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Dynamics of soil organic carbon pools following conversion of savannah to cocoa agroforestry systems in the Centre region of Cameroon

Eltson Eteckji Fonkeng [a,b,c,*], Tiphaine Chevallier [c], Marie Sauvadet [c,d], Seguy Enock [a], Nancy Rakotondrazafy [c], Lydie Chapuis-Lardy [c], Bertin Takoutsing [a], Oben Tabi Fritz [b], Jean-Michel Harmand [a,c,e,**]

[a] World Agroforestry (ICRAF), Yaoundé, Cameroon
[b] University of Dschang, Dschang, Cameroon
[c] Eco&Sols, IRD, CIRAD, INRAE, l'Institut Agro Montpellier, University of Montpellier, Montpellier, France
[d] CIRAD, UMR Eco&Sols, Montpellier, France
[e] CIRAD, UMR Eco&Sols, Yaoundé, Cameroon

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ABSTRACT

Afforestation of gramineous-woody savannah with cocoa agroforestry systems (cAFS) is a common farmer practice in Cameroon considered as sustainable. Nevertheless, the effects of afforestation of savannah with cAFS on soil organic carbon (SOC) turnover and content, and the factors controlling SOC accumulation and stabilization are unknown. SOC content at 0–10 cm soil layer, and SOC distribution in soil particle size fractions (0–20 µm fraction considered as mineral-associated organic carbon, MAOC; 50–2000 µm considered as particulate organic carbon, POC; and 20–50 µm), were compared in different systems settled on degraded savannah (orthic ferralsols). These systems included annual cropland (≈ 5 years old), cocoa monoculture (≈10 years old), and cAFS (from 20 to 60 years old) including different shade tree species such as *Albizia adianthifolia, Canarium schweinfurthii, Dacryodes edulis, Milicia excelsa and Ceiba pentandra*. Savannah and nearby secondary forest patches were also included in the design as controls. Soil ^{13}C was analysed to investigate the soil carbon turnover after afforestation (C3 plants) of gramineous savannah (C4 plants).

SOC significantly increased in the 0–10 cm depth from 10.6 ± 3.1 g C kg^{-1} in degraded savannah to 17.9 ± 5.6 g C kg^{-1} in cAFS reaching similar levels as in nearby secondary forests (16.3 ± 5.8 g C kg^{-1}), while annual cropland and cocoa monoculture presented a non-significant decrease in SOC content. These changes were due to rapid loss of SOC derived from savannah plants (C4) – about 76% within the first 15 years after conversion, and higher gain of SOC derived from C3 plants in cAFS than in the other land uses (e.g. from 3.4 ± 1.5 g C kg^{-1} in savannah to 17.8 ± 5.7 g C kg^{-1} in cAFS). This SOC enrichment in cAFS was distributed in POC (64%), MAOC (30%) and the intermediate 20–50 µm soil fraction (6%). The higher annual litter input accumulated on a longer period in cAFS (20 to 60 years) than in cocoa monoculture (10 years) concomitant with the lower litter recalcitrance of associated trees compared to cocoa could explain the higher enrichment of SOC in all fractions in cAFS. The soil pH and exch. Ca^{2+} differed under the different shade tree species, and were positively correlated to SOC content. The highest contents of soil exch. Ca^{2+} induced by *Ceiba* and *Milicia* in the top 10 cm soil layer could contribute to increase SOC enrichment under those species through soil aggregation and related C stabilization. We found no strong evidence of the effect of soil texture on additional soil carbon accumulation in cAFS, especially for the more stable C pool (MAOC). Our results evidenced that savannah afforestation with cAFS appears as a valuable option for top soil carbon enrichment and should consider tree species associated to cocoa to enhance soil C sequestration, soil quality and cocoa production sustainability.

* Corresponding author at: World Agroforestry (ICRAF), Yaoundé, Cameroon.
** Corresponding author at: Centre for International Cooperation in Agricultural Development Research, France
E-mail addresses: eteckjieltson@yahoo.com (E.E. Fonkeng), jean-michel.harmand@cirad.fr (J.-M. Harmand).

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1. Introduction

Increasing the capacity of soils to act as a major terrestrial carbon sink for climate change mitigation is a major preoccupation nowadays. Afforestation, the practice of planting trees where they do not naturally occur (Putz and Redford, 2009), is believed to have positive impacts on long-term carbon storage by the ecosystem (Strand et al., 2021) and is promoted as a climate mitigation strategy (IPCC, 2007; Smith et al., 2014). Afforestation of croplands increases soil organic carbon (SOC) content, however, there are inconsistent reports on the effects of afforestation of savannahs and grasslands on SOC (Don et al., 2011). Some authors reported an increase in SOC after afforestation of savannahs (Li et al., 2012; Nijmeijer et al., 2019a; Dubiez et al., 2019), which they attributed to higher litter inputs from the new vegetation especially in the case of highly degraded savannah, depleted in SOC because of frequent bush fire and/or occasional cultivation (Sauer et al., 2012). On the other hand, other authors reported a decrease (Berthrong et al., 2009; Mao and Zeng, 2010; Sandoval López et al., 2020; Strand et al., 2021) or no net change (Epron et al., 2009; Marin-Spiotta et al., 2009) in SOC contents after the afforestation of savannahs or grasslands. These trends were attributed to the short time considered after afforestation or by the high initial SOC content of the previous land use before afforestation (Hong et al., 2020). In addition, we might observe no to negative changes in SOC if the afforested savannahs are in tropical regions, with highly weathered soils like Ferralsols or with a sandy texture, that provide fewer mineral surfaces for physical protection and stabilization of SOC (Feller and Beare, 1997). As such, a better understanding of the mechanisms that control the accumulation and stabilization of SOC in sandy tropical Ferralsols after savannah afforestation is necessary.

Changes in SOC contents after afforestation may depend on several factors: the land-use (Lei et al., 2019; Cunningham et al., 2012), the pedoclimatic conditions (Hong et al., 2020; Wu et al., 2019), disturbances caused during afforestation (Zerva et al., 2005), and the tree species (Harmand and Njiti, 1998; Tang and Li, 2013; Abou Rajab et al., 2016; Morazzo et al., 2021; Hou et al., 2020; Sauvadet et al., 2020; Wartenberg et al., 2020). All these factors could affect biomass production, microbial activity, and SOC turnover, i.e., either the intensity of the C inputs (accumulation of new C) or the intensity of the C losses (mineralization vs. stabilization of the old and new C).

After land use change, the SOC from the previous vegetation will be progressively lost and replaced by the C inputs from the new vegetation (Hu et al., 2016). The SOC from the previous vegetation might decompose rapidly due to high biological activities in soils under tropical humid environments, and possibly due to priming effect after C inputs from the new vegetation (e.g., Bernard et al., 2022). On the other hand, thanks to regular and new C inputs, soil aggregation and physical protection of SOC against mineralization could increase (Barthès et al., 2008; **Razafimbelo et al.,** 2008) especially in the case of perennial agroecosystems. The use of δ^{13}C natural abundance makes possible the partition between old SOC from the native vegetation and new SOC from the new vegetation as SOC has a carbon isotope composition comparable to that of the source plant material (Epron et al., 2009; Blagodatskaya et al., 2011; Ghafoor et al., 2017; Poeplau et al., 2018; Rong et al., 2021). In our study, the afforestation of savannah with cocoa agroforestry systems (cAFS) should result in the substitution of mainly C4 gramineous plant inputs to the soil by C3 plant inputs from cocoa, associated shade trees and dicotyledonous weeds. Because the C4 plants have lower lignin and tannin contents than C3 plants, the SOC derived from C4 plants might decompose faster than the SOC derived from C3 plants (Ye and Hall, 2020; Wynn and Bird, 2007), especially under a tropical humid environment. We thus expected a rapid decline in SOC derived from C4 plants and an increase in SOC derived from C3 plants after savannah conversion to cAFS.

Considering only bulk SOC limits the understanding of the dynamics of SOC as affected by land use and management because soil organic matter is considered a heterogeneous mixture of compounds with different properties and turnovers (Lehmann and Kleber, 2015; Kögel-Knabner and Rumpel, 2018). Bulk SOC can be physically separated into carbon pools with different turnovers. Physical organic matter fractionation by particle size serves as an important tool in breaking down SOC into different pools which react differently to land use and management (Balesdent et al., 1998; Puget et al., 2000; Cardinael et al., 2015; Lavallee et al., 2020) and in turn affect the SOC turnover (Golchin et al., 1995). The dynamics of carbon exchanges between and within these pools is still shrouded in uncertainty especially concerning the size of each pool, the capacity of each pool to store additional carbon and how these pools respond to land use, management changes and climate change in different contexts. Usually, the labile pool is considered as the SOC associated with sand or coarse particles (50–2000 μm) called particulate organic carbon (POC) mainly made up of plant debris and fungal materials (Six et al., 2001), while a rather stable pool is considered as the SOC associated to the clay + fine silt (0–20 μm fraction), called mineral-associated organic carbon (MAOC) (Cotrufo et al., 2019). The labile pool POC is important in soil aggregation and serves as an energy source for soil microbial biomass (Zotarelli et al., 2007). These POC and microbial biomass fractions were considered as essential in terms of soil fertility as they rapidly release nutrients and be positively correlated to crop yield (Wood et al., 2016. The MAOC, on the other hand, consists of organic compounds resulting from organic matter decomposition, microbial metabolism, necromass and plant root exudation (Cotrufo et al., 2019). This C pool is a mixture of particles or micrometer-sized microaggregates, where organic matter is encrusted by minerals (Chenu and Plante, 2006), physically and chemically protected from degradation. They were considered as the major site of organic matter stabilization (Chenu et al., 2019, Lavallee et al., 2020) and hence important for land restoration.

Generally, the change in SOC in the early years after afforestation is expected to be pronounced more in the coarser particle-size fractions (POC) (DeGryze et al., 2004; d'Annunzio et al., 2008) than in the finer fractions (MAOC) due to higher recent C inputs. In MAOC fractions, some studies reported a persistence of C4 plants derived C (Epron et al., 2009) while others found a significant loss of C4 plants derived C and an increase of C3 plants derived C (Teixeira et al., 2019; Santos et al., 2020) in quite the same context of recently (<20 years) afforested soils with eucalyptus. These results showed the potential of afforestation for long-term carbon sequestration by increasing SOC in all SOC pools. We thus expected evolution of C3 and C4 derived C amounts in all the soil fraction after afforestation.

