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

B. Barthès 1,*, A. Azontonde 2, E. Blanchart 1, C. Girardin 3, C. Villenave 4, S. Lesaint 1, R. Oliver 1 & C. Feller 1

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 13C. 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, 13C natural abundance, Benin

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 (Vocíkner 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 plot 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 cultivations (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 (Blesdent et al. 1987).

**MATERIALS AND METHODS**

**Description of the site and treatments**

The trials were conducted from 1988 to 1999 at Agonkanmey (8°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. DMK) 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.

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**Above-ground biomass of maize and mucuna**

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 1993, 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 × 0.30 m. Litter was simultaneously and similarly sampled. Root sampling was also carried out in November 1999 on 6 raonoliths per plot, each of 0.25 × 0.25 × 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 Kjeldahl 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 606).

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:

$$\delta^{13}C(\%) = \left(\frac{^{13}C_{Sample}}{^{12}C_{Ref}}\right)/\left(\frac{^{13}C_{Ref}}{^{12}C_{Ref}}\right) - 1 \times 1000 \quad (1)$$

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 G and $^{13}$C balances, respectively (Mariotti 1991):

$$C_t = C_{rem} + C_{max} + C_{weed} + C_{muc} \quad (2)$$

$$\delta^{13}C_t = \delta^{13}C_{rem} + \delta^{13}C_{max} + \delta^{13}C_{weed} + \delta^{13}C_{muc} \quad (3)$$
Table I. Soil clay content, pH in water, total carbon content (C\textsubscript{t}), C:N ratio, and total carbon stock in 1988 and 1999 (mean ± standard deviation where available).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>T</th>
<th>NPK</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay (g 100 g\textsuperscript{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>14.7 ± 0.1</td>
<td>21.6</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td>10-20</td>
<td>n.d.</td>
<td>33.9</td>
<td>n.d.</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>5.6 ± 0.1</td>
<td>5.1</td>
<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>10-20</td>
<td>5.4 ± 0.2</td>
<td>4.7</td>
<td>5.4 ± 0.2</td>
</tr>
<tr>
<td>C\textsubscript{t} (g kg\textsuperscript{-1})</td>
<td></td>
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<tr>
<td>0-10</td>
<td>5.5 ± 0.2</td>
<td>5.3 ± 0.1</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>10-20</td>
<td>4.6 ± 0.3</td>
<td>4.0 ± 0.7</td>
<td>4.8 ± 0.4</td>
</tr>
<tr>
<td>20-30\textsuperscript{a}</td>
<td>4.1 ± 0.2</td>
<td>3.5 ± 0.5</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>30-40\textsuperscript{a}</td>
<td>3.2 ± 0.1</td>
<td>n.d.</td>
<td>3.2 ± 0.1</td>
</tr>
<tr>
<td>50-60</td>
<td>n.d.</td>
<td>2.4 ± 0.1</td>
<td>n.d.</td>
</tr>
<tr>
<td>C:N ratio</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0-10</td>
<td>10.2 ± 1.0</td>
<td>12.2 ± 0.4</td>
<td>10.8 ± 0.5</td>
</tr>
<tr>
<td>10-20</td>
<td>10.9 ± 1.4</td>
<td>10.1 ± 0.6</td>
<td>10.7 ± 1.5</td>
</tr>
<tr>
<td>20-30\textsuperscript{a}</td>
<td>11.4 ± 1.2</td>
<td>8.7 ± 0.5</td>
<td>10.6 ± 1.9</td>
</tr>
<tr>
<td>30-40\textsuperscript{a}</td>
<td>8.2 ± 0.8</td>
<td>8.8 ± 1.4</td>
<td>8.5 ± 1.4</td>
</tr>
<tr>
<td>50-60</td>
<td>n.d.</td>
<td>7.0 ± 0.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>C\textsubscript{t} stock at sampling (t C ha\textsuperscript{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10</td>
<td>7.7 ± 0.7</td>
<td>8.4 ± 0.3</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>10-20</td>
<td>13.6 ± 0.9</td>
<td>14.5 ± 0.4</td>
<td>14.6 ± 1.0</td>
</tr>
<tr>
<td>20-40\textsuperscript{a}</td>
<td>25.9 ± 1.5</td>
<td>24.2 ± 0.5</td>
<td>27.0 ± 1.8</td>
</tr>
<tr>
<td>50-60</td>
<td>n.d.</td>
<td>32.0 ± 0.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>C\textsubscript{t} stock in March at fixed mass (t C ha\textsuperscript{-1})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10\textsuperscript{b}</td>
<td>7.7 ± 0.7</td>
<td>8.1 ± 0.3</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>10-20\textsuperscript{b}</td>
<td>13.6 ± 0.9</td>
<td>13.4 ± 0.2</td>
<td>14.6 ± 1.0</td>
</tr>
<tr>
<td>20-40\textsuperscript{b}</td>
<td>25.9 ± 1.5</td>
<td>23.9 ± 0.5</td>
<td>27.0 ± 1.8</td>
</tr>
<tr>
<td>0-10</td>
<td>n.d.</td>
<td>2.4 ± 0.1</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*20-40 cm in 1988; \textsuperscript{a}depth layers in 1988; \textsuperscript{b}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.

