

Biological soil health indicators are sensitive to shade tree management in a young cacao (*Theobroma cacao* L.) production system

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ABSTRACT

Cacao agroforestry systems can offer important benefits, such as greenhouse gas mitigation, microclimate regulation and improved soil health. The selection of tree species for cacao agroforestry systems is a critical step impacting cacao yields, as well as the environmental and economic sustainability of the production system. However, the effects of different tree species on soil processes and functions have been poorly studied. We assessed a series of soil health indicators in a five-year-old agroforestry trial located in the Ecuadorian Amazon. The following treatments: “control” (cacao monoculture), “timber” (cacao with *Cedrelinga cateniformis* Ducke; a leguminous tree), “fruit” (cacao with *Bactris gasipaes*), “N-fix” (cacao with *Erythrina velutina* Wild) and “mixed” (cacao with *C. cateniformis* + *E. velutina*) were replicated 3 times in randomized blocks. The experiment was managed organically with low levels of external inputs. We collected soil, litter and leaf samples of cacao trees at two different distances (~2 m and ~6 m) from the shade tree. Biological (potential soil respiration, and macrofauna abundance, richness and Shannon diversity), chemical (pH, CEC, total C and N, macro and micro-nutrients and Cd), and physical (bulk density and water holding capacity) soil health indicators were measured. Additionally, we analysed nutrients and Cd in cacao leaves and litter. Results showed positive effects of the “timber” and “mixed” treatments on biological soil health indicators, primarily earthworm abundance and potential respiration, as compared to cacao monocultures. Treatment effects on potential respiration showed different trends in litter versus soil, indicating that effects of shade trees on litter respiration do not transfer directly to soil respiration. Physical and chemical soil indicators, including available Cd which is irrelevant in the context of food safety regulations, did not show any differences among the treatments. Five years after establishment, no significant differences in yields were found among the control and any of the shade tree treatments.

1. Introduction

Cacao (*Theobroma cacao* L.) is an important global commodity, grown mainly by smallholder farmers throughout the tropics. Most cacao is produced in West Africa and Ecuador is the largest cacao producer in Latin America. Despite growing market demand, global total cacao production is declining (Fountain and Hütz-Adams, 2022). Numerous factors have contributed to this trend, declining soil fertility, increases in pests and diseases and climate change are commonly reported (Richard and Røed, 2016; Clough et al., 2009). Furthermore, social factors such as low profit for smallholder farmers and shortage of labour and technical assistance limit farmers’ engagement in sustainable

cacao production practices (Franzen and Mulder, 2007). Widespread impoverishment of soil health (Giller et al., 1997), is threatening cacao supply, the wellbeing of smallholder cacao producers and contribute to deforestation and biodiversity loss (Tscharntke et al., 2005; Pretty, 1997).

Theobroma cacao is an understory tree species native to humid tropical regions of northern South America, and according to some reports, central America (Motamayor et al., 2002). Most cacao cultivation has traditionally taken place in agroforestry systems in the lower story of the evergreen forest (Boyer, 1973). Agroforestry management involves the combination of different vegetation strata and species (including shade trees) and is gaining advocacy in the context of global crises such

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as climate mitigation, adaptation and biodiversity loss. Agroforests can also contribute to the restoration and maintenance of soil health in cacao production systems (Jose, 2009). In the second half of the 20th century monocrop plantations have been introduced as the main production model for cacao. However, nowadays cacao AFS receive renewed interest (Blaser et al., 2018; Niether et al., 2020). Shading is especially crucial in the establishment phase of cacao production (de Almeida and Valle, 2007), but higher yields have been reported when shade is progressively removed during production plateau (Ofori-Frimpong et al., 2007; Blaser et al., 2017) as cacao trees have a high self-shading capacity once mature (Hartemink, 2005). Nonetheless, agroforestry systems are nowadays increasingly promoted by supply chain actors to improve the sustainability of cacao production systems (Blaser et al., 2018; Niether et al., 2020). The selection of shade tree species for cacao agroforestry systems is a critical step determining the performance of the cacao production system but has been poorly studied, especially when it comes to effects on soil health (Niether et al., 2020). Both Steffan-Dewenter et al. (2007) and Niether et al. (2020) highlight that the effects of shade trees are highly context-dependent and influenced by species' specific traits that affect interactions with the main crop due to resource competition and complementary. Therefore, although shade trees offer a number of key services, little is known about the effects of the presence and species of shade trees in cacao cultivation on soil functions and the net impacts on crop productivity (Marshall, 2004; Niether et al., 2020).

The term soil health refers to “the continued capacity of a soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plants, animals and humans” (Doran and Zeiss, 2000). A healthy soil supports multiple soil functions: carbon cycling and storage, nutrient cycling, contaminant mitigation, pest control, water regulation and plant production (Creamer et al., 2022). Soil health is an abstraction of a holistic concept that cannot be measured directly (Janzen et al., 2021). Instead, we assess soil health based on sets of indicators that are proxies of soil processes relevant to the abovementioned soil functions. Indicator sets and assessments allow monitoring of soil health within agroecosystems, in response to different management strategies, and inform interventions to be implemented to restore and maintain soil health (Bünemann et al., 2018). Soil health integrates biology, physics, and chemistry, therefore any indicator set aimed at assessing soil health should ideally reflect all three aspects (Creamer et al., 2022). Most indicator schemes to assess soil health focus on temperate climates and annual cropping systems or pastures, yet few existing approaches for monitoring soil health have been applied to cacao (or other tropical perennial crops) rising to contextualise them for applicability in these production systems (Amponsah-Doku et al., 2022). Tropical perennial systems, such as cacao, distinctively rely on the substantial recycling of tree biomass into surface litter, which is crucial for maintaining soil health by enhancing organic matter content and nutrient cycling (Asigbaase et al., 2021).

Over the past decade, a number of studies have assessed biological aspects of soil health in cacao plantations, expanding beyond traditional chemical and physical indicators. For example, Rousseau et al. (2012) explored soil macrofauna assemblages as a soil health indicator within cacao agroforestry systems in Costa Rica. Alfaro-Flores et al. (2015) reported that in Bolivia's “Alto Beni” region, agroforestry and fallow plots exhibit higher soil microbial biomass than monoculture cocoa farms, with no significant difference between organic and conventional management. In Peru, Buyer et al. (2017) found that soil microbial communities in cacao plantations are significantly affected by agroforestry management and cover cropping. Natural agroforestry systems altered less the microbial community structures compared to soils in slash-and-burn methods, and specific cover crops, like centro (*Centrosema macrocarpum*) and perennial peanut (*Arachis pintoi*), were found to alter bacterial ratios and fungal biomass. Yao et al. (2021) conducted a study in West Africa assessing carbon and macronutrient cycling in cocoa and teak (*Tectona grandis*) plantations using indicators such as leaf litterfall, carbon and macronutrient inputs, and soil chemical and

microbial parameters. This study highlighted the importance of integrating trees that provide quality litter for enhanced cocoa nutrient availability and accelerated soil carbon storage. Further supporting this approach, a study by Koné and Yao (2021) in Ivory Coast demonstrated that mixed tree species stands, like agroforestry systems, can parallel natural forests in maintaining soil organic carbon stocks and microbial activity, underscoring their potential in the development of sustainable timber systems in West and Central Africa. These studies collectively emphasize the utility of biological indicators in detecting variations in soil health due to agroforestry, meanwhile highlighting a pivotal shift towards more sustainable cocoa farming practices that prioritize both crop yield and environmental conservation. However, these studies place less emphasis on the impact of shade tree arrangement and young agroforestry trials on soil health, leaving a knowledge gap.

Our study was aimed at quantifying the effect of different shade tree species and species arrangements on soil health and its relations to cacao production in a young plantation situated in the Ecuadorian Amazon. Special attention was placed on the role of different shade tree species (representing legume, fruit or timber trees, or combinations) and effect of distance of the shade trees in affecting: i) litter composition and soil health at different distance from the shade tree in cacao agroforestry systems, ii) the spatial tree diversity within the productions systems and iii) indicators with relevance for the following soil functions were prioritized in this context: nutrient cycling, carbon concentration, contaminant mitigation and cacao pod yield. The objective of this study was to evaluate a set of soil health indicators, that include biological, chemical, and physical aspects, and are linked to one or more soil functions in cacao agroecosystems. Another key objective was to determine which indicators are sensitive to changes in management, in particular different shade tree arrangements in a young agroforestry system, and to assess whether the proximity to these shade trees influences these indicators. We hypothesized that: i) the early effects of shade tree species on key soil functions are most prominently captured by soil biological indicators; ii) the inclusion of shade trees, especially leguminous trees, in a low-input organic cacao production system has a positive impact on cacao yields; iii) soil health indicator values change with distance from the shade trees and this effect can be related to differences in shading, litter quality and quantity.

2. Materials and methods

2.1. Site description

The study was carried out in the Northeastern part of the Ecuadorian Amazon region in an existing replicated field experiment established in 2015 at the Central Amazon Research Center (ECCA) of the Instituto Nacional de Investigaciones Agropecuarias (INIAP). The trial is located at Joya de los Sachas in the province of Orellana (0°21'31.9"S, 76°51'51.1"W; 265 m asl.). Mean annual temperature is 24.0 °C with variation of 1.3 °C and a mean annual precipitation of 3217 mm, corresponding to an Köppen-Geiger climate classification of tropical wet (Aw). The soil at the site has been classified as an Alfisol, according to USDA Soil Taxonomy (López et al., 2021). The soil texture was characterized as a clay loam (22.7% sand, 37.5% clay) in the top layer of the A horizon, changing to silty clay (15.5% sand, 42.3% clay) in the AB horizon (Table 1). The soil was slightly acidic, with an average pH of 5.4 and SOM content of 87.6 g kg⁻¹ in the A horizon. The trial includes four different treatments (low input organic, high input organic, high input conventional and medium input conventional). The current study only focused on the low-input organic treatment. Organic inputs for this treatment are mostly derived from internal resources such as litter fall and prunings from cacao and shade trees, which is representative of smallholder practices in the area.

The shade treatments were established in a domized Block Design ($n = 3$) in 2015, in an area that was previously under oil palm production. All shade trees were recently planted and carefully chosen for their

Table 1

Data from a soil profile at the INIAP cocoa agroforestry trial in the Ecuadorian amazon. Field work in this regard was performed by dr. E. Chavez (ESPOL) and dr. A. Margenot (University of Illinois) in 2018 at the same research site.

