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Bernard Barthès, Anastase Azontonde, Eric Blanchart, Cyril Girardin, Cécile Villenave, et al.. Effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on soil carbon in an Ultisol under maize cultivation in southern Benin. *Soil Use and Management*, 2004, 20 (2), pp.231-239. 10.1111/j.1475-2743.2004.tb00363.x . bioemco-00175352

HAL Id: bioemco-00175352

<https://hal-bioemco.ccsd.cnrs.fr/bioemco-00175352>

Submitted on 17 Jul 2023

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**Effect of a legume cover crop (*Mucuna pruriens* var. *utilis*) on soil carbon
in an Ultisol under maize cultivation in southern Benin**

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Keywords

Soil organic carbon, legume cover crop, mucuna, ¹³C natural abundance, Benin

Abstract

Long-term fallow is no longer possible in densely populated tropical areas, but legume cover crops can help maintain soil fertility. Our work aimed at studying changes in soil carbon (C) in a sandy loam Ultisol (Benin), in a 12-year experiment including three maize cropping systems with manual tillage: traditional no-input cultivation (T), fertilized cultivation (NPK), and association with *Mucuna pruriens* (M). The origin of soil C was also studied, through the determination of natural abundance of soil and biomass ¹³C.

In T, NPK and M, changes in soil C at 0-40 cm were -0.2, +0.2 and +1.3 Mg C ha⁻¹ yr⁻¹, with residue C amounting to 3.5, 6.4 and 10.0 Mg C ha⁻¹ yr⁻¹, respectively. After 12 years of experimentation, maize-originating C in litter plus soil (0-40 cm) represented less than 4% of both total C and overall maize residue C. In contrast, mucuna-originating C in litter plus soil represented more than 50% of both total C and overall mucuna residue C in M, possibly due to accelerated mineralization of native soil C (priming effect) and slow mulch decomposition. Weed-originating C in litter plus soil represented ca. 10% of both total C and overall weed residue C in T and NPK. Thus mucuna mulch was very effective in promoting C sequestration in the soil under study.

Introduction

Soil organic matter fulfils both the “fertility” functions required by farmers and the “environmental” functions related to carbon (C) sequestration required by society. In many rural areas in tropical countries, the environmental challenge consists in limiting deforestation, increasing organic matter storage in cultivated soils, and reducing current erosion, and is thus closely linked with the organic C balance in the plant-soil-atmosphere system. Due to the economic conditions that prevail in many developing countries, this challenge can only be met through the emergence of alternative land-uses involving high levels of organic inputs and soil organic C sequestration at the plot level as well as at wider scales (Feller et al. 2001).

In his synthesis on soil fertility in semi-arid to subhumid areas of Africa, Pieri (1989) reported the need for organic inputs to ensure the sustainability of plant productivity, even in the case of intensive cropping systems involving mineral fertilization. Furthermore, numerous works have demonstrated the direct or indirect positive effects of soil organic matter on various chemical, physical and biological properties of soil related to plant behaviour (Sanchez 1976; Pieri 1989).

Natural fallow has long been the main practice to maintain soil fertility in tropical areas. However, as its effects only become significant after a period of at least 5 years, natural fallow is no longer possible in contexts of increasing population, e.g. in southern Benin, where the population density is as high as 300 to 400 inhab km⁻² (Azontonde 1993). Many authors have underlined the advantage of legume-based cover crops in Africa (isohyet > 800 mm), in order to replace natural fallow, help to control weeds and erosion, and enrich the soil in organic matter and nitrogen (Voelkner 1979; Raunet et al. 1999; Carsky et al. 2001). In southwestern Nigeria, higher maize yields were measured in live mulch plots covered with *Centrosema pubescens* or *Psophocarpus palustris* than in conventionally tilled and no-till plots over four consecutive seasons (Akobundu 1980).

The effect of the association (or more specifically, of relay-cropping) of the legume cover crop *Mucuna pruriens* var. *utilis* with maize has been assessed in southern Benin since 1988 in terms of plant productivity, soil fertility, and erosion control (Azontonde 1993; Azontonde et al. 1998; Barthès et al. 2000). The maize-mucuna system (M) was compared with traditional maize cultivation (T), and with fertilized maize cultivation (NPK). This paper focuses on changes in soil organic C and on its origin (mucuna, maize, weeds, initial soil organic C), which was studied through the measurement of natural ¹³C abundance (Balesdent et al. 1987).

Materials and methods

Description of the site and treatments

The trials were conducted from 1988 to 1999 at Agonkanmey (6°24'N, 2°20' E), near Cotonou in southern Benin. The climate is subhumid-tropical with two rainy seasons (March-July and September-November). Mean annual rainfall and temperature are 1200 mm and 27°C, respectively. The landscape is dominated by low plateaux. The soils are classified as Typic Tropudults (USDA) or Dystric Nitosols (FAO), and have a sandy loam surface layer overlying a sandy clay loam layer at a depth of about 50 cm. Most of the land is cultivated with maize, beans, cassava or peanuts, often associated with oil palm.

The study was carried out on three 30-m long by 8-m wide experimental plots with a 4% slope. Plot replication was not implemented in this demonstration trial, as it is usually impossible in long-duration trials (Shang & Tiessen 2000), especially when these include runoff plots, as was the case in this experiment. Three cultivation treatments were compared: T, traditional pure maize cropping system without any inputs; NPK, pure maize cropping system with mineral fertilizers (200 kg ha⁻¹ of NPK 15-15-15, and 100 kg ha⁻¹ of urea); M, relay-cropping of maize and a legume cover crop, *Mucuna pruriens* var. *utilis*, with no fertilizer. Maize (*Zea mays* var. *DMR*) was always cropped during the first rainy season with superficial hoe cultivation by hand (hoeing depth was about 5 cm). On the M plot, maize was sown in the mucuna mulch from the previous year. Mucuna was sown one month later, and, once maize had been harvested, its growth as a relay-crop continued until the end of the short rainy season. During the short rainy season, the T and NPK plots were left under natural fallow. Further information on the site and soil has been provided by Azontonde (1993) and Azontonde et al. (1998). However, precise records of cropping systems on the experimental plots prior to 1988 are not available.

