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CARBON SEQUESTRATION RESEARCH IN BRAZILIAN SAVANNAH SYSTEMS: PROBLEMS AND RESULTS

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Abstract. The natural abundance of ^{13}C is a good tool to study soil organic matter dynamics in systems where the photosynthetic path of the dominant vegetation is changed. Still, considerable uncertainty remains concerning isotope discrimination during humification of C4-litter, the representativity of sites in relation to spatial variation, and procedures of humus fractionation. In the Brazilian savannah, the *cerrado*, humus dynamics is relatively fast and amounts of 30-40% of the native humus can be replaced within decades. It is not clear, though, whether the fractions that have not been replaced within that time span have a significantly different dynamics. The fact that a significant portion of organic matter in such soils is not extractable by the regular procedures, suggests that its turnover time may be an order of magnitude slower than that of the easily replaced fractions addressed in this paper. Any students investigating the effects of land use change on soil organic matter should be aware that equilibrium situations cannot be reached in decades or even centuries, and that the predictive value of such studies is necessarily limited, even if the correct procedures are used.

Resumen. La abundancia natural de ^{13}C es una buena herramienta para estudiar la dinámica de la materia orgánica en sistemas en los que la ruta fotosintética de la vegetación dominante ha cambiado. Sin embargo, hay grandes incertidumbres respecto a la discriminación isotópica durante la humificación de la hojarasca de plantas C4, la representatividad de los sitios respecto a las variaciones espaciales y los procedimientos de fraccionamiento del humus. En el *cerrado* brasileño, la dinámica del humus es relativamente rápida y cantidades del orden del 30-40% del humus nativo pueden ser reemplazados en décadas, pero no está claro, cuando las fracciones que no han sido reemplazadas tienen una dinámica diferente. El hecho de que una porción significativa de la materia orgánica no es extraíble por los procedimientos usuales sugiere que el tiempo de reciclaje puede ser un orden de magnitud más lento que el de las fácilmente reemplazables. Por ello, los estudios sobre los efectos del cambio de uso sobre la materia orgánica del suelo deben tener en cuenta que la situación de equilibrio no se alcanza en décadas ni en siglos, por lo que su valor predictivo es necesariamente limitado incluso cuando se utilizan procedimientos correctos

INTRODUCTION

The Brazilian *cerrado* is a savannah ecosystem that comprises dense forests, open forests with grassy undergrowth, and open grassland with scattered trees. It is one of the major biomes of Brazil. During the last half century, the cerrado has been increasingly used for grazing and cultivation. This has raised concern as to the environmental impact, especially with regard to carbon storage and sustainability, of such land use changes. Much research is carried out with regard to the impact of agricultural practices on soil structure and soil organic matter storage (Abdala, 1993; Corazza et al., 1999; Resck et al.,1999; Freitas et al., 2000).

On the other hand, forested lands along river basins are being lost to water basins. Construction of such basins has led to the loss of considerable areas of riparian forest (Davide & Botelho, 1999). Locally, tree mixtures mimicking the composition of riparian forests have been planted close to the shores of artificial water reservoirs.

Both the conversion of cerrado forest to grassland or agricultural land, and the conversion of grassy savannah to riparian forest involve a change in photosynthetic pathway of the predominant vegetation. Forests have a C3-dominated vegetation, while tropical grasslands and some crops (e.g. maize) are dominated by C4-species. This allows the investigation of carbon dynamics of such systems with the aid of the natural abundance of the stable carbon isotope ^{13}C. Soil organic matter is largely formed from the remains of the vegetation standing on and rooting in the soil. Soil organic matter therefore reflects the chemical composition of the vegetation input. This is especially true of the isotopic signal.

Plants make their tissue from CO_2 that they take up from the air. The carbon in the air consists mainly of the 'light' isotope ^{12}C, but about 1% consists of the heavy stable isotope ^{13}C, and an even smaller part of the radioactive isotope ^{14}C. Plants prefer the lighter isotopes over the heavy ones, so that the relative amounts of ^{13}C (and ^{14}C) in the plant are slightly lower than those in the atmosphere. C4 plants discriminate less against heavy isotopes (and therefore have higher relative contents) than C3 plants. Because the ^{13}C content of the atmosphere is not stable over time, ^{13}C contents in plants or humus are not expressed with respect to that of the atmosphere, but with respect to a standard geological sample, the PeeDee belemnite, or its proxy the Vienna PeeDee Standard. The difference with the reference sample is expressed as the δ^{13}C of a given sample, as follows:

$$\delta^{13}C\ (‰) = 1000 \times (^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard}) / {}^{13}C/^{12}C_{standard}$$

The δ^{13}C value for the atmosphere is approximately -8 ‰; values for C3 plants range from -32 to -22 ‰ (mean value of -27) and for C4 plants from -16 to -9 ‰ (mean value of -13 ‰).

