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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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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 to study changes in soil carbon in a sandy loam Ultisol in Benin, which involved a 12-year experiment on three maize cropping systems under manual tillage: traditional no-input cultivation (T), mineral fertilized cultivation (NPK), and association with *Mucuna pruriens* (M). The origin of soil carbon was also determined through the natural abundance of soil and biomass ^{13}C. In T, NPK and M changes in soil carbon at 0–40 cm were –0.2, +0.2 and +1.3 t C ha^{-1} yr^{-1}, with residue carbon amounting to 3.5, 6.4 and 10.0 t C ha^{-1} yr^{-1}, respectively. After 12 years of experimentation, carbon originating from maize in litter-plus-soil (0–40 cm) represented less than 4% of both total carbon and overall maize residue carbon. In contrast, carbon originating from mucuna in litter-plus-soil represented more than 50% of both total carbon and overall mucuna residue carbon in M, possibly due to accelerated mineralization of native soil carbon (priming effect) and slow mulch decomposition. Carbon originating from weeds in litter-plus-soil represented c. 10% of both total carbon and overall weed residue carbon in T and NPK. Thus mucuna mulch was very effective in promoting carbon sequestration in the soil studied.

Keywords: Soil organic carbon, legume cover crop, mucuna, ^{13}C natural abundance, Benin

INTRODUCTION

Soil organic matter fulfils both the 'fertility' functions required by farmers and the 'environmental' functions related to carbon 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 carbon 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 large amounts of organic inputs and soil organic carbon sequestration at the field level as well as at wider scales (Feller *et al.* 2001).

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In his synthesis of soil fertility in semiarid 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 studies 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 five years, natural fallow is no longer possible in contexts of increasing population, for example, in southern Benin, where the population density is as high as 300–400 km^{-2} (Azontonde 1993). Many authors have underlined the advantage of legume-based cover crops over natural fallow in Africa (isohyet >800 mm) for controlling weeds and erosion, and enriching 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 carbon and on its origin (mucuna, maize, weeds, initial soil organic carbon), which was studied through the measurement of natural abundance of ^{13}C (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 for growing maize, beans, cassava or peanut, 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. There was no treatment replication in this demonstration trial, as it is usually impossible in long-duration trials (Shang & Tiessen 2000), especially when these include runoff plots, as 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 a compound fertilizer NPK 15–15–15, and 100 kg ha^{-1} of urea); and 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 using superficial hoe cultivation by hand (hoeing depth about 5 cm). On the M plot, maize was sown in the mucuna mulch from the previous year. Mucuna was sown 1 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 from 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 (D_b) 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.

Above-ground biomass of maize and mucuna was determined every year from five replicate quadrates (1×1 m) per plot at maize harvest (August) and at the maximum growth of mucuna (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 above-ground biomass determined in 1995, and annual above-ground biomass. Sampling of above-ground biomass of weeds was carried out in November 1999 at 9 locations per plot, using a 0.25×0.25 m quadrate. Litter was simultaneously and similarly sampled. Root sampling was also carried out in November 1999 on 6 monoliths per plot, each of $0.25 \times 0.25 \times 0.30$ m, which were cut into three depth layers (0–10, 10–20 and 20–30 cm), and visible roots were hand sorted. We assumed that roots and litter sampled in T and NPK plots originated from weeds, whereas those sampled in M plot were from mucuna. All plant samples were dried at 70 °C, weighed and finely ground for carbon 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 ^{13}C abundance.

Carbon and nitrogen determination, and other analyses

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

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

$$\delta^{13}C\,(‰) = (((^{13}C/^{12}C_{sample})/(^{13}C/^{12}C_{ref})) - 1) \times 1000 \quad (1)$$

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

$$C_t = C_{rem} + C_{mai} + C_{wee} + C_{muc} \quad (2)$$

$$\delta^{13}C_t \times C_t = (\delta^{13}C_{rem} \times C_{rem}) + (\delta^{13}C_{mai} \times C_{mai}) + (\delta^{13}C_{wee} \times C_{wee}) + (\delta^{13}C_{muc} \times C_{muc}) \quad (3)$$

Table 1. Soil clay content, pH in water, total carbon content (C_t), C:N ratio, and total carbon stock in 1988 and 1999 (mean ± standard deviation where available).