Soil and plant sampling

Individual soil samples were collected within pits (i) in March, June, August and October 1988 and 1995, at 18 locations per plot at depths of 0-10, 10-20 and 20-40 cm, using 0.2-L cylinders, and (ii) in November 1999, at three locations per plot at 0-10, 10-20 (in two replicates), 20-30, 30-40 and 50-60 cm (one replicate), using 0.5-L cylinders. Samples were simultaneously collected with a knife at different places on the walls of the pits, and with an auger below the pits. Soil bulk density (Db) was determined after oven-drying of cylinder-samples, whereas the other samples were air-dried, sieved (2 mm) or finely ground for carbon (C) and nitrogen (N) analyses.

Aboveground biomass of maize and mucuna was determined every year from five-replicate sampling (1 × 1 m) at maize harvest (August) and at mucuna maximal growth (October), respectively. In 1995, following the same pattern, roots of maize and mucuna were collected at depths of 0-10, 10-20 and 20-40 cm, and hand-sorted (Azontonde et al. 1998). Annual root biomass was calculated using the ratio of below- to aboveground biomass determined in 1995, and annual aboveground biomass. Sampling of weed aboveground biomass was carried out in November 1999 at nine locations per plot, using a 0.25 × 0.25-m frame. Litter was simultaneously and similarly sampled. Root sampling was also carried out in November 1999 on six 0.25 × 0.25 × 0.30-m monoliths per plot: monoliths were cut into three depth layers (0-10, 10-20 and 20-30 cm), and visible roots were hand-sorted; with respect to the vegetation cover, we assumed that roots and litter sampled in T and NPK originated from weeds, whereas those sampled in M were from mucuna. All plant samples were dried at 70°C, weighed for biomass measurement, and finely ground for C determination. Additionally, root, stem and leaf samples of maize and mucuna as well as fruits of mucuna were collected in 1999, air-dried and finely ground for the determination of natural ¹³C abundance.

Carbon and nitrogen determination, and other analyses

Total carbon content (Ct) of soil samples collected in 1988 and 1995 was determined by the Walkley and Black method (WB) and total nitrogen content (Nt) by the Kjeldahl method. Ct and Nt of soil samples collected in 1999 were determined by dry combustion (DC) using an Elemental Analyzer (Carlo Erba NA 1500). Ct was analyzed on 60 samples using both WB and DC methods, leading to a relationship ($r = 0.971$) that was used to convert WB data into DC data. All Ct data are thereafter expressed on a DC basis. The C content of plant samples was determined by dry combustion using an Elemental Analyzer (CHN LECO 600).

Stable C isotope ratios of plant and soil samples collected in 1999 were measured by dry combustion in an Elemental Analyzer (Carlo Erba NA 1500) coupled with an Isotope Ratio Mass Spectrometer (VG-Instruments SIRA 10). They are expressed as $\delta^{13}\text{C}$ values:

$$\delta^{13}\text{C} (\text{‰}) = \left(\left(\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{reference}}} \right) - 1 \right) \times 1000, \quad (1)$$

reference being the international standard NBS (Girardin & Mariotti 1991). For each plot and each soil layer, proportions of Ct originating from different sources were calculated according to equations (2) and (3), which refer to C and ¹³C balances, respectively (Mariotti 1991):

$$\text{Ct} = \text{C}_{\text{rem}} + \text{C}_{\text{mai}} + \text{C}_{\text{wee}} + \text{C}_{\text{muc}} \quad (2)$$

$$\delta^{13}\text{Ct} \times \text{Ct} = (\delta^{13}\text{C}_{\text{rem}} \times \text{C}_{\text{rem}}) + (\delta^{13}\text{C}_{\text{mai}} \times \text{C}_{\text{mai}}) + (\delta^{13}\text{C}_{\text{wee}} \times \text{C}_{\text{wee}}) + (\delta^{13}\text{C}_{\text{muc}} \times \text{C}_{\text{muc}}) \quad (3)$$

with C_{rem} , C_{mai} , C_{wee} and C_{muc} being remaining initial soil C (i.e. present in 1988), maize-derived C, weed-derived C and mucuna-derived C, respectively.

Particle-size analysis was performed by the pipette method after destruction of organic matter with H_2O_2 and total dispersion. Soil pH in water was determined using a 1:2.5 volumetric soil:solution ratio.

Statistical analyses

Differences in mean soil total carbon content (Ct, in $g\ C\ kg^{-1}$), Ct stock ($Mg\ C\ ha^{-1}$) and $\delta^{13}Ct$ (‰) between plots or between years were tested by Student unpaired *t*-tests; no assumptions were made on normality and variance equality (Dagnélie 1975).

Ct was determined through 18- and three-replicate sampling in March 1988 and November 1999, respectively. The validity of the latter was assessed using 18-replicate sampling carried out in October 1995 as a reference, i.e. assuming that it exhibited a normal distribution and provided an unbiased estimation of Ct. Following Dagnélie (1975) and Shang & Tiessen (2000), we calculated that at 95% confidence level, whatever the plot and the depth layer, three-replicate sampling in 1995 would have led to a less than 5, 8 and 7% relative error in Ct estimation in T, NPK and M, respectively. Thus we considered Ct determined in 1999 by three-replicate sampling as representative of the mean of the plot. Similarly, we considered Ct stock estimated in November 1999 as representative of the mean of the plot.

Results

General properties of bulk soil

The soil was sandy loam at a depth of 0-10 cm, and its clay ($< 2\ \mu m$) content increased with depth. At 0-10 cm, clay content greatly increased from 1988 to 1999 in T (around 50%), but not in NPK and M (increase $< 15\%$) (Table 1). In 1999, clay content was greater in T at 0-10 cm than in NPK and M at 10-20 cm. The sand ($> 50\ \mu m$) content was between 60 and 80 $g\ 100g^{-1}$ to a depth of 20 cm, mainly in the form of coarse sand ($> 200\ \mu m$). Soil pH was acidic (< 6) and decreased with time, especially in T and NPK (-0.5 over a decade).