In humus that consists of a mixture of C3- and C4-derived litter, the relative amount of each contribution can be calculated by a simple linear mixing model (Balesdent and Mariotti, 1996):

$\delta^{13}C_T = F_{C3} \times \delta^{13}C_{C3} + F_{C4} \times \delta^{13}C_{C4}$, in which:

$\delta^{13}C_T = \delta^{13}C$ of the total sample,

F_{C3} and F_{C4} = fractions of C3- and C4-derived humus ($F_{C3} + F_{C4} = 1$)

$\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ = the mean δ^{13}C of C3- and C4-derived humus, respectively.

There are various problems attached to the indiscriminate use of this method for calculating changes in humus contributions upon change of land use. First, under C3-vegetation, a relative increase in ^{13}C content is noted when fresh litter is converted to humus; it is not known whether this increase also occurs in C4-humus. Second, calculations are sometimes carried out for separate organic matter fractions while fractionation procedures lack sufficient standardisation. Third, because observations over time are virtually absent, changes are always calculated with respect to a reference plot. These three factors need some scrutiny to understand the value of replacement calculations. The three items will be illustrated with data from samples collected at the Maize and Sorghum National Research Centre (EMBRAPA-CPMS), Sete Lagoas, Minas Gerais, Brazil (Roscoe, 2002).

^{13}C ENRICHMENT UPON HUMIFICATION OF LITTER

Under native C3 vegetation, the humus is always slightly enriched with ^{13}C with respect to the litter. Roscoe et al. (2000a) noted the following differences under native cerrado forest: Both the higher values and the lower variability of the soil humus are clearly illustrated by Table 1.

TABLE 1. Mean $\delta^{13}C$ values and standard deviations of litter and topsoil humus of five 100 m^2 plots under native forest.

Vegetation	$\delta^{13}C$ – litter	$\delta^{13}C$ – topsoil
111	-27.12 (0.13)	-23.87 (0.02)
112	-28.52 (0.21)	-23.72 (0.09)
113	-25.86 (0.17)	-23.65 (0.08)
114	-26.91 (0.12)	-23.60 (0.17)
115	-27.03 (0.23)	-23.64 (0.11)

Such changes in ^{13}C contents have not been properly documented for native C4-systems, such as tropical grasslands. Most authors suppose that the increase for C4 litter is similar to that for C3 litter, but this point of view can be criticised. To predict the possible effect for C4-derived litter and humus, the reason of the increase should be understood. Various mechanisms have been used to explain the increase in ^{13}C content in soil humus. The first possibility is a preferential decay of compounds that have relatively low ^{13}C contents (isotopic discrimination). This mechanism cannot explain the increase, because the relatively stable organic components (aliphatics, aromatics) tend to have lower ^{13}C contents than easily decomposable ones nitrogen compounds, polysaccharides. Another explanation is the relative depletion over time of the atmosphere with respect to ^{13}C, due to burning of fossil fuels (Suess effect). The Suess effect supposes that the humus represents a longer stretch of time than the litter, and may therefore largely reflect the higher ^{13}C contents in the atmosphere of the past. If at all, the Suess effect should influence C3 plants, which discriminate more strongly against the heavy isotope, less than C4 plants, the heavy isotope contents of which are closer to those of the atmosphere. In systems with rapid soil organic matter dynamics, such as a tropical savannah, the Suess effect should be negligible because both litter and soil organic matter are in equilibrium with the recent atmospheric isotopic composition. A third explanation is the admixture of relatively ^{13}C-rich microbial-derived organic matter to the soil humus. Most microbes do not discriminate against heavy isotopes. Microbes build their biomass from soil CO_2, which contains both CO_2 due to decay of soil organic matter, and atmospheric CO_2. Soil CO_2, therefore, has a higher ^{13}C content than plant-derived soil organic matter, and admixture of microbial biomass may increase the ^{13}C content of the total soil organic matter. This increase, however, is smaller for C4-plant derived humus, because the isotopic composition of microbial biomass is closer to that of C4 litter than to that of C3 litter. This means that the increase in $\delta^{13}C$ between C4-litter and its humus should be lower than in C3-litter. To establish whether this is true, native grassland systems still need to be studied. Assuming equal increase for C3 and C4 litter probably underestimates replacements calculated for C3→C4 systems.

HUMUS FRACTIONATION AND ISOTOPIC SIGNAL

Because soil organic matter fractions such as density or grain-size fractions are increasingly used as proxies for carbon pools in dynamic models, replacement studies are sometimes carried out for such fractions separately. Apart from the fact that the sum of the separated fractions does not always equal the total organic matter of the soil, the fractionation procedure itself may induce uncertainties in the results.

To separate grain-size or density-fractions, soil aggregates need to be destroyed, after which particle size fractions are separated by sieving or settling velocity, and density fractions are obtained by using heavy liquids. Destruction of aggregates is usually carried out by sonication, but in the past, few authors specified, or standardised their sonication procedures.