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

[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. For treatment key, see Materials and Methods and Figure 1.
n.d. = not determined.

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

Particle size analysis was 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 soil : solution ratio.

Statistical analyses

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

C_t was determined from 18- and 3-fold replicate samples 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, that is, assuming that it exhibited a normal distribution and provided an unbiased estimation of C_t. Following Dagnélie (1975) and Shang & Tiessen (2000), we calculated that at 95% confidence level, whatever the plot and the depth layer, 3-replicate sampling in 1995 would have led to a less than 5, 8 and 7% relative error in C_t estimation in T, NPK and M plots, respectively. Thus we considered C_t determined in 1999 by 3-replicate sampling as representative of the mean of the plot. Similarly, we considered C_t 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 µm) content increased with depth. At 0–10 cm, clay content greatly increased from 1988 to 1999 in T (around 50%), but not in NPK or M (increase <15%) (Table 1). In 1999, clay content was greater in T at 0–10 cm than in NPK or M at 10–20 cm. The sand (> 50 µm) content was between 60 and 80 $g\,100\,g^{-1}$ to a depth of 20 cm, mainly in the form of coarse sand (>200 µm). 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 C_t ($g\,C\,kg^{-1}$) between plots were small in March 1988 (<6% at 0–10 cm), though sometimes significant (Table 1). From March 1988 to November 1999, C_t 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, C_t 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 C_t differed slightly between plots, but a marked

increase in M and slight changes in T and NPK led to greater final C_t in M, especially at 0–20 cm.

Changes in C_t stock (t 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%, $P<0.01$); greater final C_t stock in M than in T (70%, $P<0.01$), and NPK (45%, $P<0.05$). C_t stock at 0–40 cm finally reached 24, 29 and 41 t C ha^{-1} in T, NPK and M, respectively. Referring to initial soil mass (Ellert & Bettany 1995), and considering seasonal variations in C_t and D_b, as indicated by seasonal sampling carried out in 1995, we also calculated C_t 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 C_t stocks at sampling dates and on a depth basis. Between March 1988 and March 1999, mean (± standard deviation) annual changes in C_t 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) t C ha^{-1} yr^{-1} in T, +0.2 (±0.4) and +0.2 (±0.6) t C ha^{-1} yr^{-1} in NPK, and +1.3 (±0.4) and +1.3 (±0.5) t C ha^{-1} yr^{-1} in M, respectively.

Residue carbon above and below ground returned to the soil

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

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

The $\delta^{13}C$ of maize roots, stems and leaves was –11.3, –11.4 and –11.9‰, respectively, that is, a mean of –11.5‰. The $\delta^{13}C$ of mucuna roots and stems (together), leaves, seeds and husks was –24.4, –24.8, –23.6 and –25.3‰, respectively, that is, a mean of –24.5‰. The $\delta^{13}C$ of weed above-ground biomass varied to a certain extent within plots: for 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, weeds were almost completely absent in M, which was entirely covered by mucuna.

For November 1999, $\delta^{13}C_t$ of bulk soil was maximum in T at 0–10 cm (–21.4‰) and minimum in M at 0–20 cm (c. –24‰) (Figure 2). Variations in $\delta^{13}C_t$ with depth were rather limited in NPK (from –22.5 to –23.1‰) and below 30 cm depth in T (from –22.7 to –22.9‰) and M (from –22.1 to –22.6‰). $\delta^{13}C_t$ 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.

