 (Fig. 4). PC 2 was positively correlated with cluster 2 and 3, and negatively correlated to the cluster 5. The cluster 5 grouped plots that all belonged to different age classes, but they were geographically very close to each other. The cluster 2 grouped plots 7 and 10 from the same age and slope classes, and geographically close to each other. The cluster 1, 3 and 4 grouped plots from different age classes and not geographically close to each other.

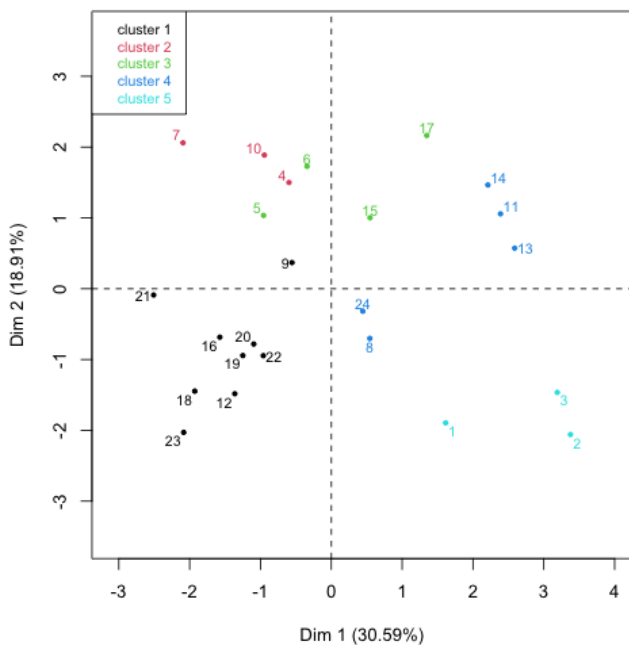


Figure 4 - Plot clustering according to the first two axes of the PCA

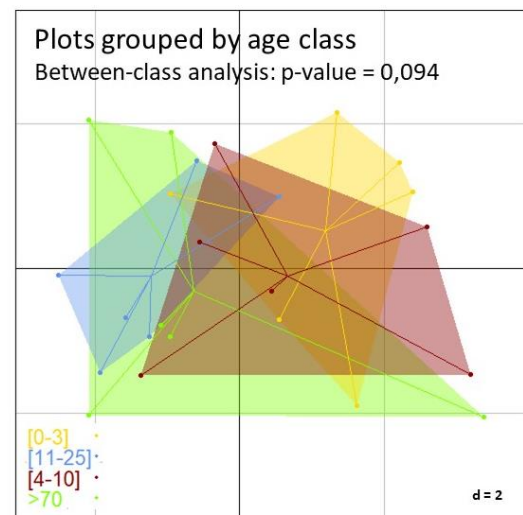


Figure 5 - Plots grouped by age class according to the first two axes of the PCA.

The between-class analysis showed that the groups of plots according to their age class were not significantly different from each other in the hyperspace created by the taxa densities (p-value= 0.094) (Fig. 5).

4.1.1.1.2 Sampling unit level

The statistical units of the second PCA are the sampling units (Fig.6).

The first three axis of the PCA accounted for 53.83 % of the variance. The first axis (eigenvalue = 26.32%) represented a positive gradient for *Dermaptera*, *Others*, *Coleoptera* and *Hemiptera* abundances. The second axis (eigenvalue = 14.91%) represented a positive gradient for *Scolopendrida* and *Isopoda*. The third axis (eigenvalue = 12.61%) represented a positive gradient for *Hymenoptera* abundance.

Sampling units were separated into 3 cluster along the first two axis (Fig.7). The cluster 2 was slightly positively correlated with the second axis and the cluster 3 was positively correlated with the first axis. All the sampling units in the cluster 3 have a soil cover of cocoa pods.

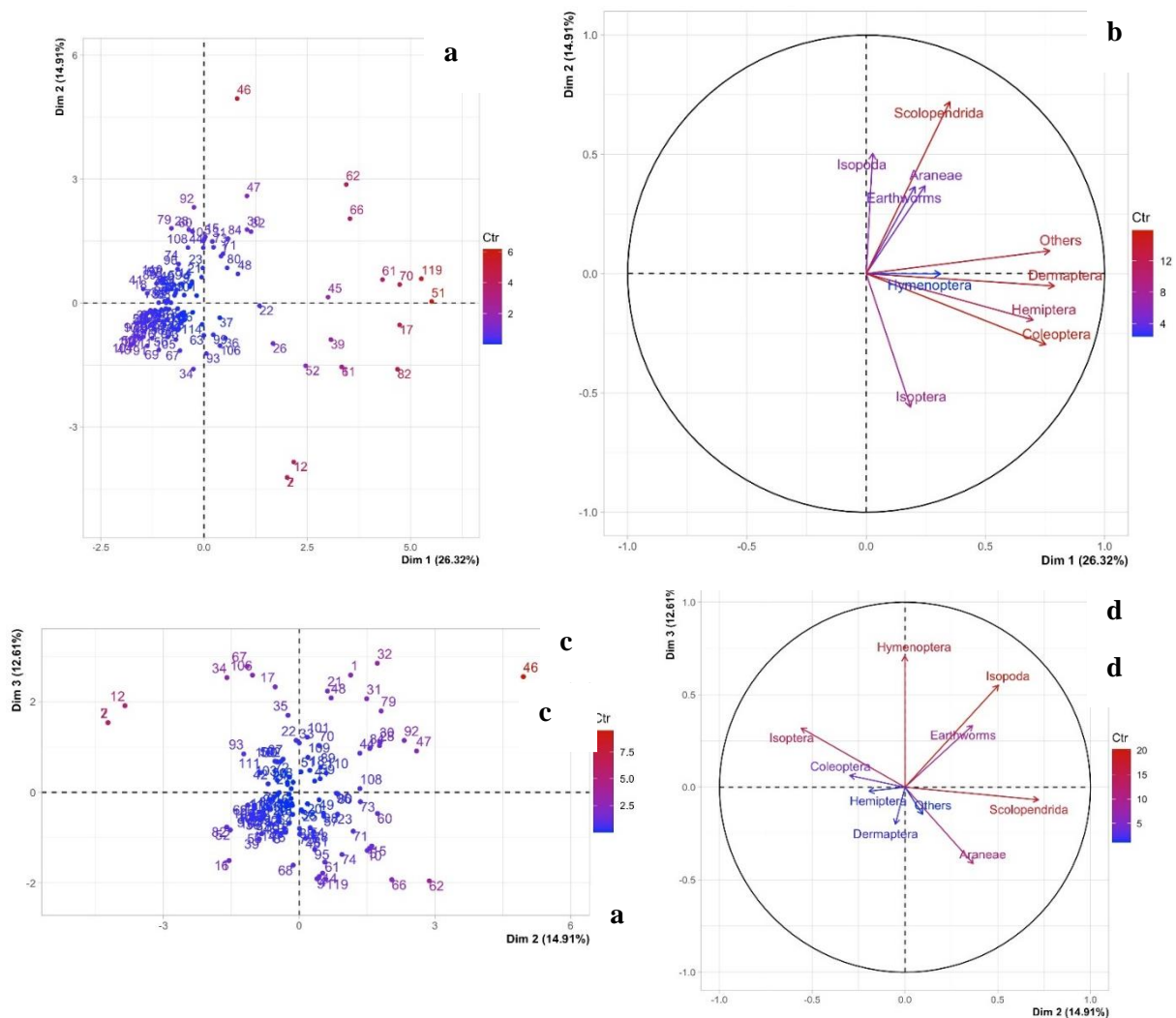


Figure 6-PCA of the taxa abundances (per sampling unit). (a) Graph of sampling units according to the axes 1 and 2 (b) Correlation circle according to the axes 1 and 2 (c) Graph of sampling units according to the axes 2 and 3 (d) Correlation circle according to the axes 2 and 3

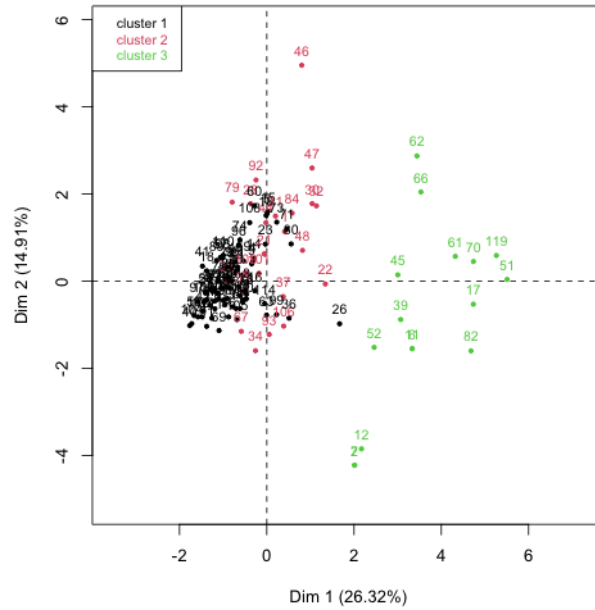
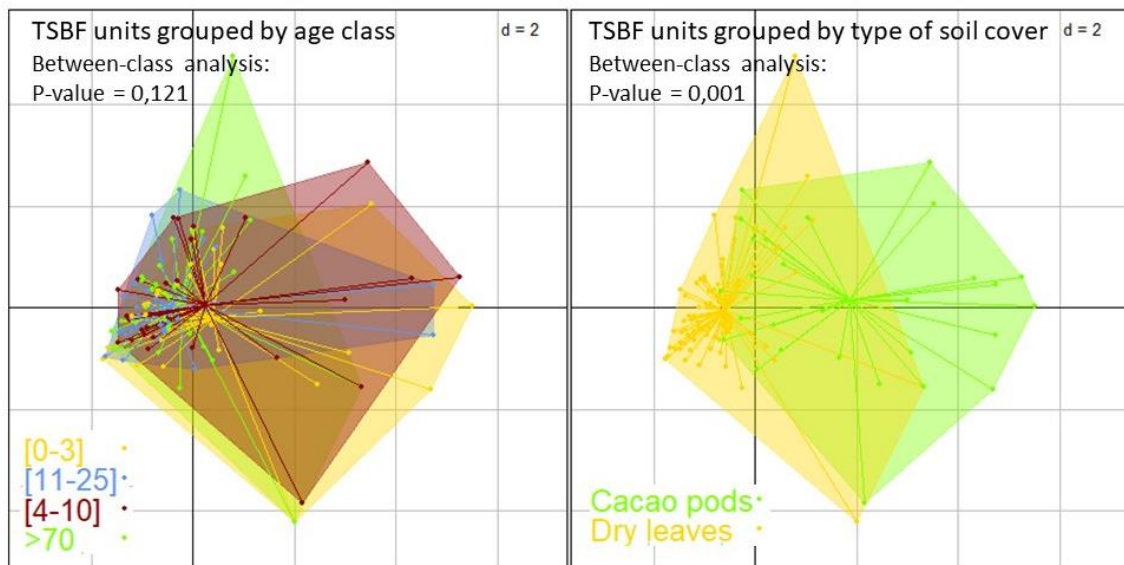


Figure 7 - Sampling units clustering according to the first two axes of the PCA

The between-class analysis showed no difference between the groups of sampling units according to their age class (p-value = 0.121) It showed a highly significant difference between the groups of sampling units according to their type of soil cover (p-value = 0.001) (Fig.8).



b

Figure 8 – Sampling units grouped by age class (a) and by type of soil cover (b) according to the first two axes of the PCA.

4.1.1.2 Functional approach

The results of our classification by functional groups are presented in table 5.

Table 5 – Sampled macrofauna data by functional groups

	Predators	Phytophagous	Detritivores	Engineers
Relative abundance (%)	15.1	7.8	2.7	74.4
Min (ind.m ⁻²)	0	0	0	0
Max (ind.m ⁻²)	1584	1296	736	9232
Mean (ind.m ⁻²)	265.3	135.2	43.1	1338.5
Standard error (ind.m ⁻²)	22.4	19.4	7.5	166.6

A PCA was performed on the functional groups. The first three axes of the PCA accounted for 88,6 % of the variance (Fig.9). The first axis (eigenvalue = 47.95%) represented a positive gradient for Phytophagous and Predator abundance. The second axis (eigenvalue = 23.67%) represented a positive gradient for Ecosystem Engineers abundance. The third axis (16.98%) represented a negative gradient for Detritivore abundance.

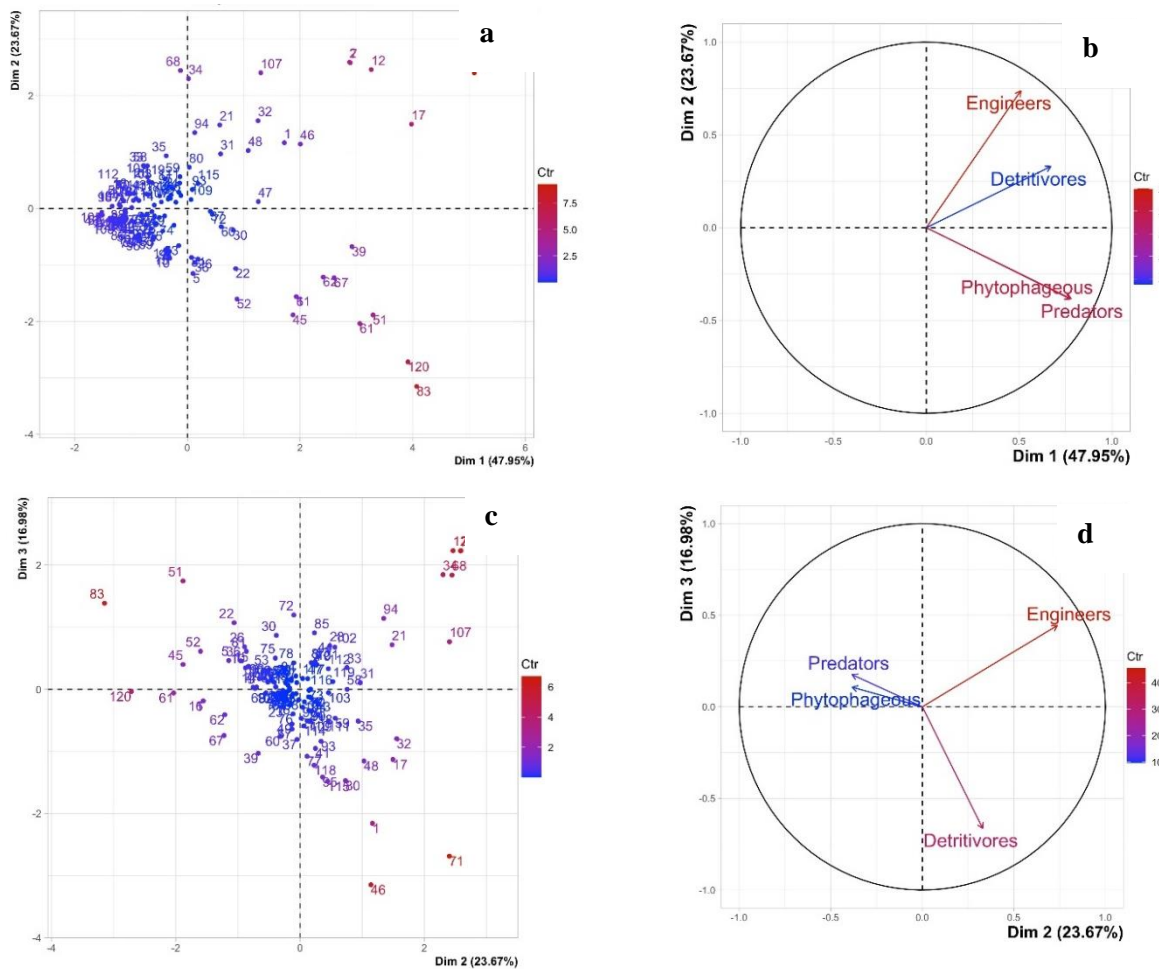


Figure 9 - PCA of the functional groups abundances. (a) Graph of sampling points according to the axes 1 and 2 (b) Correlation circle according to the axes 1 and 2 (c) Graph of sampling points according to the axes 2 and 3 (d) Correlation circle according to the axes 2 and 3

Sampling points were separated into 3 cluster along the two first axis (Fig.10). The cluster 2 was positively correlated with the second axis and the cluster 3 was positively correlated with the first axis. All the sampling points grouped in the cluster 3 had a soil cover of cocoa pods.