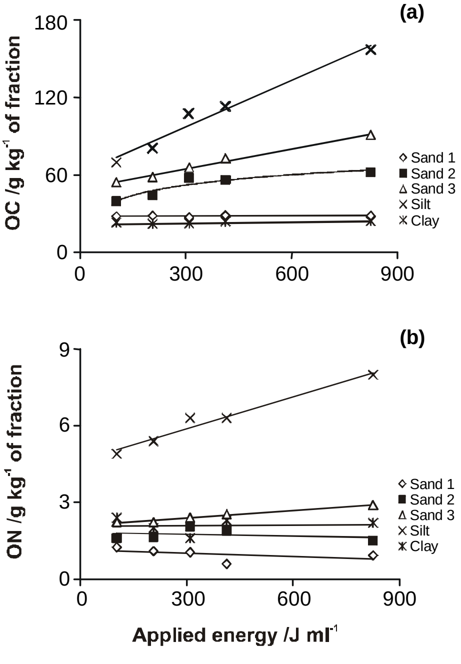

FIGURE 1. Effect of applied ultrasonic energy on carbon (a) and nitrogen (b) in particle-size fractions of a Brazilian Oxisol (>80% clay fraction). From Roscoe et al., (2000b).

Figure 1 indicates the effect of applied sonic energy to carbon and nitrogen encountered in a number of grain-size fractions. As shown by this figure, the amount of carbon and nitrogen encountered in fine fractions strongly increases with applied ultrasonic energy. The curves suggest that destruction of aggregates is not complete, even at high energies. From Figure 1 it is clear that for comparing soils, experimental conditions have to specified in detail, and that comparison of different soils may be extremely difficult.

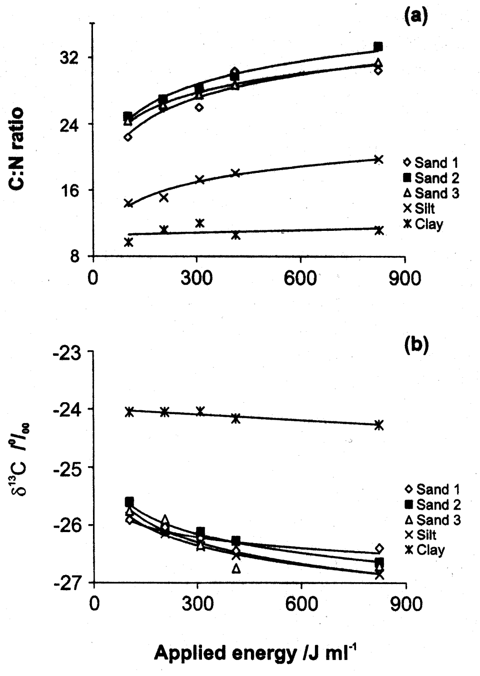

FIGURE 2. Changes in C/N ratio and δ^{13}C in the grain-size fractions of Figure 1. From Roscoe et al. (2000 b).

It is worse, if the isotopic signal also changes with the applied ultrasonic energy, as shown in Figure 2. At first sight, it seems illogical that the δ^{13}C of all grain-size fractions would decrease with increasing energy, but one has to keep in mind that the graph does not show the amount and composition of 'free' organic matter such as roots and leaf remnants. In the present case, the 'free' fraction contains a considerable amount of C3-derived charcoal, which has a low ^{13}C content. Sonication destroys litter fragments and charcoal, and transfers it to increasingly fine fractions. The figure shows that in such situations, the amount of energy applied to destroy aggregates will influence isotope-based replacement calculations.

REPRESENTATIVITY OF REFERENCE AND EXPERIMENTAL SITES

In paired plots, changes in the experimental plot are calculated with reference to an 'unchanged' or 'original' plot, which should represent the state of the experimental plot before any induced changes. Few authors take any effort to determine the representativity of their reference plots. They assume that fields have a homogeneous isotopic distribution, and most replacement calculations are based on one to one comparison of experimental and reference plots or profile pits. To investigate the validity of such an approach, Roscoe (2002) investigated the spatial variability of carbon contents and isotopic signal of a native cerrado forest with locally different incidence of fire (Figure 3).

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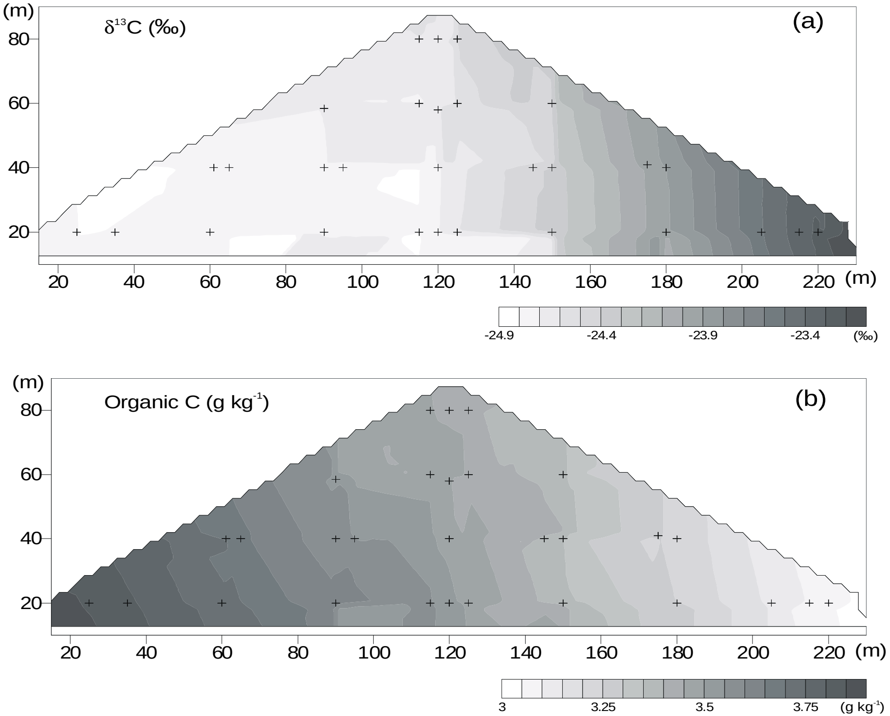