In Central Africa, and particularly in Cameroon, the natural expansion of forest into savannah, in the forest-savannah transition area, has been widely documented (Guillet et al., 2001; Sagang et al., 2022 among several authors). This area, which extends over a strip of about 150 km wide North–South at the border of the semi-deciduous forest (Guillet et al., 2001) is also a cocoa producing area, where farmers have set up for decades diversified cAFS on savannah (Jagoret et al., 2012; Camara et al., 2012; Nijmeijer et al., 2019a, 2019b). In the Bokito district within this area, savannahs are annually burnt and periodically cultivated for about 5–7 years, after which they return to fallow land or are converted to perennial cropland such as cAFS in which cocoa, the main component, is planted in association with a diversity of trees species. This afforestation of savannah with cAFS was previously described as a sustainable production option associated with an increase of ecosystem services, including soil carbon storage and soil fertility (Jagoret et al., 2012; Saj et al., 2017; Madountsap et al., 2017; Nijmeijer et al., 2019a, 2019b), which seemed dependent on the shade tree species (Sauvadet et al., 2020). Compared to cAFS and forests, cocoa monoculture systems have been shown to have lower SOC levels due to lower fine root production and less litter inputs (Niether et al., 2019). However, to our knowledge, no study has assessed soil carbon in cocoa monocultures settled on savannahs. The conversion of savannahs to annual croplands usually showed a decline in SOC (Deng et al., 2016; Guo and Gifford, 2002; Harmand et al., 2017). This loss can be attributed to the constant soil disturbance due to tillage in these systems which exposes SOC hitherto physically and chemically protected in soil aggregates to bacterial mineralization and erosion (Bronick and Lal, 2005; Dignac et al., 2017).

In addition, the processes and factors of increasing SOC contents after afforestation of savannah with cAFS, such as time since conversion, shade tree species, amount of litterfall and soil texture which drive SOC contents in the labile and stable pools and their turnover are still unknown and could give a clue on the SOC accumulation and stabilization in the long term.

This study was thus designed to investigate how the conversion of savannah to cAFS affects SOC in bulk soil and SOC pools compared to other land uses. We hypothesized that (i) cAFS are more efficient than cocoa monocultures or annual cropping systems, and could be as efficient as nearby secondary forest in building up SOC, (ii) afforestation of savannah with cAFS resulted in an increase in both the labile (POC) and the more stable (MAOC) C pools, and (iii) the SOC derived from savannah grasses (C4 plants) is rapidly lost and replaced by SOC derived from trees (C3 plants) with time (< 20 years) in all the soil fractions. We tested these hypotheses by comparing different systems set-up on savannah (cocoa agroforestry associating different shade tree species, cocoa monoculture and annual cropland) and nearby savannahs and forests for total SOC and SOC in different particle size fractions. We used natural stable isotopes ($\delta^{13}C$ analysis) in bulk soil and particle size fractions to partition SOC between C4 and C3 plants derived SOC.

2. Materials and methods

2.1. Description of study site

The study was carried out in the villages of Bakoa and Guéfigué, located in a forest-savannah transition zone in the Bokito subdivision (4°30 N, 11°10 E), Centre region of Cameroon. In this area, the landscape consists of hills and plateaus with gentle slopes at altitudes ranging from 400 to 550 m a.s.l. and is characterized by a mosaic of different land uses: herbaceous savannahs, forests, cocoa agroforests and croplands (Jagoret et al., 2012; Nijmeijer et al., 2019a, 2019b). Annual average temperature is about 25 °C, and annual rainfall ranges from 1300 to 1400 mm, with a main dry season lasting from mid-November to the beginning of March (Jagoret et al., 2012; Nijmeijer et al., 2019b). Soils are Orthic Ferralsol (Jones et al., 2013). The main herbaceous species in the savannah is *Imperata cylindrica* (Jagoret et al., 2011). In this area, cocoa plantations are mainly diversified cAFS created, either on forest or savannah land (Jagoret et al., 2012; Nijmeijer et al., 2019a, 2019b). However, the reduced availability of forestland in recent years led to an increase of the proportion of cocoa plantations established on former savannah.

2.2. Experimental design

We selected in the landscape (same topographic level, between bottom and mid slope) plots associated to different land uses: two references - savannah (12 plots), forest (12 plots) - and different cropping systems established on savannah: (i) 5-years-old annual cropland (6 plots), (ii) 10-years-old cocoa monoculture (6 plots), and 20- to 60-years-old cAFS (8 plots) (Fig. 1, Table 1). In our synchronic approach (Junior et al., 2013), we compared systems at a given time and we used the natural savannah ecosystems as a surrogate of previous land use to infer changes in SOC after conversion of savannahs to croplands or cocoa systems of different ages. Savannahs in the area are annually burned and periodically cultivated with maize, roots, and tubers, therefore we chose savannah plots where no agricultural activity could be noticed for the last seven years.

We found in this area only young cocoa monocultures (10 years old), which had been established in cropland, cultivated for about 5 years after conversion of savannah. Therefore, cocoa monocultures were sampled 15 years after conversion of savannah (5 years of cropland +10 years of cocoa cultivation).

The 8 diversified cAFS were chosen according to the presence of five associated shade tree species with contrasting characteristics and uses, regularly occurring in these systems in this area: *Canarium schweinfurthii* and *Dacryodes edulis* (evergreen, fruit trees), *Milicia excelsa* and *Ceiba pentandra* (deciduous, timber trees) and *Albizia adianthifolia* (deciduous, N_2-fixing tree). One individual tree per plot could be found for *Dacryodes* and *Ceiba*, whereas only seven, six and five individuals could be found across all eight plots for *Milicia, Albizia* and *Canarium,* respectively (Table 1). The age of the cAFS (two plots of 20 years and 6 plots of ≈ 60 years) resulted mainly from this choice (Fig. 1, Table 1, Supplementary material Table 1). The density of associated shade trees in cAFS ranged from 41 to 134 trees ha^{-1}. In all cocoa plots (monoculture and cAFS), cocoa tree density was on average 1400 ± 300 ha^{-1}.

The area of the selected cocoa plots, either in monoculture or in cAFS, ranged from 1025 m^2 to 4833 m^2 with an average of 2691 m^2. The area occupied by the canopy of associated tree species (Supplementary material Table 1) ranged from 24% to 75% (average of 57%) of the surface area of the cocoa plots. Of the 57% of shade cover, *Albizia, Canarium, Dacryodes, Milicia, Ceiba* and other species accounted for 6%,

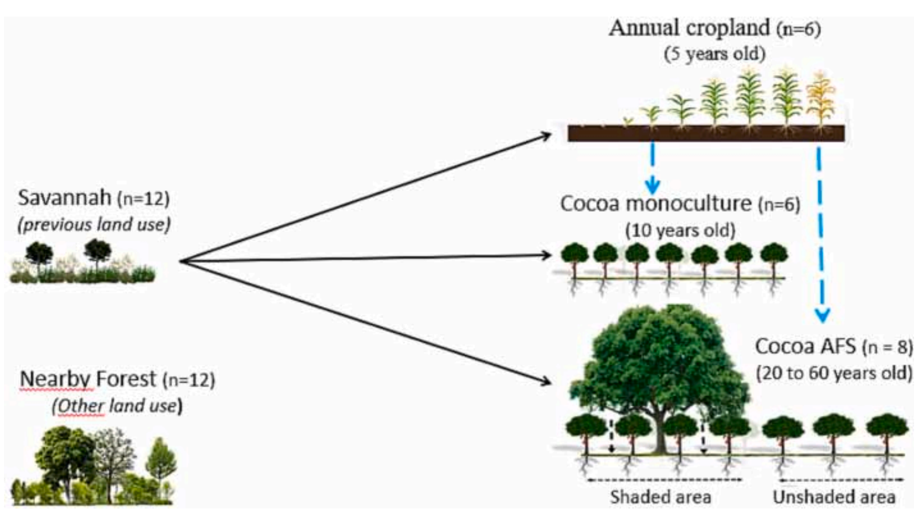

Fig. 1. Trajectories of land use change from savannah to annual cropland, cocoa monoculture, and cocoa agroforestry system (cAFS). The black lines show the primary conversion from savannah to other land use; The dotted blue lines indicate that annual crop land is often the first stage in the conversion of savannah to cacao monoculture or cAFS (n = number of plots).

Table 1
Experimental design: land uses; age of the systems; number of plots; number of soil samples, litter collectors and analyses carried out.

Land uses	System age (years)	Number of plots	Number of replicates for each analysis			
			Bulk soil	Soil organic matter fractionation	Soil fractions	Litter collection
Forest	n.d.	12	12	n.d.	n.d.	n.d.
Savannah	n.d.	12	12	5	15	n.d.
Annual cropland	5	6	6	n.d.	n.d.	n.d.
Cocoa monoculture	10	6	6	5	15	6
Cocoa agroforestry (cAFS)	20–60	8				
Unshaded area			8	5	15	8
Under *Albizia*			5	5	15	6
Under *Canarium*			5	n.d.	n.d.	5
Under *Dacryodes*			8	5	15	8
Under *Milicia*			7	5	15	7
Under *Ceiba*			8	5	15	8
Total			77	35	105	48

Note: n.d. = not determined. Bulk soil analyses correspond to soil organic carbon (SOC) content, N content, pH_{water}, exch. Ca^{2+} and $\delta^{13}C$. For each component studied, the numbers in the columns represent the number of samples analysed. For savannah, cocoa monoculture and cAFS modalities (expect *Canarium*), 5 soil samples were fractionated into three particle size fractions, and C and N contents and $\delta^{13}C$ in each soil fraction was analysed. Litter was collected in all cocoa monoculture plots and cAFS modalities.

10%, 4%, 8%, 19% and 10%, respectively. By deduction, the unshaded area (area outside the canopy of shade trees) ranged from 25% to 76% (average of 43%) of the surface area of the cocoa plots. To investigate the specific effect of shade trees on SOC, SOC pools and related properties, we selected 5 to 8 individuals of each tree species mentioned above according to their occurrence within the 8 cAFS. Finally, the sampling design resulted in an unbalanced replication scheme (Table 1).