Horizon	Top depth	Bottom depth	Thickness	Sand	Clay	Silt	USDA textural class
	(cm)	(cm)	(cm)	(%)	(%)	(%)	
A	0	8	8	22.7	37.5	39.8	clay loam
AB	8	13	5	15.5	42.3	42.3	silty clay
Bt	13	20.5	7.5	16.5	58	25.5	clay
Bt	20.5	28	7.5	18.3	53.7	28	clay
Bt	28	49	21	15.6	58.6	25.8	clay
Bt	49	62	13	12.4	64.6	23.1	clay
Bt	62	103	41	15.6	66.2	18.2	clay
C	103	165	62	62	22.3	15.6	sandy clay loam
C	165	170	5	33.1	36.9	30	clay loam
C	170	177	7	55.1	18	26.9	sandy loam
C	177	215	38	81.5	16.2	2.3	sandy loam

functional significance in the local context, including fruit production, nitrogen fixation, and wood production. The shade treatments consist of four agroforestry systems: “timber” (*Cedrelinga cateniformis* Ducke; a leguminous timber tree also/locally known as tornillo or cedrorana), “fruit” (*Bactris gasipaes*; peach palm), “N-fix” (*Erythrina velutina* Wild; a widely used leguminous shade tree) and “mixed” (*C. cateniformis* + *E. velutina*). A fifth “control” treatment is included (monoculture without shade; MONO). Shade tree spacing in the agroforestry systems was 12 × 12 m in timber and fruit, and 6 × 6 m in *Erythrina*. Cacao trees were

newly planted at a density of 3 × 3 m. Net plots contained 6 cacao plants and were surrounded by 3 rows of cacao plants as borders (Fig. 1 and 2). The cacao cultivars in the trial are two national clones EET 95 and EET 103, alternated in strips (Fig. S1). Differences between cacao genotype were not determined in this study. The cacao trees received 0.5 kg Ecoabonaza” (organic fertilizer with chicken manure) and 0.35 kg lime per tree before planting. No pesticides were applied. Pruning of the cacao trees is carried out biannually, with the clippings subsequently redistributed across the soil. Shade trees were pruned once per year and weed was controlled with a weed wacker monthly. Harvest took place manually once per month.

2.2. Selection of soil health indicators

The selection of indicators was driven by both practical and scientific considerations as the predictor should be meaningful, robust, interpretable, sensitive, low-cost and relatively easy to measure considering local field and laboratory conditions. We identified indicators that included at least one or more biological, chemical, or physical soil indicators, with a particular emphasis on those that provide information about the following soil functions: nutrient cycling, carbon concentration, contaminant mitigation (cadmium) and primary production. We conducted measurements of the biological indicators (potential soil and litter respiration, total macrofauna and earthworm abundance, and taxonomic richness and Shannon diversity of macrofauna); chemical indicators (pH, cation exchange capacity (CEC), total soil organic carbon (SOC), total nitrogen (N), macro and micronutrients and cadmium (Cd));



Fig. 1. Visual representation of the experimental trial in northern the Ecuadorian Amazon, which aimed to evaluate the performance of different shade tree arrangements on predictors of soil health and cacao productivity. The satellite image indicates the location of the experimental sites where the agroforestry systems were amended traditionally, while the tree icons indicate the type of agroforestry system within the sampled plot. The treatments consist of five agroforestry systems which are distinguished as: “monoculture” (without shade trees; control), “timber” (*Cedrelinga cateniformis* Ducke; a leguminous timber tree also/locally known as tornillo or cedrorana), “fruit” (*Bactris gasipaes*; peach palm), “N-fix” (*Erythrina velutina* Wild; a widely used leguminous shade tree) and “mixed” (*C. cateniformis* + *E. velutina*).

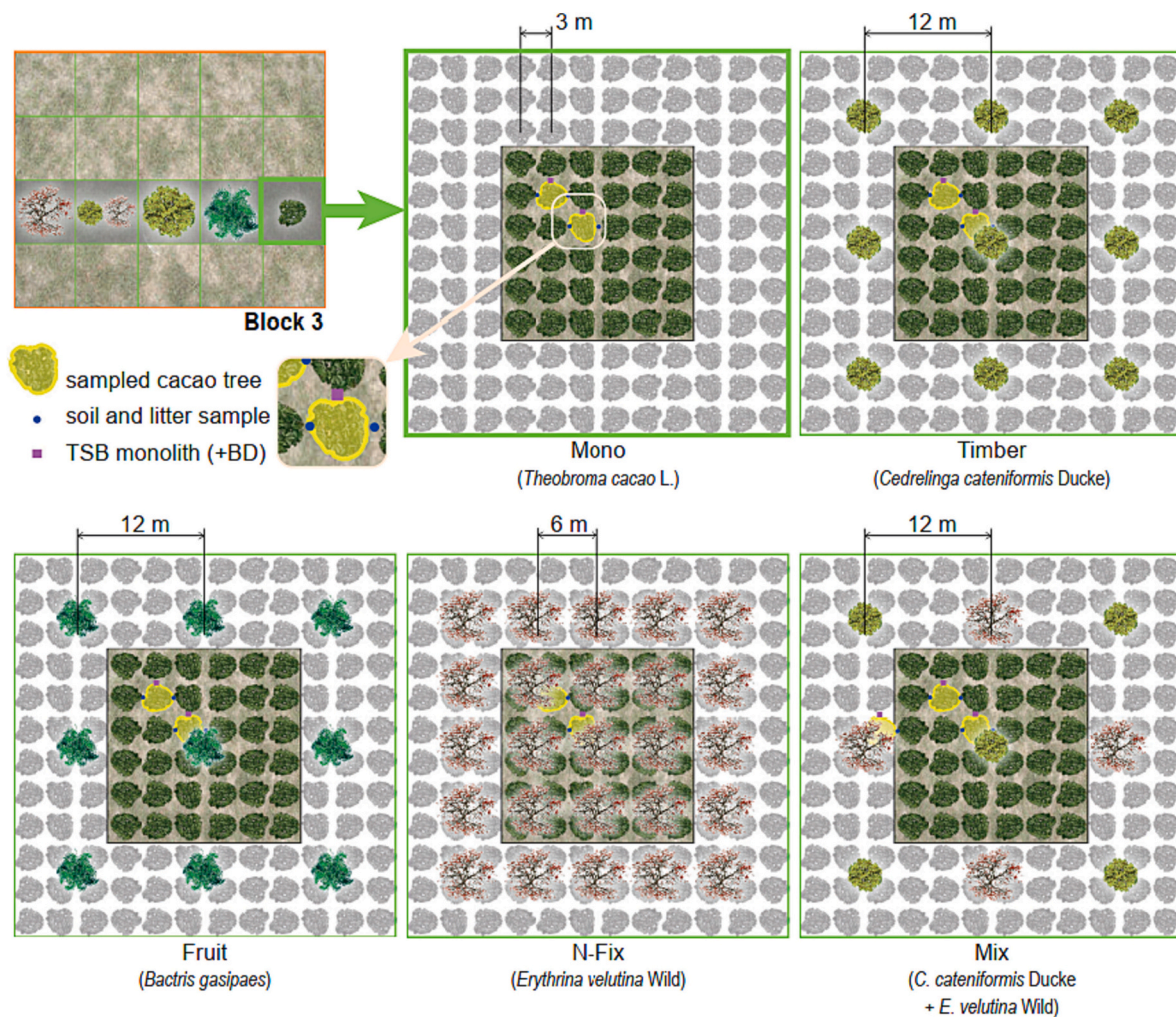


Fig. 2. Layout of the sampling strategies in the different agroforestry treatment.

and physical indicators (bulk density and water holding capacity). Furthermore, we assessed nutrient and trace metal levels in cacao leaves and litter.

2.3. Soil, plant sampling, and yield data collection

Soil, litter and leaf samples were collected in October 2019. In total, 15 plots were sampled (5 shade tree treatments x 3 replicate blocks). To account for the effects of distance from the shade trees on litter and soil characteristics, samples were taken around the cacao trees at two different locations within the net plot (~2 m and ~6 m from the shade tree). In the monoculture “control” plot, samples were taken in the center of the net plot (Fig. 2). Soil and litter samples were taken on opposite sides of the selected cacao tree (in an angle of 180°). Sampling was conducted within the rooting zone of the tree, at 60 cm from the trunk, as closer to the trunk of the cacao tree roots are not abundantly. First, surface litter was collected using a 20.3 cm diameter circular sampling frame after removal of living biomass. Then, the underlying soil was collected at depths of 0–7.5 cm and 7.5–15 cm using a spade. Litter and soil samples from the same tree were individually combined, yielding one composite soil sample and one composite litter sample per layer for each cacao tree. Additionally, the second pair of mature leaves on a flowering branch was collected in four cardinal directions, to obtain a total of 8 leaves per plant (2 leaf samples per plot) for foliar analysis. Immediately after sampling, field moist soil samples from the first soil depth were homogenized and split into two subsamples. About 250 g of

moist soil was stored in a cooler with ice for transport to the laboratory. The remaining soil, leaf and litter samples, were transported at ambient temperature. Pod yield data for the years 2017, 2018, 2019 were collected as follows: Pods were collected once every month during the period January–September for 9 out of 144 cacao trees per plot. Fresh pod weight in kg per plant was recorded. Shade cover in the different shade trees (at two distances) was estimated using a spherical densitometer. Readings took place in the week of 21–26 October 2019, always by the same person to avoid bias. The device was held level above the cacao tree and the number of uncovered blocks on the mirror were counted. On each location four readings were made facing north, east, south, and west. Later, the average was calculated to determine over-story density (%).

2.4. Measurement of biological soil health indicators

Soil macrofauna community abundance and diversity were assessed using the Tropical Soil Biology and Fertility (TSBF) method (Anderson and Ingram, 1993), with a modified sampling design considering small experimental plots. A soil monolith (25 × 25 × 20 cm deep) was excavated with a flat shovel adjacent to each sampled tree (two per plot) and put in a bag (Fig. 2). All macrofauna visible with the naked eye (>2 mm) was hand-sorted in a nearby barn and stored in 70% ethanol (or 4% formalin for earthworms). Blocks 1 and 2 of the trial had extremely high densities of ants dominated by the species *Nylanderia fulva*; commonly known as “crazy ants”. Therefore, for practical reasons, we collected

100 ants from each monolith with >100 ants present, which refers to an equivalent of >1000 (ind. m⁻²). All macrofauna was transported in a cooler to the lab. Specimens from each sampling point were identified, generally, to the order level (depending on the taxonomic group), for calculation of diversity indices and further analyses. Macrofauna abundance and diversity were expressed as taxonomic richness (S, number of distinct taxonomic groups) and Shannon Index (H; Shannon, 1948).