FIGURE 3. Contour maps of topsoil δ^{13}C (a) and C-content (b) in a native cerrado forest. Interpolation between observations by conventional kriging. Crosses indicate measurements. *From Roscoe (2002).*

In Figure 3, the right-hand part of the forest plot had a higher fire incidence - and consequently more grassy (C4) undergrowth than the left-hand part. The gassy part had a lower topsoil organic carbon content and a higher $\delta^{13}C$ than the pure forest. The difference in $\delta^{13}C$ was almost 2 ‰ over a distance of 200 metres, and the maximum difference in carbon content was close to 0.9 %.

For comparison, a nearby plot on the same soil type that had been cultivated for 30 years with a rotation of maize (C4) and beans (C3), with conventional tillage and no-tillage, was also sampled geostatistically. Figure 4 shows the carbon contents and Figure 5 the $\delta^{13}C$ distribution pattern of this plot. Both figures show interpolation by conventional kriging (top) and by within-strata kriging (bottom). The latter emphasises the differences between the two tillage systems.

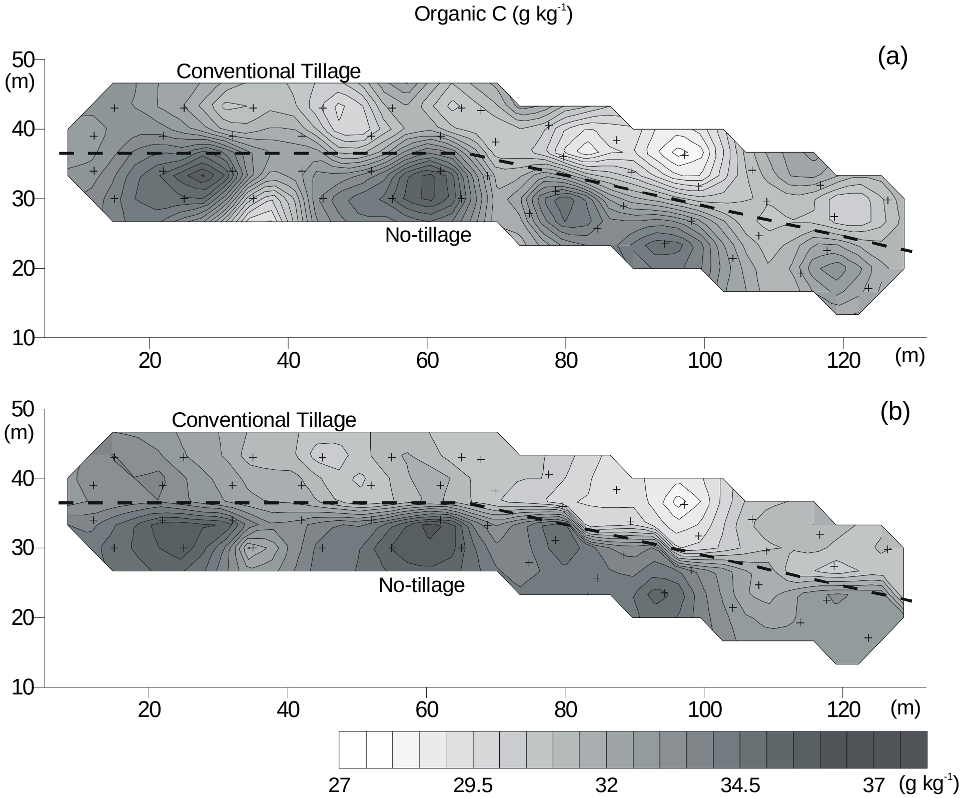

FIGURE 4. Contour maps of organic C in the topsoil, predicted by (a) ordinary kriging, and (b) within-strata kriging of a plot cultivated for 30 years with a maize-beans rotation. Crosses indicate measurements. The last ten years, the top of the field has been under conventional tillage, the bottom under no-tillage. *From Roscoe (2002)*

Figure 4 shows that the carbon contents do not show a specific distribution, although contents under no tillage are slightly higher than under conventional tillage. The absence of clear within-field trends of carbon distribution is in agreement with relatively fast carbon dynamics and a homogenous agricultural practice. Within each type of cultivation, measured carbon contents vary about 0.5%. Taking into account the irregular distribution of roots in maize and bean fields, this is a very small variation.