Soil total carbon content and stock in 1988 and 1999

Differences in Ct ($g\ C\ kg^{-1}$) between plots were small in March 1988 ($< 6\%$ at 0-10 cm), though sometimes significant (Table 1). From March 1988 to November 1999, Ct slightly decreased in T (-4 to -18%), did not change significantly in NPK (change $< 20\%$), and increased considerably in M at 0-10 and 10-20 cm ($+120$ and $+50\%$; $p < 0.01$) but not at 20-

40 cm (-7%). In November 1999, Ct at 0-10 and 10-20 cm was thus about 70 and 90% greater in M than in NPK ($p < 0.05$), and 120 and 80% greater in M than in T ($p < 0.01$), respectively. Differences between plots were rather small below this depth, as were differences between NPK and T ($< 30\%$ in general). In short, initial Ct differed slightly between plots, but a marked increase in M and slight changes in T and NPK led to greater final Ct in M, especially at 0-20 cm.

Changes in Ct stock (Mg C ha^{-1}) at 0-40 cm were similar (Table 1): small initial differences between plots ($< 7\%$); from March 1988 to November 1999, slight or non-significant changes in T and NPK ($< 15\%$) but a considerable increase in M (+50%, $p < 0.01$); higher final Ct stock in M than in T (70%, $p < 0.01$) and NPK (45%, $p < 0.05$). Ct stock at 0-40 cm finally reached 24, 29 and 41 Mg C ha^{-1} in T, NPK and M, respectively. Referring to initial soil mass (Ellert & Bettany 1995) and considering seasonal variations in Ct and Db, as indicated by seasonal sampling carried out in 1995, we also calculated Ct stocks in March 1999 on a mass basis (Table 1). This calculation led to similar overall differences between plots or between March 1988 and March 1999 as calculating Ct stocks at sampling dates and on a basis of depth. Between March 1988 and March 1999, mean (\pm standard deviation) annual changes in Ct stock within the masses corresponding to initial 0-20 and 0-40 cm soil layers were -0.0 (± 0.1) and -0.2 (± 0.1) $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ in T, +0.2 (± 0.4) and +0.2 (± 0.6) $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ in NPK, and +1.3 (± 0.4) and +1.3 (± 0.5) $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ in M, respectively.

Aboveground and belowground residue C returned to the soil

In T, NPK and M, respectively, mean annual residue biomass returned to the soil reached 8.0, 13.0 and 19.9 $\text{Mg ha}^{-1} \text{ yr}^{-1}$ (dry matter); it represented 3.5, 6.4 and 10.0 $\text{Mg C ha}^{-1} \text{ yr}^{-1}$, with 39, 74 and 84% as aboveground biomass (Figure 1). In T, returned C mainly originated from weeds (17% as aboveground biomass and 55% as roots). In contrast, returned C in NPK was mainly from maize (61% as aboveground biomass and 14% as roots). In M, maize and mucuna accounted for similar values of residue C, either as aboveground biomass (about 40% each) or roots (8% each).

Natural ^{13}C abundance of plant material and bulk soil

The $\delta^{13}\text{C}$ of maize roots, stems and leaves was -11.3, -11.4 and -11.9‰, respectively, i.e. a mean of -11.5‰. The $\delta^{13}\text{C}$ of mucuna roots and stems (together), leaves, seeds and husks was -24.4, -24.8, -23.6 and -25.3‰, respectively, i.e. a mean of -24.5‰. The $\delta^{13}\text{C}$ of weed

aboveground biomass varied to a certain extent within plots: in November 1999, in T, it varied from -20.9 to -13.4‰ depending on the sample (0.25 × 0.25 m), and its weighted mean over nine replicates was -16.1‰ (standard deviation: 2.2‰); in NPK, it varied from -26.6 to -17.4‰, and its weighted mean reached -23.1‰ (±2.3‰). In contrast, it was almost impossible to sample weeds in M, which was entirely covered by mucuna.

In 1999, $\delta^{13}\text{C}_t$ of bulk soil was maximal in T at 0-10 cm (-21.4‰) and minimal in M at 0-20 cm (ca. -24‰) (Figure 2). Variations in $\delta^{13}\text{C}_t$ with depth were rather limited in NPK (from -22.5 to -23.1‰) and below a 30-cm depth in T (from -22.7 to -22.9‰) and M (from -22.1 to -22.6‰). $\delta^{13}\text{C}_t$ was significantly greater in T than in NPK and M at 0-10 cm ($p < 0.01$), and in NPK than in M at 0-10 ($p < 0.05$) and 10-20 cm ($p < 0.01$). Differences between T and NPK were small ($< 0.6‰$) except at 0-10 cm.

The origin of soil carbon

At each depth layer, we determined the proportion of C_t in the form of C_{rem} (remaining initial soil C), C_{mai} (maize-derived C), C_{wee} (weed-derived C) and C_{muc} (mucuna-derived C) in T, NPK (denoted N in the equations) and M in November 1999. This determination involved the resolution of the following system of equations, resulting from the application of equations (2) and (3) to each plot (C_t and $\delta^{13}\text{C}_t$, which were measured, are denoted C_{tT} and $\delta^{13}\text{C}_{tT}$ in T, C_{tN} and $\delta^{13}\text{C}_{tN}$ in NPK, and C_{tM} and $\delta^{13}\text{C}_{tM}$ in M):

$$C_{tT} = C_{\text{rem}T} + C_{\text{mai}T} + C_{\text{wee}T} + C_{\text{muc}T}$$