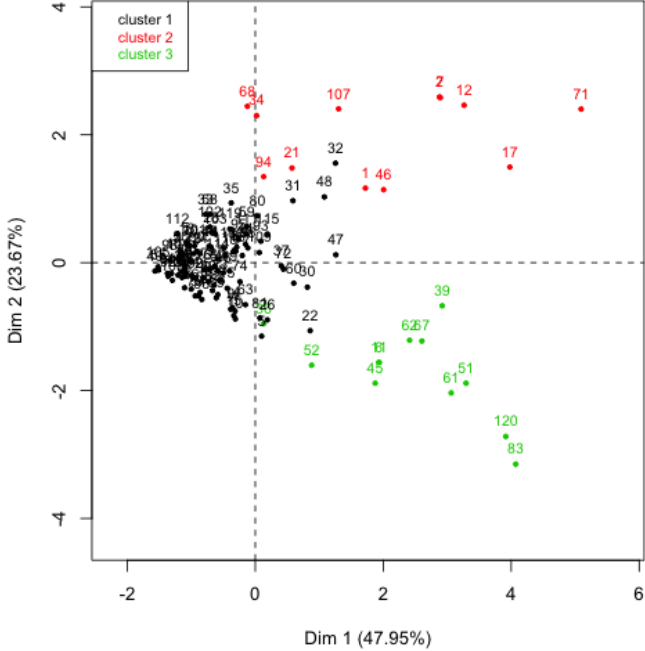


Figure 10 - Sampling units clustering according to the first two axes of the PCA

The between-class analysis showed no significant difference between the age classes (p-value = 0.071). However, it revealed a highly significant difference between the type of soil cover (p-value = 0.001) (Fig.11)..

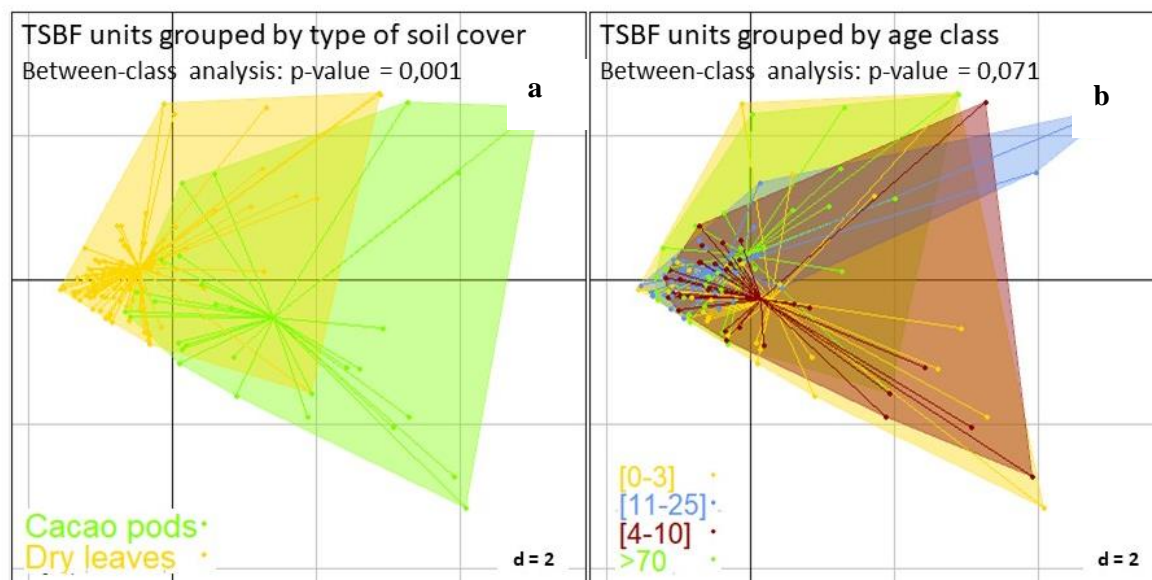


Figure 11 – Sampling units grouped by age class (a) and by type of soil cover (b) according to the first two axes of the PCA

1.1.1 Exploration of the soil dataset

A PCA was performed on the soil parameters dataset (Fig. 12). The 38 individuals of the PCA (statistical units) were the soil composite samples. The first three axis accounted for 65.3% of the variance. The first axis (eigenvalue = 31.43%) represented a positive gradient for OC, OM and N contents. The second axis (eigenvalue = 20.1%) represented a positive gradient of conductivity opposed to clay content. The third axis (eigenvalue = 13.76%) represented a positive gradient of CaCO₃ and P content.

Composite samples were separated into three cluster along the first two axis (Fig. 15). The cluster 1 was negatively correlated to the first axis. It had high values of sand, bulk density and pH and low values of silt, clay, OC, OM and N. It. The cluster 2 had low values of CE, Sand, Ca²⁺ and Na⁺ and high values of clay and silt. The cluster 3 was positively correlated with the first axis. The three composite samples of this cluster all have a soil cover of cocoa pods.

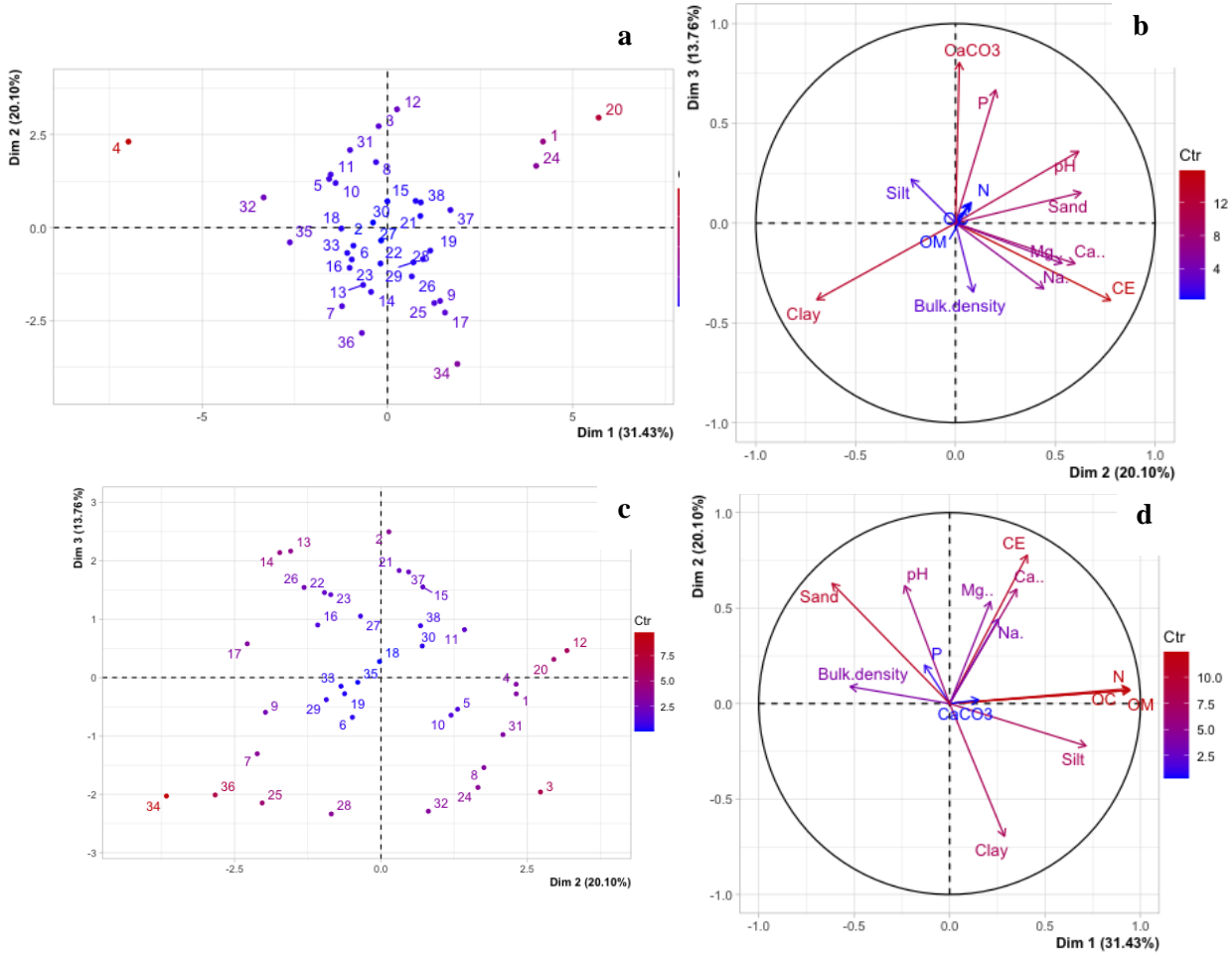


Figure 12 - PCA of the soil chemical and physical parameters (a) Graph of the composite samples according to the axes 1 and 2 (b) Correlation circle according to the axes 1 and 2 (c) Graph of the composite samples according to the axes 2 and 3 (d) Correlation circle according to the axes 2 and 3

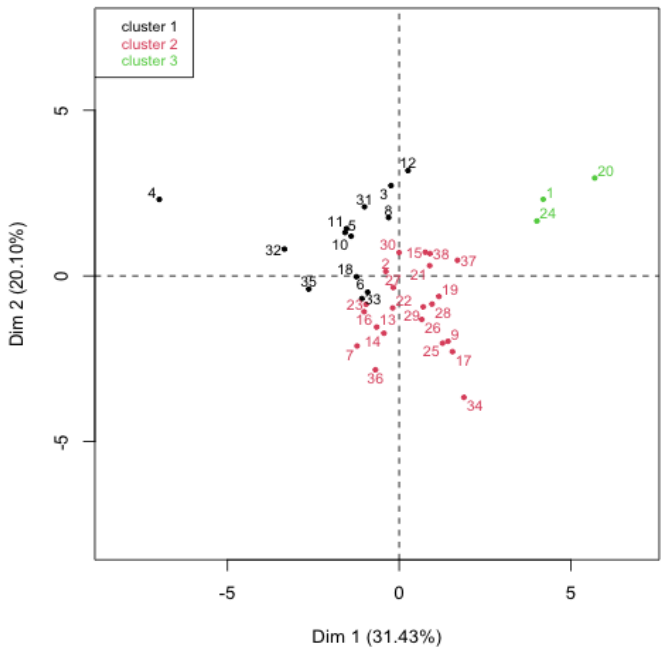


Figure 13 - Composite samples clustering according to the first two axes of the PCA

The between-class analysis did not show significant differences neither between the age classes (p-value = 0.166) nor between the type of soil cover (p-value = 0.126). However, it revealed a very significant difference between the degree of slope (p-value = 0.006) (Fig.14).

1.1.2 Interactions between the soil quality and the soil macrofauna

In this section, the interactions between the soil quality and the soil macrofauna were tested.

A PCA of the macrofauna with the composite soil samples was performed preliminary to the co-inertia analysis. The co-inertia analysis (Fig. 15) revealed significant covariations between the macrofauna dataset and the soil physical and chemical properties dataset. The first two axis of the co-inertia accounted for 77.99% of the variance. The RV coefficient was 0.26 and a permutation test revealed its significantly (p-value=0.049). It means that there was a significant correlation with the macrofauna dataset and the soil dataset.

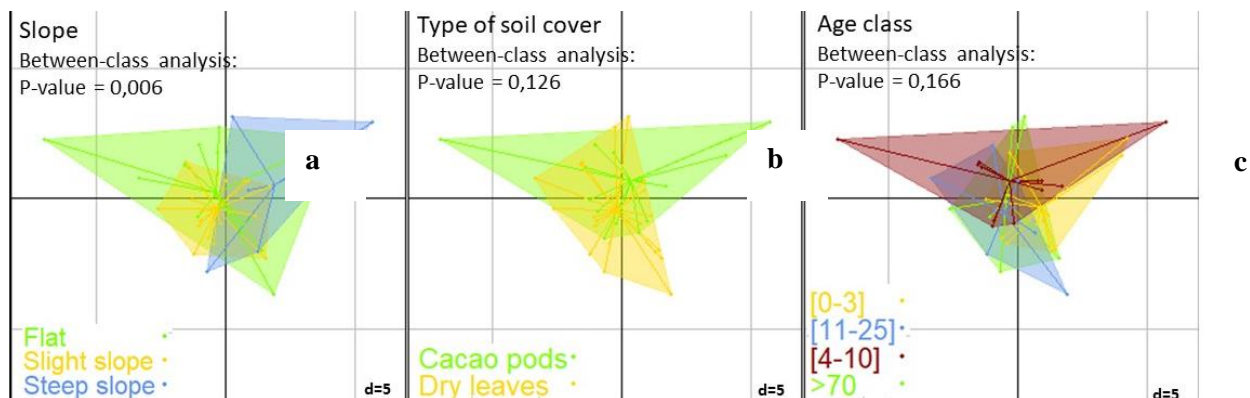


Figure 14 - Composite samples grouped by degree of slope (a), type of soil cover (b) and by age class (c) according to the first two axes of the PCA.

The correlation analysis based on Pearson coefficient (Fig.16) revealed which variables were correlated together. The density of *Isoptera* was positively correlated with the sand content ($r^2=0.57$, p-value <0.001) and negatively correlated with the silt and clay content ($r^2= -0.44$ and p-value < 0.01 for both). It was also negatively correlated with the organic carbon (OC), organic matter (OM) and Nitrogen (N) contents ($r^2= -0.45$ and p-value <0.01 for all).