The distribution of isotopic signal (Figure 5), however, tells a different story.

There is virtually no difference between the two cultural practices, but a very clear trend over the length of the field. Because the cultural practice cannot have induced such a trend, we have to conclude that it reflects a trend in the natural vegetation, similar to that found in the native forest, that was present *before* the plot was cultivated. As in the native forest plot of Figure 3, the maximum variation in δ^{13}C is about 2 ‰. For calculations of carbon replacement, it is tempting to compare the right-hand side of the cultivated plot with the right-hand side of the forest plot, and vice versa, but there is no guarantee that such a selection of reference and trial sites would be correct. Obviously, comparing a random site from the cultivated plot with another random site from the native forest will not give meaningful results. Both gross underestimates and overestimates of carbon replacements could results from such a procedure. Nevertheless, most published carbon replacement studies are based on comparison of one trial site with one reference site, without landscape context

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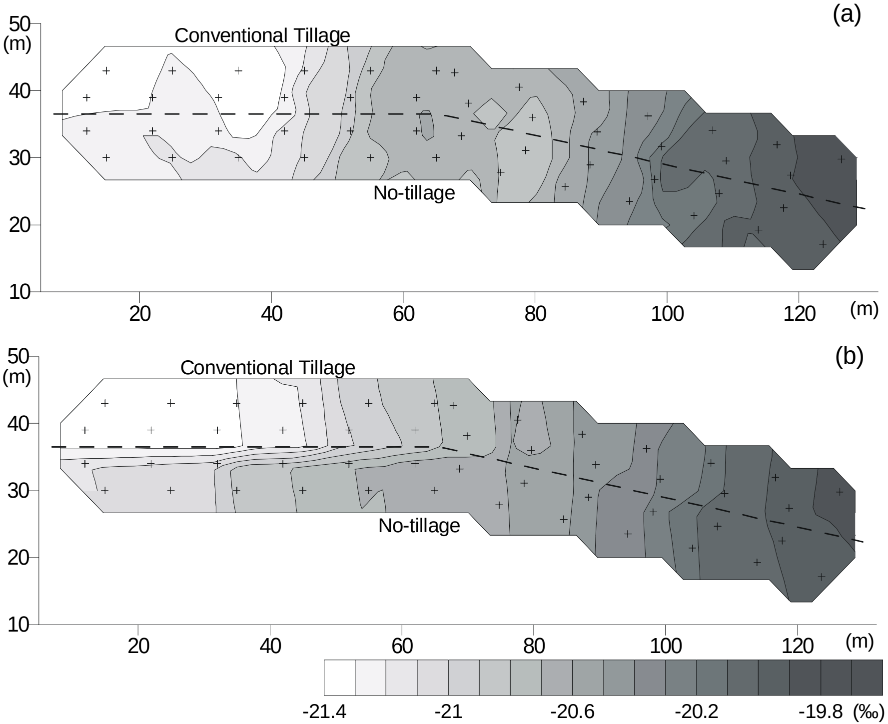

FIGURE 5. Contour maps of δ^{13}C predicted by (a) ordinary kriging, and (b) within-strata kriging of a plot cultivated during 30 years with a maize-beans rotation. Crosses indicate measurements. *From Roscoe et al, (2002)*.

RESULTS OF CARBON REPLACEMENT STUDIES

A number of carbon replacement studies in were carried out in the cerrado system. For a cerrado → permanent grassland conversion, this was done on grain-size fractions under controlled conditions of ultrasonic aggregate destruction. Spatial variation of isotopic composition may have played a role, but this was not accounted for in the experiment. Consequently, the actual amounts of calculated replaced carbon may be somewhat different, but the trends should be valid. For a grassy cerrado → riparian forest conversion, the calculations were was based on bulk samples and grain-size fractions. Spatial variation of isotopic signal was not investigated.

Cerrado → grassland conversion

A field that had been converted to permanent pasture 23 years before sampling was compared with the forested cerrado plot (Roscoe et al., 2001). Trends of carbon contents and isotopic signal with depth are illustrated in Figure 6. Because the forest produces much of its litter *on* the soil, carbon concentrations in the top few centimetres of the soil are considerably higher than just below. The forest soil shows a significant decrease in $\delta^{13}C$ in the top few centimetres, which is due to the admixture of relatively undecomposed litter (see discussion above). Under grassland, the $\delta^{13}C$ is higher throughout the profile. The depth of the increased isotopic signal reflects the importance of deep root litter from the (*Brachyaria*) grass vegetation.