Potential soil respiration (CO₂ evolution during incubation under standardized conditions, homogenized soil sample, optimal temperature, and moisture) is used as indicator for microbial activity. This indicator was measured following a modified method from Hess and Schmidt (1995). Air-dried soil samples from the first soil layer were homogenized by passing 50 g of soil through a 2 mm sieve for removal of debris and large particles. The moisture content of dry soil was then gradually adjusted to 60% of the water holding capacity (determined by adding water to an approx. 50 g dry soil sample with a plant sprayer until the soil appeared shimmering and water was noticeably accumulated in the openings). The re-wetted soil was subsequently transferred to polyethylene bottles (300 mL), sealed with parafilm and stored in a dark place at constant temperature (23 °C). The bottles were opened for 10 min prior to each measurement to refresh the headspace. A Go Direct® CO₂ Gas Sensor was used to determine increase in gaseous CO₂ concentration in the headspace (ppm CO₂s⁻¹) of the bottles. Measurements were repeated at 24, 48, 72 and 96 h time intervals and lasted for 10 min. To avoid the disturbance effect due to rewetting, we excluded the measurements taken after 24 and 48 h from the data set and focused on respiration measurements once the CO₂ curve had stabilized following the first flush of gas (Pulleman and Tietema, 1999). The C respiration in µg C-CO₂ per gram dry soil per hour was calculated applying the following formula:

$$C \text{ respiration} = 0.273 \times \frac{[\text{change CO}_2 \text{ in headspace (mg.m}^{-3}\text{.h)} \times \text{headspace volume (m}^{-3}\text{)}]}{\text{air dry sample weight (g)}}$$

where 0.273 is the conversion factor from C to CO₂ in mg.

2.5. Measurement of chemical and physical soil health indicators

Bulk density (BD) and gravimetric soil moisture content (W) at the time of sampling were measured at a depth of 10 cm by inserting PVC rings (diam. 7.5 cm; height 7.8) into the center of this layer capturing undisturbed soil using one of the sides of the pit following excavation of the monoliths. This resulted in a total of two measurements per plot (one per cacao tree). The ring was then placed in an air-tight plastic bag within a protective container for transport. Soils in the PVC rings were carefully removed by cutting the edge of the ring with an iron saw. The diameter of each ring was measured (in cm) prior to its removal. The moist soil held by the ring was then weighed and a representative subsample (40 g) was used to determine the gravimetric moisture content by oven drying at 105 °C until constant weight. The subsamples were used to determine dry weight of the moist samples in the rings. Rocks and large roots from each ring were dried and weighed separately. Then, the calculated dry soil weight (g) was divided by the soil volume (cm³) to calculate bulk density (g.cm⁻³).

The disturbed soil samples (see Section 2.2) were air-dried, manually crushed and sieved (< 2 mm) prior to determination of nutrient availability and other chemical soil properties. Soil pH was measured in deionized water (1:2.5) after 30 min of equilibration. Availability of soil macro-, micro- nutrients and Cd was quantified by ICP-OES after Mehlich III extractions (Mehlich, 1984). Analyses included phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), zinc (Zn),

copper (Cu), iron (Fe), cadmium (Cd). Total soil carbon (TSC, also known as TOC) and total soil nitrogen (TSN) were quantified using dry combustion. This process involved using 150 mg of dry soil, which was homogenized through a 250 µm sieve to create a uniform and testable sample. We employed a CN Analyzer (Elementar Analyzer, Germany) for the analysis.

2.6. Litter and leaf characterization

The total weight of the litter samples collected in the field was measured when still moist and corrected for moisture content (see below) to calculate dry litter mass per m² present in the field at the time of sampling. One part of the moist litter sample was directly stored in the fridge for 30 d until further analysis. Potential litter respiration was measured following the same protocol mentioned for soil samples (Section 2.4), but adjusted for fresh organic material using the method described in Pulleman and Tietema (1999). A fixed amount of moist litter, corresponding to 4.4 g of dry weight, was brought to the desired initial gravimetric water content of 272% by adding demineralized water using a plant sprayer. Measurements of respiration lasted 10 min as described above for soil respiration measurements.

The cacao leaves were cleaned in a bath of deionized water prior to drying and processing. The remaining part of the litter sample and the cacao leaf samples were oven-dried at 60 °C until stable weight. Dry litter and leaf samples were ground and sieved (850 µm) before determination of total element concentrations. In short, 350 mg of tissue was weighed in a 50 mL reaction tube. Then, samples were digested with 6 mL of concentrated HNO₃ and 1 mL of H₂O₂. The samples were left to digest for 12 h at 80 °C in an open digestion system (DigiPREP, SCP Science). Macro and micronutrients and trace metals were then quantified by ICP-OES. 50 mg of pulverized material (< 2 mm) were used to

prepare a homogeneous testable sample to determine total carbon (TC) and total nitrogen (TN) content through dry combustion using an CN analyzer (Elementar Analyzer, Germany).

Lignin concentrations in litter was measured using the Klason method in which lignin is determined as 'klason lignin' by quantifying the wood component insoluble in a 72% sulphuric acid solution (Kirk and Obst, 1988). No pre-treatment, such as ethanol-benzene or 80% aqueous ethanol (1:2), was applied for lignin determination as the effects of different pre-treatments are still widely disputed (Toda et al., 2015). The concentrations of extracted total polyphenol were determined according to method described by (Anderson and Ingram, 1993), after extraction with 20 mL methanol. Briefly, 0.75 g of litter was weighted into a 50 mL glass tube. Then, 20 mL of a 50% methanol solution was added. Accordingly, the tube was covered with parafilm and placed in a water bath at 70–75 °C for 1 h. The extract was then filtered. In a new flask were then Folin-Denis reagent, sodium 17% carbonate, the extract and water well mixed and put to rest for 20 min. Afterwards the absorbance of the extracts was measured at 761 nm using a UV-VIS spectrophotometer (Biotek synergy HT).

2.7. Statistical analysis

All analyses were carried out using JMP pro 14.0.1 software package (SAS Institute 2015). Data analyses involved all soil, leaf and litter samples taken from the 5 different agroforestry treatments and 3 blocks, at 2 distances nested within the main plots (*n* = 33). Before starting any analyses, an ANOVA was used to rule out any block effect on leaf, litter,

and soil indicators. Then, a mixed model approach was applied to examine the main and interaction effects of the treatments (*Treat.*; fixed factor) and distance from the shade trees (*Dist.*; a random factor to account for spatial autocorrelation) on the different soil health indicator values, litter quality and quantity, shade cover and leaf nutrient content. Additionally, ANOVA was used to see if distance had an immediate effect on the C and N contents in the soil samples from different depths. This was not the case. Two-way ANOVA was used to explore relationships between soil C and N contents, *Treat.* and soil depth, to understand the impact of shade tree arrangement on these soil health indicators. Fixed factors included *Treat.*, soil depth and *Treat.* by soil depth interactions. Where there were significant effects of *Treat.*, interactions between *Treat.*Dist.* and *Treat.*depth*, Tukey post-hoc analysis ($\alpha = 0.05$) was carried out. Another mixed model approach was applied to examine the main and interactive effects of treatment (*Treat.*) on cacao yield of 2017, 2018 and 2019 and block effect (as random factor). A principal component analysis (PCA) was performed to identify which soil health indicators and litter characteristics are most strongly associated with each other, determine which of those variables contribute most to the variation in the data, and how they related to the different agroforestry arrangements of the trial. When needed, data was log transformed to comply with statistical assumptions of homoscedasticity and normality of the residuals. This was done for i) Litter: K, Cd, polyphenols, respiration (96 h), ii) Leaf: P, Fe, Zn and Cd and iii) Soil: K, P, Zn, Cd and respiration (76 h), species richness, total macrofauna, macro fauna diversity and earthworm abundance.

3. Results

3.1. Shade and litter characteristics of the different agroforestry systems

Shade cover at the time of sampling varied from 0 to 65%, depending on shade tree treatment ($p < 0.001$; Table 2). A clear effect of distance on shade cover of the cacao trees was observed, except for “N-fix”. Estimated shade cover at 2 m from the shade tree was largest for the “N-fix” and “fruit” treatments (51% and 22%, respectively), and further decreased in the order “timber” = “mixed” > “control”. At 6 m distance

the shade cover followed the order “N-fix” > “fruit” = “mixed” = “timber” > “control”. There was no interaction effect of treatment and sampling distance on shade cover.

Litter dry weight, C/N ratio and macronutrient content were not significantly affected by treatment or sampling distance (Table 2). The average litter dry weight was 11.1 Mg ha⁻¹ and litter contained 275C, 19 N, 1.5 P, 5.3 K (g kg⁻¹). Concentrations of Zn ($p = 0.027$) and Cd ($p = 0.038$) in litter differed significantly among treatments with concentrations being highest in the “control” with only cacao trees (178 and 17.5 mg kg⁻¹, for Zn and Cd respectively) and lowest in “N-fix” (130 and 5.5 g kg⁻¹, respectively), but no distance or interaction effects were found.

Potential litter respiration taken as point measurement after 96 h differed ($p = 0.047$) among treatments and was marginally significant after 168 h ($p = 0.062$; Fig. 3 & S2). Moreover, distance affected potential litter respiration after 168 h ($p = 0.013$). Overall, potential litter respiration rates were higher in samples collected closer to the shade trees than in the samples that were taken at larger distance. There was no interactive effect of shade tree and distance on potential litter respiration.

3.2. Soil health indicators

3.2.1. Biological soil health indicators

A total of 2899 macro-invertebrates were collected, representing 15 different taxonomic groups (Table S1). Samples from two out of the three blocks were infested by ants with counts of more than >1000 individuals per m². This specific ant was identified as *Nylanderia fulva* Mayr (1862), commonly known as crazy ant. When excluding crazy ants from the total macrofauna count, we counted a total of 376 specimens of arthropods and earthworms. The most abundant groups were *Annelida* (earthworms, 61.6%) and *Hymenoptera* (wasps, bees and ants, 15.9% of total individuals). All other taxonomic groups, including *Isopoda* (woodlice), *Chilopoda* (Centipedes) and *Coleoptera* (Beetles) counted each for <5% of total abundance.

Earthworm abundance was affected by shade trees ($p = 0.043$), such that earthworm abundance was a factor 3.2 higher in “mixed”

Table 2

Means and standard errors for shading and litter data observed in the different cocoa agroforestry systems ($n = 5$). *P*-values for the effect of treatment (*Treat.*), Distance (*Dist.*) and their interaction (*Int.*) are provided. Samples were taken in October 2019 in a cocoa agroforestry trial in Coca, Amazon, Ecuador. Means followed by the same letter in the column do not differ as per the Tukey test ($\alpha = 0.05$). Shaded headers indicate treatments that include legume trees.

