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Temporal changes in VAM fungi in the cocoa agroforestry systems of central Cameroon

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Abstract In Cameroon, cocoa trees are mostly grown in forests and without fertilization. Our aim was to learn more about the temporal dynamics of soils in cocoa agroforests by comparing young (1–4 years old) and old (over 25 years old) cocoa agroforests. Short fallow and secondary forest were used as treeless and forest references. The numbers and diversities of soil vesicular arbuscular mycorrhizal (VAM) fungi on 60 cocoa producing farms in the Central province of Cameroon were assessed based on the classical morphotyping of spore morphology. We also observed the soil organic matter, nitrogen and major soil nutrients. VAM spore density was significantly lower in the young cocoa agroforests (16 spores g^{-1} dry soil) than in the old cocoa agroforests (36 spores g^{-1} dry soil). Levels in the nearby secondary forest (46 spores g^{-1} dry soil) were not significantly different from old cocoa. The spore density was significantly highest in the short fallow (98 spores g^{-1} dry soil). The Shannon–Weaver index also showed significantly lower biodiversity in young cocoa (0.39) than in old cocoa agroforests (0.48), secondary forest (0.49) and short fallow (0.47). These observations were supported by significant differences in the C:N ratio, Ca, Mg, and cation exchange capacity between young and old cocoa agroforests. We concluded that unfertilized cocoa agroforests could be sustainable, despite a decrease in some soil characteristics at a young stage, due to traditional land-conversion practices based on selective clearing and burning of secondary forest.

Keywords Cocoa agroforestry · Mycorrhiza · On-farm research · Soil fertility · *Theobroma cacao* L

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Introduction

In most cocoa producing countries, and particularly in Cameroon, farmers grow cocoa trees (*Theobroma cacao* L.) in forests without fertilization. These types of agroforestry systems are currently considered to be of high economic and environmental value (Duguma et al. 2001; Johns 1999; Rice and Greenberg 2000; Schroth and Harvey 2007). Other studies in Cameroon or in Ghana have revealed the potential interest of such agroforestry systems for carbon sequestration

and management of soil organic matter (Bisseleua et al. 2008; Kotto-Same et al. 1997; Laird et al. 2007). But few studies have been conducted on soil fertility trends in cocoa agroforests, in comparison with other ecosystems (Hartemink 2005; Isaac et al. 2007). In that respect, the soil microbiological richness of cocoa agroforests is poorly documented, even though it might be a good indicator of microbiological activity and soil quality.

In terms of soil microbiology, the positive role of vesicular arbuscular mycorrhizal (VAM) fungi in the growth of numerous crops is of great interest (Muchane et al. 2009). This positive role has also been observed in young cocoa trees (Azizah Chulan 1991). In addition, cocoa trees are very dependent on phosphorus, which is usually difficult to mobilize (Jadin and Snoeck 1984), but phosphorus uptake could be facilitated by using VAM fungi to enhance tree root activity (Habte and Bittenbender 1999; Janos 1996; Tchabi et al. 2008). In that context, VAM fungi could be used as an indicator of soil fertility changes between cocoa agroforests at different stages and with neighbouring natural forest ecosystems (Musoko et al. 1994; Ngonkeu 2003).

The purpose of our study was thus to learn more about soil biological dynamics along the cocoa chronosequence in cocoa agroforests. To that end, we studied the density and the diversity of vesicular arbuscular mycorrhizal (VAM) fungi under four ecosystems: young cocoa agroforests, old cocoa agroforests, short fallow in which *Chromolaena odorata* predominated as treeless control, and natural secondary forest as forest control. As background and for confirmation of the results, we also compared temporal changes in soil organic matter and major soil nutrients (C, N, P, K, Ca, Mg, and pH).

Materials and methods

Site description

The study was carried out in the forest area of Central province of Cameroon located between 2.1° to 5.8° North and 10.5° to 16.2° East, at an altitude of 600–800 m. The climate is hot and humid with an average annual temperature of 25°C. It is divided into two distinct wet and dry seasons (bimodal rainfall regime), representing an average total annual rainfall of 1,542 mm (Santoir and Bodpa 1995).

Central province is largely covered by dense semi-deciduous forests (Zapfack et al. 2002). The farms were selected in the departments of Lékié, Nyong and Mfoumou, Nyong and Soo, Mbam and Kim and Mvila. The soils of the study area fell mainly into the FAO class of Orthic Ferralsols. These soils are unsaturated, ferralitic, clayey-sandy, highly leached and acidic (pH = 4.3–5.5). They are generally low in organic matter and total nitrogen, and low cation exchange capacity (Santoir and Bodpa 1995).