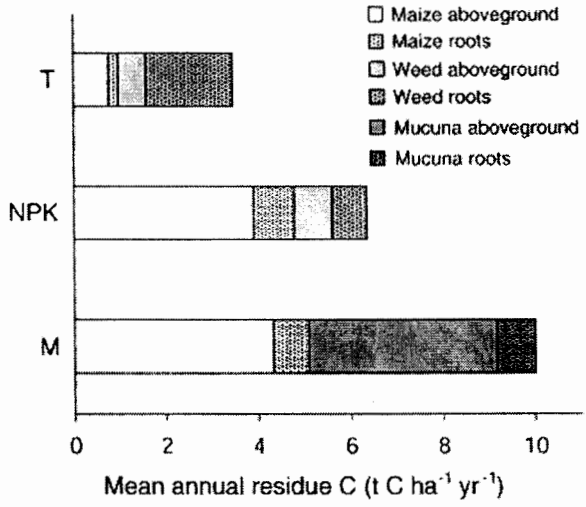

Figure 1. Mean annual above-ground and below-ground residue carbon returned to the soil. T = traditional maize production with no inputs, NPK = T + fertilizer, M= maize in association with *Mucuna pruriens*.

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 by 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 $\delta^{13}C_t$, which were measured, are denoted by 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. Thus:

$$C_{tT} = C_{remT} + C_{maiT} + C_{weeT} + C_{mucT}$$

$$\delta^{13}C_{tT} \times C_{tT} = (\delta^{13}C_{remT} \times C_{remT}) + (\delta^{13}C_{maiT} \times C_{maiT}) + (\delta^{13}C_{weeT} \times C_{weeT}) + (\delta^{13}C_{mucT} \times C_{mucT})$$

$$C_{tN} = C_{remN} + C_{maiN} + C_{weeN} + C_{mucN}$$

$$\delta^{13}C_{tN} \times C_{tN} = (\delta^{13}C_{remN} \times C_{remN}) + (\delta^{13}C_{maiN} \times C_{maiN}) + (\delta^{13}C_{weeN} \times C_{weeN}) + (\delta^{13}C_{mucN} \times C_{mucN})$$

$$C_{tM} = C_{remM} + C_{maiM} + C_{weeM} + C_{mucM}$$

$$\delta^{13}C_{tM} \times C_{tM} = (\delta^{13}C_{remM} \times C_{remM}) + (\delta^{13}C_{maiM} \times C_{maiM}) + (\delta^{13}C_{weeM} \times C_{weeM}) + (\delta^{13}C_{mucM} \times C_{mucM})$$

To reduce the number of variables, the following assumptions were made:

(i) deep soil layers contained C_{rem} only, and $\delta^{13}C_{rem}$ was thus estimated as $\delta^{13}C_t$ of the 90–100 cm soil layer in 1999 (–22.9‰ in T, –23.1‰ in NPK and –22.6‰ in M);

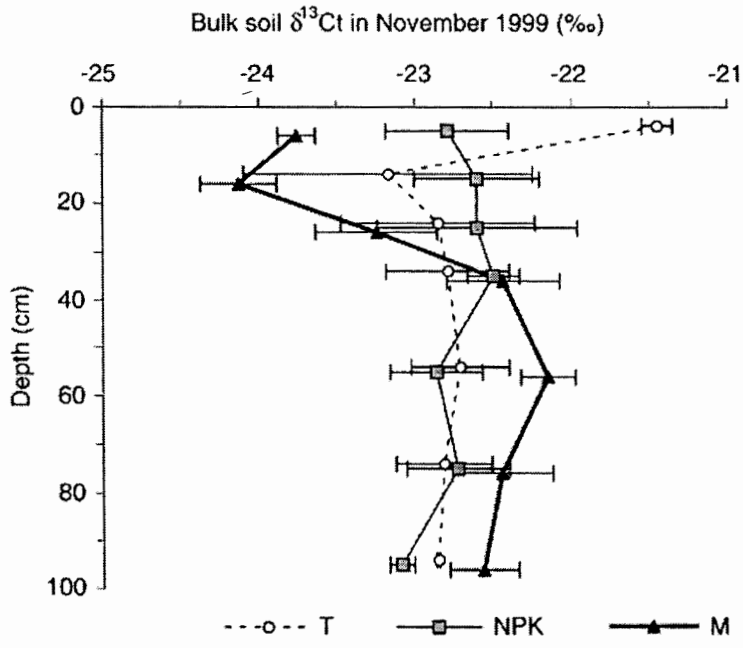

Figure 2. Bulk soil natural ^{13}C abundance in November 1999 (mean and standard deviation). ○, traditional maize production with no inputs (T); ▨, T + mineral fertilizer (NPK); ▲, maize in association with *Mucuna pruriens* (M).