Indicator	Unit	Cocoa agroforestry systems										p-values		
		FRUIT		MIX		MONO		N-FIX		TIMBER		Treat.	Dist.	Int.
		mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)			
Shading														
Shade cover	%	22.2b	(11.05)	14.53bc	(3.51)	0 c	(0.00)	51.08a	(6.05)	13.42bc	(5.05)	<0.001	<0.001	0.109
Litter														
Litter dry weight	Mg ha ⁻¹	9.8	(1.89)	11.1	(1.16)	12.3	(1.74)	12.4	(1.54)	9.9	(1.12)	0.645	0.570	0.525
C	g kg ⁻¹	271.6	(34.80)	280.9	(21.80)	275.4	(38.40)	289.1	(37.60)	264.4	(28.90)	0.975	0.374	0.417
N	g kg ⁻¹	18.0	(1.40)	20.0	(1.50)	19.3	(2.00)	22.4	(2.30)	19.0	(1.80)	0.412	0.504	0.082
P	g kg ⁻¹	1.6	(0.10)	1.5	(0.10)	1.6	(0.10)	1.4	(0.10)	1.5	(0.10)	0.668	0.953	0.944
K	g kg ⁻¹	6.0	(0.90)	4.6	(0.50)	5.7	(1.10)	4.2	(0.60)	6.3	(0.80)	0.260	0.713	0.590
Mg	g kg ⁻¹	4.1	(0.10)	4.0	(0.20)	3.6	(0.30)	3.8	(0.20)	3.8	(0.40)	0.734	0.348	0.115
Zn	mg kg ⁻¹	166.1ab	(7.50)	155.6ab	(9.20)	177.8a	(11.90)	130.1b	(5.80)	144.2ab	(10.80)	0.027	0.912	0.449
Fe	mg kg ⁻¹	16,698	(3282.90)	16,286	(1435.30)	14,726	(2361.50)	14,715	(2904.10)	16,819	(2273.90)	0.905	0.638	0.675
Cd	mg kg ⁻¹	8.2ab	(1.30)	11.7ab	(2.90)	17.5a	(4.10)	5.5b	(0.60)	6.8ab	(1.40)	0.038	0.592	0.452
Total Polyphenols	μg ml ⁻¹	18.7	(1.75)	17.0	(1.40)	24.5	(2.90)	17.9	(1.80)	21.4	(3.20)	0.114	0.914	0.246
Klason lignin														
Residue	% SR*	49.3	(1.30)	48.6	(2.20)	50.5	(1.60)	44.8	(2.10)	48.8	(1.40)	0.375	0.289	0.782
Litter Respiration	ug CO2-C g ⁻¹ dry soil hr ⁻¹													
96 h		84.5ab	(14.83)	60.2ab	(8.38)	62.8ab	(12.44)	39.4b	(4.20)	89.9a	(17.90)	0.047	0.091	0.552
Litter Respiration	ug CO2-C g ⁻¹ dry soil hr ⁻¹													
168 h		69.9	(6.30)	54.2	(8.80)	62.2	(13.00)	35.7	(4.60)	70.6	(12.80)	0.062	0.013	0.397

* Solid Residue.

**Ca, Co, Mn, Cu and S were collected in this study but were not displayed in the main analyses as focus was placed on the macro and common micronutrient discussion, there were no significant differences found for these nutrients in this mixed model analyses. Al was not reported as result returned by ICP, furthermore Al results were also not reliable according to the reference sample. Na and Mo were reported but mostly below detection limit. (n.d = non defined).

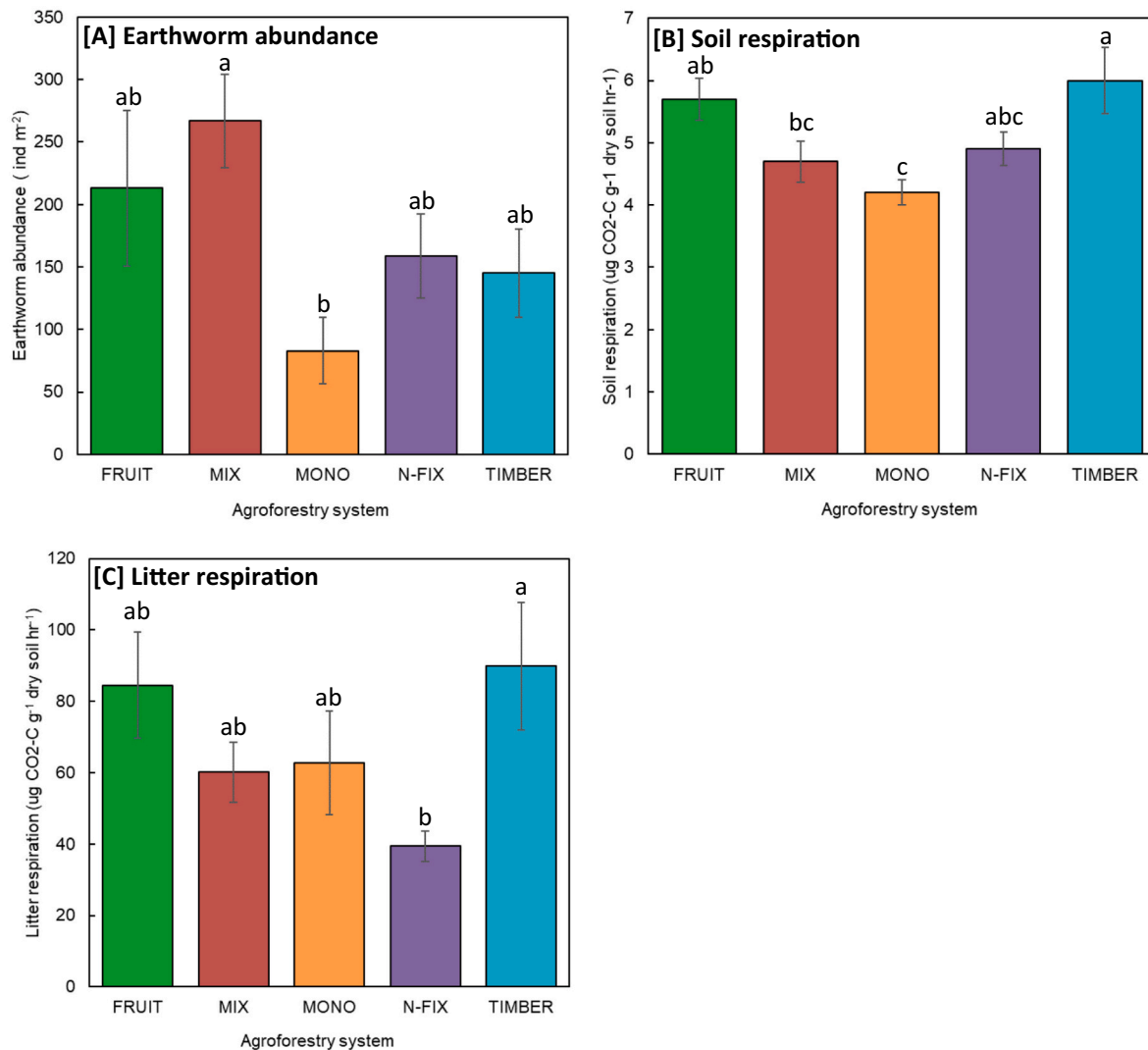


Fig. 3. Biological indicators of soil health and litter characteristics analysed under different cocoa agroforestry systems in Coca, Amazon Ecuador. The bar graph shows the means with std. err. From left to right: A) showing mean values of earthworm abundance sampled in the trial, B) showing mean values of soil respiration after 96 h of incubation in the lab, C) showing mean values of litter respiration after 96 h of incubation in the lab.

agroforests as compared to "control" monocultures. Earth worm abundance was not affected by distance from the shade trees nor by the interaction between the two (Fig. 3; Table 4).

Treatment and distance did not influence macrofauna diversity, taxonomic richness, or other indicators of macrofauna abundance ($p > 0.05$). Taxonomic richness (number of orders) ranged from 4 to 6 taxa per monolith, while diversity, based on the Shannon Index (H), ranged from 0.57 to 0.90 per monolith.

Potential soil respiration, as measured in lab conditions, was significantly affected by shade tree after 72 h ($p < 0.01$) and 96 h ($p < 0.01$; Fig. 3). After 96 h, soil respiration was a factor 1.43 higher in the "timber" treatment compared to the "control". These findings are consistent with respiration rates at 72 h. There was no effect of distance or its interaction with tree species on soil respiration ($p > 0.05$).

3.2.2. Physical and chemical soil health indicators

Carbon and N contents and C/N ratios were analysed in two soil layers and showed significant depth effects ($P < 0.01$; Table 4). The C and N content in the first soil layer was two-fold greater than in the deeper soil layer in all treatments, but we found no effect of treatment on soil C and N content in both 0–7.5 and 7.5–15 cm layers.

The soil C/N ratio varied ($p = 0.021$) with soil depth (D) and with

treatment. The C/N ratio of the first soil layer was a factor 1.28 higher in "fruit" agroforestry systems as compared to "mixed". Moreover, higher C/N ratios were found in the upper soil layer (11.2 on average) compared to the deeper layer (7.9 on average) in all treatments. Tree species did not affect C/N ratio at the 7.5–15 cm depth.

Total soil N was on average $4.7 (\pm 0.13)$ g N kg⁻¹ in the first soil layer (Table 4), and Mehlich 3-extractable macronutrient concentration was on average $29.1 (\pm 1.63)$ g P kg⁻¹, $0.54 (\pm 0.04)$ g K kg⁻¹ and Fe and Zn were $156.4 (\pm 3.7)$ and $4.7 (\pm 0.32)$ mg kg⁻¹, respectively, with no treatment effect for any of the measured nutrients (Table 3). Average Mehlich 3-extractable Cd in the soil across all treatments was $0.3 (\pm 0.01)$ mg Cd kg⁻¹. Nutrients and Cd were not significantly affected by treatment, distance, nor their interaction.

Bulk density in the first soil layer was on average 0.73 g cm⁻³ and the water holding capacity was 48% (gravimetric) across treatments (Table 3). Bulk density and the water holding capacity were not significantly affected by treatment, distance, nor their interaction.

3.3. Relationships between litter characteristics and soil health indicators

Relations between the litter characteristics, soil health indicators and shade trees were explored through principal component analysis (PCA).

Table 3
Means and standard errors for biological, chemical and physical soil health indicators observed in the different cocoa agroforestry systems ($n = 5$). P-values for the effect of treatment (*Treat.*), Distance (*Dist.*) and their interaction (*Int.*) are provided. Samples were taken in October 2019 in a cocoa agroforestry trial in Coca, Amazon, Ecuador. Means followed by the same letter in the column do not differ as per the Tukey test ($\alpha = 0.05$). Shaded headers indicate treatments that include legume trees.