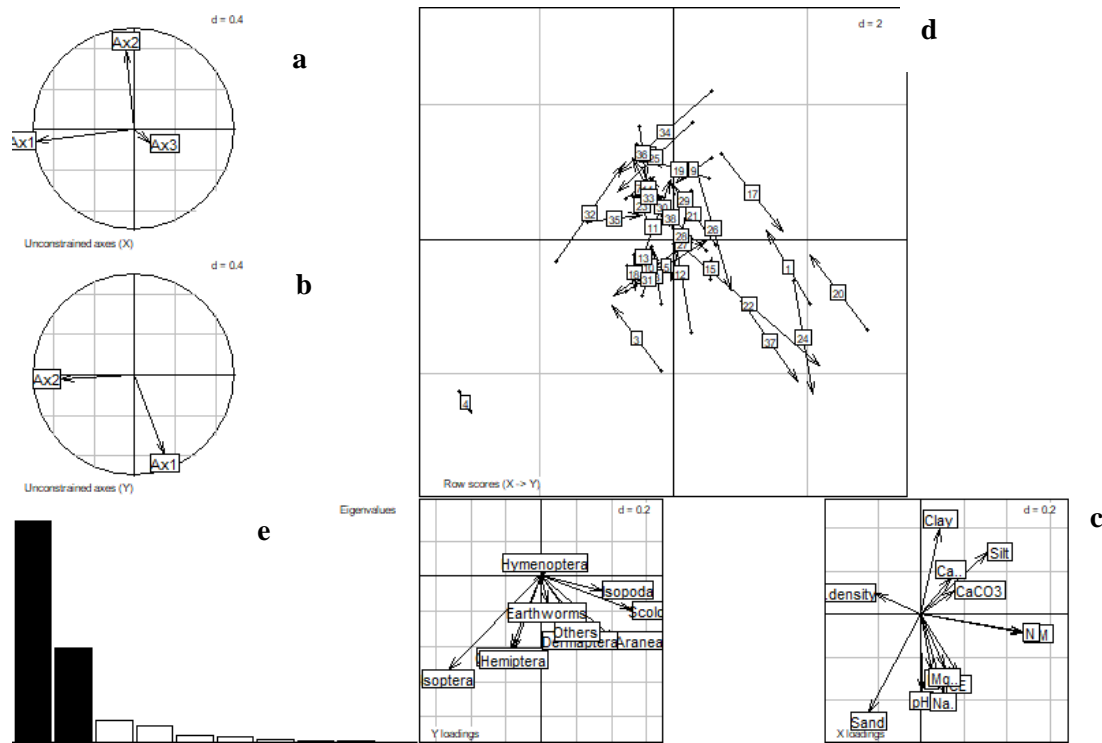


Figure 15 - Co-inertia analysis between the PCA on soil chemical and physical parameters and the PCA on macrofauna taxa abundances. (a) Projection of the soil PCA axes into the axes of the co-inertia analysis. (b) Projection of the macrofauna PCA axes into the axes of the co-inertia analysis. (c) Contribution of the variables to the co-inertia analysis. (d) Position of each composite sample described by the soil dataset (beginning of the arrow) and by the macrofauna dataset (end of the arrow). (e) eigenvalues.

On the contrary, the density of *Scolopendrida* and *Araneae* were positively correlated with the organic carbon, organic matter ($r^2=0.53$ and $r^2=0.39$ for both, respectively) and Nitrogen contents ($r^2=0.52$ and $r^2=0.40$, respectively), with p-values < 0.05 for all correlations with *Araneae* and p-values <0.001 for all correlations with *Scolopendrida*.

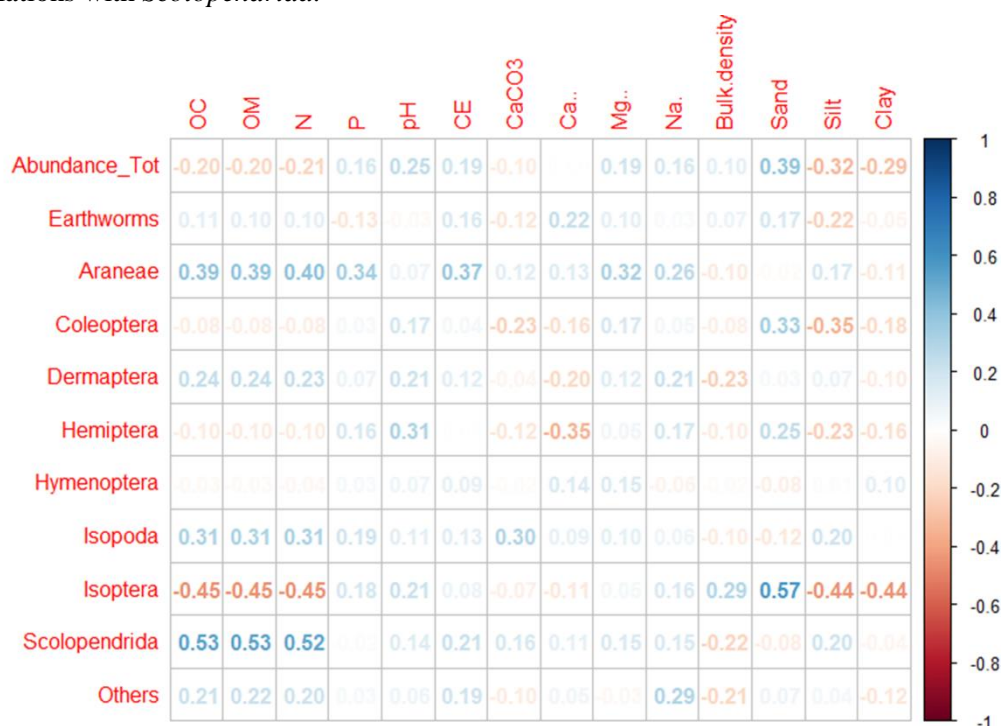


Figure 16 - Correlations between macrofauna densities and soil chemical and physical parameters

4.2 Explicative univariate analysis

4.2.1 Evolution of the plant density and diversity at plot scale during the production cycle (hypothesis 2)

The age class [11-25] had the lowest limit (D = 0.253) and the lowest mean (D = 0.0331) of diversity index (Fig. 17), while the age class [0-3] has the highest value (D = 0.674) and the highest mean (D = 0.597). The model of response variable “Diversity of the cultivated vegetation (Simpson index)” and independent variable “age of the plantation” was statistically significant (F-value=9.9, p-value= 7.28e-

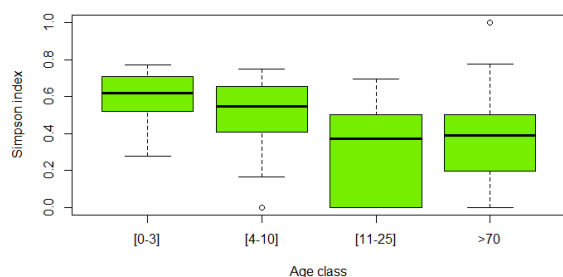


Figure 17 - Diversity of the cultivated vegetation for each age class

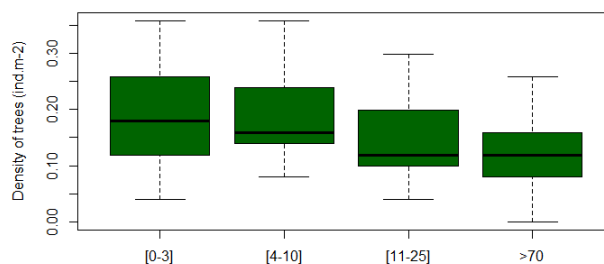


Figure 132 - Density of the cultivated vegetation in each age class.

06). The diversity was highly significantly higher in the age class [0-3] than in the age classes [11-25] (p-value = 0.00003) and >70 (p-value = 0.0002). It was significantly higher in the age class [4-10] than in the age class [11-25] (p-value = 0.021). The age class >70 had the lowest limit (0.103 ind.m⁻²) and the lowest mean (0.130 ind.m⁻²) of density (Fig. 18), while the age class [0-3] had the highest limit (0.216 ind.m⁻²) and the highest mean (0.191 ind.m⁻²). The model of response variable “density of the cultivated vegetation” and independent variable “age of the plantation” was statistically significant (F-value=5.4, p-value= 0.00154). The density of trees at the sampling unit level was significantly higher in the age class [0-3] than in the age class >70 (p-value = 0.008). There was no difference between the other age classes.

4.2.2 Influence of the plant density and diversity on the soil macrofauna (hypothesis 2)

No effect of the vegetation diversity or density was revealed, neither on the macrofauna diversity nor on the macrofauna density (Fig.19). (i) Total density of macrofauna and density of vegetation: coefficient of correlation = 0.10, p-value = 0.27 (ii) Total density of macrofauna and diversity (Simpson index) of vegetation: correlation coefficient = -0.02, p-value = 0.83 (iii) Diversity of macrofauna and density of vegetation: coefficient of correlation = -0.09, p-value = 0.29 (iv) Diversity of macrofauna and diversity of vegetation: coefficient of correlation = -0.10, p-value = 0.27.

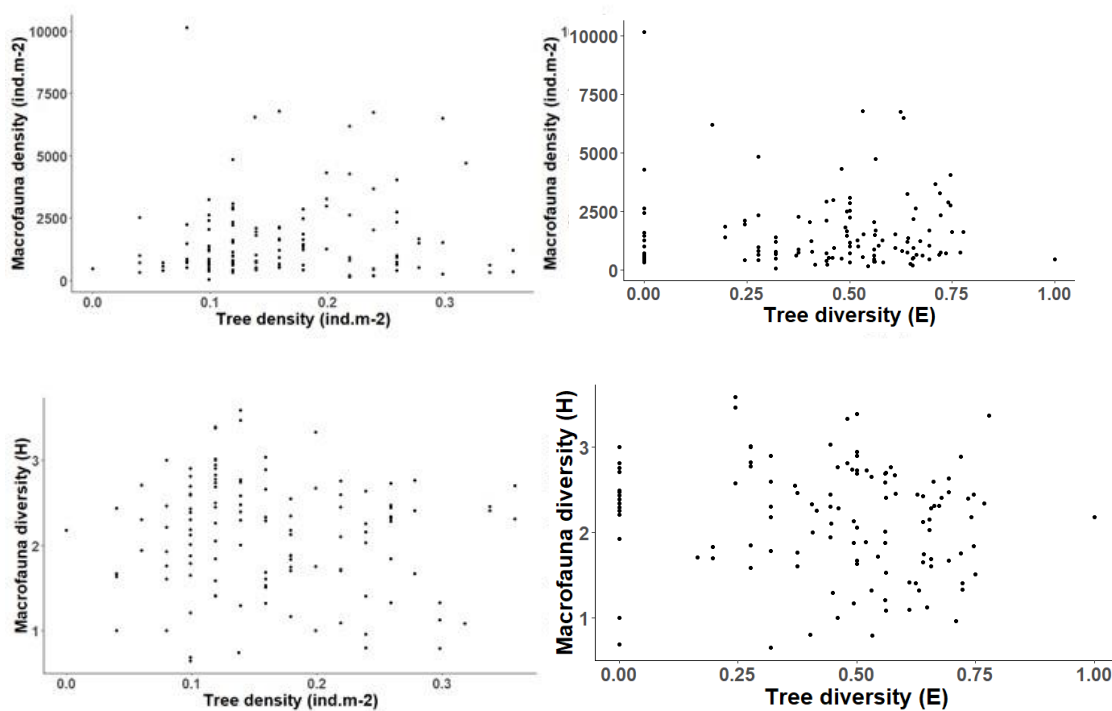


Figure 19 - Macrofauna density and diversity as functions of tree density and diversity

4.3 Explicative multivariate analysis

4.3.1 Modeling the macrofauna parameters according to the age of the plantation and the presence of cacao pods (hypotheses 1, 3 and 4)

In this section the hypotheses 1, 3 and 4 were tested. The influence on the soil macrofauna abundance and diversity of the age of the plantation (hypothesis 1), the presence of cacao pods husks (hypothesis 3) and the interactions between the age of the plantation, and the presence of cacao pods husk (hypothesis 4) were tested.

The total abundance, the abundance of each taxon and the species richness are presented in appendix 7.

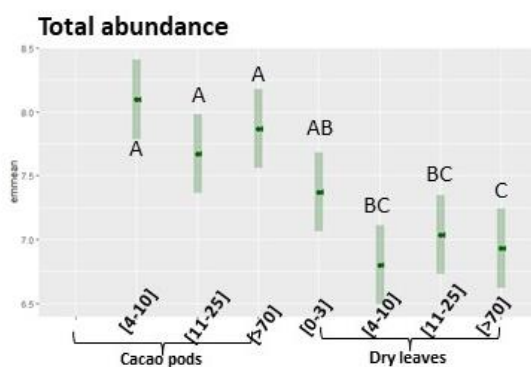


Figure 20 - Total abundance of macrofauna under each type of soil cover in each age class. The circle represents the marginal mean, the arrow represents the standard errors and the light green line indicates the lower and upper limits

The best fitted model for the total abundance was the full model. The total abundance was significantly higher in all age classes under cacao pods than in all age classes except from the age class [0-3] under leaf litter. Under leaf litter, the total abundance was significantly higher in the age class [0-3] than in the age class >70 (Fig. 20). The best fitted model for *Dermaptera* was the model with “Age class” and “type of soil cover” as factors, but without their interaction. The marginal mean for *Dermaptera* abundance of the age class [0-3] was significantly higher than in the age classes [4-10] and >70, and under cacao pods (Fig.21).

The best fitted model for earthworms, *Hymenoptera*, *Coleoptera* and *Hemiptera* abundances was the model with only the fixed parameter “type of soil cover”. For all, the marginal mean was significantly

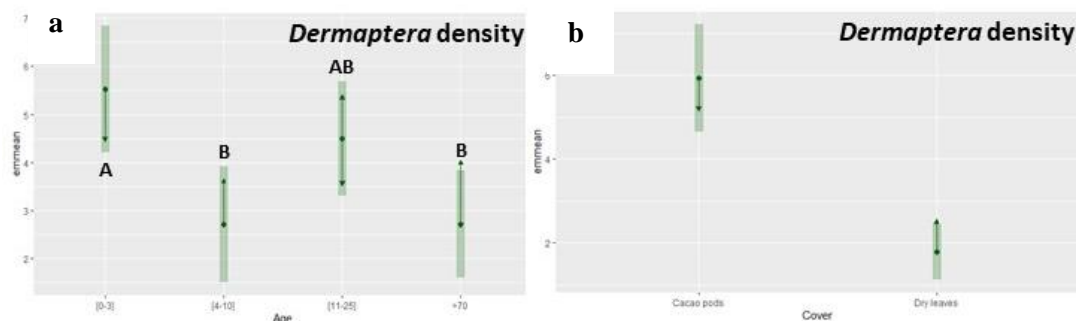


Figure 21 - Total abundance of *Dermaptera* in each age class (a) and under each type of soil cover (b). The circles represents the marginal means, the arrow represents the standard errors and the light green line indicates the lower and upper limits

higher under cacao pods (Fig.22). The best fitted models for *Araneae* and *Scolopendrida* were the null models (with the plot as random effect).