$$\delta^{13}\text{C}_{tT} \times C_{tT} = (\delta^{13}\text{C}_{\text{rem}T} \times C_{\text{rem}T}) + (\delta^{13}\text{C}_{\text{mai}T} \times C_{\text{mai}T}) + (\delta^{13}\text{C}_{\text{wee}T} \times C_{\text{wee}T}) + (\delta^{13}\text{C}_{\text{muc}T} \times C_{\text{muc}T})$$

$$C_{tN} = C_{\text{rem}N} + C_{\text{mai}N} + C_{\text{wee}N} + C_{\text{muc}N}$$

$$\delta^{13}\text{C}_{tN} \times C_{tN} = (\delta^{13}\text{C}_{\text{rem}N} \times C_{\text{rem}N}) + (\delta^{13}\text{C}_{\text{mai}N} \times C_{\text{mai}N}) + (\delta^{13}\text{C}_{\text{wee}N} \times C_{\text{wee}N}) + (\delta^{13}\text{C}_{\text{muc}N} \times C_{\text{muc}N})$$

$$C_{tM} = C_{\text{rem}M} + C_{\text{mai}M} + C_{\text{wee}M} + C_{\text{muc}M}$$

$$\delta^{13}\text{C}_{tM} \times C_{tM} = (\delta^{13}\text{C}_{\text{rem}M} \times C_{\text{rem}M}) + (\delta^{13}\text{C}_{\text{mai}M} \times C_{\text{mai}M}) + (\delta^{13}\text{C}_{\text{wee}M} \times C_{\text{wee}M}) + (\delta^{13}\text{C}_{\text{muc}M} \times C_{\text{muc}M})$$

In order to reduce the number of variables, the following assumptions were made:

- (i) deep soil layers contained C_{rem} only, and $\delta^{13}\text{C}_{\text{rem}}$ was thus estimated as $\delta^{13}\text{C}_t$ of 90-100 cm soil layer in 1999 (-22.9‰ in T, -23.1‰ in NPK and -22.6‰ in M);
- (ii) $\delta^{13}\text{C}_{\text{mai}}$ was the same in all plots, and equal to mean $\delta^{13}\text{C}$ calculated for maize (-11.5‰); similarly $\delta^{13}\text{C}_{\text{muc}}$ was the same in all plots and equal to mean $\delta^{13}\text{C}$ calculated for mucuna (-24.5‰);
- (iii) $\delta^{13}\text{C}_{\text{wee}}$ was equal to weighted mean $\delta^{13}\text{C}$ of aboveground biomass of weed samples collected in November 1999 (-16.1‰ in T and -23.1‰ in NPK);

- (iv) due to the vigorous development of mucuna, weeds did not grow in M ($C_{weeM} = 0$);
- (v) there was no mucuna in T and NPK ($C_{mucT} = C_{mucN} = 0$);
- (vi) C_{mai} was proportional to total C input by maize over the experimental period ($C_{maiT} = 0.18 C_{maiM}$ and $C_{maiN} = 0.93 C_{maiM}$);
- (vii) C_{wee} was proportional to weed biomass C measured in November 1999 ($C_{weeT} = 1.60 C_{weeN}$).

Using these assumptions reduced the number of variables to six (C_{remT} , C_{remN} , C_{remM} , C_{maiM} , C_{weeN} , C_{mucM}) and allowed the system of six equations to be solved at each depth layer (Table 2; as an example, the steps leading to the solution of this system of equations at 0-10 cm are presented in Annex 1). Considering each depth layer separately, C_{rem} accounted for more than 95% of C_t in T and NPK, except at 0-10 cm where C_{wee} represented 21% and 10% of C_t , respectively. In contrast, in M, more than 50% of C_t was in the form of C_{muc} to a depth of 30 cm and in the form of C_{rem} below, C_{rem} contribution being remarkably small at 10-20 cm (7% of C_t ; we checked the effects of uncertainties linked to weed sampling on C_{remM} : changing assumptions (iii) and (vii), i.e. changing $\delta^{13}C_{weeT}$, $\delta^{13}C_{weeN}$ and C_{weeT} -to- C_{weeN} ratio, always yielded C_{remM} lower than 10% of C_{tM} at 10-20 cm). Whatever the depth, C_{mai} represented less than 6% of C_t in NPK and M, and less than 1.5% in T.

The origin of C present in litter and soil (soil mass corresponding to the initial 0-40 cm layer) in November 1999 was determined assuming that litter originated from weeds only in T and NPK, and from mucuna only in M (considering vegetation cover), and that litter had the same C content as corresponding living plants. In November 1999, C in litter plus soil was mainly in the form of C_{rem} in T and NPK (90%), and in the form of C_{muc} in M (70%); C_{wee} accounted for 9 and 7% of soil plus litter C in T and NPK, respectively, and C_{mai} for less than 4% whatever the plot (Table 3). Overall, recent C (i.e. originating from biomass grown during the period of the experiment) represented 9, 11 and 72% of litter plus soil C in T, NPK and M, respectively (considering initial 0-20 cm layer, these proportions were 17, 15 and 83%, respectively).

The amount of C from each origin in litter plus soil (initial 0-40-cm mass) in November 1999 was compared with its source, i.e. initial C_t stock (1988) or total above- and belowground residue C returned to the soil over the period of the experiment (considering the litter and vegetation cover in November 1999, annual C input from maize, weeds and mucuna was multiplied by 12, 11.5 and 11, respectively, to account for total C input from March 1988 to November 1999). In November 1999, C_{mai} in soil plus litter represented 2% of C input from maize, whatever the plot, and C_{wee} , 7 and 11% of estimated C input from weeds in T and

NPK, respectively. In contrast, C_{muc} represented 57% of C input from mucuna in M (Table 3). Overall, in November 1999, litter plus recent soil C represented 6, 4 and 27% of total residue C over the period of the experiment in T, NPK and M, respectively.