2.3. Soil and litter sampling

In each treatment, one composite soil sample of the 0–10 cm depth was prepared from 10 sampling points. For savannah, forest and annual cropland, the ten sampling points were distributed in a zig zag manner covering the whole surface of the plot. For cocoa monoculture plots and unshaded area of cAFS, the ten sampling points spaced at least 30 cm apart were located at 1 m away from the base of cocoa stems. Under each selected tree in cAFS, the ten sampling samples were also equally gathered in one composite sample. They were spaced at least 30 cm apart and located at intermediate distance between the shade tree trunk and its canopy edge and also at 1 m away from the base of cocoa stems. A first soil sampling was done in May 2017 for savannah, cocoa monoculture and cAFS and an additional soil sampling was done, at the same season, one year later in June 2018 for forest, annual cropland, and additional savannah plots.

Leaf litterfall was measured in cAFS and cocoa monoculture during 7 months, from September 2017 to March 2018, when most of the annual litterfall occurs (Saj et al., 2021). As previously described in Sauvadet et al. (2020) for cAFS litterfall measurements, one 0.45 m^2 collector was set above each soil sampling location in cocoa monoculture plots and cAFS modalities (Table 1). Leaf litter was harvested every 15 days from September 2017 to March 2018. For each collector and sampling date, the collected leaf litter was dried in the laboratory at 37 °C for 1 week, and litter dry weights from all sampling periods were then summed.

2.4. Laboratory analysis

The collected soil samples were analysed at the ISO9001:2015 certified LAMA laboratory of Dakar (UAR IMAGO, IRD, Senegal) after air-drying and sieving soils at 2 mm. Soil texture analysis was done after destruction of soil organic matter separating soil into five mineral particle size fractions (μm): clay 0–2, fine silt 2–20, coarse silt 20–50, fine sand 50–200 and coarse sand >200) with the Robinson pipette method (NF X 31–107; AFNOR, N., 2003). Total carbon and nitrogen contents were determined by dry combustion of dry soil subsamples (50–100-mg) ground to 0.25 mm, using an elemental analyser (Thermo Fisher Scientific CHN NA2000, Waltham, MA, US). The pH in water was determined using a soil distilled water ratio of 1/2.5 (NF ISO 10390; AFNOR, 2005). The concentration of exchangeable Ca was determined by the cobaltihexamine chloride method and using a microwave plasma atomic emission spectrometer (MP-AES 4210 Agilent; according to the NF ISO 23470– by AFNOR, 2018). Soil stable isotopic abundances ($\delta^{13}C$) were measured in ISO9001:2015 certified LAMA laboratory of Noumea (UAR IMAGO, IRD, New Caledonia) using a stable isotope analyser (SERCON Integra 2 (2015). The C isotope ratio is expressed relative to the international PDB limestone standard (Balesdent and Mariotti, 1996) with $\delta^{13}C$ expressed in ‰ according to the following equation:

$$\delta^{13}C = [(Rsample - Rstandard)/Rstandard] \times 1000 \quad \text{(Eq. 1)}$$

where R is the ratio of the heavy (^{13}C) to light (^{12}C) isotopes of carbon. A simple mixing model (Eqs. 2 and 3) (Nyberg and Högberg, 1995) was used to calculate the amount of SOC derived from C4 plants (mainly grasses of savannah) (SOC_{C4}) and that derived from C3 plants (mainly trees and dicotyledonous grasses) (SOC_{C3}) according to the following equations:

$$SOC_{C4} = \left[\left(\delta^{13}C - \delta^{13}C_{C3}\right)/\left(\delta^{13}C_{C4} - \delta^{13}C_{C3}\right)\right] \text{ x C} \quad \text{(Eq. 2)}$$

$$SOC_{C3} = \left[\left(\delta^{13}C - \delta^{13}C_{C4}\right)/\left(\delta^{13}C_{C3} - \delta^{13}C_{C4}\right)\right] \text{ x C} \quad \text{(Eq. 3)}$$

where $\delta^{13}C$ is the isotopic composition (^{13}C) of the soil at time of sampling, $\delta^{13}C_{C4}$ is the $\delta^{13}$of gramineous savannah plants, $\delta^{13}C_{C3}$ is the $\delta^{13}$of trees and C is SOC content. We used the $\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ values of -28.01 ‰ and - 11.8 ‰, respectively, previously measured by Guillet et al. (2001) in the forest-savannah transition zone in Cameroon.

The organic matter fractionation method performed was adapted from Feller (1979), Balesdent et al. (1991) and Gavinelli et al. (1995) protocols. Five soil samples (from the 2017 sampling campaign) from savannah, cocoa monoculture, unshaded areas in cAFS, and under each shade tree species except *Canarium* in cAFS (Table 1) were selected. For each of these soil samples, 10 g of 2 mm soil was added with 10 glass beads of 4 mm diameter, 250 ml of deionised water, and 10 ml of sodium hexametaphosphate at 50 g/l. The pre-soaked samples were let to sit in a refrigerator overnight at 4 °C and then shaken in a rotary shaker at 43 rpm for 2 h. The suspension obtained was wet sieved through 200-, and 50- μm mesh-size sieves, successively, then the suspension that went through the 50-μm sieve was transferred to a 1800 ml beaker in which ultrasound waves were applied for 10 min using a probe type ultrasound generating unit (Fisher Bioblock Scientific, Illkirch, France) with a power output of 600 W and working in 0.7:0.3 operating: interruption intervals. After sonication, the suspension was passed through a 20-μm sieve. The 200–2000, 50–200 and 20–50 μm fractions were oven dried at 40 °C until constant weight and the weight taken. The remaining suspension that passed through the 20 μm sieve was transferred to a 1 l glass cylinder and filled with deionised water and shaken by hand in 30 rotations. A 100 ml aliquot was collected immediately using a Robison pipette dipped at 10 cm into the cylinder. The 0–20 μm fraction obtained was oven dried at 40 °C until constant weight and the weight taken. We obtained three particle size fractions: (i) sand-size particles (50–2000 μm), (ii) coarse silt (20–50 μm) and (iii) clay + fine silt (0–20 μm). All the dried soil fractions were finely grounded and analysed for carbon concentration previously described for total C in bulk soil. The $\delta^{13}C$ was analysed for each soil fraction for savannah, cocoa monoculture, unshaded cocoa area, under *Albizia, Milicia, Dacryodes* and *Ceiba* in cAFS (Table 1). The amount of SOC derived from C3 plants and C4 plants was determined using the simple mixing model equations in section 2.4.

The *Canarium* modality in cAFS was not fractionated and so its POC and MAOC values were predicted using midinfrared spectroscopy (MIRS) and Partial Least Square Regression (PLSR) method. For this, the soil samples collected in cAFS and cocoa monoculture were analysed for midinfrared (MIR) absorbance. MIR absorbance was obtained using a Tensor 27 HTS-XT instrument from Bruker Optics and the measured wavebands ranged from 4000 to 601 cm^{-1} with a resolution of 4 cm^{-1}. Due to the limited number of samples, 80% of the samples analysed in the laboratory using conventional methods (including POC and MAOC contents) were considered as reference samples and the remaining 20% of samples were used for validation. Using the Partial Least Square Regression (PLSR), all processed soil spectral data were translated into MAOC and POC values by relating soil spectroscopy calibration models to the set of reference soil samples for which both spectral and soil fractionation measurement were available. The calibration models developed were then applied to the MIR spectra to predict MAOC and POC values for all the samples (Sila et al., 2016). The accuracies of the predictions were assessed by computing the validation metrics using leave-one-out cross-validation. The metrics included the coefficient of determination (R^2), the root mean square error (RMSE), residual prediction deviation (RPD) and the ratio of performance to inter quartile distance (RPIQ). The best model was sufficiently accurate for C prediction in soil fractions (Supplementary material Table 2).

2.5. Statistical analysis

The distribution of each variable was tested using the Shapiro-Wilk Test while the Levene's test was used to check the homogeneity of variance with the entire data set for each variable. The logarithmic transformation was used to bring the distribution to approximate normality and resolving variance homogeneity when the data did not show a normal distribution and variances were not equal. Given that a linear relationship between soil texture and soil carbon had been established for these soils (Nijmeijer et al., 2019a), a one-way analysis of covariance (ANCOVA – clay + silt as covariate) was carried out to test the effects of land use type, age and modality of cAFS on SOC, $\delta^{13}C$, C4 and C3 plants derived SOC in bulk soil and in the different soil particle size fractions.

For each variable studied in the cAFS, a weighted mean was obtained by considering the relative unshaded area and the relative area occupied by each selected species in the plot. For the unselected species, an average value of all species was used and applied to the relative area occupied by those species (Supplementary material Table 1). The means were presented with their standard deviations. The Student Newman-Keuls test was used to compare the mean differences between land uses and stand age. The Pearson correlation coefficient and linear regressions were conducted to investigate the relationship between SOC in bulk soil, different soil particle size fractions, clay and clay + fine silt contents. All Analysis were performed using XLSTAT by Addinsoft software version 2021. The significance of the treatment effects was tested at 5% probability level.

3. Results

3.1. Variations of bulk soil properties among land uses

Considering all samples for all land uses, the soil clay (0–2 μm) content ranged between 76 g kg^{-1} soil and 164 g kg^{-1} soil, and the clay + fine silt (0–20 μm) content ranged between 140 g kg^{-1} soil and 244 g kg^{-1} soil (Table 2), with no significant differences between land uses, age after conversion, and shade tree species within the cAFS (Table 2 and Supplementary material Table 3). When all soil samples were plotted, SOC content presented positive correlations with clay content ($r = 0.59$, $P < 0.0001$), clay+ fine silt content ($r = 0.49$, $P < 0.0001$), soil N content ($r = 0.97$, $P < 0.0001$), soil pH ($r = 0.55$, $P < 0.0001$) and soil exchangeable Ca^{2+} ($r = 0.31$, $P = 0.04$) (Table 3).

We found no significant difference of SOC and N contents between the age of 20 years and the age of 60 years in cAFS, neither in unshaded areas nor in areas shaded by each different tree species (Supplementary material Table 3). Therefore, bulk SOC and N content values for the subsequent analyses do not consider age categories of cAFS.

Land use affected the SOC content values in the top 10 cm layer. The SOC contents ranged in the following increasing order: annual cropland - 5 years old (5.5 ± 2.2 g C kg^{-1}) ≤ cocoa monoculture −10 years old (6.9 ± 3.7 g C kg^{-1}) ≤ savannah (10.6 ± 3.1 g C kg^{-1}) < forest (16.3 ± 5.8 g C kg^{-1}) = cAFS 20–60 years old (18.2 ± 1.8 g C kg^{-1}) (Table 2).