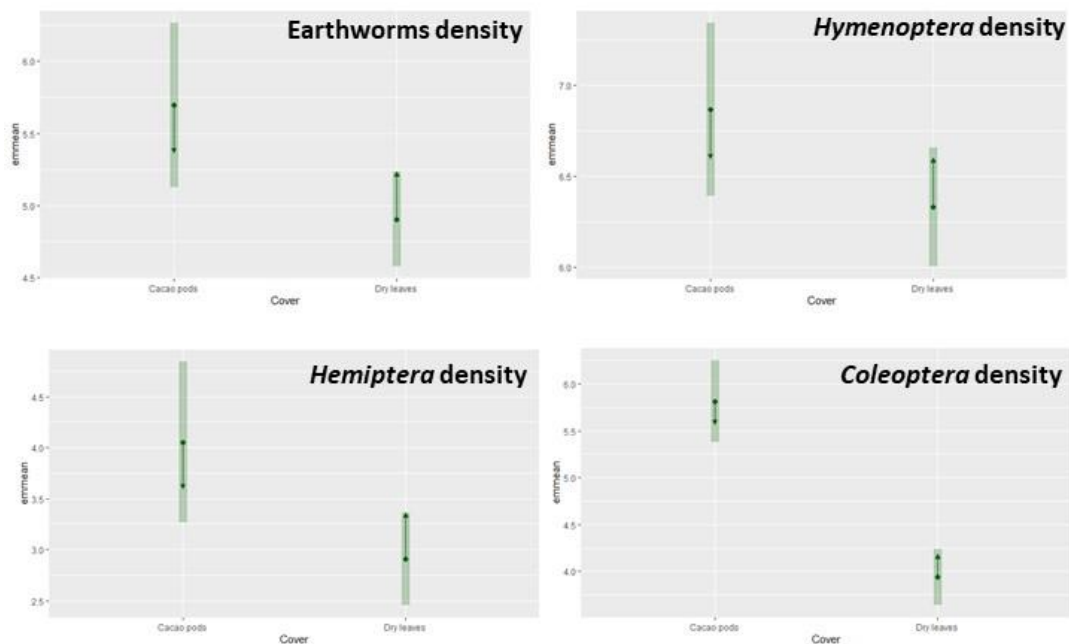


Figure 22 - Total abundance of the different taxa under each type of soil cover. The circle represents the marginal means, the arrow represents the standard errors and the light green line indicates the lower and upper limits

The best fitted model to predict the specific richness (S) was the model with only the fixed parameter “type of soil cover”. The specific richness was significantly higher under cacao pods than under leaf litter (Fig.23). The best fitted model to predict the Shannon index was the model including both the factor “age class” and “type of soil cover”, and their interaction. The Shannon index was significantly higher under leaf litter in the age class [11-25] (Fig.24). The best fitted model for the Simpson index was the null model, without the random effect of the plot.

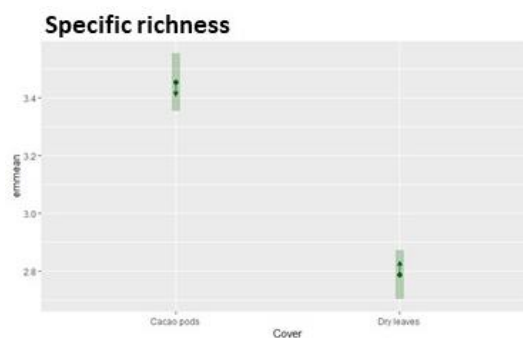


Figure 23 - Specific richness under each type of soil cover. The circle represents the marginal mean, the arrow represents the standard errors and the light green line indicates the lower and upper limits

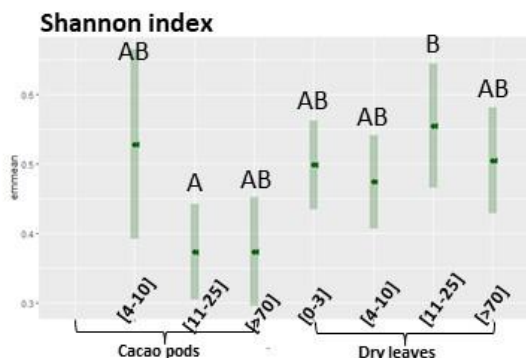


Figure 24 – Shannon index in each age class and under each type of soil cover

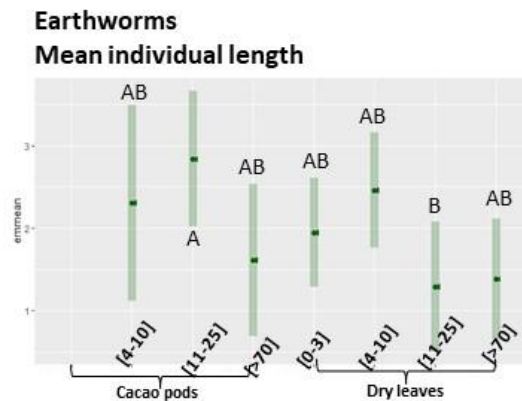


Figure 25 - Earthworms mean individual length in each age class and under each type of soil cover. The circles indicate the marginal means, the light green lines indicates the lower and upper limits

The earthworms functional traits are presented in appendix 8. The best fitted model for the total biomass and the mean body mass of earthworms was the complete model, but no marginal mean was significantly different from the other. It was also the complete model for the mean individual length, and there was a significant difference: the length was higher under cacao pods than under leaf litter in the age class [11-25] (Fig.25).

1.1.3 Modeling the soil parameters according to the age of the plantation and the presence of cacao pods (hypotheses 1, 3 and 4)

In this section the hypotheses 1, 3 and 4 were tested. The influence on the soil quality of the age of the plantation (hypothesis 1), the presence of cacao pods husks (hypothesis 3) and the interactions between the age of the plantation, and the presence of cacao pods husk (hypothesis 4) were tested.

The soil chemical and physical parameters according to the age class and the type of soil cover are presented in appendix 9 and 10.

The PCA and the between-class analysis revealed a highly significant effect of the type of soil cover on the macrofauna abundances (p-value = 0.001). Although the BCA on soil parameters could not reveal any significant difference between the types of soil cover, we hypothesized that some of the soil parameter could be impacted by the type of soil cover, and explain the impact of type of soil cover on the macrofauna abundances

The best fitted models for the organic carbon content, the organic matter content, the nitrogen content, the pH, the conductivity, the calcium carbonate content, the calcium content, the magnesium content, the sodium content, and the clay content, were the null model without the random effect of the plot. The best fitted models for the phosphorus content, the sand content, the silt content were the full model. The phosphorus content was significantly higher in the age class [11-25] than in the age class >70 under cacao pods (Fig.26a). Even if the full model were the ones that best fitted to the sand content and to the

silt content data, they did not reveal any significant effect (Fig26c and d). For the bulk density, it was hard to choose between the model with only the plot as random effect, and the model with the type of soil cover as fixed effect and the plot as random effect. In the second one, the bulk density was significantly lower under cacao pods (Fig 26b).

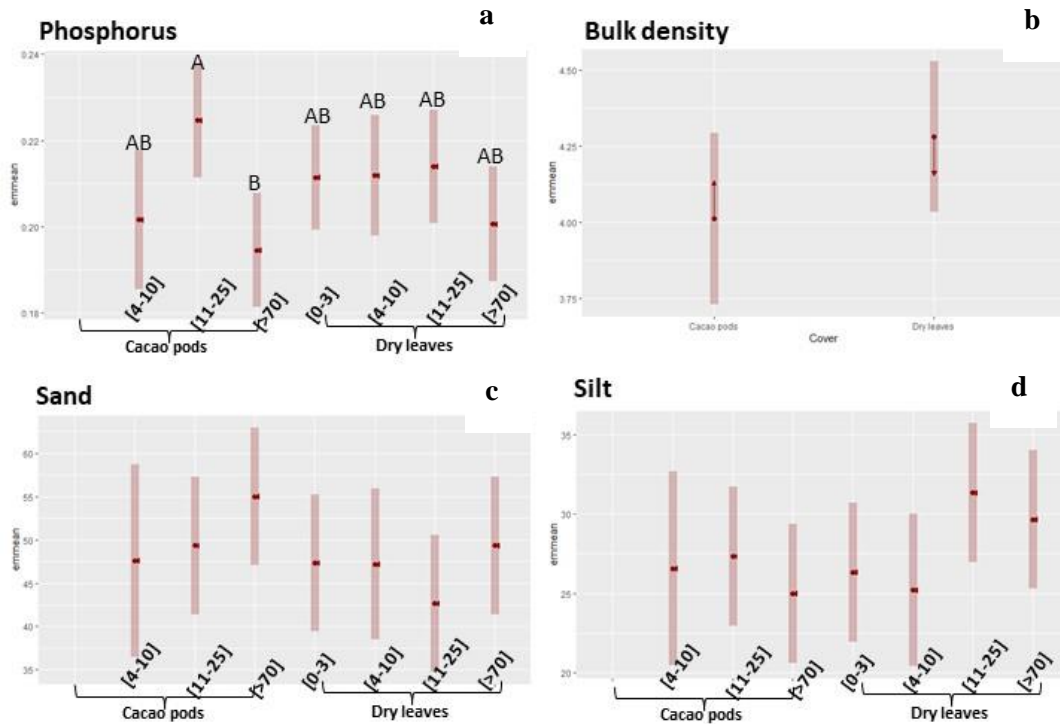


Figure 26 - Phosphorus content (a), sand content (c) and silt content (d) in each age class and under each type of soil cover. Bulk density (b) under each type of soil cover. The circles represent the marginal mean, the arrows represent the standard errors and the light green line indicates the lower

5 Discussion

5.1 Supporting and questioning the hypotheses

The average density of total macrofauna at the sampling points ($1655.1 \text{ ind.m}^{-2}$) was half lower (3368 ind.m^{-2}) than under diverse cacao-based AFS in the Colombian Amazon (Suárez et al. 2021). However it was similar to the total density of macrofauna found in soils characterized by high bases (1620 ind.m^{-2}) and high carbon levels (1626 ind.m^{-2}) in cacao-based AFS in the Talamanca region of Costa Rica (Rousseau et al. 2012). The average Shannon index ($H= 2.13$) was lower than in all types of soils sampled in this same study, but the average Simpson index ($D = 0.77$) was higher. The overall species richness (27 taxa) was higher in our study than in the study of Suárez et al. (2021) who found 12 taxa, including the pasture and forest plots.

5.1.1 The temporal heterogeneity did not influence the soil quality (hypothesis 1).

This study could not reveal any effect of the age class on most of the soil parameters. In a different environment (Kenya) and in different AFS, Kamau et al. (2017) revealed significant effects of the age of the plantation on soil nutrient levels. Thus, we could expect that different age classes could have led to different results (Appendix 11).

5.1.2 The temporal heterogeneity influenced only one taxon abundance (hypothesis 1)

No diversity index of the macrofauna was influenced by the age of the plantation alone. *Dermaptera* was the unique taxon whose density was affected by the age class. It was higher in the age class corresponding to the initial stage compared to two other age classes. It is in accordance with the study of Pauli et al. (2011) which could not reveal any difference in soil macrofauna density and diversity in 2 years old AFS compared with 10 years old AFS in Honduras. However, it does not concord with Kamau et al. (2017) who found that soil macrofauna total abundance was influenced by the age of the plantation, in maize-based agroforestry systems in Kenya.

The age classes corresponded to the mean age of the cacao trees population, but in almost each parcel there was some heterogeneity due to conservation of some old trees and to replanting, especially after hurricanes episodes (Siegel and Alwang 2004), This increased the variance of the mean age of the cacao trees population.

The analysis revealed few effect of the age on the macrofauna, but a rearrangement of age classes could lead to different results. The limits of the age classes were very close to each other and one plot could have more age difference with a plot of the same age class than with a plot of another age class. It would be interesting to repeat the experiment with age classes of narrow range and more spaced from each other (Appendix 11). Different results could be obtained through assessing more thoroughly the diversity. We calculated the Shannon and Simpson indices, which are indices of the alpha-diversity. The alpha-diversity is the local diversity, measured independently from any reference outside the community (here, the sampling unit). We did not calculate the gamma and beta-diversity. The gamma diversity is a measure of the overall diversity of a large region including different ecosystems (here, the soil macrofauna of the Dominican Republic). The beta diversity is the difference between gamma and alpha diversity, it compares the diversity of the local ecosystem to the diversity of the larger ecosystem that include it (Marcon 2014). It would allow to estimate the level of diversity of each sampling unit, compared to a regional reference.

Furthermore, the comparison of marginal means for the abundance of *Isoptera* according to the age of the plantation and the type of soil cover (corresponding to the model with the lowest AIC) provided

obviously erroneous results. This may be due to the high occurrence of 0, but no certain explanation could be found at the date.

5.1.3 The plant composition and distribution at plot scale evolved during the production cycle of the cacao plantation (hypothesis 2)

A significant effect of the age class on the density and diversity of the vegetation was revealed. It is in accordance with Notaro (2019) who found that there were less associated plants in older plantations.

The inventory of the plot was realized by the intern, with the help of the producers, the Cacao Forest Project's technician, and the local sampling team. It was not realized by a professional botanist, and the trees were identified by their local common names. Although producers and local inhabitant have a good knowledge of the trees grown in the cacao-based AFS, they often use different common names to refer to the same species. To limit this, the scientific name of each identified tree was searched on the following databases: Abbott and Leakey (2006), Orwa et al. (2009), Kern (2014), and Food plants international (2021). The same species named by several common names were grouped together. Local people may also use the same common name for actually different species, so it is important to bear in mind that different species could have been named the same, underestimating the diversity.

The criteria to include a plant in the census has weaknesses because it was chosen before seeing all the plantations. A strict compliance to this criterion would have led to the exclusion of the majority of the plants in the age class [0-3] years old. Indeed, the cacao trees and associated crops were seldom above the height limit of 1m and it was reduced to 40cm in this age class. Even if most of the plots in the three other age class had almost no plant under 1 m, it would have been more coherent to include all plants above 40cm in all plots.

Several agricultural practices evolve during the production cycle of the cacao plantation, and the modification of composition and disposition of cultivated plants is only one among them. Moreover, the pruning practices evolve during the production cycle of the cacao plantation, influencing the architecture of the canopies. This evolution might influence the soil quality and the soil macrofauna, but could not be addressed in this study.