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

(ii) $\delta^{13}C_{mai}$ was the same in all plots, and equal to mean $\delta^{13}C$ calculated for maize (–11.5‰); similarly, $\delta^{13}C_{muc}$ was the same in all plots and equal to mean $\delta^{13}C$ calculated for mucuna (–24.5‰);

(iii) $\delta^{13}C_{wee}$ was equal to weighted mean $\delta^{13}C$ of above-ground biomass of weed samples collected in November 1999 (–16.1‰ in T and –23.1‰ in NPK);

(iv) owing 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 carbon 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 carbon 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 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. By contrast, more than 50% of C_t in M was in the form of C_{muc} to a depth of 30 cm and in the form of C_{rem} below, the C_{rem} contribution being remarkably small at 10–20 cm viz. 7% of C_t. (We checked the effects of uncertainties linked to weed sampling on C_{remM} by changing assumptions (iii) and (vii): changing $\delta^{13}C_{weeT}$, $\delta^{13}C_{weeN}$ and C_{weeT}-to-C_{weeN} ratio always yielded C_{remM} less than 10% of C_{tM} at 10–20 cm.)

Table 3. Origin of carbon (remaining initial soil C, maize, weeds, mucuna) and proportion of carbon from each source remaining in litter-plus-soil in November 1999.[a]

	Units	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	$t\,C\,ha^{-1}$	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	$t\,C\,ha^{-1}$	-	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	$t\,C\,ha^{-1}$	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

[a]Soil mass corresponding to initial 0–40 cm layer; in November 1999 its carbon stock amounted to 23.4, 27.2 and 40.6 $t\,C\,ha^{-1}$ in T, NPK and M, respectively.

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 carbon present in litter and soil, that is the 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, and that litter had the same carbon content as corresponding living plants. In November 1999, carbon 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 litter-plus-soil carbon in T and NPK, respectively, and C_{mai} for less than 4% irrespective of treatment (Table 3). Overall, recent carbon originating from biomass grown during the period of the experiment represented 9, 11 and 72% of litter-plus-soil carbon in T, NPK and M, respectively. (Considering the initial 0–20 cm layer, these proportions were 17, 15 and 83%, respectively.)

The amount of carbon from each origin in litter-plus-soil (initial 0–40 cm mass) in November 1999 was compared with its source, that is, initial C_t stock (1988) or total above- and below-ground residual carbon 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 litter-plus-soil represented 2% of carbon input from maize, irrespective of treatment, and C_{wee} amounted to 7 and 11% of estimated C input from weeds in T and NPK, respectively. By contrast, C_{muc} represented 57% of carbon input from mucuna in M (Table 3). Overall, in November 1999, litter plus recent soil carbon represented 6, 4 and 27% of total residual carbon over the period of the experiment in T, NPK and M, respectively.

DISCUSSION

Changes in soil carbon

At the end of the experiment, C_t stock at 0–40 cm reached 24, 29 and 41 $t\,C\,ha^{-1}$ under unfertilized maize, fertilized maize and maize–mucuna, respectively. This is consistent with C_t stocks measured in similar soil conditions in southern Benin, which amounted to 27, 30 and 48 $t\,C\,ha^{-1}$ at 0–35 cm under palm tree plantation, food crops (with fallow) and forest, respectively (Djegui *et al.* 1992). Our data on changes in C_t stock were also consistent with other published data. In a 3-year experiment on an Alfisol in southwestern Nigeria, rates of 0.2 $t\,C\,ha^{-1}\,yr^{-1}$ at 0–10 cm were recorded under fertilized maize (Lal 2000), the same as in NPK. Under maize–mucuna, we measured a 1.3 $t\,C\,ha^{-1}\,yr^{-1}$ increase in C_t stock, compared with rates of around 1 $t\,C\,ha^{-1}\,yr^{-1}$ at 0–20 cm 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 $t\,C\,ha^{-1}\,yr^{-1}$ 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 greater than 2 t $C\,ha^{-1}\,yr^{-1}$ 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 promotes carbon sequestration in tropical soils, especially in cropping systems that include legume cover crops.

