PROPOSITIONS

1. Although carbon turnover is relatively rapid in clayey Cerrado soils, stocks of soil organic matter are rather stable and reclamation for agriculture has little impact on carbon emissions.
   (*This thesis*)

2. A better understanding of the processes responsible for ^{13}C discrimination during decomposition is essential to clarify assumptions embedded in linear mixing models used to estimate soil organic matter dynamics based on ^{13}C natural abundance.
   (*This thesis*)

3. If the whole area of Cerrado in Brazil (2 million km^2) were completely burned at once, the CO_2 released to the atmosphere would roughly equal the mean annual emission due to fossil fuel burning worldwide in the last decade (6.3 ± 0.6 Gt C yr^{-1}). The difference is that the Cerrado would start regrowing in the next rainy season.

4. Researchers, policy makers, and world leaders should be aware that overemphasizing carbon sequestration may offset the Kyoto Protocol, instead of Climate Change.

5. Weak assumptions often become unproven "truths" by repeated citations in scientific publications.

6. "Life was thus an almost utterly improbable event with almost infinite opportunities of happening" (about the origin of life in *Lovelock, J.E. 1979. Gaia: a new look at life on Earth. Oxford University press, Oxford*).

7. Living in the Netherlands provides Brazilians an opportunity to learn a lot about themselves.

8. War against terrorism cannot alleviate hunger and anger.

Propositions belonging to the Doctoral Thesis entitled
"Soil organic matter dynamics in a Cerrado Oxisol"
By Renato Roscoe.

Wageningen, January 23, 2002

Soil organic matter dynamics in a Cerrado Oxisol

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Soil organic matter dynamics in a Cerrado Oxisol

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Contents

Chapter 1

General introduction

The Brazilian territory is divided basically into six large biomes: Amazon Forest, Cerrado, Atlantic Forest, Meridional Forests and Grasslands, Pantanal, and Caatinga (Figure 1.1). The Cerrado Biome (see Appendix 1) spreads over 2 million square kilometres or 23% of the country. In terms of area, it is second only to the Amazon Forest. Most of the Cerrado is concentrated in a "core area" located in the central part of the country, but some significant fragments are found north of the Amazon Forest. Its central position brings the Cerrado Biome in contact with the other five biomes.

Besides its importance as the second major biome in the country, in the last three decades the Cerrado region has increasingly contributed to the national agricultural, pastoral and forestry production (Macedo, 1995; Resck et al. 1999). Being an almost empty region in the 1960s, its population increased rapidly, and reached more than 21 million inhabitants in 1992 (Pereira et al., 1997). In 1998, from a total of 136 million ha potentially suitable for agriculture (Macedo, 1995), 40 million ha had been converted into pasture and about 15 million ha into annual crops and forestry (Resck, 1998). The land use systems are characterised by large farm units, monocultures, heavy mechanisation, and high external inputs (Cadavid-Garcia, 1995; Klink et al., 1995). The major crops are soybeans (for export) and maize, rice, and beans (internal market). Most of the planted area is under conventional tillage. In 1995, the Cerrado region was responsible for about 25% of the national agricultural production and sheltered 40% of the cattle flock (Macedo, 1995).

The relatively fast occupation, and especially the strategy of rural development (based on strongly mechanised and intensive agriculture, large properties, high external inputs, and crop export) has raised important questions about the sustainability of the agro-ecosystems in the region (Resende et al., 1996). Losses of indigenous and local knowledge, impact on biodiversity, water and air pollution, soil degradation, erosion, and siltation of rivers and lakes are among the impacts reported in literature (Macedo, 1995; Resende et al., 1996; Resck, 1998; Resck et al., 1999).

Cerrado soils are dominated by low-activity clays (kaolinite, gibbsite and iron oxihydroxides) (Adámoli et al., 1985; Reatto et al., 1998; Resck et al., 1999). In such environments, soil organic matter (SOM) is particularly important to chemical, biochemical, and physical-chemical processes related to nutrient cycling, biological activity, aggregation, and water availability (Resck, 1998; Resck et al., 1999; Resende et al., 1996). Inappropriate technologies associated with rapid occupation may dramatically decrease SOM contents (Silva et al., 1994). This has consequences, not only for soil quality, but also for the global carbon cycle. Maintaining or increasing SOM levels is crucial for a sustainable use of these ecosystems. Besides, such a strategy may also be important for preventing carbon emission, or even promoting sequestration of atmospheric CO_2 (IPCC, 2000).

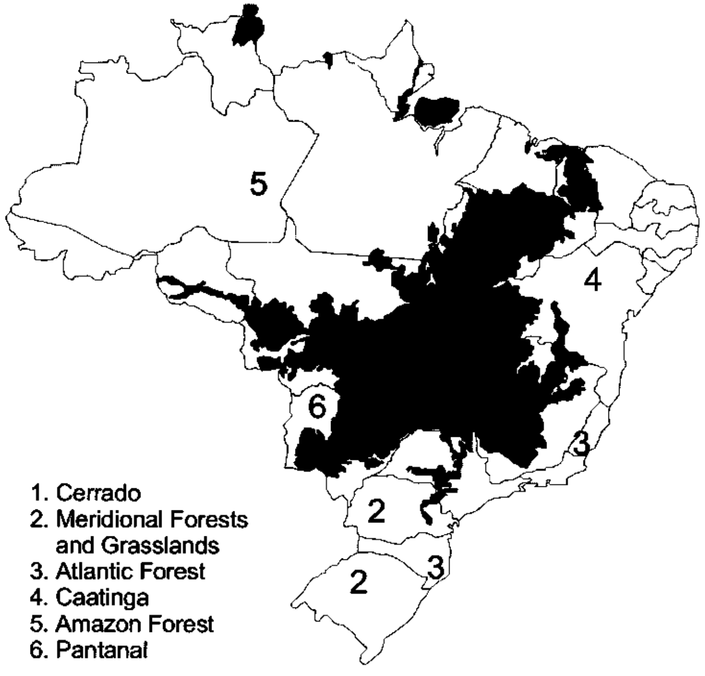

Figure 1.1. Geographical distribution of the principal biomes in Brazil. Grey area represents the Cerrado Biome.

1.1. Carbon Stocks in Cerrado

Inorganic and organic forms of carbon are continuously exchanged between the atmosphere, oceans and terrestrial biosphere. The stock of carbon as CO_2 in the atmosphere (760 Gt C) is about 50 times lower than in the oceans (39,000 Gt C) and three and a half times lower than in the terrestrial biosphere (2,500 Gt C) (IPCC, 2000). In the period between 1850-1998, the total anthropogenic emissions of CO_2 was about 405 ± 60 Gt C. Two thirds of this total was emitted as a result of fossil fuel burning and cement production. The remaining one third is attributed to emission due to land use and land-use changes, predominantly in forest ecosystems (IPCC, 2000). During the last decade (1989-1998), the annual emission of CO_2 due to fossil fuel burning and cement production was 6.3 ± 0.6 Gt C yr^{-1}, and that due to land-use change 1.6 ± 0.8 Gt C yr^{-1}. In the same period, 3.3 ± 0.2 Gt C yr^{-1} was stored in the atmosphere, and 2.3 ± 0.8 Gt C yr^{-1} was taken up by the oceans. The final balance results in a residual terrestrial uptake of 2.3 ± 1.3 Gt C yr^{-1}, known as the "missing carbon sink" (IPCC, 2000). According to Schlesinger (1997) the current emission of CO_2 from fossil fuel burning and cement production would be entirely taken up by the oceans if the oceans were well mixed. However, only surface waters are in a relatively fast equilibrium with atmospheric CO_2. While the turnover time of dissolved C in these superficial waters is about 11 years, the turnover time in the whole ocean is about 350 years as a result of the slow mixing of deep waters (Schlesinger, 1997). For this reason, in the last decade, only about 29% of the annual CO_2 emission (considering fossil fuel, cement and use-land change) was taken up by the oceans (IPCC, 2000). Another 42 % was stored in the atmosphere, and 29 % was taken up by terrestrial ecosystems. If all anthropogenic CO_2 emission were halted, the ocean uptake would re-equilibrate the CO_2 concentration of the atmosphere to values close to the pre-industrial period in a couple of hundred years (Schlesinger, 1997). However, according to the IPCC's IS92a scenario[1] ("business-as-usual"), anthropogenic emissions will reach 20.3 Gt C yr^{-1} by 2100 (IPCC, 1995). Within that time, the absorption of carbon by the oceans will also increase, but in a slow rate. According to Cox et al. (2000), the annual increments in ocean uptake will decrease continuously in the next century, tending to an asymptotic value of 5.0 Gt C yr^{-1} by 2100. Therefore, the unique alternatives to reduce CO_2 concentration in the atmosphere will be either to reduce emissions (substitution of the energetic matrix and decrease in deforestation), or to promote carbon sequestration in the terrestrial ecosystems.

The International Panel on Climate Change recently summarized data of carbon stocks in the major terrestrial ecosystems (IPCC, 2000) (Table 1.1). Tropical savannas occupy about 15% of the total terrestrial area and store about 13% of the total carbon. With an area of about 2.0 million square kilometres (Ribeiro & Walter, 1998) the Cerrado Biome represents about nine percent of the total area of tropical savannas in the world. Considering average values of C stocks for savannas (Table 1.1), Cerrado's stocks would be about 5.9 Gt C and 23.8 Gt C in vegetation and soil, respectively. This would represent a stock of about 29 t ha^{-1} of carbon for the vegetation and 117 t ha^{-1} for the soils. However, the variation between savanna types is very large, as can be inferred by the diversity of vegetation types in the Cerrado (Table A1.1-Appendix 1). Estimates for the total carbon stocks in this biome were not made so far, although some information is available for specific physiognomic forms.

Table 1.1. Global carbon stocks in vegetation and top 1m of soils (IPCC, 2000; based on WBGU, 1998).

Biome	Area (10^6 km^2)	Carbon Stocks (Gt C) Vegetation	Soil	Total
Tropical forests	17.6	212	216	428
Temperate forests	10.4	59	100	159
Boreal forests	13.7	88	471	559
Tropical savannas	22.5	66	264	330
Temperate grasslands	12.5	9	295	304
Deserts and semideserts	45.5	8	191	199
Tundra	9.5	6	121	127
Wetlands	3.5	15	225	240
Croplands	16.0	3	128	131
Total	151.2	466	2011	2477

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[1] IPCC = Intergovernmental Panel on Climate Change. In its 1994 report (IPCC, 1995), IPCC evaluated several scenarios of CO_2 emission for the XXI century. Th IS92a scenario, known as "bisiness-as-usual", assumed population of 11.3 billion inhabitants by 2100 and economic growth of 2.3% from 1990-2100.

Abdala (1993) estimated carbon stocks in different compartments of a *cerrado sensu stricto* from Central Brazil. The total carbon stock in the vegetation plus soil (1 m depth) was 265 t ha^{-1}, divided into arboreal (28.5 t ha^{-1}) and herbaceous (4 t ha^{-1}) strata, litter (5 t ha^{-1}), roots and detritus (42.5 t ha^{-1}), and soil organic matter (185.0 t ha^{-1}). Abdala's results emphasise the importance of the soil for C storage in savanna systems. According to IPCC (2000), 80% of the total carbon in tropical savannas is stored in the soil (Table 1.1).

Table 1.2. Estimates of carbon stocks in soils under different vegetation types and management systems in the Cerrado Biome.

Vegetation Type[a] and Land-Use	Soil Type[c]	Clay (g kg^{-1})	Organic C (t ha^{-1})[d]	Reference
All types	LV	< 150	75	Tognon et al. (1998)
All types	LV	150-250	128	"
All types	LV	250-350	172	"
All types	LV	350-600	188	"
All types	LV	>600	188	"
All types	LE	< 150	87	"
All types	LE	150-250	109	"
All types	LE	250-350	172	"
All types	LE	350-600	180	"
All types	LE	>600	182	"
Cerradão	LE	707	143	Brossard et al. (1997)
Cerrado sensu stricto	LE	707	148	"
Campo limpo	LE	616	208	"
Campo sujo	LV	540	190	"
Mata ciliar	LA	634	165	"
Grazed *cerrado sensu stricto*	LE	689	174	"
Pasture	LE	532	161	"
Cerrado sensu stricto	LE	490	154	Corazza et al. (1999)
Eucalyptus (12 years)[b]	LE	720	170	"
Cultivated pasture (18 years)[b]	LE	590	173	"
Crops, no-tillage (15 years)[b]	LE	410	148	"
Crops, conventional tillage (15 years)[b]	LE	410	178	"
Cerrado *sensu stricto*	LE	880	150	Roscoe et al. (2000c)
Pasture (23 year)[b]	LE	880	170	"

[a] for a description of the vegetation see Table A1-1, Appendix 1;

[b] the previous vegetation was cerrado *sensu stricto*.

[c] LE – Dark Red Latosol; LV – Red Yellow Latosol; LA – Yellow Latosol (Table A1.2);

[d] stock for one metre depth. Roscoe et al (2000c) used elemental analyser for organic C determinations. Tognon et al. (1998) used wet oxidation (EMBRAPA, 1979) and their data was corrected by a factor of 1.5, as suggested by Appendix F in EMBRAPA (1999). Brossard et al (1997) and Corazza et al. (1999) also used wet oxidation (Nelson & Sommers, 1982), but their data was corrected by a factor of 1.15, according to Brossard et al. (1997).

According to available data, carbon stocks in the soil organic matter may vary form 75 to 208 t ha^{-1} for different vegetations and soils of the Cerrado Biome (Table 1.2). This range encompasses the average value of 117 t ha^{-1} estimated for the savanna ecosystems (IPCC, 2000). Organic C content tends to increase with clay content, as already shown for several other ecosystems (e.g. Feller and Beare, 1997, for tropical ecosystems; Christensen, 2000, for temperate ecosystems). The Red-Yellow Latosols (LV – 123 profiles) in the study of Tognon et al. (1998) tended to store more carbon than the Dark Red Latosol (LE – 121 profiles) for all textures, suggesting a influence of other edaphic processes. According to Corazza et al. 1999), the grassland vegetations (*campo sujo* and *campo limpo*) tend to store more carbon than forests (*Cerradão* and *mata ciliar*) and savanna (*cerrado sensu stricto*). This was attributed to the dense root system of grasses in these ecosystems.

The net ecosystem production (NEP) represents the amount of carbon stored in a defined ecosystem in a given period of time (IPCC, 2000). NEP measurements have been intensified for the principal biomes of the Globe in an attempt to better understand the uptake potential of terrestrial ecosystems (Wigley and Schimel, 2000; IPCC, 2001). At present, it is accepted that the combined terrestrial ecosystems of the world take up about 2.3 Gt C yr^{-1}, but very little is known about the role of each individual biome in this process (IPCC, 2000). A better knowledge of the NEP of different ecosystems is an important issue on the political agenda. In Article 3.1 of the Kyoto Protocol, the industrialised nations (Annex I Parties) agreed to reduce their emissions of greenhouse gases between 2008 and 2012 (IPCC, 2000). According to Article 3.3 of the Protocol, the NEP of ecosystem in individual countries (resulting from afforestation, reforestation, and other land-use changes) may be taken into account for meeting these commitments (IPCC, 2000).

Recent measurements have shown NEPs of 2.5 to 7 t C ha^{-1} yr^{-1} and up to 2.5 t C ha^{-1} yr^{-1} in temperate and boreal forests, respectively (IPCC, 2000). For tropical rain forests, current measurements have shown values of 0.7 to 5.9 t C ha^{-1} yr^{-1} (IPCC, 2000) and 1.0 t C ha^{-1} yr^{-1} (Grace et al. 1995ab) for moist and seasonal forests, respectively. A pioneer studied was carried out in a natural reserve of *cerrado sensu stricto* from Central Brazil (Miranda et. al, 1996), where carbon fluxes were estimated by eddy covariance. Although the experiment covered only one year (1993) and the vegetation had been burned (fire of low intensity that affected mainly the herbaceous stratum) seven years before the experiment, the ecosystem absorbed about 2.5 t C ha^{-1} yr^{-1}. Due to evident limitations, conclusive interpretations of a such value can not be made, but the measurements suggest that this vegetation can, at least temporarily, sequester as much carbon as some boreal, temperate, and tropical forests.

Undoubtedly, better estimates of organic carbon stocks and fluxes in Cerrado are needed for more definitive conclusions. Nevertheless, considering the Cerrado area and the average carbon stocks for tropical savannas (Table 1.1), this biome contains about one percent of the total C in terrestrial ecosystems (about 30 Gt C), contributing very little to global carbon stocks (about 2577 Gt C). On the other hand, the first measurements showed a potential of carbon sequestration comparable with that of most of the other biomes in the world, which may be important for understanding the "missing carbon sink" and for carbon sequestration programs at a national level in the scope of the Kyoto Protocol.

This thesis address the dynamics of carbon in one of the most widespread soil classes in the Cerrado Biome, covering about 320,000 km^2. Better understanding of the carbon cycle in this ecosystem may provide useful tools for a better use of the soils, avoiding extra CO_2 emissions, or even promoting C sequestration.

1.2. Impact of Land Use

The first major anthropogenic impact on Cerrado ecosystems was the increase in fire frequency, which is part of the management of native pastures (Eiten, 1972). Fire is a common ecological component in these ecosystems, and is held responsible for the maintenance of some grassland forms (Eiten, 1972, 1992; Mistry, 1998). However, increasing fire intensity in more arboreal forms (e.g. *cerrado sensu stricto*, see Table A1.1 – Appendix 1) may significantly change vegetation composition and structure, reducing the tree canopy layer in favour of the grass stratum (Eiten 1972, 1992; Miranda & Klink, 1996a,b; Mistry, 1998). The effect of increasing fire incidence on soil organic matter has not been assessed so far. Reducing above ground biomass (Eiten 1972, 1992) may have an important impact on supply of residues. On the other hand, tropical grasses produce large quantities of below-ground litter (Fisher et al., 1994; Corazza et al., 1999), which would maintain soil organic matter levels. Brossard et al. (1997) showed that, for a sequence of Cerrado soils, the stocks of carbon (to 1-m depth) tended to be higher in grasslands than in woodland forms (Table 1.2).

Reclamation of Cerrado soils has a potentially much higher impact on soil organic matter than fire alone. Soils may loose considerable amounts of SOM by reduction of litter supply or increasing decomposition rate and erosion (Andreux, 1996; Piccolo, 1996; Christensen, 2000; Carter, 2001). Conventional tillage (plowing and/or harrowing) has been considered one of the most degradative systems of land use, with high chances of reducing carbon stocks in soil (Bayer & Mieniczuk, 1999; Resck et al., 1999). In Cerrado, evidence of soil degradation and reduction of water infiltration and holding capacity as a result of conventional tillage, has been reported by several authors (Silva et al., 1994; Lepsch et al., 1994; Resende et al., 1996; Resck, 1998; Resck et al., 1999). Silva et al. (1994) sampled 220 Cerrado topsoils continuously cultivated with soybean (heavy disk harrow). The authors observed a severe reduction of SOM contents in 5 years of cultivation. The decreases were 80% of the initial contents for Quartz sands (< 15% clay), 76% for loamy Red-Yellow Latosols (15-30% clay), and 41% for clayey Red-Yellow Latosols (> 30% clay) (see Table A1.2 – Appendix 1 for equivalence in FAO Legend and Soil Taxonomy). However, a reduction is not always observed. No changes in the SOM stocks (0-40cm) was observed by Freitas et al. (2000) for a clayey Dark Red Latosol after 25 year of conventional tillage (vegetables, rice, maize, and beans). Therefore, the effects of conventional tillage on SOM losses are not completely elucidated for Cerrado soils.

In the last decade, no-tillage has been introduced in Cerrado, in an attempt to control soil and water degradation (Resck et al., 1999). In the cropping season 1988/1989, the area under no-tillage in Cerrado was insignificant. It increased exponentially and, in 1998/1999, according to the Brazilian Federation on Zero Tillage (FEBRAPDP) there was 3.3 million ha under no-tillage (http://www.agri.com.br/febrapdp/pd_area_estados.htm). Although the technology has spread quickly, very little scientific information is available, especially with respect to SOM dynamics (Resck et al., 1999). In subtropical regions of Brazil, no-tillage has led to systematic increase in SOM contents, especially when combined with rotations including crops with high residue production (Bayer & Mielniczuk, 1999; Bayer et al., 2000). For Cerrado soils, contrasting results have been reported. Higher carbon stocks in no-tillage than in conventional tillage were reported by Resck et al. (1999), but not observed by Maria & Castro (1993a,b) and Freitas et al. (2000).

Well managed cultivated pastures may provide enough organic carbon to maintain or even increase native C contents (Campos et al., 1995; Corazza et al., 1999). Pastures may also preserve the good soil physical properties, such as aggregation and soil porosity, typical of native Cerrado soils (Westerhof et al., 1999; Neufeldt et al., 1999). Pastures have been considered one of the most preservative land use systems for Cerrado soils, and are recommended for the recuperation of degraded croplands (Westerhof et al., 1999; Neufeldt et al., 1999; Corazza et al., 1999). Nevertheless, such benefits are accompained by large impacts on Cerrado biodiversity (Macedo, 1995).

The impact of different land use systems on SOM is still unclear for Cerrado soils, especially when different fractions of SOM is concerned. In this thesis, the effect of pasture, conventional, and no-tillage systems were analysed. The focus was on how these systems would interfere in SOM stocks and dynamics in different physically fractionated soil separates.

1.3. SOM location and dynamics

Several descriptive models deal with the spatial organization of mineral and organic particles in soil (Oades, 1984; Oades & Waters, 1991; Golchin et al., 1994a, 1997; Christensen, 1996 a, b, 2000). These models can be very complex, as the one described by Golchin et al. (1997), but in general a relatively simple model (Figure 1.2) may well represent this arrangement (Christensen, 1996a, b, 2000).

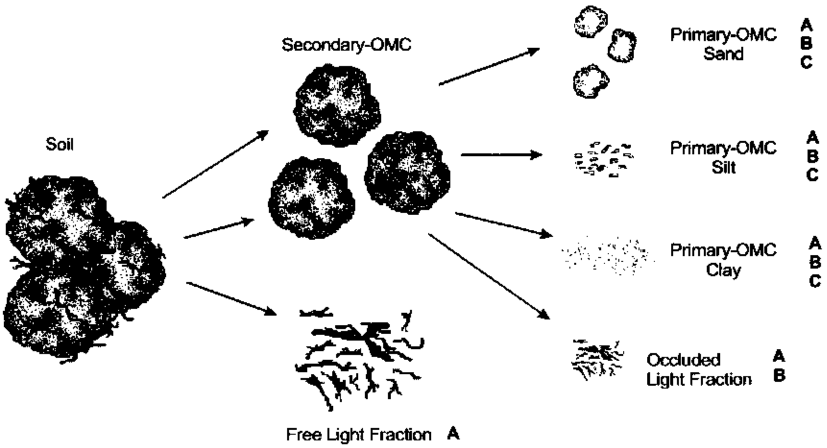

Figure 1.2. Descriptive model of the spatial orgnanization of mineral and organic particles in soil. Bulk soil is composed by secondary organo-mineral complexes (Secondary-OMC) and free uncomplexed organic matter or free light fraction. Secondary-OMC are formed by the aggregation of primary organo-mineral complexes (Primary-OMC). In this process of formation, some uncomplexed organic matter is trapped inside aggregates, the occluded light fraction. Letters next to each fraction represents mechanisms of protection against decomposition: A, intrinsic recalcitrance; B, occlusion inside aggragates, and C binding/complexation with the mineral particles. Further details in the text.

According to Christensen (1996a, b, 2000), SOM can be found in pools with different degrees of association with the soil mineral matrix (Figure 1.2). SOM can be free or only weakly associated with soil particles (uncomplexed organic matter or *free light fraction*), or strongly associated to mineral particles as *organo-mineral complexes*. These complexes are called *primary*, when formed by direct interaction of primary mineral particles and organic compounds. At a second hierarchical level, primary complexes may bind together into secondary complexes or aggregates. In this process of aggregation, some uncomplexed organic material may be encapsulated, the *occluded light fraction*.

In the model of Figure 1.2, SOM pools have a different availability for decomposers, due to distinct mechanisms of protection and stabilization. These mechanisms can be summarised in three categories: intrinsic chemical recalcitrance of the organic molecules, occlusion inside aggregates, and binding/complexation with mineral particles (Christensen, 1996a).

Different components of animal and vegetal residues differ in complexity, requiring specific enzymatic processes for their decomposition (Stevenson & Cole, 1999). More complex molecules (e.g. lignin, cutin, suberin) are less susceptible to decay than more simple molecules (e.g. carbohydrates, proteins), being more recalcitrant in the environment (Baldock et al., 1992; Schulten & Leinweber, 2000). This intrinsic recalcitrance of molecules is a protecting mechanism common for all pools represented in Figure 1.2, but it is the exclusive mechanism only for the *free light fraction*.

The capture of light fraction inside aggregates (occlusion) may substantially reduce its availability for microbiota (Christensen, 2000). Occlusion creates a peculiar environment inside aggregates, with reduced water, air, and nutrient diffusion, limiting enzymatic and biological activity (Burns, 1982; Nannipieri et al., 1990; Collins et al., 1997; Hassink & Whitmore, 1997). Therefore, for the occluded light fraction two of the protective mechanisms are operational: recalcitrance and occlusion (Figure 1.2).

Organic compounds may strongly bind to mineral particles forming stable organo-mineral complexes. This complexation has a high protective effect on soil organic matter (Schulten & Leinweber, 2000; Christensen, 2000). Several binding mechanisms (e.g. cationic and hydrogen bounds, electrostatic and Van der Waals interaction) obstruct the access of decomposers (Schulten & Leinweber, 2000). As shown in Figure 1.2, all three protective mechanisms may be operational for this fraction. Recalcitrance and binding/complexation for all particles, and occlusion only for the complexes located inside aggregates, in inaccessible locations.

As suggested by the model, the intensity of protection would increase in the order: free light fraction < occluded light fraction < organo-mineral complexes (Baldock et al., 1992; Golchin et al., 1997; Christensen, 1996a).

In this thesis, the model of Figure 1.2 was applied in the methodology of soil fractionation to study the dynamics of soil organic matter. It was hypothesised that in the studied Oxisol, soil organic matter was divided in the different dynamic pools assumed for this model.

1.4. Objectives of this thesis

The initial objective of this thesis was to evaluate the impact of different management systems on SOM dynamics in one of the most representative soil classes in

Cerrado, the Dark Red Latosol (Table A1.2 – Appendix 1). The research strategy was to compare, in the same soil unit, paired plots with different management systems (native vegetation, pasture, no-tillage and conventional tillage). To assess SOM dynamics, a combination of two methodologies was used, (i) physical fractionation of soil into particle-size and density separates, and (ii) natural abundance of the ^{13}C isotope. Physical fractionation aimed to separate SOM pools with different chemistry, location, and turnover, which hypothetically would be more sensitive to soil alterations than total SOM. The natural abundance of ^{13}C was used to assess the turnover time of SOM and its fractions. As these methodologies have so far hardly been applied in Cerrado ecosystems, the initial focus of the thesis was changed: general objective was extended to three specific objectives:

- (i) to adapt the methodology of physical fractionation of soil and ^{13}C natural abundance to SOM studies in a Cerrado ecosystem;
- (ii) to contribute to a better understanding of the distribution and dynamics of different SOM pools in this specific environment; and
- (iii) to evaluate the long-term effect on SOM of converting a *cerrado sensu stricto* in: a) cultivated pasture; and b) annual crops (maize and beans) under conventional tillage and no-tillage.

1.5. Relevant methodologies

Two methodologies were widely applied in this research: the separation of soil organic matter by physical fractionation of soil, and the estimate of carbon origin by the ^{13}C natural abundance approach. A general description of the methods is given here, but specific points are detailed in the introduction of each chapter.

Physical Fractionation

Physical fractionation of soil has been widely used in SOM studies (Feller & Beare, 1997; Christensen, 2000). Several fractionation schemes were proposed to separate SOM fractions with different degree of association with mineral particles and location in the soil matrix (Golchin et al., 1997; Feller & Beare, 1997; Christensen, 2000). The methodology involves density and particle-size separation, together or separately. Although good results were obtained for temperate soils (Golchin et al., 1997; Christensen, 2000), very little has been done on tropical soils (Feller & Beare, 1997). The conceptual model of SOM distribution in soil, considered in this thesis (Figure 1.2), is compatible with a fractionation scheme where density and particle-size separation are applied. Density separation may be used to initially separate the *free light fraction*, before complete destruction of aggregates. For this separation, a solution of sodium iodide (NaI) with density 1.7 g cm^{-3} is recommended (Gregorich & Ellert, 1993). The light fraction has a density lower than this value. After complete destruction of aggregation, the same solution is used for separating the *occluded light fraction*. The fraction, with a density higher than 1.7 g cm^{-3}, is called the *heavy fraction* and is subsequently divided into different particle-size classes, the *primary organo-mineral complexes*. Dispersion of soil before fractionation is an important and controversial step in the methodology (Christensen, 1992). Ultrasonic energy has been recommended for the dispersion, because it allows the quantification of the applied energy (Schmidt et al., 1999). However, few researchers adequately calibrate their equipment (Raine & So, 1993). The amount of energy to be used for dispersion depends on soil type, and almost no information is available for Cerrado soils (Sá, 1998). In this thesis, special attention was given to the process of dispersion, as well as to the distribution and dynamics of the pools/fractions hypothetically defined in Figure 1.2.

^{13}C *natural abundance*

The majority of carbon in nature (98.9%) is present as the stable isotope ^{12}C. The ^{13}C stable isotope represents about 1.1%. During the assimilation of atmospheric CO_2 via the photosynthesis, plants discriminate against ^{13}C. As a consequence plants are depleted in ^{13}C, compared to the CO_2-C in the atmosphere. Plants with different photosynthetic pathways have different ^{13}C discrimination; C_3 plants are more efficient in discriminating ^{13}C than C_4 plants. Therefore, C_4 plants have significantly more ^{13}C in their tissue and residues than C_3 plants. This difference is used as a natural tracer in SOM studies, whenever a C_4- replaces a C_3-dominated vegetation or vice-versa (Balesdent & Mariotti, 1996). The methodology can be applied to bulk soil samples or to different SOM fractions, which is an advantage in distinguishing fractions according to their dynamics. The proportion of ^{13}C in a given sample is conventionally expressed in units of δ^{13}C (‰), which is defined as the relative difference between the isotope ratios ($^{13}R={}^{13}C/{}^{12}C$) of sample and standard: $\delta^{13}C = [{}^{13}R_{\text{sample}} - {}^{13}R_{\text{standard}})/({}^{13}R_{\text{standard}})] \times 1000$. The international standard is the Vienna-Pee Dee Belemnite (V-PDB). The present δ^{13}C value of the atmosphere is -8‰. C_3 plants show a δ^{13}C ranging from –32 to –22‰ (mean of –27‰) and C_4 plants have values of –16 to –9‰ (mean of –13‰) (Boutton, 1996; Balesdent & Mariotti, 1996). Linear mixing models are used for the calculations of source (C_3 or C_4) contributions for samples of any mixed system (Balesdent & Mariotti, 1996). Several assumptions are made when using such a methodology. In most studies these assumptions are not explicit and are incorporated in the final error of the calculations. The calculations involve a considerable number of input variables, which complicates the estimate of output uncertainties (Veldkamp & Weitz, 1994; Phillips & Gregg, 2001). Recently, Phillips & Gregg (2001) successfully applied Taylor series approximations to assess these uncertainties. This technique was used in the last two chapters of this thesis to give an impression of the reliability of estimates.

1.6. Outline of the thesis

This thesis is composed of six major chapters (2 to 7) written as independent papers. For this reason some gaps and overlaps were inevitable. The papers are organised chronologically, and important finds in the initial chapters are used in the subsequent ones.

Chapter 2 deals with the identification and characterization of a reference vegetation and soil profile for comparison with the other management systems. The chapter describes a native reserve of *cerrado sensu stricto* and how different fire regimes affect SOM contents and the establishment of a ^{13}C-reference profile for dynamic studies.

In Chapter 3, the focus is on establishing a physical fractionation procedure to SOM in the Cerrado oxisol under investigation. Special attention is given to the dispersion by ultrasonic energy, a crucial point in the methodology. A procedure to calibrate the ultrasonic equipment and to determine the minimum energy required for efficient dispersion is proposed. Important questions about aggregation are also discussed.

Chapter 4 applies the results of Chapter 2 and 3 to a study of the effect of cultivated pasture (*Brachiaria* spp.) on the dynamics of particle-size and density fractions of SOM, 23 years after reclamation. Important issues related to the dynamics of the fractions are discussed. The capacity of carbon storage and dynamics in the system as a whole are also addressed.

Chapter 5 deals with the spatial variability of SOM and $\delta^{13}C$ in the studied soil. The effects of fire and cultivation (conventional tillage and no-tillage) are addressed. The implications of using paired plots for dynamic studies based on ^{13}C natural abundance are discussed in the light of spatial variability.

Chapter 6 combines the results of Chapters 2, 3, 4, and 5 to analyse the effect of 30 years of maize/beans cultivation on SOM. The possible interference of compaction in the cultivated area on C stock calculations is discussed. Uncertainty analysis is used to assess the variability of carbon replacements.

In chapter 7, data from Chapters 5 and 6 were used to evaluate the effect of assuming equal discrimination for C_3 and C_4 plants in calculations of replacements. Uncertainty and sensitivity analyses were applied to assess the significance of such an assumption and its sensitivity to different combinations of input parameters. The theoretical background of assuming equal discrimination for both C_3 and C_4 plants was discussed in the light of present hypotheses of ^{13}C fractionation with decomposition.

Chapter 8 presents a general discussion of the results obtained in the previous chapters and summarises the main conclusions. Some recommendations for further research are given.

Chapter 2

Effects of fire on soil organic matter in a "cerrado sensu-stricto" from Southeast Brazil as revealed by changes in $\delta^{13}C$

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Geoderma (2000) 95: 141-160.

Abstract

We studied the effect of increasing fire incidence on litter and soil organic matter in a Dark Red Latosol (Typic Haplustox) under a "cerrado sensu-stricto" vegetation in Southeast Brazil. After 21 years, C_4-grass population significantly increased in the plot under high fire incidence ("cerrado 3") compared to the plots of low fire incidence ("cerrado 1" and cerrado 2"). In general, the variability of organic carbon content, organic nitrogen content and $\delta^{13}C$, both in litter and in the topsoil, was higher in "cerrado 3" than in the other plots. The higher fire incidence in "cerrado 3" decreased the litter stock of carbon and nitrogen by 3703 ± 930 and 104 ± 26 $Kg.ha^{-1}$, respectively, in relation to the other plots. However, no difference in carbon (195.0 ± 8.2 $Mg.ha^{-1}$) and nitrogen (13.4 ± 1.0 $Mg.ha^{-1}$) stock in the first meter of soil was observed between the plots. From litter to topsoil, the values of $\delta^{13}C$ increased between 2.06 and 3.39 ‰ under C_3-plants, and decreased 8.31‰ under C_4-plants. For all plots, the $\delta^{13}C$ profile showed an increase in $\delta^{13}C$ with depth. In "cerrado 1" and "cerrado 2", the $\delta^{13}C$ values ranged from -25.80 to -23.19 ‰, typical for a C_3-dominated vegetation profile. In "cerrado 3", the replacement of C_3- by C_4-derived C was in average 34.6% throughout the profile. We conclude that; (i) the studied undisturbed "cerrado sensu-stricto" is a C_3-dominated vegetation; (ii) biannual fire incidence for 21 years reduced the C_3 dominance and decreased the stock of C and N in litter, but not in the soil; (iii) in the same period, at least about one third of the C stock in the soil, or 67.5 $Mg.ha^{-1}$ (to 1 m depth), was replaced by C_4-derived below ground litter; and (iv) in choosing a $\delta^{13}C$ reference profile for soils under cerrado it is necessary to take fire incidence into account.

2.1. Introduction

A huge area of Central Brazil is covered by a savanna-like vegetation generically referred to as "Cerrado". It spreads for about 1.8 million Km^2 or 20% of the country, and is characterised by a seasonal climate (Adámoli et al., 1985). The mean annual temperature ranges from 22°C in the South to 27°C in the North, the mean annual precipitation from 1200 to 1800 mm, and the dry season has a duration of 5 to 6 months. The "cerrado" vegetation results from the combination of basically two ecologically limiting factors: (i) soils of low fertility and relatively high acidity, and (ii) a seasonal climate (Silva & Resck, 1997). The vegetation is composed of a basement of grass, with a variable cover of shrubs and trees (Eiten, 1972, 1992). It tends to be more open (fewer trees and shrubs) with decreasing soil fertility and water availability (Adámoli et al., 1985). There are several physiognomic forms, varying from the most dense type called "cerradão"(similar to a woodland savanna, with trees to a height of 20 m); through the more common "cerrado sensu stricto" (a species-rich dense vegetation of shrubs and trees, 8-10 m high, with a grass undergrowth); to the grassland "campo cerrado" forms (Eiten, 1972, 1992; Miranda et al., 1997). The "cerrado sensu-stricto" covers 53% of the area (Miranda et al., 1997).

Because of its possible effect on global climate change, in recent years, special attention has been given to CO_2 emission due to land use changes (Houghton et al., 1995; Schlesinger, 1997). Increasing burning of vegetation causes a significant reduction in aboveground carbon stock and constitutes an important source of atmospheric CO_2, particularly in tropical rainforests (Sudquist, 1993; Schimel, 1995; Detwiler & Hall, 1988). The carbon flux in "cerrado" ecosystems is still unclear (Miranda et al., 1996). Although fire is a natural event in this environment (Eiten, 1972), increasing anthropogenic pressure has intensified its occurrence. Frequent fires cause changes in vegetation composition and carbon stock (Eiten 1992). Considering the large area of the "cerrado" ecosystem, changes in aboveground biomass and in SOM as a result of intensive burning may provide a considerable addition of CO_2 to the atmosphere, even at a global scale.

Differences in photosynthetic pathway of C_3 and C_4 plants give a distinct discrimination of ^{13}C during assimilation of atmospheric CO_2. This discrimination can be measured by $^{13}C/^{12}C$ ratio (^{13}R) differences. The data are usually expressed as $\delta^{13}C$, which is defined as the relative difference between the isotope ratios of sample and standard: $\delta^{13}C = (^{13}R_{sample} - ^{13}R_{standard}) / (^{13}R_{standard}) \times 1000$. The international standard is V-PDB (Vienna-Pee Dee Belemnite). The present $\delta^{13}C$ value of the atmosphere is -8‰. C_3 plants show a $\delta^{13}C$ ranging from –32 to –22‰ (mean of –27‰) and C_4 plants have values of –16 to –9‰ (mean of –13‰) (Boutton, 1996; Balesdent & Mariotti, 1996). This difference is used to estimate SOC dynamics, when a C_3 vegetation is replaced by a C_4 or vice-versa. Most of the studies have been done in cultivated areas, where crops with a photosynthetic pathway different from the original vegetation are used (Balesdent & Mariotti, 1996).

In natural savanna areas, the vegetation is a mix of C_3 (trees and scrubs) and C_4 (grasses) plants. The spatial variability of the species may hinder the establishment of reference profiles for shifting-vegetation studies using the natural ^{13}C abundance in these ecossystems. However, there are some evidences that the "cerrado sensu-stricto" is a C_3-dominant vegetation (Miranda et al., 1997; Pressada et al. 1996), what may give a homogeneous $\delta^{13}C$ signature for the soil organic matter.

Martin et al. (1990) found that fire protection in a savanna from Ivory Coast for 25 yrs changed the C_3/C_4 ratio of the vegetation resulting in significant changes in $\delta^{13}C$ of SOC. In Brazil, increasing incidence of fire in "cerrado sensu-stricto" reduces the arboreal population and favours colonisation by grasses (Eiten, 1972, 1992; Miranda & Klink, 1996a and 1996b). If soils under "cerrado sensu-stricto" (in a specific area) have a homogeneous $\delta^{13}C$ signature and increasing fire incidence increases the C_4-grass population, ^{13}C can be used as a natural tracer to understand the SOM dynamics in this environment.

The aims of this study were to evaluate: (i) the spatial variability of $\delta^{13}C$ of litter and topsoil organic matter in a "cerrado senso-stricto" under different fire intensity; (ii) the effect of fire on litter and soil organic matter with depth; and (iii) to establish a $\delta^{13}C$ reference profile for the studied vegetation.

2.2. Materials and Methods

2.2.1. Description of the studied area

The study was carried out at the Maize and Sorghum National Research Centre – Brazilian Institute of Agricultural Research (EMBRAPA-CPMS), Sete Lagoas-MG, Brazil. The area is located at latitude 19°26'S and longitude 44°10' W, and has a mean altitude of 730 m. The climate was classified by Sans (1986) as Aw in Köppen's classification. The mean annual temperature is 22.1°C. The mean annual precipitation is 1340 mm, with a wet season from October to March, and a dry season from April to September.

The sampled soil was a Dark Red Latosol (Typic Haplustox), previously described by Sans (1986). All plots were located on the upper slope of a hill with less than 2% slope. The clay content is about 600 g kg^{-1} in the A_h (0-2/3cm) horizon, increasing gradually to 750 g kg^{-1} in the B_{w2} (90-100cm) horizon. The pH_{water} (soil:water ratio 1:2.5) varies from 5.1 in the topsoil to 4.4 in the subsoil; the base saturation is 22 % in the A_h horizon and decreases to 1 % in the B_{w2}; exchangeable aluminium increases from 0.55 to 1.18 $cmol_+ kg^{-1}$ (Sans, 1986).

The studied area is part of a natural reserve of the EMBRAPA-CNPMS. The research centre (previously called "Instituto de Pesquisa do Oeste") was installed in a virgin cerrado area in 1958 and the studied place was kept as a natural reserve since then. The surrounded region has been used for pasture since the 1970s. Fire is a common management practice in these pastures, and accidental burns in parts of the reserve have been registered in the last decades.

Three plots of 10x10m (50 to 100 meters from each other) were selected in representative sites with different fire incidence. In the first plot, referred to as "cerrado 1", fires occur sporadically, very close to the natural situation. This area is protected against periodical accidental fires by a road and there is no fire registered since 1977 (21 years before sampling). The road also protects the second plot (cerrado 2), but it was burned two times in the same period. The last fire was in the dry season of 1997 (one year before sampling). The third area (cerrado 3) is not protected by the road, and is burned frequently. From 1977 to 1998 ten fires were registered, given a biannual frequency. The last fire happened also in the dry season of 1997.

2.2.2. Vegetation description

The vegetation in each of the 10x10 plots was described by the number of individuals of each species in three groups: trees of 4-8 m high; shrubs and young trees of 1-4 m high; and herbaceous (only grasses were described). The results are given in Figure 2.1 and Tables 2.1, 2.2 and 2.3.

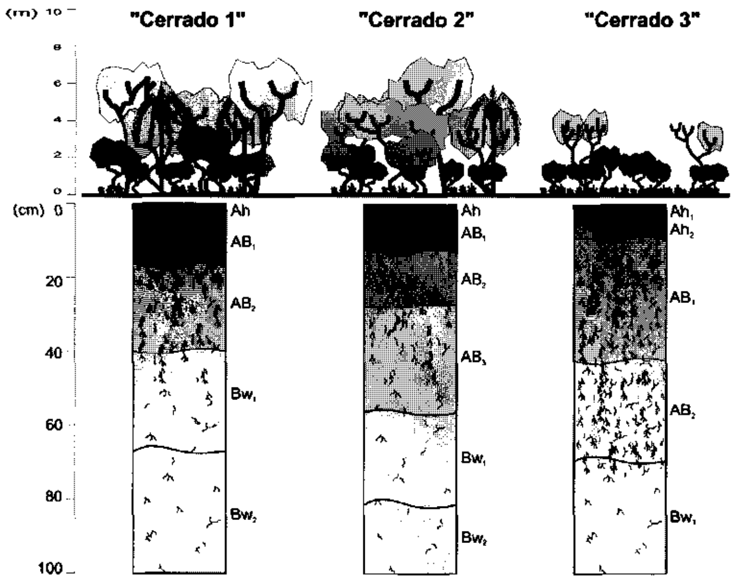

Figure 2.1. Schematic representation of the changes in a "cerrado sensu-stricto" vegetation and root distribution in the subjacent soil profile as a function of fire incidence in 21 years: "cerrado 1" – no fire; "cerrado 2" – two fires; and "cerrado 3" – ten fires in the period.

2.2.3. Litter and Soil Sampling

Litter and soil were sampled in May 1998, in the beginning of the dry season. In "cerrado 1" and "cerrado 2" plots, the amount of litter was estimated by collecting all litter from one square meter soil surface. Duplicate samples were oven-dried at 75°C until constant weight, and weighed. Five random samples of litter and of the topsoil (Ah horizon, 0-2/3 cm) directly below were collected in each plot to estimate the spatial variability of total C, total N and $\delta^{13}C$. Species in litter samples were identified when possible, oven-dried, milled and stored for analysis. Soil samples were air-dried, passed through a 2 mm sieve and stored for analysis.

Table 2.1. Vegetation description of "cerrado 1" (100 m^2 plot).

Species (number of individuals)	No. Species	No. Individ.
Trees (4-8 meters)		
Austroplenckia populnea (1); Cabralea Polytrica (3); Caryocar brasiliense (2); Cecropia hololeuca (1); Bowdichia virgilioides (2); Hymenaea stigonocarpa (2); Plathymenia reticulata (1); Qualea parviflora (1); Siparuna guianensis (3); Syagrus sp. (3); Tabebuia ochracea (2); Xylopia aromatica (5).	12	26
Shrubs and Young Trees (1-4 meters)		
Bowdichia virgilioides (2); Cabralea Polytrica (1); Eugenia dysenterica (4); Kielmeyera coriacea (1); Miconia albicans (2); Olyra sp. (6); Peritassa campestris (1); Qualea grandiflora (1); Qualea parviflora (1); Senna sp. (1); Siparuna guianensis (3); Stryphnodendron adstringens (1); Xylopia aromatica (2).	13	26
Herbaceous[1]		
Melinis minutiflora	1	Few[2]

[1] only the predominant grass was described;

[2] less than 5% of grass cover;

Table 2.2. Vegetation description of "cerrado 2" (100 m^2 plot).

Species (number of individuals)	No. Species	No. Individ.
Trees (4-8 meters)		
Austroplenckia populnea (1); Bowdichia virgilioides (2); Caryocar brasiliense (4); Connarus sp. (1); Dimorphandra mollis (1); Hymenaea stigonocarpa (1); Plathymenia reticulata (2); Qualea grandiflora (1); Siparuna guianensis (1); Syagrus sp. (1); Tabebuia ochracea (1); Tabebuia sp. (1); Xylopia aromatica (1).	13	18
Shrubs and Young Trees (1-4 meters)		
Bauhinia bongardill (1); Eugenia dysenterica (2); Hymenaea stigonocarpa (2); Miconia albicans (3); Peritassa campestris (4); Roupala montana (2); Rudgea viburnoides (1); Siparuna guianensis (2); Smilax sp (4); Tabebuia aurea (1); Vernonia polyanthes (3); Xylopia aromatica (5); Zeyheria digitalis (1).	13	31
Herbaceous[1]		
Melinis minutiflora	1	Few[2]

[1] only the predominant grass was described;

[2] less than 5% of grass cover;

Table 2.3. Vegetation description of "cerrado 3" (100 m^2 plot).

Species (number of individuals)	No. Species	No. Individ.
Trees (4-6 meters)[1] *Caryocar brasiliense (2); Eugenia dysenterica (1); Lafoensia pacari (2); Magonia pubescens (1); Plathymenia reticulata (3); Qualea grandiflora (1); Qualea parviflora (1); Schefflera macrocarpa (1); Tabebuia ochracea (2).*	9	14
Shrubs and Young Trees (1-4 meters) *Aspidosperma sp. (1); Brosimum gaudichaudii (3); Byrsonima coccolobifolia (3); Byrsonima verbascifolia (1); Cabralea Polytrica (5); Caryocar brasiliense (2); Cestrum coriaceum (1); Enterolobium ellipticum (1); Jacaranda caroba (1); Lafoensia pacari (2); Myrcia sp. (3); Peritassa campestris (1); Peixotoa sp. (1); Protium heptaphyllum (1); Qualea grandiflora (1); Roupala montana (1); Rudgea viburnoides (1); Schefflera macrocarpa (1); Senna sp. (1); Styrax camporum (2); Vernonia polyanthes (1); Xylopia aromatica (2); Zeyheria digitalis (3).*	23	39
Herbaceous[2] *Brachiaria spp.; Melinis minutiflora.*	2	Many[3]

[1] no tree higher than 6 meters was found

[2] only the predominant grass was described;

[3] 40 – 80% of grass cover.

The amount of litter in "cerrado 3" was not quantitatively measured because it was negligible as a result of frequent burning. However, 10 samples of litter and respective topsoil, five under C_3 plants and five under C_4 plants, were collected to evaluate the variability of the total C, N and $\delta^{13}C$. Litter and soil samples were processed in the same way described above.