5.1.4 The plants heterogeneity did not influence the soil macrofauna (hypothesis 2)

Our study could not reveal any effect of the intra-plot vegetation density and diversity on the density and diversity of soil macrofauna. This is not in accordance with Brussaard (1998) who suggest that a higher density of trees induces a denser rhizosphere, producing more food for the detritivore soil macrofauna organisms. Similarly, Tsufac et al. (2020) who found that the tree diversity in cacao-based AFS positively influenced the soil fertility, and explained it by the fact that diverse tree species attract diverse soil fauna. Similarly, Suárez et al. (2021) showed that the diversity and density of soil

macrofauna was influenced by the diversity of trees in cacao-based AFS, and Pauli et al. (2010) suggested that a higher diversity of trees induced a higher activity of earthworms. However, these studies compared different agroforestry systems, while we tested the intra-plot heterogeneity, at the sampling unit level. It may reveal that the macrofauna density and diversity are impacted by the inter plot heterogeneity but not by the intra-plot heterogeneity. Still, other studies revealed direct relations between the specific richness of the vegetation and the specific richness of soil organisms (Decaëns 2010). Bisseleua et al. (2009) found that ant species richness was positively related to tree species richness and densities, in cacao-based AFS in Cameroon. Among cacao-based AFS, animal diversity is generally higher in the more diversified systems (Schroth and Harvey 2007)

Nevertheless, different experimentations could have highlighted some effects of the vegetation on the macrofauna. The permanence of the plants was not taken into account, while they may have different short-term and long-term effects on the macrofauna. Effects may be revealed taking into account only the individuals above an age limit. It would require information about the planting date of each individual, which may be difficult in plots that are not dedicated to experimentations. Beyond the cost advantage, conducting experiments in farmer's parcels offers real conditions and enables to test factors that are not always rapidly reproducible (i.e. the age class of plantation above 70 years old), but it is not as controllable as an experimental plot.

The influence of the vegetation density and diversity at the sampling point level was only tested with correlation tests, which could not reveal any effect. It would have been more appropriate to include these variables into linear models together with the other variables, in order to test if they improved the models. However, generalized linear models only allows for fixed factors that are categorical variables.

The influence of plants heterogeneity on the soil quality was not tested, because the experimental plan did not allow for it. Indeed, the soil data were collected for composite samples made of different sampling points (see paragraph 3.1.5.4) while the plant density and diversity data were collected at each sampling point (see paragraph 3.1.5.2). In order to test the correlations between both dataset, it would have been necessary to use the means of plant density and diversity of the sampling points used to realize the composite soil samples. Such distortion of the data would have generated inaccuracy in the results.

5.1.5 The decomposition of cacao pod husks influenced the soil physical properties (hypothesis 3)

The bulk density was the unique soil parameter influenced by the heaps of decomposing cacao pod husks. It was lower under cacao pod husks than under leaf litter. We may hypothesize that this was due to the significantly higher densities of ants and earthworms, and the larger length of earthworms, under cacao pods. Indeed, it has been proven that earthworms are “important actors in the regeneration of compacted soils” (Capowiez et al. 2012) and that ants decrease the bulk density of agricultural soils (De Bruyn and Conacher 1990, Cerdà and Jurgensen 2008). Also, longer earthworms may have a greater

influence on the soil structure due to a higher burrowing capacity. The soil sampling method used to determine the bulk density has some weaknesses: the small cylinders method induces risk of compression by hammering on the edges of the cylinder, and it is time consuming, as one must realize the sample again when a big element (stone, root) interferes. Plus, the small volume sampled implies high relative errors (Baize 2018).

The absence of influence of cacao pod husks on the soil chemical parameters in this study is surprising, knowing that cacao pods are mainly made of organic carbon (Brito-Vega et al. 2018), thus may increase the organic carbon content of the soil. High variances of organic carbon, organic matter and nitrogen contents in the soil were observed, that may be explained by the kinetic of nutrient leaching from the cacao pods. Indeed, all cacao pods heaps were selected at the same decomposition level, but their age varied between three weeks and two months. Hougni et al (2021) found that cacao pods lose most of their dry weight after 30 days of decomposition, followed by a phase of slower weight loss and lower decomposition rates. Assuming that the organic carbon of decomposed cacao pods is included in the soil after a duration d , it would imply that the carbon content is higher in soils under cacao pods less than 30 days + d old, compared to soil under more decomposed cacao pods. These variances could also be explained by the recurrence of cacao pods heaps at the same place. It can vary between 2 weeks (interval between two harvests during the main producing period) and several years, according to the choices of the producers. A complementary study could test the effects of the frequency of cacao pods application on the soil and the soil macrofauna. It would require a control of these applications over several years. The high variances observed could also be due to other factors that were not measured, like local temperature and humidity.

Moreover, some impacts of the cacao pods on the soil parameters could have been revealed with more exhaustive soil analysis. More specifically, the potassium content could not be measured because the measuring equipment of the INDRHI laboratory was damaged when we delivered the samples. This missing data could have contributed to reveal a link between the cacao pods cover and the soil chemical parameters, as cacao pods have a high potassium content (Sodré et al. 2012, Lu et al. 2018, Tsai et al. 2018), that can reach 86.45 % of the mineral elements (Ofori and Awudza 2017). In a cacao agroforestry system in Costa Rica, the K harvest in pods was 28.4 kg/ha⁻¹.yr⁻¹, which was higher than Ca (5.3kg/ ha⁻¹.yr⁻¹) and Mg (4.3kg/ ha⁻¹.yr⁻¹) (Fassbender et al. 1988). It was estimated that for 1000 kg of dry cacao beans, 34,5 kg of potassium, 11.5 kg of N and 1.8 kg of P were removed from the agroecosystem through the pods, in a cacao-based AFS in Costa Rica (Hartemink 2005).

5.1.6 The decomposition of cacao pod husks influenced the soil macrofauna density and diversity (hypothesis 3)

The specific richness (S) was higher under cacao pods. The other diversity indices (Simpson and Shannon indices) were not impacted by the cacao pods alone.

The total abundance was generally larger under cacao pods than under leaf litter. The abundance of the taxa earthworms, *Hymenoptera*, *Hemiptera*, *Dermaptera* and *Coleoptera* were higher under cacao pods. Regarding the earthworms taxon, it is coherent with a recent study (Prastowo 2020) that compared earthworms abundances under cacao pods, cacao leaves and another type of amendment at different quantity levels and found out that the earthworms abundance was higher under the highest quantity of cacao pods, compared to the other treatments. The earthworms length was higher under the cacao pods. Our results regarding the ants abundance (the order *Hymenoptera* being almost only represented by the family *Formicidae* in our study) are also coherent with the study of Fowler (1993) who showed that ants use decomposing cacao pods for nesting and foraging. Later, Castaño-Meneses et al. (2015), revealed a high richness of species in decomposing fallen cacao pods: up to 10 different ant species and 37 different other taxa inhabited them. The most abundant ant species reported in the fallen cacao pods were *Wasmania auropunctata* and *Solenopsis* spp. Even if the study referred above focused on decomposing fallen cacao pods and not on empty cacao pods heaps as harvest wastes, it is interesting to note that the two ant species mentioned were also the most abundant in our study.

Similar findings occurred in different agroecosystems, under different types of pruning or crops residues. Lavelle et al. (2001) stated that returning crop residues to the soil maintain high level of soil invertebrates activity. De Aquino et al. (2008) showed that no-till agroecosystems where the residues of the previous crop are left on place have the more diverse soil macrofauna communities, compared to conventional tillage systems. García-Tejero and Taboada (2016) found that litter-trapping microhabitats, including piles of pruning residues, increased the density and diversity of soil detritivores and predators communities.

We chose to gather the macrofauna from the litter, the 0-10 cm layer and the 10-20 cm layer together, because we wanted to test the effect of the variables on the soil system as a whole. Our research question did not imply to separate the litter and the different horizons from each other. Further, the soil analysis by horizon would not have been affordable, and the same approach needed to be adopted for the soil physical and chemical parameters and for the macrofauna. However, the macrofauna was sampled and identified by horizon, so it would be possible to perform new analyses in order to answer different research questions. For the sake of coherence with this approach of the soil as an undivided system, the volume and the biomass of the litter were not measured. It could have been done to relativize the macrofauna density, as there might be a positive correlation between the volume and biomass of the litter and the density of the macrofauna. Chemical analysis of the leaf litter and of the cacao pod husks would have brought insight about the impact of cacao pods on the soil macrofauna independently from the soil belowground. These impacts could have been tested by modeling the macrofauna from the litter layer (leaves and cacao pod husks) as function of the litter layer chemical parameters and of the litter layer biomass.

5.1.7 The interactions between the temporal heterogeneity and the decomposition of cacao pod husks influenced the soil quality and the soil macrofauna (hypothesis 4).

Regarding the results on the soil macrofauna, the Shannon diversity index was higher under leaf litter in the age class corresponding to the stabilized stage. The species richness being higher under cacao pods in all age classes, may suggest that the species evenness was higher under leaf litter in the stabilized stage. It would be coherent with the highly significantly higher densities of some taxa (*Coleoptera* and *Dermaptera*) under cacao pods, and with the nesting of ants inside decomposing cacao pods (Fowler 1993). If one species nests in the cacao pods, it will reduce the evenness. However, it is not in accordance with Castaño-Meneses et al. (2015) who revealed a high richness of species in decomposing fallen cacao pods. It would be necessary to calculate the Pielou evenness index, in order to confirm this assumption. Still, it is not easily interpretable why the species evenness was higher under leaf litter only in one age class.

The interaction of both factors had a significant effect on the total abundance of soil macrofauna. The abundance of macrofauna in all age classes under cacao pods was larger than the abundance of all age classes except from the age class [0-3] under leaf litter. It must be remembered that no data could be collected in the age class [0-3] under cacao pods. The study should be done again with a previous preparation of the plots from this age class by application of cacao pods at known frequencies.

Regarding the results on the soil parameters, the phosphorus content was higher in the age class corresponding to the stabilized stage compared to the age class corresponding to the senescent stage, only under cacao pods. The average P content of our study site is substantially lower than the P content of cacao agroforestry soils in Brazil (that have not received P fertilization for 10 years) (Zaia et al. 2012). Aleixo et al. (2019) found that the phosphorus content in the soils of cacao-based AFS in Brazil was impacted by the tree species and density. As there is no significant difference of density between the age classes [11-25] and >70, it could be assumed that cacao pods release phosphorus, but that the species in the age class [11-25] do not absorb much phosphorus. We would need to characterize the species found in each age class and realize a literature review on the phosphorus requirements of each species. This result could also be due to sampling errors.

5.1.8 The soil quality and the soil macrofauna influenced each other.

The co-inertia analysis and the correlation test revealed significant correlations between the soil parameters and the macrofauna. The texture cannot (or very slightly) be modified by the macrofauna. Hence, it might be assumed that a correlation between the density of a taxon and a soil texture parameter reveals a strict cause-effect impact of the soil on the macrofauna. For example, it might be assumed that the soil sand content affected positively the density of *Isoptera*. The test also revealed a negative

correlation between the soil OM, OC and N contents and the density of *Isoptera*. It might be assumed that sandy soil are less rich in OM, OC and N, as these elements are stabilized by silt and clay particles (Six et al. 2002). These soils may be less favorable to soil macrofauna taxa which have high requirements of OM, OC and N, and favor more adaptable taxa, such as the *Isoptera*. *Isoptera* form the basis of the food web, they convert nitrogen-poor material into nitrogen-rich bodies, on which other taxa of the soil macrofauna feed, such as spiders and ants (Takuya and Masahiko 2001)

However, there were interactions between the OM, OC and N content of the soil and the macrofauna, and the correlation, therefore, cannot be strictly interpreted as a cause-effect relationship.

The positive correlation between the predators *Scolopendrida* and *Araneae* abundances and the OM, OC and N contents may have different explanations. It may be another factor that covary with both elements, without direct relation between them. For example, a thicker litter would increase the OM, OC and N content of the soil and it would also provide more habitats for *Araneae* and *Scolopendrida*. It is unlikely that these taxa would increase the OM, OC and N content of the soil, as they do not feed on litter.

Explaining the correlations between the soil quality and its macrofauna is particularly delicate, because strong interactions between both have already been proven (Rousseau et al. 2013) and it is known that the macrofauna also impacts the soil (Jouquet et al. 2006). Besides, the soil parameters were measured for the 0-20cm soil layer, while the macrofauna was recorded for the whole soil system (litter + 0-20cm soil layer). The litter influences the soil macrofauna directly as shelter and food source, and indirectly through the modifications it causes on the soil quality. This study could not consider these effect separately. For this purpose, it would have been necessary to perform the tests only with the macrofauna from the soil layers 0-10cm and 10-20cm, but it would not even have ensure to separate the direct and indirect effects of the litter, as some organisms live, feed or move in the litter and in the soil below-ground.

The functional traits of earthworms (length and body mass) were only analyzed as response factors of the type of soil cover and the age class. It would be interesting to make multivariate descriptive analysis of the bulk density (as a response factor) depending on the length of earthworms and other fixed and random factors. Indeed, it is well known that earthworms affects the soil porosity through their burrowing activities (Brown et al. 2004). Furthermore, it would have been interesting to measure other functional traits on earthworms, such as their diameter and musculature, to link them with soil parameters, but we could not access the necessary equipment.

5.2 Limits of the experimental protocol

The attempt at constituting a control set of plots failed. Only one forest plot could be sampled out of the six planned in the time and space limits of this study. Moreover, the delimitation and inventory of a

forest plot required much more time and plant knowledge than a cacao-based AFS plot. This forest control set is missing to the study, as well as a second control set of plots in *conucos*, the other land use that generally precedes the plantation of cacao trees. According to the owners, the land use before cacao plantations was generally a *conuco*, which is a parcel of annual crops for family consumption. However, no certain information could be obtained for the oldest plantations (over seventy years old). Those control plots would have represented the age class <0 in the chronosequence. The five TSBF sample points realized in the forest plot were excluded from the statistical analysis because the number of samples was not sufficient. We could still use the macrofauna and soil data to establish a qualitative comparison, but it would not be statistically valid. Moreover, the analysis adapted to unbalanced experimental designs accept more probably the null hypothesis when the factor actually has an effect, because it does not take the risk of attributing variance to one factor when it could be attributed to the other factor. Plus, the initial experimental protocol planned on the sampling of 48 plots equally distributed in two areas, to have a sample of sufficient size and to test the effect of the area. Indeed, an effect of the area on the density and on the diversity of the macrofauna can be expected as it depends on topography (Liu and Li 2008), microclimate, (Rozen et al. 2013) and distance to the sea (Zheng et al. 2020). Plus, 48 plots would have resulted in 240 sampling points, a number that would have allowed to 30 repetitions of each treatment (in the case of a balanced experimental plan) Unfortunately, the sampling in the second area could not be done due to time constraints.

The macrofauna sampling method that was adopted has weaknesses : The TSBF extraction method is “time-consuming, laborious and prone to researchers bias” (Gongalsky 2021). The time and labor required have been reduced by our adaptation, but there is inevitably a bias due to the sampling team, when comparing with other studies’ results. However, efforts have been made to keep the same sampling team with the same motivation level the whole time, and to keep every one’s role in the sampling process. For example, the same two persons have been extracting the monolith together, as different persons would have taken more or less time. Only two persons have been replaced because they lost motivation and meticulousness. There is finally low heterogeneity due to the sampling team inside the study, and it is included in the error of the statistical models. Moreover, hand-sorting is well adapted for earthworms, but it may lead to omit not easily visible species from the smaller macrofauna. Other sampling methods leading to different results can be used for macrofauna sampling, like thermoextractors (Gongalsky 2021). The TSBF hand-sorting method may still be more exact than other methods as pitfall traps that were discarded for their low precision: in a study in Indonesian tropical agroforests, 50% more species were found with the TSBF hand sorting method than with the pitfall method on the same sampling point (Prayogo et al. 2019).