In this region, cocoa agroforestry systems are dominant and adopted by almost all cocoa growers. Such systems, well described by Rice and Greenberg (2000) and by Sonwa et al. (2007), are characterized by a wide range of species with many different uses (Bisseleua et al. 2008; Kotto-Same et al. 1997). In this type of traditional farming systems, planting is done by clearing a portion of the forest before planting cocoa trees. Then, the undergrowth is eliminated using the slash and burn technique. Only a few trees are left, either to provide slight shade over the cocoa trees, or for their economic value (fruit, medicinal, timber, etc.). In the first years after planting, cocoa trees are mixed with annual cash crops (corn, macabo, peanuts, etc.) and perennials (plantain, cassava).

The cocoa trees are mainly of the Amelonado species (80%). They are planted at an average density of 1,200 trees per hectare. The shade trees consist mainly of large native forest trees ($\pm$ 110 trees ha^{-1}) and medium size fruit trees ($\pm$ 371 trees ha^{-1}). A minimum level of management is used; in particular, none of the farmers used fertilizers and they only applied low levels of fungicide treatments against black pod disease (Paulin et al. 2003). In our study, the influence of possible fungicide residues was not considered because no effect of the commonly used chemical fungicide (Ridomil^{TM}) on soil VAM has been demonstrated in situ in cocoa agroforests, though some toxicity can be observed in the laboratory, or on annual crops (S. Nyasse, personal communication). Likewise, no evidence of any negative effect of fungicide use in a cocoa plot has been demonstrated on either litter decomposition or soil bulk density or earthworm activity (Norgrove 2007).

Experimental design and soil sampling

We selected 60 cocoa farms according to three criteria. First, each farm had the four treatments:

secondary forest (control), old cocoa agroforest (25 years old or more), young cocoa agroforest (1–4 years old: immature phase), and short fallow (<3 years) hosting many herbaceous plants, but mainly *Chromolaena odorata*. Intermediate age categories of the cocoa plots were not considered as it has already been demonstrated that the ecological balance is only reached after 25 years in such cocoa agroforests (Isaac et al. 2005; Jagoret et al. 2008). Second, the soils of the farms had to be of a similar soil group: Ferric Acrisol. Third, farmers used the same cultivation methods in establishing their cocoa plots (same types of shade trees over the cocoa trees) and for management, and no mineral fertilization.

One composite soil and root sample was taken in each of the four treatments at the 60 sites during the 2004 main dry season (February–April). The litter was removed before sampling to prevent any influence of chemicals. Only the topsoil horizon (0–20 cm) was sampled because it has been demonstrated that VAM fungi colonize only young and small roots and are not normally found in deeper cocoa tree roots (Burle 1961). About 95% of feeder roots are concentrated in the topsoil (Jadin and Vaast 1990). In each young or old cocoa plot, ten individual soil cores were taken randomly at a rate of two soil samples 0.5 m from the base of five randomly chosen cocoa trees and at two symmetrical points. In each fallow or secondary forest plot, the ten samples were taken at random points in the plots. All ten samples taken in the same plot were pooled and mixed to obtain one composite sample per treatment, making a total of 240 composite samples.

Extraction of VAM fungus spores and soil chemical analysis

The soil samples were air-dried and sieved (2 mm^2 mesh). The spores of VAM fungi were studied following Brundrett et al.'s (1996) recommendations. The spores were extracted using the wet sieving and decanting method (Gedermann and Nicolson 1963) followed by differential water/sucrose centrifugation (Jenkins 1964). A 100 g sample of dry soil was suspended in 500 ml of water, then left to settle for a few seconds. The suspension was poured through three overlapping sieves with decreasing mesh sizes (500–200–50 μm). The operation was repeated four times. Spores retained by the 200 and 50 m^{-6} sieves were mixed and suspended in distilled water. The spores were sorted by hand under a binocular microscope and separated into groups according to their morphological similarities. The VAM fungi were identified down to the genus level using the classical morphotyping of spore morphology (Franke 1992). For each sample, the numbers of spores per genus were counted and the diversity of VAM fungi was calculated using the Shannon–Weaver index that combines two components of population diversity: genus richness and the homogeneity of individuals (Krebs 1985).