In the centre of each plot, a profile was dug to 1m depth and described according to FAO (1990). Figure 2.1 shows a schematic representation of the root distribution based on the profile description. Soil bulk density was determined in duplicate for each horizon, using volumetric rings. In the topsoil (0-5 cm) two samples were taken from 0 to 2/3 cm and from 2/3-5 cm, according to the thickness of the Ah horizon (Figure 2.1). From 5 to 50 cm, samples were taken of each 5 cm layer and from 50-100 cm, of each 10 cm layer. Soil samples were processed as described above. OC and ON contents were expressed on volumetric base (g dm^{-3}).

2.2.4. C, N, and $\delta^{13}C$ analysis

Carbon and nitrogen was determined by flash combustion in an EA 1108 Elemental Analyzer, according to Van Lagen (1996). ^{13}C abundance was determined after the conversion of total C to CO_2, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer at the Center for Isotope Research at the University of Groningen, the Netherlands. Results were expressed as $\delta^{13}C$ (‰). $\delta^{13}C$ was determined in duplicate for each sample. The analytical precision for the pure-carbon standard sample GS7 was 0.05‰. We determined the analytical precision for the soil samples by 16 measurements of a homogenized standard soil sample (Labex 12) which was replicated throughout the measurements.

2.2.5. Carbon origin estimations

For the "cerrado 3" plot, the proportion of carbon derived from C4 and C3 plants in the SOM was estimated for each depth. For the calculations, we used a mass balance equation, according to Balesdent and Mariotti (1996):

$$\mathrm{TOC} \times \delta^{13}\mathrm{C}_{\mathrm{T}} = \mathrm{SOC}_{\mathrm{C3}} \times \delta^{13}\mathrm{C}_{\mathrm{C3}} + \mathrm{SOC}_{\mathrm{C4}} \times \delta^{13}\mathrm{C}_{\mathrm{C4}}$$

Where: TOC = total soil organic carbon; $\mathrm{SOC}_{\mathrm{C3}}$ = soil organic carbon derived from C_3 vegetation; $\mathrm{SOC}_{\mathrm{C4}}$ = soil organic carbon derived from C_4 vegetation; $\delta^{13}\mathrm{C}_{\mathrm{T}}$ = δ^{13}C of total soil organic carbon; $\delta^{13}\mathrm{C}_{\mathrm{C3}}$ = δ^{13}C of soil organic carbon derived from C_3 vegetation; $\delta^{13}\mathrm{C}_{\mathrm{C4}}$ = δ^{13}C of soil organic carbon derived from C_4 vegetation.

We considered the δ^{13}C value of the SOM at the various depths in "cerrado 1" as a reference for C_3-derived carbon. For the C_4-derived carbon, we used the value of litter from C_4 plants and a similar increase of the δ^{13}C value from litter to topsoil as observed for C_3-derived carbon in "cerrado 1" (Balesdent et al., 1988). The same calculated value was used for all depths, because no additional discrimination with depth of the C_4-derived carbon was expected, since the increase in C_4 plant population took place in the last 21 years (a short period of time for a significant accumulation of humified carbon from C_4 plants).

2.3. Results

2.3.1. Organic carbon and nitrogen

The quantity of litter was not significantly different between "cerrado 1" (8767.9 ± 2919.0 kg ha^{-1}) and "cerrado 2" (7060.9 ± 564.0 kg ha^{-1}). The same was observed for the contents of carbon (4107.7 ± 1367.6 kg ha^{-1} and 3298.8 ± 26.35 kg ha^{-1}, respectively), and nitrogen (115.7 ± 38.5 kg ha^{-1} and 93.2 ± 7.4 kg ha^{-1}, respectively). The amount of litter in "cerrado 3" was negligible. Considering the average of the plots "cerrado 1" and "cerrado 2", the higher fire intensity in "cerrado 3" reduced the litter storage of OC and ON by 3703 ± 930 and 104 ± 26 kg ha^{-1}, respectively.

Table 2.4 shows the total organic carbon (OC), total organic nitrogen (ON), and C:N ratio for the litter and topsoil samples (Ah horizon). No significant differences could be noticed for these three parameters between the samples from "cerrado 1" and "cerrado 2". The coefficient of variation (V) ranged from 0.01 to 0.18 for samples from litter and topsoil. For the litter samples, the highest V was observed for ON content and C:N ratio. OC of topsoil samples showed a significantly higher variability than that of litter, but this was not found for ON. In contrast, topsoil C:N ratio showed a low variability.

In "cerrado 3", the V value for OC in litter was relatively small (Table 2.4). Considering all the samples together (C_3 plus C_4 plants), the variability of the ON and, consequently, of the C:N ratio were higher, with a coefficient of variation of 0.39 and 0.44, respectively. When the samples were grouped according to the photosynthetic pathway of the predominant vegetation, the variability of these parameters was significantly lower. Litter under C_3 plants showed a nitrogen content of 9.6 ± 0.74 g kg^{-1} and under C_4 plants of 4.5 ± 0.66 g kg^{-1}, and a C:N ratio of respectively 43.6 ± 1.69 and

Table 2.4. Means and coefficient of variation (n=5) of total organic carbon (TC), total nitrogen (TN), and C:N ratio for litter layer and topsoil (0-5 cm), of "cerrado 1", "cerrado 2", and "cerrado 3".

Plot	Layer	***TC*** ($g.Kg^{-1}$)	TN ($g.Kg^{-1}$)	C:N ratio
Cerrado 1	Litter	468.5 (0.02)[1]	13.2 (0.14)	35.9 (0.13)
	Topsoil	64.7 (0.14)	4.54 (0.10)	14.2 (0.06)
Cerrado 2	Litter	467.2 (0.01)	13.2 (0.11)	35.8 (0.11)
	Topsoil	61.4 (0.18)	4.3 (0.13)	14.2 (0.04)
Cerrado 3	Litter (n=10)	434.2 (0.07)	7.05 (0.39)	72.6 (0.44)
	Topsoil (n=10)	61.4 (0.22)	3.66 (0.18)	13.6 (0.05)
	Litter (C_3)	418.2 (0.09)	9.6 (0.08)	43.6 (0.03)
	Topsoil (C_3)	50.6 (0.18)	3.66 (0.15)	13.8 (0.04)
	Litter (C_4)	450.2 (0.01)	4.5 (0.15)	101.7 (0.14)
	Topsoil (C_4)	49.24 (0.27)	3.66 (0.24)	13.4 (0.06)

[1] numbers between parentheses indicate the coefficient of variation (V) calculated by dividing the standard deviation by the average.

101.7 ± 0.80 g kg^{-1}. These differences in litter composition (ON and C:N ratio) were not reflected by differences in topsoil composition. Although the variability in topsoil samples was higher than in litter, no significant differences were observed in OC, ON and C:N ratio in relation to the covering vegetation. Similar to the results for "cerrado 1" and "cerrado 2", the variability of OC and ON in topsoil followed the same trend and was significantly higher than the V values for C:N ratio.

The contents of organic carbon (OC) and organic nitrogen (ON) as a function of depth are shown in Figure 2.2. In "cerrado 1", the values of these parameters in the topsoil (Ah horizon, 0-3cm) of the described profile were higher than the average of the samples taken to estimate the spatial variability of the plot. We observed values of 104.0 g kg^{-1} of OC and 6.9 g kg^{-1} of ON for the Ah horizon (0-3cm) in the profile, in contrast to the average (n=5) of 64.7 g kg^{-1} and 4.54 g kg^{-1} obtained for the same depth using the random samples (Table 2.4). It is possible that the topsoil sample from "cerrado 1" profile was contaminated by litter material. Strong evidence for such contamination is the low $\delta^{13}C$ of this sample (-27.42‰), which is closer to the litter signature (-27.86 ± 0.51‰) than to that of the topsoil (-25.80 ± 0.51‰) (Table 2.5). For this reason we decided to use, for the uppermost horizon, the mean values of the topsoil samples (0-3 cm) of each plot (Table 2.4) instead of the value from the described profile.

The content of OC expressed on a volumetric basis showed a decrease with depth and did not differ significantly between the three plots (Figure 2.2b). The OC content decreases sharply in the first 15 cm and then gradually through the next 80 cm. Although the ON content on a weight base in the topsoil (0-3 cm) was lower in "cerrado 3" than in the other plots (Table 2.4), due to the high soil density in this plot (data not shown), the ON content expressed on a volumetric base did not differ between the three plots. The content of ON (Figure 2.2c) followed the same trend as OC. Using the average for the three plots, the amount of OC and ON stored in the soil to 1 m depth was 195.0 ± 8.2 and 13.4 ± 1.0 Mg ha^{-1}, respectively.

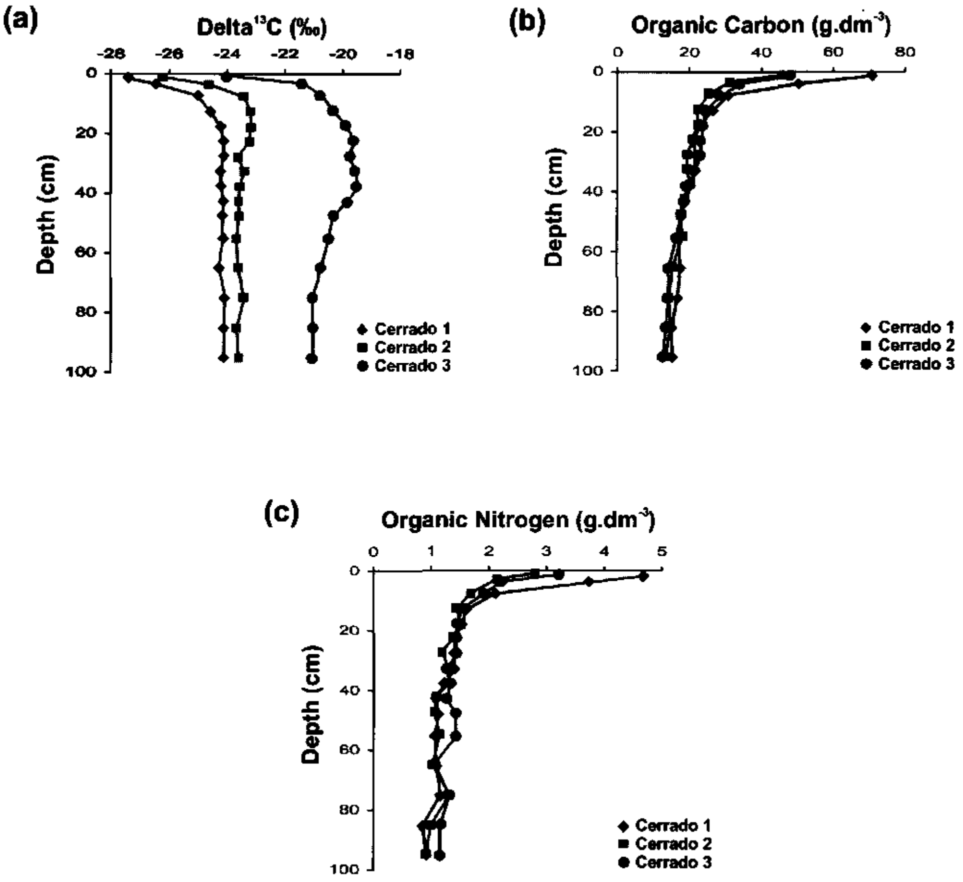

Figure 2.2. Distribution of (a) δ^{13}C values of the soil organic matter (‰); (b) content of organic carbon (g dm^{-3}); and (c) content of organic nitrogen (g dm^{-3}); as a function of depth, in "cerrado 1, 2 and 3" (Typic Haplustox).

2.3.2. δ^{13}C of litter and soil

To evaluate the effect of vegetation on δ^{13}C, leaf fragments in the litter were identified if possible. The results are shown in Tables 2.5, 2.6 and 2.7. In "cerrado 1" and "cerrado 2" litter, fragments of 64 and 54% (respectively) of the species present in the plots (Table 2.1 and 2.2) were identified. In "cerrado 3" litter (considering only C_3 plants), 60% of the species (Table 2.3) were identified.

Table 2.5. Litter description and $\delta^{13}C$ value of litter and topsoil (0 – 5cm) for samples from "Cerrado 1".

Sample	Identified Species	Litter $\delta^{13}C$ (‰)	Topsoil $\delta^{13}C$ (‰)
101A	*Caryocar brasiliense, Qualea grandiflora, Bowdichia virgilioides, Xylopia aromatica, Siparuna guianensis, Olyra sp.*	-27.76 (± 0.10)	- 26.46 (± 0.04)
102A	*Caryocar brasiliense, Qualea grandiflora, Plathymenia reticulata, Xylopia aromatica, Siparuna guianensis, Banisteriopsis sp.*	-27.05 (± 0.12)	- 25.10 (± 0.06)
103A	*Caryocar brasiliense, Qualea grandiflora, Xylopia aromatica, Siparuna guianensis, Cecropia hololeuca, Banisteriopsis sp.*	-28.26 (± 0.04)	- 25.54 (± 0.06)
104A	*Caryocar brasiliense, Qualea grandiflora, Xylopia aromatica, Cecropia hololeuca, Tabebuia ochracea, Hymenaea stigonocarpa, Syagrus sp., Banisteriopsis sp.*	-28.54 (± 0.12)	-26.31 (± 0.02)
105A	*Caryocar brasiliense, Qualea grandiflora, Xylopia aromatica, Siparuna guianensis, Plathymenia reticulata, Kielmeyera coriacea, Senna sp., Olyra sp., Banisteriopsis sp.*	-27.67 (± 0.18)	-25.61 (± 0.02)
	Average ± standard deviation	- 27.86 (± 0.51)	- 25.80 (± 0.51)

The mean and standard deviation of the $\delta^{13}C$ measurements of the standard soil sample Labex 12 was –25.36 ± 0.17 ‰. The measurement of $\delta^{13}C$ for each litter and topsoil sample was performed in triplicate to assess the variability related to aliquot preparation and analysis. The SD of the measurements were in average 0.14 ‰ for litter and 0.07 ‰ for soil samples (Tables 2.5, 2.6, and 2.7).

Although differences in litter origin could be observed (Tables 2.1 and 2.2), $\delta^{13}C$ of the litter showed a low variability and did not differ between "cerrado 1" (-27.86 ± 0.57 ‰) and "cerrado 2" (-27.81 ± 0.21 ‰) (Tables 2.5 and 2.6).

In "cerrado 3" (Table 2.7), the variability of the litter $\delta^{13}C$ value, considering all the samples (under C_3 and C_4 plants), was very high (-20.65 ± 6.49 ‰). When the samples were grouped according to the photosynthetic pathway of the species identified in the litter, the variability of the groups was considerably smaller.

The variability of the $\delta^{13}C$ signature of the topsoil in "cerrado 1" (-25.80 ± 0.51 ‰) and "cerrado 2" (-25.29 ± 0.58 ‰) plots was slightly higher than in the litter (Tables 2.5 and 2.6). In "cerrado 3", the $\delta^{13}C$ of the topsoil, considering samples under C_3 and C_4 plants together, was lower than in litter, with a mean of –23.11 ± 1.11 ‰ (Table 2.7). When the samples were separated into the different groups, the values of $\delta^{13}C$ were – 23.70 ± 0.10 ‰ and – 22.52 ± 1.33 ‰ for topsoil under C_3 and C_4 plants, respectively. In contrast to litter results, $\delta^{13}C$ for topsoil samples did not differ significantly according to the photosynthetic pathway of the covering vegetation.

Table 2.6. Litter description and $\delta^{13}C$ value of litter and topsoil (0 – 5cm) for samples from "Cerrado 2".

Sample	**Identified Species**	**Litter $\delta^{13}C$ (‰)**	**Topsoil $\delta^{13}C$ (‰)**
106A	*Tabebuia aurea, Caryocar brasiliense, Qualea grandiflora, Xylopia aromatica, Plathymenia reticulata, Peritassa campestris, Hymenaea stigonocarpa, Bowdichia virgilioides, Rudgea viburnoides, Smilax sp*	-27.87 (± 0.15)	- 25.43 (± 0.05)
107A	*Tabebuia aurea, Caryocar brasiliense, Qualea grandiflora, Xylopia aromatica, Zeyheria digitalis.*	-27.98 (± 0.18)	- 25.09 (± 0.12)
108A	*Qualea grandiflora, Qualea parviflora, Xylopia aromatica, Bowdichia virgilioides, Siparuna guianensis, Smilax sp*	-28.00 (± 0.05)	- 26.30 (± 0.04)
109A	*Qualea grandiflora, Xylopia aromatica, Plathymenia reticulata, Banisteriopsis sp., Siparuna guianensis, Smilax sp*	-27.78 (± 0.01)	- 25.08 (± 0.10)
110A	*Xylopia aromatica, Peritassa campestris, Hymenaea stigonocarpa, Banisteriopsis sp., Siparuna guianensis, Smilax sp, Roupala montana, Eugenia dysenterica*	-27.42 (± 0.01)	- 24.53 (± 0.09)
	Average ± standard deviation	- 27.81 (± 0.21)	- 25.29 (± 0.58)

The topsoil samples of "cerrado1", "cerrado 2", and "cerrado 3" (under C_3 plants) showed a significant enrichment in ^{13}C compared to litter (Tables 2.5, 2.6, and 2.7). The average increases in $\delta^{13}C$ were of 2.06, 2.52, and 3.39 ‰, respectively. On the other hand, for the samples from "cerrado 3" under C_4 plants, the $\delta^{13}C$ values decreased 8.31 ‰ from the litter to the topsoil (Table 2.7).

Figure 2.2a shows the variation of the $\delta^{13}C$ signature with depth for the three profiles. In all profiles the topsoil value is the mean of the random samples.

In "cerrado 1", the $\delta^{13}C$ values of the topsoil increased by 1.80 ‰ in the first 15 cm, stabilising after that at –24.15 ± 0.06‰ until 100cm. In "cerrado 2", the $\delta^{13}C$ was –25.29 ± 0.58 ‰ in the topsoil; it increased to –23.19 ± 0.09 ‰ at 10 cm depth (remaining stable until 25 cm) and stabilised at –23.57 ± 0.09 ‰ from 25 to 100 cm. In "cerrado 3", the topsoil showed a value of –23.11± 1.11 ‰, which increased sharply to reach –19.71 ± 0.16 ‰ at 15 cm depth. This value remained stable until 45 cm, and decreased to an almost stable value of –21.08 ± 0.02 ‰ between 70 and 100 cm.

Table 2.7. Litter description and $\delta^{13}C$ value of litter and topsoil (0 – 5 cm) for samples from "Cerrado 3".

Sample	Identified Species	Litter $\delta^{13}C$ (‰)	Topsoil $\delta^{13}C$ (‰)
111A	*Caryocar brasiliense, Xylopia aromatica, Peritassa campestris, Qualea parviflora, Cestrum coriaceum, Serjania sp.*	-27.12 (± 0.13)	- 23.87 (± 0.02)
112A	*Qualea grandiflora, Qualea parviflora, Roupala montana, Caryocar brasiliense, Protium digitalis*	-28.52 (± 0.21)	- 23.72 (± 0.09)
113A	*Peixotoa sp., Brosimum gaudichaudii, Serjania sp., Xylopia aromatica, Qualea grandiflora, Caryocar brasiliense, Roupala montana.*	-25.86 (± 0.17)	- 23.65 (± 0.08)
114A	Serjania sp., Senna sp., Xylopia aromatica, Cabralea Polytrica, Rudgea virbunoides	-26.91 (± 0.12)	- 23.60 (± 0.17)
115A	*Caryocar brasiliense, Vernonia polyanthes, Banisteriopsis sp., Xylopia aromatica, Hymenaea stigonocarpa, Serjania sp.*	-27.03 (± 0.23)	- 23.64 (± 0.11)
	Average ± standard deviation (for samples 111A to 115A)[1]	- 27.09 (± 0.85)	- 23.70 (± 0.10)
116A	*Melinis minutiflora*	-15.41 (± 0.31)	- 24.48 (± 0.17)
117A	*Melinis minutiflora, Brachiaria decumbens, Brachiaria brizantha.*	-13.43 (± 0.13)	- 23.64 (± 0.04)
118A	*Melinis minutiflora, Brachiaria decumbens, Brachiaria brizantha.*	-14.77 (± 0.13)	- 22.12 (± 0.01)
119A	*Melinis minutiflora, Brachiaria decumbens, Brachiaria brizantha.*	-13.93 (± 0.12)	- 21.33 (± 0.120
120A	*Melinis minutiflora, Brachiaria decumbens, Brachiaria brizantha.*	-13.53 (± 0.19)	- 21.03 (± 0.04)
	Average ± standard deviation (for samples 116A to 120A)[2]	- 14.21 (± 0.76)	- 22.52 (± 1.33)
	Average ± standard deviation (for samples 111A to 120A)	- 20.65 (± 6.49)	- 23.11 (± 1.11)

[1] group of samples where the identified species in the litter follows the C_3 photosynthetic pathway; [2] group of samples where the identified species in the litter follows the C_4 photosynthetic pathway

2.3.3. Carbon origin estimates in "cerrado 3"

We calculated the contribution of C_4-derived carbon in "cerrado 3" (Figure 2.3). The $\delta^{13}C$ values of the SOM in "cerrado 1" (Figure 2.1) were used as a reference for C_3

derived carbon. The calculated $\delta^{13}C$ value of the C_4-derived carbon was –13.17‰. The amount of C_4-derived carbon in the profile was of 8.68 g dm^{-3} in the topsoil (0-3 cm), increasing to 12.9 g dm^{-3} (3-5 cm), and decreasing gradually to 3.7 g dm^{-3} (90-100 cm) (Figure 2.3a). The C_4-derived carbon varied from 21.3 to 42.5% (average of 34.6%) of the total carbon along the profile (Figure 2.3b). The smallest value was found in the topsoil (21.3%). The highest relative contribution of C_4-derived carbon was observed between 15 and 45 cm depth (average of 40.5%). At 1 meter depth, still 27.6% of the total carbon came from C_4 plants.

2.4. Discussion

2.4.1. Organic carbon and nitrogen

Although "cerrado 1" showed a slightly higher quantity of litter than "cerrado 2", the difference was not significant because of the high variability of the measurements in the first plot (coefficient of variation of 0.33). The small difference in the vegetation composition of these plots (Figure 2.1; Tables 2.1 and 2.2) suggests a similar litter deposition and a low effect of fire in "cerrado 2".

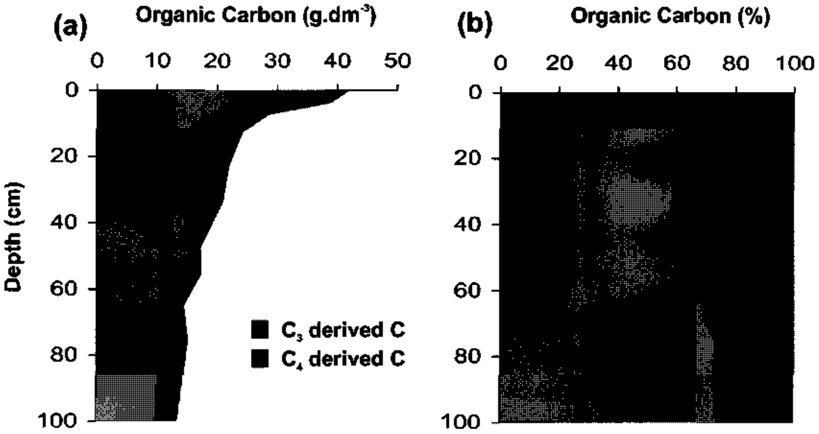

Figure 2.3. Distribution of organic C with depth in "cerrado 3": (a) expressed in g of C per dm^{-3} of soil; and (b) as a percentage of the total organic C.

The contents of OC and ON in the litter and topsoil samples were in accordance with previously reported data for cerrado areas (Adámoli et al., 1985; Pressada et al., 1996; Assad, 1997; Silva & Resck 1997). For the litter samples, the highest variability was associated to ON concentrations and may possibly be attributed to differences in nitrogen content of the different species. Miranda et al. (1997), for instance, found that the leaf nitrogen content of 44 arboreal species from the cerrado varied from 7 to 50 g kg^{-1} of dry matter.

In the topsoil samples, both OC and ON showed a higher variability than C:N ratio and $\delta^{13}C$. This suggested that in the topsoil the variability is more related to the spatial distribution of the total soil organic matter than to its nature (chemical composition).

The storage of C in the first 100 cm depth (195 ± 13 Mg ha^{-1}) was slightly higher than the values calculated from Sans (1986) for the same cerrado area (171 Mg ha^{-1}), and significantly higher than the 127.2 and 125.5 Mg ha^{-1} of OC found under a "cerradão" and a "cerrado sensu-stricto", respectively on a similar Oxisol from Central Brazil (Assad, 1997).

2.4.2. *$\delta^{13}C$ in litter and topsoil*

The variability of $\delta^{13}C$ measurements (related to aliquot preparation and analysis) was higher in litter (1 SD = 0.14‰) than in soil samples (1 SD = 0.07‰). However, both showed a smaller variability than the standard soil sample Labex 12 (1 SD = 0.17‰). Although the standard Labex 12 shows a high homogeneity in relation to other analytical determinations (e.g. total carbon and nitrogen, data not shown), it had a relatively high variability in $\delta^{13}C$ values compared to values of 0.07 ‰ reported by Balesdent & Mariotti (1996). Therefore, although the analytical precision of the pure-carbon standard sample GS7 measurement in our laboratory was 0.05 ‰, we estimate the analytical precision of the soil samples to be 0.17 ‰.

The variability of the $\delta^{13}C$ was low for litter and topsoil in "cerrado 1" and "cerrado 2". In average, the uncertainty of the $\delta^{13}C$ values in these plots was ± 0.5‰ for both litter and soil samples. Van Kessel et al. (1994) reported a similar variability of the isotopic composition of topsoil samples of (± 0.5‰) in an area of 2.25 ha of homogeneous vegetation. Balesdent et al. (1993) found a significantly higher variability of $\delta^{13}C$ (± 1.5‰) along a transect 2 Km long, in a homogeneous temperate forest. Although our plots were of only 100 m^2, the variability of $\delta^{13}C$ was comparable to these previous works. Despite the relatively high diversity of the vegetation composition in these plots (Tables 2.1 and 2.2), the predominance of C_3 plants gives a homogeneous isotopic composition for the carbon added to the system. In "cerrado 3", however, the variability was much higher than in the other two plots (±12.88 and ±1.58 ‰ for litter and topsoil, respectively), which is explained by the effect of C_4-derived carbon.

Under low fire intensity ("cerrado 1" and "cerrado 2"), the $\delta^{13}C$ of the litter and topsoil samples (Tables 2.5 and 2.6) showed a predominant contribution of C_3-derived material, with values typically observed in woodlands systems (Martin et al, 1990; Balesdent et al., 1993; Balesdent & Mariotti, 1996). Although the "cerrado sensu-stricto" is described as a mix of C_3 and C_4 plants (Eiten, 1972, 1992; Miranda et al., 1997), there is some evidence in the literature that it is a C_3-dominated vegetation. Pressada et al. (1996), studying a "cerrado sensu-stricto" with edaphoclimatic conditions very similar to that of the present study, compared the $\delta^{13}C$ value of SOM and charcoal to ^{14}C dates at various depths, and postulated that the vegetation has been dominated by C_3 plants at least for the last 12,000 years.

In "cerrado 3", variations in $\delta^{13}C$ values of litter and the topsoil could be directly related to the photosynthetic pathway of the species identified in the litter samples (Table 2.6). However, while the difference in $\delta^{13}C$ between C_3 and C_4 litter was 12.88‰, it was only 1.58‰ in the subjacent topsoil. This suggests that litter origin changed significantly locally (under C_4 vegetation), but the effect of C_4-carbon on the topsoil's SOM appears to be more evenly distributed. There are two possible explanations for this phenomenon: (i) the high content of charcoal observed in the topsoil (data not shown) may be predominantly derived from C_3-plant material; and (ii) a large portion of the SOM has a slow turnover, so that it remains dominated by C_3-derived carbon, even after C_4-plant colonisation. Because C_4 plants do not have woody tissue, charcoal is dominated by C_3 material. At the same time, the high clay content of the studied soil (600 g kg^{-1}) may increase SOM stability, since clay-bound organic matter is relatively recalcitrant (high turnover time) and usually represents the major carbon pool in most soils (Christensen, 1992; Feller & Beare, 1997; Shang & Tiessen, 1997). In fact, clay-bound organic carbon (OC) was dominant in "cerrado 1" topsoil (Chapter 3).

The $\delta^{13}C$ values in "cerrado 1" and "cerrado 2" showed a increasing trend from litter to topsoil (Table 2.5 and 2.6) and downwards in the profile (Figure 2.2a). Similar trends were reported for several profiles under C_3 vegetation (Vitorello et al., 1989; Balesdent et al, 1988; Nadelhoffer & Fry, 1988; Balesdent et al., 1993; Balesdent & Mariotti, 1996; Boutton, 1996; Pressada et al., 1996). There are three possible explanations for such enrichment with depth (Nadelhoffer & Fry, 1988; Balesdent et al., 1993; Balesdent & Mariotti, 1996): (i) the decrease in $^{13}C/^{12}C$ isotope ratio of atmospheric CO_2 over the last 150 yrs as a result of fossil fuel burning and additional biosphere mineralization; (ii) the difference in decomposition rates of organic compounds with different $\delta^{13}C$ signature; and (iii) the fractionation during the microbial decomposition of organic compounds, leading to high $\delta^{13}C$ values for microbial products compared to plant material. In "cerrado 3", however, under C_3 plants the difference between litter and topsoil is also related to the incorporation of some C_4-derived carbon, and under C_4 plants the higher value for litter than for topsoil samples is probably associated to the dominant effect of the C_3-carbon signature as discussed above.

Although the vegetation composition of "cerrado 1" and "cerrado 2" slightly differed from each other (Figure 2.1; Tables 2.1 and 2.2), some residual C_4-derived organic material (from possible C_4 invasion as a consequence of the two fires registered) may be responsible for the higher $\delta^{13}C$ values in "cerrado 2". However, the average difference between $\delta^{13}C$ values of the two plots through the profile (0.73‰) was only slightly higher than the standard deviation of the topsoil samples (0.57 and 0.65‰ for "cerrado 1" and "cerrado 2", respectively), suggesting that this difference may be also a result of spatial variability.

In "cerrado 3", the $\delta^{13}C$ values throughout the profile (0-100 cm) were in average 3.72‰ and 2.99‰ higher than in "cerrado 1" and "cerrado 2", respectively. These differences were too large to be attributed to spatial variability and were clearly related to the influence of C_4-derived carbon.

2.4.3. Carbon origin estimates in "cerrado 3"

The increase of grass population in "cerrado senso-stricto" vegetation as a consequence of fire is well documented, although its dynamics is still unclear (Eiten, 1972, 1992; Rosa, 1990; Miranda & Klink, 1996a, 1996b). The impact of this change in grass population is not always accompanied by a change in photosynthetic pathway of the vegetation, since *Ecnolaena inflexa* (a C3 grass) is dominant in some cerrado areas (Klink & Solbrieg, 1996; Miranda & Klink, 1996a, 1996b). In "cerrado 3", some *Ecnolaena inflexa* was observed, but the grass basement was widely dominated by two exotic C_4-grasses, *Brachiaria spp.* and *Melinis minutiflora* (Table 2.3), and the litter $\delta^{13}C$ was typical of C_4-derived material (Table 2.7). For this reason, the majority of the carbon input from the grass population was considered to be C_4-derived carbon. In addition, this C_4-derived carbon can be assumed to be young carbon, since the grass population increased only in the last 21years. Based on this assumption, and knowing that the total OC content to 1 meter (195 Mg ha^{-1}) did not differ between the plots (Figure 2.3b), we postulate that at least 34.6% of the total OC has a turnover time smaller than 21 years. This represents an amount of 67.5 Mg ha^{-1} of carbon that can be lost in a relatively short period of time, if the land use system adopted for this environment does not provide sufficient replacement of organic carbon.

According to Figure 2.3, the smallest replacement of C_3-derived carbon by C_4-derived carbon happened in the topsoil. This seems to be related to the high quantity of C_3-charcoal in this horizon (as discussed above). The high percentages of C_4-derived carbon at deeper horizons suggest that the major contribution of C_4-derived material to SOM came from below ground carbon production (Figure 2.3). In fact, it was observed in the field that the amount of roots was significantly higher in "cerrado 3" profile than in the other plots (Figure 2.1). According to Assad (1997) the native cerrado grasses concentrate their root system in the first 50 cm depth. However, under *Brachiaria sp.* pasture, they found an abundant and homogeneous root distribution until 1.75 m depth. In "cerrado 3", native grasses showed a low density and the dominant grasses were *Melinis minutiflora* and *Brachiaria spp.* (Table 2.3), which means that a large root density extended to considerable depth can be expected in this soil.

Martin et al. (1990) studied the gradual changes in $\delta^{13}C$ of SOC in a savanna from Ivory Coast with an increasing C_3-plants population as a result of protection against fire. They observed a significant reduction in $\delta^{13}C$ until 1m deep and the highest replacement of C_4-derived carbon by C_3-derived carbon in the topsoil. Then, according to Martin and co-workers' data, above ground litter seams to be the major source of new carbon in a savanna system, where a C_4-dominated vegetation is replaced by a C_3-dominated one. According to our results, the below ground carbon production appears to be the major source of new SOC in systems where a C_3-dominated savanna is replaced by a C_4-dominated one, as a consequence of increasing fire incidence.

5. Conclusion

Under low fire incidence ("cerrado 1" and "cerrado 2"), the $\delta^{13}C$ of litter and topsoil in the studied "cerrado sensu-stricto" was typical of C_3-dominated vegetation and the variability was relatively small, with standard deviations of about 3 times the analytical precision of measurements (1 SD = 0.17‰). Under high fire incidence ("cerrado 3"), the variability was much higher due to C_4-derived carbon input.

The higher fire incidence in "cerrado 3" increased the C_4-plant population and, consequently, the $\delta^{13}C$ of SOC in the first 100 cm depth. In "cerrado 2", the fire intensity was not sufficient to significantly change the vegetation dominance (C_3-plants), and the $\delta^{13}C$ depth profile.

Increasing fire incidence for 21 years in "cerrado 3" decreased the amount of carbon and nitrogen stored in litter by 3703 ± 930 and 104 ± 26 kg ha^{-1}, respectively, but no change in the contents of these elements was observed in soil. The amount of carbon (195.0 ± 8.2 Mg ha^{-1}) and nitrogen (13.4 ± 1.0 Mg ha^{-1}) in soil (to 1 m depth) did not differ between the plots.

Nevertheless, the SOM origin in the first meter of depth changed significantly in 21 years under high fire incidence. In this period, 34.6% or 67.5 Mg ha^{-1} of the C_3-derived carbon was replaced by C_4-derived carbon, suggesting a rapid turnover of part of the SOM in this soil despite its elevated clay content. In the studied system, the high C_4-

root production was sufficient to replace this relatively labile carbon. If the land-use system adopted on this type of soil is not able to provide sufficient replacing-carbon, significant losses will occur in a short period of time.

We also observed that changes of vegetation composition as a function of fire could significantly interfere in the establishment of a reference $\delta^{13}C$ profile and, consequently, generate misinterpretations in studies of SOM dynamics.

Chapter 3

Disruption of soil aggregate by different amounts of ultrasonic energy in SOM fractionation of a clay Latosol: carbon, nitrogen, and $\delta^{13}C$ distribution in particle-size fractions

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Abstract

Ultrasonic energy has been widely used to disrupt soil aggregates before fractionating soil physically when studding soil organic matter (SOM). Nevertheless, there is no consensus about the optimum energy desirable to disrupt the soil. We therefore aimed (i) to quantify the effect of different ultrasonic energies on the recovery of each particle-size fraction and their C, N, and $\delta^{13}C$ *distribution, and (ii) to determine an ideal energy to fractionate SOM of a specific soil. Our results show that the 2000-100 µm particle-size fraction was composed mainly of unstable aggregates and the 100-2 µm fraction of stable aggregates. Energies of 260-275* $J\ ml^{-1}$ *were sufficient to disrupt most of the unstable aggregates and leave stable aggregates. The use of this threshold energy combined with particle-size fractionation was not satisfactory for all proposes, since litter-like material and relatively recalcitrant organic carbon present in stable aggregates > 100 µm were recovered in the same pool. An ultrasonic energy of 825* $J\ ml^{-1}$ *was not sufficient to stabilize the redistribution of soil mass and organic matter among particle-size fractions, but at energies above 260-275* $J\ ml^{-1}$ *relatively stable aggregates would fall apart and cause a mix of carbon with varied nature in the clay fraction.*

3.1. Introduction

The Dark Red Latosol covers more than 360 thousands km^{-2} or about 18% of the total area of Brazilian Cerrado (Adámoli et al. 1985). Originally under savanna vegetation, these soils have been rapidly reclaimed for agriculture, what may profoundly impact their carbon balance. Muggler (1998) observed that aggregation (specially micro-aggregates) can be very strong in these soils, due to high iron oxides content. The high stability of aggregates will affect SOM dynamics and distribution among particle size fractions. Understanding the dynamics of aggregate disruption is crucial for using particle size fractionation to elucidate SOM dynamics in this iron-rich environment.

Physical fractionation of soil organic matter (SOM) has provided useful information about its dynamics in natural and agricultural environments. Density and particle-size analysis have been used individually or together to separate SOM with significant differences in chemical composition, location in the soil matrix, and degree of association with clay particles (Christensen, 1992; Feller & Beare, 1997). In combination with carbon isotopic analysis, physical fractionation has provided insight into the dynamics SOM (Balesdent & Mariotti, 1996).

When organic matter is fractionated according to particle-size classes, large aggregates of the soil have to be disrupted to separate primary particles from aggregates of the same size (Christensen, 1992). The energy input has to be sufficiently large to disrupt large secondary aggregates into primary particles and primary aggregates (mainly strong organo-mineral complexes). However, if too much energy is used then plant debris (fraction >50 μm) may be broken down, resulting in a transfer of C from coarse to fine fractions (Balesdent *et al.*, 1991). Since the dynamics of SOM may be strongly related to its location in, and degree of association with, soil particles (Christensen, 1992), an energy just sufficient to disrupt unstable aggregates but leave stable aggregates intact seems to be desirable.

Edward & Bremner (1967a,b) first introduced the concept of micro- and macro-aggregates, suggesting a limit at 250 μm to distinguish them. Further studies consolidated their concept and showed that macro-aggregates were less stable than micro-aggregates and, therefore, were more sensitive to environmental changes (Tisdall & Oades, 1982; Elliott, 1986). Elliott (1986) and Christensen (1992) suggested that SOM associated with macro-aggregates was more easily decomposable than that present in micro-aggregates. From this concept, it follows that the optimum energy input level for particle-size fractionation of SOM is the quantity sufficient to disrupt macro-aggregates into micro-aggregates and primary particles. Thus, SOM associated with micro-aggregates (theoretically more recalcitrant and stable) will be removed from the coarse particle-size fractions, leaving in these fractions only the relatively easily decomposable litter-like organic particles. Christensen (1992), after a comprehensive review on physical fractionation of soil for SOM studies, proposed that the energy of maximum disruption of the sand-size aggregates (beyond which no further change occurs in the recovered sand-size fraction) should be used as the optimum for fractionating SOM. Christensen's approach is somewhat related to the aggregate hierarchy theory (Edward & Bremner, 1967a,b), since the largest changes in sand-size fraction due to increasing energy input probably come from disruption of macro-aggregates.

Ultrasonic vibration has been widely used in particle-size determinations and clay separation since the pioneer work of Edward & Bremner (1967a,b). More recently, ultrasonic energy has been applied to determine aggregate stability, and to disrupt soil aggregates before SOM fractionation in particle-size classes (Christensen, 1992). The greatest advantage of ultrasonication is the possibility to quantify the amount of energy put into the soil suspension (North, 1976; Christensen, 1985, 1992; Raine & So, 1993, 1994). However, despite its widespread use in soil studies, there is no standard procedure for ultrasonication (Christensen, 1992; Schmidt *et al.*, 1999). This makes the comparison between different studies difficult. A first attempt to standardize the energy input in ultrasonic disruption was made by North (1976).

North (1976) emphasized that the power output of most sonicators differs from the real power applied to the suspension during operation. Therefore, to normalize a procedure for ultrasonic disruption, the equipment has to be calibrated. North (1976) proposed to quantify the real ultrasonicator power output calorimetrically, by measuring the temperature increase due to ultrasonic emission of a certain mass of water per unit time. The method was slightly modified after critical remarks by Koenigs (1978), but it constitutes the basis of later studies (Christensen, 1992; Raine & So, 1993, 1994; Schmidt *et al.*, 1999).

Carbon and nitrogen contents usually increase with decreasing particle-size class (Christensen, 1992; Feller & Beare, 1997). In most of the soils studied, clay-size contains the majority of the C and N, although some workers found most C and N in the silt-size fraction (Christensen, 1992; Feller & Beare, 1997). However, the comparison of reported results is frustrated by the use of different class limits (mainly of the silt class) and the common omission of the energy used to disrupt the aggregates (Christensen, 1992; Schmidt et *al.*, 1999). Gregorich *et al.* (1988) showed that with increasing ultrasonic energy, the amount of C and N recovered in the clay fraction tends to increase. So unless the energy input is standardized, the pools of SOM associated with particle-size fractions have little meaning.

Many researchers have used the natural abundance of ^{13}C in plant residues as a tracer to elucidate SOM dynamics in particle-size separates (Balesdent & Mariotti, 1996). Even in ecosystems dominated by C_3 plants, the $^{13}C/^{12}C$ ratio tends to increase with decreasing particle size, as a result of the humification process (Balesdent & Mariotti, 1996). However, scant attention has been given to possible variation in $\delta^{13}C$ signature of a fraction as a consequence of the amount of energy used in the fractionation.

We have therefore studied ultrasonic fractionation of soil (i) to quantify the effect of different ultrasonic energies levels (calibrated calorimetrically) on the recovery of each particle-size fraction and their C, N, and $\delta^{13}C$ distribution, and (ii) to determine an ideal amount of energy for fractionating the SOM in a specific soil.

3.2. Materials and Methods

The soil used in this experiment was a Dark Red Latosol (Typic Haplustox) from the Cerrado region in Brazil. Topsoil samples were collected from 0 to 5 cm depth in a native reserve of cerrado (savanna vegetation) at the EMBRAPA-CPMS (Maize and Sorghum Research Centre – Brazilian Institute of Agricultural Research), in Sete Lagoas-MG. The vegetation was dominated by C_3 plants, and no addition of C_4 plant material was detected in the litter and soil profile (to 1 m depth) (Roscoe et al., 2000). General soil characteristics are given in Table 3.1.

Table 3.1. Chemical, physical and mineralogical properties of the topsoil (0-5 cm) of a Dark Red Latosol from EMBRAPA-CNPMS, Sete Lagoas, Brazil.

Depth	pH_{water}	pH_{KCl}	OC[a]	ON[b]	C:N	$CEC_{pH7.0}$	Sand	Silt	Clay
/cm			------/g kg^{-1}----			/$cmol_c$ kg^{-1}	---------/g kg^{-1}---------		
0-5	5.1	4.1	32.5	2.4	13.5	16.15	100	278	622

	Total content[c]					Clay minerals[d]			
Depth	SiO_2	Al_2O_2	Fe_2O_3	TiO_2	P_2O_5	Ka.	Gb	Hm	Gt
/cm	--------------------/g kg^{-1}--------------------					----------------/g kg^{-1}----------------			
0-5	242.9	272.7	129.2	5.8	2.2	530	89	56	80

[a] OC – organic carbon concentration; [b] ON – organic nitrogen concentration; [c] total content after H_2SO_4 digestion; [d] Ka – kaolinite; Gb – gibbsite; Hm – hematite; Gt – goethite.

3.2.1.Sonicator Calibration:

The sonicator used was a Vibracell probe-type model VC100, with a probe of 115 mm length and 13 mm diameter, operating at a frequency of 20 kHz. Three combinations of probe depth and output control position were tested: (i) 15 mm (depth) and position 60; (ii) 15 mm (depth) and position 70; (iii) 30 mm (depth) and position 90.

To calibrate the sonicator, the real power output was calculated for the three combinations described above by the methodology adapted from North (1976) as described by Morra *et al.* (1991), using the equation:

$$P_c = \{(m_w c_w + C_{cont})\, \Delta T/t_s\} + H \qquad (1)$$

where P_c is the calculated power (W), m_w is the mass of water (g); c_w is the specific heat of water (4.186 J g^{-1} $^{o}C^{-1}$), C_{cont} is the heat capacity of the container (J $^{o}C^{-1}$); ΔT is the temperature change (^{o}C), t_s is the time of sonication (s), and H is the rate of energy loss (J s^{-1}).

The container used for the measurements was a centrifuge tube of 200 ml, placed inside a polystyrene container of 10 cm x 10 cm x 15 cm, covered by a polystyrene lid of 10 cm x 10 cm x 5 cm (Figure 3.1). Through a small orifice in the lid, the probe of a digital thermopar thermometer was introduced to measure temperature change.

To measure the heat capacity of the container (C_{cont}), the mixture method according to Duncan & Starling (1939, cited by Raine & So, 1993) was used. About 50 g of distilled water was heated to 50-60 ^{o}C, the exact mass (m_1) of water and temperature (T_1) was recorded. This mass of water was quickly added to the container that had a previous ± 50 g of distilled water (m_2) at room temperature (T_2), and the lid replaced immediately. The equilibrium temperature was recorded (T_3) and the heat capacity was determined by equation (2) (Morra *et al.*, 1991; Sá, 1998):

$$C_{cont} = \{m_1 c_w\, (T_1 - T_2)/(T_3 - T_2)\} - (m_2 c_w) \qquad (2)$$

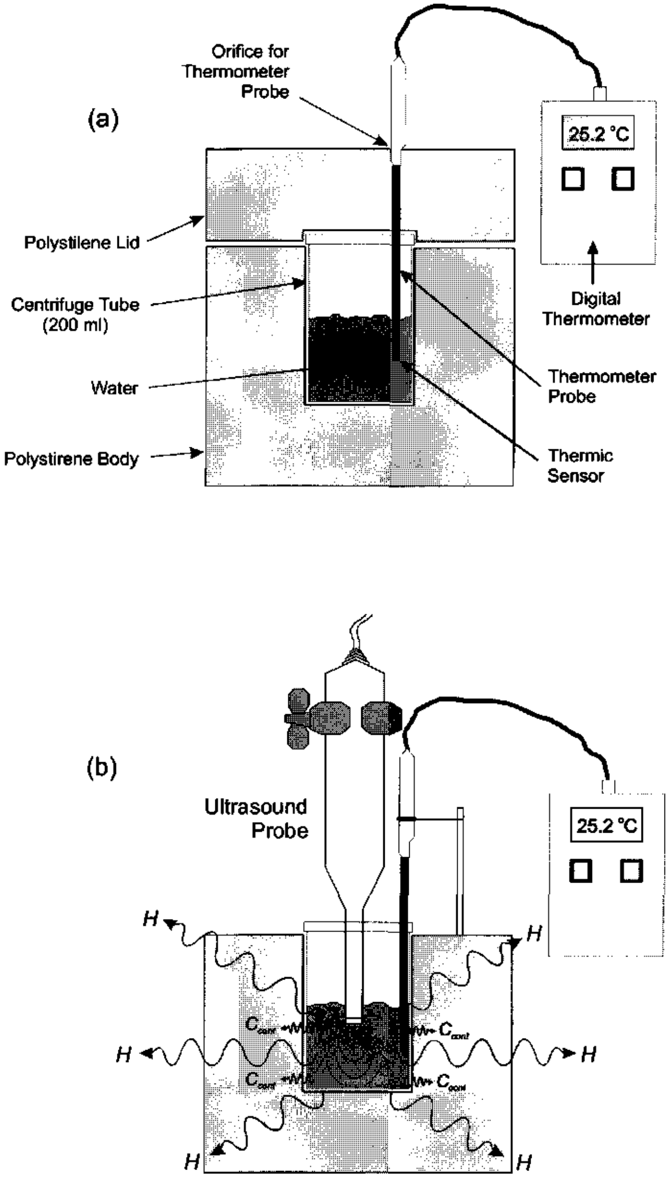

Figure 3.1. Schematic representation of the calorimeter used to calibrate the ultrasound equipment: (a) during the determination of the heat capacity of the container (C_{cont}); and (b) during the quantification of energy output of the ultrasound equipment. Arrows in (b) represents the energy loss (H) and the absorption of energy by the container or the heat capacity of the container (C_{cont}).

where C_{cont} and c_w are as above, m_1 is the mass of water (g) heated to T_1, m_2 is the mass of water (g) at T_2, and T_3 is the final equilibrium temperature of the water in the container (°C). The heat capacity was measured ten times, giving an average and standard deviation of 73.14 ± 2.86 J $°C^{-1}$.