Discussion

Changes in soil carbon

At the end of the experiment, Ct stock at 0-40 cm reached 24, 29 and 41 Mg C ha⁻¹ under unfertilized maize, fertilized maize and maize-mucuna, respectively. This is consistent with Ct stocks measured in similar soil conditions in southern Benin, which amounted to 27, 30 and 48 Mg C ha⁻¹ at 0-35 cm under palm tree plantation, food crops (with fallow) and forest, respectively (Djegui et al. 1992). Our data on changes in Ct stock were also consistent with other published data. In a three-year experiment on an Alfisol in southwestern Nigeria, rates of +0.2 Mg C ha⁻¹ yr⁻¹ at 0-10 cm were recorded under fertilized maize (Lal 2000), as was the case in NPK. Under maize-mucuna, we measured a 1.3-Mg C ha⁻¹ yr⁻¹ increase in Ct stock, whereas rates of around +1 Mg C ha⁻¹ yr⁻¹ at 0-20 cm have been recorded in Brazilian Ultisols and Oxisols under long-term no-till cropping systems (Bayer et al. 2001; Sá et al. 2001). In Honduras, rates from +0.2 to +1.4 Mg C ha⁻¹ yr⁻¹ at 0-10 cm have been reported from a set of experiments on various Alfisols, Inceptisols and Ultisols under maize-mucuna systems (Triomphe 1996a), and rates beyond +2 Mg C ha⁻¹ yr⁻¹ at 0-20 cm have even been measured in a Nigerian Alfisol under a two-year *Pueraria* cover (Lal 1998). These results confirm that residue mulching promote C sequestration in tropical soils, especially in cropping systems including legume cover crops.

Residue biomass

In M, the high rates of Ct increase were linked with the great residue biomass returned to the soil. Mucuna aboveground biomass was 8 Mg ha⁻¹ yr⁻¹ in M vs. 6-7 Mg ha⁻¹ yr⁻¹ in one-year mucuna fallows studied in Nigeria (Vanlauwe et al. 2000), and an average of 11 Mg ha⁻¹ yr⁻¹ in mucuna-maize systems in Honduras (> 2000-mm annual rainfall; Triomphe 1996b). The ratio of change in Ct stock to residue C measured in our plots also agreed with data in the literature: in 12-year no-till maize-legume rotations on a sandy clay loam Ultisol in Brazil, Ct stock increase at 0-17.5 cm represented 11-15% of aboveground residue C (Bayer et al. 2001), vs. 15% in M (and 5% in NPK). In contrast, in long-term no-till cereal-legume rotations on clayey Oxisols also in Brazil, the increase in Ct stock at 0-40 cm represented 22-25% of total residue C (Sá et al. 2001), vs. 13% in M (and 3% in NPK). This difference underlines the

increasing C sequestration resulting from increasing clay content, which promotes stable aggregation and hence organic matter protection (Feller & Beare 1997).

Due to the absence of crop during the short rainy season, the contribution of weeds to residue biomass was important in T and NPK: they represented 49 and 20% of aboveground residue biomass, respectively. Weeds also represented about 50% of aboveground residue biomass in non-fertilized maize plots studied in Nigeria (Kirchhof & Salako 2000). These data underline the need for systematic sampling of weed biomass when it represents a noticeable proportion of residues returned to the soil. In our experiment, weeds were sampled at one date only, and it is likely that it led to some uncertainties. Weed biomass was negligible in M: proportions of aboveground residue biomass from maize, mucuna and weeds were 49, 51 and 0%, respectively, vs. 49, 42 and 9% in one-year maize-mucuna plots studied in Nigeria (Kirchhof & Salako 2000). Indeed, weed suppression is recognized as the most important factor that determines adoption of mucuna fallow systems by farmers (Carsky et al. 2001).

Nitrous oxide emissions

Fertilizers supplying the soil with nitrogen determine nitrous oxide (N₂O) emissions, which can be roughly estimated using equation (4) (Bouwman 1996):

$$\text{N-N}_2\text{O emissions (kg ha}^{-1}\text{ yr}^{-1}\text{)} = 1 + [0.0125 \times \text{N-fertilizer (kg ha}^{-1}\text{ yr}^{-1}\text{)}]. \quad (4)$$

In NPK, N supply by fertilizers was 76 kg N ha⁻¹ yr⁻¹ (Azontonde et al. 1998). Following equation (4), it resulted in 2-kg N-N₂O ha⁻¹ yr⁻¹ emissions. As the global warming potential of N₂O is about 300 times that of CO₂ (IPCC 2001), these N₂O emissions were equivalent to more than 0.2-Mg C-CO₂ ha⁻¹ yr⁻¹ emissions, and thus offset Ct increase (0.2 Mg C ha⁻¹ yr⁻¹). In M, mucuna residues supplied the soil with more than 250 kg N ha⁻¹ yr⁻¹ (Azontonde et al. 1998). In this case, equation (4) lead to an overestimation of N₂O emissions, as it was established from a set of experiments excluding legume cover crops, which provide N that is less directly available than mineral fertilizers. However, it may give an order of magnitude: following equation (4), N supply by mucuna residues could result in 4-kg N-N₂O ha⁻¹ yr⁻¹ emissions, equivalent to 0.5-Mg C-CO₂ ha⁻¹ yr⁻¹ emissions (vs. 1.3 Mg C ha⁻¹ yr⁻¹ as Ct increase). Though overestimated, these data point out that from an environmental point of view, Ct increase in soils under legume cover crops could be partly offset by N₂O emissions.

The origin of soil carbon

Our results indicate that at the end of the experimental period, recent C represented a small proportion of soil plus litter C in T and NPK (ca. 10%, mainly originating from weeds in T).