Residue biomass

In M, the high rates of C_t increase were linked with the large residue of mucuna returned to the soil. Mucuna above-ground biomass was 8 $t\,ha^{-1}\,yr^{-1}$ in M versus 6–7 $t\,ha^{-1}\,yr^{-1}$ in 1-year mucuna fallows studied in Nigeria (Vanlauwe *et al.* 2000), and an average of 11 $t\,ha^{-1}\,yr^{-1}$ in mucuna–maize systems in Honduras (> 2000 mm annual rainfall; Triomphe 1996b). The ratio of change in C_t stock to residue carbon measured in our plots also agrees with data in the literature. In 12-year, no-till maize–legume rotations on a sandy clay loam Ultisol in Brazil, the C_t stock increase found at 0–17.5 cm represented 11–15% of above-ground residue carbon (Bayer *et al.* 2001), versus 15% in our plot M (and 5% in NPK). In contrast, in long term, no-till cereal–legume rotations on clayey Oxisols also in Brazil, the increase in C_t stock at 0–40 cm represented 22–25% of total residue carbon (Sá *et al.* 2001), versus 13% in our plot M (and 3% in NPK). This difference underlines the increasing

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

The contribution of weeds to residue biomass was important in T and NPK, owing to the absence of crop during the short rainy season. Weeds represented 49 and 20% of above-ground residue biomass in T and NPK, respectively. They also represented about 50% of above-ground 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 on one date only, and it is likely that this led to some error. Weed biomass was negligible in M: proportions of above-ground residue biomass from maize, mucuna and weeds were 49, 51 and 0%, respectively, versus 49, 42 and 9% in 1-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 (N_2O) emissions, which can be roughly estimated using equation 4 (Bouwman 1996):

$$\text{N-}N_2O \text{ emissions } (\text{kg ha}^{-1}\text{ yr}^{-1}) = 1 + [0.0125 \times \text{N-fertilizer } (\text{kg ha}^{-1}\text{ yr}^{-1})]. \quad (4)$$

In NPK, nitrogen supply by fertilizers was $76\,\text{kg N ha}^{-1}\text{ yr}^{-1}$ (Azontonde *et al.* 1998), which according to equation 4, resulted in emissions of $2\,\text{kg N-}N_2O\,\text{ha}^{-1}\text{ yr}^{-1}$. As the global warming potential of N_2O is about 300 times that of CO_2 (IPCC 2001), these N_2O emissions were equivalent to more than $0.2\,\text{t C-}CO_2\,\text{ha}^{-1}\text{ yr}^{-1}$ emissions, thus offsetting C_t increase ($0.2\,\text{t C ha}^{-1}\text{ yr}^{-1}$). In M, mucuna residues supplied the soil with more than $250\,\text{kg N ha}^{-1}\text{ yr}^{-1}$ (Azontonde *et al.* 1998). In this case, equation 4 led to an overestimation of N_2O emissions because it had been derived from a set of experiments that excluded legume cover crops, which provide nitrogen in a less directly available form than mineral fertilizers. Following equation 4, nitrogen supply by mucuna residues could result in emission of $4\,\text{kg N-}N_2O\,\text{ha}^{-1}\text{ yr}^{-1}$, equivalent to $0.5\,\text{t C-}CO_2\,\text{ha}^{-1}\text{ yr}^{-1}$ emissions (vs. $1.3\,\text{t C ha}^{-1}\text{ yr}^{-1}$ as C_t increase). Though overestimated, these data suggest that from an environmental viewpoint the C_t increase in soils under legume cover crops could be partly offset by N_2O emissions.