The rate of energy loss (H) from the system was measured by heating about 100 g of water (m_w) to ± 50 °C in the container using the ultrasonic probe. After that, the system was left at room temperature (21 ± 1°C), and the water temperature was recorded each 10 seconds during the first 3 minutes, each 30 s until 60 minutes, and each 5 minutes until 3 hours. The rate of energy loss was calculated by:

$$H = \{(m_w c_w)\ \Delta T/t_d\} \tag{3}$$

where H , c_w, and m_w are as above, ΔT is the temperature change of 1°C, and t_d is the time necessary to decrease temperature by 1°C.

The energy loss, H, was calculated for each temperature interval of 1°C and plotted against temperature. There was a linear decrease in energy loss with decreasing temperature ($H = 0.129\ T - 3.22$, $r^2 = 0.99$). In contrast, North (1976) considered the energy loss to be constant with temperature. However, variations in energy loss as a function of temperature have been reported in several works (e.g. Christensen, 1985). Sá (1998) showed that, for his container, the energy loss in relation to temperature was exponential: $H = 0.0078 \exp(0.1131\ T)$, $r^2 = 0.98$. The isolation capacity of the container strongly determines the energy loss of the system and probably explains the different results in literature. North's (1976) and Sá's (1998) containers showed a better isolation than ours (smaller H). Since the isolation of the container is sufficient to maintain a low variability of the measurements (small effect of external temperature fluctuations), differences in the pattern of energy loss are not relevant. We used the fitted value of H to calculate the power output (P_c) in equation (1), and found little variation for the three settings tested (Table 3.2), suggesting that the container was sufficiently isolated. We used a P_c value of 34.39 ± 2.06 W (probe depth of 30 mm and setting position 90) for further particle-size dispersion.

The applied energy, E_a, was expressed in J ml^{-1}, as proposed by Christensen (1985) and calculated by:

$$E_a = P_c \cdot t_s / V \tag{4}$$

where P_c is the calculated power (W), t_s is the time of sonication (s), and V is the volume of suspension in ml.

3.2.2. Particle-size fractionation:

Soil samples were collected, air-dried, and passed through a 2-mm sieve. Before particle-size fractionation, light SOM was removed twice by flotation in water. This procedure was adopted to avoid redistribution of plant debris among the fractions as a consequence of breakdown by ultrasonic energy (Balesdent *et al.*, 1991). The light SOM fraction was recovered, dried, and weighed. The remaining soil was then oven-dried (60°C for 2 days) and stored at room conditions. Sub-samples of 10 g (oven-dry base) were placed in a 200 ml centrifuge tube with 100 ml of distilled water and submitted to the different sonication treatments. Sand size fractions (> 50 μm) were separated by wet-sieving. The sand fraction was then fractionated by dry-sieving into sand 1 (2000-250 μm), sand 2 (250-100 μm), and sand 3 (100-50 μm). Clay and silt were separated by sedimentation by the method of Van Doesburg (1996).

Table 3.2. Nominal and calculated power output as a function of probe depth (mm) and the output setting of the Vibracell CS100 ultrasound processor. Values in brackets are the standard error (n=10).

Probe depth (mm)	Output Setting	Nominal Power[a] /W	Calculated Power /W
15	60	22	21.40 (0.37)
15	70	23	24.25 (0.48)
30	90	25	34.39 (0.65)

[a] Power output shown in the equipment screen.

3.2.3. Recovery of particle-size fractions:

Curves of weight recovery of each particle-size fraction were accumulated using the values of applied energy per ml, calculated by equation (4), against the amount of sand 1 (2000-250 μm), sand 2 (250-100 μm), sand 3 (100-50 μm), silt (2-50 μm), and clay (< 2 μm) fractions. In the disruption procedures we used five different periods of sonication: 180, 300, 600, 900, 1200, and 2400 seconds. Using a *Pc* of 34.39 ± 2.06 W (Table 3.2) and 100 ml of soil suspension (10 g of soil in 100 ml of water) the equivalent energies from equation (4) were respectively: 62, 103, 206, 310, 413, and 825 J $\mathrm{ml^{-1}}$. We performed the measurements for each energy in triplicate. Temperature was kept below 35°C during sonication to avoid reduction in cavitation (Christensen, 1985). The power input was sufficiently large to homogenize the suspension during the procedure, and stirring was not used.

3.2.4. Disruption index (DI)

We calculated the same disruption index (D_1) by equation (5) used by Sá (1998) and an alternative dispersion index (D_2), equation (6) at each energy (see the 'Results and discussion'). We plotted both dispersion indices as a function of *E*a (J $\mathrm{ml^{-1}}$) and fitted a non-linear model composed of two partial linear regressions. The data were divided in two groups, with a minimum of two energy levels (9 observations) in the smallest group. Linear equations were fitted for each of the two groups. In the total, three combinations were tested: (i) two energy levels in the first group and four in the second; (ii) three energy levels in each group; and (iii) four in the first and two in the second. We used the least-squares method to choose the better combination of the two linear equations. The model's ANOVA was calculated using the sum of the variation of the two individual linear models. The coefficient of determination (r^2) of the model was calculated dividing the variation due the model by the total variation. The significance was tested by the F test.

$$D_1 = 100 \times (\text{clay} + \text{silt}) / (\text{clay} + \text{silt} + \text{sand 1} + \text{sand 2} + \text{sand 3}) \qquad (5)$$

D_2 = 100 x (clay + silt + sand 3)/ (clay + silt + sand 1 + sand 2 + sand 3) (6)

3.2.5. C, N, and $\delta^{13}C$ analysis

Carbon and nitrogen were determined by flash combustion in an EA 1108 Elemental Analyser, as described by Van Lagen (1996). The ^{13}C abundance was determined as $^{13}CO_2$ after the conversion of total C to CO_2, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer at the Centre for Isotope Research at the University of Groningen. Results were expressed in $\delta^{13}C$ (‰), which represents the $^{13}C/^{12}C$ ratio (R_{sample}) of the sample in relation to the $^{13}C/^{12}C$ ratio of the VPDB standard ($R_{standard}$) (Craig, 1957, cited by Boutton, 1996), where $\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1000$. The three replicates of each particle-size fraction (for each energy level) were combined for analysis. Organic C and N, and $\delta^{13}C$ were determined in duplicate for each fraction and energy level.

We studied the trends of each of the analysed variables (organic C and N, C:N ratio, and $\delta^{13}C$) as a function of applied energy (Table 3.5 and Figures 3.4, 3.5, and 3.6). For this purpose, we tested the fitness of three different models:

$Y = b_0 + b_1X$,
$Y = b_0 + b_1 \ln X$, and
$Y = b_0 + X/b_1$.

To test whether the average values of organic C and N, C:N, and $\delta^{13}C$ for each fraction differed from each other, we used an one-way analysis of variances where the independent variable was the particle-size class. The different energy levels were used as 'pseudo-replicates', since real replicates were not available.

3.3. Results and discussion

3.3.1. Fraction recovery curves:

The boundaries selected between different particle-size classes were based on literature and previous observations. The fractionation of soil into sand (50-2000 μm), silt (50 – 2 μm), and clay (< 2 μm) has been conventionally used in studies of soil organic matter (Christensen, 1992). However, our subdivision of sand into three different fractions was based on the macro-micro-aggregate model (Edwards & Bremner, 1967a,b) for the 250μm boundary, and on laser-grain sizing results of Muggler (1998) for a similar soil and on our previous observations using the same technique for our soil, for the 100 μm boundary (data not shown). We observed that with increasing ultrasonic energy, aggregates larger than 80-100 μm tended to disrupt, transferring mass to smaller particle-size fractions.

The total recovery of soil after fractionation ranged from 86.3 to 100.1 %, with a mean of 95.3%. The recovery of each fraction was plotted against the applied ultrasonic energy (Figure 3.2). Inverse equations were fitted to the data of sand 1 (2000-250 μm) and sand 2 (250-100 μm), showing a sharp decline in the fractions recovered at small energies (Figure 3.2a and Table 3.3). With increasing applied energy, the rate of decline decreased, and only small changes occurred at energies exceeding 300 J ml^{-1}. Asymptotic values of the recovery of sand 1 and sand 2 fractions were 51.2 and 13.8 g per kg of soil, respectively. On the other hand, the recovery of sand 3 and silt (Figure

3.2b) decreased linearly with applied energy, but with relatively small slopes. The recovery of the clay size fraction (Figure 3.2c) increased steeply at small applied energies and stabilized asymptotically at large values.

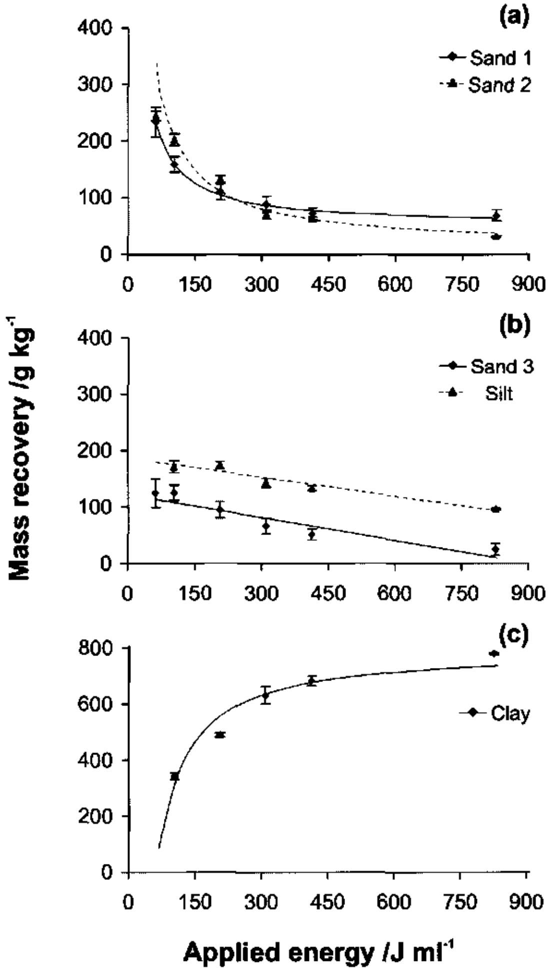

Figure 3.2. Mass recovery of each fraction as a function of applied ultrasonic energy, E_a, for (a) sand 1 (2000-250 μm) and sand 2 (250-100 μm), (b) sand 3 (100-50 μm) and silt (50-2 μm), and (c) clay (< 2 μm). The symbols represent the mean of observed values (n=3), the bars represent the standard error of the means, and the lines represent the fitted models (Table 3).

Morra *et al.* (1991) and Gregorich *et al.* (1988) also reported a similar trend of decreasing sand fraction and increasing clay fraction with increasing amounts of ultrasonic energy. Silt-sized aggregates, in contrast, showed no trend; they remained almost stable at all energies used. Gregorich *et al.* (1988) suggested that the constant mass of the silt-sized fraction was due to a balance between gain of material from sand and loss to clay fraction. Morra *et al.* (1991) postulated that in such case the diameter of silt-sized particles would change. However, they did not find any redistribution in the silt-sized fraction as determined by Coulter counter, and they suggested that the silt-sized aggregates represented a more stable assemblage of primary particles than aggregates in the sand-sized fraction.

Table 3.3. Relations between the recovery of sand 1 (2000-250 μm), sand 2 (250-100 μm), sand 3 (100-50 μm), silt (50-2 μm) and clay (< 2 μm) and the ultrasonic applied energy (E_a).

Fraction	Equation	Coefficient of Determination, r^2
Sand 1	$Y = 51.2 + 11165.9/E_a$	0.84
Sand 2	$Y = 13.8 + 20096.2/E_a$	0.95
Sand 3	$Y = 121.9 - 0.14 \times E_a$	0.81
Silt	$Y = 184.9 - 0.11 \times E_a$	0.81
Clay	$Y = 793.0 - 49168.7/E_a$	0.92

Figure 3.2 shows that sand fractions 1 and 2 lost most of their mass at fairly small ultrasonic energies. On the other hand, sand 3 and silt lost mass slowly and continuously during all the ultrasonic treatment. This suggested that the aggregates present in these two groups have a different stability against ultrasonic disruption, somewhat similar to the hypothesis of Morra *et al.* (1991), although the boundary between the fractions was different. Thus, we postulate that the aggregates in our soil can be divided into two groups according to their stability against ultrasonic energy: unstable (2000-100 μm), and stable (100-2 μm).

Our results confirm previous observations of Oades & Waters (1991) that no aggregate hierarchy can be applied to Oxisols if the boundary between micro and macro-aggregates of 250 μm (Edwards & Bremner, 1967a,b) is considered. However, a hierarchical differentiation is observed if a boundary of 100 μm is used.

3.3.2. Disruption Index

Based on North's (1976) method to calculate the optimum applied energy, Christensen (1992) proposed the energy of maximum disruption of the sand-size aggregates (represented by a stabilization in the proportion of sand-size particles recovered) as the ideal for SOM fractionation. Sá (1998) applied this concept and proposed a dispersion index, D_1, equation (5). Based on our results, we propose an alternative dispersion index for our soil, D_2, equation (6). The D_2 measures the mass of particles in the fraction smaller than 100 μm in relation to the total soil mass. This index defines the proportion of soil mass found in unstable 2000-100 μm aggregates. Plotting the calculated D's against the energy input, one gets a dispersion curve (North, 1976) in which the initial point of a linear, almost plateau region may represent the maximum disruption of the larger aggregates.

We plotted the calculated D_1 and D_2 against the applied energy and fitted the data with a non-linear model using the least-squares method (Figure 3.3). Neither of the two indices reaches a plateau. This means that aggregate disruption continues beyond the maximum energy used (825 J ml^{-1}), without reaching stabilization. The models fitted the data very well, showing coefficients of determination $r^2 = 0.93$ and $r^2 = 0.94$ for D_1 and D_2, respectively. The points of interception were at 276 and 260 J ml^{-1}, respectively. The rate of aggregate disruption (represented by the slope of the linear partial equations) sharply decreased after the points of interception for both indices. Considering 825 J ml^{-1} as the maximum of disruption (100 %), 78 and 83% of the aggregates larger than 50 and 100 μm respectively were disrupted at the intercept points.

Although a plateau was not reached, both indices showed a clear differentiation in the disruption of aggregates below and above the threshold energy, represented by the intercept point of the two partial linear regressions (Figure 3.3). An ultrasonic energy around 260-275 J ml^{-1} appeared to be appropriate to disrupt unstable aggregates in the soil studied.

The small difference between the two indexes was directly related to the sand 3 fraction (50-100 μm). Considering the decreasing rate of mass in this fraction, only about 26-28% of the disrupted aggregates disintegrated at less than 260-275 J ml^{-1} (Table 3.3). This suggests that the sand 3 fraction was dominated by stable aggregates.

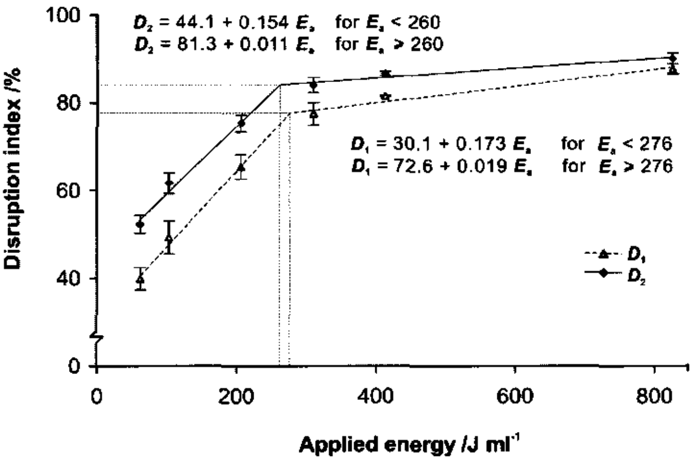

Figure 3.3. Disruption index D_1 and D_2 calculated according to equations (5) and (6), as a function of applied energy (E_a). The straight lines represent the estimated values of *D*s according to the models fitted to the data.

3.3.3. Carbon, Nitrogen, C:N ratio, and $\delta^{13}C$ distribution

The concentrations of organic C and N (in fractions and whole soil), C:N ratio, and $\delta^{13}C$ of litter, whole soil, light OM, and of the soil after removal of light OM can be seen in Table 3.4. The average recovery of organic C and N was 97 and 81%, respectively. About 30 and 20%, respectively, of the recovered organic C and N was in the light OM fraction. The C:N ratio and $\delta^{13}C$ of the light OM were intermediate between litter and soil, but closer to those of litter. The light OM had a composition similar to litter, since it comprised partly decomposed plants residues (Christensen, 1992).

Table 3.4. Concentration of organic carbon (OC) and nitrogen (ON), C:N ratio, and $\delta^{13}C$ of litter, whole soil, light organic matter (light OM), and soil after removal of light OM (Soil - light OM). Values in brackets are the standard error (n=3).

	Concentration /g kg^{-1} of fraction		Concentration /g kg^{-1} of whole soil		C:N	$\delta^{13}C$
	OC	ON	OC	ON	ratio	/‰
Litter	456.4	13.2	-	-	35.9	-27.84
	(4.6)	*(1.03)*				*(0.28)*
Whole soil	-	-	58.5	3.95	14.8	-25.88
			(0.3)	*(0.04)*		*(0.33)*
Light-OM	291.4	11.05	17.2	0.6 5	26.4	-27.04
	(11.1)	*(0.21)*	*(0.4)*	*(0.01)*		*(0.05)*
Soil – (light-OM)	-	-	39.8	2.53	15.7	-25.38
			(0.3)	*(0.08)*		*(0.08)*

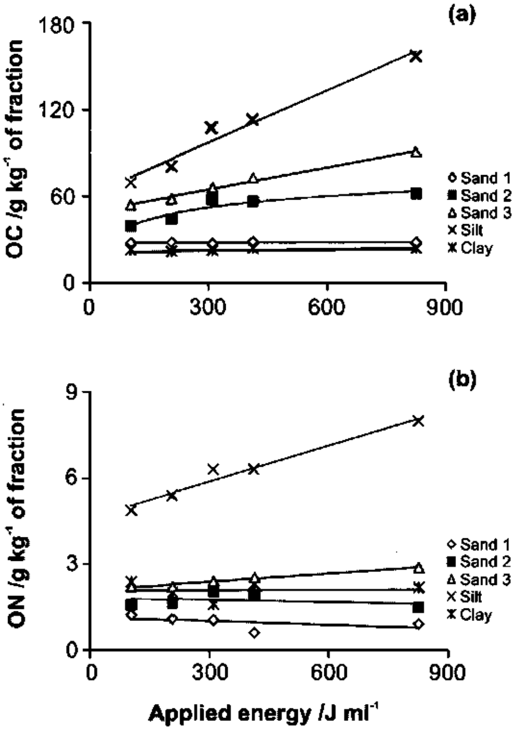

Figure 3.4. Effect of the ultrasonic applied energy on (a) the concentration of total organic carbon (OC), and (b) the concentration of total organic nitrogen (ON). Values are expressed as the mass fraction of OC and ON (g) per mass of each particle-size fraction (kg).

The $\delta^{13}C$ values significantly increased from litter to soil (Table 3.4). The $\delta^{13}C$ enrichment was 0.8, 2.04, and 2.45 ‰ from litter to light OM, whole soil, and whole soil minus the light OM, respectively. Since there was no change of vegetation composition with regard to photosynthetic pathway, this sequence suggested that there was an isotopic enrichment upon decomposition. The increase of $\delta^{13}C$ from litter to SOM has been reported in several works (Balesdent & Mariotti, 1996). Although the phenomenon is still unclear, it could be related to a selective persistence of compounds rich in ^{13}C or to an isotopic fractionation during microbial respiration (Balesdent & Mariotti, 1996).

Ultrasonic energy input had no significant effect on organic C in sand 1 (2000-250 μm) and clay (< 2 μm), on organic N in sand 1, sand 2 (250-100 μm), and clay (Table 3.5 and Figure 3.4). In sand 2, sand 3 (100-50 μm) and silt (50-2 μm) organic C, and in sand 3 and silt organic N increased significantly with energy input. The concentration of organic C increased by 56, 67 and 126% in sand 2, sand 3 and silt, respectively, from the smallest to the largest energy. The increase in organic N was 30 and 67% in sand 2 and silt, respectively.

The amounts of organic C and N in each particle-size class (expressed as the mass fraction of organic C and N per mass of whole soil) recovered with increasing ultrasonic energy can be seen in Figure 3.5a. The organic C and N concentrated in silt and clay fraction (Table 3.6). At the lowest energy (103 J ml^{-1}), up to 51 % of the organic C and 68% of the organic N was in the silt + clay fraction. At the highest level, 85 and 88% of the organic C and N, respectively, were in the same fractions. For energies less than 310 J ml^{-1}, the silt-size class held more organic C than the other fractions. However, at the larger energies (413 and 825 J ml^{-1}), the quantity of organic C in the clay-fraction surpassed that in the silt-size fraction. For organic N (Figure 3.5b) the same behaviour was observed, but the organic N in the clay-fraction exceeded silt-sized N at the level of 206 J ml^{-1}. Gregorich *et al.* (1988) observed a similar pattern for organic C distribution, although the clay-sized fraction exceeded the silt-sized fraction only at an energy of 1000 J ml^{-1}.

Under increasing ultrasonic energy input, organic C and N content decreased in the larger fractions (sand 1, sand 2, and sand 3), and increased in the clay fraction (Table 3.5 and Figure 3.5). The silt-sized fraction, however, showed a different pattern. The organic C content (Figure 3.5a) increased to 310 J ml^{-1}, and after that tended to stabilize close to the asymptotic value of 16 g kg^{-1}. The organic N content did not show any trend with increasing energy (Figure 3.5b and Table 3.5). Gregorich *et al.* (1988) and Morra *et al.* (1991) reported a similar behaviour in the sand and clay fractions of different soil types, but not in silt-sized fractions. The persistence of large amounts of organic carbon in the silt-sized fraction and its relatively stability suggest that this fraction was probably composed of micro-aggregates strongly stabilized by organic matter.

Figure 3.6 shows the values of C:N ratio and $\delta^{13}C$ for each fraction as a function of applied energy. The C:N ratio (Figure 3.6a and Table 3.6) tended to decrease with particle-size, suggesting that a more humified material was present in finest fractions. Clay-sized fractions had the smallest ratios, followed by silt-sized fractions, and finally by sand-sized fractions. The C:N ratio in sand-sized fractions tended to increase with ultrasonic energy (Table 3.5 and Figure 3.6a), suggesting that a more humified material, probably associated to clay particles and silt-sized stable aggregates, was removed. A less humified and more litter-like material (large C:N ratio, Table 3.4) tended to accumulate in these coarse fractions. Another possibility was the accumulation of large charcoal fragments containing little N in those fractions. Although the disruption characteristics of the sand 3 (100-50 μm) and silt fractions were quite similar (Figure 3.2b), these two fractions showed a significant difference in C:N ratio (Table 3.6 and Figure 3.6a). Sand 3 had a C:N ratio more similar to the other sand fractions, suggesting that the organic matter present in these fractions was comparable.

The $\delta^{13}C$ values were larger in the clay fraction than in the other fractions at all energies (Table 3.6 and Figure 3.6b). With increasing energy, the $\delta^{13}C$ value in the silt and sand fractions decreased logarithmically (Table 3.5 and Figure 3.6b). In the clay fraction, $\delta^{13}C$ was almost stable, showing a slightly linear decrease at larger inputs of energy. This suggested that the organic matter leaving the coarse fractions and accumulating in clay fractions had $\delta^{13}C$ values very close to those of this fraction. We also observed that this transferred organic matter had a small C:N ratio, suggesting a high degree of humification. These results support the hypothesis of Nadelhoffer & Fry (1988) that isotope fractionation during decomposition results in an accumulation of ^{13}C in more humified material.

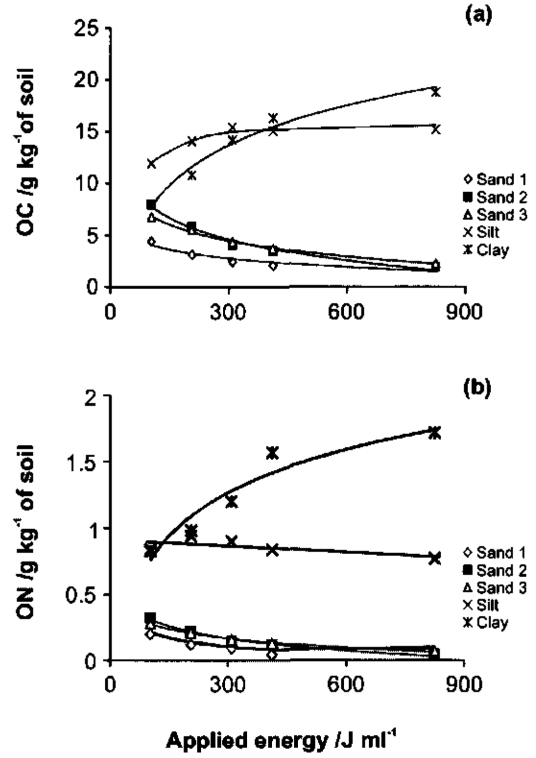

Figure 3.5. Effect of the ultrasonic applied energy on (a) the concentration of total organic carbon (OC), and (b) the concentration of total organic nitrogen (ON). Values are expressed as the mass fraction of OC and ON (g) per mass of total soil (kg).

Table 3.5. Effect of ultrasonic applied energy (E_a) on the contents of OC and ON, expressed as the mass fraction of organic carbon (OC) and nitrogen (ON) (g) per mass of each particle-size fraction (kg) and per mass of total soil (kg); and on C:N ratio and δ^{13}C (‰).

Fraction[1]	Equation	Coefficient of Determination, r^2
	OC /g kg^{-1} of fraction	
Sand 1	-	-
Sand 2	$Y = -13.6 + 11.5 \ln (E_a)$	0.88
Sand 3	$Y = 49.1 + 0.052 (E_a)$	0.99
Silt	$Y = 61.0 + 0.120 (E_a)$	0.97
Clay	-	-
	ON /g kg^{-1} of fraction	
Sand 1	-	-
Sand 2	-	-
Sand 3	$Y = 2.10 + 0.001 (E_a)$	0.97
Silt	$Y = 4.63 + 0.004 (E_a)$	0.97
Clay	-	-
	OC /g kg^{-1} of total soil	
Sand 1	$Y = 1.46 + \{306.9/(E_a)\}$	0.99
Sand 2	$Y = 21.56 - 2.97 \ln (E_a)$	0.98
Sand 3	$Y = 17.22 - 2.27 \ln (E_a)$	0.99
Silt	$Y = 16.09 - \{417.7/(E_a)\}$	0.92
Clay	$Y = -17.93 + 5.54 \ln (E_a)$	0.98
	ON /g kg^{-1} of total soil	
Sand 1	$Y = 0.24 + \{18.3/(E_a)\}$	0.93
Sand 2	$Y = 0.92 - 0.132 \ln (E_a)$	0.98
Sand 3	$Y = 0.75 - 0.102 \ln (E_a)$	0.99
Silt	-	-
Clay	$Y = -1.43 + 0.47 \ln (E_a)$	0.92
	C:N ratio	
Sand 1	$Y = 3.42 + 4.121 \ln (E_a)$	0.87
Sand 2	$Y = 5.36 + 4.064 \ln (E_a)$	0.98
Sand 3	$Y = 8.10 + 3.407 \ln (E_a)$	0.99
Silt	$Y = 0.97 + 2.773 \ln (E_a)$	0.95
Clay	-	-
	δ^{13}C /‰	
Sand 1	$Y = -24.66 - 0.277 \ln (E_a)$	0.83
Sand 2	$Y = -23.42 - 0.484 \ln (E_a)$	0.97
Sand 3	$Y = -23.22 - 0.544 \ln (E_a)$	0.84
Silt	$Y = -23.61 - 0.487 \ln (E_a)$	0.99
Clay	$Y = -24.00 - 0.0003 (E_a)$	0.88

[1] the fractions are sand 1 (2000-250 µm), sand 2 (250-100 µm), sand 3 (100-50 µm), silt (50-2 µm) and clay (< 2 µm);

To compare the transfer of soil mass and carbon between fractions, we used 260-275 J ml^{-1} as the threshold energy between unstable and stable aggregates. The difference in mass recovery of each fraction (Figure 3.2) between the energy levels of 310 and 825 J ml^{-1} showed that about 6 % of the total soil mass was transferred to the clay fraction from the courser fractions. At the same time, 12 % of the total carbon (Figure 3.5a) was transferred from the sand fractions to the clay fraction. The mass of soil and carbon transferred to the clay fraction resulted from the disruption of fairly stable aggregates present in the coarse fractions. If the threshold energy of 260-275 J ml^{-1} was used for particle-size fractionation of the SOM, at least 41% of the organic carbon present in the coarsest fractions (2000-100 μm) would be still associated with relatively stable aggregates. As the carbon in these stable aggregates was mainly present as organic matter bound to clay, the SOM associated to that coarse fraction is a combination of young litter-like material and relatively old recalcitrant organic matter. Organic carbon of different characteristics in the same pool is likely to lead to misinterpretation of the dynamics of soil organic matter. Christensen (1992) recommended that all the secondary aggregates be disrupted to primary particles and strong organo-mineral complexes. For this reason, larger energies should be used in the fractionation of organic matter in particle-size separates. The disadvantage of using such large energies is a possible mix of carbon originally belonging to unstable and stable aggregates in the clay fraction.

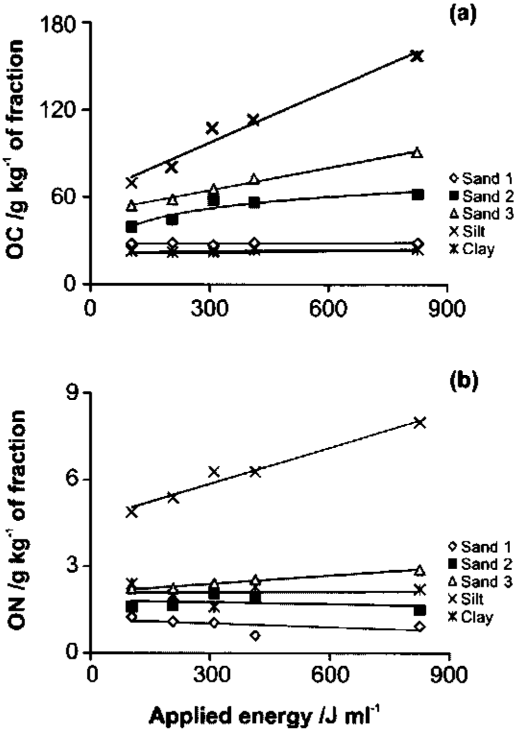

Figure 3.6. Effect of the ultrasonic applied energy on (a) C:N ratio, and (b) δ^{13}C (‰).

Table 3.6. Mean values of the contents of organic carbon (OC) and nitrogen (ON), expressed as the mass fraction of OC and ON (g) per mass of each particle-size fraction (kg) and per mass of total soil (kg), and on C:N ratio and $\delta^{13}C$ (‰) for the different particle-size fractions. Values in brackets are the standard error (n=5).

Fractions	Contents in fraction		Contents in soil		C:N ratio	$\delta^{13}C$
	OC	ON	OC	ON		
	---g Kg^{-1} of fraction--		---g Kg^{-1} of total soil-			----‰---
Sand1	2.77 b[1]	0.10 c	6.99 b	4.12 c	26.84 a	- 26.23 b
(2000-250 μm)	*(0.46)*	*(0.02)*	*(1.21)*	*(1.13)*	*(1.53)*	*(0.11)*
Sand2	4.64 b	0.17 c	11.74 b	6.76 c	28.48 a	- 26.17 b
(250-100 μm)	*(1.27)*	*(0.04)*	*(2.72)*	*(1.93)*	*(1.43)*	*(0.17)*
Sand3	4.50 b	0.17 c	11.36 b	6.74 c	27.48 a	- 26.32 b
(100-50 μm)	*(0.78)*	*(0.04)*	*(2.04)*	*(1.50)*	*(1.19)*	*(0.21)*
Silt	14.30 a	0.86 b	35.89 a	33.64 b	16.74 b	- 26.38 b
(50-2 μm)	*(0.60)*	*(0.04)*	*(1.39)*	*(1.65)*	*(0.98)*	*(0.17)*
Clay	13.58 a	1.26 a	34.01 a	48.75 a	10.74 c	- 24.12 a
(< 2 μm)	*(1.95)*	*(0.17)*	*(4.70)*	*(5.69)*	*(0.38)*	*(0.04)*

[1] means followed by the same letter in the columns did not differ from each other according to the Tukey's test at a significance level of 0.05.

3.4. Conclusions

In the soil studied the particle-size fraction between 2000-100 μm was composed mainly of unstable aggregates and the 100-2 μm fraction by stable aggregates. However, the silt-sized (50-2 μm) fraction showed a persistent content of organic matter, with a fairly small C:N ratio, suggesting that this fraction was stabilized by a recalcitrant organic material, distinct from that in the sand 3 fraction (100-50 μm). Sand 3 seemed to be an intermediary fraction, composed dominantly by stable aggregates, but also by some litter-like material or perhaps charcoal in small quantity.

The threshold ultrasonic energy for the disruption of unstable aggregates was about 260-275 J ml^{-1}. However, 41% of the carbon recovered in the coarse fractions (2000-100 μm) at 310 J ml^{-1} was still associated with stable aggregates. Thus, although the differential disintegration of unstable and stable aggregates is desirable in studies of SOM dynamics, the use of this threshold energy combined with particle-size fractionation is not satisfactory: litter-like material and relatively recalcitrant organic C present in stable aggregates may be recovered in the same pool.

The maximum amount of ultrasonic energy used in this study (825 J ml^{-1}) was not sufficient to reach a stabilization of soil mass and organic matter recovery in the particle-size fractions. On the other hand, the results indicate that energies exceeding 260-275 J ml^{-1} will disrupt fairly stable aggregates and may promote the mix of carbon with different natures in the clay fraction, as suggested by the C:N ratio and $\delta^{13}C$ changes.

Chapter 4

Soil organic matter dynamics in density and particle size fractions as revealed by the $^{13}C/^{12}C$ isopotic ratio in a Cerrado's Oxisol

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Abstract

In order to better understand the dynamics of soil organic matter SOM in Oxisols and the impact of converting native cerrado (savannah) into pasture, we studied the dynamics of different physically separated SOM pools at different depths in a cerrado Oxisol (Typic Haplustox), under natural conditions and after 23 years of cultivated pasture (Brachiaria spp.) via the replacement of the native C (C_3-derived) by pasture C (C_4-derived). Organic C stocks of the original cerrado (15 ± 3 kg m^{-2}) and pasture (17 ± 3 kg m^{-2}) were not significantly different, which was attributed to the high biomass production of the tropical grasses and the protective effect of the high clay content (> 800g kg^{-2}). We observed that 89-91% of the total organic C accumulated in the clay + silt fraction. The replacement of cerrado-derived C by pasture-derived C was in average 36%, 34%, and 19% for A_p, AB_1, and B_{w2} horizons respectively, suggesting a fast turnover rate of organic C regardless of the high clay content. The replacement decreased in the order: free low-density organic matter (LDOM) > heavy fractions (sand, silt, clay) > occluded-LDOM. The lower replacement of the occluded-LDOM compared to the heavy fractions was attributed to protection inside aggregates and to a possible accumulation of C_3-derived charcoal (black carbon). After 23 years of pasture, about 50% of the total organic C in the free-LDOM in the topsoil was still from cerrado, indicating that a significant part of this fraction was relatively recalcitrant. Charcoal fragments observed in the fraction suggested that the recalcitrance was probably due to charred material.

4.1. Introduction

As a consequence of food and energy demand, and governmental incentives, reclamation of native cerrados (savannahs) for agriculture and pasture in the Central Region of Brazil increased substantially in the last three decades (Macedo, 1995). In 1995, the Cerrado Region (24.4 % of Brazilian territory) was already responsible for about 25% of the national grain production and sheltered 40% of the cattle flock (Macedo, 1995). Considering the large environmental and socio-economic importance of the region, the consequences of land-use changes in this ecosystem must be addressed and better understood.

Cerrado soils (mostly Oxisols) are well known for their stable microstructure (Muggler et al., 1997). However, heavy mechanisation and high levels of fertiliser/lime application may substantially decrease soil organic matter (SOM) contents, macro-aggregation, and soil porosity (Neufeldt et al., 1999; Resck et al., 2000). Losses of up to 80% of the total SOM in 5 years of soybean cultivation were already reported (Silva et al., 1994). These losses are mainly related to changes in carbon input and quality, nutrient status and soil structure (Resck et al., 2000). On the other hand, pasture and no-tillage may have a minimal impact or even increase SOM contents (Campos et al., 1995; Corazza et al., 1999). Aggregation and porosity might also be maintained and improved by no-tillage (Campos et al. 1995; Resck et al., 2000) and pasture (Westerhof et al., 1999; Neufeeldt et al, 1999).

Location in the soil matrix and degree of association with mineral particles strongly affects the dynamics of SOM (Schulten & Leinweber, 2000). Density and particle-size separation have been used to obtain SOM fractions related to these two aspects: location (inside or outside aggregates) and association with mineral particles (bound or not to clay, silt and sand) (Christensen, 1992).

Carbon isotopic composition has been successfully used in studies of SOM dynamics (Balesdent & Mariotti, 1996). The $^{13}C/^{12}C$ ratio of plants that follow the C_3- and C_4-photosynthetic pathways strongly differ, providing a natural tracer when a C_3 vegetation is substituted by a C_4 one (Balesdent & Mariotti, 1996). Cerrado vegetation presents a large variety of physionomic forms, varying from grassland to woodland savannahs (Eiten, 1972). Therefore, cerrado vegetation would be a mix of C_3 and C_4 plants, making it difficult or even impossible to establish ^{13}C reference profiles. There is increasing evidence, however, that cerrado "sensu-stricto" is a C_3-dominated vegetation (Pessenda et al., 1996; Miranda et al., 1997; Chapter 2). This allows the use of the isotopic approach for assessing SOM dynamics, when a cerrado "sensu-stricto" is converted into C_4-pasture.

In order to better understand the dynamics of SOM in Oxisols and the impact of converting native cerrado into pasture, we studied the dynamics of different physically separated SOM pools at different depths in a cerrado Oxisol (Typic Haplustox), under natural conditions and after 23 years of cultivated pasture (*Brachiaria spp.*) via the replacement of the native C (C_3-derived) by pasture C (C_4-derived).

4.2. Material and methods

4.2.1. Site description

The study was carried out at the Maize and Sorghum National Research Centre – Brazilian Institute of Agricultural Research (EMBRAPA-CPMS), Sete Lagoas-MG, Brazil. The area is located at latitude 19°26'S and longitude 44°10' W, and has a mean altitude of 730 m. The mean annual temperature is 22.1°C, and annual precipitation is 1340 mm, with a dry season from April to September. Two representative sites were selected in a homogeneous Dark Red Latosol (Typic Haplustox) soil unit. Site 1 was a reserve of native "cerrado sensu-stricto" vegetation, dominated by C_3-plants (Chapter 2). Site 2 was an adjacent cultivated pasture, where the native cerrado was replaced by *Brachiaria spp.* 23 year before sampling. In the last year, however, the pasture was ploughed (10 cm) and cultivated with millet. The possible influence of the millet cropping would be restricted to the upper soil layer (Ap horizon). At the sampling time (two months after harvest), *Brachiaria spp.* covered about 60% of the soil. As millet is also C_4 plant, we neglected its influence and considered the last year as equivalent to the previous ones. Duplicate profiles were described per site and sampled to 1m depth (Table 4.1).

4.2.2. Soil fractionation

SOM was separated by density and particle-size fractionation. Air-dried and sieved (< 2 mm) soil samples of the Ah, AB_1, AB_2 and Bw_2 horizons (Site 1), and Ap, AB, and Bw_2 horizons (Site 2) were fractionated in triplicate (see Table 4.1 for depths). Low-density organic matter (LDOM) was separated by flotation using a NaI solution (1.7 g cm^{-3}) before and after aggregate disruption, according to Gregorich & Ellert (1993). In short, 20 g of sample was placed in a 200ml centrifuge tube with 100 ml of NaI solution, gently shaken by hand, and left standing at room temperature for about 15 minutes. After centrifugation (15 min, 3500 rpm), the supernatant was filtered through a membrane filter (Whatman GF/A) into a millipore vacuum unit. The fraction recovered on the filter was washed with 0.01 M $CaCl_2$ solution (100ml) and distilled water (200ml). This fraction was called "free-LDOM". The sediment was re-suspended in NaI and centrifuged for two more times as described above. The three sub-fractions were joined, oven-dried at 50°C, and stored for analysis. The sediment was re-suspended and ultra-sonicated at 270 J ml^{-1}, with a Vibracell VCX 600 probe-type model, calibrated calorimetrically as described in Chapter 3. The centrifugation and filtration procedure was repeated three times as described above and the fraction recovered from the supernatant was referred as "occluded-LDOM". The heavy fraction (sediment) was washed one time with 0.01 M $CaCl_2$ and about 10 times with distilled water until the clay fraction remained in suspension after 24 hours. The volume was adjusted to 100ml and 10ml of NaOH 1M were added to the suspension as dispersant (to a final concentration of 0.1 M) as recommended for clayey Oxisols (EMBRAPA, 1997). Finally, the sand fraction (2000 - 50 μm) was separated by wet-sieving (50 μm), and the silt (50 - 2 μm) and clay (< 2 μm) by sedimentation according to van Doesburg (1996).

4.2.3. Organic C and $\delta^{13}C$

Organic C was analysed for all samples and fractions, using an Interscience Elemental Analyser EA1180. ^{13}C abundance was determined after the conversion of total C to CO_2, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer at the Centre for Isotope Research at the University of Groningen, the Netherlands. Results were expressed as $\delta^{13}C$ (‰). For the calculations, we used a mass balance equation (1), according to Balesdent & Mariotti (1996):

Table 4.1. Soil properties for different horizons of a Typic Haplustox under cerrado and pasture.

Sites	Hor.	Depth Range	Soil Bulk Density	Texture^{a} Clay	Texture^{a} Silt	Texture^{a} Sand	OC	TN	Clay Mineralogy^{b} Ka	Clay Mineralogy^{b} Gb
		---/cm---	/g cm^{-3}	---------/g kg^{-1}---------					------------	
Cerrado 1	A_h	0-3	0.68	870	50	80	64.7	5.9	++++	+
	AB_1	3-17	0.82	870	50	80	34.6	1.9	++++	+
	AB_2	17-40	0.80	880	40	80	26.7	1.7	++++	+
	B_{w1}	40-67	0.78	na	na	na	22.0	1.6	na	na
	B_{w2}	67-100+	0.86	900	30	70	17.9	1.3	++++	+
Cerrado 2	A_h	0-2	0.63	870	50	80	61.4	6.1	++++	+
	AB_1	2-13	0.75	870	50	80	31.0	2.3	++++	+
	AB_2	13-28	0.81	900	20	80	24.1	2.1	++++	+
	AB_3	28-57	0.81	na	na	na	23.0	1.5	na	na
	B_{w1}	57-82	0.83	na	na	na	17.7	1.4	na	na
	B_{w2}	82-110+	0.89	900	30	70	15.9	1.2	++++	+
Pasture 1	A_p	0-8	0.84	880	40	80	32.3	2.6	++++	+
	AB_1	8-24	0.89	na	na	na	30.6	2.5	++++	+
	AB_2	24-52	0.90	880	40	80	25.1	1.9	na	na
	B_{w1}	52-75	0.85	na	na	na	20.1	1.9	na	na
	B_{w2}	75-120+	0.80	900	30	70	16.0	1.5	++++	+
Pasture 2	A_p	0-10	0.84	840	50	110	34.9	2.4	++++	+
	AB_1	10-25	1.01	na	na	na	28.5	2.3	++++	+
	AB_2	25-40	0.98	850	40	110	20.5	1.5	na	na
	B_{w1}	40-75	0.94	na	na	na	17.1	1.6	na	na
	B_{w2}	75-110+	0.83	900	30	70	12.9	1.1	++++	+

[a] clay (< 2μm), silt (2-50 μm), and sand (50-2000 μm); [b] Ka is kaolinite and Gb is gibbsite; na not analysed.

$$C_T \times \delta^{13}{}_T = C_{C3} \times \delta^{13}{}_{C3} + C_{C4} \times \delta^{13}{}_{C4} \qquad (1)$$

where C_T is the total soil organic carbon, C_{C3} is the soil organic carbon derived from C_3 vegetation, C_{C4} is the soil organic carbon derived from C_4 vegetation, $\delta^{13}{}_T$ is the $\delta^{13}C$ of total soil organic carbon, $\delta^{13}{}_{C3}$ is the $\delta^{13}C$ of soil organic carbon derived from C_3 vegetation, and $\delta^{13}{}_{C4}$ is the $\delta^{13}C$ of soil organic carbon derived from C_4 vegetation.

The reference values for C_3-derived carbon were those observed for the cerrado plot in all the depths and fractions. The reference $\delta^{13}C$ value for the C_4-derived carbon (C from pasture) was calculated for each depth and organic C fraction using the $\delta^{13}C$ of the pasture litter (-13,52 ± 0.07‰) and the same ^{13}C proportional discrimination (*D*) during the decomposition as observed in the C_3 reference profile, according to equation (2):

$$D = (\delta^{13}{}_{\text{litter}} - \delta^{13}{}_{\text{fraction}}) * 100 / \delta^{13}{}_{\text{litter}} \qquad (2)$$

where $\delta^{13}{}_{\text{litter}}$ is the $\delta^{13}C$ of the cerrado litter (-27.84 ± 0.50‰) and $\delta^{13}{}_{\text{fraction}}$ is the $\delta^{13}C$ of each fraction at a given depth.

4.2.4. Statistical analyses

Linear and non-linear models were fitted to the data of $\delta^{13}C$ and OC contents as a function of depth. The least square criterion was used to select the best-fitted model for each of these two parameters. Fitted curves for cerrado and pasture were compared by the F test at 5% of probability according to Snedecor & Cochran (1967). Stocks of organic C were calculated by integration of the fitted models to 1m depth.

4.3. Results

4.3.1. Organic C contents in soil and fractions

The contents of organic C with depth (Figure 4.1a) were calculated for each horizon using the values of soil bulk density and organic C concentration (g kg^{-1}) of Table 4.1. The models fitted for the two cerrado profiles did not differ significantly according to the F test at 5% of probability (Snedecor & Cochran, 1967). The same was observed for the two models fitted to the pasture data. Thus, one combined model for cerrado and one for pasture (Figure 4.1a) represented the distribution of organic C with depth. They were significantly different according to the F test at 5% of probability. The calculated C stocks to 1-meter soil depth were 15 ± 3 kg m^{-2} and 17 ± 3 kg m^{-2} (mean and 95% confidence interval) for cerrado and pasture, respectively.

The recovery of organic C after fractionation ranged from 86% to 100%, with an average of 93% (Table 4.2). The losses of organic C during the fractionation occurred due to (i) loss of material during the manipulation of the sample in the various steps, (ii) losses of LDOM that passed through the filter membrane (<1.6μm), and (iii) co-extraction of humic substances during the dispersion due in NaOH 0.1M. The possible co-extraction of humic substances was probably small since the samples were not shaken vigorously after the addition of the dispersing agent, and decreasing the pH to 3 with HCl 2M to flocculate the clay fraction re-precipitated the humic acids. Therefore, only small losses of fulvic acids would be expected. Most of the total loss, though, was probably related to manipulation of the sample through the fractionation (loss of particles).

The relative distribution of organic C among the fractions was similar for all profiles (Table 4.2 and Figure 4.2). Organic C concentrated in the clay fraction for all horizons of the profiles, except the A_h horizons of cerrado 1 and 2. The fraction of organic C present in the clay fraction in relation to the total organic C increased with depth from about 30% to 80%, mostly as a consequence of a decrease in light fractions (Figure 4.2).

We plotted the contents of organic C in the clay faction as a function of the total organic C in the soil (Figure 4.3). A quadratic model was fitted to the data. As the clay mineralogy was constant in the first meter (Table 4.1), the asymptotic value of 32.5 g of organic C kg^{-1} of clay can be considered as the value of carbon saturation of the clay fraction.

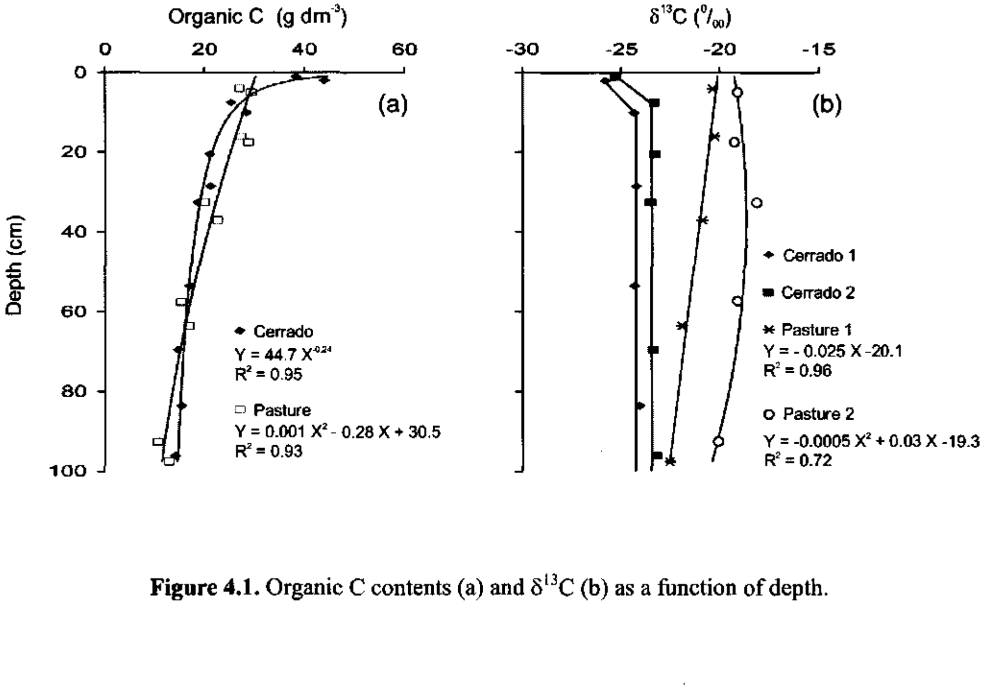

Figure 4.1. Organic C contents (a) and $\delta^{13}C$ (b) as a function of depth.