The results of the soil chemical and physical analysis may not be totally reliable. Indeed, the INDRHI is not specialized in soil analysis and is not part of the Global Soil Laboratory Network (GLOSOLAN). GLOSOLAN is an international partnership that fosters harmonization of methods, units, data and

information. It trains its member to quality control, standard operating procedures, use and maintenance of equipment. We could have obtained more reliable results from one of the soil laboratory which are part of this partnership in the Dominican Republic: the private *Laboratorio Agroempresarial Dominicano* and the *Laboratorio De Suelos, LABOAGRO* of the *Universidad Autónoma De Santo Domingo*.

5.3 Limits of the identification process

All the morphotypes were identified at the order level, and it was decided to rather take the risk of separating the same species into two different morphotype, than to take the risk of grouping together two different species in the same morphotype. This issue was quite strong due to sexual dimorphism, cast polymorphism, and heterometaboles orders. Sexual dimorphism occurs for *Coleoptera* (body size and shape), *Arachnida* (males are usually smaller and brighter than females, with differences in buccal pieces), more than half the known *Myriapoda* (body size), and to a lesser extent *Diptera* (wings and eyes size) (Mori et al. 2017). Ants and termites populations have different casts: ants can be queen, worker or male and termites can be reproductive or sterile, and among sterile, soldier, presoldier or workers. Each cast have a different morphology. Furthermore, the few male ants could not be identified by the specialists, so they were classed alone in one morphotype. The heterometaboles, such as *Dermaptera*, *Isoptera* and *Orthoptera*, do not perform total metamorphosis and their nymph resemble the adult. It was sometimes difficult to find out if a morphotype was the nymph of another one, or if it was a different species. Also, larvae and adults of the same species may have been separated into two different morphotypes. This would lead to overestimation with the taxonomical approach, but this separation is necessary for an adequate functional approach. Indeed, the different ontogenetic levels of the same species may have different functions. “On the one hand, adults and larvae of the same species are taxonomically undivided since they form a continuous life cycle. On the other, they do play different roles in a soil ecosystem at different life stages. For example, predatory larvae of cantharid beetles become phytophagous as adult” (Gongalsky 2021). In the present work, the species richness and diversity indices may be overestimated. Identification to the species level would be necessary. Two entomologists already identified the ant morphotypes at species level, and one identified the *Diplopoda* at order level. However, he could only communicate his identification after all the statistical analysis were done, so the *Diplopoda* were still considered as one big taxon in this study. It induced underestimation of the species richness and of the diversity index, because this taxa actually included 6 different orders. However it did not modify the classification according to both taxonomic and functional approach. Indeed, the taxa *Diplopoda* only represented 0.8% of the total abundance (earthworms excluded), so it was not included in the PCA, and it made sense to group them in the other analysis. Plus, all *Diplopoda* belong to the detritivores. Collaborations with specialists for further identification still need to be done. There are several obstacles to such completion of the study: the number of soil taxonomists for all groups is decreasing worldwide due to the development of molecular disciplines

(Eisenhauer et al. 2017, cited in Gongalsky 2021) and there is a clear lack of entomologists in the Dominican Republic, Rosina Taveras Macarrulla estimates that they are less than 10 in the whole country. Plus, we may have sampled species that are not taxonomically identified yet. Thanks to the contribution of specialized entomologists, we also expect to discover new species. It would be interesting to perform further analysis to test if the factors of the study influence the proportion of native vs exotic ant species, and so on with all orders after the species identification. Once all the morphotypes will be identified at the species level, a more precise and sure functional classification will be possible: “The attribution to a functional group require genus or even species level identification” (Marsden et al. 2020). The functional classification realized in this study is unperfect because one order can actually include species with different alimentation strategies. The orders were attributed to the functional groups to which most of its species belonged. The *Hymenoptera* were attributed to the group “engineer”, while only the family *Formicidae* of this order actually belong to the engineers group. The *Dermaptera* were attributed to the group “phytophagous” when most of them are omnivorous, and some are predators (Ren et al. 2019). The *Thysanoptera* were also attributed to the group “phytophagous”, to which most belong, but some are palynivore, mycophagous and few are even predators (INRAE 2021). Lastly, *Psocoptera* were excluded from the functional classification, as they are totally omnivore and represented only 0.2% of the total abundance of macrofauna in the study.

We will participate to the international project GlobalSoilMacrofauna through sharing the data obtained on the soil macrofauna. Among several goals, this project aims at creating a “large-scale synthesis of the effects of human activity on soil macrofauna and to explore the links to ecosystem services delivery, especially primary productivity” (Mathieu et al. 2021).

Beside the possible overestimation of the macrofauna diversity and underestimation of the plot plants diversity, the indices that were calculated have their own limits. The Shannon and Simpson indices were selected because they estimate both the richness and the evenness of the species. They are the most commonly calculated and enable to compare the results with other studies. The Shannon index has a smaller relative root-mean-square error, and the Simpson index is less biased than the Camargo evenness index. However, the Shannon index is sensitive to changes in species abundance pattern, and has a bias for small sample size of less than 1000 individuals (Mouillot and Lepretre 1999). Simpson index is more reliable for small samples.

5.4 Unconsidered variation factors

The delineation of the plots had to be adapted to the constraints of the small area and irregular shapes of the parcels. It was not possible to ensure a consistent distance from the edges. Hence, border effects and influences of the neighboring ecosystems may have influenced the results.

The slope factor was only included in the descriptive analysis, that revealed a very significant effect of the slope on the soil physical and chemical parameters. It was not tested by explicative analysis, nor included in the predictive models, due to time constraints. It would be necessary to include the slope as

a random factor in the GLMMs to predict the macrofauna density and diversity, and not as a fixed factor, because this study did not aim at testing this factor. However it would have been necessary to have it as a second random factor, because it may impact the response variables. Though, a random factor must have at least 10 modalities to be statistically valid and the slope was only categorized in 3 class. We should have measured the exact slope of each plot in order to be able to include it as a random effect of 24 modalities in the GLMMs. The slope factor may be included as a fixed parameter in the GLMMs to predict the soil chemical and physical parameters, because it has been proven that the slope has an effect on them: slope is positively correlated with erosion in cacao plantations, and the erosion reduces the litter thickness and the nutrient storage capacity of the soil (Munir et al. 2019). Even if there is usually few erosion in cacao-based AFS, it can be observed on steep slope and when the cacao is young (Hartemink 2005). We may hypothesize that the differences in soil parameters according to the slope revealed by the PCA were due to lower OM, OC, N and minerals content in steeper slope classes. In this study, we can consider that the slope is taken into account as a random effect, nested into the random effect of the plot. Some other factors that may be sources of variation could be neither controlled nor measured, like the proximity to water sources and the eventual presence of hens on the plot, the local temperature and the local humidity. These factors are the reason why it was necessary to include a random effect of the plot in the predictive models.

6 Conclusion

This study revealed no influence of the temporal heterogeneity on the soil quality, and a limited influence on the soil macrofauna (only one taxon density was influenced). It revealed an evolution of the agricultural practices in terms of plant composition and disposition during the production cycle of the cacao plantation, but no influence of this evolution on the soil macrofauna density and diversity. This study revealed positive influences of the agricultural practice of making post-harvest heaps of cacao husks inside the plantation, on the soil physical properties and on the soil macrofauna density and diversity.

It would be hazardous to affirm the genericity of the results to other contexts of tropical diversified cacao-based AFS of Central America and Africa. Even in similar contexts in equatorial, organically grown with no tillage cacao-based AFS, it would depend on the nature of the parent material and of the soils. However, as exposed above, many studies in different agroecosystems already revealed the positive effect of crop residues application on the density and/or diversity of the soil macrofauna. Thus, the part of the results of this study relative to the influence of the crop residues on the soil macrofauna, integrated in the larger body of literature on the subject, could be generalizable to other agroecosystems. It would be interesting to realize similar studies in these contexts and perform meta-analysis to confirm the genericity of our results, or to reveal higher effects of the variables dependent on the context (geography, geology, pedology, microclimate, cultivated species).

As mentioned above, a complementary study on the effects of frequency of cacao pods application would be necessary. Then, the producers should be advised to distribute the cacao pods inside the plantation throughout the successive harvests, at a set frequency, in order to stimulate the soil macrofauna in the whole plantation. Besides, it would be interesting to set an experimentation to test the effects of cacao pods in an adaptation of the Bio-Organic Fertilization (FBO) method. The FBO method is a need-based and location-specific fertilization method for tree plantations: trenches are dug between trees and filled-up with organic material (in this case, it would be decomposing cacao pods), vermicompost and inoculated with earthworms in order to locally enhance the ecological functionality of the soil (Lavelle et al. 2014). The FBO method showed a positive influence on yields but it also has potential for soil conservation (Senapati et al. 2002) that could be tested in the context of cacao-based AFS with decomposing cacao-pods. However, the decomposing cacao pods heaps can host the fungus *Phytophthora palmivora* that is responsible for the black pod rot, causing from 20% to 30% of cacao yield loss worldwide (Lu et al. 2018, Cedeño Moreira et al. 2020, Hougni et al. 2021). This is the unique disease that affect the Dominican cacao-based SAF (Notaro 2019). Therefore, the managed use of decomposing cacao pods should be associated to the health monitoring of the plantations, focused on this disease. It would include the exclusion of host species from the AFS, and management of shade, as

excessive shade favors the development of *Phytophthora*, but a high level of shade favors cacao endophytes that are antagonists of the black pod rot (Mortimer et al. 2018).

The results of this study are not agroecologically directly applicable in the field. It is necessary to integrate them into a transdisciplinary approach of the whole food system in which the study site and actors belong. The results of this ecological study must be put into perspective with the social, economic, cultural and political issues of the cacao production, before to take action.

7 References

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8 Appendices

Appendix 1 – Variables collected and not used in this study.

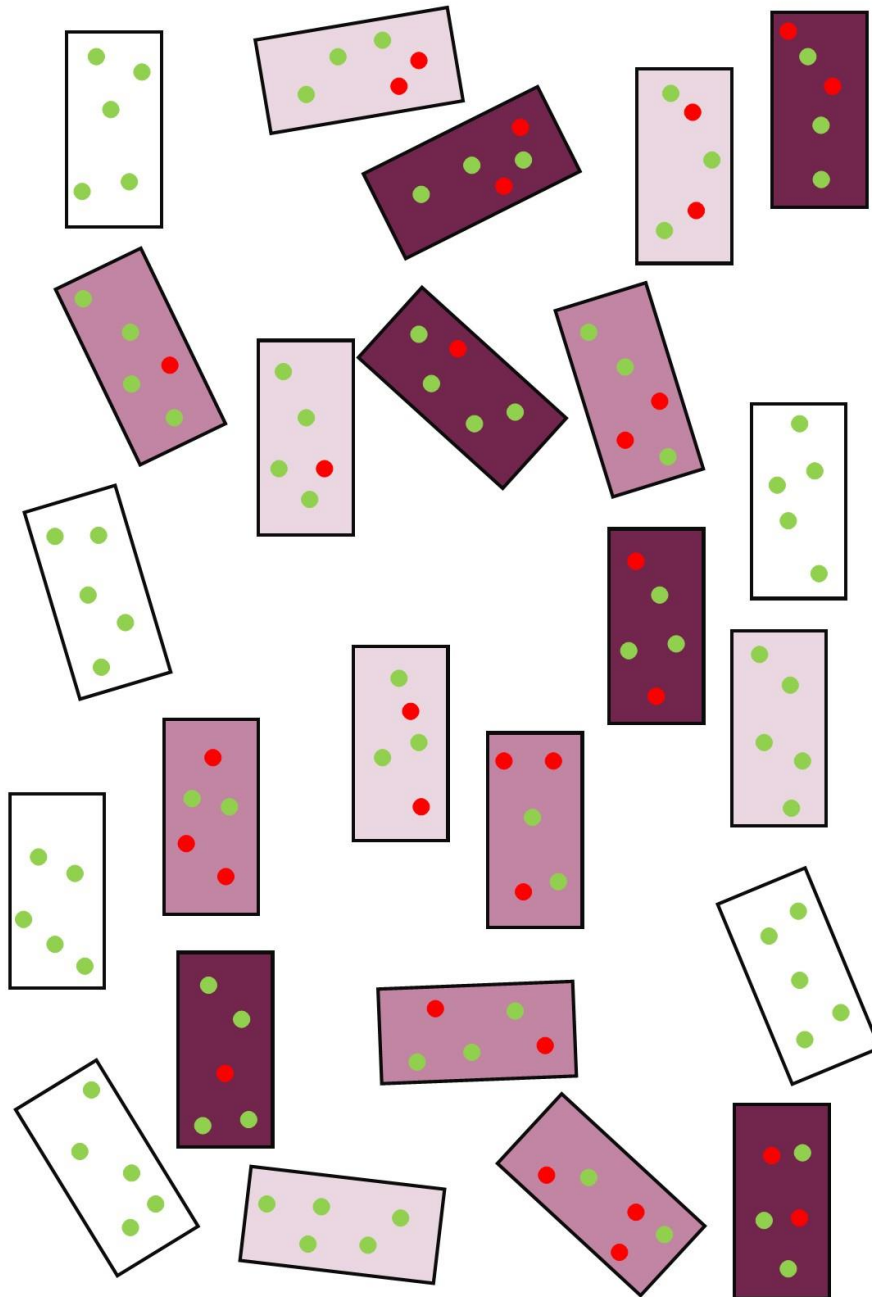
Number	Variable	Mean	Standard deviation	Min	Max	Unit	Method	
1	Vegetation density of the plot	0,125	0,039	0,061	0,208	ind.m-2	Inventory	
2	Vegetation specific richness of the plot	10,6	4,1	5	20	Number of species	Inventory	
3	Vegetation diversity of the plot	1,274	0,343	0,66	1,893	Shannon index	Inventory	
4	Slope	3 categories: <i>flat</i> , <i>slight slope</i> and <i>steep slope</i>						Naked eye appreciation
5	Proximity to a <i>Persea Americana</i>					Boolean yes/no	Measuring tape	
6	Proximity to a <i>Citrus</i>					Boolean yes/no	Measuring tape	

All the data could not be analyzed yet. The experiment protocol included two binary factors of proximity to the species *Persea Americana* and to the genus *Citrus*. It was hypothesized that the species would influence the abundance and the diversity of the soil macrofauna, based on other studies that revealed different abundances of earthworms, beetles, termites and centipedes according to the closest tree species, with an interaction effect of the tree's age (Kamau et al. 2017). The literature review preliminary to this study enlightened many evidences for the effect of specific tree species on the soil macrofauna, even if no study was found about the specific effect of the species *Persea Americana* and the genus *Citrus* in cacao-based agroforestry. These hypothesis will be tested later in the frame of a scientific publication with Cacao Forest