\( n.d. = \text{not determined.} \)

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 I). 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\textsuperscript{-1} to a depth of 20 cm, mainly in the form of coarse sand (>200 μm). 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 \( \delta^{13}C \) (‰) between plots or between years were tested by Student unpaired \( t \)-tests; no assumptions were made on normality and variance equality (Dagnelie 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 Dagnelie (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.

where \( C_{rem} \), \( C_{maiz} \), \( C_{wee} \) 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.

\( C_t \) stock at sampling date and fixed depth: 0-10 cm in 1988; \textsuperscript{a}depth layers in 1988; \textsuperscript{b}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.

\( n.d. = \text{not determined.} \)
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 I): 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 I). 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 \(-0.0 (\pm 0.1) \) and \(-0.2 (\pm 0.1)\) t C ha\(^{-1}\) yr\(^{-1}\) in T, \(+0.2 (\pm 0.4) \) and \(+0.2 (\pm 0.6)\) t C ha\(^{-1}\) yr\(^{-1}\) in NPK, and \(+1.3 (\pm 0.5) \) and \(+1.3 (\pm 0.5)\) 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 C ha\(^{-1}\) yr\(^{-1}\) (dry matter); it represented 25, 64 and 10.4 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 \(^{13}\)C of maize roots, stems and leaves was \(-11.3, -11.4\) and \(-11.9\)‰, respectively, that is, a mean of \(-11.5\)‰. The \(^{13}\)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 \(^{13}\)C of weed above-ground biomass varied to a certain extent within plots: for weeds (17% as above-ground biomass and 55% 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).

\[ \text{Origin of soil carbon} \]

At each depth layer, we determined the proportion of \( C_t \) in the form of \( C_{\text{rem}} \) (remaining initial soil \( C_t \)), \( C_{\text{mai}} \) (maize-derived \( C_t \)), \( C_{\text{wee}} \) (weed-derived \( C_t \)) and \( C_{\text{muc}} \) (mucuna-derived \( C_t \)) 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 \( C_{\text{rem}} \), which were measured, are denoted by \( C_{\text{rem}} \) and \( \delta^{13} C_{\text{rem}} \) in T, \( C_{\text{rem}} \) and \( \delta^{13} C_{\text{rem}} \) in NPK, and \( C_{\text{rem}} \) and \( \delta^{13} C_{\text{rem}} \) in M. Thus:

\[ C_{\text{T}} = C_{\text{rem}} + C_{\text{mai}} + C_{\text{wee}} + C_{\text{muc}} \]

\[ \delta^{13} C_{\text{T}} = \delta^{13} C_{\text{rem}} + \delta^{13} C_{\text{mai}} + \delta^{13} C_{\text{wee}} + \delta^{13} C_{\text{muc}} \]

\[ C_{\text{N}} = C_{\text{rem}} + C_{\text{mai}} + C_{\text{wee}} + C_{\text{muc}} \]

\[ \delta^{13} C_{\text{N}} = \delta^{13} C_{\text{rem}} + \delta^{13} C_{\text{mai}} + \delta^{13} C_{\text{wee}} + \delta^{13} C_{\text{muc}} \]

\[ C_{\text{M}} = C_{\text{rem}} + C_{\text{mai}} + C_{\text{wee}} + C_{\text{muc}} \]

\[ \delta^{13} C_{\text{M}} = \delta^{13} C_{\text{rem}} + \delta^{13} C_{\text{mai}} + \delta^{13} C_{\text{wee}} + \delta^{13} C_{\text{muc}} \]