Conversion of savannah to annual cropland or to cocoa monoculture did not change significantly the SOC content. By contrast, the afforestation of savannah with cAFS significantly increased SOC content in the top 10 cm layer. This increase was significant for all sampling positions within the cAFS farms – in unshaded area, and under the canopies of all studied shade tree species except *Albizia*. However, the amplitude of the increase varied with the shade tree species, with SOC ranging from 13.9 ± 2.8 g C kg^{-1} under *Albizia* and 16.8 ± 6.5 g C kg^{-1} under *Dacryodes* and up to 23.5 ± 5.5 g C kg^{-1} under *Ceiba* (Table 2). Furthermore, cAFS set up after savannah showed similar levels of SOC as under nearby secondary forests.

The soil N ranged from 0.4 ± 0.2 g N kg^{-1} soil in annual cropland to 1.9 ± 0.5 g N kg^{-1} under *Ceiba* in cAFS which was similar to under *Milicia* but significantly higher than in all other cAFS modalities with 1.2 ± 0.2 g N kg^{-1} on average. The topsoil C/N ratios ranged from 10.8 ± 2.0 in forest, 12.0 ± 0.8 in cocoa monoculture, 12.1 ± 1.4 in cAFS, 12.4 ± 0.6 in annual cropland to 14.2 ± 1.5 in savannah (Table 2).

Land use affected the soil pH, which ranged from 5.9 to 7.2 (Table 2) with the lowest values in annual croplands, cocoa monoculture and cAFS under *Albizia* (about 6), intermediate (around 6.3–6.6) under forest, savannah and a few cAFS modalities: under unshaded area, *Canarium* and *Dacryodes*, and significantly higher (7.1–7.2) in cAFS under *Milicia* and *Ceiba*.

Land use affected the soil exchangeable Ca^{2+} contents; which ranged from 1.2 ± 1.0 cmol kg^{-1} soil in annual cropland, to a value almost 10 times higher in cAFS under Milicia. Soil exchangeable Ca^{2+} presented similar values for forest, savannah, annual cropland and cocoa monoculture but tended to increase under cAFS (average of 7.9 ± 5.0 cmol kg^{-1}). However, soil exchangeable Ca^{2+} under cAFS highly depended on shade tree species with significantly highest values under *Ceiba*, *Milicia*, *Dacryodes* and Unshaded area (about 8.3 to 11.5 cmol kg^{-1} soil) and lowest values under *Albizia* and *Canarium.*

3.2. Soil $\delta^{13}C$ values and proportions of SOC in bulk soil derived from C-4 and C-3 plants in land uses

The soil $\delta^{13}C$ decreased significantly among land uses, in the following order (corresponding to a decreasing proportion of SOC derived from C4 plants), from −16.9 ± 1.9 ‰ in savannah, −19.2 ± 2.2 ‰ in annual cropland, −22.9 ± 0.6 ‰ in cocoa monoculture, −25.7 ± 1.4 ‰ and − 26.9 ± 0.6 ‰ in 20-year-old and 60-year-old cAFS respectively. The latter two values were similar to values under forest

Table 2
Organic carbon content in bulk soil and other selected soil properties of the top 10 cm layer in all land uses.

Variables	Forest	Savannah	Annual cropland	Cocoa monoculture	Cocoa agroforestry system						
					Unshaded area	Under *Albizia*	Under *Canarium*	Under *Dacryodes*	Under *Milicia*	Under *Ceiba*	Weighted average cAFS
Clay (g kg^{-1} soil)	164 ± 9.2 a	132 ± 67 a	76 ± 37 a	98 ± 73 a	92 ± 60 a	118 ± 41 a	158 ± 100 a	97 ± 50 a	101 ± 23 a	137 ± 4.5 a	95 ± 43 a
Clay + fine silt (g kg^{-1} soil)	244 ± 89 a	201 ± 74 a	140 ± 36 a	203 ± 139 a	192 ± 42 a	209 ± 35 a	238 ± 101 a	171 ± 46a	166 ± 35 a	215 ± 62 a	177 ± 47 a
SOC (g C kg^{-1} soil)	16.3 ± 5.8 b	10.6 ± 3.1 cd	5.5 ± 2.2 d	6.9 ± 3.7 d	15.4 ± 5.3 b	13.9 ± 2.8 bc	17.8 ± 5 b	16.8 ± 6.5 b	18.9 ± 3.9 ab	23.5 ± 5.5 a	18.2 ± 1.8 b
Soil N (g N kg^{-1} soil)	1.4 ± 0.6 b	0.78 ± 0.3 c	0.4 ± 0.2 c	0.6 ± 0.4 c	1.2 ± 0.4 b	1.1 ± 0.2 b	1.4 ± 0.4 b	1.2 ± 0.4 b	1.5 ± 0.3 ab	1.9 ± 0.5 a	1.2 ± 0.2 b
C/N	10.8 ± 2 c	14.2 ± 1.5 a	12.4 ± 0.6 b	12 ± 0.8b c	12.5 ± 1.1 b	12.3 ± 1.2 b	12.7 ± 0.7 b	13.6 ± 1.1 ab	12.3 ± 0.9 b	12.2 ± 0.5 b	12.1 ± 1.4 b
pH H_2O	6.4 ± 0.5 b	6.3 ± 0.3 b	5.9 ± 0.5 c	6.2 ± 0.1 c	6.6 ± 0.1 b	6 ± 0.3 c	6.7 ± 0.1 b	6.6 ± 0.1 b	7.1 ± 0.2 a	7.2 ± 0.2 a	6.5 ± 0.7 b
Exch. Ca^{2+} (cmol kg^{-1} soil)	4.4 ± 2.2 c	2.1 ± 0.8 c	1.2 ± 1.0 c	5.3 ± 4.1 bc	8.3 ± 4 ab	2.7 ± 1.5 c	2.1 ± 0.6 c	9.3 ± 3.8 ab	11.5 ± 3.7 a	10.4 ± 5.9 a	8.3 ± 3.2 b

Note: Means separation was done by Neumann Keuls; data are presented as mean ± SD, Values on each row followed by a different letter are not significantly different ($p < 0.05$).

Table 3
Pearson's correlation coefficients between soil properties (all samples considered) of the top 10 cm layer.

Variables	Clay	Clay + fine silt	SOC	Soil N	C/N	pH	P	Exch. Ca^{2+}
Clay	**1**							
Clay + fine silt	**0.89**	**1**						
SOC	**0.59**	**0.49**	**1**					
Soil N	**0.59**	**0.52**	**0.97**	**1**				
C/N	0.08	−0.05	0.28	0.06	**1**			
pH	0.12	−0.03	**0.55**	**0.57**	−0.06	**1**		
Exch. Ca^{2+}	−0.01	−0.14	**0.31**	0.25	0.18	**0.51**	0.17	**1**

Values in bold are different from 0 with a significance level α = 0.05.

(−25.8 ± 1.5 ‰) (Fig. 2). Within the cAFS, $\delta^{13}C$ ranged from −25.8 ± 2.1 ‰ under *Albizia* to −27.1 ± 0.4 ‰ under *Ceiba* (Fig. 3). In cAFS, the time after afforestation did not significantly impact the $\delta^{13}C$ (Fig. 2).

The SOC derived from C4 plants represented 69% of the total SOC in savannah and decreased to 55%, 34% and 6–14% in annual cropland, cocoa monoculture and cAFS, respectively. This corresponded to a significant decrease in SOC amount derived from C4 plants, from 7.2 ± 2.2 g C kg^{-1} in savannah to 2.8 ± 0.9 g C kg^{-1} in annual cropland, 1.7 ± 0.9 g C kg^{-1} in cocoa monoculture and on average 2.04 ± 0.7 g C kg^{-1} in 20-year-old and 1.2 ± 0.6 g C kg^{-1} in 60-year-old cAFS (Fig. 2 and supplementary material Table 4). After the conversion of savannah, the initial SOC derived from C4 plants was rapidly lost in a proportion of 76% within 15 years (5 years of annual cropland +10 years of cocoa monoculture) and then remained almost unchanged over time, reaching a loss of about 83% in 60-year-old cAFS (Fig. 2). This decrease in SOC derived from C4 plants in cAFS did not depend on shade tree species.

By contrast, the SOC derived from C3 plants increased from 31% of the total SOC in savannah to 45%, 66% and 86–94% in annual cropland, cocoa monoculture and cAFS modalities respectively. This corresponded to an increase of SOC derived from C3 plants from 3.4 ± 1.5 g C kg^{-1} in

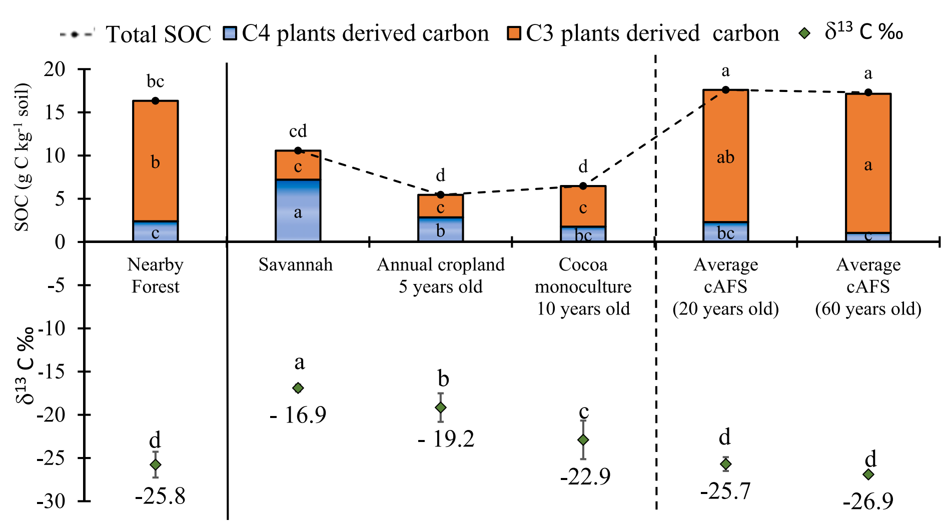

Fig. 2. Total SOC content and SOC content derived from C4 and C3 plants and $\delta^{13}C$ of the top 10 cm layer for all land uses. For each parameter, values associated to the same letter are not significantly different at $p < 0.05$.