Chemical analysis was performed using standard extraction methods: total C by acid digestion and spectrophotometric analysis (Heanes 1984), total N (Kjeldahl), pH H_2O, available P (Olsen and Sommers), extraction of exchangeable cations (K, Ca, Mg) and cation exchange capacity (CEC) in ammonium acetate.

Statistical analyses

Analyses of soil nutrients and numbers of spores were performed by analysis of variance (ANOVA) using a general linear model. Statistical analysis of numbers of spores required a log transformation (X +1) to eliminate zero values and thus reduce variability between repetitions of the counts of the same treatment. Tests of significance between treatments were performed according to the Fisher test. When significant differences were observed, the Newman-Keuls test was used to compare the treatment averages between them.

Results

Soil VAM fungus counts and diversity

Spores of vesicular arbuscular mycorrhizal (VAM) fungi were found in all soils. Five genera were identified: *Glomus* spp. was the most abundant; *Scutellospora* spp. and *Acaulospora* spp. were found in smaller quantities (Table 1). *Gigaspora* spp. and *Archaospora* spp. were found in very small quantities and were consequently not reported in Table 1.

Under young cocoa agroforests, the total number of spores of all VAM fungi was significantly lower (at $P < 0.05$), than those under the three other systems (Table 1). Similarly, the Shannon–Weaver index of

Table 1 Characterisation of the three dominant VAM fungi *Glomus* spp., *Scutellospora* spp., *Acaulospora* spp., and average number of spores, Shannon–Weaver biodiversity index of four ecosystems in the cocoa region of Central Cameroon (mean ± SE)

Ecosystems	Number of spores per gram of soil				Biodiversity indexes	
	Total	*Glomus* spp.	*Scutellospora* spp.	*Acaulospora* spp.	Shannon index (H′)	Homogeneity (E, in %)
Secondary forest (control)	46 ± 12.3b	39 ± 10.8b	1 ± 6.50b	6 ± 4.63a	0.49 ± 0.02a	44.5
Old cocoa (>25 years old)	36 ± 15.8b	30 ± 13.8b	4 ± 2.80b	2 ± 2.48b	0.48 ± 0.01a	43.8
Young cocoa (0–4 years old)	16 ± 12.3c	13 ± 10.2 c	2 ± 2.45b	1 ± 2.38b	0.39 ± 0.03b	35.7
Short fallow	98 ± 27.5a	85 ± 29.6a	9 ± 4.50a	4 ± 2.63a	0.47 ± 0.02a	43.1
$F_{obs.}$	18*	18*	3*	11*	16**	NS

$F_{obs.}$ coefficient of Fischer test ($n = 60$)

* Treatments significantly different at $P < 0.05$ (Newman-Keuls test)

** Treatments significantly different at $P < 0.01$ (Newman-Keuls test)

Table 2 Total soil carbon (C), total soil nitrogen (N), and C:N ratio of four ecosystems in the cocoa region of Central Cameroon (mean ± SE)

Ecosystems	C_{total} (%)	N_{total} (%)	C/N (%)
Secondary forest (control)	1.46 ± 0.52ab	0.11 ± 0.036ab	13.27 ± 0.45a
Old cocoa agroforest (>25 years)	1.78 ± 0.63a	0.13 ± 0.047a	13.69 ± 0.47a
Young cocoa agroforest (0–4 years)	1.09 ± 0.39b	0.07 ± 0.036b	15.57 ± 0.53b
Short fallow	1.29 ± 0.46ab	0.08 ± 0.041b	16.13 ± 0.55b
$F_{obs.}$	2*	4*	4*

$F_{obs.}$ coefficient of Fischer test ($n = 60$)

* Treatments significantly different at $P < 0.05$ (Newman-Keuls test)

the young cocoa agroforests was significantly the lowest (at $P < 0.01$).

Soil organic and chemical properties

Young cocoa agroforests showed significantly lower total C and N levels (at $P < 0.05$), than in old cocoa (Table 2). C and N levels of secondary forest and fallow soils were intermediate values.

The C:N ratio significantly differentiated the two types of decomposition rates for soil organic matter (at $P < 0.05$) (Table 2). On the one hand, old cocoa agroforests and secondary forest had C:N values around 13, showing normal organic matter decomposition rates. On the other hand, fallow and young cocoa agroforests had higher C:N values around 16, showing slower organic matter decomposition rates.