Origin of soil carbon

Our results indicate that at the end of the experimental period, recent carbon represented a small proportion of litter-plus-soil carbon in T and NPK (c. 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 carbon represented the main contribution to final amounts of litter-plus-soil carbon. 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 carbon represented a great proportion of litter-plus-soil carbon in M (c. 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 decomposed slowly. Carbon originating from mucuna was mainly limited to the top 30 cm of the soil profile, due to its rather superficial rooting, as confirmed by root counts (Carsky *et al.* 2001; B. Barthès, unpublished data). The low proportion of initial carbon remaining in the topsoil of M is questionable. It may be explained by a priming effect consecutive to the enhancement of biological activity resulting from the huge N-rich residues of 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 carbon origin from measurements of ^{13}C natural abundance. In sandy and clayey Brazilian Oxisols, the proportion of recent carbon 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 from maize plots in temperate areas (Balesdent *et al.* 1987; Clapp *et al.* 2000), the proportion of recent carbon at 0–20 cm ranged from 15 to 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, that is, 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 can be much greater: recent carbon represented c. 60% of C_t at 0–30 cm after 50 years cultivation of sugarcane on 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 carbon to C_t (c. 70% at 0–40 cm), especially considering that N-rich mucuna residues strongly promoted mineralization of native soil carbon.

Our results also show that recent carbon in litter-plus-soil (initial 0–40 cm mass) represented 6, 4 and 27% of total residue carbon 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 for maize plots more than 10 years old in temperate areas, the proportion of recent soil carbon (0–30 or 0–40 cm) to total residue carbon ranged from 12 to 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, the proportion tends to increase with greater residue biomass, possibly due to a priming effect (Kuzyakov *et al.* 2000) of the clay content due to its physical protection of organic matter in stable aggregates (Feller & Beare 1997), and with a decrease in the intensity of tillage, since tillage promotes carbon 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 the small amount of residue and low clay content.

CONCLUSION

In the sandy loam Ultisol studied here, maize–mucuna relay-cropping was very effective in promoting soil carbon sequestration (1.3 t 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 carbon, either positive when the crop was supplied with fertilizer (+0.2 t C ha^{-1} yr^{-1}) or negative without fertilizer (–0.2 t C ha^{-1} yr^{-1}). However, rough estimates revealed that from a global change standpoint, N_2O emissions resulting from nitrogen supply by mucuna could partly offset carbon sequestration in soil. In cropping systems that include legume cover crops, N_2O fluxes need further investigation in order to establish greenhouse gas balances.

Measurements of ^{13}C natural abundance showed that at the end of the experiment, irrespective of treatment, carbon originating from maize in litter-plus-soil represented a small proportion (<4%) of both litter-plus-soil carbon and total maize residue carbon returned to the soil over the period of the experiment. In contrast, under maize–mucuna, mucuna-derived carbon represented a great proportion (>50%) of both litter-plus-soil and mucuna residue carbon. It is likely that mulching of nitrogen-rich mucuna residues promoted accelerated mineralization of native soil organic matter, the amount of which decreased dramatically during the experimental period, whereas the mineralization of mulch carbon remained slow. The proportion of carbon originating from weeds in litter-plus-soil represented about 10% of the total under fertilized and non-fertilized pure maize. Overall, under pure maize and maize–mucuna, recent carbon accounted for about 10 and 70% of litter-plus-soil carbon, and represented about 5 and 27% of total residue carbon, respectively. However, due to weed sampling at one date only, these results are uncertain, and further research should include more systematic weed sampling.

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ANNEX 1

Determination of the contribution of remaining initial soil carbon (C_{rem}), maize-originating carbon (C_{mai}), weed-originating carbon (C_{wee}) and mucuna-originating carbon (C_{muc}) to total soil carbon (C_t) at 0–10 cm in T, NPK (denoted by 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:

$$C_{tT} = 5.3 = C_{remT} + C_{maiT} + C_{weeT} + C_{mucT} = C_{remT} + 0.18\,C_{maiM} + 1.60\,C_{weeN} + 0$$

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

In NPK:

$$C_{tN} = 6.7 = C_{remN} + C_{maiN} + C_{weeN} + C_{mucN} = C_{remN} + 0.93\,C_{maiM} + C_{weeN} + 0$$

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

In M:

$$C_{tM} = 11.5 = C_{remM} + C_{maiM} + C_{weeM} + C_{mucM} = C_{remM} + C_{maiM} + 0 + C_{mucM}$$

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

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).