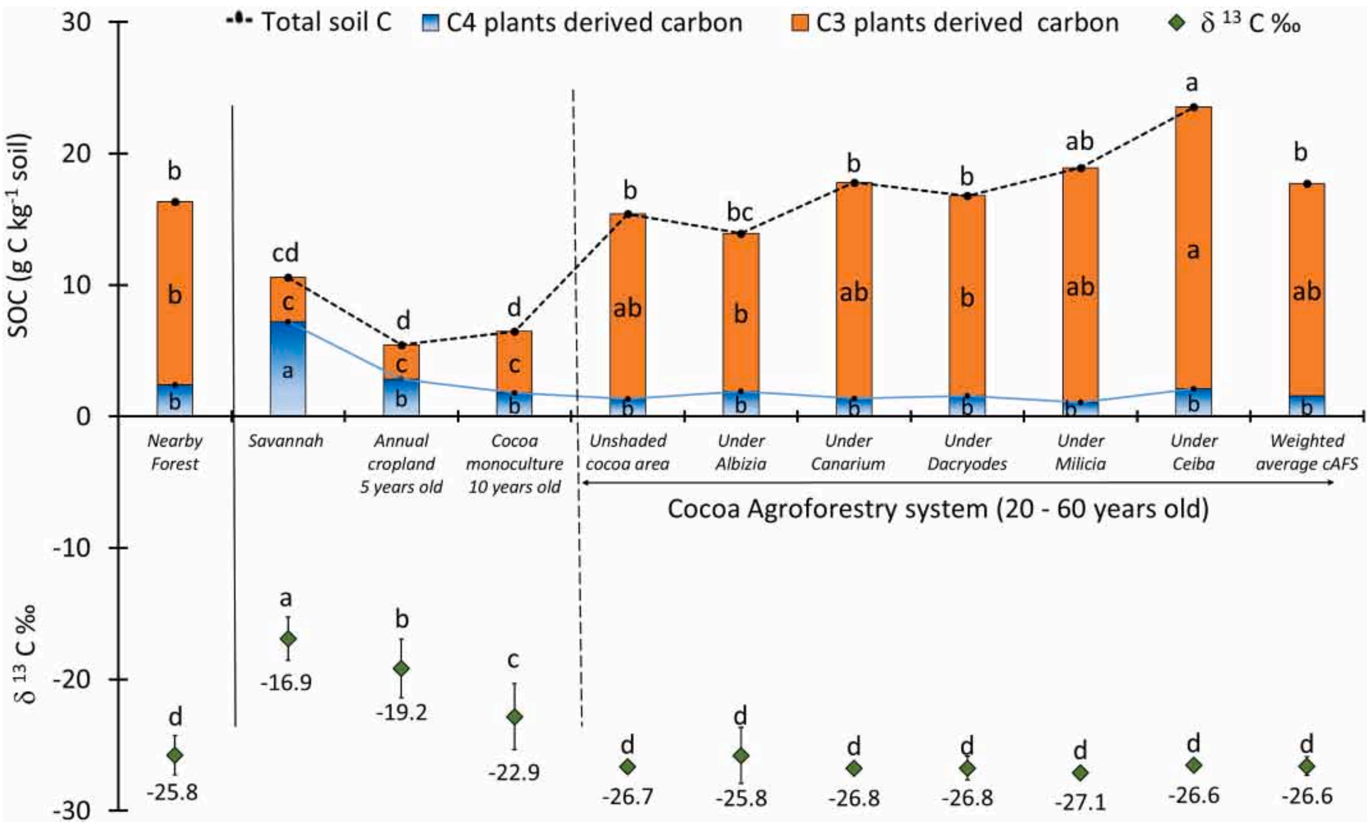

Fig. 3. Total SOC content, SOC content derived from C3 and C4 plants and δ^{13}C of the top 10 cm layer for all land uses. For each parameter, values associated to the same letter are not significantly different at $p < 0.05$. Values for cAFS are weighted average values of the 8 cAFS modalities.

savannah, 5.1 ± 4.7 g C kg^{-1} in cocoa monocultures after 10 years, to 15.5 ± 4.1 g C kg^{-1} and 16.9 ± 4.6 g C kg^{-1} on average in 20- and 60-year-old cAFS, respectively (Fig. 2, Supplementary material Table 4). If we consider the SOC content derived from C3 plants in annual cropland as an initial value when cocoa monoculture and cAFS were set up, the newly incorporated carbon from the cocoa system, can be estimated at least as the difference between current and initial values in SOC derived from C3 plants. Therefore, this newly incorporated carbon from the cocoa system accounted for at least 37% and 77% of total SOC in cocoa monoculture of 10 years old and cAFS of 20–60 years old, respectively. The amounts of SOC derived from C3 plants ranged from 11.9 ± 3.5 to 20.1 ± 4.5 g C kg^{-1} in 20 years old cAFS and from 12.1 ± 2.7 to 22.2 ± 5.8 g C kg^{-1} in 60 years old cAFS according to the shade tree species (Supplementary material Table 2). The increase in C3 plants derived C

Table 4
Carbon contents and concentrations in fractions of the top 10 cm soil layer.

Parameters of soil fractions	Savannah	Cocoa monoculture	Cocoa agroforestry system					
			Unshaded area	Under *Albizia*	Under *Dacryodes*	* Under *Canarium*	Under *Milicia*	Under *Ceiba*
C content (g C kg^{-1} soil)								
50–2000 μm (POC) (g C kg^{-1} soil)	3.45 ± 0.2 c	2.61 ± 0.7 c	6.7 ± 2.3 bc	6.84 ± 2.8 bc	6.95 ± 3.4 bc	5.2 ± 1.6 bc	9.46 ± 2 ab	12.38 ± 5 a
20–50 μm (g C kg^{-1} soil)	0.89 ± 0.5 ab	0.60 ± 0.4 b	1.64 ± 0.7 ab	0.96 ± 0.3 ab	1.51 ± 0.6 ab	0.9 ± 0.4 ab	1.21 ± 0.4ab	1.98 ± 1 a
0–20 μm (MAOC) (g C kg^{-1} soil)	4.63 ± 0.6 ab	3.68 ± 2.7 b	7.80 ± 1.9 a	6.23 ± 0.6 ab	8.08 ± 3.2 a	7.6 ± 2.3 a	7.32 ± 1.8 a	8.57 ± 1.7 a
SOC (0–2 mm) (g C kg^{-1} soil)	9.2 ± 0.9 cd	6.9 ± 3.7 d	17.5 ± 4.2 abc	14.4 ± 2.9bc	16.9 ± 7.1 abc	17.5 ± 5.7 abc	19.4 ± 3.8 ab	24.2 ± 5.8 a
C recovery (g C kg^{-1} soil)	8.97 ± 1.2 cd	6.9 ± 3.8 d	16.2 ± 4.2 abc	14.03 ± 3.02 bcd	16.5 ± 7.1 abc	13.6 ± 3.9 bcd	18 ± 3.6 ab	22.9 ± 6.1 a
C recovery (%)	97.5 ± 3.8	100 ± 5.1	92.6 ± 1.8	97.4 ± 2.9	97.6 ± 4.9	n.d	92.8 ± 1.5	94.6 ± 2.8
Mass recovery (%)	99.4 ± 0.5	98.5 ± 1.0	99.2 ± 0.5	99.5 ± 0.4	99.2 ± 0.7	n.d	99.4 ± 0.9	99.3 ± 0.6
POC / SOC (%)	38 b	41 ab	41 ab	47 ab	42 ab	n.d	53 a	53 a
MAOC / SOC (%)	52 a	51 a	49 a	45 ab	49 a	n.d	41 bc	38 c
C concentration in MAOC (g C kg^{-1} fraction)	27.9 ± 5.1 c	22.4 ± 1.7 c	42.8 ± 4.3 ab	37.4 ± 10 b	46.2 ± 5.03 ab	n.d	53.9 ± 7.9 a	51.2 ± 11.4 a

Note: Data are presented as mean ± SD. Means separation was done by Neumann Keuls; for each parameter, in each row, values associated to the same letter are not significantly different at $p < 0.05$. The C and mass recovery were calculated based on values in Table 2. SOC = soil organic carbon; POC = Particulate organic carbon; MAOC = Mineral associated organic carbon; n.d. = not determined.

* SOC in particle size fractions for cAFS under *Canarium* was predicted using the partial least squares regression model based on NIR spectrums.

was significantly higher under *Ceiba* than under *Albizia*, both of which did not differ from the other cAFS modalities showing intermediate values.

3.3. SOC distribution in soil particle-size fractions for the different land uses

Approximately 98.5 ± 1.0% to 99.5 ± 0.4% of the whole-soil mass and 92.6 ± 1.8% to 100 ± 5.1% of the total SOC were recovered in the soil physical fractionation (Table 4). While the mass of the clay + fine silt fraction (0–20 μm) was about 20% of the bulk soil mass, MAOC (mineral associated organic carbon) in this fraction accounted for 38% to 52% of bulk SOC depending on the land use. On the other hand, while the mass of the sand fraction (50–2000 μm) was about 70% of the bulk soil mass, POC (particulate organic carbon) in this fraction accounted for 38% to 53% of bulk SOC. The intermediate coarse silt fraction (20–50 μm) represented 10% of the bulk soil mass only and accounted for only 7% to 10% of bulk SOC (Table 4). The proportions of POC in total SOC significantly shifted from about 38% in savannah to 53% in cAFS under *Milicia* and *Ceiba* while the proportion of MAOC in total SOC significantly shifted from 52% in savannah to 41% and 38% under *Milicia* and *Ceiba* respectively. For cocoa monoculture and other cAFS modalities the proportions of MAOC and POC in total SOC did not differ significantly from savannah. (Table 4). Savannah afforestation with cAFS significantly increased ($p < 0.0001$) the POC contents under *Milicia* (multiplied by 2.7) and *Ceiba* (multiplied by 3.5). Only a tendency of increase was observed under *Albizia, Canarium, Dacryodes* and unshaded area, and a negative tendency under Cocoa monoculture but these differences were not significant at a *p* probability level of 0.05. The intermediate particle-size fraction (20-50 μm) showed less contrasted trends, yet presented the lowest value under cocoa monoculture, and the highest under Ceiba in cAFS. The MAOC contents were significantly higher in cAFS (except under *Albizia*) than in cocoa monoculture, but there was only a non-significant trend of increase in MAOC contents in cAFS compared to savannah (although significant for *Ceiba* at $P < 0.07$) (Table 4). However, for limiting the bias because of the role of mineral mass in the amount of MAOC, we also compared the C concentration in the fine 0–20 μm soil fraction (g C kg^{-1} fraction) for the different treatments. We observed significant ($p < 0.0001$) increases of MAOC concentration in all cAFS modalities in comparison to Savannah (from +34% under *Albizia* to +93% under *Ceiba*). Therefore we can conclude that cAFS increased significantly the MAOC fraction with the highest positive effects for *Milicia* and *Ceiba* (Table 4).