Appendix 2: Table summarizing the data collected during the study

Variable	Unit	Minimum	Maximum	Mean	Standart deviation	Measuring method
For each sampling unit						
Tree density	ind.m ⁻²	0,040	0,358	0,162	0,075	Inventory of a 8m diameter area
Species richness within a 4 meters radius	Number of species.50,27m ⁻²	1	6	2,9	1,3	Inventory of a 8m diameter area
Distance of each tree from the sampling point m						Measuring tape
Shannon index		0,00	1,61	0,77	0,44	$H = \sum(p_i \cdot \ln p_i)$
Simpson index						$D = 1 - \sum p_i^2$
Macrofauna						
Total density	ind.m ⁻²	48	10144	1655,1	1665,0	TSBF sampling
Species richness	Number of morphotypes	40	66	20,2	9,9	TSBF sampling
Shannon index		0,65	3,59	2,13	0,66	$H = \sum(p_i \cdot \ln p_i)$
Simpson index		0,25	0,96	0,77	0,18	$D = \sum(p_i)^2$
Density of each big taxon	ind.m ⁻²					TSBF sampling
Earthworms		0	1568	199,6	253,0	
<i>Acari</i>		0	368	11,4	44,0	
<i>Araneae</i>		0	544	100,9	100,6	
<i>Blattodea</i>		0	48	3,2	7,3	
<i>Coleoptera</i>		0	1376	127,6	202,8	
<i>Collembola</i>		0	16	0,5	2,8	
<i>Dermaptera</i>		0	1024	61,1	151,4	
<i>Diplopoda</i>		0	656	12,9	61,0	
<i>Diplura</i>		0	16	0,3	2,0	
<i>Diptera</i>		0	320	12,8	37,2	
<i>Embioptera</i>		0	16	0,1	1,4	
<i>Stylommatophora</i>		0	112	8,4	19,6	
<i>Pulmonata</i>		0	16	0,3	2,0	
<i>Hemiptera</i>		0	208	28,0	43,5	
<i>Hymenoptera</i>		0	5536	774,0	1076,6	
<i>Isopoda</i>		0	240	20,6	40,3	
<i>Isoptera</i>		0	7600	393,6	1281,5	
<i>Lepidoptera</i>		0	48	2,8	7,9	
<i>Neuroptera</i>		0	16	0,1	1,4	
<i>Orthoptera</i>		0	32	1,2	4,6	
<i>Pseudoscorpionida</i>		0	16	0,4	2,5	
<i>Psocoptera</i>		0	128	3,1	13,4	
<i>Schizomida</i>		0	112	6,0	16,6	
<i>Scolopendrida</i>		0	240	47,2	49,2	
<i>Scorpiones</i>		0	16	0,1	1,4	
<i>Thysanoptera</i>		0	32	1,2	5,4	
<i>Uropygi</i>		0	16	0,1	1,4	
Color and pigmentation of individual earthworms						Pictures
Total biomass of earthworms	mg.m ⁻²	0,0	51161,6	6374,8	8880,5	Scale (0,1mg accuracy)
Individual biomass of earthworms	mg	0,0	355,4	36,6	49,8	Scale (0,1mg accuracy)
individual length of earthworms	mm	0,0	6,3	2,0	1,4	Ruler (1mm accuracy)
Soil						
Potential hydrogen	Logarithmic scale	5,06	6,98	6,20	0,37	Potentiometer
Conductivity (indicates the quantity of sals (K+, Ca+, Mg+, Na+, Cl-, HCO3-))	mS.cm ⁻¹	0,23	0,60	0,37	0,10	Conductimetry
Percentage of calcium carbonate	%	0,28	1,39	0,50	0,22	Calcimetry
Percentage of organic matter	%	0,70	5,12	2,77	0,81	Walkley-Black method
Percentage of organic carbon	%	0,41	2,97	1,61	0,47	Walkley-Black method
Percentage of Nitrogen	%	0,04	0,26	0,14	0,04	Walkley-Black method
Content of Phosphorus	mg.Kg ⁻¹	57,50	250,00	133,33	49,14	Spectroscopy
Quantity of exchangeable Calcium cations	meq/L ⁻¹	1,12	2,56	1,86	0,38	Ammonium acetate method
Quantity of exchangeable Magnesium cations	meq/L ⁻¹	0,36	2,08	1,20	0,45	Ammonium acetate method
Quantity of exchangeable Sodium cations	meq/L ⁻¹	0,03	3,38	0,67	0,61	Ammonium acetate method
Bulk density (Dry weight per volume unit)	g/cm ⁻³	1,09	2,69	2,09	0,30	Sampling cylinder and oven-drying.
Percentage of sand	%	28,00	82,00	48,56	9,24	Bouyoucos method
Percentage of silt	%	10,00	36,00	27,44	5,29	Bouyoucos method
Percentage of clay	%	8,00	42,00	24,00	6,70	Bouyoucos method

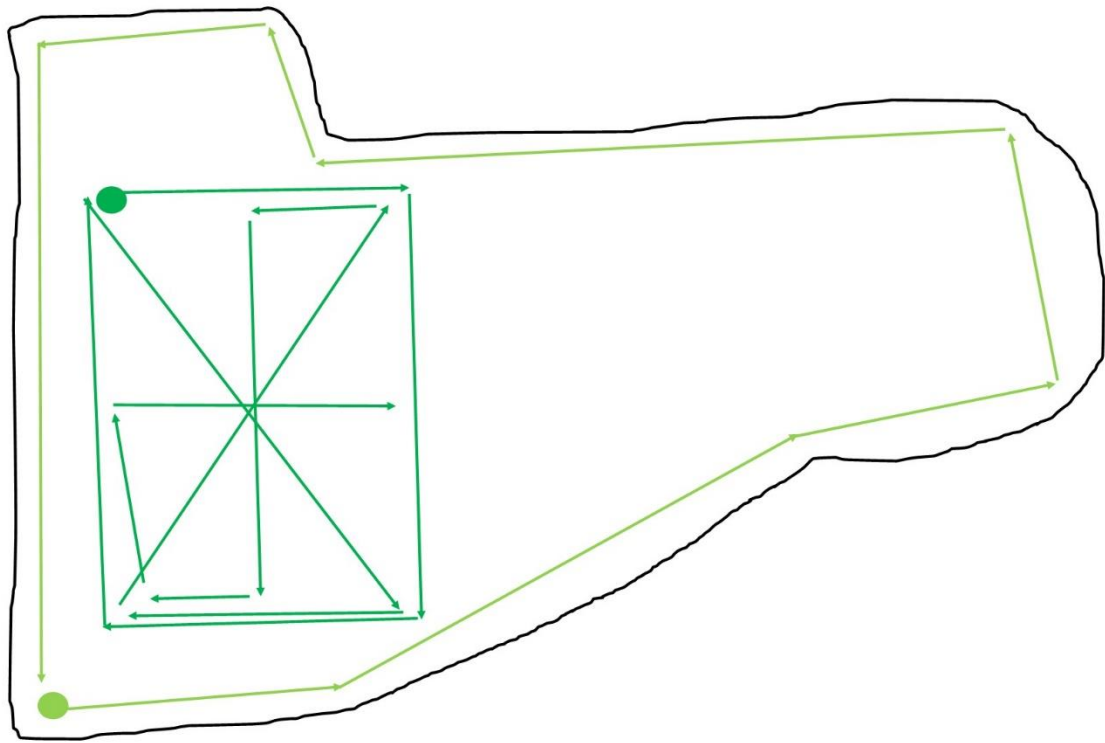
Appendix 3 – Schematization of the experimental plan.








Legend :

- | | | | | | |
|---|--|---|---|---|------------------------------------|
|  | Plot of the age class [0-3] years old |  | Plot of the age class [11-25] years old |  | Sampling point under leaves litter |
|  | Plot of the age class [4-10] years old |  | Plot of the age class > 70 years old |  | Sampling point under cacao pods |

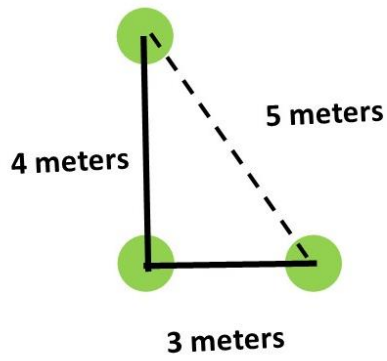
Appendix 4 – Schematization of the transect walk process







Legend:

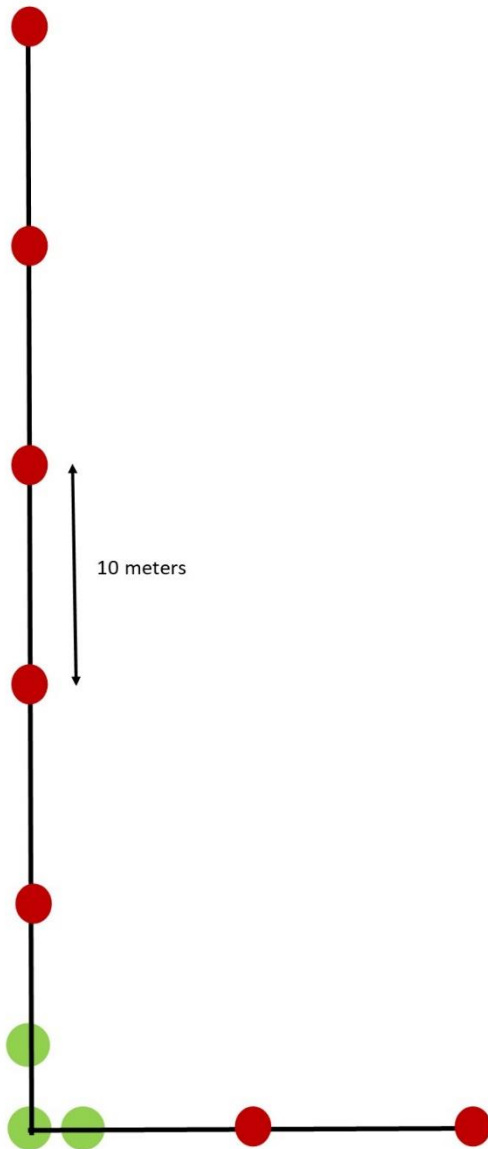
-  Start of the first transect walk
-  Start of the second transect walk
-  Direction of the first transect walk
-  Direction of the second transect walk
-  Delimitation of the parcel

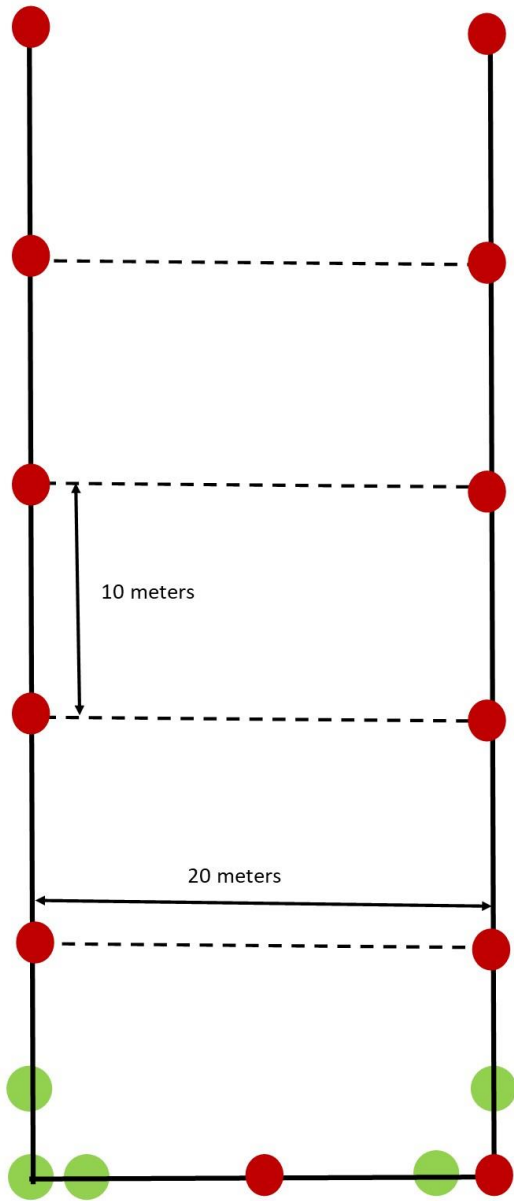
Appendix 5 – Method used for delineation of the plots. (A) Establishment of perpendicularity with the Pythagorean theorem. (B) Establishment of linearity. (C) Correction.



Legend :

-  Stake used for the establishment of perpendicularity
-  Stake used for the establishment of linearity
-  String delineating the plot
-  Measuring tape used temporarily