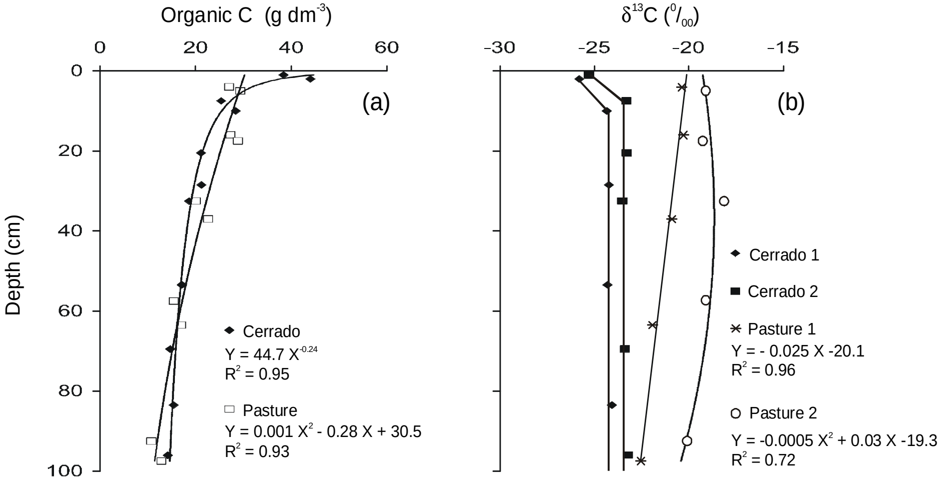

FIGURE 6. Organic C contents (a) and $\delta^{13}C$ (b) as a function of depth under cerrado forest and 23-year old Brachyaria pasture.

From the same soils, the following grain-size fractions were separated, quantified, and their isotopic signal was measured: sand (50-2000 μm), silt (2-50 μm), clay (<2 μm), free light organic matter (not in aggregates), free occluded organic matter (in aggregates). From the isotopic signal, the replacement of cerrado (forest) humus by grassland humus was calculated (Table 2). The experiments were carried out in triplicate, but the table reports the mean values alone.

TABLE 2. Relative replacement (%) of cerrado-C by grassland-C in grain-size and free fractions of a 23-year old Brachyaria pasture. Occl. and Free refer to occluded and free (non mineral bound) organic fractions. From Roscoe et al., 2001.

Horizon	Depth	Clay	Silt	Sand	Occl.	Free	Bulksoil
Ap	0-10	34	38	32	19	51	36
AB2	10-40	35	36	31	5	24	34
Bw2	40-110	19	19	19	nd	20	19

Table 2 indicates that in the topsoil of the grassland, about 35% of all carbon is derived from the grassland, while the other 65 % is still due to the former forest vegetation. Replacement below 40 cm depth is significantly lower, probably due to fewer grass roots. The free carbon fraction of the topsoil consists for about 50% of grassland litter. This seems low, but the fraction contains some charcoal, which is stable over a long period of time. The fact that the 'occluded' fraction shows less input of grass-derived organic matter clearly illustrates that this fraction is at least partially isolated (protected) from recent humus contributions. A bulk replacement of some 35% in 23 years cannot be extrapolated to the future, because more recalcitrant fractions may take much longer to replace. The similar replacement in the three grain-size fractions suggests absence of special protection in any of these fractions.

Cerrado → arable land conversion

For the maize-beans rotational system, replacement calculations were based both on total soil and on density fractions. Using a potassium iodide solution of density 1.7 kg.dm^{-3}, three fractions were separated: free light fraction (FLF), occluded light fraction (OLF) and heavy fraction. The heavy fraction is bound to mineral particles. Figure 7 gives the relative contribution of C4-humus in the bulk soil, and the absolute amount of this contribution. Both the relative replacement by- and the absolute amount of- C4-derived organic matter is roughly the same in conventional tillage and no-tillage. The trend with depth is statistically significant under no-tillage alone.

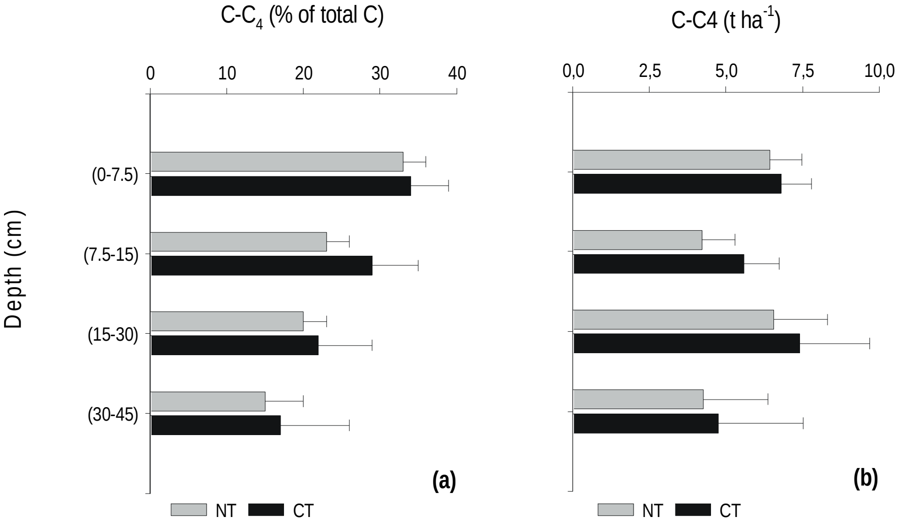

FIGURE 7. Proportions (a) and stock (b) of maize-derived carbon (C4-C) as a function of depth in the maize-beans rotation plots, after thirty years of cultivation. NT = no tillage; CT = conventional tillage. *From Roscoe & Buurman (2003)*