4.3.2. $\delta^{13}C$ of soil and fractions

In the cerrado profiles, $\delta^{13}C$ increased from A_h (0 – 2/3 cm) to AB_1 (2/3 – 13/17 cm) horizons and remained stable towards the bottom (Figure 4.1b). The increases were of 1.56‰ and 1.93‰, respectively for cerrado 1 and 2. The average stable values were –24.24 ± 0.06 ‰ (mean ± standard error) and –23.36 ± 0.05 ‰, for cerrado 1 and cerrado 2, respectively. In pasture 1, the values of $\delta^{13}C$ decreased continuously with depth. However, in pasture 2, the values first increased to a maximum at 35 cm and, then, decreased towards the bottom of the profile. A larger difference in $\delta^{13}C$ values was observed between pasture profiles than between cerrado profiles.

Table 4.2. Organic C contents in relation to total soil (g kg^{-1} of soil) for different horizons of each profile studied.

Sites	Hor.	Range	Fractions					OC
			Clay (< 2μm)	Silt (2-50 μm)	Sand (50-2000μm)	Occl-LDOM	Free-LDOM	Recovery
		---/cm---	---/g kg^{-1}---					--/ % --
Cerrado 1	A_h	0-3	22.0 ± 0.3^a	10.9 ± 0.3	6.2 ± 0.1	0.6 ± 0.1	24.8 ± 0.7	100
	AB_1	3-17	19.6 ± 0.3	4.8 ± 0.2	2.4 ± 0.1	0.4	3.8 ± 0.4	90
	AB_2	17-40	17.6	3.7 ± 0.1	0.9	0.1	1.2 ± 0.1	88
	B_{w2}	67-100+	13.7 ± 0.2	1.6	0.5	nd	0.3 ± 0.1	91
Cerrado 2	A_h	0-2	22.0 ± 0.2	11.1 ± 0.5	6.2 ± 0.1	1.0 ± 0.2	20.9 ± 0.1	100
	AB_1	2-13	19.1 ± 0.4	5.5 ± 0.2	2.8 ± 0.1	0.5	2.9 ± 0.1	100
	AB_2	13-28	15.9 ± 0.2	3.2	0.6	0.1	0.8 ± 0.1	86
	B_{w2}	82-110+	12.2 ± 0.1	1.4	0.5	nd	0.2 ± 0.1	90
Pasture 1	A_p	0-8	18.5 ± 0.6	6.6 ± 0.5	2.7 ± 0.1	0.3	2.2 ± 0.7	94
	AB_2	24-52	17.7 ± 0.1	3.3 ± 0.1	1.0	0.1	0.8 ± 0.2	91
	B_{w2}	75-120+	13.1 ± 0.1	1.4	0.4	nd	0.1 ± 0.1	94
Pasture 2	A_p	0-10	18.6 ± 0.4	7.0 ± 0.1	3.7	0.8 ± 0.1	3.6 ± 0.2	97
	AB_2	25-40	15.0 ± 0.1	2.3	0.5	0.1	0.4	90
	B_{w2}	75-110+	10.9 ± 0.1	0.9	0.3	nd	0.2	95

^{a} means ± standard errors (n=3); values of standard error smaller than 0.1 was omitted; nd not determined.

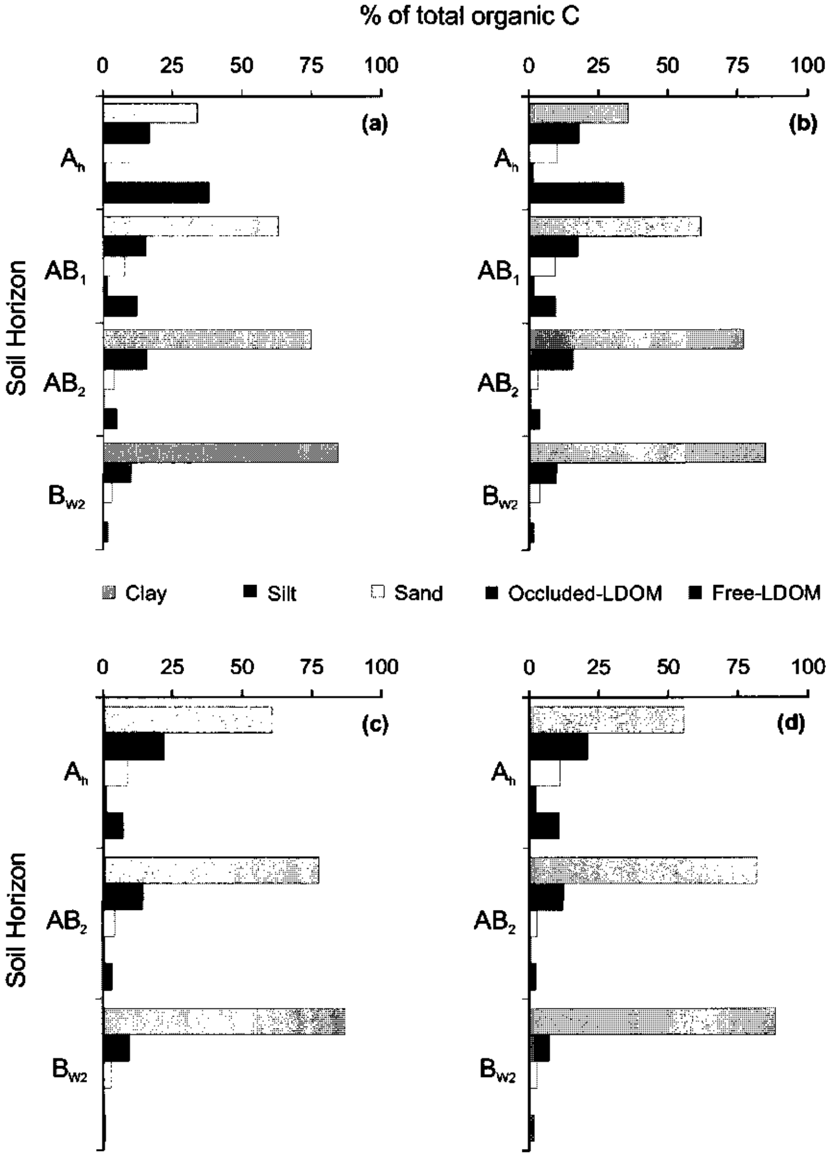

Figure 4.2. Organic C distribution (% of the total organic C) among the density and particle size fractions, in the different horizons of (a) cerrado 1, (b) cerrado 2, (c) pasture 1, and (d) pasture 2. The standard error due to fractionation was smaller than 1% of the total organic C for all the fractions and could not be represented.

Figure 4.4 shows the $\delta^{13}C$ values for each fraction of the different profiles as a function of depth. In cerrado profiles (Figure 4.4a and Figure 4.4b), the free- and occluded-LDOM tended to be depleted in ^{13}C compared to the other fractions and bulk soil. Those fractions showed $\delta^{13}C$ values closed to the litter of cerrado (–27.84 ± 0.50 ‰). The silt and sand fractions were in general slightly depleted and the clay fraction slightly enriched in ^{13}C in respect to the bulk soil. For all the fractions but free-LDOM, changes with depth were similar to those in the bulk soil. In pasture profiles (Figures 4c and 4d) the occluded-LDOM was remarkably depleted in ^{13}C compared to the other fractions. The free-LDOM tended also to be depleted in ^{13}C in the AB and B_{w2} horizons. In pasture 1, the heavy fractions showed $\delta^{13}C$ values very close to those of the total soil (Figure 4.4c). However, in pasture 2, the values of the heavy fractions were more spread and higher than in pasture 1 (Figure 4.4d).

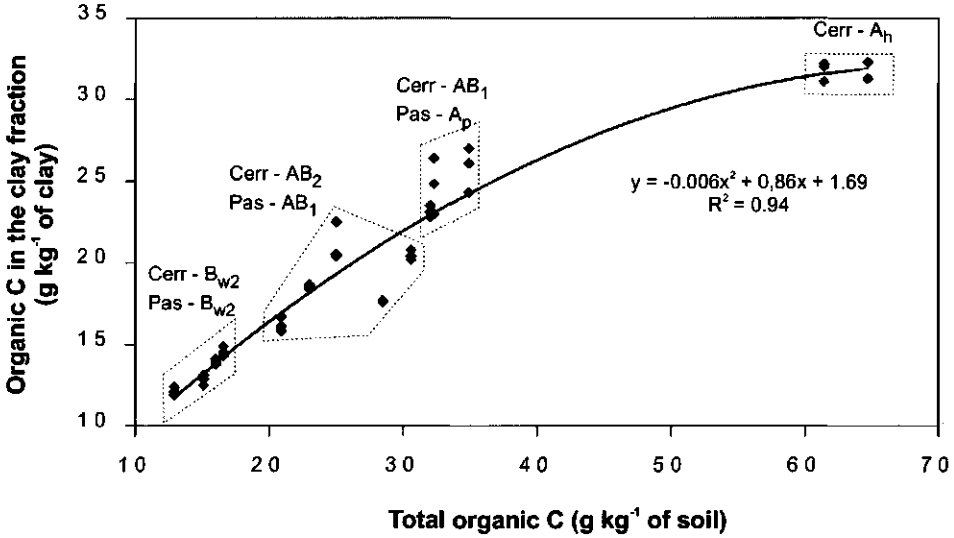

Figure 4.3. Organic C content in the clay-size fraction (g kg^{-1}) as a function of the total organic C content in the bulk soil (g kg^{-1}). Solid line represents the fitted quadratic model. Dashed lines group observed values by horizon (see Table 4.1) in cerrado (Cerr) and pasture (Pas).

4.3.3. Replacement of organic C

The percentage of organic C derived from pasture (C_4) and cerrado (C_3) present in the soil after 23 year of pasture was estimated for total SOM and for each density and particle-size fraction, using equations (1) and (2) (Table 4.3). Due to the availability of only two profiles per site, the variability of $\delta^{13}C$ values could not be estimated. To obtain a rough idea of the variability, we calculated a "replacement range". For the calculation of this "range", we compared data from cerrado 2 (Figure 4.4b) with pasture 1 (Figure 4.4c) and cerrado 1 (Figure 4.4a) with pasture 2 (Figure 4.4d). The first combination gave the lowest and the second the highest values of organic carbon substitution. In general, the substitution was higher in the topsoil, decreasing to deeper horizons. The occluded-LDOM exhibited the lowest replacement of cerrado organic C among all fractions. The heavy fractions (clay, silt and sand) showed a similar substitution in each horizon. In the A_p horizon, the free-LDOM showed a substitution higher than all other fractions. However, in the AB_2 horizon, the substitution was smaller than in the heavy fractions and, in B_{w2}, the replacement was similar to that of the heavy fractions.

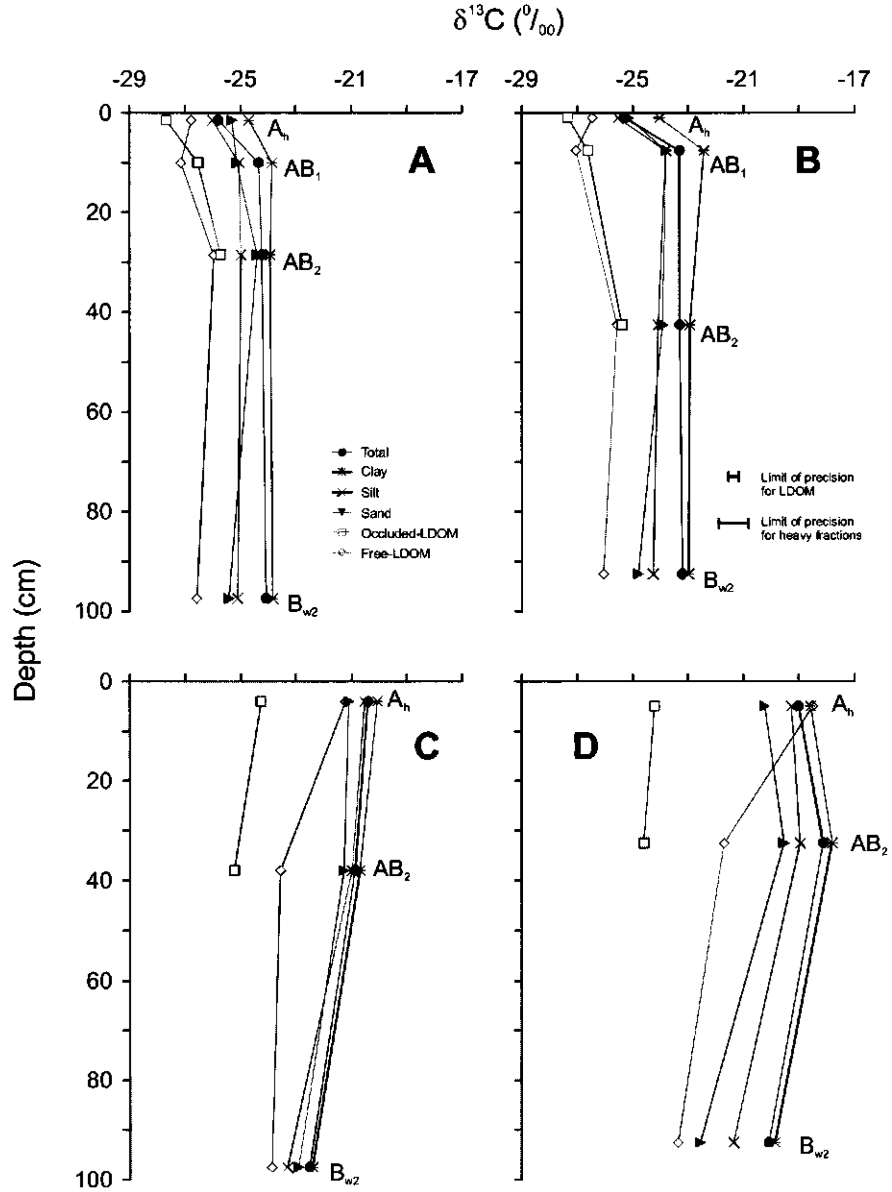

Figure 4.4. Values of $\delta^{13}C$ (‰) of the density and particle size fractions, in the different horizons of (a) cerrado 1, (b) cerrado 2, (c) pasture 1, and (d) pasture 2. The variability of $\delta^{13}C$ values for each fraction due to fractionation was close to the limit of precision for LDOM (0.17‰) and for all heavy fractions (0.50‰). These limits are shown in (b).

Table 4.3. Replacement range (%) of cerrado organic C by pasture organic C, in each fraction for different horizons of pasture.

Site	Hor.	Fractions					Bulk Soil
		Clay (< 2μm)	Silt (2-50 μm)	Sand (50-2000μm)	Occl-LDOM	Free-LDOM	
		/ %					
Pasture	A_p	24-44 (*34*)[a]	30-46 (*38*)	25-38 (*32*)	19 (*19*)	41-61 (*51*)	27-45 (*36*)
	AB_2	19-50 (*35*)	25-47 (*36*)	22-39 (*31*)	1-8 (*5*)	15-32 (*24*)	20-48 (*34*)
	B_{w2}	5-32 (*19*)	8-29 (*19*)	15-22 (*19*)	nd	16-24 (*20*)	6-32 (*19*)

[a] the lowest limits of replacement were calculated taking the values of $\delta^{13}C$ of cerrado 2 and pasture 1;

4.4. Discussion

4.4.1. Organic C contents in soil and fractions

The calculations based on the fitted models for each site (Figure 4.4.1a) suggested that no significant change in organic C stock occurred after 23 years of cultivated pasture. Nevertheless, a change of organic C distribution with depth was observed, which is probably due to a higher contribution of below (roots) than above ground litter in pasture. The high contents of clay in the studied soil (Table 4.1) may be responsible for the maintenance of the organic C stocks after conversion to pasture, due to the protective effect of clay (Hassink & Whitmore, 1997; Schulten & Leinweber, 2000). At the same time, the high biomass production (especially roots) of the tropical C_4-grass *Brachiaria spp.* (Corazza et al. 1999) was probably responsible for maintaining an organic C supply comparable to that in cerrado. Similar studies in cerrado have shown that cultivated pastures maintain or even increase the organic C stocks (Westerhof et al., 1999; Neufeldt et al., 1999; Carazza et al., 1999).

The relationship between organic C in the clay faction and the total organic C in the soil (Figure 4.3) suggested that the ratio of sorptive clay surface to "available" reactive organic matter increased with depth (Schulten & Leinweber, 2000). In the upper horizon of cerrado (A_p), the sorptive clay surfaces are probably saturated with organic C (close to the asymptotic value of 32.5 g of organic C kg^{-1} of clay), suggesting that all the other horizons (in both plots) still had available sorptive clay surface.

The tendency of clay saturation with organic C as suggested by our data (Figure 4.3) was in agreement with Hassink (1996), who showed that the physical capacity of soil to preserve SOM (defined as the maximum amount of C that can be associated with clay and silt particles) was limited. In a later study, Hassink & Whitmore (1997) developed a model, based on principles of adsorption-desorption kinetics, to study the capacity of soil to physically protect SOM against decomposition. The authors showed that, for a group of soils from the Netherlands covering a large textural range, the preservation of applied C was controlled by the degree of saturation of the clay- and silt-size fractions with SOM, rather than by soil texture per se. As suggested in Figure 4.3, most of the clay in the soil profile was unsaturated, which gave for the studied a relatively large potential for organic C preservation, if more reactive organic C became available (e.g. change in C supply due to change in vegetation).

4.4.2. $\delta^{13}C$ of soil and fractions

The cerrado profiles were typical for soils under C_3 vegetation, where $\delta^{13}C$ values of total soil usually increase with depth (Balesdent & Mariotti, 1996). This increase has been attributed to (i) isotopic discrimination during decomposition, (ii) differences in decomposition rates of organic compounds with different $\delta^{13}C$ signature, and (iii) decrease in $^{13}C/^{12}C$ isotopic ratio of atmospheric CO_2 over the last 150 year, as a result of fossil fuel burning and additional biosphere mineralization (Balesdent & Mariotti, 1996). However, the enrichment in ^{13}C with depth was observed only from A_h to AB_l. In AB_l, the lower amount of organic C in the LDOM than in the heavy fractions (Figure 4.2a and Figure 4.2b) indicated that most of the carbon was present as organo-mineral complexes. This suggested a high degree of humification already in this horizon. Since the studied soil is very homogeneous to 1-meter depth (Table 4.1), further enrichment in ^{13}C would not be expected. The same behaviour was reported for similar soils under cerrado (Pessenda et al., 1996).

The difference between the $\delta^{13}C$ values of the cerrado profiles was in average 0.88‰. This difference may be attributed to the variability of the organic material added because of two major evidences. First, the distribution of C among the fraction is very similar for both cerrado profiles (Figure 4.2) (if not, the variability could be the result of different distribution of C with different degrees of humification, since $\delta^{13}C$ tends to increase with decomposition). And second, the difference is slightly higher than the uncertainty of the $\delta^{13}C$ values of the litter (± 0.5‰) estimated in Chapter 2 for cerrado 1 and cerrado 2. In addition, as the difference is more pronounced for the heavy fractions than for the LDOM (Figure 4.4), the variability in the C added could also come from a previous addition of C_4-material during the accumulation process of the current SOM.

In pasture, the values of $\delta^{13}C$ were significantly higher than in cerrado throughout the profile and for all fractions (Figure 4.4). A decreasing trend in these values with depth was also observed. These results suggested that substantial addition of pasture organic C (richer in ^{13}C) occurred in all fractions and was highest in the first horizons. The difference between profiles was higher than for cerrado, which could be attributed to an additional source of variation, the distribution of pasture-derived material. Because of the relatively short period of time (only 23 years of pasture), we may not expect that this distribution is spatially homogeneous.

4.4.3. Replacement of organic C

Due to evident limitation of our data (based only in two observations per site), quantitative interpretations of the data should be handled with care. For this reason the replacement data was discussed in general terms and higher attention was given to differences between fractions.

In general, a high replacement of organic C from cerrado by pasture material was observed in the soil (Table 4.3), suggesting that organic C dynamic is relatively fast, notwithstanding the high clay content (Table 4.1). These findings were in accordance with previous studies suggesting a fast turnover rate of SOM in tropical Oxisols (Silva et al., 1994; Shang & Tiessen, 1997).

Only in the first horizon the free-LDOM showed a higher replacement of organic C than the heavy fractions. Higher turnover rates for free-LDOM than for heavy fractions have been widely reported in the literature (Gregorich & Ellert, 1993; Golchin et al., 1995). However, the replacement of about 50% of the topsoil in 23 years is relatively low for a fraction usually considered labile (Gregorich & Ellert, 1993; Golchin et al., 1995). Additionally, in the lower horizons, the free-LDOM showed replacements close to that of the heavy fractions and even lower. This suggests the presence of recalcitrant material in the fraction. Actually, optical microscopy indicated abundant fragments of charcoal in the free-LDOM throughout the pasture profile. This charcoal showed a $\delta^{13}C$ of $-27.94 \pm 0.29‰$ (average and standard error) and was probably responsible for a substantial part of the remaining cerrado carbon in this fraction. Cerri et al. (1985) also attributed the relative stability of the SOM associated to the 500-2000μm fraction of an Oxisol to the presence of charcoal debris left after burning. Similar observations were reported by Skjemstad et al. (1990) and Feller & Beare (1997).

The occluded-LDOM showed the lowest replacement of cerrado-derived carbon of all SOM fractions. Lower turnover rates of occluded-LDOM in relation to free-LDOM were extensively reported (Gregorich & Ellert, 1993; Golchin et al., 1995), but turnover rates lower than those of the heavy fraction have not been reported so far (Gregorich & Ellert, 1993; Golchin et al., 1995; Feller & Beare, 1997). We considered two explanations for the slower turnover of this fraction: (I) the occluded LDOM was chemically recalcitrant, or (II) its location in the soil matrix preserved LDOM against decomposition. The first explanation is at variance with reported evidence that occluded LDOM is usually less decomposed than the heavy fraction (Gregorich & Ellert, 1993; Golchin et al., 1995; Feller & Beare, 1997). Proof for a lesser decomposition of the occluded LDOM fraction is also found in its $\delta^{13}C$ values. The $\delta^{13}C$ values of the occluded material were lower than those of the heavy fractions and close to the typical values for C_3 litter (Figure 4.4). The fact that the values were not higher than those of C_3 litter suggests a low decomposition, because $\delta^{13}C$ values tend to increase with advancing decomposition (Balesdent & Mariotti, 1996). This is compatible with a relative abundance of recalcitrant charcoal in the occluded-LDOM fraction, similar to its occurrence in the free-LDOM. Recalcitrant components such as charcoal could be relatively accumulated in the occluded fraction, because the occlusion itself is due to the fact that the material probably past through the gut of mesofauna, which by digestion selectively removes more decomposable material.

The second possible explanation is the protection of occluded-LDOM due to its location in the soil matrix (Christensen, 1992; Gregorich & Ellert, 1993; Golchin et al., 1995; Hassink & Whitmore, 1997). In addition to the mechanism discussed above, this offers a good explanation of selective preservation of C_3 organic matter inside aggregates.

4.5. Conclusions

After 23 years of pasture, the levels of organic carbon in the first 100cm of the studied soil did not change significantly, partly due to the high biomass production of the tropical grasses, and especially to the protective effect of the high clay content. Nevertheless, a substantial replacement of the original organic C was observed, especially in the topsoil, which suggests a high turnover rate of C in this system notwithstanding its high clay content.

The lower replacement of organic C in the occluded-LDOM fraction was due to protection inside aggregates, in combination with a relative recalcitrance of this fraction due to the presence of charcoal from the C_3 vegetation. However, a significant influence of charred material would be expected only if a preferential accumulation (in relation to the other fractions) of charred material occurred in this fraction.

The relatively low substitution of organic C in the free-LDOM (after 23 year of pasture) showed that a significant part of this fraction was recalcitrant. The observation of charcoal fragments in the fraction indicated that this recalcitrant fraction was mainly charred material. These results suggested that, in cerrado soils, quantification of charcoal (black carbon) would be essential in fractionation schemes for SOM dynamics studies.

Chapter 5

Effects of fire and cultivation on $\delta^{13}C$ and soil organic matter spatial variability in a cerrado *sensu stricto* (Brazil)

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Abstract

We studied the spatial continuity of $\delta^{13}C$ *and other soil organic matter (SOM) related variables (organic C, total N, and C:N ratio) in a plot of native cerrado senso-stricto partially affected by fire and in a nearby cultivated area with neighbouring plots under conventional and no-tillage systems. Our aims were to describe the spatial variability of these properties in the cerrado, with especial attention to fire effects, and to analyse how the management systems affect their spatial structure. We used global, within strata, and stratified kriging to model the spatial variability in the areas. In cerrado, the total variability of all variables was relatively small, which was attributed to the high textural and mineralogical homogeneity of the clayey soil. Nevertheless, part of the variability was spatially structured. In cerrado locations with more open vegetation, long-term cumulative effect of repeated fires seemed to determine the spatial structure of* $\delta^{13}C$ *and SOM-related variables. Cultivation reduced the variability of most of the variables and changed their spatial structure. The variables tended to be less spatially structured in no-tillage than in conventional tillage, due to small-scale variability. The spatial structure observed for* $\delta^{13}C$ *in the cultivated area was probably inherited from the former cerrado vegetation. This implies that, in studies of SOM dynamics, the variability of the replacements would be overestimated if the trends in both areas were not taken into account.*

5.1. Introduction

The Cerrado Biome covers a vast area (about 2 million km^2) of the Central Brazil region. It is composed of different savannah types, ranging from grassland ("campo" forms) to woodland ("cerradão") (Ribeiro & Walter, 1998). As a result of increasing food demand and governmental incentives, large areas of cerrado have been quickly converted to agriculture and pasture during the last 30 years (Klink et al., 1993).

Soil organic matter (SOM) is an important component of Cerrado soils, especially because these soils are dominated by low-activity clays (kaolinite, gibbsite, and iron oxi-hydroxides) (Resck et al., 2000). Different management systems may strongly affect the dynamics of SOM. Conventional tillage usually has the highest impact, increasing the decomposition rate and, consequently, reducing SOM stocks. On the other hand, more conservative practices, as no-tillage or minimum tillage systems, may maintain or even increase SOM stocks in soils (Feller & Beare, 1997). Although information about the mean effect of such management systems on SOM has significantly increased in the last decades, very little is known about changes in its spatial variability (Souza et al., 1998).

The ^{13}C stable isotope is currently used as a tracer in SOM dynamics studies when a C_3-dominant vegetation is replaced by a C_4-dominant one or vice-versa (for a comprehensive review we refer to Balesdent & Mariotti, 1996). The technique enables estimation of turnover rates for total or specific pools of SOM at time scales varying from days to decades. Due to the high costs of the analysis, most of the studies are based on few "representative" samples and very little is known about the spatial variability of ^{13}C in soils (Veldkamp & Weitz, 1994).

The cerrado *sensu stricto* is a mixed vegetation composed of a herbaceous stratum of grasses (most of them C_4) and an arboreal stratum of trees and shrubs (all C_3) (Miranda et al., 1997; Ribeiro & Walter, 1998). The density of the arboreal stratum regulates the population of grasses. Therefore, the proportion of C_3 and C_4 plant material added to the soil may vary spatially, as a function of arboreal density. The variability in the density of this stratum is related to soil attributes (e.g. texture, mineralogy, effective depth, fertility), hydrology, and fire intensity (Ribeiro & Walter, 1998). On a given soil unit, where hydrology and soil attributes are homogeneous, fire may be the major factor in defining the vegetation density (Mistry, 1998; Ribeiro & Walter, 1998). Roscoe et al. (Chapter 2) observed that increasing frequency of anthropogenic fires over 21 years reduced the canopy density of a cerrado *sensu stricto* and increased the population of C_4 grasses. However, the natural frequency of fire in cerrado *sensu stricto* does not change the dominance of C_3 plants, as suggested by $\delta^{13}C$ values of SOM typical for soils under C_3 vegetation (Pesenda et al., 1996; Miranda et al., 1996; Chapter 2). Nevertheless, once C_4 plants tend to cluster in areas were the arboreal density is smaller (Mistry, 1998; Ribeiro & Walter, 1998), the $\delta^{13}C$ values of SOM may be highly spatially structured. Such spatial structure may affect the interpretation of SOM dynamics based on $\delta^{13}C$ values.

The objective of this paper was to study the spatial continuity of $\delta^{13}C$ and SOM-related variables (organic C, total N, and C:N ratio) in a plot of native cerrado *senso-stricto* partially affected by fire and in a nearby cultivated area with neighbouring plots under conventional and no-tillage systems. Our aims were to describe the spatial variability of these properties in the cerrado, with especial attention to fire effects, and to analyse how the management systems affect their spatial structure.

5.2. Material and Methods

5.2.1. The studied area

The study was carried out at the Maize and Sorghum National Research Centre – Brazilian Institute of Agricultural Research (EMBRAPA-CPMS), Sete Lagoas-MG, Brazil. The area is located at latitude 19°26'S and longitude 44°10' W, and has a mean altitude of 730 m. The mean annual temperature is 22.1°C, and annual precipitation is 1340 mm, with a dry season from April to September. In a homogeneous Dark Red Latosol (Typic Haplustox) soil unit, we selected an experimental field with two neighbouring treatments: conventional tillage (1 time disk plough + 2 times light disk harrow) and no-tillage (directly sown, without ploughing or harrowing) systems, and a nearby (1 km) plot of native vegetation. The latter was supposed to be representative of the former vegetation in the cultivated area. The vegetation was classified as cerrado *sensu stricto*, a dense savannah vegetation dominated by C_3 plants (Chapter 2). The conventional tillage (*CT*) plot was cultivated with maize/beans in succession for 30 years. The no-tillage (*NT*) plot was cultivated as the *CT* plot for the first 20 years and, another 10 years as a no-tillage system.

The cerrado area is part of a natural reserve and was protected against fire for the last 21 years. However, in this period, two accidental fires partially burned the area. Both fires reached only the right-side corner of the area (Figure 5.1a), but the exactly extension is unknown. These fires were of low intensity, with only the litter and herbaceous stratum completely burned. The dry season of 1999 was unusually severe which increased the susceptibility of the reserve to fire and one week before sampling a third fire burned the whole area for the first time in 21 years. This fire was also of low intensity. Parts of the litter layer remained unburned in locations close to the left-side corner (Figure 5.1a).

5.2.2. Sampling strategy

In the cerrado plot (*CER*) 29 samples were collected in an incomplete grid design, with sampling distances ranging from 5 to 210 m (Figure 5.1a). In the cultivated area, 49 soil samples were collected (24 in the no-tillage and 25 in the conventional tillage), also following an incomplete grid design, with sampling distances ranging from 6 to 120 m (Figure 5.1b). The sampling design was based on the optimisation procedure described by Van Groeningen & Stein (1998), where the maximum distance between an arbitrary point in the field and its closest point of the sampling scheme is minimised. Adaptations of the optimised sampling design were made according to field observations and limitations. In the cultivated field, samples were taken form 0 to 7.5 cm. In *CER* the sampling depth was from 2.5 to 10 cm. The upper A_h horizon (0-2.5 cm) was avoided because it is composed by a mixture of litter and loose soil which tends to disappear after the first year of cultivation, as consequence of ploughing. In addition, an accidental fire incorporated a large amount of charcoal and ashes into this layer one week before sampling.

We quantified the relative abundance of ^{13}C in the total organic C ($\delta^{13}C$) expressed in parts per mil (‰) in relation to the V-PDB international standard (Balesdent & Mariotti, 1996). Three SOM-related variables were also analysed: contents of organic C (C_m in g kg^{-1}), total N contents (N_m in g kg^{-1}), and C:N ratio. The descriptive statistics of the variables were summarised in Table 5.1.

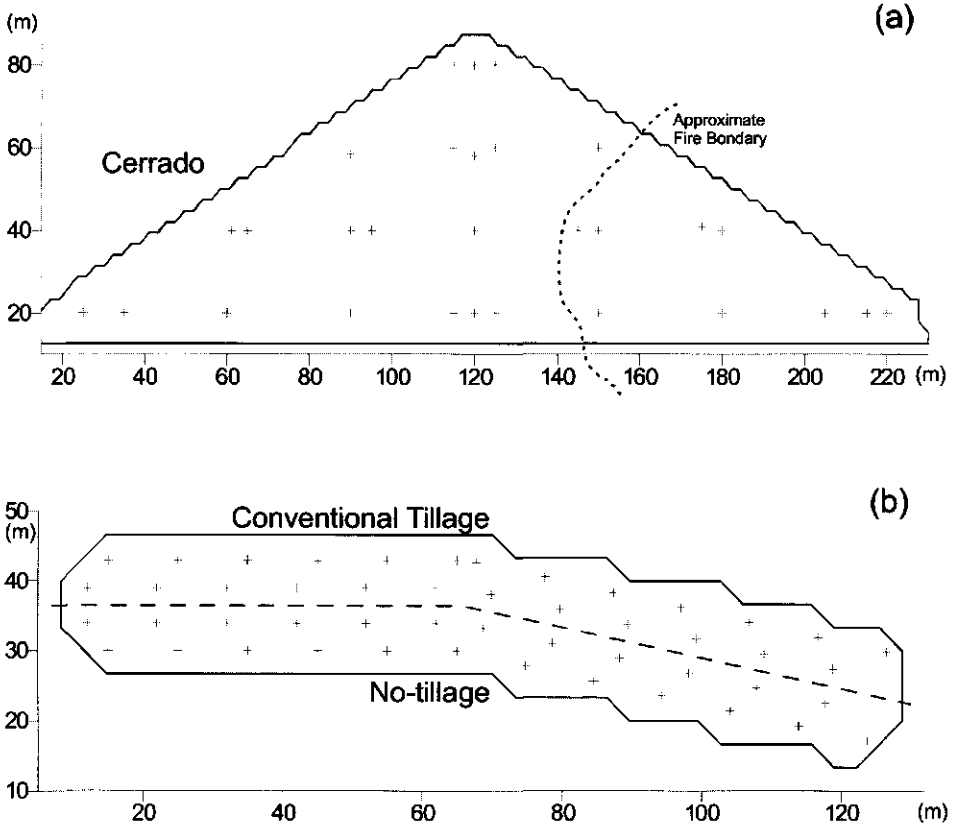

Figure 5.1. Sampling design applied in (a) the cerrado area and (b) the cultivated area. Dashed line in (a) represents the approximate fire boundary and in (b) the boundary between the conventional (above) and no-tillage (bellow) systems.

5.2.3. Chemical Analyses

Organic C and total N were analysed with an Interscience Elemental Analyser EA1180. ^{13}C abundance was determined after the conversion of total C to CO_2, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer at the Centre for Isotope Research at the University of Groningen, the Netherlands. Results were expressed as $\delta^{13}C$ (‰), which is defined as the relative difference between the isotope ratios of sample and standard: $\delta^{13}C = (^{13}R_{sample} - ^{13}R_{standard}) / (^{13}R_{standard}) \times 1000$, where ^{13}R is the ratio $^{13}C/^{12}C$. The international standard is V-PDB (Vienna-Pee Dee Belemnite) (Balesdent & Mariotti, 1996).

Table 5.2. Descriptive statistics for organic C (C_m), total N (N_m), $\delta^{13}C$ (‰), and C:N ratio in cerrado (*CER*) and cultivated areas (*NT* and *CT*).

Statistics	C_m (g kg^{-1})	N_m (g kg^{-1})	$\delta^{13}C$ (‰)	C:N ratio
		Cerrado (*CER*)		
n	29	29	28	29
mean	34.5	2.30	-24.4	15.1
variance	13.0	0.06	0.92	0.25
CV(%)	10.5	10.9	3.9	3.5
Skewness	-0.11	-0.32	1.24	0.27
Kurtosis	0.99	0.54	1.50	-0.31
		No-tillage + Conv. tillage (*NT* + *CT*)		
n	49	49	46	49
mean	32.5	2.06	-20.6	15.8
variance	4.70	0.04	0.34	0.57
CV(%)	6.7	9.7	2.8	4.8
Skewness	0.03	-0.10	0.18	0.17
Kurtosis	-0.55	-1.19	-0.87	-1.31
		No-tillage (*NT*)		
n	24	24	24	24
mean	34.0	2.23	-20.6	15.2
variance	2.64	0.01	0.32	0.12
CV(%)	4.8	4.5	2.8	2.3
Skewness	-0.18	-0.75	0.27	-0.36
Kurtosis	-0.73	0.93	-0.90	-0.51
		Conv. tillage (*CT*)		
n	25	25	22	25
mean	31.0	1.89	-20.7	16.4
variance	2.31	0.01	0.37	0.34
CV(%)	4.9	5.8	2.9	3.6
Skewness	-0.11	-0.004	0.15	-1.10
Kurtosis	0.42	-0.87	-0.90	0.40

5.2.4. Spatial Statistics

We use two-dimensional variograms to analyse the spatial continuity of the studied variables. Variograms ($\gamma_{(h)}$) were calculated for each *lag* distance (*h*) according to equation (1).

$$\gamma_{(h)} = \frac{1}{2N_{(h)}} \sum_{i=1}^{N(h)} \{V(\mathrm{x}_i) - V(\mathrm{x}_i + \mathrm{h})\}^2 \qquad (1)$$

where, $\gamma_{(\mathrm{h})}$ is the semi-variance for pair of points separated by the distance **h**, $N_{(\mathrm{h})}$ is the number of points separated by **h**, $V(\mathbf{x}_i)$ is the value of the variable under study in the location $\mathbf{x}_i$, and $V(\mathbf{x}_i + \mathbf{h})$ is the value of the variable in the location $\mathbf{x}_i + \mathbf{h}$.

For the cerrado plot, all variograms were estimated using all pairs of points per lag distance, referred to as global variograms. For the cultivated area, variograms were estimated in three different ways. Global variograms were estimated using all the pair of points in the whole area separated by each specific *lag* distance (*h*). Within-strata variograms were estimated using all pairs of points (both plots), but without considering pairs of points located over the boundary between plots (one point in each management system). Stratified variograms were estimated by the pairs of points located in each specific plot. Only isotropic variograms were used, since the shape of the fields and the number of sampling locations did not allow anisotropy analysis (Voltz & Webster, 1990). Permissible theoretical models (Webster & Oliver, 1990) were automatically fitted to the variograms trough the weighted least-squares procedure. We used the ratio between the square sum of deviances (SSD) and the total sum of squares (SST) to chose the best fitted variogram model, defined as:

$$\frac{SSD}{SST}=\sum_{i=1}^{n}\frac{(g_i-\gamma_i)^2}{(g_i-m_\gamma)^2} \qquad (2)$$

where γ_i are the n variogram estimates, m_γ is the average of γ_i, and g_i are the values of the variogram model.

We used equation (3) to calculate the proportion of the total modelled variability that could be explained by the lag distance (*VED*) as an index to evaluate how much of the variation was spatially structured.

$$VED=\frac{(S-C_0)*100}{S} \qquad (3)$$

where S is the sill of the modelled variogram, when a sill is reached, or S is the semi-variance for the maximum lag distance (h) considered in the variogram estimation, when a sill is not reached; and C_0 is the nugget.

Ordinary kriging (*OK*) was used for prediction when the global and stratified variograms were considered. In the latter, *OK* was performed in each plot separately. When using the within-strata variogram, kriging was performed using only data within a stratum (plot) for prediction within its boundaries (Voltz & Webster, 1990). This procedure was referred as within-strata kriging (*WK*). A neighbourhood of eight points was considered for kriging calculation.

5.2.5. Cross-validation

The performance of each kriging procedure was assessed by cross-validation according to Isaaks & Srivastava (1989). Each observation was eliminated in turn and the remaining observations were used to predict the value in that particular location. The predicted and the observed values were then compared. The prediction errors (*r*) were calculated by subtracting the observed value from the predicted value for all the point locations. We analysed error distribution and Person's correlation between observed and predicted values ($\rho_{\hat{z}z}$). We also calculated two indices according to Voltz & Webster (1990). The mean error (*ME*) was given by equation (4).

$$ME=\frac{1}{n}\sum_{i=1}^{n}\{\hat{z}(\mathrm{x}_i)-z(\mathrm{x}_i)\} \qquad (4)$$

where $z(\mathbf{x}_i)$ was the observed value at location $\mathbf{x}_i$ and $\hat{z}(\mathbf{x}_i)$ was the predicted value. This indices measures the bias of the prediction, and it should be close to zero for unbiased methods. The mean square error (*MSE*) which measures the precision of the predictions was given by equation (5).

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\{\hat{z}(x_i) - z(x_i)\}^2 \qquad (5)$$

5.3. Results

5.3.1. Descriptive statistics

Summary statistics of the variables were calculated for the cerrado (*CER*) and for the whole cultivated area (*NT* and *CT*) (Table 5.1). Coefficients of skewness and kurtosis suggested that variables were normally distributed, except $\delta^{13}C$ in *CER*, and C:N ratio in *CT*. In general the variances and coefficient of variation (*CV*) were rather low for all the variables and were higher for the cerrado than for the cultivated plots. The largest variation was observed for organic carbon (C_m) and total nitrogen (N_m). Conversely, the variability of C:N ratio and $\delta^{13}C$ was remarkably lower.

Stratifying the cultivated area into no-tillage (NT) and conventional tillage (CT) reduced variances of C_m, N_m, and C:N ratio in both plots. No changes in $\delta^{13}C$ variances were observed with stratification. These results suggest that part of the variation of C_m, N_m, and C:N ratio in the pooled area (*NT* + *CT*) is related to differences between management systems.

5.3.2. Spatial variability

In the cerrado plot, all variables showed spatial structure (Table 5.2 and Figure 5.2). The relatively high values of the SSD/SST ratio, especially for $\delta^{13}C$, C_m, and N_m, suggest that the best fitted-models do not properly describe the variograms. The *VED* values suggested that for $\delta^{13}C$, C_m, N_m, and C:N ratio 38 to 45% of the variability was spatially structured. In general, variogram values for these variables were higher in *CER* than in the cultivated area.

Table 5.2. Parameters of the models fitted to data the global variograms from cerrado plot (*CER*) of $\delta^{13}C$ (‰), organic C (C_m), total N (N_m), and C:N ratio.

Variable	Model	Parameters			SSD/	*VED*
		C_0	A	b	SST	(%)
$\delta^{13}C$	Gaussian	0.545	2.350	224	0.468	39
C_m	Gaussian	7.71	31.4	224	0.423	38
N_m	Gaussian	0.031	0.171	224	0.526	45
C:N ratio	Gaussian	0.201	0.145	40.4	0.363	42

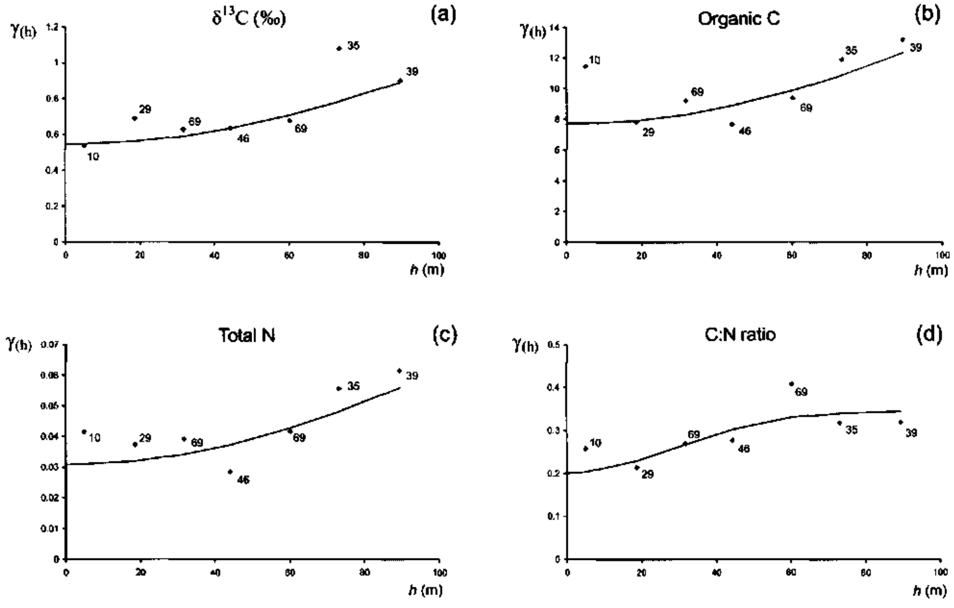

Figure 5.2. Global variograms of (a) $\delta^{13}C$, (b) organic C (C_m), (c) total N (N_m), and (d) C:N ratio in the cerrado plot.

The models fitted to the global variograms of C_m, N_m, and C:N ratio showed a good agreement with the estimated semi-variances (low *SSD/SST* ratio) (Table 5.3). When the whole cultivated-area was considered without taking the boundary between the management systems into account, the high *VED* values suggested a strong spatial structure in those variables. For the three variables a sill was reached at very similar ranges (Table 5.3 and Figure 5.3bcd). Although the *SSD/SST* ratio was also low for $\delta^{13}C$, the global variogram was remarkably different from that of the other variables. The $\delta^{13}C$ variogram showed a high nugget, low *VED*, and did not reach a sill within the limits of lag distances considered (Table 5.3 and Figure 5.3a).

Within-strata variograms were successfully fitted to C_m, C:N ratio, and $\delta^{13}C$ (Table 5.3). For N_m, no spatial structure could be observed within the limits of lag distances considered. A substantial reduction in the maximum semi-variances of the within-strata variograms for C_m, N_m (nugget), and C:N ratio was observed in relation to the global variograms (Figure 5.3abd). Conversely, the modelled global and within-strata variograms for $\delta^{13}C$ were very similar (Table 5.3 and Figure 5.2a).

As observed for the within-strata variograms, the stratified variograms showed remarkably lower semi-variances for C_m, N_m, and C:N ratio than the global variograms, and no difference for $\delta^{13}C$ (Figure 5.3 and Figure 5.4). For the stratified variograms, in no-tillage the variables C_m and N_m did not show any detectable spatial structure, while in conventional tillage they were structured reaching a sill at similar ranges (Table 5.3 and Figure 5.4bc). For C_m, the nugget effect observed in *NT* was higher than the sill in *CT*, whereas for N_m it was slightly lower. On the other hand, $\delta^{13}C$ and C:N ratio showed spatial dependence for both management systems (Figure 5.4ad). In *NT*, $\delta^{13}C$ semi-variances were more continuous than in *CT*, but they were also larger at most of the considered lag distances (Figure 5.4a). The *VED* index suggested that the lag distance explained substantially more of the δ^{13}C variability in *CT* (77 %) than in *NT* (44 %). For the C:N ratio, the semi-variances in *NT* were also more continuous, but, in contrast to δ^{13}C, they were always lower than in *CT*. Nevertheless, the *VED* values indicated the lag distance could explain a larger proportion of the variability in *CT* (89 %) than in *NT* (68 %).

Table 5.3. Parameters of the models fitted to the stratified, global and within-strata variograms of organic C (C_m), total N (N_m), δ^{13}C (‰), bulk density (D_b), and C:N ratio.