Considering the scanty vegetation covering T and NPK plots after maize harvest and during the short rainy season, it is not surprising that initial soil C represented the main contribution to final soil plus litter C. In the same way, the noticeable contribution of weeds is consistent with the fact that they covered T and NPK plots during the short rainy season. In contrast, recent C represented a great proportion of soil plus litter C in M (ca. 70%) and originated mainly from mucuna. This substantial contribution resulted from the great residue biomass provided by mucuna, and we may assume that the thick mulch it formed had a slow decomposition. Mucuna-originating C was mainly limited to the top 30 cm of the soil profile, due to rather superficial rooting, as confirmed by root counting (Barthès, unpublished data) and published data (Carsky et al. 2001). The low proportion of remaining initial C in the topsoil of M is questionable. It may be explained by a priming effect consecutive to the enhancement of biological activities resulting from huge N-rich restitution by mucuna. Indeed, several authors have reported that addition of easily decomposable plant residues could greatly stimulate the mineralization of native organic matter (Jenkinson & Ayanaba 1977; Kuzyakov et al. 2000).

Several studies have also reported results on soil C origin from measurements of ^{13}C natural abundance. In sandy and clayey Brazilian Oxisols, the proportion of recent C at 0-20 cm ranged between 20 and 30% of C_t after 10-12 years' cultivation involving reduced or no tillage without a cover crop (Feller et al. 1991; Shang & Tiessen 2000; Sá et al. 2001). Also including maize plots from temperate areas (Balesdent et al. 1987; Clapp et al. 2000), the proportion of recent C at 0-20 cm ranged between 15 and 30% of C_t after a decade, and tended to increase with increases in residue biomass and clay content, whereas tillage and climate effects were unclear. This proportion was smaller in our study, i.e. 14% in T and 10% in NPK (0-20 cm layer, litter being excluded), possibly due to small residue biomass and clay content. In long-term experiments involving residue return, the proportion of recent C could be much greater: recent C represented ca. 60% of C_t at 0-30 cm after 50-yr cultivation of sugarcane in an Inceptisol in Ecuador (Rhoades et al. 2000). Thus we may assume that in M, though it occurred only over a period of a decade, high residue return similarly resulted in the substantial contribution of recent C to C_t (ca. 70% at 0-40 cm), especially considering that N-rich mucuna residues strongly promoted mineralization of native soil C.

Our results also show that recent C in litter plus soil (initial 0-40 cm mass) represented 6, 4 and 27% of total residue C over the period of the experiment in T, NPK and M, respectively; this proportion was 2% for maize, 7-11% for weeds, and 57% for mucuna. Data in the literature indicate that in maize plots (> 10 yr) from temperate areas, the proportion of recent

soil C (0-30 or 0-40 cm) to total residue C ranged between 12 and 20% under conventional tillage (Balesdent et al. 1987; Gregorich et al. 2001), but was 41% under no tillage (Clapp et al. 2000). In a clayey Oxisol under no-till cereal-legume rotations, this proportion reached 60% after 10 years (Sá et al. 2001). Overall, it tended to increase with increases in residue biomass (possibly due to priming effect; Kuzyakov et al. 2000), with clay content (due to physical protection of organic matter in stable aggregates; Feller & Beare 1997), and with a decrease in the intensity of tillage (since tillage promotes C mineralization; Six et al. 2002). In M, the proportion of residue C remaining in the soil was within the range of published data; in T and NPK, it was lower than published data, possibly due to low residue biomass and clay content.

Conclusion

In the sandy loam Ultisol under study, maize-mucuna relay-cropping was very effective in promoting soil C sequestration (+1.3 Mg C ha⁻¹ yr⁻¹ over the 12-year period of the experiment), due to the great residue biomass provided by mucuna. The study shows that the tropical savannahs have great potential for carbon sequestration. Pure maize cultivation resulted in smaller changes in soil C, either positive when the crop was supplied with fertilizers (+0.2 Mg C ha⁻¹ yr⁻¹) or negative otherwise (-0.2 Mg C ha⁻¹ yr⁻¹). However, rough estimations revealed that from a global change standpoint, nitrous oxide emissions resulting from N supply by mucuna could partly offset C sequestration in soil. In cropping systems including legume cover crops, N₂O fluxes should thus be further investigated in order to establish greenhouse gas balances.

Measurements of ¹³C natural abundance showed that at the end of the experiment, whatever the treatment, maize-originating C in soil plus litter represented a small proportion (< 4%) of both soil plus litter C and total maize residue C returned to the soil over the period of the experiment. In contrast, under maize-mucuna, mucuna-derived C represented a great proportion (> 50%) of both soil plus litter C and mucuna residue C. It is likely that mulching of N-rich mucuna residues promoted the accelerated mineralization of native soil organic matter, whose amount decreased dramatically during the experimental period, whereas the mineralization of mulch C remained slow. Weed-originating C in soil plus litter represented about 10% of both soil plus litter C and total estimated weed residue C under fertilized and non-fertilized pure maize. Overall, under pure maize and maize-mucuna, recent C accounted for about 10 and 70% of soil plus litter C, and represented about 5 and 27% of total residue C,

respectively. Due to weed sampling at one date only, there were however some uncertainties in these results, and further research should include more systematic weed sampling.

Acknowledgements

We thank Barthélémy Ahossi, Maurice Dakpogan, Charles de Gaulle Gbehi, Nestor Gbehi, Branco Sadoyetin and André Zossou for field work, and Jean-Yves Laurent and Jean-Claude Marcourel for technical assistance. This work was financially supported by a French PROSE grant (CNRS-ORSTOM program on soil and erosion).

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Annex 1. Determination of the contribution of remaining initial soil C (C_{rem}), maize-originating C (C_{mai}), weed-originating C (C_{wee}) and mucuna-originating C (C_{muc}) to total soil C (C_t) at 0-10 cm in T, NPK (denoted N in the equations) and M in November 1999.