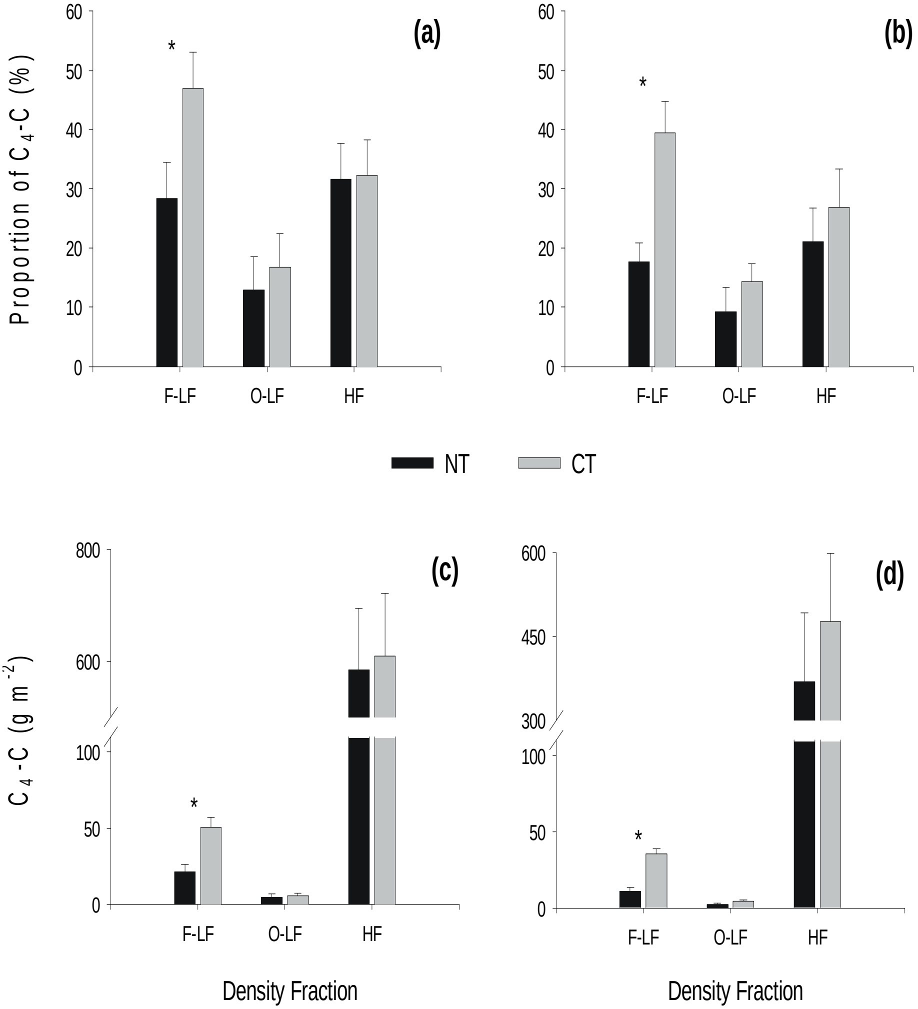

FIGURE 8**.** Proportions of C4-derived C (a,b) and absolute amounts of C4-derived C (c,d) in fractions of organic matter in a 30-year old maize-beans rotation. FLF = free light fraction; OLF = occluded light fraction; HF = heavy fraction. Depth is 0-7.5 cm for a and c, and 7.5-15 cm for b and d. Vertical bars represent standard error. Asterisks above the bars indicate significant (5% probability) differences between NT (no tillage) and CT (conventional tillage). *From Roscoe & Buurman(2003).*

The beans-maize rotation produces both C3 (beans) and C4 (maize) litter. The calculations are based on the C4 input alone, and may therefore not give a complete picture of the changes in the system. Figure 8 gives the relative proportions and absolute amounts for two depths of C4-derived soil organic matter in each of the fractions. The figures 6c and 6d indicate that the largest absolute amount of C-4 derived organic matter is present in the heavy fraction. This is not surprising, because the clay fraction of the soil (>80%) carries most of the organic matter. Only in the free light fraction (FLF), conventional tillage has significantly more C4-carbon, both in proportion and in amount, than no-tillage. The proportion of C4-carbon in the heavy fraction is slightly over 30% in the top horizon (Fig. 8a) and between 20 and 30% in the second horizon (Fig. 8b). These values are close to the replacements found after 23 years of pasture (see above). Although the reason can only be guessed at, it is possible that maize contributes the bulk of the subsurface litter due to the two crops, and that therefore the replacement is dominated by maize-derived material.

Both the grassland system and the maize-beans rotation point to a relatively rapid replacement of 30-35% of the organic matter in the topsoil. Neither in the cerrado → grassland, not in the cerrado → cropland conversion a significant loss of total carbon stocks was found (Roscoe, 2002).