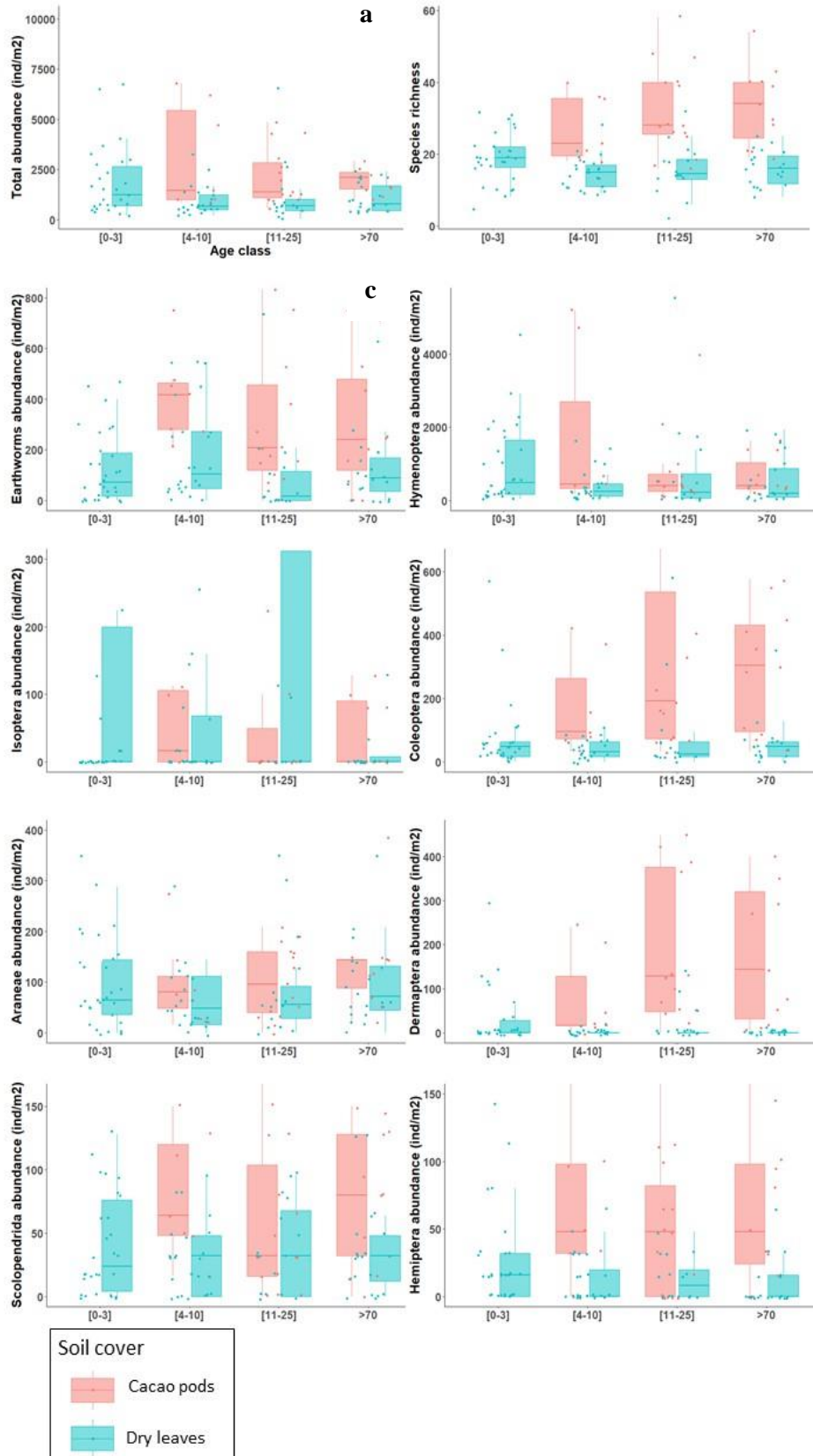




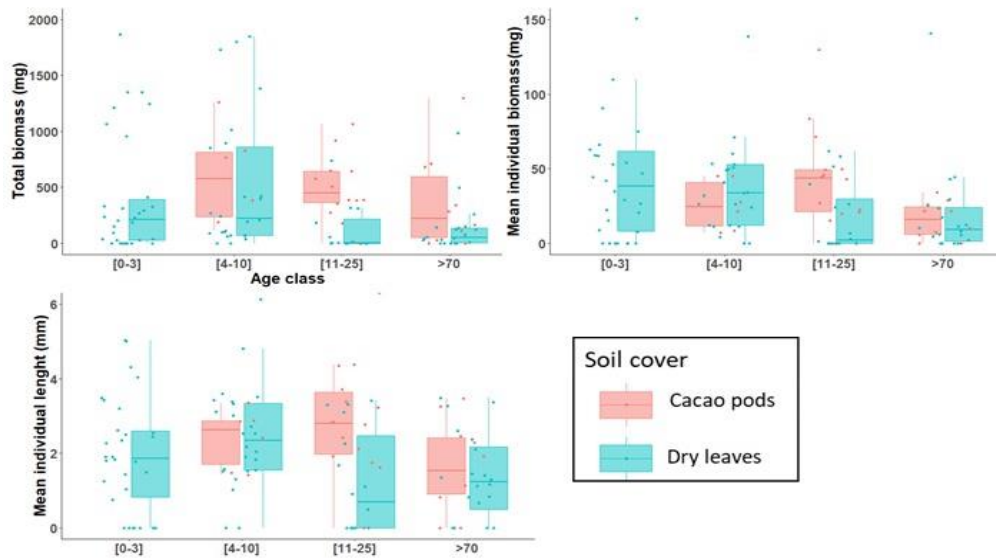
Appendix 6: Mean abundances and diversity of soil macrofauna in the four age classes and two types of soil cover of the study. Standard errors are included next to each value in small letters

Macrofauna taxon or variable	[0-3] (ind.m-2)		[4-10] (ind.m-2)		[11-25] (ind.m-2)		>70 (ind.m-2)				
	Dry/leaves	Cacao pods	Dry/leaves	Cacao pods	Dry/leaves	Cacao pods	Dry/leaves	Cacao pods			
Earthworms	127,5	352,0	185,3	451	38,0	299,4	81,3	262,4	74,1	120,0	31,9
<i>Hymenoptera</i>	951,5	1912,0	405,3	988,4	90,0	780,6	281,7	689,6	176,5	551,2	144,4
<i>Isopiera</i>	593,1	288,5	576,0	550,7	65,2	122,3	78,6	729,0	476,4	756,9	84,3
<i>Colleoptera</i>	75,7	21,2	192,0	65,7	40,7	396,6	104,1	84,0	37,4	315,2	17,0
<i>Araeneae</i>	96,5	16,4	90,7	37,8	80,7	97,1	192	91,0	26,2	171,2	18,7
<i>Dermoptera</i>	31,5	11,8	88,0	43,7	2,0	237,7	73,5	18,0	10,6	248,0	0,8
<i>Scolopendrida</i>	45,3	9,8	69,3	17,4	30,0	57,1	17,2	3,0	8,9	73,6	8,4
<i>Hemiptera</i>	32,0	8,8	66,7	28,1	12,7	48,0	14,4	13,0	3,9	65,6	3,8
<i>Isopoda</i>	34,1	8,7	18,7	13,3	10,7	10,3	6,4	13,0	10,0	19,2	3,9
<i>Diplopoda</i>	9,1	4,3	0,0	0,0	7,3	50,3	46,7	6,0	4,1	19,2	2,6
<i>Diptera</i>	3,7	2,0	98,7	50,8	3,3	25,1	11,3	2,0	2,0	12,8	3,9
<i>Acari</i>	1,6	1,2	2,7	2,7	1,3	42,3	26,4	14,0	10,1	43,2	1,1
<i>Stylommatophora</i>	10,7	3,7	10,7	5,3	2,0	12,6	7,0	3,0	1,6	22,4	21,7
<i>Scizomida</i>	5,3	1,9	2,7	2,7	5,3	11,4	7,0	3,0	1,6	16,0	2,0
<i>Blattodea</i>	2,1	1,0	2,7	2,7	1,3	4,6	3,5	3,0	1,6	4,8	1,7
<i>Lepidoptera</i>	4,8	1,7	5,3	5,3	1,3	3,4	3,4	3,0	1,6	3,2	0,8
<i>Psooptera</i>	1,6	6,4	2,7	2,7	4,0	1,1	1,1	3,0	1,6	12,8	1,6
<i>Orthoptera</i>	1,1	0,7	0,0	0,0	2,0	1,1	1,1	2,0	1,4	0,0	0,8
<i>Thysanoptera</i>	1,6	1,2	0,0	0,0	0,7	0,0	0,0	2,0	2,0	0,0	1,6
<i>Pseiscorpiionida</i>	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	1,6	0,8
<i>Collembola</i>	1,1	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0
<i>Diphura</i>	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0
<i>Embiopiera</i>	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0
<i>Neuroptera</i>	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
<i>Scorpiones</i>	0,0	0,0	0,0	0,0	0,0	1,1	1,1	0,0	0,0	0,0	0,0
<i>Uropygi</i>	0,5	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
<i>Pulmonata</i>	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Total abundance	1836,3	306,5	3506,7	1107,2	930,7	2218,3	378,1	1245,0	405,9	2784,0	1066,4
Specific richness (S)	19,0	1,2	25,2	3,4	14,9	33,3	3,1	15,8	1,8	32,9	1,1
Shannon index (H)	2,0	0,1	1,9	0,3	2,1	2,7	0,1	1,8	0,1	2,7	0,1

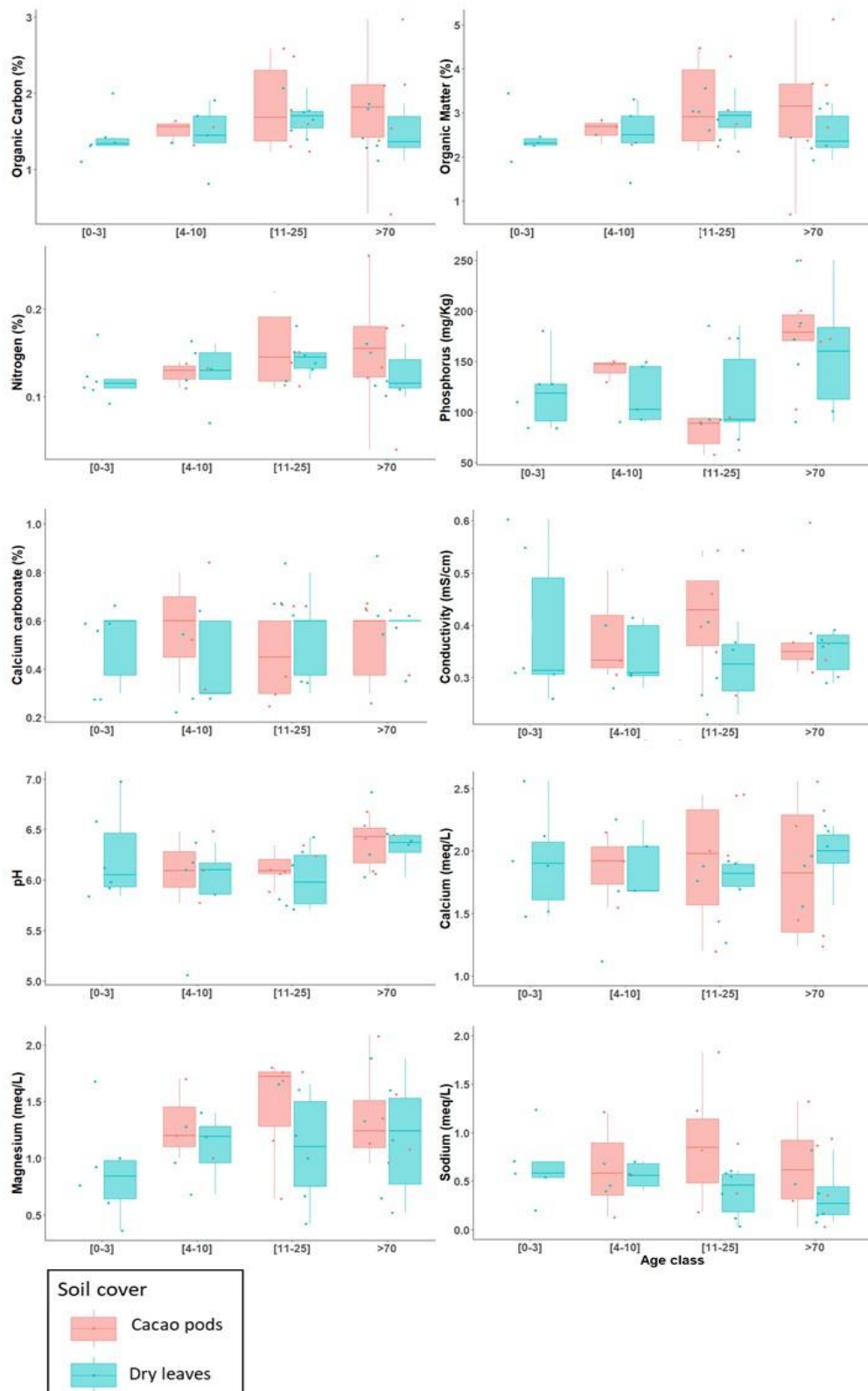
Appendix 7 – Total abundance, species richness and abundance of each taxon according to the type of soil cover and to the age class



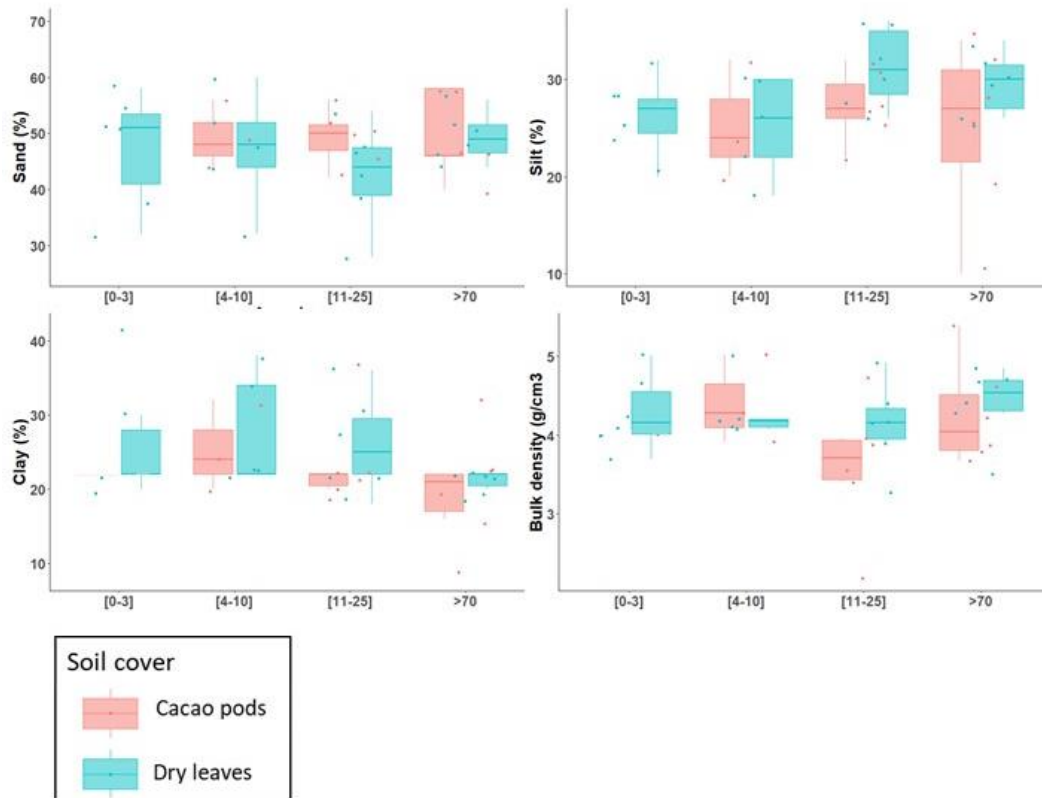
Appendix 8 – Earthworms functional traits according to the age class and to the type of soil cover.



Appendix 9: Soil chemical parameters according to the age class and to the type of soil cover



Appendix 10: Soil physical parameters according to the age class and to the type of soil cover



Appendix 11: Alternative age classification for the plantations

Age class	Description			
[0-2]	Initial stage, the trees are young and unproductive			
[5-7]	Transition stage, the trees are growing, and some are already producing			
[20-22]	Stabilized stage, the fruit trees are productive			
>70	Senescent stage, the production is reduced			

9 Abstract

Diversified tropical cacao-based agroforestry systems are part of the agroecological movement aiming, *inter alia*, at creating food systems that do not undermine the ecosystem services. However, the temporal and intra-system practices heterogeneity and their effects on the soil and soil macrofauna as ecosystem services providers still remain unclear. This study aims at exploring relationships between temporal heterogeneity, agricultural practices that generate spatial heterogeneity at plot level, and the soil quality and its macrofauna density and diversity. This study was led in cacao-based diversified AFS in the Dominican Republic. 120 macrofauna samples under leaf litter or cacao pod husks were conducted in 24 plots of 4 age classes. The intra-plot density and diversity was measured and soil analysis were realized. The macrofauna was identified at order level. Statistical analysis were performed to explain the influences of the age of the plantation and the agricultural practices (type of soil cover, tree density and diversity), on the soil chemical and physical parameters, and the macrofauna abundance and diversity. A limited effect of the age of the plantation and no effect of the evolution of tree density and diversity were revealed. Under cacao pods, the macrofauna density and specific richness were higher and the bulk density was lower. Therefore, a managed use of decomposing cacao pods in cacao-based tropical SAF of the Dominican Republic could enhance soil health and secure ecosystem services



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