Indicator		Unit	Cocoa agroforestry systems										p-values			
			FRUIT		MIX		MONO		N-FIX		TIMBER		Treat.	Dist.	Int.	
			mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)				
Soil Biological																
	Soil Respiration 72 h	$ug\ CO_2-C\ g^{-1}\ dry\ soil\ hr^{-1}$	6.34 ab	(0.30)	6.07 b	(0.39)	5.69 b	(0.18)	5.90 b	(0.41)	8.53 a	(1.04)	0.009	0.462	0.462	
	Soil Respiration 96 h	$ug\ CO_2-C\ g^{-1}\ dry\ soil\ hr^{-1}$	5.72 ab	(0.34)	4.70 bc	(0.33)	4.22 c	(0.20)	4.90 abc	(0.27)	6.02 a	(0.53)	0.007	0.112	0.112	
	Macrofauna diversity	<i>Shannon-index</i>	0.75	(0.18)	0.90	(0.20)	0.57	(0.23)	0.71	(0.20)	0.78	(0.21)	0.920	0.838	0.838	
	Species Richness	#	4	(0.43)	5	(0.96)	4	(0.88)	4	(0.42)	6	(1.28)	0.355	0.941	0.941	
	Annelida	$ind\ m^{-2}$	213 ab	(62.49)	267 a	(37.33)	83 b	(26.83)	159 ab	(33.65)	145 ab	(35.45)	0.043	0.865	0.865	
	Total macrofauna abundance (without <i>Nylanderia fulva</i>)	$ind\ m^{-2}$	282.0	(44.9)	366.7	(49.1)	304.0	(184.7)	215.3	(40.8)	272.7	(56.9)	0.820	0.486	0.486	
Soil chemical & physical																
	pH	–	>5	5.57	(0.12)	5.36	(0.09)	5.41	(0.14)	5.27	(0.12)	5.65	(0.16)	0.332	0.965	0.874
	CEC	$mol\ kg^{-1}$	–	9.22	(1.26)	8.00	(0.49)	7.22	(0.83)	7.23	(0.72)	9.32	(1.20)	0.354	0.930	0.679
	avP	$g\ kg^{-1}$	>25	29.21	(4.46)	29.87	(3.37)	29.82	(4.55)	27.86	(3.94)	28.27	(3.01)	0.997	0.676	0.835
	K	$g\ kg^{-1}$	>0.15	0.42	(0.06)	0.59	(0.11)	0.43	(0.06)	0.51	(0.06)	0.69	(0.17)	0.503	0.745	0.647
	Mg	$g\ kg^{-1}$	>3	0.16	(0.01)	0.15	(0.01)	0.17	(0.02)	0.17	(0.02)	0.19	(0.02)	0.651	0.886	0.168
	Zn	$mg\ kg^{-1}$	>1	6.40	(0.86)	3.81	(0.29)	4.49	(0.36)	4.26	(0.72)	5.16	(1.05)	0.087	0.904	0.456
	Fe	$mg\ kg^{-1}$	>4.5	171.08	(6.64)	149.90	(3.92)	156.34	(8.59)	159.74	(14.68)	147.94	(6.62)	0.370	0.787	0.816
	melich.av Cd	$mg\ kg^{-1}$	n.a	0.36	(0.03)	0.32	(0.03)	0.37	(0.04)	0.30	(0.04)	0.31	(0.03)	0.322	0.487	0.345
	Bulk Density	$g\ cm^{-3}$		0.75	(0.02)	0.73	(0.02)	0.69	(0.04)	0.75	(0.02)	0.75	(0.03)	0.586	0.590	0.753
	WHC	%		50.22	(0.40)	47.8	(0.86)	47.82	(1.52)	49.12	(0.72)	47.88	(1.29)	0.187	0.303	0.809

*Indicative reference values for available soil nutrients to support production (van Vliet and Giller, 2017; Wessel, 1970).
**Ca, Co, Mn, Cu and S were collected in this study but were not displayed in the main analyses as the focus was not mainly on chemical soil analyses, there were no significant differences found for these nutrients in this mixed model analyses. Al was not reported as result returned by ICP, furthermore Al results were also not reliable according to the reference sample. Na and Mo were reported but mostly below detection limit.

Table 4
C/N ratio, C & N content in the 0–7.5 cm, 7.5–15 layers in soils under different cocoa agroforestry systems in Coca, Amazonia, Ecuador (means). P-values for the effect of treatment (*Treat.*), Soil depth and their interaction (*Int.*) are provided. In each treatment, lowercase letters compare means on the columns. Means followed by the same letter in the column do not differ as per the Tukey test ($p < 0.05$). Shaded headers indicate treatments that include legume trees.

Indicator		Soil layer (cm)	Cocoa agroforestry systems										p-values		
			FRUIT		MIX		MONO		N-FIX		TIMBER		Treat.	Soil depth	Int.
			mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)			
C content	%	0–7.5	5.43	(0.26)	4.61	(0.33)	5.01	(0.18)	5.40	(0.13)	5.25	(0.27)	0.296	<0.001	0.411
		7.5–15	1.99	(0.03)	2.48	(0.19)	2.60	(0.33)	2.40	(0.15)	2.33	(0.20)			
N Content	%	0–7.5	0.45	(0.03)	0.51	(0.03)	0.44	(0.02)	0.45	(0.01)	0.46	(0.02)	0.548	<0.001	0.069
		7.5–15	0.25	(0.01)	0.30	(0.02)	0.33	(0.03)	0.30	(0.01)	0.29	(0.01)			
C/N ratio	–	0–7.5	12.08 a	(0.39)	9.38 b	(0.94)	11.33 ab	(0.38)	11.85 ab	(0.26)	11.23 ab	(0.22)	0.043	<0.001	0.021
		7.5–15	7.79 c	(0.28)	8.02 c	(0.19)	7.71 c	(0.22)	8.12 c	(0.21)	7.92 c	(0.26)			

Each principal component (PC) was interpreted according to the magnitude of their eigenvalues (Fig. 4). The first principal component (PC1) explained 34.4% of the variance in the data and was mainly associated with litter parameters, such as litter respiration (0.97), lignin content (0.91), N content (–0.96), C content (–0.91) litter dry weight (–0.98), but at the same time also strongly associated to chemical soil health indicators such as pH (0.92) and CEC (0.91). Litter dry weight, C and N concentrations clustered mostly around "N-fix", while soil biological parameters seem to cluster in the opposite direction of the cacao monocultures ("control"). Cadmium content in soil and litter appears to cluster with the cacao monocultures. The second principal component (PC2) explained 27.2% of the variance in the data and was mainly associated with soil health indicators, mainly positively with biological indicators such as macrofauna diversity (0.95), earthworm abundance (0.73) and macrofauna richness (0.71) and negatively with soil Cd (–0.81). Litter characteristics and soil health indicators do not seem to cluster in clear separated groups.

3.4. Cacao productivity, leaf nutrient, and Cd content

A significant variation in foliar nitrogen (N) content was observed across different treatments ($p < 0.01$, Table 5). Specifically, lower foliar N contents were recorded in both the "control" ($20.3\ g\ kg^{-1}$) and "fruit" treatment ($20.0\ g\ kg^{-1}$) as compared to the "N-fix" treatment ($25.7\ g\ kg^{-1}$). No significant single or interactive effects of treatment and distance were found for other nutrients. Average foliar nutrient contents were $1.18\ (\pm 0.03)\ g\ P\ kg^{-1}$ and $79.11\ (\pm 4.99)\ mg\ Fe\ kg^{-1}$, which is under the optimal value of $2\ g\ kg^{-1}$ and $175\ mg\ kg^{-1}$ as indicated by van Vliet and Giller (2017, Table 14). The foliar Cd content, averaging $10.87\ (\pm 0.83)\ mg\ kg^{-1}$, exhibited no variation across treatments ($p = 0.556$). No difference was observed between the agroforestry systems ($p = 0.667$; Table 6), nor did we find a block ($p = 0.338$) effect on cacao bean yield of all 3 years establishment. Pod counts between year 2 and 5 after planting was variable in time, with 2018 showing the highest yields. Average pod production was $47\ kg\ ha^{-1}$, 515 and $142\ kg\ ha^{-1}$ in 2017, 2018 and 2019, respectively.

Table 5

Means and standard errors for leaf data observed in the different cocoa agroforestry systems (n = 5). P-values for the effect of treatment (*Treat.*), Distance (*Dist.*) and their interaction (*Int.*) are provided. Samples were taken in October 2019 in a cocoa agroforestry trial in Coca, Amazon, Ecuador. Means followed by the same letter in the column do not differ as per the Tukey test ($\alpha = 0.05$). Shaded headers indicate treatments that include legume trees.

			Cocoa agroforestry systems										p-values		
Indicator	Unit		FRUIT		MIX		MONO		N-FIX		TIMBER		Treat.	Dist.	Int.
			mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)			
Leaf		*													
C	g kg ⁻¹	n.d	442.2	(5.13)	445.2	(3.30)	445.8	(3.90)	452.8	(3.60)	449.4	(3.00)	0.458	0.472	0.932
N	g kg ⁻¹	>20	20.0b	(0.72)	22.1ab	(1.20)	20.3b	(0.70)	25.7a	(0.80)	21.4ab	(1.40)	0.019	0.771	0.795
P	g kg ⁻¹	>2	1.2	(0.10)	1.1	(0.04)	1.1	(0.06)	1.4	(0.10)	1.1	(0.00)	0.105	0.195	0.730
K	g kg ⁻¹	>12	11.7	(1.33)	12.3	(0.87)	11.9	(0.95)	15.2	(1.60)	11.6	(0.70)	0.245	0.517	0.907
Mg	g kg ⁻¹	>2	5.9	(0.44)	4.9	(0.26)	4.9	(0.47)	4.8	(0.30)	5.9	(0.70)	0.308	0.718	0.964
Zn	mg kg ⁻¹	>80	254.4	(49.05)	211.3	(31.80)	227.4	(15.80)	182.8	(24.90)	191.3	(21.70)	0.631	0.973	0.881
Fe	mg kg ⁻¹	>175	85.9	(15.01)	70.4	(10.40)	88.8	(13.00)	68.9	(5.20)	85.9	(10.80)	0.653	0.594	0.086
Cd	mg kg ⁻¹	<1.68	10.6	(1.14)	13.1	(2.55)	10.9	(1.29)	9.4	(1.22)	9.3	(1.33)	0.556	0.965	0.936

* Indicative reference values for nutrients in plant leaves to support cocoa production P/K according to (van Vliet and Giller, 2017; table 14), C/N/Mg/Zn/Fe according to Chávez et al. (2019; slide 27), Cd calculated from Argüello et al., 2019 (Section 4.3).

**Ca, Co, Mn, Cu and S were collected in this study but were not displayed in the main analyses as focus was placed on the macro and common micronutrient discussion, there were no significant differences found for these nutrients in this mixed model analyses. Al was not reported as result returned by ICP, furthermore Al results were also not reliable according to the reference sample. Na and Mo were reported but mostly below detection limit. (n.d = non defined).

Table 6

Means and standard errors for cacao pod yield data observed in the different cocoa agroforestry systems (n = 5). P-values for the effect of treatment (*Treat.*), block and their interaction (*Int.*) are provided. Samples were taken in October 2019 in a cocoa agroforestry trial in Coca, Amazon, Ecuador. Means followed by the same letter in the column do not differ as per the Tukey test ($\alpha = 0.05$). Shaded headers indicate treatments that include legume trees.