Contributions were calculated using equations (2) and (3), assumptions (i) to (vii), and C_t and $\delta^{13}C_t$ measured in November 1999 (C_t and $\delta^{13}C_t$ are denoted C_{tT} and $\delta^{13}C_{tT}$ in T, C_{tN} and $\delta^{13}C_{tN}$ in NPK, and C_{tM} and $\delta^{13}C_{tM}$ in M, respectively):

- in T:

$$\begin{aligned} C_{tT} = 5.3 &= C_{remT} + C_{maiT} + C_{weeT} + C_{mucT} = C_{remT} + 0.18 C_{maiM} + 1.60 C_{weeN} + 0 \\ \delta^{13}C_{tT} \times C_{tT} &= -21.4 \times 5.3 = -113.4 \\ &= (\delta^{13}C_{remT} \times C_{remT}) + (\delta^{13}C_{maiT} \times C_{maiT}) + (\delta^{13}C_{weeT} \times C_{weeT}) + (\delta^{13}C_{mucT} \times C_{mucT}) \\ &= -22.9 C_{remT} + (-11.5 \times 0.18 C_{maiM}) + (-16.1 \times 1.60 C_{weeN}) + 0 \\ &= -22.9 C_{remT} - 2.1 C_{maiM} - 25.8 C_{weeN} \end{aligned}$$

- in NPK:

$$\begin{aligned} C_{tN} = 6.7 &= C_{remN} + C_{maiN} + C_{weeN} + C_{mucN} = C_{remN} + 0.93 C_{maiM} + C_{weeN} + 0 \\ \delta^{13}C_{tN} \times C_{tN} &= -22.8 \times 6.7 = -152.8 \\ &= (\delta^{13}C_{remN} \times C_{remN}) + (\delta^{13}C_{maiN} \times C_{maiN}) + (\delta^{13}C_{weeN} \times C_{weeN}) + (\delta^{13}C_{mucN} \times C_{mucN}) \\ &= -23.1 C_{remN} + (-11.5 \times 0.93 C_{maiM}) - 23.1 C_{weeN} + 0 \\ &= -23.1 C_{remN} - 10.7 C_{maiM} - 23.1 C_{weeN} \end{aligned}$$

- in M:

$$\begin{aligned} C_{tM} = 11.5 &= C_{remM} + C_{maiM} + C_{weeM} + C_{mucM} = C_{remM} + C_{maiM} + 0 + C_{mucM} \\ \delta^{13}C_{tM} \times C_{tM} &= -23.8 \times 11.5 = -273.7 \\ &= (\delta^{13}C_{remM} \times C_{remM}) + (\delta^{13}C_{maiM} \times C_{maiM}) + (\delta^{13}C_{weeM} \times C_{weeM}) + (\delta^{13}C_{mucM} \times C_{mucM}) \\ &= -22.6 C_{remM} - 11.5 C_{maiM} + 0 - 24.5 C_{mucM} \end{aligned}$$

These equations led to $C_{remT} = 4.2$, $C_{remN} = 5.8$, $C_{remM} = 3.0$, $C_{maiM} = 0.2$, $C_{weeN} = 0.7$, and $C_{mucM} = 8.4$ (in g C kg⁻¹ soil).

Table 1. Soil clay content, pH in water, total carbon content Ct, C:N ratio, and total carbon stock in 1988 and 1999 (mean \pm standard deviation when available).

	Depth cm	T		NPK		M	
		1988	1999	1988	1999	1988	1999
Clay g 100 g ⁻¹	0-10	14.7 \pm 0.1	21.6	11.1 \pm 0.6	12.8	12.7 \pm 0.6	13.6
	10-20	nd	33.9	nd	19.8	nd	17.9
pH	0-10	5.6 \pm 0.1	5.1	5.6 \pm 0.1	5.2	5.2 \pm 0.1	5.0
	10-20	5.4 \pm 0.2	4.7	5.4 \pm 0.2	5.0	5.1 \pm 0.2	5.0
Ct g kg ⁻¹	0-10	5.5 \pm 0.2	5.3 \pm 0.1	5.4 \pm 0.1	6.7 \pm 1.8	5.2 \pm 0.1	11.5 \pm 2.0
	10-20	4.6 \pm 0.3	4.0 \pm 0.7	4.8 \pm 0.4	3.8 \pm 1.2	4.8 \pm 0.4	7.3 \pm 0.9
	20-30 ^a		3.5 \pm 0.5		3.6 \pm 1.1		4.4 \pm 0.1
	30-40 ^a	4.1 \pm 0.2	3.2 \pm 0.1	4.0 \pm 0.4	4.1 \pm 0.7	4.6 \pm 0.3	4.2 \pm 0.2
	50-60	nd	2.4 \pm 0.1	nd	3.5 \pm 1.8	nd	3.3 \pm 0.5
C:N	0-10	10.2 \pm 1.0	12.2 \pm 0.4	10.8 \pm 0.5	11.3 \pm 0.1	11.5 \pm 0.5	11.9 \pm 0.8
	10-20	10.9 \pm 1.4	10.1 \pm 0.6	10.7 \pm 1.8	9.9 \pm 0.7	12.0 \pm 1.8	11.6 \pm 0.8
	20-30 ^a		8.7 \pm 0.5		9.3 \pm 1.0		10.0 \pm 1.2
	30-40 ^a	11.4 \pm 1.2	8.2 \pm 0.8	10.6 \pm 1.9	8.8 \pm 1.4	12.8 \pm 1.7	8.9 \pm 1.3
	50-60	nd	7.0 \pm 0.4	nd	8.8 \pm 3.2	nd	8.1 \pm 1.4
Ct stock at sampling date and fixed depth, Mg C ha ⁻¹	0-10	7.7 \pm 0.7	8.4 \pm 0.3	7.3 \pm 0.5	10.6 \pm 3.4	6.8 \pm 0.3	17.4 \pm 3.3
	0-20	13.6 \pm 0.9	14.5 \pm 0.4	14.6 \pm 1.0	17.0 \pm 3.9	13.8 \pm 0.8	28.7 \pm 3.9
	0-40	25.9 \pm 1.5	24.2 \pm 0.5	27.0 \pm 1.8	28.8 \pm 5.7	27.7 \pm 1.7	41.4 \pm 4.9
	0-60	nd	32.0 \pm 0.3	nd	39.7 \pm 3.6	nd	51.7 \pm 4.1
Ct stock in March at fixed mass, Mg C ha ⁻¹	0-10 ^b	7.7 \pm 0.7	8.1 \pm 0.3	7.3 \pm 0.5	9.7 \pm 3.1	6.8 \pm 0.3	15.6 \pm 2.9
	0-20 ^b	13.6 \pm 0.9	13.4 \pm 0.2	14.6 \pm 1.0	16.4 \pm 4.0	13.8 \pm 0.8	27.7 \pm 3.9
	0-40 ^b	25.9 \pm 1.5	23.9 \pm 0.5	27.0 \pm 1.8	29.0 \pm 6.0	27.7 \pm 1.7	42.5 \pm 5.0