Grassy cerrado → riparian forest conversion

Planting a selection of forest species in the grassy cerrado on the banks of large water reservoirs constitutes a change opposite to the conversion of forested cerrado to agricultural land. In addition to reconstituting native riparian vegetation, such initiatives were thought to have a positive effect on carbon sequestration. The research was done on 10-year old planted riparian forest in the Itutinga/Camargos reservoir, Minas Gerais, Brazil (de Alcantara, 2002). Carbon budgets were based on four sampling lines, each consisting of ten composite samples, both in the cerrado and in the planted forest. Composite samples were necessary because of the large number of tree species and the spatial variability of litter deposition and litter quality in the forest. $\delta^{13}C$ was used to calculate relative replacement of grassland (C4) carbon by forest (C3) carbon.

Litter production in grassy and tree-dominated vegetation is very different. Measured above-ground litter production in the two systems is given in Table 3

TABLE 3**.** Litter production and litter properties in grassy cerrado and 10-year old planted riparian forest. *From de Alcântara (2002)*

Area	Dry matter kg/ha	C %	N %	C/N	C addition kg/ha/yr	N addition kg/ha/yr
Cerrado	352	41	0.65	63	145	2.3
Forest	699	42	1.19	35	298	8.4

Reading Table 3, one has to take into account that in grassland, most of the litter production is underground. The real litter contribution of grassy cerrado is therefore underestimated. Table 3 indicates that the forest contributes twice as much *surface* litter as the grassland, and that this litter has almost the double nitrogen content. In contrast to forests of temperate areas, the C/N ratio of the riparian forest is very low due to the large proportion of leguminous trees. Surface litter addition under forest appears therefore to be both larger in amount and of better quality than under grass.

Using soil bulk density measurements and carbon contents of the horizons 0-2.5, 2.5-5, 5-10, and 10-20 cm depth, total stocks of carbon under grassy cerrado and planted riparian forest were calculated (Table 4).

TABLE 4. Carbon stocks in the upper 20 cm of the soil under cerrado and under riparian forest, 10 years after planting. *From de Alcântara (2002).*

	C-stock (g.m-^{2})	N-stock (g.m^{-2})
Forest	5520	380
Cerrado	5980	440
Loss upon reforestation	460	60

Instead of a gain in organic carbon, table 4 suggests a decrease in both carbon an nitrogen stock. This conclusion, however, needs some qualification. The litter layer has not been accounted for. As the previous table shows, the difference in the litter layer would compensate about 10% of the calculated losses. Forest litter is predominantly accumulated *on* the soil instead of *in* the soil. It requires biological activity to reach downward, and this is a slower process than the accumulation and decomposition to humus of grass roots. Furthermore, a forest needs a long time for full growth, and an even longer time to reach equilibrium with the organic matter in the soil. The figures, therefore, do not indicate that carbon and nitrogen sequestration is necessarily lower under forest.

The ^{13}C values under the planted forest give an idea of the amount of carbon replacement effected in ten years (Table 5). The standard deviations of the calculated losses are not given in the table, but they indicate that the losses may not be significant. Table 5 indicates significant replacement of C4 by C3 carbon within 10 years. It is not surprising that the highest replacement (65%) was found very close to the surface. There was no difference in the contributing fresh organic matter to the layers between 2.5 and 20 cm depth. Chemical analysis of the extractable humified fraction by pyrolysis-GC/MS, however, indicated a more gradual decrease with depth of the C3-derived litter than suggested by the replacement data of Table 5 (de Alcântara, 2002). The most obvious chemical change due to the addition of the forest litter was a strong increase in lignin-derived moieties.

TABLE 5. Relative contribution of C3 (new) carbon at various depths, and losses of C4-carbon under a 10-year old planted riparian forest. *From de Alcântar (2002).*

Depth	C3-carbon	C4-carbon	C4-carbon loss	Carbon balance
cm	%		g.dm^{-3}	
0-2.5	65	12.85	11.21	+1.03
2.5-5	43	13.47	8.34	-3.16
5-10	47	13.46	7.70	-5.80
10-20	43	13.80	4.50	-2.87

CONCLUSIONS

The natural abundance of ^{13}C is a good tool to study soil organic matter dynamics in systems where the photosynthetic path of the dominant vegetation is changed. Still, considerable uncertainty remains concerning isotope discrimination during humification of C4-litter, the representativity of sites in relation to spatial variation, and procedures of humus fractionation.

In the Brazilian savannah, the *cerrado*, humus dynamics is relatively fast and amounts of 30-40% of the native humus can be replaced within decades. It is not clear, though, whether the fractions that have not been replaced within that time span have a significantly different dynamics. The fact that a significant portion of organic matter in such soils is not extractable by the regular procedures, suggests that its turnover time may be an order of magnitude slower than that of the easily replaced fractions addressed in this paper. Any students investigating the effects of land use change on soil organic matter should be aware that equilibrium situations cannot be reached in decades or even centuries, and that the predictive value of such studies is necessarily limited, even if the correct procedures are used.

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