Total SOC, POC and MOAC contents were plotted as a function of soil texture (clay + fine silt content). Savannah and cocoa monoculture treatments were grouped, because they did not show any significant differences for SOC, POC and MAOC (Table 4), and compared to all the cAFS modalities (Fig. 4). Total SOC, MAOC and in a lesser extent POC contents increased significantly with the clay + fine silt content for both group of systems "savannah + cocoa monoculture" and cAFS (Fig. 4). In addition, we found significant differences in the regression slopes between savannah + monoculture and cAFS for total SOC ($P < 0.0001$) and POC ($P < 0.0001$), but surprisingly not for MAOC ($P = 0.4$) (Fig. 4, Table 5). Those results suggest a positive significant effect of soil texture on total SOC and POC accumulation in cAFS. However, we found that the accumulation of MAOC under cAFS compared to savannah + cocoa monoculture was not different at low and at high clay contents, which indicates that the soil texture had no apparent effect in accumulation of MAOC (Fig. 4, Table 5).

The C/N ratios decreased from the plant litter (from 70 for savannah grasses to 18 for *Albizia* leaf litter; Sauvadet et al., 2020) to the soil fractions, and with the size of particle soil fractions for all land uses (Fig. 5, Supplementary material Table 5). Land uses affected soil C/N ratio especially for POC. The highest POC values were observed in savannah (23.2), followed by cocoa monoculture (18.6) and lowest in *Milicia* (16.3), *Albizia* (16) and *Ceiba* (15.9) (Fig. 5). However, these

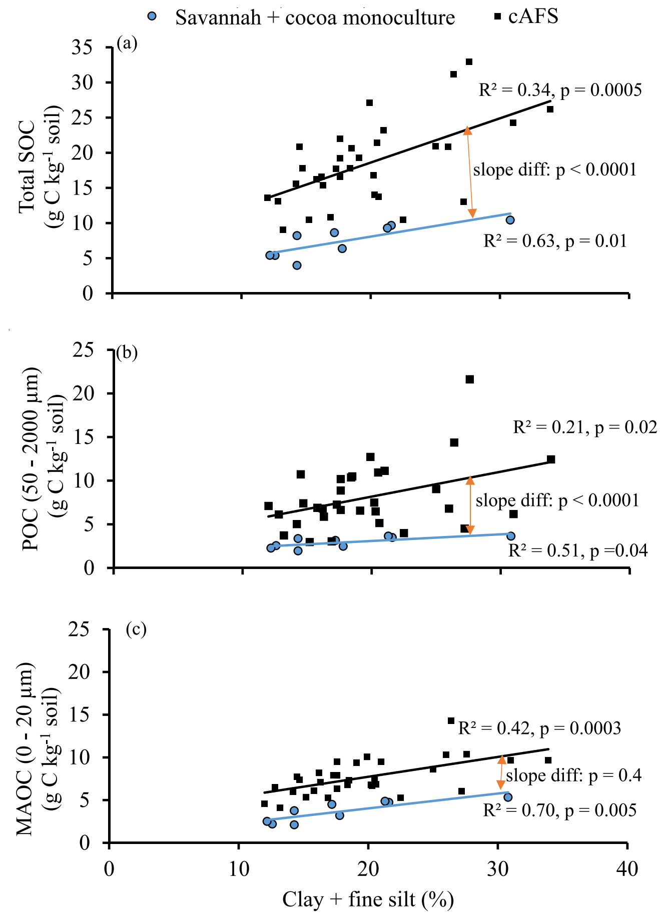

Fig. 4. Relationships between clay + fine silt content and SOC content of the top 10 cm layer in different pools: (a) Total SOC, (b) Carbon content in coarse soil fraction (POC) (50–2000 μm), and (c) carbon content in fine soil fraction (MAOC) (0–20 μm). The difference between slope values of the relationships were significant in Total SOC and POC but not in MAOC.

differences became less noticeable in the fine soil fractions (C/N of 8.7 to10.4) (Supplementary material Table 5).

3.4. Variations of $\delta^{13}C$ and proportions of SOC derived from C4 and C3 plants in the different soil fractions

Land use change had significant impacts on $\delta^{13}C$ values of all soil fractions ranking in the significant decreasing order: Savannah > Cocoa monoculture > cAFS in all soil fractions (Table 6). There was no effect of the shade tree species on the $\delta^{13}C$ values of any soil fractions ($\delta^{13}C$ in Unshaded area = $\delta^{13}C$ under *Albizia* = $\delta^{13}C$ under *Ceiba*). On the other hand, $\delta^{13}C$ values increased in the finer fractions, yet this trend was only significant in Savannah (−18.1 ± 0.5 ‰ in the coarse fraction vs. -15.5 ± 0.7 ‰ in the fine fraction) and Unshaded area of cAFS (−26.9 ± 0.3 ‰ in the coarse fraction vs. -25.3 ± 0.4 ‰ in the fine fraction).

Savannah conversion to cocoa monoculture or cAFS significantly decreased the amount of SOC derived from C4 plants in all the soil fractions (Table 6). Within each land use, the proportions of SOC derived from C4 plants were rather similar in POC and MAOC soil fractions: the C derived from C4 plants in respectively POC and MAOC accounted for 76–77%, 19–33%, 9–18%, 8–12% and 5–16% of total C fraction for savannah, cocoa monoculture, under *Albizia,* under *Ceiba* and in unshaded area of cAFS, respectively.

As C4 was depleted, C3 plant derived SOC increased after savannah conversion to cocoa systems. SOC derived from C3 plants in POC and MAOC did not differ between savannah and cocoa monoculture and yet

Table 5
Regression equations between SOC, POC, MAOC and clay + fine silt content and predicted SOC values at 15%, 22% and 30% clay + fine silt contents for each land use considered.

C Pool	Land use	R^2	*P* Value	Equation	Δ C (g kg^{-1}) at 15% clay + fine silt	Δ C (g kg^{-1}) at 22% clay + fine silt	Δ C (g kg^{-1}) at 30% clay +fine silt
SOC (g C kg^{-1} soil)	Cocoa monoculture + savannah cAFS	0.6 0.34	0.01 0.0005	Y = 0.303 x + 2.0148 * Y = 0.629 x + 6.1017 *	8	11.2	13.8
POC (g C kg^{-1} soil)	Cocoa monoculture + savannah cAFS	0.5 0.2	0.04 0.02	Y = 0.076 x + 1.5675 * Y = 0.285 x + 2.4714 *	3.4	5.5	7.1
MAOC (g C kg^{-1} soil)	Cocoa monoculture + savannah cAFS	0.7 0.4	0.005 0.0003	Y = 0.174 x + 0.5542 y = 0.2321× + 3.0905	3.3	3.8	4.3

Note: SOC = soil organic carbon; POC = Particulate organic carbon; MAOC = Mineral associated organic carbon. In each equation, Y = carbon in pool, x = clay + fine silt content. For each carbon pool, slopes of both equations followed by a * are significantly different at P < 0.05.

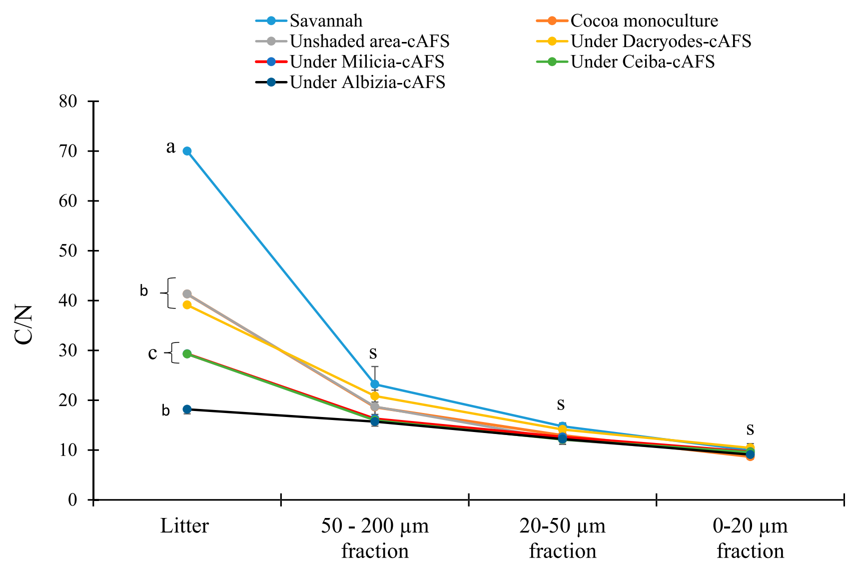

Fig. 5. Variations of C/N ratio from litter to different soil particles. Letter a, b, and c represent significant differences (*P* < 0.05) of litter C:N ratio between land uses; S: significant difference between land uses for the same soil fraction.

was significantly higher in cAFS. (Fig. 6).

In Cocoa monoculture, the low increase in SOC derived from C3 plants in both fractions partially compensated for the significant loss of SOC derived from C4 plants, resulting in an overall non-significant loss of SOC in both fractions (Fig. 6). The newly incorporated carbon from the cocoa system in the different soil fractions, can be estimated, at least as the difference in SOC derived from C3 plants, between current (cocoa monoculture) and initial values (savannah). Almost half (48%) of the newly incorporated C in the topsoil was included in MAOC after 15 years of savannah conversion (5 years of annual crop +10 years of cocoa monoculture) (Table 6).

In cAFS, a significant ($p < 0.0001$) increase in C derived from C3 plants in all soil fractions largely exceeded the loss of SOC derived from C4 plants, resulting in overall increase of MAOC and POC (Fig. 6). Regarding newly incorporated C from the cAFS in the topsoil, 31% and 28% were included in MAOC while 62% and 65% were included in POC under unshaded area and *Ceiba* respectively (Table 6). The MAOC increased in cAFS but less than POC, then its proportion relative to total SOC decreased in comparison to savannah.