		Parameters			SSD/	*VED*
Variable	Model	C_0	A	b	SST	(%)
		Global Variogram				
δ^{13}C	Gaussian	0.134	0.360	62.50	0.091	59
C_m	Gaussian	0	4.91	7.55	0.092	100
N_m	Gaussian	0	0.044	7.30	0.110	100
C:N ratio	Gaussian	0.0035	0.544	8.07	0.056	99
		Within-strata Variogram				
δ^{13}C	Gaussian	0.138	0.825	106	0.085	58
C_m	Exponential	0	2.23	5.86	0.385	100
N_m	Nugget Effect	0.011	-	-	-	0
C:N ratio	Linear	0.049	0.0033	-	0.152	79
		Stratified Variogram 1 (No-tillage)				
δ^{13}C	Gaussian	0.179	0.973	136	0.108	44
C_m	Nugget Effect	2.52	-	-	-	0
N_m	Nugget Effect	0.012	-	-	-	0
C:N ratio	Linear	0.045	0.0018	-	0.202	68
		Stratified Variogram 2 (Conventional Tillage)				
δ^{13}C	Gaussian	0.078	0.485	61.3	0.105	77
C_m	Spherical	0.37	1.73	35.1	0.066	82
N_m	Spherical	0.003	0.010	30.4	0.154	81
C:N ratio	Linear	0.041	0.006	-	0.169	89

* Linear Model: $\gamma_{(h)} = C_0 + A * (h)$

Exponential Model: $\gamma_{(h)} = C_0 + A\,[1 - \mathrm{EXP}\,\{-(h/b)\}]$; for $h > 0$;

Gaussian Model: $\gamma_{(h)} = C_0 + A\,[1 - \mathrm{EXP}\,\{-(h/b)^2\}]$; for $h > 0$;

Spherical Model: $\gamma_{(h)} = C_0 + A\,\{(3/2)\,(h/b) - (1/2)\,(h/b)^3\}$ for $h\ \varepsilon\ (0,b]$; and

$\gamma_{(h)} = C_0 + A$; for $h > b$

5.3.3. *Kriging and Cross Validation*

In *CER*, the modelled variograms were used for predictions by ordinary kriging. The observed and predicted values were compared by a cross-validation procedure (Table 5.4). The indexes *ME* and *MSE* should not be used to compare the predictions of different variables (e.g. C_m and N_m), since those indexes depend on the magnitude of the values. Thus, for comparing different variables, we used the coefficient of correlation ($\rho_{\hat{z}z}$). To compare different kriging methods for the same variables we used the three indices.

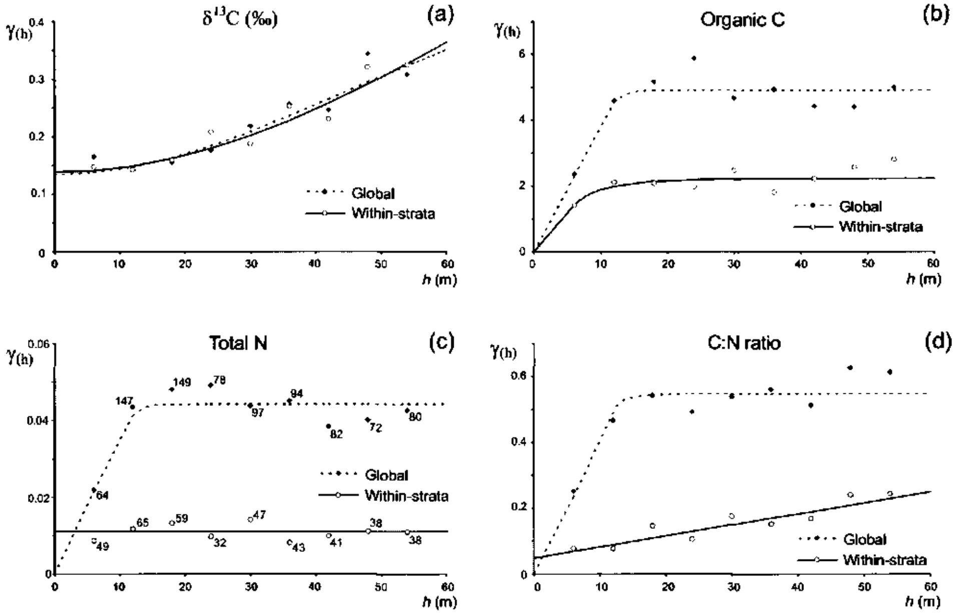

Figure 5.3. Global and within-strata variograms of of (a) δ^{13}C, (b) organic C (C_m), (c) total N (N_m), and (d) C:N ratio in the cultivated plots.

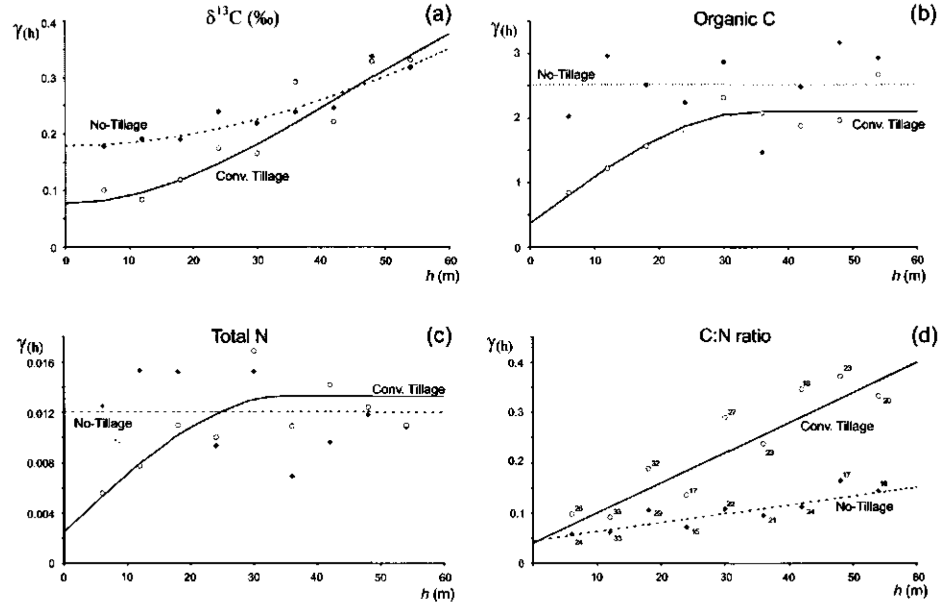

Figure 5.4. Stratified variograms of of (a) δ^{13}C, (b) organic C (C_m), (c) total N (N_m), and (d) C:N ratio in the cultivated plots.

Table 5.4. Indices calculated from the cross-validation results, representing the error distribution of predictions by ordinary kriging (*OK*) in cerrado plot (*CER*), and by ordinary kriging (*OK*), within-strata kriging (*WK*), and stratified kriging (*SK*) in the cultivated plots (*NT* and *CT*) for organic C (C_m), total N (N_m), $\delta^{13}C$ (‰), and C:N ratio.

Indices	Cerrado	Cultivated Areas (*NT* + *CT*)		
	OK	*OK*	*WK*	*SK*
		Organic C (C_m)		
ME	-0.176	-0.006	0.057	-0.007
MSE	5.178	0.034	0.269	1.403
$\rho_{\hat{z}z}$	0.77	1.00	0.98	0.83
		Total N (N_m)		
ME	-0.018	-0.002	0.010	-0.0002
MSE	0.022	0.0004	0.0030	0.0070
$\rho_{\hat{z}z}$	0.79	1.00	0.97	0.91
		$\delta^{13}C$		
ME	0.036	-0.003	0.013	0.009
MSE	0.175	0.106	0.109	0.106
$\rho_{\hat{z}z}$	0.89	0.83	0.82	0.83
		C:N ratio		
ME	0.041	-0.002	-0.028	-0.028
MSE	0.055	0.166	0.050	0.048
$\rho_{\hat{z}z}$	0.90	0.87	0.96	0.96

In *CER*, the coefficient of correlation between predicted and observed values ($\rho_{\hat{z}z}$) were higher for $\delta^{13}C$ and C:N ratio than for C_m and N_m (Table 5.4). The *ME* and *MSE* values for C_m and N_m substantially decreased from *OK* in *CER* to all the kriging procedures applied in the cultivated area. Conversely, the $\rho_{\hat{z}z}$ values increased. The same trend was also observed for $\delta^{13}C$, but the differences were smaller. For C:N ratio, the *ME* value decreased from *CER* to *NT+CT*. The *MSE* increased from *OK* (*CER*) to *OK* (*NT+CT*), but was slightly higher to *OK* (*CER*) than to *WK* and *SK*. The $\rho_{\hat{z}z}$ followed an inverse trend, increasing from *OK* (*NT+CT*) through *OK* (*CER*) to *WS* and *SK*.

Comparing the different kriging procedures applied to the cultivated area (*NT+CT*), we could distinguish three different behaviours. For C_m and N_m, *OK* showed the best predictions, with the highest $\rho_{\hat{z}z}$ and lower *ME* and *MSE*. Within-strata kriging (*WK*) was slightly worse, and *SK* showed the less accurate predictions. For $\delta^{13}C$, there were only slight differences between the procedures. For C:N ratio, the best predictions were made by *WK* and *SK*.

5.4. Dicussion

5.4.1. Kriging and Cross-validation

In general, the cross-validation results showed a good agreement between the observed and predicted values. However, according to Voltz & Webster (1990) the cross-validation method is biased and optimistic. Ideally, an independent set of samples should be used to test the accuracy of predictions. The number of available observations was not enough to separate a independent set of data for validation, without jeopardising the variogram estimations. In such situations, cross-validation is the best method for testing the predictions (Isaaks & Srivastava, 1989). Nevertheless, the high accuracy of the predictions could be partly due to the "optimistic character" of cross-validation.

In the cultivated area, the number of observations in each stratified plot did not provide enough paired points per *lag* distance for a good estimation of individual variograms. Only for one lag distance there were more than 30 pairs of points, which is the minimum number of observations recommended for estimating the variograms (Isaaks & Srivastava, 1989). In such a situation, Voltz & Webster (1990) recommended the use of within-strata variograms, which would increase the number of points per lag distance and, at the same time, would allow a different expected mean for each plot. Aware of this limitation, we tried to model the stratified variograms for comparison with the global and within-strata variograms in further predictions.

Despite the reduced number of observations, stratified variograms were successfully modelled for some of the variables, with good adjustments as indicated by the low *SSD/SST* values (Table 5.3). In general, the cross-validation results suggested that the performance of stratified kriging (*SK*) was only slightly worse or even equivalent to within-strata kriging (*WK*). These results suggest that is worthwhile to analyse the stratified variograms, even when a reduced number of points is available per stratum.

5.4.2. Fire and spatial structure in cerrado

In cerrado, the lower accuracy of C_m and N_m predictions suggested by cross-validation (Table 5.4) can be attributed to a combination of a comparatively high variability in relation to the other variables (Table 5.1), low number of point-observations (29) and low adjustment of the models to the variograms (Table 5.3). C:N and $\delta\ ^{13}C$ were better predicted, because not only the number of observations and the adjustment of the models (Table 5.3) were low, but also the total variability (Table 5.1).

In general, the variability of $\delta^{13}C$, C_m, N_m, and C:N (Table 5.1, Table 5.2, and Figure 5.2) was rather low. Because the cerrado *sensu stricto* is a mixed vegetation composed of a herbaceous stratum of grasses (most of them C_4) and a mosaic of shrubs and trees (all C_3) (Miranda et al., 1997; Ribeiro & Walter, 1998), the arrangement of the vegetation may provide a high variability for organic matter distribution (C_m, N_m, and C:N) and especially for $\delta^{13}C$, due to the mixed character of the vegetation (C_3- and C_4-plants). On the other hand, the studied soil has a very high clay content (>850 g kg^{-1}) and most of its soil organic matter (SOM) is stored in the clay plus silt compartment (Chapters 3 and 4). This means that only a small part of the SOM is in a relatively rapid dynamic equilibrium with the currently quite heterogeneous vegetation and most of its contents is controlled by protection mechanisms related to clay. This causes a large SOM-background, which is more influenced by soil variability (e.g. texture and mineralogy) than by vegetation. Latosols are well known for their high textural and mineralogical homogeneity (Oliveira et al., 1992), which would explain the relatively low variability observed for all variables studied. Nevertheless, C_m and N_m were twice more variable than C:N and $\delta^{13}C$ (Table 5.1). This suggests a higher variability in the quantitative distribution of soil organic matter (SOM) than in its quality (chemical composition). Similar behaviour was reported for C:N ratio and $\delta^{13}C$ in the same cerrado area in 10 x 10 m plots (Chapter 2) and for $\delta^{13}C$ in Canadian (Kessel et al., 1994) and Scotch soils (Marriott et al., 1997).

Despite the relatively low variability, all variables were spatially structured. The long range for C:N ratio (Figure 5.2d) and the absence of a sill for $\delta^{13}C$, C_m, and N_m (Figure 5.2abc) suggested that the modelled spatial structure was probably related to large-scale variability, and was independent of small-scale variability related to individuals or small groups of trees and shrubs. This small-scale variability was incorporated in the nugget effect.

Analysing the trends observed for $\delta^{13}C$ (Figure 5.5a) and C_m (Figure 5.5b) in the cerrado plot, we postulate that these trends were related to the higher incidence of fire registered in the right corner of the area. This particular corner was burned two times in the last 21 years, while the rest of the area remained unburned. The fires could have reduced the carbon stocks and decreased the density of the canopy (Mistry, 1998). With a less dense arboreal/shrub stratum, the C_4 grass population would increase, rising the $\delta^{13}C$ values of the input carbon in the system. This would explain the trends of lower values of C_m (Figure 5.5b) and N_m (data not shown) and higher $\delta^{13}C$ (Figure 5.5a) values in the right corner of the area. However, in cerrado *sensu stricto* the arboreal/shrub stratum tends to recover very fast, decreasing the C_4 population within a couple of years without fire (Mistry, 1998; Ribeiro & Walter, 1998). This means that the $\delta^{13}C$ signature of the input carbon would be enriched in ^{13}C (C_4 material) only temporarily. As discussed before, in this soil, SOM is mostly present in organic-mineral complexes forming a relatively stable background pool. In addition, we observed that the light fraction of SOM (< 1.7 g cm^{-3}) has a signature linked to the present vegetation in the area (C_3 dominant plants) but that the heavy fraction followed the increasing trend of $\delta^{13}C$ values observed in Figure 5.5a (Chapter 4). These results suggested that the observed trends in $\delta^{13}C$ and C_m were not entirely a consequence of the two fires observed in the last 21 years, but probably related to a long-term, cumulative effect of repeated fires during SOM accumulation/stabilisation processes. This long-term effect of fire may have generated small differences in the vegetation Although the whole area was dominated by C_3 plants (Chapter 2), small differences in the canopy may influence the moisture content of the litter and its susceptibility to ignition during the dry season (Mistry, 1998).

5.4.3. Conventional and No-tillage systems

Cultivation homogenised the distribution of organic matter and reduced the total variability of C_m, N_m, C:N, and $\delta^{13}C$ (Table 5.1, Figure 5.1, Figure 5.2, and Figure 5.3). This decrease supports the hypothesis that the large nugget observed for those variables in *CER* probably incorporated the structure given by individual (or groups of) trees and shrubs that could not be modelled within the limits of lag distance analysed (15 to 105 meters).

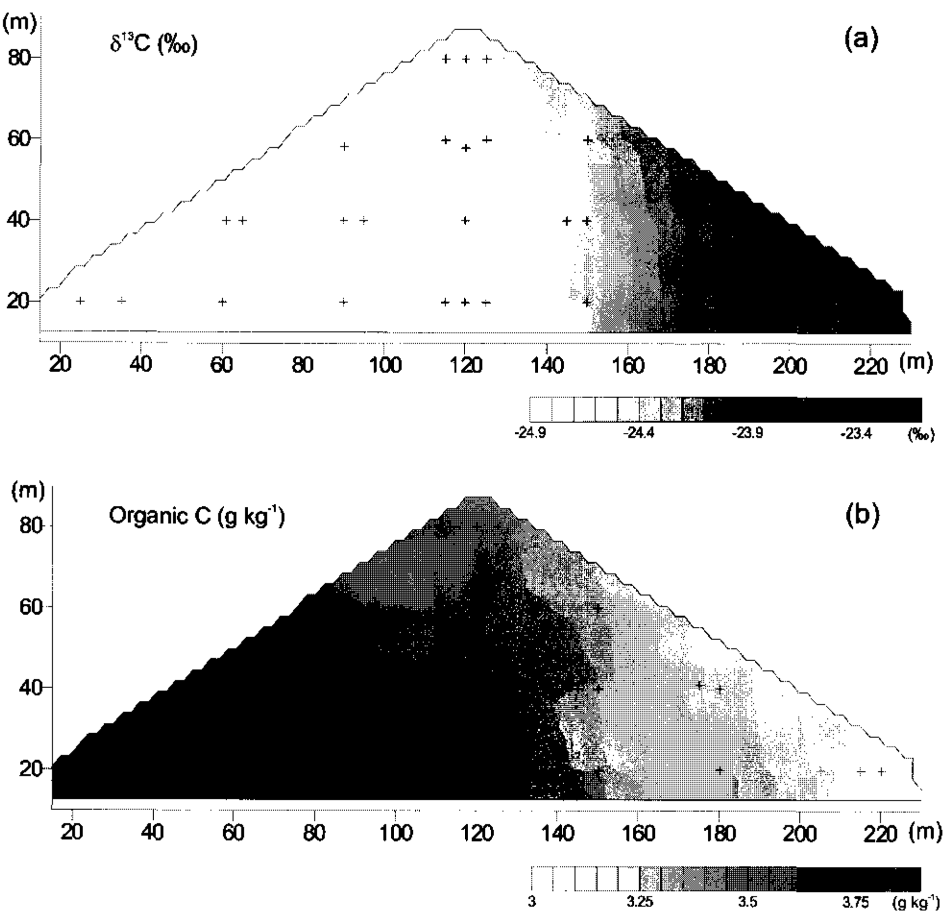

Figure 5.5. Contour maps of (a) δ^{13}C and (b) organic C (C_{m}).

In general, all the variables showed higher unexplained variability (not spatially structured) in no-tillage than in conventional tillage (Table 5.1 and Figure 5.4). In no-tillage, the soil is not homogenised by ploughing and soil variables tend to show spatial structure related to the cultivation rows (Souza et al., 1998). This would generate variability at short distance, in the order of half to one metre (space between rows for beans and maize, respectively). As the sampling strategy adopted did not follow the rows and the minimum lag distance analysed was 6 meters, the small-scale variability was incorporated to the nugget. This explains the pure nugget effect for C_{m} and N_{m}, and the lower amount of spatially structured variability for δ^{13}C and C:N ratio in relation to conventional tillage (Figure 5.4).

Analysing the performance of the different kriging methods in the cultivated area, we could assess the effect of management systems on spatial variability. Three different behaviours were observed. First, C_{m} and N_{m} showed the best prediction by *OK*, which suggested spatial dependence between plots, since the total stratification of the area by *SK* or the partial stratification by *WK* decreased the accuracy of the predictions (Table 5.4). Comparing the maps of *OK* and *WK* (Figure 5.6), we observe that the *OK* procedure captured most of the difference between the plots, but at the same time allowed some spatial structure over the boundary. As discussed before for *CER*, most of the C_m and N_m dynamics in this Latosol seems to be controlled by clay protection mechanisms (Chapters 3 and 4), which are hardly affected by cultivation (Feller & Beare, 1997). This allowed us to postulate that the spatial structure over the boundary was probably related to the variation of a background pool of SOM linked to more stable soil characteristics as texture and mineralogy.

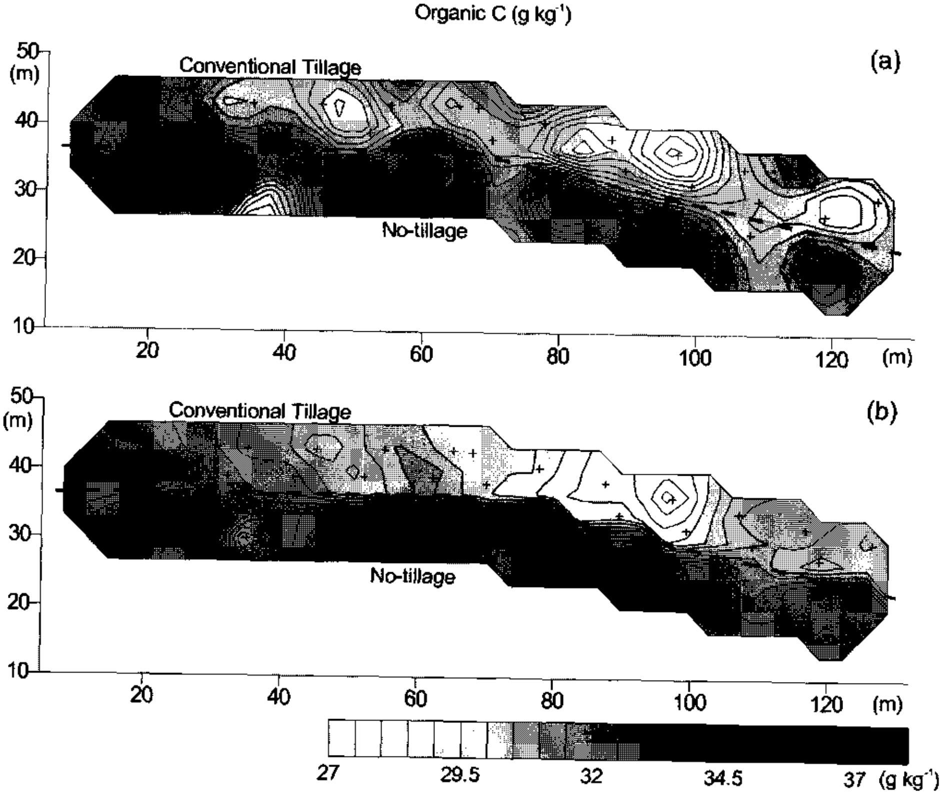

Figure 5.6. Contour maps of organic C (C_m) predicted by (a) ordinary kriging and (b) within-strata kriging for the cultivated area.

A second behaviour was observed for C:N ratio, which showed no spatial dependence between plots. C:N ratio showed a clear spatial structure for all the methods of variogram estimation (Figures 5.3 and 5.4). In contrast with C_m and N_m, the better performance of both stratification methods (*SK* and *WK*) indicated no spatial dependence between the plots and a high effect of the management system. The sharp boundary that arose in the *WK* map comparatively to the *OK* map (Figure 5.7) clearly illustrates these observations. The difference in C:N ratio between plots suggested that *NT* system changed the composition of SOM. In a previous study, Roscoe et al. (2000c) observed differences in the N-dynamics for the same area. The authors observed that, although the C and N contents were not different, *NT* had significantly higher contents of nitrogen in the microbial biomass, higher urease activity, and was more efficient in using N-urea fertiliser than *CT*.

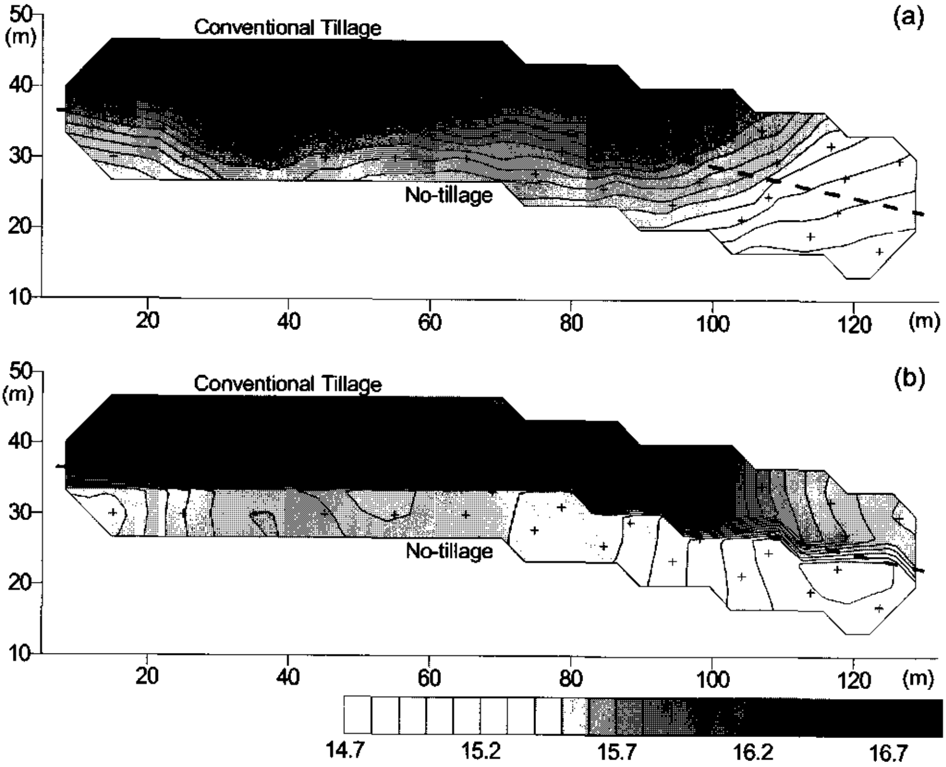

Figure 5.7. Contour maps of C:N ratio predicted by (a) ordinary kriging and (b) within-strata kriging for the cultivated area.

Finally, similar to C_m and N_m, $\delta^{13}C$ showed spatial structure over the whole area, but there was no difference in the performance of predictions with (*SK* and *WK*) or without (*OK*) stratification. This happened because there were no apparent differences between the treatments and the spatial variability was basically unidirectional (Figure 5.8), toward the length of the plots. The difference between the average values of *CER* and *NT+CT* indicated an evident addition of maize material to the soil organic matter (enriched in ^{13}C). However, the structure of the spatial variation in the cultivated fields suggested that the incorporation of maize-derived material was not different between the treatments. The similarity of the spatial structure of $\delta^{13}C$ in *CER* (Figure 5.2a) and in *NT+CT* (Figure 5.3a and 4a) was remarkable. The combination of these results suggests that the spatial structure of $\delta^{13}C$ in the cultivated area was probably inherited from the former cerrado vegetation.

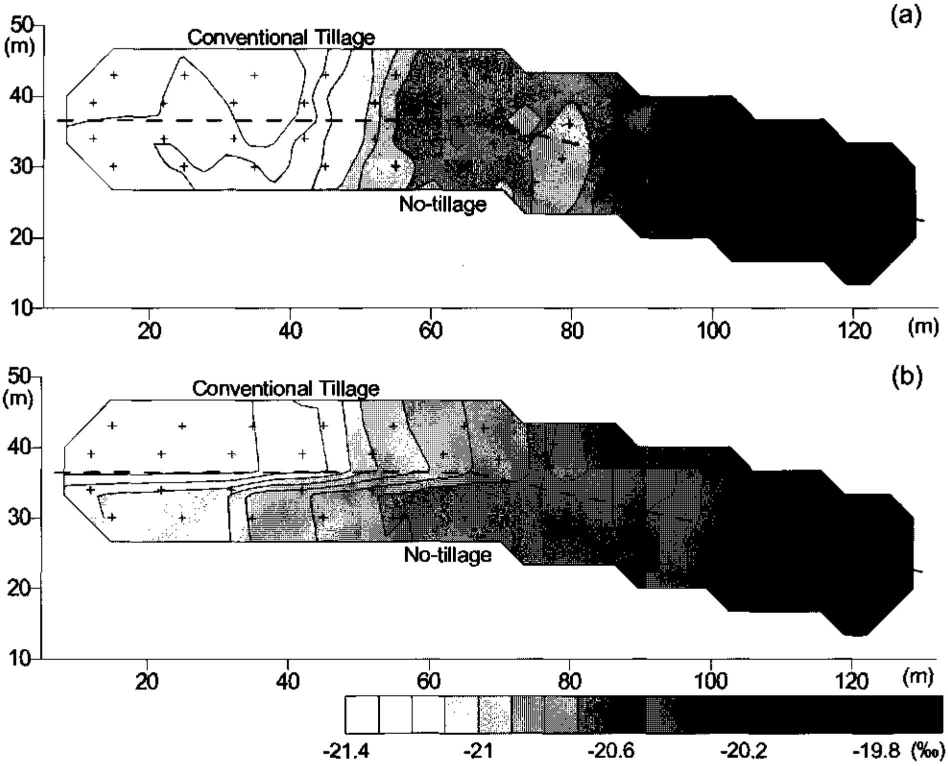

Figure 5.8. Contour maps of $\delta^{13}C$ predicted by (a) ordinary kriging and (b) within-strata kriging for the cultivated area.

5.5. Conclusions

In the studied cerrado sensu-stricto, the total variability of $\delta^{13}C$ and SOM-related variables (C_m, N_m, and C:N) were relatively small, which was attributed to the high textural and mineralogical homogeneity of clayey Latosols. Nevertheless, part of the variability was spatially structured.

In cerrado, the spatial structure observed for $\delta^{13}C$ and SOM-related variables was probably related to long-term cumulative effect of repeated fires during the SOM accumulation/stabilisation processes.

Cultivation reduced the variability of most of the variables and changed the spatial structure. The variables tended to be less spatially structured in no-tillage than in conventional tillage, which was attributed to small-scale (smaller than the minimum lag distance) variability in that system. C_m and N_m showed spatial dependence across the boundary, suggesting a large background SOM content independent of the management system, probably related to soil characteristics. No-tillage altered the composition of SOM as revealed by the C:N ratio.

The spatial structure observed for $\delta^{13}C$ in the cultivated area was probably inherited from the former cerrado vegetation. This means that, in studies of SOM replacement, if the trends in both areas were not taken into account, the variability of the replacements would be overestimated.

Chapter 6

Effect of tillage and no-tillage on soil organic matter dynamics in density fractions of a Cerrado Oxisol

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Soil and Tillage Research (accepted).

Abstract

We used density fractionation and the ^{13}C natural abundance to assess changes in soil organic matter (SOM) in an cultivated Oxisol after 30 years of reclamation of a cerrado sensu-stricto. The objectives of the study were (i) to evaluate the long-term impact of conventional and no-tillage systems on SOM stocks in a Dark Red Latosol (Oxisol) from the Cerrado, and (ii) to better understand the dynamics of SOM in different density fractions of this soil. We observed that cultivation led to compaction, as indicated by increased soil bulk density. This resulted in systematic overestimate of C and N stocks in cultivated areas when compared to the natural cerrado. Conversion of cerrado into conventional tillage (CT) or no-tillage (NT) system did not alter the total C (~100 Mg ha^{-1}) and N (~7 Mg ha^{-1}) stocks of the first 45cm depth in 30 years of cultivation. However, about 22% of the total carbon was replaced by maize material in the period. The relative replacement of carbon decreased following the order: free light fraction > heavy fraction > occluded light fraction. The low substitution in the occluded light fraction was attributed to a possible presence of charcoal. The free light fraction was more sensitive to changes in the management systems, and converting cerrado into cropland significantly decreased its quantity. The proportions of C replacement in this fraction were higher in CT than NT, suggesting a faster turnover in the first. Nevertheless, because most carbon (~95%) was held in the HF, carbon dynamics in the whole soil was controlled by the behavior of this fraction. The maintenance of C levels even after 30 years of cultivation and the absence of differentiation between no-tillage and conventional tillage was attributed to the high clay contents and Fe+Al oxi-hydroxides concentrations of the studied soil as well as the adequate C supply by the maize crop.

6.1. Introduction

Reclamation of native cerrados (savannas) for agriculture has substantially increased in the last three decades. From a total of 136 million ha potentially suitable for agriculture, 47 million ha was under cultivation in 1995 (Macedo, 1995). The widespread use of intensive mechanisation in Cerrado soils has arisen serious concerns about the possible impact of cultivation on soil quality and sustainability (Resck, 1998).

Latosols (Oxisols) are the most representative group of soils in the Cerrado Biome, spreading over 46% of the region, mainly on gently rolling and flat uplands (Resck, 1998). From this total, Dark Red Latosols cover about 40%, or more than 360,000 km^2. Due to a high degree of weathering, these soils are characterised by low-activity clays (kaolinite, gibbsite and iron oxi-hydroxides), low nutrient content, high toxic aluminium concentration, and relatively high acidity (Resende et al., 1997; Resck, 1998; Resck et al., 2000). In such an environment, SOM is an important component of soil with significant impact on nutrient cycle, biological activity, cation exchange capacity, and aggregate stability (Silva et al., 1994; Resck, 1998; Resck et al., 2000).

Conventional tillage (based on plowing and/or harrowing) is considered the most degradative system of land use (Resck et al., 2000). In this system, plant residues are split and mixed to soil, aggregates are disrupted (releasing physically protected organic materials), temperature, aeration, and biological activity increase. Consequently, conventional tillage may substantially increase SOM losses. Conversely, in no-tillage systems, residues are not mixed to soil and accumulate on the surface. The decomposition rate of SOM tends to be lower, and C stocks may remain unchanged or even increase (Resck et al., 2000).

Physical fractionation of SOM is a powerful tool in land-use change studies (Christensen, 1992). Density fractionation allows the separation of SOM fractions with different location in the soil matrix and degree of association with mineral particles (Golchin et al., 1997). The free light fraction (F-LF), defined as the fraction with density lower than 1.7g cm^{-3}, separated from soil before disaggregation (Gregorich & Ellert, 1993), is chemically very close to litter and has, in general, a very fast decomposition rate (Golchin et al., 1997). The occluded light fraction (O-LF) is defined as the material with a density lower than 1.7 g cm^{-3}, separated after ultrasonic dispersion. This fraction shows a more advanced degree of decomposition and a slower turnover. The heavy fraction (HF), with density higher than 1.7 g cm^{-3}, is composed mainly of highly decomposed material, with a very slow decomposition rate (Golchin et al., 1997). Changes in these different fraction, especially in the low-density fractions, can be more sensitive to management-induced impacts on soil than the total SOM.

The ^{13}C stable isotope represents about 1.1% of the total carbon in nature. As atmospheric $^{13}CO_2$ is discriminated during photosynthesis, plants are depleted in ^{13}C compared to the atmosphere. The degree of discrimination is higher for C_3 than for C_4 plants and, therefore, C_4 plants are comparatively enriched in ^{13}C. This difference is used as a natural tracer for SOM dynamics studies, when a C_3- is replaced by a C_4-dominated vegetation or vice-versa (Balesdent & Mariotti, 1996).

As the cerrado sensu-stricto investigated in this study is a C_3-dominated vegetation (Chapter 2), we followed the replacement of carbon in fields cultivated with a C_4 vegetation (maize) for 30 years. The objectives of the study were (i) to evaluate the long-term impact of conventional and no-tillage systems on SOM stocks in a Dark Red Latosol (Oxisol) from the Cerrado, and (ii) to better understand the dynamics of SOM in different density fractions of this soil.

6.2. Materials and Methods

6.2.1. Site description and sampling

The study was carried out at the Maize and Sorghum National Research Centre – Brazilian Institute of Agricultural Research (EMBRAPA-CPMS), Sete Lagoas-MG, Brazil. The area is located at latitude 19°26'S and longitude 44°10' W, at an altitude of 730 m. The mean annual temperature is 22.1°C, and annual precipitation is 1340 mm, with a dry season from April to September. Three different areas where sampled in a homogeneous Dark Red Latosol (Typic Haplustox) soil unit. The first was an area of native "cerrado sensu-stricto" vegetation, taken as a standard. The area was dominated by C_3-plants (Chapter 2). The second was a nearby experimental area cropped with maize/beans under irrigation. In the area, two treatments were sampled. The first, referred to as conventional tillage (CT), was cropped for 30 years with maize/beans in succession. For each crop the soil was plowed once (disk plow) and harrowed twice (light disk harrow). In the second treatment, referred as no-tillage (NT), the soil was initially cultivated for 20 years as the CT plot and, in the last 10 years, the soil was sown directly without plowing or harrowing.

The contribution of beans material, a C_3 plant, for SOM during the period of cultivation (30 years) could not be assessed. However, its contribution for the total C_3-derived material present in the cultivated soil is negligible. Firstly, because it has a very fast decomposition rate and contributes very little to build up SOM. And secondly, because most of the material produced annually (all above ground biomass) was harvested. We assumed, therefor, that the C_3-derived material originated from the previous cerrado vegetation.

The soil was sampled in October 1999, just before the start of the rainy season. Preliminary geostatistical studies in the area (Chapter 5) showed that, although the total variability was rather small, organic C, organic N, and $\delta^{13}C$ were spatially structured in the topsoil (0 – 7.5 cm). In the cerrado, there was a clear trend for these three variables towards the east corner of the area. In the cultivated fields, the same trend was observed only for $\delta^{13}C$. In order to capture the variability of the areas, we selected three sampling locations in each plot, following the observed trends. Thus, for each treatment (CT, NT, and Cerrado), three pits (50 x 50 x 50 cm) 50 m from each other were dug in a transect along the trend direction. Disturbed samples were taken for four different depths (0 - 7.5; 7.5 – 15; 15 – 30 and 30 – 45 cm) for organic C, total N and $\delta^{13}C$ analysis. From the same depths, duplicated volumetric samples (metallic rings) were taken for bulk density analysis.

6.2.2. Density fractionation

Soil organic matter for 0-7.5 and 7.5-15 cm depths was separated by density fractionation, based on Gregorich & Ellert (1993). The light fraction was separated by flotation in a NaI solution (1.7g cm^{-3}), before and after aggregate disruption. In short, 100 g of sample was placed in a 1-litre beaker with 500 ml of NaI solution, gently shaken by hand, and left standing at room temperature for about 1 hour. The supernatant was aspirated with an vacuum pomp, centrifugated (15 min, 3500 rpm), and filtered through a membrane filter (Whatman GF/A) into a millipore vacuum unit. The fraction recovered on the filter was washed with 100 ml of 0.01 M $CaCl_2$ followed by 200 ml of distilled water. The sediment in the centrifuge tubes was placed back in the 1-litre beakers, re-suspended in NaI (the solution previously filtered) and gently shaken by hand. The same procedure was repeated two times as described above. The three sub-fractions were joined, oven-dried at 50°C, and stored for analysis. This fraction was called "free light fraction" (F-LF). The sediment was re-suspended and ultra-sonicated at 800 J ml^{-1}, with a Vibracell VCX 600 probe-type model, calibrated calorimetrically as described in Chapter 3. The centrifugation and filtration procedure was repeated three times as described above and the fraction recovered from the supernatant was referred to as "occluded light fraction" (O-LF). The heavy fraction (sediment) was washed one time with 0.01 M $CaCl_2$ and about 10 times with distilled water until the clay fraction remained in suspension after 24 hours, freeze dried, and stored for analysis.

6.2.3. C, N, and ^{13}C analyses

Organic C was analysed for all samples and fractions, using an Interscience Elemental Analyser EA1180. ^{13}C abundance was determined after the conversion of total C to CO_2, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer at the Centre for Isotope Research at the University of Groningen, the Netherlands. Results were expressed as $\delta^{13}C$ (‰), which is defined as the relative difference between the isotope ratios of the sample and a given standard: $\delta^{13}C = (^{13}R_{sample} - ^{13}R_{standard}) / (^{13}R_{standard}) \times 1000$; where $^{13}R_{sample}$ and $^{13}R_{standard}$ are the $^{13}C/^{12}C$ isotope ratio for the sample and the standard, respectively. The international standard is V-PDB (Vienna-Pee Dee Belemnite). The present $\delta^{13}C$ value of the atmosphere is -8‰. C_3 plants show a $\delta^{13}C$ ranging from –32 to –22‰ (mean of –27‰) and C_4 plants have values of –16 to –9‰ (mean of –13‰) (Balesdent & Mariotti, 1996).

6.2.4. Replacement calculations

For calculating the relative contribution of C derived from maize (C_4), we used equation (1), according to Balesdent & Mariotti (1996):

$$F_{C4} = (\delta_M - \delta_{C3})/(\delta_{C4} - \delta_{C3}) \qquad (1)$$

where, F_{C4} is the proportion of C_4 derived material, δ_M is the $\delta^{13}C$ of the total mixed SOM (in the cultivated fields), δ_{C3} is the $\delta^{13}C$ of SOM derived from C_3 vegetation, and δ_{C4} is the $\delta^{13}C$ of litter carbon derived from C_4 vegetation (maize).

The reference values for C_3-derived carbon were those observed for the various depths and fractions of the cerrado plot. The reference $\delta^{13}C$ value for the C_4-derived carbon was the $\delta^{13}C$ of the maize litter (-12.8 ± 0.09‰, average ± standard error, n=3).

Equation (2) gave the stock of C_4-derived C (S_{C4}) in each fraction and depth by:

$$S_{C4} = S_T \times F_{C4} \qquad (2)$$

where , S_{C4} is the stocks of C_4-derived carbon (kg m^{-2}), S_T is total C stocks (kg m^{-2}), and F_{C4} is the proportion of C_4-derived carbon in a given fraction and depth.

6.2.5. Statistical analyses

Linear and quadratic models were fitted to data of soil mass (SM), organic C and N stocks by the least square criteria. The coincidence of lines was tested by the F test according to Snedecor & Cochran (1967). Tukey's and t tests were used to compare means of proportion of C in each fraction and proportions of C_4-derived C between treatments within each fraction, respectively.

The uncertainty in the calculations of proportions of C_4-derived C (F_{C4}) in each depth and fraction was estimated by the Taylor series approximation, according to Phillips & Gregg (2001). The variance of F_{C4} ($\sigma^2_{F_{C4}}$) may be estimated using partial derivatives (Taylor, 1982) as:

$$\sigma^2_{F_{C4}} = \left(\frac{\partial F_{C4}}{\partial \delta_M}\right)^2 \sigma^2_{\delta_M} + \left(\frac{\partial F_{C4}}{\partial \delta_{C4}}\right)^2 \sigma^2_{\delta_{C4}} + \left(\frac{\partial F_{C4}}{\partial \delta_{C3}}\right)^2 \sigma^2_{\delta_{C3}} \qquad (3)$$

which reduces to:

$$\sigma^2_{F_{C4}} = \frac{1}{(\delta_{C4-}\delta_{C3})}\left[\sigma^2_{\delta_M} + F^2_{C4}\,\sigma^2_{\delta_{C4}} + (1-F_{C4})^2\,\sigma^2_{\delta_{C3}}\right] \qquad (4)$$

where, $\sigma^2_{\delta_M}$, $\sigma^2_{\delta_{C4}}$, and $\sigma^2_{\delta_{C3}}$ are the variances of δ_M, δ_{C4}, and δ_{C3}, respectively.

The variances of C_4-derived C stocks were also estimated by partial derivatives, according to the Taylor series approximation (Taylor, 1982) as:

$$\sigma^2_{S_{C4}} = \left(\frac{\partial S_{C4}}{\partial S_T}\right)^2 \sigma^2_{S_T} + \left(\frac{\partial S_{C4}}{\partial F_{C4}}\right)^2 \sigma^2_{F_{C4}} \qquad (5)$$

which reduces to:

$$\sigma^2_{S_{C4}} = S^2_{C4}\left(\frac{\sigma^2_{S_T}}{S^2_T} + \frac{\sigma^2_{F_{C4}}}{F^2_{C4}}\right)^2 \qquad (6)$$

where, $\sigma^2_{S_{C4}}$, $\sigma^2_{S_T}$, and $\sigma^2_{F_{C4}}$ are the variances of S_{C4}, S_T, and F_{C4}, respectively.

6.3. Results

6.3.1. Total soil

Soil bulk density was higher in the cultivated plots than in cerrado (Table 6.1). This was probably caused by compaction due to cultivation, because Sans (1986) observed bulk densities of 0.77 (0-13cm depth) and 0.79 g cm^{-3} (13-45 cm depth) in the same cultivated area, 16 years before our sampling. We calculated the accumulated soil mass as a function of depth for each plot (Figure 6.1a). As we had differences in the bulk densities (Table 6.1), the SM for the same depth interval was higher in NT and CT than in CER (Figure 6.1a). Taking the mass of soil in each depth interval in CER, we calculated the equivalent depths in NT and CT, with the same soil mass (Figure 6.2).

Table 6.1. Soil bulk density, organic C and total N contents, and C:N ratio of a Typic Haplustox under native cerrado (Cerr), no-tillage (NT) and conventional tillage (CT).

	Depth (cm)	Bulk Density (g cm^{-3})	Organic C (g kg^{-1})	Total N (g kg^{-1})	C:N	δ^{13}C (‰)
Cerr.	0 – 7.5	0.79 (0.03)[a]	41.8 (3.5)	2.9 (0.3)	14.5	-24.8 (0.5)
	7.5 – 15	0.83 (0.01)	30.9 (3.6)	2.0 (0.3)	15.1	-24.0 (0.6)
	15 – 30	0.80 (0.01)	24.6 (3.0)	1.6 (0.2)	15.5	-23.9 (0.4)
	30 – 45	0.86 (0.02)	21.1 (2.1)	1.3 (0.1)	16.5	-23.8 (0.3)
NT	0 – 7.5	1.04 (0.03)	31.4 (0.6)	2.2 (0.1)	14.5	-20.8 (0.6)
	7.5 – 15	1.08 (0.01)	27.3 (0.1)	1.8 (0.0)	15.5	-21.4 (0.5)
	15 – 30	1.06 (0.00)	25.0 (0.8)	1.6 (0.0)	16.1	-21.7 (0.5)
	30 – 45	0.95 (0.03)	20.8 (2.2)	1.2 (0.1)	17.8	-22.1 (0.7)
CT	0 – 7.5	0.98 (0.00)	31.1 (0.4)	2.0 (0.0)	15.6	-20.7 (0.5)
	7.5 – 15	1.02 (0.01)	31.2 (0.9)	2.0 (0.0)	15.8	-20.7 (0.6)
	15 – 30	1.07 (0.02)	26.8 (2.0)	1.7 (0.1)	15.9	-21.5 (0.7)
	30 – 45	0.95 (0.04)	20.8 (2.3)	1.2 (0.1)	17.8	-22.0 (1.0)

[a] mean and standard error (between brackets) for three replicates.

The stocks of organic C (Figure 6.1b) and N (Figure 6.1c) as a function of depth were described for each plot by a single quadratic model for both systems. For organic C, the lines fitted to NT and CT were not significantly different at a significance level of 0.05. The curve for NT and CT was significantly different from the model fitted to CER. For organic N stocks, there were no significant differences among the models fitted for the three plots.

We calculated the stocks of C and N as a function of depth with and without the correction for bulk density (Table 6.2). Without correction, the stocks of C tended to be higher in NT and CT than in CER. Corrected values for NT and CT were 15-20% lower throughout the profile, and differences in relation to CER disappeared. For N stocks, the opposite was observed. When corrected, the values tended to be smaller in the first three depths.

The δ^{13}C of the total soil was lower in CER than in the cultivated fields for all depths (Table 6.1). There was no difference between NT and CT. The values tended to increase and decrease with depth in CER and NT+CT, respectively.

The amount of C_4 material incorporated into soil did not differ between NT and CT (Figure 6.3). In the topsoil (0-7.5 cm), about 33% of total C came from maize after 30 year of cultivation (Figure 6.3a). At 30-45 cm, this proportion decreased to 15-17%. The C_4-C stocks were also not different between the management systems (Figure 6.3b). In the upper 45 cm of soil 21.5 ± 3.1 Mg ha^{-2} of C_4-C was incorporated in NT and 24.5 ± 3.9 Mg ha^{-2} in CT. About half of this amount was present in the upper 15 cm.

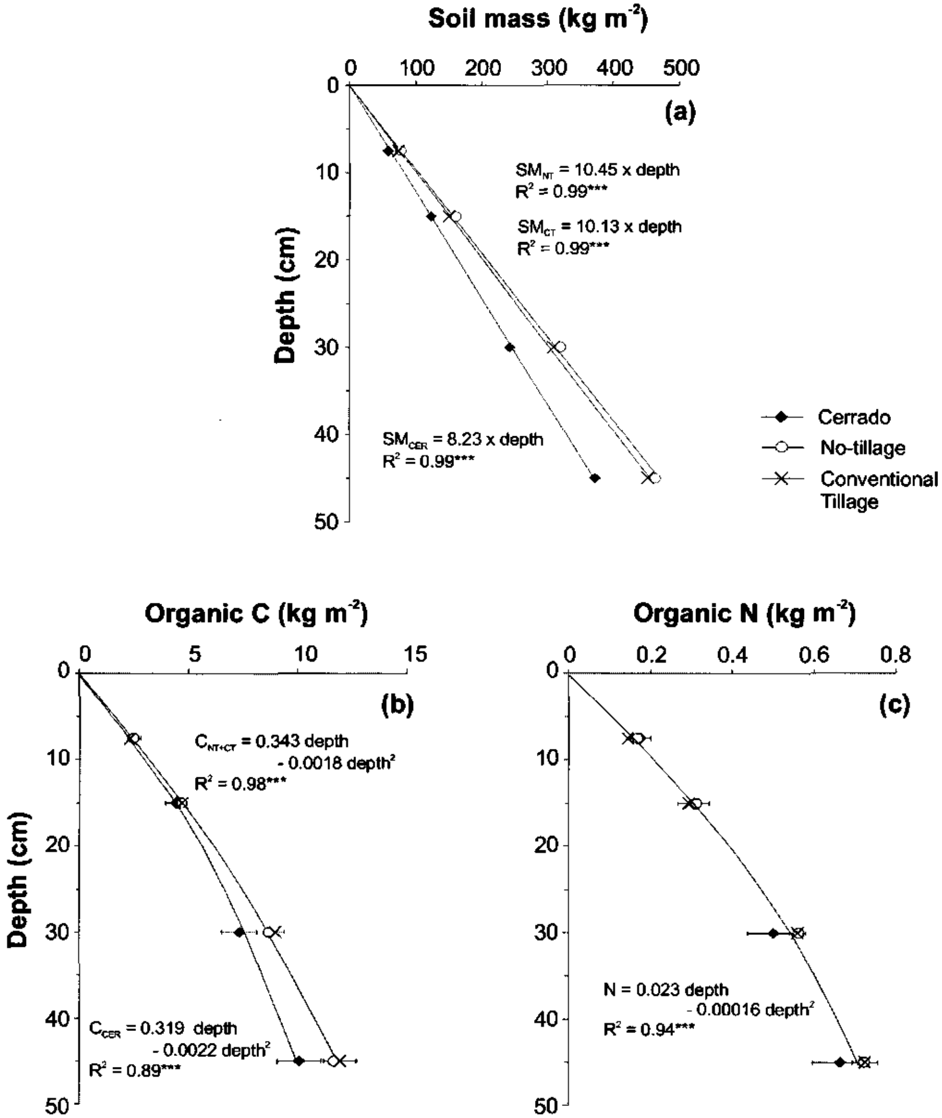

Figure 6.1. Cumulative mass of (a) soil and stocks of (b) carbon and (c) nitrogen, as a function of depth in cerrado (CER), no-tillage (NT), and conventional tillagg (CT).