		Cocoa agroforestry systems										p-values		
		FRUIT		MIX		MONO		N-FIX		TIMBER		Treat.	Block	Int.
Indicator	Unit	mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)	mean	(s.e)			
Cocoa pod yield														
Yield 2017	kg ha ⁻¹	29.6	(29.62)	48.9	(30.71)	50.6	(30.76)	79.91	(46.97)	29.4	(29.39)	0.667	0.338	0.964
Yield 2018	kg ha ⁻¹	631.7	(116.12)	627.7	(189.67)	545.1	(237.34)	436.6	(74.59)	336.3	(47.06)	0.636	0.501	0.227
Yield 2019	kg ha ⁻¹	79.1	(32.27)	260.2	(111.09)	122.0	(68.37)	116.1	(63.59)	132.5	(58.26)	0.806	0.218	0.508

4. Discussion

4.1. Shade tree species in cacao agroforestry systems influenced biological soil health indicators

Our findings showed that agroforestry influenced some of the biological soil health indicators, mainly earthworm abundance and soil respiration, even at early stage of establishment (5 years). Yet, other important physical and chemical soil health indicators were not affected by agroforestry systems. This confirms our first hypothesis that the early effects of shade tree species on soil health are mostly captured through biological soil indicators. Earthworms' abundances were a factor three higher in cacao intercropped with *C. cateniformis* + *E. velutina* than in the cacao monoculture. Similar findings were found in a coffee agroforestry in Costa Rica, where earthworm abundance was lowest in the organic monoculture system compared to the organic agroforestry systems with either *Erythrina poeppigiana* or *Terminalia amazonia* trees (Johnson-Maynard and Lugo-Perez, 2006). This positive effect of shade trees on earthworms and soil respiration may be explained by a higher litter quality in the system as compared to monoculture cacao. For instance, Cesarz et al. (2007) demonstrated in their study that earthworm density and diversity is generally positively correlated with tree species diversity, reflecting the importance of litter quality attributes for decomposer fauna. Despite the minimal variation in litter quality across treatments observed in our study, possibly because of the homogeneous litter layer dominated by cacao leaves, our data indeed suggests that shade trees can influence litter respiration. For instance, the highest rate of litter respiration was recorded in the plot with *C. cateniformis*, a leguminous timber tree species. This species may provide a more nutritionally rich litter layer, potentially enhancing the food source and

therefore presence for earthworms as observed in the "mixed" treatment.

Potential soil respiration was a factor 1.4 higher in agroforests that contained only *C. cateniformis* or *B. gasipaes* trees compared to "control", indicating that shade tree species may provide better quality litter inputs than cacao only, thus stimulating soil microbial activity and nutrient cycling compared to unshaded cacao. Lori et al. (2022) found similar results in a 15-year-old cacao trial in Bolivia, where organic management within a long-term agroforestry trial significantly enhanced the microbial community diversity in comparison to conventional monoculture. It's plausible that soil respiration rates are elevated in agroforestry systems due to enhanced temperature regulation coupled with a greater quantity and diversity of litter, these factors collectively foster an optimal niche, promoting a more varied microbial community (Araújo et al., 2014; Rodrigues et al., 2015). A more diverse microbial community with more abundant microbial community can result in higher respiration rates in agroforestry systems. It would therefore be recommended to test in a future study the total microbial biomass and community composition to better understand the dynamics between agroforestry systems and varying respiration rates in soil and litter layer within this study. We expected that N-fixing species (*C. cateniformis* and *E. velutina*) would have a greater positive effect on biological soil indicators, compared to other treatments, but this was not confirmed in our study. While potential respiration trends somewhat aligned between litter and soil in response to treatments, an anomaly arose with the 'n-fix' and 'control' treatment. This suggests that the influence of shade trees on litter respiration in some cases may directly impact soil respiration. Such linkage carries implications for soil health, as the litter layer plays a pivotal role as an interface connecting above-ground and below-ground ecosystems.

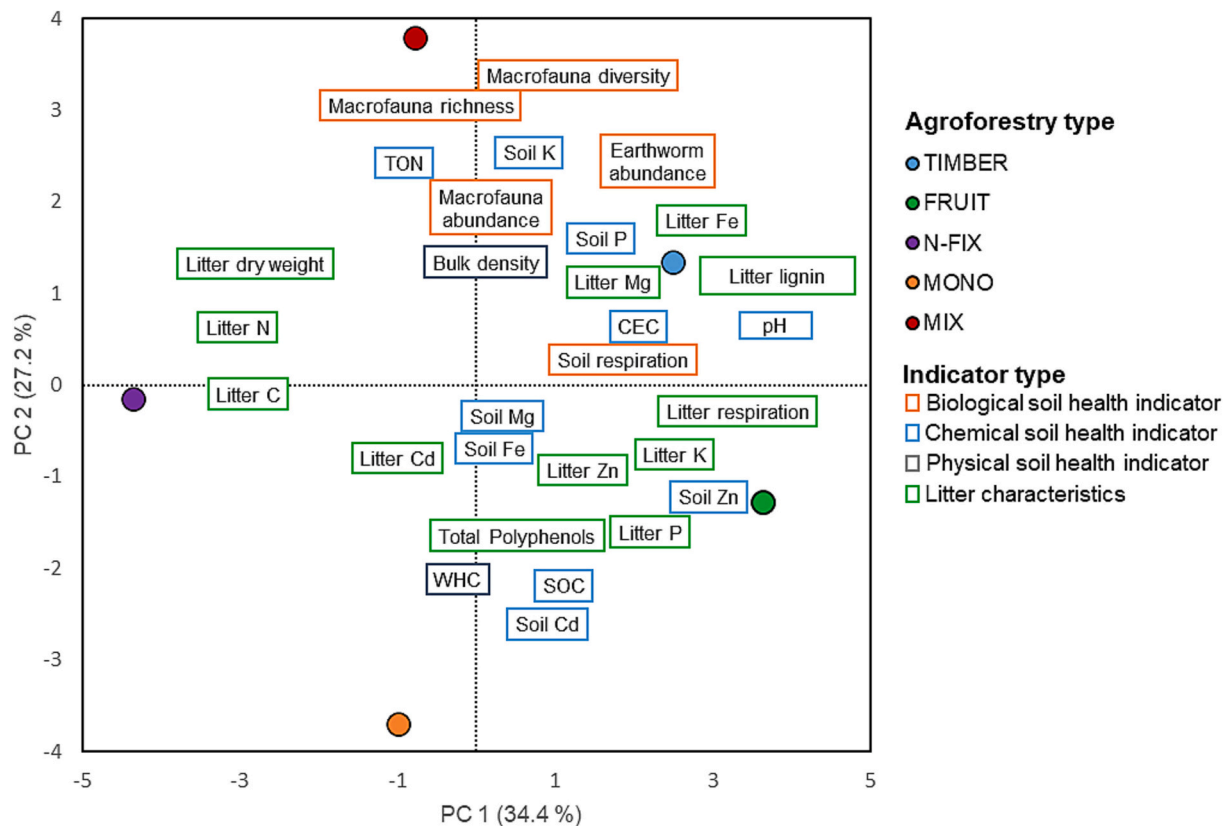


Fig. 4. Bi-plot showing how different agroforestry systems relate to litter characteristics and a set of biological, chemical and physical soil health indicators. Samples were collected within a cacao agroforestry trial within the Ecuadorian Amazon. Principal component 1 and 2 explain 34.4% and 27.2% of total variation, respectively.

Shade trees had no effect on the selected chemical and physical soil health indicators, except C/N ratio in the upper soil layer (0–10 cm). Our results demonstrate that agroforestry systems influence soil health first through changes in biological indicators which over time could potentially lead to alter soil physical-chemical parameters and impact agronomic traits in cacao. For instance, a continued high earthworm density during a prolonged period of time may greatly influence soil organic matter dynamics and soil structure and associated physical conditions in a plot which positively affects nutrient cycling and plant uptake (Barois et al., 1999). However, the impact of shade trees on soil health can vary greatly between scientific studies. For instance, our results align with finding of Ayres et al. (2009) who confirmed in their study conducted in the Southern Rocky Mountains, North America, that tree species traits mainly influenced leaf litter quality, soil respiration, and macrofauna abundance. Contrastingly, de Souza et al. (2012), who compared a 13-year-old coffee monoculture with agroforestry systems with differing shade-tree arrangements in the Atlantic Forest biome of Brazil, found that chemical and biological soil health in agroforestry systems did not differ significantly from full-sun coffee production systems.

4.2. Distance from shade tree does not influence litter quality or soil indicators

We hypothesized that soil health indicator values differ with distance from the shade trees and this effect can be related to differences in shading, litter quality and quantity. Recent studies confirm that soil health indicators could be affected by tree species in the agroforestry systems and the distance of the cacao to the shade tree (Niether et al., 2020). Nevertheless, our results showed that distance from the shade tree did not change leaf, litter or soil health indicators. This finding could be attributed to the fact that cacao trees produce a substantial

amount of litter (5–10 t/ha/y; van Vliet and Giller, 2017). However, litter samples closer to the shade tree showed overall higher microbial respiration compared to samples taken further away, after one week of incubation. A higher litter respiration rate implies greater microbial activity and decomposition activity within the litter layer. This may affect nutrient cycling, ecosystem productivity, carbon emissions, and soil health, contingent upon the broader ecological context and prevailing environmental conditions. As a result, it raises the possibility that, over time, proximity to certain shade trees (such as *C. cateniformis* and *E. velutina*) may increase nutrient availability in the soil. For instance, in a global meta-analysis on agroforestry, Ma et al. (2020) highlight that beside tree-biomass also the soil organic carbon stock in agroforestry systems (relative to cropland or pasture) increases with tree age and that such patterns vary with tree species richness and regional climate. However, this finding was not observed in the soil samples analysed in our study.

4.3. Shade tree species affected nutrient content, but not Cd in cacao leaves

The planting density of *E. velutina* within the “N-fix” treatment was higher than in the other treatments, which explains the elevated shade level observed within the treatment which could cause competition effects with cacao for other sun and essential nutrients (Blaser et al., 2018). Nonetheless, agroforestry treatments did not affect cacao yields, however the shade tree species arrangements impacted foliar nutrient content of cacao trees. For instance, foliar N content of cacao trees grown in the agroforest with only *E. velutina* trees was overall a factor 1.3 higher than foliar N content of cacao trees in the monoculture (“control”) or agroforests with the fruit tree *B. gasipaes*. This might result from biomass inputs of the *E. velutina* trees. For instance, the higher N

content in the leave tissue could be caused by the N-fixing capacity of the *E. velutina* shade trees in this system and the higher density of these trees compared to other arraignment. Oelbermann et al. (2004), who studied the decomposition of *E. poeppigiana* in alley cropping systems in Costa Rica, observed that N inputs ranged from 73.84 kg ha⁻¹ in younger productions system (< 5 years) to 220.85 kg ha⁻¹ in full grown production systems (> 15 years). Hence, tree species from the *Erythrina* genus can contribute significantly to both SOM storage and to the N capital of soils leading to higher N available for uptake compared to cacao monocultures (Heuvelink et al., 1988).