4. Discussion

4.1. Soil organic carbon in Savannah

The savannahs in our study area (Bokito, Cameroon) had lower SOC contents in the top 10 cm (10.6 ± 3.2 g C kg^{-1}) than those reported by Guillet et al. (2001) in another area in the forest-savannah mosaic of Cameroon at Kandara (about 25 g C kg^{-1}). The lower SOC contents in Bokito savannahs may be due to lower soil clay content (8–16%) than in undisturbed Kandara savannahs (>40%). In the same way, in Kondi, Congo, d'Annunzio et al. (2008) and Epron et al. (2009) reported lower SOC content (6 to 6.4 g C kg^{-1}) in savannah associated with very low soil clay content (4–6%). However, besides soil texture, savannah management could also explain the SOC content levels. Annual burning and periodical cultivation of the Bokito savannahs may also have depleted SOC contents. This could explain the similar SOC content in Bokito savannahs compared to savannahs in Mampu, Democratic Republic of Congo (12.4 ± 0.1 g C kg^{-1}) associated with less top soil clay contents (4–9%) (Dubiez et al., 2019). For the topsoil of non-woody savannahs in Congo, Epron et al. (2009) reported $\delta^{13}C$ values of - 14.8 ± 0.3‰ close to the - 13 ± 0.3 ‰ value thought to represent better the isotopic composition of SOC in non-woody savannahs (Schwartz et al., 1996; suspense Averti, 2017; Saiz et al., 2018; Sanaiotti et al., 2002). The savannahs in Bokito and those in Kandara, both in the forest-savannah mosaic of Cameroon, had lower and similar topsoil $\delta^{13}C$ values (−16.9 ± 1.7 ‰ and − 17.8 ± 1.7 ‰, respectively) that can be explained by the presence of trees, shrubs and C3 herbs in those savannahs. Furthermore, in Bokito savannahs, the $\delta^{13}C$ value was higher, and closer to typical values of non-woody savannahs, in the fine soil fraction (−15.5 ± 0.7 ‰) compared to the other soil fractions. It suggests that those savannahs were more dominated by C4 grasses and then less influenced by C3 trees/shrubs and dicotyledonous species, in the past than nowadays.

Table 6
Soil δ13C (‰) and origin of carbon in the fractions (derived from C4 or C3 plants).

Variables	Savannah	Cocoa monoculture	cAFS in unshaded area	cAFS under *Albizia*	cAFS under *Ceiba*	Δ C Savannah vs cocoa monoculture*	Δ C Savannah vs cAFS unshaded area	Δ C Savannah vs cAFS under Ceiba
δ^{13}C (‰)								
50–2000 μm (POC)	−18.1 ± 0.5 a	−24.6 ± 1.7 b	−26.9 ± 0.3 c	−26.5 ± 0.7 c	−26.6 ± 1.1 c			
20–50 μm	−16.3 ± 1.2 a	−23.4 ± 2.4 b	−26.4 ± 0.6 c	−26.4 ± 2.3 c	−26.2 ± 0.5 c			
0–20 μm (MAOC)	−15.5 ± 0.7 a	−21.1 ± 2.5 b	−25.3 ± 0.4 c	−24.9 ± 2.2 c	−25.7 ± 0.3 c			
0-2 mm (SOC)	−15.6 ± 1 a	−22.9 ± 2.5 b	−26.5 ± 0.4 c	−26.3 ± 2.01 c	−26.5 ± 0.4 c			
C4 plants derived C (g kg $^{-1}$)								
50–2000 μm (POC)	2.7 ± 0.4 a	0.5 ± 0.2 b	0.5 ± 0.4 b	0.9 ± 0.4 b	1.3 ± 0.5 b	- 2.2 (45%)	- 2.2 (43%)	- 1.4 (33%)
20–50 μm	0.6 ± 0.4 a	0.1 ± 0.0 b	0.1 ± 0.1 b	0.1 ± 0.2 b	0.2 ± 0.1 b	- 0.5 (10%)	- 0.5 (10%)	- 0.4 (9%)
0–20 μm (MAOC)	3.4 ± 0.4 a	1.2 ± 0.2 b	1 ± 0.4 b	0.7 ± 0.9 b	0.9 ± 0.3 b	- 2.2 (45%)	- 2.4 (47%)	- 2.5 (58%)
total fractions	6.7 ± 0.9 a	1.8 ± 0.1 b	1.6 ± 0.5 b	1.6 ± 1 b	2.4 ± 0.6 b	- 4.9 (100%)	- 5.1 (100%)	−4.3 (100%)
0-2 mm (SOC)	7.1 ± 0.7 a	1.7 ± 0.5 b	1.6 ± 0.7 b	1.6 ± 1.7 b	2.4 ± 0.8 b	- 5.4	−5.5	- 4.7
C3 plants derived C (g kg $^{-1}$)								
50–2000 μm (POC)	0.9 ± 0.3 b	2.2 ± 1 b	9.3 ± 6.1 a	8.9 ± 2.8 b	14.2 ± 5.9 a	+1.4 (48%)	+ 8.4 (62%)	+ 13 (65%)
20–50 μm	0.2 ± 0.2 b	0.5 ± 0.5 b	1.2 ± 0.7 a	0.5 ± 0.2 b	1.6 ± 0.8 a	+ 0.2 (8%)	+ 1 (7%)	+ 1.4 (7%)
0–20 μm (MAOC)	1 ± 0.3 c	2.5 ± 2.8 c	5.2 ± 1.4 ab	3.3 ± 0.7 b	6.8 ± 1.6 a	+1.5 (44%)	+ 4.2 (31%)	+ 5.8 (28%)
total fractions	2.1 ± 0.9 b	5.1 ± 4.2 b	15.7 ± 6 a	12.8 ± 3.3 b	22.6 ± 5.3 a	+ 3 (100%)	+ 13.6 (100%)	+ 20.5 (100%)
0-2 mm (SOC)	2.2 ± 0.6 c	5.1 ± 4.1 c	15.7 ± 3.6 b	12.8 ± 2.8 b	22.6 ± 5.8 a	+ 2.9	+ 13.5	+ 20.4

Note: Only five samples per treatment were analysed for δ^{13}C, which explains the discrepancy of the SOC values from those of Table 4 where all soil samples were considered. Data are presented as mean ± SD; means separation was done by Neumann Keuls test; for each parameter, values in each row followed by the same small letter are not significantly different ($p < 0.05$).

* ΔC represent the variations of C content relative to the savannah and are given in g C kg $^{-1}$ soil; values in brackets represent the percentage change of C in soil fractions.

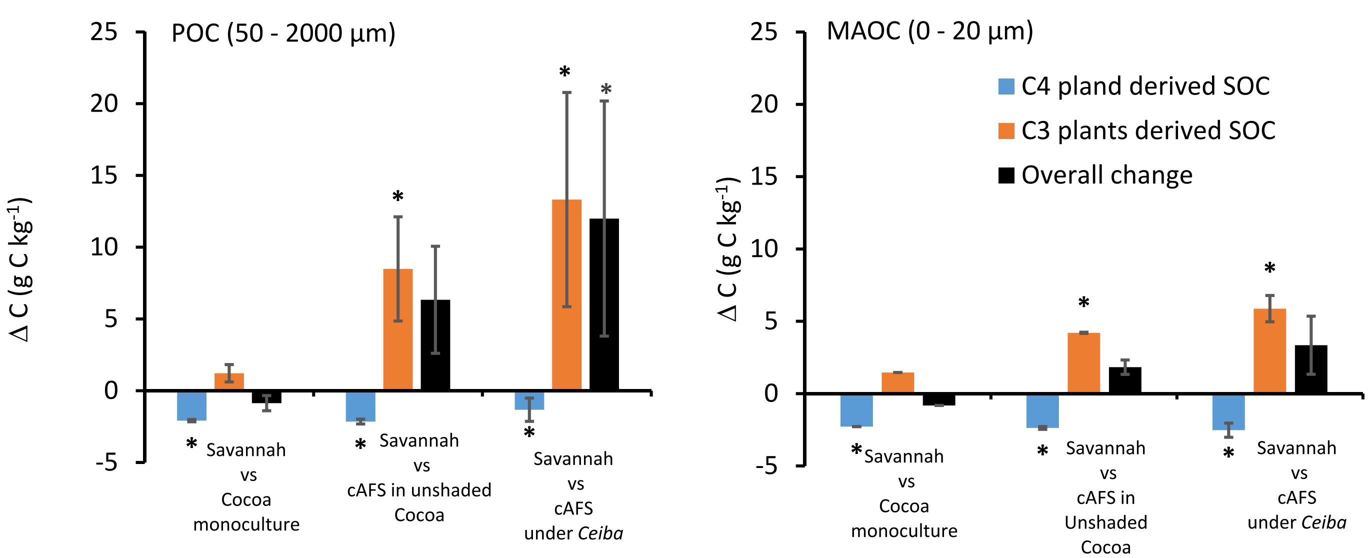

Fig. 6. Changes in C4 and C3-plants derived carbon and overall change in carbon in both soil fractions (POC and MAOC) after conversion of savannah to cocoa monoculture and cAFS modalities. * Significant change ($P < 0.05$).

4.2. Conversion of Savannah to different land uses affects SOC

Five years after conversion of savannah to annual cropland (a mixture of C3 and C4 plants), we observed a significant decrease in soil δ^{13}C associated with a significant loss of the initial SOC content derived from C4 plants present in the savannah (− 61%) and no significant change in SOC derived from C3 plants (Fig. 2). These results suggest high mineralization of initial savannah SOC resulting from soil tillage and low C restitution by crops because of products' export including roots and tubers. Therefore, the lost SOC through mineralization and erosion in annual croplands was not compensated by inputs from C4 and C3 crops.