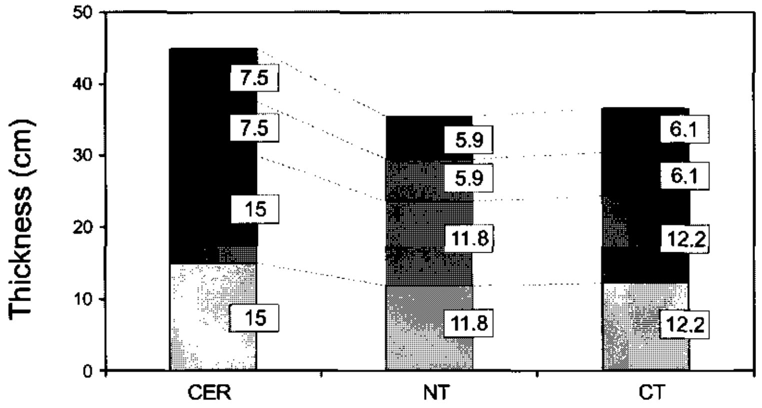

Figure 6.2. Equivalent depths in no-tillage (NT) and conventional tillage (CT) after correction for compaction of the cerrado (CER) soil. Values in boxes represent the thickness (cm) of each layer.

Table 6.2. Stocks of C and N in cerrado (CER), no-tillage (NT), and conventional tillage (CT). For NT and CT, the values were calculated for the same depths of CER – not corrected; and for equivalent depths in CER, corrected for differences in soil bulk density.

Depth in		Not corrected		Corrected	
CER	CER	NT	CT	NT	CT
----/ cm----	------------------------------C stock (kg m^{-2})------------------------------				
0 – 7.5	2.3 (0.1)[a]	2.5 (0.0)	2.5 (0.0)	2.0 (0.0)	2.0 (0.0)
7.5 – 15	2.0 (0.3)	2.3 (0.0)	2.3 (0.0)	1.9 (0.0)	1.9 (0.0)
15 – 30	3.3 (0.8)	3.9 (0.0)	3.9 (0.3)	3.3 (0.1)	3.4 (0.1)
30 – 45	2.4 (1.4)	3.1 (0.2)	3.1 (0.7)	2.8 (0.5)	2.9 (0.6)
0 – 45	10.0 (1.2)	11.8 (0.2)	11.8 (0.6)	9.9 (0.5)	10.2 (0.5)
	-----------------------------N stock (kg m^{-2})-----------------------------				
0 – 7.5	0.16[b]	0.16	0.16	0.13	0.13
7.5 – 15	0.15	0.15	0.15	0.12	0.12
15 – 30	0.23	0.23	0.23	0.20	0.21
30 – 45	0.16	0.16	0.16	0.16	0.16
0 – 45	0.70	0.70	0.70	0.61	0.62

[a] means predicted by the models from Figure 1 and standard error (between brackets) estimated from the residues of three replicates;

[b] standard error were equal or smaller than 0.01 kg m^{-2}.

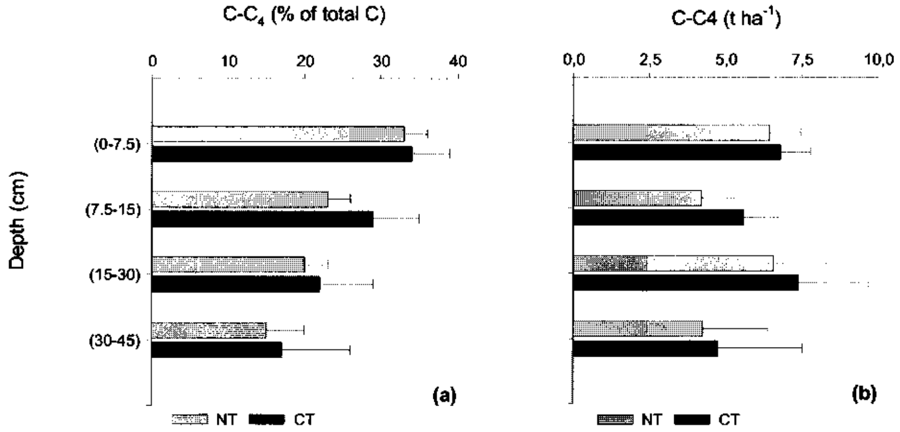

Figure 6.3. Proportions (a) and (b) stock of maize derived carbon (C_4-C) as a function of depth. Horizontal bars represent the standard error estimated by the Taylor series approximation (see Material and Methods for details).

3.3.2. Density fractions

After fractionation, we recovered 90-99% (average 96%) of all soil C and 89-101% (average 97%) of all N.

In general, the highest C contents (396-436 g kg^{-1}) were observed in the occluded light fraction (O-LF), while they varied from 212-278 g kg^{-1} and 25.8-33.6 g kg^{-1} in the free light fraction (F-LF) and the heavy fraction (HF), respectively (Table 6.3). The contents of N followed the same trend, although the differences between the light fractions were smaller (Table 6.3).

Between 80 and 95% of the C and 87-97% of the N accumulated in the HF (Figure 6.4). The O-LF held the lowest quantities of C and N (1-2% of total) and no difference was observed between treatments and depths. The F-LF showed a different pattern. In the first layer (0-7.5 cm), about 18% and 12% of the total C and N, respectively, were in the F-LF of CER. Upon cultivation, these amounts significantly dropped to values between 4-5% and 2-3% of the total organic C and N, respectively. The same trend was observed for the second depth (7.5-15cm), but with lower magnitude.

The C:N ratio of the fractions were remarkably different (Table 6.3). The O-LF showed the highest values (31-42), the F-LF intermediate (23-35), and the HF the lowest values (14-15).

In CER, $\delta^{13}C$ values of the F-LF and O-LF for both depths (between –26.7‰ and –26.9‰) were very close to the value observed for the litter (-27.8 ± 0.5‰), showing a enrichment of about 1‰ (Figure 6.5). The HF was more enriched in ^{13}C, with values 3.8‰ and 4.3‰ higher than the litter in the 0-7.5 cm depth (Figure 6.5a) and 7.5-15 cm depth (Figure 6.5b), respectively.

Table 6.3. Organic C, N and C:N ratio of the different density fraction for 2 depths in cerrado (CER), no-tillage (NT), and conventional tillage (CT). The density fractions are free light fraction (F-LF), occluded light fraction (O-LF), and heavy fraction (HF)

Plot	Depth	Organic C			Organic N			C:N ratio		
	Interval	F-FL	O-FL	HF	F-FL	O-FL	HF	F-FL	O-FL	HF
	---/cm---	------------/g kg^{-1}------------								
CER	0-7.5	25.8 (1.2)[a]	40.4 (3.2)	33.6 (2.0)	11.6 (0.8)	13.3 (1.4)	2.5 (0.2)	23 (2)	31 (2)	14 (0.3)
	7.5-15	28.3 (0.9)	39.5 (1.4)	27.1 (3.4)	9.4 (0.5)	11.2 (1.3)	1.9 (0.2)	30 (1)	36 (4)	14 (0.1)
NT	0-7.5	21.2 (2.1)	40.7 (2.4)	26.9 (2.0)	8.1 (0.9)	11.0 (0.6)	1.9 (0.2)	26 (2)	37 (2)	14 (0.3)
	7.5-15	27.3 (1.2)	39.6 (2.5)	25.8 (1.1)	7.9 (0.1)	9.5 (0.8)	1.8 (0.0)	35 (1)	42 (2)	15 (0.4)
CT	0-7.5	24.0 (0.5)	43.6 (1.4)	28.2 (1.0)	10.1 (0.3)	12.3 (0.8)	1.9 (0.1)	24 (1)	36 (4)	15 (0.2)
	7.5-15	25.2 (0.7)	41.1 (1.4)	28.2 (1.3)	10.0 (0.9)	11.1 (1.1)	1.9 (0.0)	26 (2)	38 (3)	15 (0.5)

[a] means and standard error (between brackets) for three replicates.

In NT and CT, values of $\delta^{13}C$ was always higher than in CER for all depths and fractions (Figure 6.6). Among the fractions, the lowest values were observed for the O-LF. The signature of F-LF was lower in NT than in CT for both depths. The other two fractions showed very similar values in the two cultivated fields.

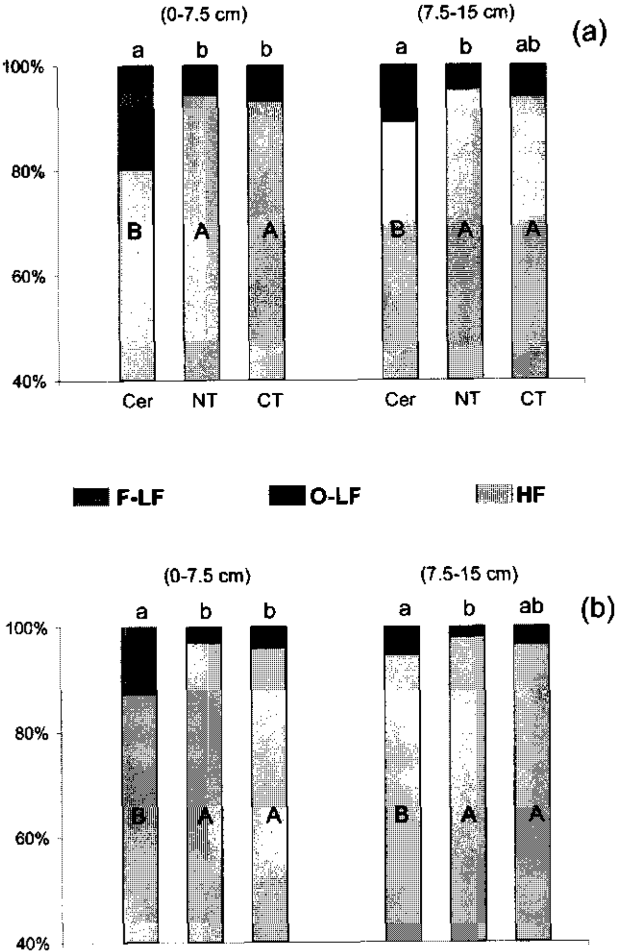

Figure 6.4. Proportions of total recovered organic C (a) and N (b) in free light fraction (F-LF), occluded light fraction (O-LF), and heavy fraction (HF) in cerrado (CER), no-tillage (NT), and conventional tillage (CT). Lower case letters compare the proportions of C and N in the F-LF between plots within the same depth. Upper case letters compare HF. Bars assigned with different letters are different by the Tukey test at 5% probability.

In the different fractions, the accumulation of C derived from maize (C_4 vegetation) tended to be higher at 0-7.5 cm (Figure 6.6ac) than at 7.5-15 cm (Figure 6.6bd). In CT, the F-LF showed the highest proportions of maize-derived material for both depths. However, in NT, no difference was observed between F-LF and HF. For both cultivated areas and depths, the O-LF showed the lowest proportional accumulation of C_4 derived material. A significantly higher proportion (Figure 6.6ab) and quantity (Figure 6.6cd) of C_4-derived carbon was observed in the F-LF of CT when compared to NT. In the two first depths, from the total amount of C_4-derived C (~ 10 t ha^{-1}) accumulated in 30 years of cultivation, about 92 to 97% of it was stored in the HF (Figure 6.6cd).

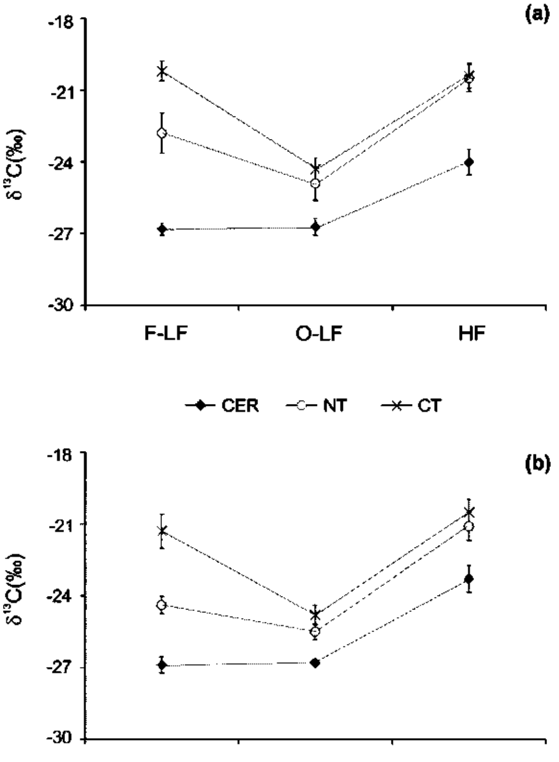

Figure 5. Values of $\delta^{13}C$ of the different density fractions in cerrado (CER), no-tillage (NT), and conventional tillage (CT), in (a) 0-7.5 cm and (b) 7.5-15 cm. Vertical bars are the standard error (n=3).

6.4. Discussion

6.4.1 Changes in C and N stocks

Veldkamp (1994) previously reported the possible influence of compaction on calculations of C stocks. The author observed a substantial increase in soil bulk density of an Eutric Hapludand and an Oxic Humitropept, 25 years after conversion from rainforest (Costa Rica) into pasture. Due to this compaction, C stocks in the first 50cm depth were systematically overestimated. We observed the same trend for C and N

(Tables 2, Figures 1 and 2). As the carbon accumulates in the heavy fraction (Figure 6.4), the relative increase of this fraction per volume of soil (compaction) will also concentrate C and N. The light fraction should be less influenced by compaction, since it is more connected to the vertical distribution of litter, which is linked to depth and mechanisms of incorporation. Therefor, in systems where C and N stocks are predominantly in the heavy fraction, we recommend to correct for compaction and express the stocks on a mass basis.

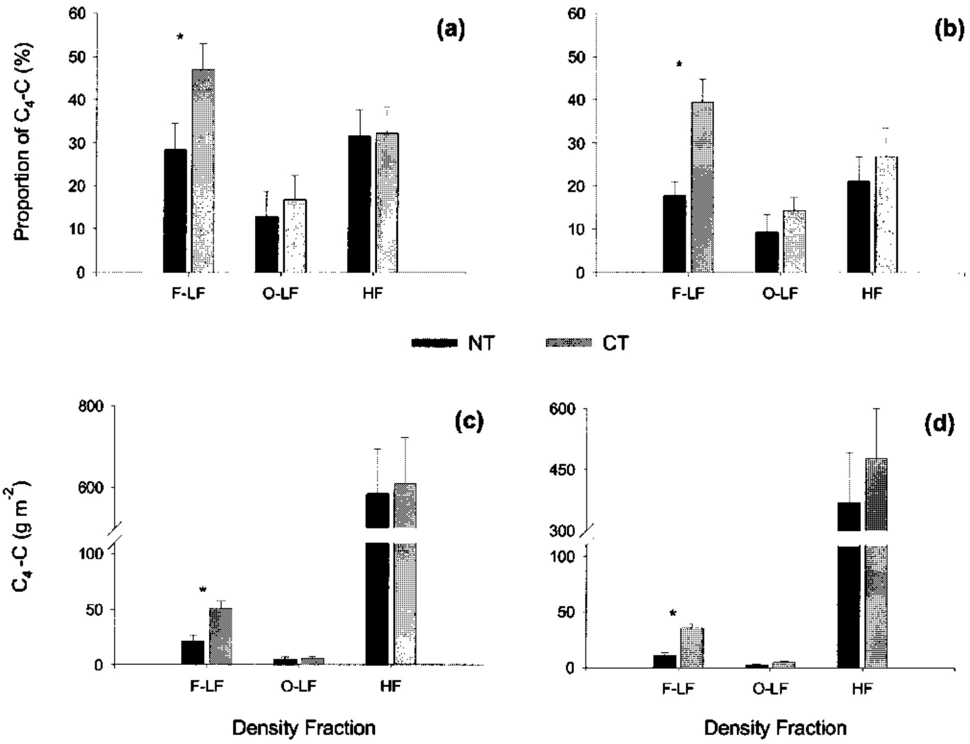

Figure 6.6. Proportions of C_4-derived C in relation to the total C for free light fraction (F-LF), occluded light fraction (O-LF), and heavy fraction (HF) at (a) 0-7.5 cm and (b) 7.5-15 cm. Stocks of C_4-derived carbon for the same fractions at (c) 0-7.5 cm and (d) 7.5-15 cm. Vertical bars represents the standard error. The Y-axes are interrupted in (c) and (d) to magnify the differences in the light fractions. Asterisks above the bars indicate significant difference between the cultivation system (t-test at 5% probability).

When corrected, stocks of C were virtually the same under native vegetation (CER) and in the fields cultivated for 30 years (NT and CT). In temperate climates, soils under conventional tillage lose up to 50% of their organic matter in about 50 or 100 years of cultivation, and that losses can be significantly reduced by no-tillage (Feller & Beare, 1997). For Cerrado soils, the effect of management systems does not allow general conclusions (Resck et al., 2000). The most comprehensive study, by Silva et al. (1994), involved 220 Cerrado topsoils under conventional soybean cultivation (heavy disk harrow). The soils were separated in three different groups: Quartz sand (< 15%

clay), loamy Red-Yellow Latosol (15-30% clay), and clayey Red-Yellow Latosol (> 30% clay). Silva et al. (1994) observed, respectively, losses of 80, 76 and 41% of the total initial carbon in 5 years of soybean cultivation, showing a high potential of SOM losses in those soils. By contrast, Freitas et al. (2000) did not observe changes in SOM stocks (0-40 cm) in a clayey Dark Red Latosol after 25 year of conventional cultivation (vegetables, rice, maize, and beans), when compared to natural cerrado vegetation. Similarly, examples of higher C stocks in no-tillage in relation to conventional tillage were reported by Resck et al. (2000) but not observed by Maria & Castro (1993a,b) and Freitas et al. (2000).

Hassink & Whitmore (1997) postulated that the protective effect of clay on soil organic matter involves two mechanisms, the interaction of SOM with the surface of the clay particles (cation bridges, hydrogen bounds, electrostatic and Van der Waals interactions) and the occlusion of organic material in the matrix of soil aggregates. The capacity of protecting SOM is positively correlated to clay contents. In addition, the typically elevated concentration of Fe and Al oxi-hydroxides in Oxisols may strongly affect the availability of SOM to decomposition. The considerable positive charge density of these components at normal soil pH forms strong electrostatic bounds with the negatively charged SOM (Resende et al., 1997). The high clay (>800 g kg^{-1}) and oxi-hydroxide contents of the studied Dark Red Latosol (Chapter 3 and 4) was probably an important factor in protecting C against decomposition. Nevertheless, the contrasting results obtained by Silva et al. (1994) for clayey Latosols, also rich in Fe and Al oxi-hydroxides, suggest that the residue quality may be of great importance as well. In their study, the soils were under continuous soybean cultivation, which produces less residue, with higher degradability, than maize. Then, the maintenance of carbon levels in our soil after 30 years of cultivation seems to result from a combination of high protective capacity of the soil and enough residue supply by the crop (maize).

6.4.2. Distribution of C and N among the fractions:

The accumulation of C and N in the heavy fraction of clayey soils is well documented in the literature and results from the strong interaction between clay particles and SOM (Christensen, 2000).

Clayey soils are also expected to accumulate significant amounts of occluded light fraction (O-LF), which would be entrapped in the matrix of soil aggregates (Hassink & Whitmore, 1997). For a variety of temperate and sub-tropical clayey soils, Golchin et al. (1994) and Parfitt et al. (1997) found between 16 and 39% of the total soil carbon in the O-LF. Conversely, for tropical clayey Oxisols under different vegetation (forest, cerrado, and pasture), Golchin et al. (1995) and Freixo et al. (2001) observed that only between 1 and 4% of the total C was in the O-LF, in agreement with our observations (Figure 6.4). Due to their particular mineralogy (dominated by kaolinite and high quantities of Fe and Al oxi-hydroxides), Oxisols are characterized by the presence of a very strong granular structure (diameter < 1.0 mm) as their basic structural unit (Resende et al., 1997). Compared to blocky structures that commonly dominate non-oxidic clayey soil (e.g. Golchin et al., 1994, and Parfitt et al., 1997), the microstructures of Oxisols have a limited internal volume in relation to their surface, reducing the probability of occluding organic material in locations inaccessible to the microbiota. This explains the low capacity of these soil in storing O-LF.

Although there was no significant reduction in the stocks of organic C and N in the total soil (section 4.1), HF, and O-LF (Figure 6.4), the quantities of F-LF strongly reduced upon cultivation. This decrease was also reported by several studies in temperate (Christensen, 2000) and tropical soils (Feller & Beare, 1997). This phenomenon has been attributed to a reduction in the residue supply and an increase in the decomposition rate of this fraction (Christensen, 2000). These results support the hypothesis that the F-LF is more sensitive to management-induced changes.

In relation to the different cultivation systems, our results were not in agreement with data from Cambardella & Elliott (1992) and Wander & Bidart (2000), who found significantly more F-LF under no-tillage than under conventional tillage. For our study, the absence of a difference between NT and CT (Figure 6.4) was probably related to the high potential for decomposition in this soils (high temperature, good water supply, liming, and fertilization) and the relatively short period of time under no-tillage (10 years), after 20 years of conventional tillage.

6.4.3. *$\delta^{13}C$ in total soil and fractions*

The trend of increasing $\delta^{13}C$ values with depth, as observed in CER (Table 6.1), has been widely reported in the literature for ecosystems dominated by C_3 plants (Balesdent & Mariotti, 1996). This relative enrichment is attributed to fractionation of ^{13}C during decomposition and to the reduction in atmospheric $^{13}CO_2$ concentrations due to fossil fuel burning and land-use changes in the past 150 years, the so-called Suess effect (Balesdent & Mariotti, 1996). The enrichment in ^{13}C upon cultivation, however, was clearly related to the incorporation of new C_4-carbon from the maize.

The $\delta^{13}C$ signatures for the light fractions of CER soil were closer to the litter than was the HF (Figure 6.5). This may also be explained by difference in the degree of decomposition of the material present in these fractions. Carbon in the HF is usually more altered by the microbiota than in the LF (Golchin et al., 1997), which would increase its ^{13}C signature due to fractionation during decomposition (Balesdent & Mariotti, 1996). The low C:N values in the HF (Table 6.3) supported the hypothesis of larger amounts of microbial material in this fraction than in the light fractions. Differences in $\delta^{13}C$ signature between the F-LF and O-LF would also be expected, since the O-LF usually shows a higher degree of decomposition (Golchin et al., 1997). But these light fractions showed similar signature (Figure 6.5). In a previous study, in the same cerrado area (Chapter 4), we observed abundant charcoal fragments in the F-LF with a signature of –27.9 ± 0.3‰, very close to that of litter (-27.8 ± 0.5‰). We also observed that the O-LF was composed of very dark and amorphous material. Although only a few fragments of charcoal could be distinguished and most of the particles could not be identified, it is very likely that this fraction has also a large quantity of charred material. The relatively high C:N ratio of the O-LF (Table 6.3) supports this hypothesis. As charcoal has the same $\delta^{13}C$ signature as litter, the presence of high amounts of these recalcitrant charred particles in the O-LF would explain the absence of ^{13}C fractionation in relation to the F-LF.

In the cultivated area, the $\delta^{13}C$ signature of all fractions indicated the incorporation of C_4 material (Figure 6.5). In general, the changes in $\delta^{13}C$ signature indicated a substitution of C_3 by C_4 carbon in the order O-LF < HF < F-LF. This observation is in conflict with the general idea that the O-LF has a turnover faster than the HF (Golchin et al., 1997), but it is explained by the presence of charcoal in the O-LF. Charred materials are known by their high recalcitrance and turnover time (Schmidt & Noack, 2000). The charred material present in the cultivated fields came from the previous cerrado vegetation with $\delta^{13}C$ values close to that of the C_3 litter. As the charcoal is preserved, only small changes in the $\delta^{13}C$ of the O-LF should occur.

6.4.4. Replacement calculations

Similar replacement of C_3-C by C_4-C in NT and CT (Figure 6.3) seems to contradict the general view that organic C under conventional tillage has a faster turnover time than in no-tillage (Resck et al, 2000). The high variability in the replacement calculations, due to the large number of input variables involved, may partially explain the absence of a difference between the treatments. This was suggested by the systematic trend of higher values of replacement in CT for all depths but the topsoil. Nevertheless, the high clay content and peculiar mineralogy seems to ascribe a high stability to the organic C in the studied soil. More than two thirds of the total carbon was not substituted in 30 years of cultivation (Figure 6.3a), which suggests that most of the carbon is in a relatively stable pool. Then, the combination of a high uncertainty in the calculations and a relatively small labile pool, which would be the pool mostly affected by cultivation (Feller & Beare, 1997; Christensen, 2000), might be the reason for an absence of differentiation between the treatments.

Although total C did not reflect the effect of different management systems, when analyzing the replacement in each fraction (Figure 6.6), we observed significantly higher amounts of C_4 derived material in the F-LF of the CT. The F-LF is considered the most labile density fraction and, consequently, the most sensitive to environmental changes (Golchin et al., 1997). Thus, although we did not observe significant differences between CT and NT in the total amount of C of the F-LF (Figure 6.4), there was a significantly higher C_4-substitution in CT. This suggests a faster turnover for the labile fraction of CT, which agrees with the literature (Resck et al., 2000).

Although the L-FL was sensitive to differences in the management systems, between 91 and 96% of the total C replacement occurred in the HF (Figure 6.6cd). For this reason, the total replacement (Figure 6.3) was strongly influenced by the substitution in the HF. The differences observed for the F-LF were cancelled by the uncertainty in the measurements of total replacement. In other words, they were smaller than the uncertainty of HF replacements.

8.5. Conclusion

Soil compaction may systematically lead to overestimation of C and N stocks in cultivated areas when compared to natural ecosystems. A correction for compaction is recommended, especially in soils where SOM accumulates in the heavy fraction.

Conversion of cerrado into a conventional tillage or no-tillage system did not alter the total C (~100 Mg ha^{-1}) and N (~7 Mg ha^{-1}) stocks of the first 45cm depth in 30 year of cultivation. However, about 22% of the total carbon was replaced by maize material in the period.

The "free light fraction" was most sensitive to changes in the management systems and significantly decreased after converting cerrado into cropland. The proportions of C replacement in this fraction were higher in conventional tillage than no-tillage, suggesting a faster turnover time in the first. Nevertheless, carbon dynamics in the studied soil was controlled by the behavior of the HF, because most carbon (~95%) was held in this fraction.

The maintenance of C levels even after 30 years of cultivation and the absence of differentiation between no-tillage and conventional tillage was attributed to the high clay contents and Fe+Al oxi-hydroxides concentrations of the studied soil, as well as the adequate C supply by the maize crop.

Chapter 7

Natural abundance of ^{13}C in soil organic matter studies: assumptions and uncertainties in calculations

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Abstract

We used two data sets (Chapters 5 and 6) to calculated the proportions and stocks of carbon derived from a new introduced C_4 vegetation in a C_3 ecosystem, by two different linear mixing models. One assumed no ^{13}C discrimination for the new C_4 introduced material and the other assumed equal ^{13}C discrimination for both C_3 and C_4 materials. Our aims were to evaluate (i) how the assumption of equal ^{13}C discrimination for C_3 and C_4 material may effect estimates of proportions and stocks of C_4-derived carbon for different soil depths and soil organic matter fractions; (ii) the significance of a possible difference between outputs using the different models; and (iii) the sensitivity of this difference to variations in the input parameters. We also discussed this assumption in the light of the current theoretical understanding of processes leading to ^{13}C discrimination in soils. We applied Taylor series approximation to estimate the output uncertainties of the models, and sensitivity analysis to test the influence on these outputs of the magnitude of ^{13}C discrimination with decomposition for the standard C_3 vegetation, the population standard deviation of soil and litter $\delta^{13}C$, and the sample size for soil and litter. We observed that choosing a model assuming or not equal ^{13}C discrimination for both C_3 and C_4 materials may generate significantly discrepant outputs. This difference was more sensitive to the standard deviation of soil $\delta^{13}C$ population and the number of soil samples. A critical analysis of the current understanding of processes leading to ^{13}C discrimination in soils suggests that the assumption of no ^{13}C discrimination for the new C_4 introduced material would provide the most realistic results. Nevertheless, further research is needed to give better support for the hypotheses explaining those processes.

7.1. Introduction

The natural abundance of ^{13}C in soil organic matter (SOM) has been widely used to trace carbon origin and dynamics in different ecosystems (Balesdent & Mariotti, 1996; Ehleringer et al., 2000). This natural tracer, which allows estimations over time-scales from a few years to millennia (Balesdent & Mariotti, 1996), represents a powerful tool in understanding impact of land-use changes on the global carbon cycle (Ehleringer et al., 2000).

The use of ^{13}C in studies of SOM dynamics requires a change in the dominant vegetation between plants following different photosynthetic pathways (Balesdent & Mariotti, 1996). This is possible because during photosynthesis C_3 and C_4 plants fractionate ^{13}C to a different degree. While the present atmosphere has a $\delta^{13}C$ value of –8 ‰ (in relation to the PDB international standard), C_3 isotopic signature ranges from –32‰ to –22‰ (average of –27‰) (Boutton, 1996). In contrast, C_4 plants are less efficient in discriminating ^{13}C, showing $\delta^{13}C$ values between –16‰ and –9‰ (average of –13‰). The mean difference between these two groups (~ 14‰) is used in linear mixing models to estimate the proportions of each source (C_3 or C_4 material) in any mixed system (e.g. litter, soil organic matter) (Balesdent & Mariotti, 1996).

Instead of sampling the same locations before and after vegetation change, paired samples are often used for SOM dynamic studies (Balesdent & Mariotti, 1996). Undisturbed ecosystems are used as $\delta^{13}C$ standards, assuming that they represent the former situation in the converted neighbour areas. Usually no standard profile is available for the introduced vegetation. Standard profiles exist only for the soil carbon of the previous vegetation. Therefore, the $\delta^{13}C$ signature of *soil* carbon derived from the new material is unknown. The available information is often plant or litter signature, or just the average (literature) $\delta^{13}C$ values of the photosynthetic group. This would not be a problem if carbon in soils kept the same signature of plant or litter materials from which it is derived. However, $\delta^{13}C$ values of soils may be enriched by 1–3‰ in relation to plant/litter material (Boutton, 1996; Balesdent & Mariotti, 1996; Ehleringer et al., 2000). This increase has been attributed to isotopic discrimination during decomposition and to the Suess effect (Ehleringer et al., 2000). Such fractionation can be easily assessed for the standard old vegetation, but has to be assumed for the newly entering material.

Basically, two different linear mixing models are used in the literature (Balesdent & Mariotti, 1996). One does not consider any ^{13}C discrimination from litter to SOM for the introduced vegetation. The other accounts for a fractionation equal to that observed in the old vegetation reference profile. If in the reference profile an increase of 2‰ was observed from litter to soil (say from –27‰ to –25‰), the same absolute increase (2‰) is applied to the newly introduced vegetation. Different final results are expected when using each model, the magnitude of which are directly related to the ^{13}C discrimination from litter to soil in the standard profile. This difference can be particularly large when working with samples from deeper horizons or more humified fractions of SOM (Balesdent & Mariotti, 1996). Therefore, assuming or not isotopic discrimination for the new introduced vegetation will systematically bias the final results.

Both models require a number of input variables from the old and new vegetation. Each of these variables has its own variability, related to field heterogeneity, sampling and sample preparation, analytical precision, etc (Balesdent & Mariotti, 1996). The output variables (i.e. source proportions) combine all these uncertainties, which propagate through calculations (Veldkamp & Weitz, 1994; Phillips & Gregg, 2001).

Although these output uncertainties can be significant, in most studies they have not been properly accessed. Using Taylor series approximations, Phillips & Gregg (2001) proposed an analytical solution to estimate these uncertainties and provided equations for directly assessing standard errors and confidence intervals for source proportions.

We used two data sets (Chapters 5 and 6) to calculated the proportions and stocks of carbon derived from a new introduced C_4 vegetation in a C_3 ecosystem, by two different linear mixing models. One assuming no litter-soil ^{13}C discrimination for the new C_4 introduced material and the other assuming equal ^{13}C discrimination for both C_3 and C_4 materials. Our aims were to evaluate (i) how the assumption of equal ^{13}C discrimination for C_3 and C_4 material affects estimates of proportions and stocks of C_4-derived carbon for different soil depths and soil organic matter fractions; (ii) the significance of a possible difference between outputs using the different models; and (iii) the sensitivity of this difference to variations in the input parameters. We hypothesised that the combined variability of outputs from the two models would overlap the systematic difference between them. We discuss this assumption in the light of the current theoretical understanding of processes leading to ^{13}C discrimination in soils.

7.2. Material and Methods

7.2.1. Data sets used

We use data reported from Chapters 5 and 6. Both data sets were collected in the same area, a homogeneous clayey Latosol unit (Oxisol, Soil Taxonomy; Ferrasol, FAO/UNESCO) from the experimental station of EMBRAPA-CNPMS, Sete Lagoas-MG, Brazil. Three plots were sampled: a *cerrado sensu stricto* (C_3-dominated woodland savannah) and two nearby fields cultivated with maize (C_4) for 30 years. Initially, conventional tillage was used in both cultivated fields, but one was converted to no-tillage, 10 years before sampling. The first data set (Chapter 5) consists of topsoil (0-7.5 cm) regionalized samples: 29 under cerrado, 24 in the no-tillage (NT) and 25 in the conventional tillage (CT). We only used data form the cerrado and the no-tillage system. Summary statistics of the input variables are presented in Table 7.1. The second data set is part of the detailed measurements made in Chapter 6 for the same area. Four different depths and two fractions of soil organic matter were evaluated in three profiles (Table 7.2 and 7.3). From this data set, we calculated two output variables: proportions of C_4 derived carbon (F_{C4}) and stocks of C_4-derived C (S_{C4}) in kg m^{-2}. These outputs were calculated either assuming or not ^{13}C discrimination for the introduced C_4 vegetation (see equations below).

Table 7.1. Description and summary statistics of input variables from Roscoe et al. (2001a).

Variable	Description	n	mean ($\hat{x}$)	variance (σ^2)
$\delta_{C3litter}$	$\delta^{13}C$ of cerrado litter (C3)	10	-27.8	0.172
δ_{C3soil}	$\delta^{13}C$ of cerrado soil (C3)	29	-24.4	0.882
$\delta_{C4litter}$	$\delta^{13}C$ of maize litter (C_4)	3	-12.8	0.024
δ_{Msoil}	$\delta^{13}C$ of the mixed system (no-tillage)	24	-20.6	0.322
C_M	carbon content (g kg^{-1}) in the mixed system	24	34.02	2.637
D_M	bulk density (g cm^{-3}) in the mixed system	24	1.10	0.0036

Table 7.2. Summary statistics of input variables at different depths of a cerrado standard vegetation (C_3) and a maize field (the mixed system) cultivated for 30 years (20 years of conventional tillage and 10 of no-tillage). Data are from Roscoe et al. (2001b).

Variable[a]	Depth (cm)	n	mean ($\hat{x}$)	variance (σ^2)
δ_{C3soil}	0-7.5	3	-24.8	0.640
δ_{C3soil}	7.5-15	3	-24.0	0.980
δ_{C3soil}	15-30	3	-23.9	0.410
δ_{C3soil}	30-45	3	-23.8	0.270
δ_{Msoil}	0-7.5	3	-20.8	0.922
δ_{Msoil}	7.5-15	3	-21.4	0.723
δ_{Msoil}	15-30	3	-21.7	0.774
δ_{Msoil}	30-45	3	-22.1	1.638
C_M	0-7.5	3	31.4	1.057
C_M	7.5-15	3	27.3	0.195
C_M	15-30	3	25.0	1.396
C_M	30-45	3	20.8	3.726
D_M	0-7.5	3	1.04	0.047
D_M	7.5-15	3	1.08	0.024
D_M	15-30	3	1.06	0.005
D_M	30-45	3	0.95	0.058

[a] variable description in Table 1.

Table 7.3. Summary statistics of input variables in different depths and soil organic matter fractions of a cerrado standard vegetation (C3) and a maize field (the mixed system) cultivated for 30 years (20 years of conventional tillage and 10 of no-tillage). Data are from Roscoe et al. (2001b).

Variable[a]	Fraction[b]	n	mean ($\hat{x}$)	variance (σ^2)
δ_{C3soil}	light	3	-26.8	0.194
δ_{C3soil}	heavy	3	-24.0	0.884
δ_{Msoil}	light	3	-22.8	2.103
δ_{Msoil}	heavy	3	-20.5	0.941
C_M	light	3	31.4	1.057
D_M	heavy	3	1.04	0.047
P_C	light	3	0.04	$2.15\text{x}10^{-6}$
P_C	heavy	3	0.96	$2.15\text{x}10^{-6}$

[a] P_C is the proportion of the total soil carbon in the fraction; the other variables are as in Table 1;
[b] light = free light fraction with density < 1.7 g cm^{-3}; heavy = heavy fraction with density > 1.7 g cm^{-3}.

7.2.2. Linear mixing models

We used two linear mixing models according to Balesdent & Mariotti (1996). The models were formulated from the mass balance equations (1) and (2):

$$\delta_M = F_{C3}\delta_{C3} + F_{C4}\delta_{C4} \quad (1)$$

$$1 = F_{C3} + F_{C4} \quad (2)$$

where δ_M is the $\delta^{13}C$ value of the mixed system, δ_{C3} is the $\delta^{13}C$ of C_3 derived material, δ_{C4} is the $\delta^{13}C$ value of C_4 derived material, F_{C3} is the proportion of C_3 carbon, and F_{C4} is the proportion of C_4 carbon. The proportion of C_4 derived material was then calculated by two different models. The first model did not consider fractionation between litter and soil organic matter of the C_4 derived material (the new source of C added), as formulated by equation (3):

$$F_{C4} = (\delta_{Msoil} - \delta_{C3soil})/(\delta_{C4litter} - \delta_{C3soil}) \quad (3)$$

where δ_{Msoil} is the $\delta^{13}C$ of SOM in the mixed systems, δ_{C3soil} is the $\delta^{13}C$ of SOM at a given depth or SOM fraction of the reference C_3 profile, and $\delta_{C4litter}$ is the $\delta^{13}C$ of the C_4 litter.

A second model considers, for the new introduced material (C_4 litter), the same ^{13}C discrimination observed for the standard C_3 vegetation from litter to SOM (or its fractions). Equation (3) is then slightly modified to equation (4), substituting δ_{C3soil} by $\delta_{C3litter}$ in the denominator:

$$F_{C4}' = (\delta_{Msoil} - \delta_{C3soil})/(\delta_{C4litter} - \delta_{C3litter}) \quad (4)$$

where δ_{Msoil}, δ_{C3soil}, and $\delta_{C4litter}$ are the same as in equation (3) and $\delta_{C3litter}$ is the $\delta^{13}C$ of C_3 litter. The difference between the two models (ΔF_{C4}) was obtained by subtracting F_{C4}' from F_{C4}.

We also calculated the stock (kg m^{-2}) of C_4-derived C at each soil layer (S_{C4T}) by equation (5) and in each fraction (S_{C4F}) by equation (6):

$$S_{C4T} = d \times (C_T \times D \times F_{C4}) \quad (5)$$

$$S_{C4F} = d \times (C_T \times D \times F_{C4} \times P_C) \quad (6)$$

where d is the layer thickness (m), C_T is the total carbon contents (g kg^{-1}), D is the soil bulk density (g cm^{-3}) and P_C is the proportion of total carbon presented in a given fraction (light or heavy fraction).

7.2.3. Uncertainty analysis

The variance of F_{C4} (σ^2_{FC4}) obtained in equation (3) (without ^{13}C discrimination) was estimated by using partial derivatives (Phillips & Gregg, 2001), according to equation (6):

$$\sigma^2_{F_{C4}} = \left(\frac{\partial F_{C4}}{\partial \delta_{Msoil}}\right)^2 \sigma^2_{\delta_{Msoil}} + \left(\frac{\partial F_{C4}}{\partial \delta_{C4litter}}\right)^2 \sigma^2_{\delta_{C4litter}} + \left(\frac{\partial F_{C4}}{\partial \delta_{C3soil}}\right)^2 \sigma^2_{\delta_{C3soil}} \quad (7)$$

which reduces to:

$$\sigma^2_{F_{C4}} = \frac{1}{(\delta_{C4litter} - \delta_{C3soil})^2}\left[\sigma^2_{\delta_{Msoil}} + F^2_{C4}\,\sigma^2_{\delta_{C4litter}} + (1 - F_{C4})^2\,\sigma^2_{\delta_{C3soil}}\right] \quad (8)$$

where, $\sigma^2_{\delta_{Msoil}}$, $\sigma^2_{\delta_{C4litter}}$, and $\sigma^2_{\delta_{C3soil}}$ are the variances of δ_{Msoil}, $\delta_{C4litter}$, and δ_{C3soil}, respectively. The variance of F_{C4}' ($\sigma^2_{F_{C4}'}$) obtained in equation (4) (with ^{13}C discrimination) was estimated also by Taylor Series approximation (Taylor, 1982), according to equation (9),

$$\begin{aligned}\sigma^2_{F_{C4}'} = {} & \left(\frac{\partial F_{C4}'}{\partial \delta_{Msoil}}\right)^2 \sigma^2_{\delta_{Msoil}} + \left(\frac{\partial F_{C4}'}{\partial \delta_{C4litter}}\right)^2 \sigma^2_{\delta_{C4litter}} \\ & + \left(\frac{\partial F_{C4}'}{\partial \delta_{C3soil}}\right)^2 \sigma^2_{\delta_{C3soil}} + \left(\frac{\partial F_{C4}'}{\partial \delta_{C3litter}}\right)\sigma^2_{\delta_{C3litter}}\end{aligned} \quad (9)$$

which reduces to:

$$\sigma^2_{F_{C4}'} = \frac{1}{(\delta_{C4litter} - \delta_{C3litter})^2}\left[\sigma^2_{\delta_{Msoil}} + (F_{C4}')^2\,\sigma^2_{\delta_{C4litter}} + \sigma^2_{\delta_{C3soil}} + (F_{C4}')^2\sigma^2_{\delta_{C3litter}}\right] \quad (10)$$

where, $\sigma^2_{\delta_{Msoil}}$, $\sigma^2_{\delta_{C4litter}}$, and $\sigma^2_{\delta_{C3soil}}$ are the same as in equation (8) and $\sigma^2_{\delta_{C3litter}}$ is the variance of $\delta_{C3litter}$. Variances of the difference between F_{C4} and F_{C4}' (ΔF_{C4}) were given by the sum of their individual variances. The variances of C_4-derived C stocks at each depth (S_{C4T}) (equation 11) and in each fraction (S_{C4F}) (equation 12) were also estimated by partial derivatives, according to the Taylor Series approximation (Taylor, 1982) as:

$$\sigma^2_{S_{C4T}} = 0.075^2 \times \left\{\left(\frac{\partial S_{C4T}}{\partial C_M}\right)^2 \sigma^2_{C_M} + \left(\frac{\partial S_{C4T}}{\partial D_M}\right)\sigma^2_{D_M} + \left(\frac{\partial S_{C4T}}{\partial F_{C4}}\right)^2 \sigma^2_{F_{C4}}\right\} \quad (11)$$

$$\sigma^2_{S_{C4F}} = 0.075^2 \times \left\{\left(\frac{\partial S_{C4F}}{\partial C_M}\right)^2 \sigma^2_{C_M} + \left(\frac{\partial S_{C4F}}{\partial D_M}\right)\sigma^2_{D_M} + \left(\frac{\partial S_{C4F}}{\partial F_{C4}}\right)^2 \sigma^2_{F_{C4}} + \left(\frac{\partial S_{C4F}}{\partial P_C}\right)^2 \sigma^2_{P_C}\right\} \quad (12)$$

which reduces to:

$$\sigma^2_{S_{C4T}} = 0.075^2 \times S^2_{C4T}\left(\frac{\sigma^2_{C_M}}{C^2_M} + \frac{\sigma^2_{D_M}}{D^2_M} + \frac{\sigma^2_{F_{C4}}}{F^2_{C4}}\right)^2 \qquad (13)$$

and

$$\sigma^2_{S_{C4F}} = 0.075^2 \times S^2_{C4F}\left(\frac{\sigma^2_{C_M}}{C^2_M} + \frac{\sigma^2_{D_M}}{D^2_M} + \frac{\sigma^2_{F_{C4}}}{F^2_{C4}} + \frac{\sigma^2_{P_C}}{P^2_C}\right)^2 \qquad (14)$$

where, $\sigma^2_{S_{C4}}$, $\sigma^2_{C_M}$, $\sigma^2_{D_M}$, and $\sigma^2_{F_{C4}}$ are the variances of S_{C4}, C_M, D_M, and F_{C4}, respectively. The Satterthwaite approximations for the degrees of freedom (γ) associated to with $\sigma^2_{F_{C4}}$ and $\sigma^2_{S_{C4}}$ were calculated according to Phillips & Gregg (2001) by equation (15) as,

$$\gamma = \frac{\left(\sum_i c_i V_i\right)^2}{\sum_i \frac{(c_i V_i)^2}{d_i}} \qquad (15)$$

where V_i are the individual variance terms, c_i are their coefficients, and d_i are their associated degree of freedom (n_i-1) on the right sides of equations (8), (10), (13), and (14).

Table 7.4. Parameter ranges and default values used in the sensitivity analyses.

Parameter	Minimum	Maximum	Default
$\delta^{13}C$ analytical precision (‰)	0.01	0.50	0.17
$\delta^{13}C$ **soil** population standard deviation (‰)	0	2.0	1.00
$\delta^{13}C$ **litter** population standard deviation (‰)	0	1.0	0.50
Sample size for **soil** (n_{soil})	3	30	–
Sample size for **litter** (n_{litter})	3	30	–
^{13}C discrimination in C_3 ($\delta_{C3litter} - \delta_{C3\ soil}$)	0	4	2

7.2.4. Sensitivity Analysis

We performed a sensitivity analysis to evaluate the influence of several factors on the difference between the two methods of calculating proportions of C_4-derived carbon (ΔF_{C4}). The factors analysed were the magnitude of ^{13}C discrimination with decomposition for the standard C_3 vegetation (δ_{C3soil} - $\delta_{C3litter}$), the population standard deviation of soil ($\sigma\delta_{Msoil}$ and $\sigma\delta_{C3soil}$) and litter $\delta^{13}C$ ($\sigma\delta_{C3litter}$ and $\sigma\delta_{C4litter}$), the sample size for soil (n_{Msoil} and n_{C3soil}) and litter ($n_{C3litter}$ and $n_{C4litter}$). The factors' ranges and default values (Table 7.4) were based on the literature (Balesdent and Mariotti, 1996; Phillips and Gregg, 2001) and our own observations (Roscoe and Buurman, 2001; Roscoe et al., 2001).

7.3. Results

7.3.1. Comparing linear mixing models

We used three data set (Tables 7.1, 7.2, and 7.3) to compare the difference of estimating source proportions and stocks by the two linear mixing models (equations 3 and 4). Except for the light fraction, the model assuming ^{13}C discrimination for the C_4 little-SOM transition (equation 4) systematically generated 20-30% lower outputs for the proportion (F_{C4}) and stocks (S_{C4}) of C_4 derived carbon (Tables 7.5, 7.6, and 7.7). These differences increased with the magnitude of discrimination from C_3 litter to fractions, and with depth. They were higher for the topsoil (Table 7.6) and for heavy fractions (Table 7.7). These results can be easily explained by comparing equations (3) and (4). The denominator of equation (4) tends to increase whenever the $\delta^{13}C$ value of C_3 litter is more negative than the soil signature.

Table 7.5. Proportions and stocks (kg m^{-2}) of C_4-derived carbon as calculated with and without litter-SOM ^{13}C discrimination for the C_4 material, standard error (SE), degrees of freedom (γ), lower and upper limits of the 95% confidence interval. Input data from Table 1, adapted from Roscoe et al. (2001). Stocks are given in kg m^{-2} for the first 7.5-cm depth.