Soil calcium and magnesium significantly identified two groups of ecosystems (at $P < 0.01$ for Ca; at $P < 0.05$ for Mg) (Table 3): lower values were found in young cocoa agroforests and short fallow, and higher values in secondary forest and old cocoa. The CEC under young cocoa agroforests was significantly lower (at $P < 0.05$) than those under old cocoa and secondary forest. Phosphorus and potassium levels were low, with no significant differences between the four treatments.

Discussion

In the cocoa agroforestry systems of central Cameroon, we were able to distinguish three types of ecosystems. The first type consisted of the young cocoa agroforests with soils having significantly lower density and diversity of VAM spores than old cocoa agroforests, secondary forest, and short fallow. This could be related to the methods of land conversion, as already demonstrated by another study in Cameroon linking the number of spores and species diversity of

Table 3 Major soil nutrient contents (P, K, Ca, Mg), CEC and pH of four ecosystems in the cocoa region of Central Cameroon compared to average optima computed for cocoa (mean ± SE)

Ecosystems	$P_{avail.}$ (ppm)	K (cmol kg^{-1})	Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	CEC (cmol kg^{-1})	pH (H_2O)
Secondary forest (control)	4.07 ± 2.12	0.22 ± 0.05a	2.49 ± 0.15a	0.56 ± 0.17a	8.48 ± 0.42a	5.1 ± 0.1
Old cocoa (>25-years old)	5.64 ± 2.26	0.17 ± 0.03b	2.36 ± 0.13a	0.69 ± 0.18a	8.80 ± 0.34a	4.9 ± 0.1
Young cocoa (0–4 years old)	4.32 ± 1.42	0.20 ± 0.04ab	2.09 ± 0.10b	0.33 ± 0.11b	7.50 ± 0.38b	5.1 ± 0.1
Short fallow	3.27 ± 1.14	0.19 ± 0.03ab	2.04 ± 0.12b	0.31 ± 0.09b	8.09 ± 0.43ab	5.0 ± 0.1
$F_{obs.}$	$<1^{NS}$	12**	10**	3	4*	$<1^{NS}$

$F_{obs.}$ coefficient of Fischer test ($n = 60$)

* Treatments significantly different at $P < 0.05$ (Newman-Keuls test)

** Treatments significantly different at $P < 0.01$ (Newman-Keuls test)

VAM fungi to various agricultural practices (Ngonkeu 2003). Similarly, the total C and N levels and C:N ratio were significantly different between young cocoa and old cocoa agroforests. Our results are consistent with those of Isaac et al. (2005) in western Ghana. These authors observed lower levels in the total soil carbon in young cocoa agroforests consecutive to the land conversion process, followed by a slow restoration of soil carbon between 15 and 25 years.

The second group contained old cocoa agroforests and secondary forest with no significant differences between them for most of the parameters. Both ecosystems had significantly higher density and diversity of VAM spores than young cocoa agroforests. A higher density and diversity of spores in old cocoa than in young cocoa agroforests might also indicate that there was actually only a limited or no effect of fungicide residues on the soil VAM fungus population, as previously suggested by Norgrove (2007). CEC values were higher than in the young cocoa and short fallow due to higher levels of Ca and Mg, confirming that soil fertility improved with the age of the cocoa trees. Our results confirm those previously observed by Musoko et al. (1994) who compared the soil characteristics on the edges of dense forest and neighbouring cocoa in the Central region of Cameroon.

The third group consisted of the short fallow. This ecosystem had the largest number of spores. This could be expected because it had a larger number of herbaceous shrubs and therefore a larger network of small roots than tree crops, thus enabling better development of mycorrhizae (Abbott and Robson 1991; Bååth and Hayman 1984).

Conclusion

Our results provided new insights into the temporal dynamics of soil fertility under unfertilized cocoa agroforests in central Cameroon. Soil microbiological and chemical properties were low in the early years after planting, but thereafter, there was a greater density and a greater diversity of vesicular arbuscular mycorrhizal (VAM) fungus spores under older cocoa (25 years and more) indicating better soil fertility than in young cocoa agroforests. The temporal dynamics of VAM fungi is supported by similar results for soil organic matter and major nutrients. These dynamics indicate that unfertilized cocoa agroforests could be sustainable, despite a decrease in some soil characteristics at a young stage, due to traditional land-conversion practices based on selective clearing and burning of secondary forest. To reduce the drawbacks of land conversion, emphasis should be placed on management practices that improve soil conservation at planting time.

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