Foliar nutrient analyses indicated a deficiency of P and Fe in the cacao leaves of all treatments, based on indicative references provided by van Vliet and Giller (2017). To achieve high productivity, cacao requires a soil with adequate levels of nutrients, however there is still a lack of knowledge on the impact of nutrient concentration on plant yields in cacao, particularly Fe. Soil pH affects the availability of nutrients, especially P, the optimum soil pH recommended to sustain adequate cacao growth is ~6.7 (Hartemink, 2005). However, most cacao soils, including the one analysed in our study, are more acidic mainly because the pedogenesis of soils in hot and humid environments (such as the one in the Amazon). Moreover, cacao cultivation tends to result in soil acidification because cacao trees, including their leaves, contain significant amounts of organic acids (Ahenkorah et al., 1987). These organic acids gradually contribute to soil acidity over time. Researchers have reported that cacao can tolerate acid soil pH provided that the supply of nutrients should be adequate (Wood, 1985). We further found that foliar Cd levels were not affected by shade trees and are too high which poses a risk for cacao farmers. Cadmium in cacao leaves is considered a good proxy for bean Cd (Shanying et al., 2017; Barraza et al., 2017). The elevated Cd content in the beans poses challenges for smallholder farmers in selling their produce, primarily due to food safety regulations (Argüello et al., 2019).

Shade tree treatments did not affect cacao yields. Agroforestry systems have been reported to differ in yield by Blaser et al. (2018) and de Heuvels et al. (2012), both studies indicated that this is mostly due to differences in shading intensity in the plots. Nevertheless, the management of nutrients can significantly influence the relationship between shade tree density and cacao yields, a pattern that has also been observed in coffee cultivation (Pulleman et al., 2023). The latter is also in line with a study performed by Jacobi et al. (2015), who found that organically managed successional or simple agroforestry systems did not impact annual cacao production compared to monoculture systems in Alto Beni, Bolivia. Plot age in their study varied from 9 to 14 years. The lack of a yield effect in our study might also be explained by the young age (5 years) of the plantation. Cacao yields usually stabilize when the plants are more mature (>5 years; Lass and Wood, 1985), meaning that effects of the added shade trees on the cacao yields might become more prominent over the years (de Heuvels et al., 2012). We observed that cacao yields were very variable over the last 3 years (2017–19), with 2018 being the peak year. Several factors determine the actual yield of cacao, causing that yields generally strongly differ from site to site even within plantations (van Vliet and Giller, 2017). Further research is therefore needed to understand in greater details which mechanism influence cacao yield in agroforestry systems over time.

In our study, we assessed various indicators to gauge their relevance in understanding the impact of shade trees on soil health and cacao production. At the forefront of usefulness, we report that earthworm abundance and soil respiration provided critical insights. These indicators showed the ecological conditions of the soil and microbial activity for better understand nutrient cycling and soil structure. Additionally, foliar nutrient content analysis played a pivotal role in optimizing cacao productivity by offering insights into nutrient availability. Chemical and physical soil health indicators, while not showing significant differences between treatments, contributed to a comprehensive view of soil health. Despite minimal variation in our study, the assessment of litter quality and quantity held relevance for long-term

considerations of nutrient inputs and decomposition. Finally, monitoring Cd levels, crucial for food safety compliance, addressed a specific concern but was somewhat less directly related to the broader assessment of soil health. These findings collectively provided a nuanced understanding of the complex interactions within agroforestry systems and their potential benefits for cacao cultivation.

5. Conclusion

This study contributes to a better understanding of the effects of shade tree management and tree species on soil health indicators and cacao yields in young cacao production systems. Our findings revealed biological indicators, mainly earthworm abundance and soil respiration, were most sensitive to early impacts of different agroforestry arrangements. Distance from shade tree or cacao genotype did not influence soil health indicators or litter characteristics. Litter respiration exhibited variations among the different shade tree arrangements, with the “timber” treatment, featuring the N-fixing tree species *C. cateniformis*, showing higher rates. This suggests a potential improvement in litter quality in this treatment. Cadmium levels in litter, leaves and soil were high and above threshold level mentioned in related literature. We conclude that biological soil health indicators are more sensitive to detect differences between shade tree species than chemical or physical indicators. However, this was not yet expressed in pod yields nor foliar nutrient levels, except foliar N content which varied among the different shade tree treatments, being highest within the “N-fix” and lowest in the “Fruit” systems. Further monitoring of this trial is recommended to capture the long-term effect of agroforestry systems in soil health, nutrient cycling, and cacao yield. Our data analysis revealed consistently high cadmium levels across all shade tree arrangements. However, we did not observe any discernible effects, which is possibly attributed to the relatively young age of the trial. Hence, for a more thorough understanding of the agroforestry system’s influence on cadmium accumulation in cacao, it is advisable to conduct a comprehensive assessment in the future when the trial is older or in similar settings with established shade trees. This research will help address the question of whether agroforestry can contribute to the mitigation of cadmium contamination in cacao plantations.

CRedit authorship contribution statement

Anna M. Visscher: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. **Eduardo Chavez:** Conceptualization, Investigation, Methodology, Resources, Supervision, Writing – review & editing, Data curation, Writing – original draft. **Carlos Caicedo:** Resources, Writing – review & editing. **Leider Tinoco:** Resources, Writing – review & editing. **Mirjam Pulleman:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.geodrs.2024.e00772>.