nd: not determined

^a 20-40 cm in 1988

^b depth layers in 1988; at equivalent masses, they corresponded in 1999 to depth layers 0-9, 0-17 and 0-36 cm in T, 0-9, 0-18 and 0-37 cm in NPK, and 0-9, 0-18 and 0-39 cm in M

Table 2. Contributions of remaining initial soil carbon (C_{rem}), maize- (C_{mai}), weed- (C_{wee}) and mucuna-originating C (C_{muc}) to soil total carbon content Ct in November 1999 (in g C kg⁻¹ soil).

Depth cm	T				NPK				M			
	C_{rem}	C_{mai}	C_{wee}	C_{muc}	C_{rem}	C_{mai}	C_{wee}	C_{muc}	C_{rem}	C_{mai}	C_{wee}	C_{muc}
0-10	4.2	0.0	1.1	0.0	5.8	0.2	0.7	0.0	3.0	0.2	0.0	8.4
10-20	4.0	0.0	0.0	0.0	3.7	0.1	0.0	0.0	0.5	0.2	0.0	6.7
20-30	3.5	0.0	0.0	0.0	3.4	0.1	0.0	0.0	1.9	0.2	0.0	2.3
30-40	3.2	0.0	0.0	0.0	3.9	0.2	0.0	0.0	3.1	0.2	0.0	0.9
50-60	2.3	0.0	0.0	0.0	3.4	0.1	0.0	0.0	3.2	0.1	0.0	0.0
70-80	2.1	0.0	0.0	0.0	2.0	0.1	0.0	0.0	1.8	0.1	0.0	0.2
90-100	2.2	0.0	0.0	0.0	1.8	0.0	0.0	0.0	1.9	0.0	0.0	0.0

Table 3. Origin of C (remaining initial soil C, maize, weeds, mucuna) and proportion of C from each origin remaining in soil plus litter in November 1999 (soil mass corresponding to initial 0-40 cm layer; in November 1999, its C stock amounted to 23.4, 27.2 and 40.6 Mg C ha⁻¹ in T, NPK and M, respectively).

		T				NPK				M			
		C _{rem}	C _{mai}	C _{wee}	C _{muc}	C _{rem}	C _{mai}	C _{wee}	C _{muc}	C _{rem}	C _{mai}	C _{wee}	C _{muc}
Stock of soil C from each origin	Mg C ha ⁻¹	21.5	0.2	1.7	0.0	25.1	1.0	1.1	0.0	12.0	1.0	0.0	27.5
Stock of litter C from each origin	Mg C ha ⁻¹	-	0.0	0.3	0.0	-	0.0	0.9	0.0	-	0.0	0.0	3.1
Proportion of C from each origin in soil + litter	%	90.5	0.8	8.7	0.0	89.4	3.5	7.1	0.0	27.5	2.4	0.0	70.1
Initial soil C and returned C from 1988 to 1999	Mg C ha ⁻¹	25.9	11.6	28.9	0.0	27.0	57.6	18.1	0.0	27.7	61.2	0.0	54.2
Proportion of initial soil C and returned C remaining in soil + litter	%	82.9	1.6	7.2	-	93.0	1.7	11.0	-	43.5	1.7	-	56.5

Figure 1. Mean annual aboveground and belowground residue C returned to the soil (mean and standard deviation).

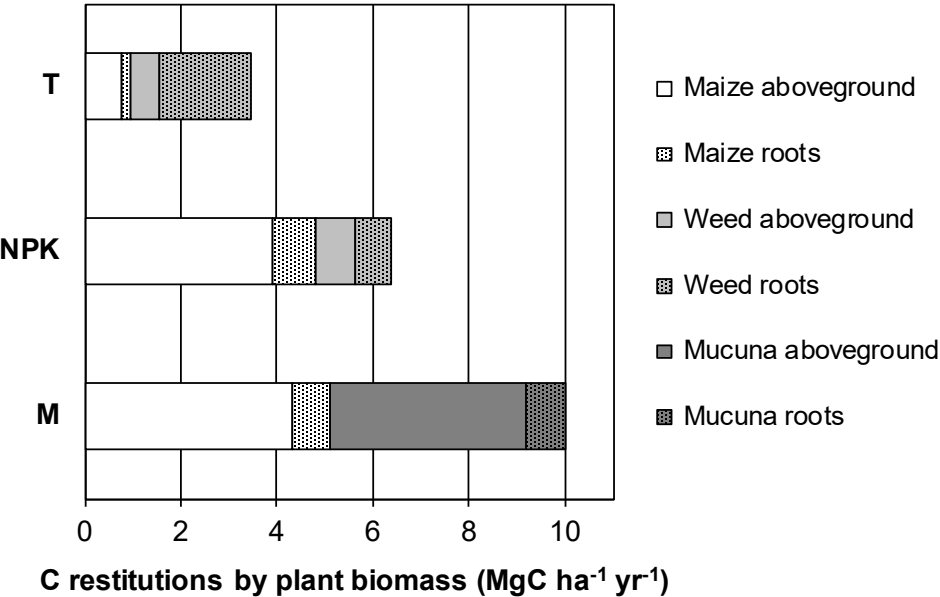


Figure 2. Bulk soil natural ¹³C abundance in November 1999 (mean and standard deviation).