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

(i) deep soil layers contained \( C_{\text{rem}} \) only, and \( \delta^{13} C_{\text{rem}} \) was thus estimated as \( \delta^{13} C \) of the 90–100 cm soil layer in 1999 (<22.9‰ in T, -23.1‰ in NPK and -22.6‰ in M);
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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 ($C_r$) in November 1999 (g C kg$^{-1}$ soil).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>T</th>
<th>NPK</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$C_{rem}$</td>
<td>$C_{mai}$</td>
<td>$C_{wee}$</td>
</tr>
<tr>
<td>0-10</td>
<td>4.2</td>
<td>0.0</td>
<td>1.1</td>
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<tr>
<td>10-20</td>
<td>4.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>20-30</td>
<td>3.5</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>30-40</td>
<td>3.2</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>40-50</td>
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<td>0.0</td>
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<td>50-60</td>
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<td>60-70</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

(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 ($C_{maiT} = 0.18C_{maiN}$ and $C_{maiN} = 0.93C_{maiM}$);

(vii) $C_{wee}$ was proportional to weed biomass carbon measured in November 1999 ($C_{weeT} = 1.60C_{weeN}$).

Using these assumptions reduced the number of variables to six ($C_{remT}$, $C_{remN}$, $C_{remM}$, $C_{maiT}$, $C_{maiN}$, $C_{mucM}$) 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 $C_r$ in T and NPK, except at 0-10 cm where $C_{wee}$ represented 21% and 10% of $C_r$, respectively. By contrast, more than 50% of $C_r$ 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 $C_r$. (We checked the effects of uncertainties linked to weed sampling on $C_{rem}$ 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 $C_{muc}$ 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.*

<table>
<thead>
<tr>
<th>Units</th>
<th>T</th>
<th>NPK</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C_{init}</td>
<td>C_{maize}</td>
<td>C_{weeds}</td>
</tr>
<tr>
<td>Stock of soil C from each origin tCha⁻¹</td>
<td>21.5</td>
<td>0.2</td>
<td>1.7</td>
</tr>
<tr>
<td>Stock of litter C from each origin tCha⁻¹</td>
<td>-</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Proportion of C from each origin %</td>
<td>90.5</td>
<td>0.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Initial soil C and returned C tCha⁻¹</td>
<td>25.9</td>
<td>11.6</td>
<td>28.9</td>
</tr>
<tr>
<td>Proportion of initial soil C and %</td>
<td>82.9</td>
<td>1.6</td>
<td>7.2</td>
</tr>
</tbody>
</table>

*Soil mass corresponding to initial 0-40 cm layer; in November 1999 its carbon stock amounted to 25.4, 27.2 and 40.6 tCha⁻¹ 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 tCha⁻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 tCha⁻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 3-year experiment on an Alfisol in southwestern Nigeria, rates of 0.2 tCha⁻¹yr⁻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 tCha⁻¹yr⁻¹ increase in Cₜ stock, compared with rates of around 1 tCha⁻¹yr⁻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 tCha⁻¹yr⁻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 Cha⁻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 Cₜ increase were linked with the large residue of mucuna returned to the soil. Mucuna above-ground biomass was 8 t ha⁻¹yr⁻¹ in M versus 6-7 t ha⁻¹yr⁻¹ in T and NPK, and an average of 11 t ha⁻¹yr⁻¹ in mucuna-maize systems in Honduras (>2000 mm annual rainfall; Triomphe 1996b). The ratio of change in Cₜ 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 Cₜ 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 Cₜ 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\textsubscript{2}O) emissions, which can be roughly estimated using equation 4 (Bouwman 1996): \[ N\textsubscript{2}O \text{ emissions (kg ha}\textsuperscript{-1} yr\textsuperscript{-1}) = 1 + [0.0125 \times \text{N-fertilizer (kg ha}\textsuperscript{-1} yr\textsuperscript{-1})]. \] (4)