Var.	Assuming ^{13}C discrimination	Mean	SE	γ	95% Confidence Interval	
					Lower Limit	Upper Limit
F_{C4}	No	0.33	0.015	53	0.30	0.36
	Yes	0.25	0.015	50	0.22	0.28
S_{C4}	No	0.92	0.012	61	0.90	0.94
	Yes	0.71	0.011	56	0.68	0.73

Standard errors did not show large differences between the models (Tables 7.5, 7.6, and 7.7). Values slightly higher were observed for calculations assuming discrimination. This was probably related to lower variances of $\delta^{13}C$ for litter than for soil, which would represent a lower variability of one of the input parameters in the denominator of equation (4) when compared to equation (3).

Satterthwaite approximations for the degrees of freedom (γ) were also barely affected by the model applied (Tables 7.5, 7.6, and 7.7). Values followed the degrees of freedom of the input variable, being considerably high for the first data set (Table 7.5).

Confidence intervals showed the same trends observed for standard errors (SE), being slightly lower when ^{13}C discrimination was considered (Tables 7.5, 7.6, and 7.7). Confidence intervals are related to SE and the degree of freedom (γ). As γ was fairly constant between models, SE trends were directly reflected in the confidence intervals. Comparing results from Table 7.5 with the first depth of Table 7.6 (two independent

Table 7.6. Mean proportions (F_{C4} and F_{C4}') and stocks (S_{C4} and S_{C4}') of C_4-derived C, standard error (SE), degrees of freedom (γ), and lower and upper limit of the 95% confidence interval, as a function of soil depth and the assumption of ^{13}C discrimination of C_4 material during litter-SOM transition. Input data are from Table 2, adapted from Roscoe and Buurman (2001). The input values for C_3 and C_4 litters are from Table 1. Stocks are given in kg m^{-2} for the each depth interval.

Var.	Depth (cm)	Assuming ^{13}C discrimination	Mean	SE	γ	95% Confidence Interval	
						Lower Limit	Upper Limit
F_{C4}	0-7.5	No	0.33	0.053	3	0.16	0.50
	7.5-15	No	0.23	0.059	4	0.04	0.42
	15-30	No	0.20	0.053	3	0.03	0.37
	30-45	No	0.15	0.071	2	-0.15	0.46
F_{C4}'	0-7.5	Yes	0.26	0.049	4	0.13	0.40
	7.5-15	Yes	0.17	0.050	4	0.03	0.31
	15-30	Yes	0.15	0.042	4	0.01	0.28
	30-45	Yes	0.11	0.053	3	-0.12	0.34
S_{C4}	0-7.5	No	0.82	0.010	4	0.78	0.85
	7.5-15	No	0.51	0.010	4	0.49	0.54
	15-30	No	0.79	0.016	3	0.74	0.84
	30-45	No	0.46	0.016	3	0.39	0.53
S_{C4}'	0-7.5	Yes	0.65	0.009	4	0.63	0.67
	7.5-15	Yes	0.38	0.009	4	0.36	0.41
	15-30	Yes	0.58	0.013	4	0.54	0.62
	30-45	Yes	0.34	0.013	3	0.28	0.39

Table 7.7. Mean proportions (F_{C4} and F_{C4}') and stocks (S_{C4} and S_{C4}') of C_4-derived C, standard error (SE), degrees of freedom (γ), lower, and upper limit of the 95% confidence interval, as a function of soil organic matter fraction and the assumption of ^{13}C discrimination of C_4 material during litter-SOM transition. Input data are from Table 3, adapted from Roscoe and Buurman (2001). The input values for C_3 and C_4 litters are from Table 1.

Var.	SOM Fraction	Assuming ^{13}C discrimination	Mean	SE	γ	95% Confidence Interval	
						Lower Limit	Upper Limit
F_{C4}	Light	No	0.28	0.060	2	0.02	0.55
	Light	Yes	0.27	0.059	3	0.01	0.52
	Heavy	No	0.32	0.060	4	0.12	0.50
	Heavy	Yes	0.23	0.052	4	0.09	0.38
S_{C4}	Light	No	0.028	0.001	2	0.022	0.034
	Light	Yes	0.026	0.001	2	0.024	0.028
	Heavy	No	0.74	0.034	5	0.64	0.83
	Heavy	Yes	0.55	0.010	5	0.52	0.57

samplings for the same area), an effect of sampling intensity on the confidence interval was evident. The 95% confidence interval of the C_4 proportion (F_{C4}) was 6 times smaller when a large number of samples was taken (Table 7.5) than for a small number of samples (Table 7.6). This happened because the sample number (*n*) influences both the standard error (SE), and the estimated degrees of freedom (γ) of the output variables.

The 95% confidence intervals for proportions of C_4 carbon (F_{C4}) in Table 7.5 did not overlap, suggesting that the difference in the output calculated by the two models was significant. Conversely, for the dataset with small *n* (Table 7.2), the 95% confidence intervals overlapped for all depths (Table 7.6). Similar behaviour was observed for light and heavy fractions (Table 7.7), despite a considerable ^{13}C discrimination in relation to litter observed for the latter (+3.8‰).

As for stocks of C_4 carbon (S_{C4}), only the light fraction (Table 7.7) showed coincident confidence intervals for the two models. For all depths (Tables 7.5 and 7.6) and the heavy fraction (Table 7.7), the values of S_{C4} were significantly different when assuming ^{13}C discrimination.

7.3.2. Sensitivity analysis

Figure 7.1 shows the sensitivity of the standard error (SE) of differences between proportions of C_4-derived carbon calculated by the two linear mixing models (ΔF_{C4}). The analytical precision (Figure 7.1a) and the magnitude of ^{13}C discrimination from litter to soil (Figure 7.1d) had little impact on the SE of the ΔF_{C4}. Litter δ^{13}C standard deviation (SD) had a intermediate influence, showing slight increases only when its values exceeded ~0.6‰ (Figure 7.1c). SD of soil δ^{13}C (Figure 7.1b) was the most important of the parameters. SE of ΔF_{C4} sharply increased for values higher than ~0.4‰, reaching values 4 times greater than the initials. Increasing the number of samples substantially decreased the SE of ΔF_{C4} for all parameters. The magnitude of this reduction increased with SD values of litter δ^{13}C (Figure 7.1c) and, specially, soil δ^{13}C (Figure 7.1b).

We separately studied the effect of increasing soil and litter sample number, keeping the default values for the other parameters (Figure 7.2). Regardless of the number of the litter samples, an increase in the number of soil samples (Figure 7.2a) reduced the SE of ΔF_{C4}. Conversely, increasing the number of litter samples had little effect on it (Figure 7.2b).

The effects of changing input parameters on means and 95% confidence intervals of ΔF_{C4} are summarised in Figure 7.3. Means were only affected by the magnitude of ^{13}C discrimination (Figure 7.3d). This happened because for varying this parameter it is necessary to change the mean signatures of soil (δ_{C3soil}) and litter ($\delta_{C3litter}$) from C_3 (equations 3 and 4). Changes in the 95% confidence intervals (Figure 7.3) followed the same trends observed for SE of ΔF_{C4} (Figure 7.1): no increase with δ^{13}C analytical SD and ^{13}C discrimination; a slight increase with SD of litter δ^{13}C; and a sharp increase with SD of soil δ^{13}C.

In general, for small sample number (n=3), confidence intervals for all variables in the evaluated range overlapped zero, suggesting no significance for ΔF_{C4} (Figure 7.3a-d). Nevertheless, if the SD of soil δ^{13}C is smaller than ~0.3‰ (Figure 7.3b) and ^{13}C discrimination higher than ~3.5‰ (Figure 7.3d), the differences slightly deviate from zero. Conversely, when sampling was intensified (n=30), the differences were significant in most cases (Figure 7.3a-d). Only for SD of soil δ^{13}C higher than ~1.5‰ (Figure 7.3b) and ^{13}C discrimination lower than ~0.8‰ (Figure 7.3d) differences were not significant.

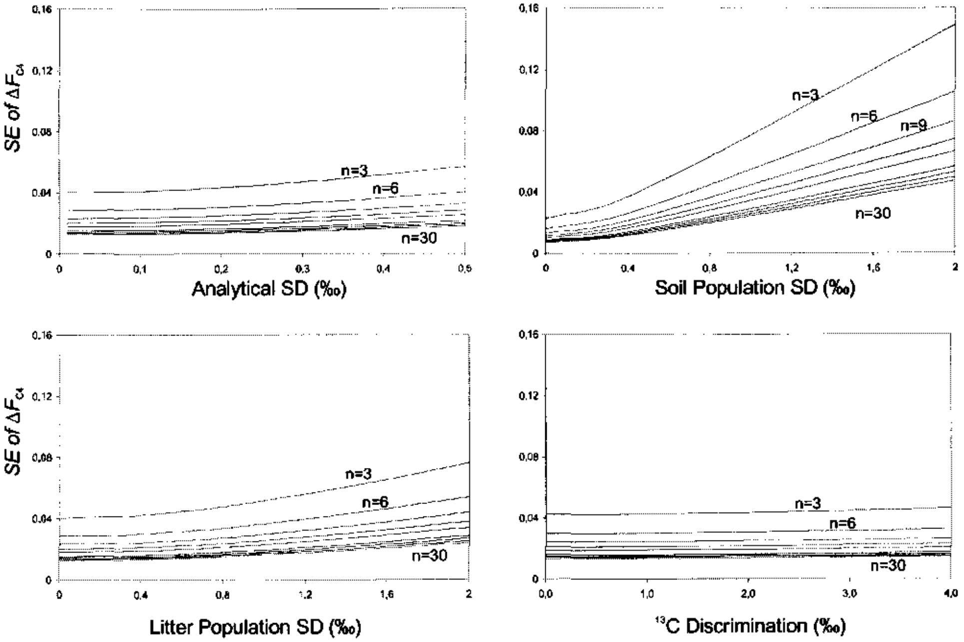

Figure 7.1. Sensitivity of the standard error (SE) of the difference between proportions of C_4-derived carbon estimated by the two different models (ΔF_{C4}), assuming or not equal ^{13}C discrimination for C_3 and C_4 materials, to: (a) $\delta^{13}C$ analytical standard error, (b) $\delta^{13}C$ population standard deviation (SD) of soil samples, (c) $\delta^{13}C$ population SD of litter samples, and (d) the magnitude of the ^{13}C discrimination for the standard C_3 vegetation ($\delta_{C3litter} - \delta_{C3soil}$). The isolines are for number of samples varying from 3 to 30. The parameters not changed in each graphic were kept at default values (Table 4).

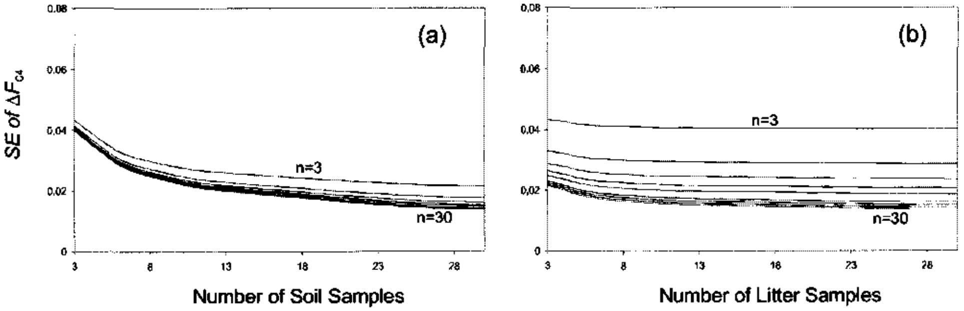

Figure 2 Sensitivity of the standard error (SE) of the difference between proportions of C_4-derived carbon estimated by the two different models (ΔF_{C4}) to: (a) the number of soil samples and (b) the number of litter samples. The isolines in (a) are for different numbers of litter samples and in (b) of soil samples. All the other parameters were kept at default values (Table 4).

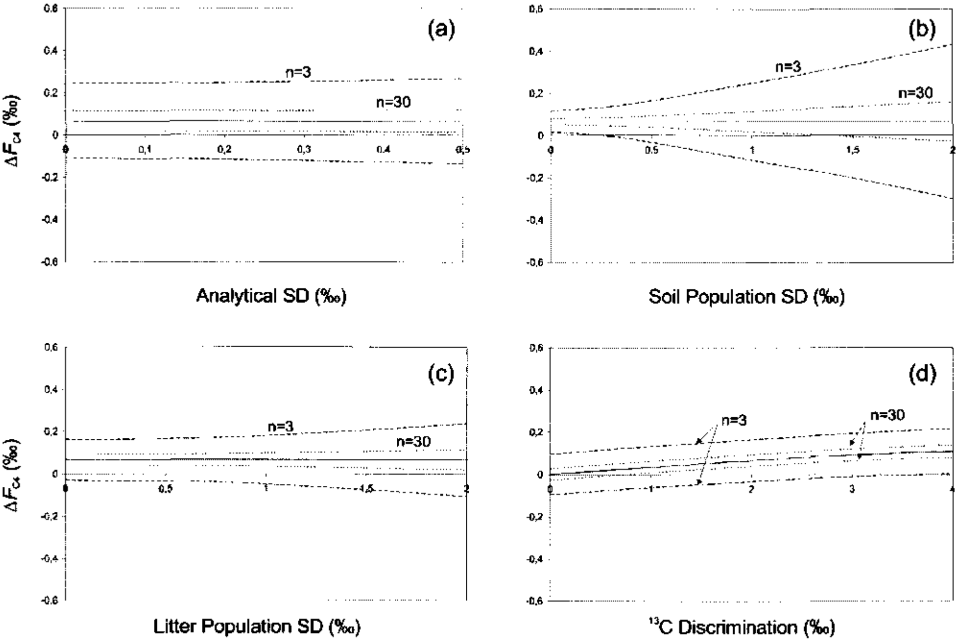

Figure 7.3. Mean difference between proportions of C_4-derived carbon estimated by the two different models (ΔF_{C4}, solid line) and the 95% confidence interval of the mean with number of sample (n) equal to 3 (dashed line) and with n equal to 30 (dotted line), as a function of: (a) $\delta^{13}C$ analytical standard error, (b) $\delta^{13}C$ population standard deviation (SD) of soil samples, (c) $\delta^{13}C$ population SD of litter samples, and (d) the magnitude of the ^{13}C discrimination for the standard C_3 vegetation ($\delta_{C3litter} - \delta_{C3soil}$). The parameters not changed in each graphic were kept at default values (Table 4).

7.4. Discussion

7.4.1. Linear Mixing models

The assumption that the ^{13}C discrimination from litter to SOM in C_4 material was the same as observed for the reference C_3 vegetation had a systematic effect on the outputs, but this difference was not always significant for F_{C4}. The combination of uncertainties of the input variables propagating through calculations seemed to exceed the systematic error induced by the different model assumptions. Only with a large sample number, the models had significantly different outputs. Uncertainty analysis has shown that source proportion variability may be substantial, especially with small sample number (Veldkamp & Weitz, 1994; Phillips & Gregg, 2001). Using sensitivity analysis, Phillips & Gregg (2001) showed that, to halve the uncertainty of source proportions, the number of samples should increase by a factor of 4. In most dynamic studies using the $\delta^{13}C$ approach, the number of samples is rather small, and rarely exceeds three samples for each input variable (Veldkamp & Weitz, 1994; Phillips &

Gregg, 2001). In such cases the assumption in the model will hardly affect the interpretations of source proportions.

Conversely, most of the time, we observed that stocks of C_4 carbon were significantly different for the two models, regardless of the sample number. The relatively high uncertainties of C_4 proportions were compensated for by the inclusion of new variables with rather low variability (carbon contents, bulk density, and proportion of carbon in the light and heavy fractions) in the calculations (Tables 7.1, 7.2, and 7.3). As shown by equation (14), the uncertainties of C_4 stocks are a linear combination of the proportional uncertainties of the input variables (Taylor, 1982). The inclusion of parameters with low variability in such expression has a high impact on the final uncertainty. Thus, where stocks of carbon are concerned, the choice of model may systematically bias the results.

7.4.2. Sensitivity Analysis

The analysed examples showed that the number of samples may increase the uncertainty of individual predictions to values high enough to overlap the systematic difference between outputs from the two linear mixing models. The sensitivity analysis confirmed the importance of the sample number for source proportion uncertainties but also highlighted other important features of the calculations. The SD of soil $\delta^{13}C$ strongly affected the difference uncertainty (SE of ΔF_{C4}), which can be explained by the high weight of this parameter in the calculations (equation 9 and 10). Conversely, SDs of the analytical and litter $\delta^{13}C$ had almost no impact on the final output uncertainty. For this reason, increasing the number of soil samples is more effective in reducing output uncertainties than taking more litter samples. It is important to notice that it is not possible to reduce the SD of soil $\delta^{13}C$, since it is fixed for a given population (Phillips & Gregg, 2001). As shown in Figure 7.3, SD of soil $\delta^{13}C$ higher than about 1.5‰ turns the difference to unsignificant (at a level of 0.05% probability) even with a relatively large sample size (n=30). Thus, for large SD of soil $\delta^{13}C$ the difference between outputs of the two models will often be not significant.

The magnitude of ^{13}C litter-SOM discrimination is directly responsible for the difference between models (equation 3 and 4). As a consequence, changing its value has a direct effect on the mean difference between model outputs. On the other hand, the uncertainty of this difference is not affected by the magnitude of ^{13}C discrimination, which suggested a low sensitivity of the SE of ΔF_{C4} to this parameter. Nevertheless, the output SE was sensitive to changes in the sample number (Figure 7.2). Combining these effects, it seemed that the magnitude of the ^{13}C discrimination may be an important factor for the final difference between models, especially for high sample numbers. This is very important when studying systems with different ^{13}C discrimination, as different depths and SOM fractions (Balesdent & Mariotti, 1996). Balesdent (1996) used the assumption of equal ^{13}C discrimination for a new introduced maize material (C_4) in several C_3 environments (equation 4). The author compared different SOM fraction, estimating mean turnover time for each of these, based on the substitution of C_3 by C_4 material. The values were of 0.5, 3, 18 and 63 years for the fractions >2000, 200-2000, 50-200, and 0-50 μm, respectively. As the discrimination increased with decreasing particle-size (Balesdent & Mariotti, 1996), the assumption made by the author may have systematically underestimated the substitution of carbon in the fine fractions. This did not happen in the course fractions, because fractionation was probably minimal. If the assumption of equal fractionation is wrong (see discussion below), the half-life of fine fractions was overestimated. This example shows that the comparison of the dynamics of different fractions (and depths) is systematically biased by the assumption of equal discrimination.

7.4.3. Should we assume ^{13}C discrimination?

The ^{13}C discrimination of C_3 material with soil depth and SOM fraction is well documented in the literature (Balesdent & Mariotti, 1996; Ehleringer et al., 2001; Chapters 5 and 6). However, very little is known for C_4 derived material (Wedin et al., 1995; Ehleringer et al., 2001). The assumption that isotopic discriminations for C_4 and C_3 materials are equal appears unsupported by literature so far (Ehleringer et al., 2001). But, the assumption of absence of discrimination for the C_4 material is also not supported by experimental results (Ehleringer et al., 2001). Several hypotheses have been suggested to explain the isotopic discrimination commonly observed in C_3 ecosystems (Nadelhoffer & Fry, 1988; Wedin et al., 1995; Boutton, 1996; Balesdent & Mariotti, 1996; Ehleringer et al., 2001). Four of these are the most commonly cited: differential decomposition of organic material; discrimination during heterotrophic respiration; depletion of ^{13}C in the atmosphere as a consequence of fossil fuel burning and land-use changes (the Suess effect); and incorporation by the microbiota of old carbon into the new introduced material (the mixing theory). The first hypothesis has been criticized because the most stable compounds derived from plant material (e.g. lignin and long-chain lipids) are depleted in ^{13}C, which would give exactly the opposite of the increase in $\delta^{13}C$ usually observed (Benner et al., 1987; Wedin et al., 1995; Boutton, 1996; Balesdent & Mariotti, 1996; Ehleringer et al., 2001). The hypothesis of discrimination upon decomposition has been questioned recently by Lin & Ehleringer (1999), who showed that there was no mitochondrial discrimination in autotrophic respiration. According to Ehleringer et al. (2001), it is very likely that the same occurs in heterotrophs. The two other hypotheses (the Suess effect and the mixing hypothesis) have been the most accepted (Ehleringer et al. 2001). But, in the light of these hypotheses, would the C_4 plant material have the same discrimination pattern as that observed for C_3 plants?

Acording to the Suess effect hypothesis, older material in soil would be in equilibrium with an atmosphere richer in ^{13}C. The atmosphere $\delta^{13}C$ had decreased about 1.5‰ in the last 200 years due to anthropogenic CO_2 emissions (Boutton, 1996; Ehleringer et al., 2001). Although this difference is smaller than the ^{13}C discrimination often observed in C_3 systems (up to 4‰), it may certainly have contributed. But, do changes in the atmospheric CO_2 signature equally influence the $\delta^{13}C$ values of C_3 and C_4 material? According to the models describing C_3 and C_4 accumulation reported by Boutton (1996), the atmospheric signature will influence both groups. However, the higher discrimination from the atmosphere to C_3 tissue suggests that these plants are more selective than C_4 plants. Then, alterations in the atmospheric signature would probably promote higher effects on C_4 than in C_3 plants. Two studies provided supporting evidences for this hypothesis. Marino et al. (1992) found a decrease of –1.6‰ (-10.7‰ to –12.3‰) in the material of a C_4 desert shrub (*Atriplex confertifolia*) over the past 200 year. On the other hand, Penuelas and Azcon-Bieto (1992) found decreases of only –0.6‰ (-25.8‰ to –26.4‰) for 12 C_3 species in Spain over the same period. Although more systematic research is needed to better clarify these processes, it seems likely that the Suess effect will cause higher effects on C_4 than in C_3 plants.

The mixing theory was proposed by Wedin et al. (1995) who observed that the ^{13}C discrimination of decomposing C_3 and C_4 material in litter bags shifted in opposite directions. While C_3 material was enriched in ^{13}C upon decomposition, C_4 litter was depleted. Their results were in conflict with the hypothesis of fractionation during respiration, which would imply in an increasing trend for both plant types. Their results were in line with Ehleringer et al. (2000) who suggested that the absence of mitocondrial fractionation for autotrophs (Lin & Ehleringer, 1997) also occurs for decomposers (heterotrophs). Wedin et al. (1995) postulated that carbon from the original litter was mixed with the native carbon during decomposition by incorporation of fungal and microbial material, which would have the same $\delta^{13}C$ of SOM. This explained the contrasting trends for C_3 and C_4 material observed by the authors: $\delta^{13}C$ values of C_3 material increased with decomposition, while $\delta^{13}C$ of C_4 material decreased. For both systems, the $\delta^{13}C$ signature tended to the value of the SOM (–23.9‰), which was intermediate between C_3- (~ –27‰) and C_4-materials (~ –13‰). Ehleringer et al. (2000) postulated that this mixing effect occurred as a consequence of incorporation of CO_2 molecules from the surrounding soil atmosphere into microbial tissue due to carboxylation reaction. They estimated that, if in a C_3 system only 5% of the microbial biomass was fixed in this manner, the microbial $\delta^{13}C$ values would be enriched about 1 and 1.5‰ in relation to the substrate. This happens because soil atmosphere is enriched in ^{13}C in relation to SOM, due to atmospheric CO_2 diffusion into soil (Ehleringer et al. 2000). So, considering the mixing hypothesis, will the ^{13}C discrimination with decomposition be the same for C_3 and C_4 plants? The answer depends on the ecosystem under study. If it is a pure C_4 system, an enrichment is also expected for the C_4 litter, but with a lower magnitude than that observed for C_3 litter in a pure C_3 system, because C_4 signature is much closer to that of atmospheric CO_2. Then, the soil atmosphere in a C_4 system is expected to have a signature not so different from the decomposing material as in a C_3 system. On the other hand, when a C_3 and C_4 litter is added to a C_3 system, it is very likely that the discrimination will differ for the two plant types, as suggested by the results of Wedin et al. (1995) and the theory of Ehleringer et al. (2000).

Back to our examples, the Suess theory suggested that the C_4 plants should have a higher discrimination than the C_3 material. However, as the C_4 material has been added only in the last 30 years, the effect of changes in atmospheric CO_2 was probably negligible. Considering the mixing hypothesis, the discrimination for C_4 would be in the opposite direction of the one observed for C_3 material. Therefore, both the assumption of no ^{13}C discrimination in C_4 material (equation 3) and that of equal ^{13}C discrimination for C_3 and C_4 residues (equation 4) would be wrong, and both equations would underestimate the proportion of C_4 material. Although more research is needed to better understand the processes involved in ^{13}C discrimination with decomposition (Ehleringer et al., 2000), the current evidence suggests that assuming absence of ^{13}C fractionation for C_4 material introduced in a C_3 ecosystem will be more close to reality.

7.5. Conclusions

Assuming either absence of ^{13}C discrimination or equal ^{13}C discrimination during litter-SOM transition for both C_3 and C_4 materials may generate contrasting outputs, especially when different depths and soil organic matter fraction are considered. These differences may not be significant for small sample sizes when proportions of sources (C_3 or C_4) are considered. But when carbon stocks from each source are concerned, the differences can be significant even with small sample numbers.

Increasing the number of soil samples is more efficient in reducing the final uncertainty of the outputs then collecting more litter samples. The difference between outputs from the two models is extremely sensitive to the population standard deviation of soil $\delta^{13}C$. For more homogeneous populations, the uncertainties of outputs are substantially reduced and the difference between the two models is very likely significant.

A critical analysis of the current understanding of processes leading to ^{13}C discrimination in soils suggests that the assumption of no litter-SOM ^{13}C discrimination for the new C_4 introduced material would provide the most realistic results. Nevertheless, further research is needed to give better support for the hypotheses explaining the processes. Especially the suggestion that no isotope fractionation takes place during heterotrophic respiration should be tested.

Chapter 8

General discussion and conclusions

In this final chapter, the general results of this thesis are discussed in relation to the specific objectives stated on the General introduction (Chapter 1), and general conclusions are given. This discussion is divided into four topics. The first deals with improvements in the two basic methodologies used throughout the previous chapters: the physical fractionation of soil and the natural ^{13}C tracer approach. In the second topic, the relation between the model of spatial organisation of mineral and organic particles in soil (Figure 1.2, Chapter 1) and soil organic matter dynamics in the studied soil is discussed. The third topic addresses the impact on carbon stocks and dynamics of the different land use systems studied. Finally, the fourth topic discusses implications and further research.

8.1. Methodological improvements

As very little information was available in literature, the two major methodologies used in this thesis (i.e. physical fractionation and natural ^{13}C tracer) had to be adapted to the studied soil. Improvements were made and are discussed separately in the next two sections.

8.1.1. Physical fractionation

Soil dispersion is a fundamental step in any physical fractionation protocol (Christensen, 1992; Gregorich & Ellert, 1993). Its intensity strongly influences the mass recovery in each density (Golchin at al. 1994) or particle-size fraction (Christensen, 1992). In Chapter 3, this was documented for the studied soil, which showed different particle-size distribution with increasing ultrasonic energy input (Figure 3.1). Sonication intensity also affected organic C and N contents, C:N ratio, and $\delta^{13}C$ of the fractions (Figures 3.3, 3.4, and 3.5, Chapter 3). Two important methodological aspects were highlighted: the necessity of a standard procedure to estimate the amount of energy effectively applied to a sample, and the definition of a procedure to determine the minimal energy necessary for dispersion.

North (1979) first discussed the necessity of a standard procedure for determining the power output of ultrasonic equipments. He postulated that most equipments do not actually apply the amount of energy shown in their display. The author suggested a calorimetric method for calibrating sonicators, which was successfully used in further investigations (Christensen, 1985; Raine & So, 1993, 1994; Schmidt et al., 1999). In Chapter 3, this method was applied with good results, and a simple calorimeter was designed for such procedure (Figure 3.1, Chapter 3). It was suggested that as far as the thermic isolation of the container is enough to allow a reproducible pattern of energy loss (*H*), without interference of the external environment, any kind of calorimeter can be used.

In the fractionation scheme, the definition of a minimum of energy necessary for efficient dispersion is essential. In Chapter 3, I observed different patterns of dispersion for distinct fractions (Figure 3.2). The amount of aggregates larger than 100μm (100-2000μm) sharply decrease up to energy values of ~310 J ml^{-1}, tending to stabilise above that. Aggregates between 50-100μm showed a linear and continuous mass loss, with a relatively gentle slope. This suggested that 100μm would be a suitable threshold value to separate stable and unstable aggregates smaller than 2000μm. However, very little difference was observed between fractions <100μm and <50μm (Figure 3.3), because the 50-100μm fraction was rather small. It was suggested that the energy for dispersing the unstable aggregates (100-2000μm), leaving intact the stable aggregates (50-100μm), was about 260-275 J ml^{-1}. Although the separation of these two types of aggregates would be desirable in soil organic matter studies (they supposedly have different SOM chemical composition and dynamics) this would cause a mix of litter-like material and relatively stable recalcitrant organic C present in the sand size fractions (>50μm). On the other hand, increasing the energy to values higher than 260-275 J ml^{-1} would mix organic material present in aggregates of different stability in the recovered clay-size fraction. To solve the problem, I combined low intensity dispersion (270 J ml^{-1}) with density and particle size separation (Chapter 4). This allowed the separation of the light litter-like material of the sand size fraction (50-2000μm) from the stable aggregates remaining after sonication. It was observed that the heavy fractions (sand, silt, and clay) had a similar dynamics (Figure 4.4 and Table 4.3), refuting the hypothesis of different material in stable (remaining after 270 J ml^{-1}) and unstable aggregates. This result suggested that most of the carbon in these fractions was bound to clay. The clay binding mechanisms were probably the same for all fractions and they were apparently the most important processes of SOM protection. All aggregates in the fraction smaller than 2000μm may well be fairly stable under natural conditions (Muggler et al., 1997; Resende et al., 1997; Ferreira et al., 1999). It has been demonstrated for Oxisols rich in Fe and Al oxi-hydroxides that the basic aggregate structure is strong very fine granular, with diameters between 60-1000μm (Ferreira et al., 1999). This structure is very resistant to cultivation and remains intact even after many years of conventional tillage (Resende et al., 1997; Resck et al., 1999). The value of 270 J ml^{-1} for separating unstable aggregates may be quite high, when compared to actual forces under natural conditions.

In conclusion, the separation of stable and unstable aggregates, as suggested in Chapter 3, appears not to distinguish different pools of SOM. Higher energies are then recommended for particle size separation in the studied soil, to obtain a complete removal of clay bound material from the course fractions. At high energy level, the possible fragmentation of the light fraction should also be considered (Balesdent et al., 1991). A separation of the light fraction (density < 1.7 g cm^{-3}) before sonication can avoid this artefact.

8.1.2. Natural ^{13}C tracer

The use of the natural abundance of ^{13}C applied as a natural tracer is a powerful tool in SOM dynamic studies (Balesdent & Mariotti, 1996). It is commonly applied when a pure C_3 or C_4 vegetation is replaced by species with a different photosynthetic pathway (Vitorello et al., 1989; Bonde et al., 1992; Balesdent et al., 1988, 1990, 1998; Balesdent, 1996; Balesdent & Mariotti, 1996; Gregorich et al., 1995; Besnard et al., 1996; Collins et al., 1999). The methodology allows estimates of short-term SOM dynamics, at time scales of days to decades (Balesdent & Mariotti, 1996). However, very few studies were carried out under mixed (C_3 + C_4) ecosystems (Martin et al., 1990). The *cerrado sensu stricto* is a mixed vegetation formed by a herbaceous C_4 vegetation with variable tree and shrub (C_3) population (Eiten, 1972, 1992; Ribeiro & Walter, 1998). The vegetation distribution pattern may strongly affect the $\delta^{13}C$ signature of SOM, since C_3 and C_4 species may occur in clusters. In a first exploratory study (Chapter 2), I observed that the studied *cerrado sensu stricto* was a C_3-dominated vegetation, with few grasses (less than 5% cover). This dominance was drastically affected by fire incidence, which increased the grass population (Figure 2.1 and Tables 2.1, 2.2, and 2.3). This was also evident from the soil $\delta^{13}C$ signature, which showed a pattern of increasing C_4 material contribution with increasing fire frequency. These results strongly suggested that, without the frequent burning of the vegetation (2-years interval), the *cerrado sensu stricto* investigated for this thesis was a C_3 dominant vegetation. This is in accordance with observations by Pessenda et al. (1996) and Miranda et al. (1997). Although fire is a common ecological event in the cerrado vegetation, the incidence at the site was not frequent enough to change the C_3 dominance. Nevertheless, the spatial pattern of $\delta^{13}C$ distribution in the topsoil of the studied natural reserve (Chapter 5) suggested that, even in a relatively homogeneous vegetation (the whole plot was classified as *cerrado senso stricto*), changes in soil ^{13}C signature may occur as a consequence of slight modifications in the vegetation. Higher values of soil $\delta^{13}C$ were found in places where the reserve was burned 2 times in 23 years than in unburned places. Slight differences in the canopy would locally allow a higher penetration of sunlight, drying out the litter layer and increasing the risk of fire (Eiten, 1972, 1992; Mistry, 1998). The causes of such variation could not be assessed, but they are probably related to a random spatial pattern of the vegetation that is continuously perpetrated by differential fire incidence. The textural and mineralogical homogeneity of the soil suggested that the variation in $\delta^{13}C$ was probably not caused by differences in soil properties. The spatial structure of $\delta^{13}C$ values observed for both natural and cultivated soils was probably related to the same processes. Therefore, the assumption that a neighbouring natural vegetation represents the reference situation for an area previous to cultivation may introduce a systematic error, which is due to the original spatial structure. This error accounted to 44-70% of the total variation of the $\delta^{13}C$ values in each plot (Table 5.3). It is difficult to correct for that error because, when trends are independent of landscape characteristics, it is hard to reconstruct the true native state of any cultivated site. Assessing this error would substantially reduce the final uncertainty of calculated replacements, because this uncertainty is very sensitive to the standard deviation of the soil ^{13}C signature (Figure 7.1).

The assessment of output uncertainties has been ignored in dynamic studies based on ^{13}C natural abundance (Veldkamp & Weitz, 1994; Phillips & Gregg, 2001). The calculation of carbon replacements by the new vegetation is estimated by mixing linear models involving several input variables (Balesdent & Mariotti, 1996). Individual uncertainties in each of these variables propagate in the final results. These uncertainties could not be assessed in Chapter 2 and Chapter 4, due to a lack of sufficient replicates. In Chapters 6 and 7, it was possible to assess these errors using Taylor series approximations as described by Taylor (1982) and Phillips & Gregg (2001). Assessing these uncertainties allowed testing for significance of differences between depths and fractions. The equations provided in Chapter 6 and 7 allow the performance of uncertainty analysis in several types of studies. Their application would substantially improve the ^{13}C-tracer methodology, increasing the reliability of conclusions.

An important assumption made during replacement calculations is related to isotopic discrimination upon decomposition. This fractionation is currently explained by four theories: the decrease of atmospheric $\delta^{13}C$ in the last 200 years as a consequence of anthropogenic CO_2 emissions (the Suess effect); differential decomposition rate of organic compounds with different $\delta^{13}C$ values; discrimination during heterotrophic respiration; and the mixing of old soil carbon with the newly introduced material through biological activity (Ehleringer et al., 2000). As discussed in Chapter 7, in most studies no reference profiles are available for the newly introduced material (e.g. maize introduced into a C_3-dominated ecosystem). There are basically two ways of calculating C_3 and C_4 contributions in a given sample: not considering $\delta^{13}C$ discrimination with decomposition for the newly introduced material, or to consider the same discrimination as observed for the old vegetation in reference profiles (Balesdent & Mariotti, 1996). In Chapter 4, I proposed a third approach. Instead of assuming an equal discrimination for the old and new vegetation, I suggested to assume the same "proportional" discrimination. This alternative assumption came from the incompatibility of expecting the same absolute discrimination for materials with signature diverging by about 14‰. It seemed more logical to assume that the discrimination should be proportional to the signature of the source (C_3 or C_4). However, this assumption can not be explained by the current hypothesis mentioned above (Balesdent & Mariotti, 1996; Ehleringer et al., 2000). As discussed in Chapter 7, the two most accepted hypotheses to explain fractionation are the Suess effect and the mixing hypothesis. Both disagree with a equal discrimination for C_3 and C_4 plants with decomposition. More conclusive data is still missing, but it appears that the assumption of absence of discrimination during decomposition for a newly C_4 introduced vegetation in a C_3 ecosystem is the most consistent.

8.2. Aggregate hierarchical model and SOM dynamics

In the *General Introduction*, a model for spatial organisation of mineral and organic particles was used (Figure 1.2). In this model, mineral particles form primary organo-mineral complexes (OMC) at a first hierarchical level of organisation. In their turn, these primary-OMC bind together forming secondary-OMC, occluding part of the light fraction. Soil organic matter dynamics would then be controlled by different protective mechanisms, namely intrinsic chemical recalcitrance, occlusion, and binding/complexation with the mineral particles. The results of Chapters 3, 4 and 6 gave good insights in the adaptability of this model for the studied soil.

In Chapter 3, the differential breakdown of soil aggregates under ultrasonic treatment suggested the existence of a boundary between stable and unstable aggregates smaller than 2 mm. In this soil, the 100-μm boundary would represent the limit between macro- and micro-aggregates, instead of the 250-μm threshold commonly used for Mollisols and Alfisols (Oades & Water, 1991; Oades, 1993). However, Chapter 4 indicates that the dynamics of SOM in aggregates larger and smaller than 100-μm were similar, suggesting that, in dynamic terms, this limit had no meaning. Carbon in all the heavy fractions (sand, silt, and clay) had the same replacement. Ferreira et al. (1999) observed that similar soils, with high oxide contents, had a typical strong fine granular structure with diameter between 60-1000 μm. It has been shown that this structure is very stable and barely affected by cultivation (Resende et al., 1997; Resck et al., 1999; Ferreira et. al., 1999). So, differentiation of stable and unstable aggregates proposed in Chapter 3 does not relate to their stability against forces brought about by cultivation. In fact, most of the aggregates analysed in this soil (all smaller than 2 mm) should be considered rather stable with cultivation.

Carbon and nitrogen usually accumulate in the heavy fraction, especially in the silt plus clay compartment (Feller & Beare, 1997; Christensen, 2000). This was confirmed for the studied soil; about 90-95% of the total carbon was stored in these fractions (Chapters 3, 4, and 6). Clayey soils are also expected to store high amounts of occluded light fraction, which would be entrapped in the aggregate matrix (Hassink & Whitmore, 1997). According to Golchin et al. (1994) and Parfitt et al. (1997), temperate and sub-tropical clayey soils may store between 16-39% of the total carbon in the occluded light fraction. However, data available for tropical clayey Oxisols show values between 1-4% (Golchin et al., 1995; Freixo et al., 2001), which agrees with our findings (1-2%, Chapters 4 and 6). This discrepancy in relation to temperate soils may be attributed to the type of structure predominant in Oxisols. Temperate and subtropical clayey soils are usually dominated by blocky structures, with relatively high volume in relation to external surface area. The strong very fine granular structure observed in Oxisols may have a small capacity to store occluded light fraction because the ratio volume/surface-area is typically small. The low accumulation of occluded light fraction can also be a function of the process of formation of such a structures.

Currently, two major hypothesis are used to explain the formation and stabilisation of the typical structure observed in Oxisols. The first, a physical-chemical hypothesis, states that this structure derives from the high contents of Fe and Al oxi-hydroxides and the long process of soil formation (Resende et al., 1997). The combination of strong binding agents and the stress caused by repeated cycles of wetting and drying for long periods of time, would tend to create very stable spherical structures (the more stable geometric solid, due to its lower volume to surface ratio) (Resende et al., 1997). Alternatively, according to a biogenic theory, the granular structures would be the product of biological activity, especially termites (Trapnell & Webster, 1986). Jungerius et al. (1999), studying the influence of termites on the granular structure of a clayey Ferrasol from Kenya, showed that the animals were responsible for building (in the subsoil) and transporting (to the surface) very stable spherical micro-aggregates, with mean diameter of 0.6 mm. These two processes are not conflicting and may happen simultaneously. Nevertheless, both processes would probably cause occlusion of organic materials with advanced stage of decomposition. The first (the physical-chemical theory) because it is a very slow process, and the occlusion of the organic compounds would not happen abruptly. According to the biogenic theory, the entrapped material would have passed through the macro- or meso-fauna guts. This would also favour the decomposition of more available organic materials, leaving behind only more recalcitrant compounds. Therefore, for both process, only small quantities of more recalcitrant materials would be occluded in granular aggregates of oxide-rich Oxisols. This would agree with the observed low amounts of occluded light fraction (1-2%) and with the lower replacement observed in this fraction in relation to the others (Table 4.3, Chapter 4; Figure 6.6, Chapter 6).

In pasture and cerrado (Chapters 4), evidence for a higher degree of decomposition in occluded than in free light fraction was provided by ^{13}C CPMAS NMR analysis (Figure 8.1). O-alkyl groups strongly decreased, and alkyl groups increased from free to occluded light fraction, which suggests a loss of more easily decomposable compounds (e.g. carbohydrates) and a relative accumulation of long chain aliphatic materials. The similar intensity of aromatic signals in both fractions appears to disagree with the hypothesis of possible charcoal accumulation in the occluded fraction, as proposed in Chapters 4 and 6. However, the poly-aromatic charred material is rich in quaternary carbon (Schmidt & Noack, 2000), which is weakly detected by CPMAS ^{13}C NMR (Piccolo & Conte, 1998). Therefore, the occluded light fraction seems to be composed of a highly decomposed material, with slow turnover, and possibly rich in charred materials (as suggested by microscopic research).

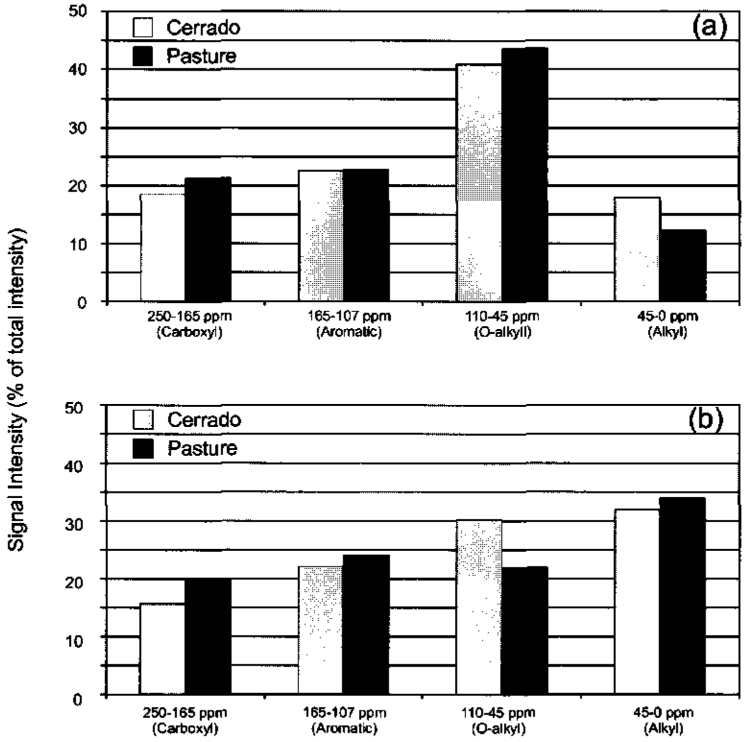

Figure 8.1. Signal intensity of CPMAS ^{13}C NMR attributed to different groups of organic compounds for (a) the free light fraction and (b) the occluded light fraction.

The free light fraction stored 9-10% of the total carbon. It presented the fastest turnover in relation to the other fractions (Chapter 4 and 6), which agreed with its composition (Figure 8.1). However, the relatively large quantities of charcoal observed in this fraction (Chapter 4) may represent an important recalcitrant pool (Schmidt & Noack, 2000). The free light fraction was also sensitive to changes due to cultivation (Chapter 6), and showed a significant decrease, while carbon in the total soil and other fractions did not change significantly.

All heavy fractions showed similar replacement (Chapter 4). This happened because most carbon present in sand- (50-2000μm) and silt-sized (2-50μm) aggregates was composed of clay-sized organo-mineral complexes (Chapter 3). About one third of the carbon in the heavy fraction was replaced by the new vegetation, both after 23 years of pasture (Chapter 4) and 30 years of arable cultivation (Chapter 6). This suggests that this fraction contains two distinct pools: a recalcitrant pool (2/3 to 3/4) and a labile pool (1/4 to 1/3). The labile pool would turnover in 23 years or less, even in a conservational system as pasture. On the other hand, the stable pool would remain unchanged after 30 years of intense cultivation (two crops per year and conventional tillage – Chapter 6).

Although most authors suggest that the heavy fraction is largely dominated by recalcitrant humified materials (Gregorich & Ellert, 1993; Golchin et al., 1997), these results show that a significant part of this fraction is composed of labile materials. This is in agreement with Baldock et al. (1992), who argued that the clay size fraction can accommodate most of the biological activity and may hold substantial quantities of labile microbial products, such as polysaccharides and proteins.

The results discussed above allow some conclusions about the model proposed in the General Introduction (Figure 1.2):

- occlusion is not an important aspect of carbon sequestration in the studied Oxisol, probably due to the formation and stabilization of the typical strong very fine granular structure;
- carbon accumulates in the free light fraction and the heavy fraction, especially in the latter;
- although the free light fraction and the heavy fraction show different dynamics, they are not homogeneous, and contain both a labile and a stable compartment;
- the stable (recalcitrant) compartment in the free light fraction is basically charred material and accounts for up to 50% of the fraction in the topsoil;
- the labile compartment of the heavy fraction constitutes about 1/4 to 1/3 of the total carbon and is probably composed of transient microbial products.

8.3. Land-use impact

The different land use systems evaluated in this thesis had no significant effect on total soil carbon stocks (Chapters 4 and 6). As discussed before, clay-bound organic matter dominates the soil organic matter, and very little material was occluded in the strong and resistant aggregates. This means that most of the carbon in this soil is in a relatively resilient pool. This may be a reflection of its high clay content (>800 g kg^{-1}) (Hassink & Whitmore, 1997; Schulten & Leinweber, 2000). The evaluation of the relationship between organic C in the clay faction and the total organic C in the soil (Figure 4.3, Chapter 4) suggests that the ratio of sorptive clay surface to "available" reactive organic matter increases with depth (Schulten & Leinweber, 2000). Only in the topsoil (0-2.5 cm) of the native cerrado plot, the clay fraction appeared to be "saturated" with organic C. In all the other horizons (in both cerrado and pasture plots), most of the clay was unsaturated, which represents a relatively high potential for organic C preservation (Hassink 1996; Hassink & Whitmore, 1997), if more reactive organic C became available (e.g. changing C supply due to conversion into a new vegetation).

The typically high concentrations of Fe and Al oxi-hydroxides in Oxisols may largely affect the availability of SOM to decomposition. These components present considerable positive charge density at normal soil pH, which forms strong electrostatic interactions with the negatively charged SOM (Resende et al., 1997). The high clay (>800 g kg^{-1}) and oxi-hydroxide contents of the studied Dark Red Latosol (Chapters 3 and 4) was probably an important factor in the maintenance of the total C levels of the studied soil.

Both pasture and maize/beans cultivation may keep a carbon supply similar to that observed in cerrado. Tropical grasses (e.g. *Brachiaria spp.*) usually produces large quantities of biomass, especially roots (Fisher et al., 1994; Corazza et al., 1999). Similar studies in cerrado have shown that cultivated pastures maintain or even increase the organic C stock (Westerhof et al., 1999; Neufeldt et al., 1999; Carazza et al., 1999). In the cultivated areas (Chapter 6), the high litter production of maize may also be responsible for the maintenance of a relatively high carbon supply. In 1995/1996, in the same experimental area, maize produced 4.2 Mg ha^{-1} of above-ground total dry matter under conventional tillage and 5.5 Mg ha^{-1} under no-tillage (Fernandes et al., 1998). As shown by Silva et al. (1994), similar clayey cerrado soils, rich in oxi-hydroxides of Fe and Al, may lose 41% of their initial carbon contents after 5 years of soybeans. This crop produces lower quantities of residues than maize, with a higher degradability. Then, the maintenance of C stocks in the studied cerrado Oxisol was probably a consequence of its high protective capacity (due to the large clay content) and the sufficient residue supply by the pasture and crops (especially maize).

8.4. Implications and further research

Soil organic matter in the studied Dark Red Latosol appears to be fairly stable. Although reclamation of cerrado ecosystems for pasture and crops may reduce the aboveground biomass, such a conversion will hardly result in additional CO_2 emissions from the soil. As 80% of the carbon stored in tropical savannas is in the soil (Table 1.1 – Chapter 1; IPCC, 2000), using the clayey Dark Red Latosol for pasture and maize/beans cultivation will have little (if any) effect on the global carbon balance. Nevertheless, one-fourth to one-third of the carbon in the first 1-m depth cycled in the period between 23-30 years, suggesting that a significant portion of the SOM has a relatively fast turnover. Some open questions remain:

- will crops with lower organic matter input (e.g. soybeans) maintain this "labile pool"?
- will land-use systems with organic matter input higher than the native cerrado, such as *Eucalyptus spp*. plantations (Corazza et al., 1999), increase the amount of SOM in this soil, favouring carbon sequestration?
- is it feasible to maintain carbon stocks higher than the present natural levels?