References

- Ahenkorah, Y., Halm, B.J., Appiah, M.R., Akrofi, G.S., Yirekyi, J.E.K., 1987. Twenty years' results from a shade and fertilizer trial on Amazon cocoa (*Theobroma Cacao*) in Ghana. *Exp. Agric.* 23, 31–39. <https://doi.org/10.1017/S0014479700001101>.
- Alfaro-Flores, A., Morales-Belpaire, I., Schneider, M., 2015. Microbial biomass and cellulase activity in soils under five different cocoa production systems in alto Beni, Bolivia. *Agrofor. Syst.* 89, 789–798. <https://doi.org/10.1007/s10457-015-9812-z>.
- Amponsah-Doku, B., Daymond, A., Robinson, S., Atuah, L., Sizmur, T., 2022. Improving soil health and closing the yield gap of cocoa production in Ghana—a review. *Sci. Afr.* 15, e01075.
- Anderson, J.M., Ingram, J.S.I., 1993. *Tropical Soil Biology and Fertility: A Handbook of Methods*, 2nd edn. CAB. International Oxfordshire, Wallingford, UK.
- Araújo, A.S.F., Borges, C.D., Tsai, S.M., Cesarz, S., Eisenhauer, N., 2014. Soil bacterial diversity in degraded and restored lands of Northeast Brazil. *Anton Leeuw. Int. J. G.* 106, 891–899. <https://doi.org/10.1007/s10482-014-0258-5>.
- Argüello, D., Chavez, E., Lauryssen, F., Vanderschueren, R., Smolders, E., Montalvo, D., 2019. Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: a nationwide survey in Ecuador. *Sci. Total Environ.* 649, 120–127. <https://doi.org/10.1016/j.scitotenv.2018.08.292>.
- Asigbaase, M., Dawoe, E., Sjogersten, S., Lomax, B.H., 2021. Decomposition and nutrient mineralisation of leaf litter in smallholder cocoa agroforests: a comparison of organic and conventional farms in Ghana. *J. Soils Sediments* 21, 1010–1023. <https://doi.org/10.1007/s11368-020-02844-4>.
- Ayres, E., Steltzer, H., Berg, S., Wallenstein, M.D., Simmons, B.L., Wall, D.H., 2009. Tree species traits influence soil physical, chemical, and biological properties in high elevation forests. *PLoS One* 4, e5964. <https://doi.org/10.1371/journal.pone.0005964>.
- Barois, I., Lavelle, P., Michel, Brossard, Jérôme, Tondoh, de los Angeles Martinez, M., Rossi, J.P., Senapati, B.K., Angeles, A., Fragosco, C., Jimenez, J.J., Thibaud, Decaens, Lattaud, C., Kanyonyo, J., Eric, Blanchart, Lydie, Chapuis, George, Brown, Moreno, A., 1999. Ecology of earthworm species with large environmental tolerance and/or extended distributions. In: Lavelle, P., Brussaard, L., Hendrix, P. (Eds.), *Earthworm Management in Tropical Agroecosystems*. CABI, Wallingford, pp. 57–85. ISBN 0-85199-270-6.
- Barraza, F., Schreck, E., Lévêque, T., Uzu, G., López, F., Ruales, J., Maurice, L., 2017. Cadmium bioaccumulation and gastric bioaccessibility in cacao: a field study in areas impacted by oil activities in Ecuador. *Environ. Pollut.* 229, 950–963. <https://doi.org/10.1016/j.envpol.2017.07.080>.
- Blaser, W.J., Oppong, J., Yeboah, E., Six, J., 2017. Shade trees have limited benefits for soil fertility in cocoa agroforests. *Agric. Ecosyst. Environ.* 243, 83–91. <https://doi.org/10.1016/j.agee.2017.04.007>.
- Blaser, W.J., Oppong, J., Hart, S.P., Landolt, J., Yeboah, E., Six, J., 2018. Climate-smart sustainable agriculture in low-to-intermediate shade agroforests. *Nat. Sustain.* 1, 234–239. <https://doi.org/10.1038/s41893-018-0062-8>.
- Boyer, J., 1973. Cycles de la matière organique et des éléments minéraux dans une cacaoyère camerounaise. *Café Cacao Thé*, 18, pp. 3–30. <https://agris.fao.org/agris-search/search.do?recordID=US201303265140>.
- Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Pulleman, M., 2018. Soil quality—A critical review. *Soil Biol. Biochem.* 120, 105–125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.
- Buyer, J.S., Baligar, V.C., He, Z., Arévalo-Gardini, E., 2017. Soil microbial communities under cacao agroforestry and cover crop systems in Peru. *Appl. Soil Ecol.* 120, 73–80. <https://doi.org/10.1016/j.apsoil.2017.09.009>.
- Cesarz, S., Fahrenholz, N., Migge-Kleian, S., Platner, C., Schaefer, M., 2007. Earthworm communities in relation to tree diversity in a deciduous forest. *Eur. J. Soil Biol.* 43, 61–67. <https://doi.org/10.1016/j.ejsobi.2007.08.003>.
- Clough, Y., Faust, H., Tschamtké, T., 2009. Cacao boom and bust: sustainability of agroforests and opportunities for biodiversity conservation. *Conserv. Lett.* 2, 197–205. <https://doi.org/10.1111/j.1755-263X.2009.00072.x>.
- Creamer, R.E., Barel, J.M., Bongiorno, G., Zwetsloot, M.J., 2022. The life of soils: integrating the who and how of multifunctionality. *Soil Biol. Biochem.* 166, 108561. <https://doi.org/10.1016/j.soilbio.2022.108561>.
- de Almeida, A.A.F.D., Valle, R.R., 2007. Ecophysiology of the cacao tree. *Braz. J. Plant Physiol.* 19, 425–448. <https://doi.org/10.1590/S1677-04202007000400011>.
- de Heuvels, O., Avelino, J., Somarriba, E., Malezieux, E., 2012. Vegetation structure and productivity in cocoa-based agroforestry systems in Talamanca, Costa Rica. *Agric. Ecosyst. Environ.* 149, 181–188. <https://doi.org/10.1016/j.agee.2011.03.003>.
- de Souza, H.N., de Goede, R.G., Brussaard, L., Cardoso, I.M., Duarte, E.M., Fernandes, R. B., Pulleman, M., 2012. Protective shade, tree diversity and soil properties in coffee agroforestry systems in the Atlantic rainforest biome. *Agric. Ecosyst. Environ.* 146, 179–196. <https://doi.org/10.1016/j.agee.2011.11.007>.
- Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. *Appl. Soil Ecol.* 15 (1), 3–11.
- Fountain, A.C., Hütz-Adams, F., 2022. Cocoa Barometer. <https://cocoabarometer.org/wp-content/uploads/2022/12/Cocoa-Barometer-2022.pdf>.
- Franzen, M., Mulder, M.B., 2007. Ecological, economic and social perspectives on cocoa production worldwide. *Biodivers. Conserv.* 16, 3835–3849. <https://doi.org/10.1007/s10531-007-9183-5>.
- Giller, K.E., Beare, M.H., Lavelle, P., Izac, A.M., Swift, M.J., 1997. Agricultural intensification, soil biodiversity and agroecosystem function. *Appl. Soil Ecol.* 6, 3–16. [https://doi.org/10.1016/S0929-1393\(96\)00149-7](https://doi.org/10.1016/S0929-1393(96)00149-7).
- Hartemink, A.E., 2005. Nutrient stocks, nutrient cycling, and soil changes in cocoa ecosystems: a review. *Adv. Agron.* 86, 227–253. [https://doi.org/10.1016/S0065-2113\(05\)86005-5](https://doi.org/10.1016/S0065-2113(05)86005-5).
- Hess, T.F., Schmidt, S.K., 1995. Improved procedure for obtaining statistically valid parameter estimates from soil respiration data. *Soil Biol. Biochem.* 27, 1–7. [https://doi.org/10.1016/0038-0717\(94\)00166-X](https://doi.org/10.1016/0038-0717(94)00166-X).
- Heuvelink, J., Fassbender, H.W., Alpizar, L., Enriquez, G., Fölster, H., 1988. Modelling agroforestry systems of cacao (*Theobroma cacao*) with laurel (*Cordia alliodora*) and poro (*Erythrina poeppigiana*) in Costa Rica II. Cacao and wood production, litter production and decomposition. *Agrofor. Syst.* 6, 37–48. <https://doi.org/10.1007/BF02344744>.
- Jacobi, J., Schneider, M., Bottazzi, P., Pillco, M., Calizaya, P., Rist, S., 2015. Agroecosystem resilience and farmers' perceptions of climate change impacts on cocoa farms in alto Beni, Bolivia. *Renew. Agric. Food Syst.* 30, 170–183. <https://doi.org/10.1017/S174217051300029X>.
- Janzen, H.H., Janzen, D.W., Gregorich, E.G., 2021. The 'soil health' metaphor: illuminating or illusory? *Soil Biol. Biochem.* 159, 108167.
- Johnson-Maynard, J., Lugo-Perez, J., 2006. Earthworm populations, microbial biomass and coffee production in different experimental agroforestry management systems in Costa Rica. *Caribb. J. Sci.* 42, 397–409.
- Jose, S., 2009. Agroforestry for ecosystem services and environmental benefits: an overview. *Agrofor. Syst.* 76, 1–10. <https://doi.org/10.1007/s10457-009-9229-7>.
- Kirk, T.K., Obst, J.R., 1988. Lignin determination. *Meth. Enzymol.* 161, pp. 87–101. [https://doi.org/10.1016/0076-6879\(88\)61014-7](https://doi.org/10.1016/0076-6879(88)61014-7).
- Koné, A.W., Yao, M.K., 2021. Soil microbial functioning and organic carbon storage: can complex timber tree stands mimic natural forests? *J. Environ. Manag.* 283, 112002. <https://doi.org/10.1016/j.jenvman.2021.112002>.
- Lass, R.A., Wood, G.A.R., 1985. *Cocoa Production: Present Constraints and Priorities for Research*. The World Bank. <https://agris.fao.org/agris-search/search.do?recordID=US2012401249>.
- López, R.E.L., Navarrete, E.F.C., Rosado, J.T.P., García, C.R.S., Margenot, A.J., 2021. Soil nitrogen cycling under contrasting management systems in Amazon *Coffea canephora* agroecosystems. *Soil Sci. Soc. Am. J.* 85, 1634–1648. <https://doi.org/10.1002/saj2.20255>.
- Lori, M., Armengot, L., Schneider, M., Schneidewind, U., Bodenhausen, N., Mäder, P., Krause, H.M., 2022. Organic management enhances soil quality and drives microbial community diversity in cocoa production systems. *Sci. Total Environ.* 834, 155223. <https://doi.org/10.1016/j.scitotenv.2022.155223>.
- Ma, Z., Chen, H.Y., Bork, E.W., Carlyle, C.N., Chang, S.X., 2020. Carbon accumulation in agroforestry systems is affected by tree species diversity, age and regional climate: A global meta-analysis. *Glob. Ecol. Biogeogr.* 29, 1817–1828. <https://doi.org/10.1111/geb.13145>.
- Marshall, E.J.P., 2004. Agricultural landscapes: field margin habitats and their interaction with crop production. *J. Crop Improv.* 12, 365–404. https://doi.org/10.1300/J411v12n01_05.
- Mehlich, A., 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* 15, 1409–1416. <https://doi.org/10.1080/00103628409367568>.
- Motamayor, J.C., Risterucci, A.M., Lopez, P.A., Ortiz, C.F., Moreno, A., Lanaud, C., 2002. Cacao domestication I: the origin of the cacao cultivated by the Mayas. *Heredity* 89 (5), 380–386.
- Niether, W., Jacobi, J., Blaser, W.J., Andres, C., Armengot, L., 2020. Cocoa agroforestry systems versus monocultures: a multi-dimensional meta-analysis. *Environ. Res. Lett.* 15, 104085. <https://doi.org/10.1088/1748-9326/abb053>.
- Oelbermann, M., Voroney, R.P., Schlönvoigt, A.M., Kass, D.C., 2004. Decomposition of *Erythrina poeppigiana* leaves in 3-, 9-, and 18-year-old alleycropping systems in Costa Rica. *Agrofor. Syst.* 63, 27–32. <https://doi.org/10.1023/B:AGFO.0000049430.52250.87>.
- Ofori-Frimpong, K., Asase, A., Mason, J., Danku, L., 2007. Shaded versus unshaded cocoa: implications on litter fall, decomposition, soil fertility and cocoa pod development. Symposium on multistrata agroforestry systems with perennial crops. In: CATIE Turrialba, 2007 Costa Rica, pp. 17–21.
- Pretty, J., 1997. The sustainable intensification of agriculture. *Nat. Res. Forum* 21, 247–256. <https://doi.org/10.1111/j.1477-8947.1997.tb00699.x>.

- Pulleman, M., Tietema, A., 1999. Microbial C and N transformations during drying and rewetting of coniferous forest floor material. *Soil Biol. Biochem.* 31, 275–285. [https://doi.org/10.1016/S0038-0717\(98\)00116-3](https://doi.org/10.1016/S0038-0717(98)00116-3).
- Pulleman, M.M., Rahn, E., Valle, J.F., 2023. Regenerative agriculture for low-carbon and resilient coffee farms: A practical guidebook. In: Version 1.0. Cali (Colombia): International Center for Tropical Agriculture (CIAT), p. 181. <https://hdl.handle.net/10568/131997>.
- Richard, A., Ræbild, A., 2016. Tree diversity and canopy cover in cocoa systems in Ghana. *New For.* 47, 287–302. <https://doi.org/10.1007/s11056-015-9515-3>.
- Rodrigues, R.C., Araújo, R.A., Costa, C.S., Lima, A.J., Oliveira, M.E., Cutrim Jr., J.A., Araújo, A.S., 2015. Soil microbial biomass in an agroforestry system of Northeast Brazil. *Trop. Grassl.* 3, 41–48. [https://doi.org/10.17138/tgft\(3\)41-48](https://doi.org/10.17138/tgft(3)41-48).
- Rousseau, G.X., Deheuvels, O., Arias, I.R., Somarriba, E., 2012. Indicating soil quality in cacao-based agroforestry systems and old-growth forests: the potential of soil macrofauna assemblage. *Ecol. Indic.* 23, 535–543.
- Shannon, C.E., 1948. A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 379–423. <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>.
- Shanying, H.E., Xiaoe, Y.A.N.G., Zhenli, H.E., Baligar, V.C., 2017. Morphological and physiological responses of plants to cadmium toxicity: a review. *Pedosphere* 27, 421–438. [https://doi.org/10.1016/S1002-0160\(17\)60339-4](https://doi.org/10.1016/S1002-0160(17)60339-4).
- Steffan-Dewenter, I., Kessler, M., Barkmann, J., Bos, M.M., Buchori, D., Erasmí, S., Guhardja, E., 2007. Tradeoffs between income, biodiversity, and ecosystem functioning during tropical rainforest conversion and agroforestry intensification. *Proc. Natl. Acad. Sci.* 104, 4973–4978. <https://doi.org/10.1073/pnas.0608409104>.
- Toda, M., Akiyama, T., Yokoyama, T., Matsumoto, Y., 2015. Quantitative examination of pre-extraction treatment on the determination of lignin content in leaves. *Bioresources* 10, 2328–2337. https://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_10_2_2328_Toda_Quantitative_Preextraction_Lignin_Leaf.
- Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecol. Lett.* 8, 857–874. <https://doi.org/10.1111/j.1461-0248.2005.00782.x>.
- van Vliet, J.A., Giller, K.E., 2017. Mineral nutrition of cocoa: A review. In: *Adv Agron.* 141, 185–270. Academic Press. <https://doi.org/10.1016/bs.agron.2016.10.017>.
- Wessel, M., 1970. Fertiliser experiments on farmers' cocoa in southwestern Nigeria. *Cocoa Growers' Bull.* 15, 22–27. <https://www.cabdirect.org/cabdirect/abstract/19710305032>.
- Wood, G.A.R., 1985. Environment. In: Wood, G.A.R., Lass, R.A. (Eds.), *Cocoa*, pp. 38–78. Longman, London.
- Yao, M.K., Koné, A.W., Otinga, A.N., Kassim, E.K., Tano, Y., 2021. Carbon and nutrient cycling in tree plantations vs. natural forests: implication for an efficient cocoa agroforestry system in West Africa. *Reg. Environ. Chang.* 21, 1–14. <https://doi.org/10.1007/s10113-021-01776-0>.