When croplands in the forest-savannah landscape of Bokito become less productive after continuous cultivation for periods of about 5–7 years, farmers let soil returned to grassy fallow, or plant cocoa either as cocoa monoculture or in association with shade tree species. In our study, soils in cocoa monocultures were sampled 15 years after conversion of savannah (5 years of cropland +10 years of cocoa monoculture), and we observed a significant decrease in soil $\delta^{13}C$ associated with a significant loss of the initial SOC derived from C4 plants (− 76%). The increase in SOC derived from C3 plants, mainly cocoa plant residues, was still not sufficient to offset this loss (Fig. 2). The newly incorporated cocoa-derived C contributed at least to 37% of the top SOC slightly depleted in comparison to savannah. Epron et al. (2009) found a similar proportion of 37% of the top SOC coming from Eucalyptus in a 14-year-old Eucalyptus plantation set up after savannah, but with a slight but non-significant increase in SOC content. The age of the plantation seems crucial to compare the effect of afforestation on savannah. It has already been shown that in the first few years after afforestation of savannah, SOC decreases due to reduced litter inputs while decomposition still occurs, and then gradually increases until a new equilibrium is reached between inputs from the new vegetation litter and outflows from decomposition (Hu et al., 2008; Epron et al., 2009). Indeed, Santos et al. (2020) reported a decrease in SOC 6 years after afforestation of grasslands with eucalyptus. Dubiez et al. (2019) found a significant increase of 13% (+1.6 g C kg^{-1}) of top SOC content after 22 years of afforestation of savannah with *Acacia auriculiformis*.

In previous studies in the same area, Jagoret et al. (2012) and Nijmeijer et al. (2019a) showed a significant increase in SOC content of cAFS established after savannah, over time. They found a mean annual increase of SOC content of 7.3 to 9.5 ‰ in cAFS aged from one to 80 years old. In our study, the SOC content in cAFS, 60 years after conversion of savannah, corresponded to a mean annual increase of 7‰, similar to the values obtained by the previous authors. However, we did not detect any difference in SOC content from cAFS of 20 years old to 60 years old. This absence of difference between SOC in 20- and 60-year-old cAFS remains difficult to explain without a good knowledge of the history of the plots. Furthermore, our study design did not sufficiently consider the whole age gradient and included a too low number of young cAFS making it difficult to draw conclusions for young cAFS. Besides, the fact that SOC and $\delta^{13}C$ values in cAFS and nearby forests were similar (Fig. 2) suggests that old cAFS have a SOC balance close to the one of forests.

4.3. Dynamics of SOC pools after savannah conversion

The conversion of savannah to cAFS showing high decrease in $\delta^{13}C$ and high increase in SOC values suggests a continuous incorporation with time of cAFS-derived C, replacing mineralized SOC derived from the old vegetation and increasing the initial level of SOC. Indeed, in 60-year-old cAFS, >80% of the initial SOC derived from C4 plants was lost while there was a 4-fold increase in SOC derived from C3 plants. Thus, the newly incorporated cAFS-derived C largely exceeded the lost SOC derived from C4 plants and contributed at least 77% of the total SOC content representing 1.8 times the SOC content of the previous savannah. In the same time in cAFS after savannah, both POC and MAOC contents increased. In both fractions, but especially in POC, the significant loss of C derived from C4 plants was largely compensated by significant inputs of new C derived from the cAFS. Total SOC derived from C3 plants present in savannah in small amount (3.4 ± 1.5 g C kg^{-1}) increased significantly to 15.7 ± 3.6 g C kg^{-1} in the unshaded part of cAFS and up to 22.6 ± 5.8 g C kg^{-1} under Ceiba. These accumulations were about 64% located in POC (9 to 14 g C kg^{-1} soil vs. 3 to 7 g C kg^{-1} soil in MAOC) (Table 6) and were the result of high cumulative total litter input (about 9.4 Mg of dry matter ha^{-1} y^{-1}; Saj et al., 2021) from cocoa and associated trees in cAFS over the years. Indeed, in our study, the amount of leaf litterfall measured during 8 months was higher in cAFS (4.2 t DM ha^{-1}) than in cocoa monoculture (3.2 t DM ha^{-1} – data not shown) (Sauvadet et al., 2020). In addition to the C inputs, SOC stabilization could be favoured in cAFS compared to cocoa monoculture. The SOC contents were positively correlated to soil pH and soil exch. Ca^{2+}, and pH and exch. Ca^{2+} were also higher in cAFS than in cocoa monoculture. Higher exch. Ca^{2+} could suggest a better aggregation and SOC stabilization (Rowley et al., 2018) in cAFS.

The high SOC content in cAFS depended on the shade tree species. The lowest values were measured under *Albizia* and the highest values under *Milicia* and *Ceiba.* This higher SOC increase under *Milicia* and *Ceiba* relatively to the other species, was mostly due to a greater C accumulation in the POC and to a lesser extent to a greater accumulation of MAOC (Table 4). Higher increase in SOC under *Milicia* and *Ceiba* could neither be linked with the size of the tree species, which was high for *Ceiba and Milicia* but also for *Canarium*, nor to the amount of litterfall, similar under all tree species, nor litter degradability, which was high under *Milicia*, *Ceiba* but also *Albizia* (Sauvadet et al., 2020). The main trait, which distinguished *Milicia* and *Ceiba* from the other tree species and could explain the high SOC content under these specific tree species, was their litter Ca content. These two species are Ca rich litter species (Sauvadet et al., 2020) and affected soil pH and soil exch. Ca^{2+} (Table 2). It is widely accepted that soil exch. Ca^{2+} significantly increases soil aggregation and structural stability by forming organo-Ca complexes contributing to SOC accumulation (Plante and McGill, 2002; Rowley et al., 2018; Huang et al., 2019). Under *Milicia* and *Ceiba*, the SOC accumulation and its possible stabilization in soil could be mediated by soil exch. Ca content which favour aggregation and possibly occlusion of POC in aggregates. Then POC inside aggregates could be protected from biological degradation (e.g., Denef et al., 2001). This assumption is further supported by the positive correlation of POC with soil clay and fine silt content (Fig. 4), which is another important factor for soil aggregation (e.g., Plante et al., 2006).

On the other hand, MAOC also presented significant differences between tree species, particularly looking at C concentration in this 0–20 μm soil with the highest positive effects of *Milicia* and *Ceiba* and the lowest effect of *Albizia* (Table 4). Furthermore, the use of isotopic signature allowed to highlight a greater C3 accumulation in MAOC under *Ceiba*, while this accumulation was the lowest under *Albizia* (Table 6). MAOC accumulation in soil is greatly dependent on its protection with strong association with clay and fine silt, which depends not only on soil texture – as evidenced by the significant correlation between MAOC and these elements in Fig. 4 – but also on the strength of the links between C and soil matrix. As MAOC-mineral particles links are ensured by bonds with Ca^{2+} (Rowley et al., 2018, 2021), we assumed that the high Ca input by leaf litterfall under *Ceiba* and *Milicia* (Sauvadet et al., 2020) favoured greater MAOC through greater association strength with soil particles, while on the contrary, soil pH decrease under *Albizia* might weaken these bonds and led to lower MAOC under this species.

By contrast to cAFS, no significant change was observed in POC and MAOC contents after conversion of savannah to cocoa monoculture (Table 4). However, isotopic measurements showed SOC dynamics in both fractions. In those fractions, the significant loss of C derived from C4 plants was partially compensated by new C derived from the cocoa monoculture and almost half of this newly incorporated C (+1.5 g kg^{-1} soil) was included in MAOC (Fig. 6 and Table 6). Epron et al. (2009) also found that half of the newly incorporated *Eucalyptus*-derived C was associated with the clay + fine silt fraction in a 14-year-old plantation set up after savannah in Congo. Those findings in tropical Ferralsols suggest that, after savannah conversion to perennial crops, a great part of the newly incorporated C in the topsoil is rapidly associated to the fine soil fraction (MAOC) before a significant change in total soil C could be detected.

As the relative mass of clay and fine silt is considered as a strong driver of SOC stabilization through the formation of organo-mineral complexes (e.g. Hassink, 1997; Feng et al., 2013), the difference of SOC content observed between systems could be due to difference in clay and fine silt content. Indeed, in our study, clay + fine silt content influenced positively all SOC pools, i.e. total SOC, POC and MAOC contents (Fig. 4). However, for a same clay+fine silt content, SOC were still higher in cAFS than in the systems “cocoa monoculture + savannah” (Fig. 4). Surprisingly, the slopes of the correlation between clay+fine silt content with total SOC and POC were higher in cAFS than in savannah + cocoa monoculture, but it was not the case for the MAOC fraction. This result suggests that the higher the clay+silt content the higher SOC and POC enrichments with no effect on the enrichment of the MAOC fraction (Table 5). This no effect of soil texture on accumulation or enrichment of MAOC after savannah afforestation in our study corroborates the results from a meta-analysis by Fujisaki et al. (2018) who did not find any dependence of additional SOC accumulation on soil texture but identified C inputs and duration of the systems as the major predictors of SOC accumulation.

5. Conclusion

Our study provides additional information on the dynamics of soil carbon after afforestation of degraded savannahs with cAFS in the humid tropics. Savannah conversion to other land uses led to high losses of SOC derived from C4 plants (gramineous plants) – 76% after 15 years. Differences in SOC contents between subsequent land uses (cropland, cocoa monoculture and cAFS) were directly linked with differential accumulation of C derived from C3 plants between the land uses. Those C inputs were insufficient under cropland and young cocoa monoculture to compensate the SOC loss derived from savannah, but in mature and old cAFS those inputs led to an increase in SOC. The similar SOC contents observed in old cAFS and adjacent secondary forest suggest that the C cycling in cAFS mimics the one in degraded forest. The higher annual litter input accumulated on a longer period in cAFS (60 years) than in cocoa monoculture (10 years) could explain the higher SOC accumulation in both POC and MAOC under cAFS which also depended on tree species within the system. The enriched litter of *Milicia* and *Ceiba* in Ca^{2+} enriched the soil in Ca^{2+} and increased soil pH, which might have induced higher POC protection by a higher level of soil aggregation and higher MAOC accumulation.

Afforestation of degraded savannah with cAFS through a careful selection of tree species, with high litter production and eventually high Ca^{2+} litter content, will have substantial benefits such as SOC accumulation and improvement of the overall soil fertility contributing to sustainable cocoa production. But it is important to consider how tree species influence other soil properties, nutritional and other interactions with cocoa plants to achieve this goal of sustainability.

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CRediT authorship contribution statement

Eltson Eteckji Fonkeng: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Tiphaine Chevallier:** Data curation, Supervision, Writing – review & editing. **Marie Sauvadet:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Seguy Enock:** Data curation. **Nancy Rakotondrazafy:** Data curation, Methodology. **Lydie Chapuis-Lardy:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Bertin Takoutsing:** Data curation, Methodology, Visualization. **Oben Tabi Fritz:** Conceptualization, Supervision, Validation, Writing – review & editing. **Jean-Michel Harmand:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, 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

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