In NPK, nitrogen supply by fertilizers was 7.6 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} (Azontonde et al. 1998), which according to equation 4, resulted in emissions of 2 kg N-N\textsubscript{2}O ha\textsuperscript{-1} yr\textsuperscript{-1}. As the global warming potential of N\textsubscript{2}O is about 300 times that of CO\textsubscript{2} (IPCC 2001), these N\textsubscript{2}O emissions were equivalent to more than 0.2 t C-C\textsubscript{0}2 ha\textsuperscript{-1} yr\textsuperscript{-1} emissions, thus offsetting 4% of CO\textsubscript{2} emissions. In M, mucuna residues supplied the soil with more than 250 kg N ha\textsuperscript{-1} yr\textsuperscript{-1} (Azontonde et al. 1998). In this case, equation 4 led to an overestimation of N\textsubscript{2}O 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 kg N-N\textsubscript{2}O ha\textsuperscript{-1} yr\textsuperscript{-1} emissions, equivalent to 0.5 t C-C\textsubscript{0}2 ha\textsuperscript{-1} yr\textsuperscript{-1} as C\textsuperscript{r} increase. Though overestimated, these data suggest that from an environmental viewpoint the C\textsuperscript{r} increase in soils under legume cover crops could be partly offset by N\textsubscript{2}O 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 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 \textsuperscript{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 of weeds, and 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\textsuperscript{r} 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\textsuperscript{r} 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 retention similarly resulted in the substantial contribution of recent carbon to C\textsuperscript{r} (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 26% 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. 2000).
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. In cropping systems that include mucuna, the tropical savannahs have great potential for carbon sequestration. In cropping systems that include mucuna, 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, N2O emissions resulting from nitrogen supply by mucuna could partly offset carbon sequestration in soil. In cropping systems that include legumes, N₂O fluxes need further investigation in order to establish greenhouse gas balances.

Measurements of ¹³C 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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Determination of the contribution of remaining initial soil carbon (Crem), maize-originating carbon (Cmaiz), weed-originating carbon (Cwee) and mucuna-originating carbon (Cmuc) to total soil carbon (Ct) 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 Ct and δ13C measured in November 1999 (C and δ13C are denoted Ct and δ13Ct in T, CtN and δ13CtN in NPK, and CtM and δ13CtM in M, respectively).

In T:

\[ C_{tT} = 5.3 = C_{\text{remT}} + C_{\text{maizT}} + C_{\text{weeT}} + C_{\text{mucT}} = C_{\text{remT}} + 0.18 C_{\text{maizT}} + 1.60 C_{\text{weeT}} + 0 \]

\[ \delta^{13}C_{tT} \times C_{tT} = -21.4 \times 5.3 = -115.4 \]

\[ \delta^{13}C_{\text{remT}} \times C_{\text{remT}} + \delta^{13}C_{\text{maizT}} \times C_{\text{maizT}} + \delta^{13}C_{\text{weeT}} \times C_{\text{weeT}} + \delta^{13}C_{\text{mucT}} \times C_{\text{mucT}} \]

\[ = -22.9 C_{\text{remT}} + (-11.5 \times 0.18 C_{\text{maizT}}) \]

\[ + (-16.1 \times 1.60 C_{\text{weeT}}) + 0 \]

\[ = -22.9 C_{\text{remT}} - 2.1 C_{\text{maizT}} - 25.8 C_{\text{weeT}} \]

In NPK:

\[ C_{tN} = 6.7 = C_{\text{remN}} + C_{\text{maizN}} + C_{\text{weeN}} + C_{\text{mucN}} = C_{\text{remN}} + 0.93 C_{\text{maizN}} + C_{\text{weeN}} + 0 \]

\[ \delta^{13}C_{tN} \times C_{tN} = -22.8 \times 6.7 = -152.8 \]

\[ \delta^{13}C_{\text{remN}} \times C_{\text{remN}} + \delta^{13}C_{\text{maizN}} \times C_{\text{maizN}} + \delta^{13}C_{\text{weeN}} \times C_{\text{weeN}} + \delta^{13}C_{\text{mucN}} \times C_{\text{mucN}} \]

\[ = -23.1 C_{\text{remN}} + (-11.5 \times 0.93 C_{\text{maizN}}) \]

\[ - 23.1 C_{\text{weeN}} + 0 \]

\[ = -23.1 C_{\text{remN}} - 10.7 C_{\text{maizN}} - 23.1 C_{\text{weeN}} \]

In M:

\[ C_{tM} = 11.5 = C_{\text{remM}} + C_{\text{maizM}} + C_{\text{weeM}} + C_{\text{mucM}} = C_{\text{remM}} + 0.2 C_{\text{maizM}} + 0.7 C_{\text{weeM}} + 8.4 (\text{in g C kg}^{-1} \text{ soil}) \]

These equations led to CremT = 4.2, CremN = 5.8, CremM = 3.0, CmaizM = 0.2, CweeN = 0.7, and CmucM = 8.4 (in g C kg^{-1} soil).