Additionally, as stated by the decision -/CMP.1 adopted by the COP 6 – UNFCC (Sixth Conference of the Parties for the United Nations Framework Convention on Climate Change) in Bonn (27 July 2001), one of the principles that should govern decisions concerning land use, land-use change and forestry activities is: "*that the implementation of these activities [should] contribute to the conservation of biodiversity and sustainable use of natural resources*". Therefore, alternatives that can improve carbon sequestration, but are incompatible with the principle above (e.g. large homogeneous *Eucalyptus spp*. plantations), should be considered carefully. Future research should concentrate on systems that integrate carbon sequestration with conservation of biodiversity and natural resources, such as diversified crop rotations, green manure, organic farming, agroforestry, etc.

An important point highlighted by this thesis is the significant presence of charcoal in Cerrado soils. This fraction can be rather recalcitrant, which would be significant in the total carbon dynamics. Further research should concentrate on the development of methodologies to assess quantity and dynamics of charred material in soils (Schmidt & Noack, 2000). Although some data is available for savannas (Kuhlbusch and Crutzen, 1995; Kuhlbuch et al. 1996), a major challenge will be to quantify the rates of charred material production in natural cerrados, which is dependent on the fire dynamics in these ecosystems. The elucidation of charcoal dynamics in these soils is essential for the adaptation and improvement of current soil organic matter dynamic models.

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Summary

The Brazilian Cerrado is the second largest biome in the country, spreading over 23 % of the national territory. In the last three decades, it has increasingly contributed to the national production, being responsible in 1995 for 25% of the national agricultural production and sheltering 40% of the cattle flock. Development strategies have stimulated strongly mechanised and intensive agricultural practices, which has raised concerns about soil organic matter (SOM) losses and soil degradation.

Cerrado soils are dominated by low-activity clays and are characterised by high acidity and low pH, cation exchange capacity (CEC), and available nutrients (especially P and N). SOM exerts important functions in these soils, which are closely related to soil quality and sustainability. It is responsible for most of the CEC, and is involved in process of soil aggregation and water dynamics. Losses of SOM due to cultivation may seriously affect these functions, and also represent an important source of CO_2 to the atmosphere. Studying the effects of different management systems on C dynamics in Cerrado soils may help to develop better ways of using these ecosystems.

The initial objective of this thesis was to evaluate the impact of different management systems on SOM dynamics in one of the most representative soil classes in Cerrado, the Dark Red Latosol (18% of the total area; Oxisol – Soil Taxonomy; Ferrasol – FAO Legend). The research strategy adopted was to compare, in the same soil unit, paired plots with different management systems (native vegetation, pasture, no-tillage and conventional tillage). The Dark Red Latosol unit (Typic Haplustox) was located in the experimental research institution of EMBRAPA-CNPMS (Brazilian Institute of Agricultural Research – Maize and Sorghum National Research Centre), in Sete Lagoas – MG, Brazil. To assess SOM dynamics, a combination of two methodologies was proposed, (i) the physical fractionation of soil in particle-size and density separates, and (ii) replacement calculations using the natural abundance of the ^{13}C isotope. The physical fractionation aimed at separating SOM pools with different chemistry, location, and turnover, which hypothetically would be more sensitive to soil alterations than total SOM. The natural abundance of ^{13}C was proposed to assess the turnover time of SOM and its fractions. However, as very little data was available about the use of these methodologies in Cerrado ecosystems, the initial focus of the thesis was changed. The initial general objective was extended to three specific objectives:

(i) to adapt the methodologies of physical fractionation of soil and ^{13}C natural abundance to SOM studies in a Cerrado ecosystem;

(ii) to contribute to a better understanding of the distribution and dynamics of different SOM pools in this specific environment; and

(iii) to evaluate the long-term effect on SOM of converting a *cerrado sensu stricto* into cultivated pasture or to annual crops (maize and beans) under conventional tillage and no-tillage.

In Chapter 2, an exploratory study was conducted to establish a $\delta^{13}C$-reference profile under the native cerrado *sensu stricto*. In a native reserve located at EMBRAPA-CNPMS, three soil profiles were analysed in plots with different fire history. Results showed that the studied cerrado was a C_3-dominated vegetation, presenting $\delta^{13}C$ values typical for soils under such a vegetation throughout the soil profile. Fire favoured the grass population and increased the amount of C_4-derived carbon in the system. No alteration in carbon stocks in soils was observed with increasing fire incidence. It was concluded that different fire regimes may interfere with the establishment of a ^{13}C

standard profile for dynamic studies. The profiles under low fire intensity were considered a good reference for the further studies.

In Chapter 3, the focus was on establishing a physical fractionation procedure for SOM in the Cerrado Oxisol under investigation. Special attention was given to the dispersion by ultrasonic energy, a crucial point in the methodology. A procedure to calibrate the ultrasonic equipment and to determinate the minimum of energy required for an efficient dispersion is proposed. Increasing ultrasonic energy significantly changed the amounts of C and N, the C:N ratio, and the $\delta^{13}C$ values of the particle size fractions analysed (0-2μm; 2-50μm; 50-100μm; 100-250μm; 250-2000μm). The results suggest that the soil (< 2 mm) can be divided into unstable (100-2000μm) and stable (50-100μm) aggregates. A threshold energy of 260-275 J ml^{-1} is proposed for the dispersion of unstable aggregates. The use of this threshold energy, combined with particle-size fractionation, was not satisfactory for all purposes, since litter-like material and relatively recalcitrant organic carbon present in stable aggregates > 100 μm were recovered in the same pool. An ultrasonic energy of 825 J ml^{-1} was not sufficient to stabilize the redistribution of soil mass and organic matter among particle-size fractions, but at energies above 260-275 J ml^{-1} relatively stable aggregates would fall apart and cause a mix of carbon with varied nature in the clay fraction.

In order to better understand the dynamics of soil organic matter (SOM) in Oxisols, and the impact of converting the cerrado *sensu stricto* into pasture, the dynamics of physically separated SOM pools at different depths in a cerrado Oxisol (Typic Haplustox), under natural conditions and after 23 years of cultivated pasture (*Brachiaria spp.*) was studied via the replacement of the native C (C_3-derived) by pasture C (C_4-derived) (Chapter 4). Organic C stocks of the original cerrado (15 ± 3 kg m^{-2}) and pasture (17 ± 3 kg m^{-2}) were not significantly different, which was attributed to the high biomass production of the tropical grasses and the protective effect of the high clay content (> 800g kg^{-1}). The clay + silt fraction accumulated 89-91% of the total organic C. The replacement of cerrado-derived C by pasture-derived C was in average 36%, 34%, and 19% for A_p, AB_1, and B_{w2} horizons respectively, suggesting a fast turnover rate of organic C, regardless of the high clay content. The replacement decreased in the order: free low-density organic matter (LDOM) > heavy fractions (sand, silt, clay) > occluded-LDOM. The lower replacement of the occluded-LDOM compared to the heavy fractions was attributed to protection inside aggregates and to a possible accumulation of C_3-derived charcoal (black carbon) in that fraction. After 23 years of pasture, about 50% of the total organic C in the free-LDOM in the topsoil was still from cerrado, indicating that a significant part of this fraction was relatively recalcitrant. Charcoal fragments observed in the fraction suggested that the recalcitrance was probably due to charred material.

In Chapter 5, the spatial continuity of $\delta^{13}C$ and other soil organic matter (SOM) related variables (organic C, total N, and C:N ratio) were analysed in the native cerrado *sensu stricto* and in a nearby cultivated area with neighbouring plots under conventional and no-tillage systems. The aims were to describe the spatial variability of these properties in the cerrado, with especial attention to fire effects, and to analyse how management systems affect their spatial structure. Global, within strata, and stratified kriging were used to model the spatial variability in the areas. In cerrado, the total variability of all variables was relatively small, which was attributed to the high textural and mineralogical homogeneity of the clayey soil. Nevertheless, part of the variability was spatially structured. In cerrado locations with more open vegetation, long-term cumulative effect of repeated fires seemed to determine the spatial structure of $\delta^{13}C$ and

SOM-related variables. Cultivation reduced the variability of most of the variables and changed their spatial structure. The variables tended to be less spatially structured in no-tillage than in conventional tillage, due to small-scale variability. The spatial structure observed for $\delta^{13}C$ in the cultivated area was probably inherited from the former cerrado vegetation. This implies that, in studies of SOM dynamics, the variability of the replacements would be overestimated if the trends in both areas were not taken into account.

In Chapter 6, as the different particle-size fraction in Chapter 4 presented similar dynamics, only density fractionation was used to assess changes in SOM upon 30 years of cultivation. The objectives of the study were (i) to evaluate the long-term impact of conventional and no-tillage systems on SOM stocks in the soil, and (ii) to better understand the dynamics of SOM in different density fractions of this soil. It was observed that cultivation led to compaction, significantly increasing soil bulk density. This resulted in a systematic overestimate of C and N stocks in cultivated areas when compared to the natural cerrado. Conversion of cerrado into conventional tillage (CT) or no-tillage (NT) system did not alter the total C (~100 Mg ha^{-1}) and N (~7 Mg ha^{-1}) stocks of the first 45cm depth in 30 years of cultivation. However, about 22% of the total carbon was replaced by maize material in this period. In accordance with results from Chapter 4, the relative replacement of carbon decreased the order: free light fraction (F-LF) > heavy fraction (HF) > occluded light fraction (O-LF). The low substitution in the O-LF was attributed to the possible presence of charcoal. The F-LF showed the highest sensitivity to changes in management system, and converting cerrado into cropland significantly decreased its quantity. The proportion of C replacement in this fraction was higher in CT than NT, suggesting a faster turnover in the first. Nevertheless, because most carbon (~95%) was held in the HF, carbon dynamics in the whole soil was controlled by the behaviour of this fraction.

In Chapter 7, results form Chapter 4 and Chapter 6 were used to calculate proportions and stocks of carbon derived from a newly introduced C_4 vegetation in a C_3 ecosystem, by two different linear mixing models. One model assumed no ^{13}C discrimination upon humification for the new introduced C_4 material, and the other assumed equal ^{13}C discrimination for both C_3 and C_4 materials. The aims were to evaluate (i) how the assumption of equal ^{13}C discrimination for C_3 and C_4 material may affect estimates of proportions and stocks of C_4-derived carbon for different soil depths and soil organic matter fractions; (ii) the significance of a possible difference between outputs using the different models; and (iii) the sensitivity of this difference to variations in input parameters. The assumption of equal discrimination was discussed in the light of the current theoretical understanding of processes leading to ^{13}C discrimination in soils. Taylor series approximation was applied to estimate the output uncertainties of the models. Sensitivity analysis was used to test the influence of these outputs in the magnitude of ^{13}C discrimination with decomposition for the standard C_3 vegetation, the population standard deviation of soil and litter $\delta^{13}C$, and the sample size for soil and litter. The two models may generate significantly discrepant outputs. This difference was most sensitive to the standard deviation of the soil $\delta^{13}C$ population and the number of soil samples. A critical analysis of the current understanding of processes leading to ^{13}C discrimination in soils suggests that the assumption of absence of ^{13}C discrimination for the newly C_4 introduced material would provide the most realistic results.

Carbon stocks in the studied soil did not change with land-use (pasture, conventional tillage, and no-tillage). The low capacity of occlusion in soil aggregates and their high stability, as well as the massive concentration of SOM in the heavy fraction (largely dominated by clay-sized organo-mineral complexes) explained the relative resilience of this soil upon disturbance. These characteristics were probably related to the large contents of oxi-hydroxides of Fe and Al in the studied soil. Nevertheless, a significant amount of SOM (about 1/4 to 1/3) was replaced in 30 years, suggesting that large part of the carbon is rather active. Conversely, the remaining 2/3 to 3/4 seemed to be fairly stable, since it was not recycled even after 30 years of intensive cultivation. This fraction probably represents the passive compartment used in dynamic models such as CENTURY and RothC.

In the studied Oxisol, density fractionation separated SOM fractions with contrasting dynamics. This could potentially be used for separating measurable SOM pools, which would represent an important advance in the verification of current carbon dynamic models. However, these fractions were a mixture of labile and recalcitrant components, and procedures for distinguishing these components are needed. Quantification of charcoal in the light fraction and non-hydrolysable materials in the heavy fractions represents a promising alternative.

The ^{13}C natural abundance approach proved efficient for tracing SOM dynamics in the Cerrado Oxisol. The spatial variability of $\delta^{13}C$ in different plots (e.g. cerrado vs. pasture or cultivated plots) is responsible for most of the final uncertainty in replacement calculations. To reduce such an uncertainty, samples should be taken at the same locations before and after conversion for a new vegetation. This implies that, ideally, experiments should be planed for long-term evaluation in the same area, instead of comparing neighbouring plots.

The assumptions in the calculations can significantly affect the outputs (e.g. source proportions). When a C_4 plant is introduced into a C_3 ecosystem, the assumption of no fractionation for the newly introduced material seems the most reliable option. Nevertheless, further research is needed to better understand the process of isotope fractionation during decomposition.

Resumo

O Cerrado brasileiro constitui o segundo maior bioma do país, cobrindo cerca de 23% do território nacional. Nas últimas três decadas, sua contribuição para a produção agropecuária nacional vem crescendo significativamente, sendo que, em 1995, era responsável por 25% da produção agrícola e abrigava 40% do rebanho bovino. As estratégias de desenvolvimento adotadas priorizaram o uso de práticas agrícolas intensivas e mecanisação pesada. Tais estratégias aumentaram os riscos de se intensificarem, também, os processos de degradação do solo e perda de sua matéria orgânica.

Os solos do Cerrado são dominados por argilas de baixa atividade e caracterizados por alta acidez e baixos pH, capacidade de trocar cátions (CTC) e disponibilidade de nutrientes (especialmente P e N). A matéria orgânica do solo (MOS) exerce funções importantes nos solos, diretamente ligadas a qualidade e sustentabilidade dos mesmos. A MOS é responsável pela maior parte da CTC e está envolvida em processos de agregação e de dinâmica de água. Perdas de MOS devido ao cultivo podem afetar seriamente suas funções no ambiente, além de representarem uma importante fonte de CO_2 para a atmosfera. Estudar os efeitos de diferentes sistemas de manejo na dinâmica da MOS no Cerrado pode ajudar no desenvolvimento de formas mais racionais de uso destes ecossistemas.

O objetivo inicial da presente tese foi o de avaliar o impacto de diferentes sistemas de manejo na dinâmica da MOS em uma das classes de solo mais representativas do Cerrado, o latossolo vermelho escuro (18% da área total; Oxisol – Soil Taxonomy; Ferrasol - Legenda da Fao). A estratégia de pesquisa adotada foi a de comparar, em uma mesma unidade de solo, parcelas pareadas com diferentes sistemas de manejo (vegetação nativa, pastagem, plantio direto e convencional). Esta unidade de latossolo vermelho escuro (Typic Haplustox) utilizada localiza-se na estação experimental da EMBRAPA-CNPMS (Empresa Brasileira de Pesquisa Agropecuária – Centro Nacional de Pesquis do Milho e Sorgo), Sete Lagoas-MG, Brasil. Para avaliar a dinâmica da MOS, foi proposta a utilização de uma combinação entre (i) o fracionamento do solo de acordo com o tamanho de partículas e densidade e (ii) o cálculo da substituição de carbono baseado na abundância natural do isótopo ^{13}C. O fracionamento físico teve como objetivo separar reservatórios de MOS com diferente composição, localização e tempo de ciclagem, os quais hipoteticamente seriam mais sensíveis a alterações no solo do que a MOS total. A abundância natural do ^{13}C foi proposta para estimar o tempo de ciclagem da MOS e de suas frações. Entretanto, como poucos dados sobre estas metodologias encontravam-se disponíveis para as condições de Cerrado, o foco inicial da tese foi alterado, sendo o objetivo inicial estendido para três objetivos específicos:

(i) adaptar as metodologias de fracionamento físico do solo e abundância natural do ^{13}C para o estudo da dinâmica da MOS em ecossistemas de Cerrado;
(ii) contribuir para um melhor entendimento da distribuição e dinâmica dos diferentes reservatórios de MOS neste específico ambiente; e
(iii) avaliar o efeito a longo prazo da conversão de um cerrado *sensu stricto* em pastagem cultivada e culturas anuais (milho e feijão) sob plantio direto e convencional.

No Capítulo 2, um estudo exploratório foi conduzido para estabelecer um perfil-referência para o $\delta^{13}C$ sob o *cerrado sensu stricto* nativo. Foram analisados perfis de solo em três parcelas de cerrado sob diferentes regimes de fogo, em uma reserva na EMBRAPA-CNPMS. Os resultados demonstraram que o cerrado estudado era dominado por plantas do tipo C_3, apresentando valores de $\delta^{13}C$ ao longo de todo o perfil típicos de solos sob este tipo de vegetação. O aumento na incidência de fogo favoreceu a população de gramíneas e aumentou a quantidade de carbono derivado de plantas C_4 no solo. Nenhuma alteração nos estoques de C total foi observada com o aumento da incidência de fogo. Concluiu-se que diferentes regimes de fogo podem influenciar na escolha de perfis de referência em estudos de dinâmica da MOS. Os perfis sob baixa intensidade de fogo foram considerados uma boa referência para os próximos estudos.

No Capítulo 3, focou-se no estabelecimento de um procedimento de fracionamento físico do solo para o estudo da MOS, buscando adaptá-lo ao Oxisol estudado. Atenção especial foi dada ao processo de dispersão por energia ultrasônica, um ponto crucial da metodologia. Foi proposto um procedimento para calibrar o equipamento de ultrasom e para determinar o mínimo de energia requerida para uma eficiente dispersão. Aumentando-se a energia ultrasônica aplicada, mudancas significativas ocorreram nos conteúdos de C e N, na relação C:N e nos valores de $\delta^{13}C$ das frações granulométricas analisadas (0-2μm; 2-50μm; 50-100μm; 100-250μm; 250-2000μm). Os resultados obtidos sugeriram que o solo (< 2mm) poderia ser dividido em agregados instáveis (100-2000μm) e estáveis (50-100μm). Um nível de energia entre 260-275 J ml^{-1} foi proposto para a separação de agregados estáveis e instáveis. O uso deste limite, em combinação com fracionamento por tamanho de partículas, não se mostrou satisfatório para todos os propósitos, pois materiais similares à liteira seriam recuperados nas mesmas frações que agregados estáveis menores que 100 μm. Uma energia ultrasônica de 825 J ml^{-1} não foi suficiente para estabilizar a redistribuição de massa de solo e de MOS entre as frações granulométricas. Contudo, níveis de energia acima de 260-275 J ml^{-1} quebrariam agregados realtivamente estáveis, causando uma mistura de carbono com natureza variável na fração argila.

Com o objetivo de melhor entender a dinâmica da MOS em Oxisols e o impacto de se converter um cerrado *sensu stricto* em pastagem, a dinâmica de frações da MOS, separadas fisicamente em diferentes profundidades do latossolo vermelho escuro (Typic Haplustox), foi estudada. Comparou-se o solo sob condições naturais e após 23 anos de pastagem cultivada (*Brachiaria spp.*) (Capítulo 4). Avaliou-se a dinâmica de carbono neste estudo através da substituição do C nativo (derivado de C_3) pelo C da pastagem (derivado de C_4). Os estoques originais de C no cerrado (15 ± 3 kg m^{-2}) e na pastagem (17 ± 3 kg m^{-2}) não foram significativamente diferentes, o que foi atribuído à alta produção de biomassa pela gramínea tropical e à proteção proporcionada pelos elevados teores de argila (> 800 g kg^{-1}). A fração silte + argila acumulou 89-91% do C orgânico total. A substituição de carbono do cerrado pelo C da pastagem foi de 36%, 34% e 19% para, respectivamente, os horizontes A_p, AB_1 e B_{W2}, sugerindo um tempo de ciclagem relativamente baixo para o C, apesar das elevadas quantidades de argila. A substituição decresceu na ordem: matéria orgânica de baixa densidade (MOBD) livre > frações pesadas (areia, silte e argila) > MOBD oclusa. A menor substituição na MOBD oclusa quando comparada à fração pesada foi atribuída a sua proteção dentro dos agregados e ao possível acúmulo de materiais carbonizados, derivados de plantas C_3. Após 23 anos de pastagem, em torno de 50% do C orgânico total presente na MOBD livre no solo superficial era ainda remanescente do cerrado, indicando que uma parte significativa desta fração é relativamente recalcitrante. Fragmentos de carvão observados

nesta fração sugerem que esta recalcitrância estava provavelmente relacionada com materiais carbonizados.

No Capítulo 5, a variabilidade espacial do $\delta^{13}C$ e de outras variávies relacionadas à MOS (C orgânico, N total e relação C:N) foram analisadas no cerrado nativo e em uma área adjascente cultivada com milho e feijão em sucessão, dividida em plantio direto e convencional. Os objetivos foram o de descrever a variabilidade espacial destas variáveis no cerrado, com atenção especial para os efeitos do fogo, e o de analisar como sistemas de manejo afetam esta estrutura espacial. A varaibilidade espacial na área foi modelada usando três diferentes metodologias: krigagem global, dentro dos estratos e estratificada. No cerrado, a variabilidade total de todas as variáveis foi relativamente pequena, o que foi atribuído à alta homogenidade textural e mineralógica deste solo argiloso. Entretanto, parte desta variabilidade encontrava-se espacialmente estruturada. Em partes do cerrado com vegetação mais aberta, o efeito cumulativo de repetidos incêndios, nos mesmos lugares, parece determinar a estrutura espacial do $\delta^{13}C$ e das variáveis relacionadas à MOS. O cultivo reduziu a variabilidade da maioria das variáveis e mudou a sua estrutura espacial. As variáveis tenderam a ser menos espacialmente estruturadas sob plantio direto comparativamente ao plantio convencional, devido a variabilidade em pequenas escalas. A estrutura espacial observada para $\delta^{13}C$ na área cultivada foi provavelmente herdada da antiga vegetação de cerrado da área. Portanto, em estudos de dinâmica da MOS, a variabilidade das substituições de carbono de diferentes vegetações será superestimada, caso as tendências não sejam consideradas.

No Capítulo 6, como as diferentes frações granulométricas no Capítulo 4 apresentaram dinâmicas similares, somente o fracionamento densimétrico foi utilizado para avaliar as mudanças na MOS, após 30 anos de cultivo. Os objetivos do estudo foram: (i) avaliar o impacto de longos períodos de cultivo, sob os sistemas de plantio direto e convencional, nos estoques de MOS; e (ii) melhor entender a dinâmica de frações densimétricas da MOS neste solo. Observou-se que o cultivo levou a uma compactação do solo, aumentando significativamente a densidade aparente. Por esta razão, os estoques de C e N nas áreas cultivadas foram sistematicamente superestimados em comparação ao cerrado. A conversão do cerrado em plantio convencional (CT) e plantio direto (PD) não alterou significativamente os estoques de carbono (~ 100 Mg ha^{-1}) e de nitrogênio (~ 7 Mg ha^{-1}) nos primeiros 45 cm de solo, após de 30 anos. Entretanto, cerca de 22% do C total foi substituído pelo material de milho neste período. Corroborando com os resultados obtidos no Capítulo 4, a substituição de carbono nas diferentes frações granulométricas seguiu a ordem: fração leve livre (FL-L) > fração pesada (FP) > fração leve oclusa (FL-O). A baixa substituição na FL-O foi atribuída a possível presença de carvão. A FL-L foi a fração mais sensível às alterações no sistema de manejo, decrescendo significativamente com a conversão do cerrado para o cultivo. A proporção de C substituído nesta fração foi superior para o PC em relação ao PD, sugerindo um tempo de ciclagem mais rápido para o primeiro. Entretanto, como a maior parte do carbono concentrou-se na fração pesada (~ 95% do total), a dinâmica do C neste solo foi controlada pelo comportamento desta fração.

No Capítulo 7, resultados dos Capítulos 4 e 6 foram usados para se calcularem, através de dois modelos lineares distintos, as proporções e estoques de carbono derivados da vegetação C_4 recentemente introduzida em um ecossistema dominado por plantas C_3. Um dos modelos assume ausência de discriminação do ^{13}C durante a decomposição do recém introduzido material de C_4. O outro modelo assume a mesma discriminação, durante a decomposição, para os materiais de C_3 e C_4. Os objetivos foram o de avaliar: (i) como as pré-suposições de cada um dos modelos afetam as estimativas

de proporções e estoques de C derivado de C_4, em diferentes profundidades do solo e frações da MOS; (ii) a significância da possível difereça entre resultados obtidos pelos diferentes modelos; e (iii) a sensibilidade desta diferença às variações nos parâmetros de entrada dos modelos. A pré-suposição de discriminação indiferenciada entre os dois grupos de plantas foi discutida na luz do presente entendimento teórico dos processos que levam à discriminação do ^{13}C em solos. Aproximações pelas séries de Taylor foram utilizadas para estimar as incertezas nos resultados dos modelos. Análises de sensibilidade foram usadas para testar a influência nestes resultados dos parâmetros: magnitude, no padrão para a vegetação C_3, da discriminação do ^{13}C com a decomposição; o desvio padrão populacional dos valores de $\delta^{13}C$ para solo e liteira; e tamanho da amostra para solo e liteira. Os resultados mostraram que os dois modelos podem gerar estimativas significativamente diferentes. Esta diferença foi mais sensível ao desvio padrão populacional do $\delta^{13}C$ e ao número de amostras de solo. Uma análise crítica do presente entendimento dos processos que levam a discriminação do ^{13}C durante a decomposição nos solos sugerem que assumir ausência de discriminação para o material de C_4 recentemente introduzido é a alternativa que geraria resultados mais realísticos.

Os estoques de carbono no solo estudado não mudou com a conversão para diferentes sistemas de uso do solo (pastagem, plantio convencional e plantio direto). A baixa capacidade de oclusão nos agregados do solo e sua alta estabilidade, da mesma forma que a massiva concentração da MOS na fração pesada (largamente dominada por complexos organo-minerais do tamanho argila) explicam a relativa resiliência deste solo quando perturbado. Estas características estão provavelmente ligadas aos grandes conteúdos de oxi-hidróxidos de Fe e Al do solo estudado. Não obstante, uma quantidade significativa da MOS (entre 1/4 e 1/3) foi substituída em 30 anos, sugerindo que uma boa parte do carbono é bem reativa. Por outro lado, a parcela restante (2/3 a 3/4) parece ser bem estável, pois manteve-se constante mesmo após 30 anos de cultivo intensivo. Esta fração provavelmente representa o compartimento passivo de modelos de dinâmica da MOS, tais como CENTURY e RothC.

No Oxisol estudado, o fracionamento densimétrico separou frações com dinâmicas contrastantes. Isso poderia ser usado para separar reservatórios mensuráveis de MOS, o que representaria um grande avanço na verificação dos modelos de dinâmica de carbono no solo. Entretanto, estas frações compunham-se de uma mistura de componentes mais lábeis e mais recalcitrantes. Metodologias para distinguir estes dois compartimentos são ainda necessárias. A quantificação de carvão nas frações leves e de compostos não hidrolisáveis nas frações pesadas podem representar uma boa alternativa.

A metodologia de análise da dinâmica da MOS baseada na abundância natural do isotopo ^{13}C mostrou-se bastante satisfatória paa o latossolo vermelho escuro estudado. A variabilidade espacial do $\delta^{13}C$ nas diferentes parcelas comparadas (e.g. cerrado vs. pastagem ou parcelas cultivadas) é responsável pela maior paste da incerteza final dos resultados nos cálculos de substituição de carbono. Para reduzir esta fonte de incerteza, o solo devem ser amostrados nos mesmos locais antes e depois da conversão para uma nova vegetação. Isso implica que, idealmente, os experimentos devem ser planejados para avaliações de longo período na mesma área, evitando-se a comparação de parcelas vizinhas.

Quando uma planta C_4 é introduzida em um sistema C_3, assumir que não existe discriminação com a decomposição para o material C_4 introduzido recentemente parece ser a melhor opção. Entretanto, o processo de fracionamento isotopico com a decomposição é um assunto que necessita de maiores investigações.

Samenvatting

De Braziliaanse *cerrado* is qua grootte de tweede bioom van het land. Het bedekt 23% van de landoppervlakte. Gedurende de laatste dertig jaar is de bijdrage van het *cerrado* gebied tot het nationale product gestadig gegroeid. In 1995 werd 25% van de agrarische productie gerealiseerd in dit gebied, en werd er 40% van het vee geweid. Interne ontwikkelingspolitiek heeft sterk gemechaniseerde en intensieve teeltwijzen gestimuleerd. Hierdoor is er bezorgdheid ontstaan over verliezen van organische stof (soil organic matter - SOM) uit de bodem en over bodemerosie.

Cerrado bodems worden gedomineerd door kleien met geringe lading, en worden gekarakteriseerd door een relatief lage pH, kationen uitwisselcapaciteit (*cation exchange capacity* - CEC), en beschikbare nutriënten (speciaal N en P). SOM vervult belangrijke functies in deze bodems, en deze functies zijn direct gerelateerd aan bodemkwaliteit en duurzaamheid. SOM is verantwoordelijk voor het grootste deel van de CEC en is zeer belangrijk voor aggregatie en water binding. Verliezen van SOM als gevolg van cultivering kunnen deze functies in belangrijke mate verstoren. Bovendien dragen verliezen van SOM uit de bodem bij tot de concentratieverhoging van het broeikasgas CO_2 in de atmosfeer. Een studie van het effect van verschillende teeltsystemen op de dynamiek van koolstof in *cerrado* bodems kan een bijdrage leveren tot beter gebruik van dit ecosysteem.

Het oorspronkelijke doel van dit onderzoek was om het effect te onderzoeken van verschillende teeltsystemen op de dynamiek van SOM in één van de meest voorkomende bodems van de *cerrado:* de *Dark Red Latosol (*Braziliaanse classification*: Latossol Vermelho Oscuro;* Soil Taxonomy*: Oxisol;* FAO Legend*: Ferralsol).* Dit type bodem bedekt 18% van het *cerrado* gebied. De opzet was de vergelijking, binnen dezelfde bodemeenheid, van gekoppelde velden met verschillend teeltsysteem (oorspronkelijke bosvegetatie, permanent grasland, akkerbouw met conventioneel ploegen en zonder ploegen. De onderzochte *Dark Red Latosol (Typic Haplustox)* was gelokaliseerd op het terrein van EMBRAPA-CNPMS (Braziliaans Instituut voor Landbouwkundig Onderzoek - Onderzoek Centrum voor Maïs en Sorghum), in Sete Lagoas, Minas Gerais, Brazilië. Een combinatie van twee methoden werd gekozen voor de studie van SOM dynamiek: (i) fysische fractionering van de bodem in korrelgrootte- en dichtheidsfracties, en (ii) berekening van vervanging van de oorspronkelijke SOM met behulp van de natuurlijke abundantie van de stabiele ^{13}C isotoop. De fysische fractionering was bedoeld om SOM fracties (*pools*) met verschillende chemie, locatie, en dynamiek van elkaar te scheiden. In theorie zouden zulke fracties gevoeliger zijn voor veranderingen dan de totale SOM. De natuurlijke abundantie van ^{13}C in zulke fracties zou dan weer gebruikt kunnen worden voor bestudering van de dynamiek. Helaas waren er erg weinig gegevens betreffende het gebruik van dergelijke methoden in *cerrado* ecosystemen. Daarom moest de oorspronkelijke focus van het onderzoek enigszins veranderd worden. Dit werd uiteindelijk:

(i) aanpassing van de fysische fractionatiemethoden en ^{13}C toepassingen aan SOM onderzoek in het *cerrado* systeem.

(ii) bijdrage tot een beter begrip van verdeling en dynamiek van SOM fracties in deze omgeving, en

(iii) evaluatie van de lange-termijn effecten op SOM van de conversie van een *cerrado sensu stricto* in permanent grasland of teeltsystemen (maïs en bonen) met en zonder ploegen.

Hoofdstuk 2 beschrijft het onderzoek ter vaststelling van een ^{13}C referentie profiel in de natuurlijke *cerrado sensu stricto*. In een reservaat van natuurlijke vegetatie in EMBRAPA-CNPMS werden drie profielen met verschillende brand frequentie geanalyseerd. De resultaten tonen aan dat de bestudeerde *cerrado* gedomineerd werd door C_3-vegetatie. De $\delta^{13}C$ waarden in de bodemprofielen vertoonden de typische waarden van bodemprofielen onder zo'n vegetatie. Brand bevoordeelde een grasvegetatie, en resulteerde in een toename in het systeem van koolstof afkomstig van C_4-planten. Er was geen verandering van de totale koolstofhoeveelheid in het profiel als gevolg van branden. De conclusie is, dat de frequentie van branden invloed heeft op het $\delta^{13}C$ profiel in de bodem, en hiermee de keuze van een referentieprofiel bemoeilijkt. De bodems onder bos met een lage brandfrequentie werden beschouwd als de juiste uitgangssituatie voor vergelijking met gecultiveerd land.

In Hoofdstuk 3 ligt de nadruk op de procedure van fysische fractionering van de onderzochte *cerrado* bodems. Dispersie met behulp van ultrasoon, een cruciale stap in de methodologie, kreeg extra aandacht. Een methode ter kalibrering van het ultrasoon apparaat wordt beschreven, en een minimum energietoevoeging voor efficiënte dispersie wordt vastgesteld. Er is een belangrijke verandering in hoeveelheden C en N, C/N verhouding, en $\delta^{13}C$ waarden van de korrelgroottefracties (0-2, 50-100, 100-250 en 250-2000 μm) met toenemende ultrasoon energie. De resultaten suggereren dat de fractie < 2 mm verdeeld kan worden in onstabiele (100-2000 μm) en stabiele (50-100 μm) aggregaten. Voor de dispersie van onstabiele aggregaten wordt een energieniveau van 260-275 J ml^{-1} voorgesteld.

De toepassing van dit energieniveau, tezamen met korrelgroottefractionering gaf niet altijd bevredigende resultaten, omdat strooiselachtig materiaal en recalcitrante C-fracties uit stabiele aggregaten >100 μm soms in dezelfde fractie werden teruggevonden. Een ultrasoon energie van 825 J ml^{-1} was onvoldoende om de herverdeling van bodemdeeltjes en SOM over de verschillede fracties te stabiliseren. Anderzijds vallen bij energieniveaus boven 260-275 J ml^{-1} relatief stabiele aggregaten uiteen en veroorzaken een mengsel van verschillende C fracties in de kleifractie.

Om de dynamiek van SOM in *Oxisols* en het effect van conversie van *cerrado sensu stricto* in permanent grasland te bestuderen, werd de dynamiek van fysische SOM fracties bestudeerd op verschillende diepten. Hiertoe werd een bodem onder natuurlijke *cerrado* vergeleken met die onder een gecultiveerd grasland (23 jaar *Brachiaria* ssp.). Voor alle fracties werd de vervanging van C_3 (bos) koolstof door C_4 (grasland)-koolstof gemeten met behulp van $\delta^{13}C$ (Hoofdstuk 4). SOM voorraden onder bos (15±3 kg C/m^2) en onder grasland (17±3 kg C/m^2) verschilden niet significant. Dit werd toegeschreven aan de hoge productie van biomassa van de grasvegetatie en het beschermende effect van het hoge kleigehalte. (>800 g/kg). De klei- en silt fracties bevatten 89-91% van de totale C hoeveelheid. Der vervanging van *cerrado* afkomstige koolstof door van gras afkomstige koolstof was gemiddeld 36%, 34% en 19% voor, respectievelijk, de A_p, AB_1 en B_{W2} horizont. Dit suggereert een snelle SOM dynamiek, niettegenstaande het hoge kleigehalte van de bodem. De vervanging nam af in de volgorde: vrije lichte organische stof (LDOM) > zware fracties (zand, silt, klei) > opgesloten organische stof (*occluded LDOM*). De geringere vervanging in de opgesloten organische stof, in vergelijking tot die van de zware fracties, werd toegeschreven aan protectie binnen aggregaten van de laatstgenoemde fractie, en aan een mogelijke ophoping van C3-afkomstige houtskool (zwarte koolstof) in die fractie. Na 23 jaar grasland was ongeveer 50% van de koolstof in LDOM nog steeds toe te schrijven aan de *cerrado*, hetgeen aanduidt dat een belangrijk deel van deze fractie tamelijk recalcitrant is. De aanwezigheid van houtskoolfragmenten in deze fractie suggereert dat deze recalcitrantie is toe te schrijven aan houtskool.

In Hoofdstuk 5 werd de ruimtelijke variabiliteit van $\delta^{13}C$ en andere variabelen (organische C, N, C/N ratio) geanalyseerd, zowel in het natuurlijke *cerrado* systeem als in een nabijgelegen gecultiveerd gebied met aangrenzende velden met conventioneel ploegen en zonder ploegen. De bedoeling was, de ruimtelijke patronen te analyseren in relatie tot het effect van (bos)branden, en te bepalen hoe verschillen in teeltwijzen deze patronen beïnvloeden. Drie soorten *kriging* werden gebruikt om de ruimtelijke patronen te modelleren: globale, binnen strata, en gelaagde. In de *cerrado* was de totale variabiliteit van alle variabelen vrij klein, hetgeen toegeschreven kan worden aan de grote texturele en mineralogische homogeniteit van deze kleiige bodem. Toch vertoonde een gedeelte van de variatie een ruimtelijke trend. In *cerrado* locaties met een meer open vegetatie, leek het cumulatieve effect van bosbranden de ruimtelijke structuur van $\delta^{13}C$ en de andere variabelen te bepalen. Cultivering verminderde de variatie van de meeste variabelen en veranderde de ruimtelijke structuur. Ruimtelijke structuur van de variabelen was minder duidelijk in de ongeploegde dan in geploegde velden, hetgeen voornamelijk te wijten was aan korte afstand variabiliteit in de eerste. De waargenomen ruimtelijke structuur van $\delta^{13}C$ was waarschijnlijk geërfd van de vroegere *cerrado* vegetatie. Dit betekent dat, bij studies van koolstofdynamiek, de variabiliteit van vervangingen als gevolg van veranderend landgebruik wordt overschat waneer de trends in beide gebieden niet meegewogen worden.

Omdat de verschillende korrelgroottefracties die besproken werden in Hoofdstuk 4 een zelfde dynamiek te zien geven, wordt in Hoofdstuk 6 alleen dichtheidsfractionering gebruikt om het effect van 30 jaar cultivering op SOM te bestuderen. De doelen van deze studie waren: (i) het lange-termijn effect van conventioneel ploegen en niet ploegen op de SOM voorraden in de bodem te evalueren; (ii) een beter inzicht te verkrijgen in de dynamiek van SOM in verschillende dichtheidsfracties. Cultivering leidde tot compactie, en een significante toename van het volumegewicht in de bouwvoor. Dit resulteert in een systematische overschatting van de C en N voorraden in gecultiveerd land, wanneer deze vergeleken worden met de *cerrado*. Conversie van *cerrado* naar traditioneel geploegd (*conventional tillage* - CT) of ongeploegde (*no-tillage* - NT) akkerbouw had in 30 jaar geen invloed op de totale hoeveelheid C (~ 100 Mg/ha) en N (~ 7 Mg/ha) voorraden in de bovenste 45 cm. Toch was in deze periode ongeveer 22% van de totale hoeveelheid koolstof vervangen door koolstof afkomstig van maïs. In overeenstemming met de resultaten van Hoofdstuk 4, nam de relatieve vervanging van C af volgens: vrije lichte fractie (F-LF) > zware fractie (HF) > opgesloten lichte fractie (O-LF). De geringe substitutie in de O-LF fractie was vermoedelijk te wijten aan de aanwezigheid van houtskool. De F-LF was het meest gevoelig voor veranderingen in management systeem. De hoeveelheid van deze fractie verminderde aanmerkelijk als gevolg van de *cerrado* - akkerbouw conversie. De vervanging in deze fractie was hoger in CT dan in NT, hetgeen een sneller omzetting in de eerste suggereert. Omdat de meeste koolstof (95 %) opgeslagen is in de zware fractie (HF), bepaalt deze fractie de dynamiek in de totale bodem.

In Hoofdstuk 7 worden resultaten uit Hoofdstukken 4 en 6 gebruikt om de relatieve hoeveelheid C_4 koolstof te berekenen die geïntroduceerd werd in het oorspronkelijke C_3 ecosysteem. Hiervoor werden twee mengmodellen gebruikt. Het eerste model veronderstelt een afwezigheid van ^{13}C discriminatie bij humificering van het geïntroduceerde C4 materiaal; het tweede model veronderstelt gelijke discriminatie voor C_3- en C_4- materiaal. De bedoeling van de vergelijking was om na te gaan (i) hoe de aanname van gelijke discriminatie de berekening beïnvloedt van relatieve vervanging en voorraden van C_4-afkomstige koolstof in verschillende fracties en diepten; (ii) wat de significantie is van de gevonden verschillen, en (iii) hoe gevoelig de verschillen zijn voor variaties in input variabelen. De aanname van gelijke discriminatie wordt besproken in het licht van huidige inzichten in processen die leiden tot ^{13}C discriminatie in bodems. Een *Taylor series* benadering werd gebruikt om de onzekerheid in de resultaten van de berekeningen te testen. Gevoeligheidsanalyse werd gebruikt om het effect te testen van deze resultaten op (i) de grootte van ^{13}C discriminatie onder invloed van decompositie voor de standaard C_3 vegetatie; (ii) de standaard deviatie van de populatie van bodem en strooiselmonsters, en (iii) de grootte van het monster voor zowel strooisel als bodemmonsters. De twee modellen kunnen significant verschillende resultaten opleveren. Het verschil was het meest gevoelig voor de standaard deviatie van de $\delta^{13}C$ populatie van de grond, en voor het aantal bodemmonsters. Een kritische analyse van de huidige inzichten omtrent processen die leiden tot ^{13}C discriminatie in de bodem suggereert dat de veronderstelling van *afwezigheid van discriminatie* voor het geïntroduceerde C_4 strooisel de meest realistische resultaten geeft.

Koolstofvoorraden in de bestudeerde gronden veranderden niet onder invloed van landgebruik (permanent grasland, conventioneel ploegen, niet ploegen). De geringe opslagmogelijkheden in bodemaggregaten, de grote stabiliteit van deze aggregaten, en de sterke concentratie van SOM in de zware fracties (die gedomineerd worden door organo-minerale complexen van kleiformaat), verklaren de relatieve weerstand van deze bodem tegen verstoringen als gevolg van landgebruik. Deze karakteristieken zijn vermoedelijk te danken aan de hoge gehalten aan oxyhydraten van Al en Fe in de bestudeerde bodem. Toch werd een aanzienlijk gedeelte van de SOM (ongeveer 1/4 tot 1/3 van het totaal) in dertig jaar vervangen, hetgeen suggereert dat een groot gedeelte van de koolstofvoorraad relatief dynamisch is. De overige 2/3 tot 3/4 bleek tamelijk stabiel, omdat het niet vervangen werd in dertig jaar van intensieve cultivering. Deze fractie vertegenwoordigt waarschijnlijk het 'passieve compartiment' dat gebruikt wordt in dynamische modellen zoals CENTURY en RothC.

In de bestudeerde Oxisol scheidde dichtheidsfractionering fracties met verschillende dynamiek. Deze methode zou potentieel gebruikt kunnen worden om meetbare SOM 'pools' te scheiden, hetgeen een belangrijke vooruitgang zou zijn bij de verificatie van huidige modellen voor koolstofdynamiek. Niettemin zijn de verkregen fracties nog steeds mengsels van labiele en recalcitrante componenten, en methoden om deze twee te scheiden moeten nog ontwikkeld worden. Kwantificering van houtskool in de lichte fractie, en van niet-hydrolyseerbare componenten in de zware fracties is een veelbelovend alternatief.

Het gebruik van de natuurlijke abundantie van ^{13}C is een efficiënte methode voor de studie van koolstofdynamiek in de bestudeerde *cerrado* Oxisol. De ruimtelijke variatie van $\delta^{13}C$ in de verschillende velden (i.e. *cerrado* versus gecultiveerde systemen) is verantwoordelijk voor het grootste gedeelte van de onzekerheid in de berekende C vervanging. Om deze onzekerheid te reduceren zouden monsters genomen moeten worden in hetzelfde veld voor en na de conversie. Dit betekent dat, in het ideale geval, lange termijn experimenten voorzien zouden moeten worden ter evaluatie van veranderingen in hetzelfde veld, in plaats van naburige velden te vergelijken.

De veronderstellingen waarvan voor de berekeningen wordt uitgegaan kan een aanzienlijk effect hebben op resultaten zoals relatief aandeel van (C_3 en C_4) koolstofbronnen. Wanneer een C_4 vegetatie wordt geïntroduceerd in een C_3 ecosysteem, lijkt de veronderstelling dat er geen fractionering optreedt bij humificering van het C_4

materiaal de beste uitkomsten te geven. Toch is verder onderzoek noodzakelijk om het proces van isotoop-fractionering gedurende decompositie beter te begrijpen.

Appendix 1

The Cerrado Biome

Area
The Cerrado area is of 2,064,676 km^2 (Pereira et al., 1997).

Population
The population in 1992 (last survey) was 21,064,010 inhabitants (Pereira et al., 1997).

Vegetation
The vegetation in the Cerrado Biome is a mix of three basic physiognomic groups: forests, savannahs, and grasslands (Table A1.1).

Table A1.1. Physiognomic groups and sub-groups of the vegetation found in the Cerrado Biome (adapted from Ribeiro and Walter, 1998).

Physiognomic Groups	***Physiognomic Sub-Groups***	Trees/ shrubs (m)	Canopy Density (% total area)
Forests	- *Mata Ciliar* (riparian forest without gallery)	20-25	50-90
	- *Mata de Galeria* (riparian forest forming gallery)	20-30	70-95
	- *Mata Seca* (dry forest)	15-25	50-95
	- *Cerradão* (woodland savannah)	8-15	50-95
Savannah	- *Cerrado sensu stricto – Denso* (dense)	5-8	50-70
	- *Cerrado sensu stricto – Típico* (typical)	3-6	20-50
	- *Cerrado sensu stricto – Ralo* (sparse)	2-3	5-20
	- *Cerrado sensu stricto – Rupestre* (with bedrock)	2-4	5-20
	- *Parque de cerrado* (occuring in *murundus*[a])	3-6	5-20
	- *Palmeiral* (only palm-trees)	8-15	30-60
	- *Vereda* (only palm-trees in a wetland)	12-15	5-10
Grasslands	- *Campo sujo* (with scattered shrubs)	2	< 5
	- *Campo rupestre* (with scattered shrubs and bedrock)	< 2	< 5
	- *Campo limpo* (almost no shrubs)	< 2	< 1

[a] *murudus* are convex elevations 0.1-1.5m high, with a diameter of 0.2-20m, common in flat depressions with moist accumulation. The origin of these structures is still unclear but, according to the most accepted theories, they are active or inactive termite nests or they result from differential erosion (Ribeiro and Walter, 1998).

Climate

The peculiar climate of the region is characterised by wet summers and dry winters. The annual precipitation varies from 600 to 2200 mm, but 65% of the area has between 1200 and 1600 mm annually. This rainfall is not well distributed, concentrating in the summer. The dry season lasts from 4 to 7 months in 88% of the area and from 5 to 6 months in 67% of the area. The mean annual temperature varies from 22°C in the South to 27°C in the North (Adámoli et al., 1985).

Soils

The major soil classes are presented in Table A1.2.

Table A1.2. Major soil classes occurring in the Cerrado Biome, according to the Brazilian System of Soil Classification (Camargo et al., 1987a,b), and their equivalents in the FAO Legend (FAO, 1974) and Soil Taxonomy (Soil Survey Staff, 1975) (adapt from Reatto et al., 1998).

Brazilian System of Soil Classification[a]	FAO Legend	Soil Taxonomy	Area (%)
Red-Yellow Latosol	Ferrasols	Oxisols	21.6
Dark Red Latosol	Ferrasols	Oxisols	18.6
Purple Latosol	Ferrasols	Oxisols	3.5
Yellow Latosol	Ferrasols	Oxisols	1.5
Una Latosol	Ferrasols	Oxisols	0.5
Quartz Sand	Arenosols	Entisols	15.2
Red-Yellow Podzolic	Acrisols, Nitosols, Luvisols	Ultisols, Alfisols	8.2
Dark Red Podzolic	Acrisols, Nitosols, Luvisols	Ultisols, Alfisols	6.9
Litolic	Lithosols, Rankers, Cambisols, Regosols, Phaeozems	Entisols, Inceptisols	7.3
Plintosol	Acrisols, Arenosols, Ferrasols, Gleysols or Planosols	Ultisols, Oxisols, Inceptsols, Entisols	6.0
Cambisol	Cambisols	Inceptisols	3.1
Plintic Plintosol	Acrisols, Arenosols, Ferrasols, Gleysols or Planosols	Ultisols, Oxisols, Inceptsols, Entisols	3.0
Others			4.6

[a] although the Brazilian System of Soil Classification was altered in 1999 (EMBRAPA, 1999), we used the old system (Camargo et al., 1987a,b) because the available survey of the Cerrado's soils is based on it.

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