

# SEÇÃO II - QUÍMICA E MINERALOGIA DO SOLO

## TRANSFORMATIONS IN OCCLUDED LIGHT FRACTION ORGANIC MATTER IN A CLAYEY OXISOL; EVIDENCE FROM $^{13}\text{C}$ -CPMAS-NMR AND $\delta^{13}\text{C}$ SIGNATURE<sup>(1)</sup>

R. ROSCOE<sup>(2)</sup>, P. BUURMAN<sup>(3)</sup>, B. VAN LAGEN<sup>(4)</sup> & E. VELTHORST<sup>(4)</sup>

### SUMMARY

We hypothesised that, during occlusion inside granular aggregates of oxide-rich soils, the light fraction organic matter would undergo a strong process of decomposition, either due to the slow process of aggregate formation and stabilisation or due to digestion in the macro- and meso-fauna guts. This process would favour the accumulation of recalcitrant materials inside aggregates. The aim of this study was to compare the dynamics and the chemical composition of free and occluded light fraction organic matter in a natural cerrado vegetation (woodland savannah) and a nearby pasture (*Brachiaria* spp.) to elucidate the transformations during occlusion of light fraction in aggregates of a clayey Oxisol. Nuclear Magnetic Resonance of the  $^{13}\text{C}$ , with Cross Polarisation and Magic Angle Spinning ( $^{13}\text{C}$ -CPMAS-NMR), and  $^{13}\text{C}/^{12}\text{C}$  isotopic ratio were combined to study organic matter composition and changes in carbon dynamics, respectively. The occluded light fraction had a slower turnover than the free light fraction and the heavy fraction. Organic matter in the occluded fraction also showed a higher degree of decomposition. The results confirm that processes of soil organic matter occlusion in the typical "very fine strong granular" structure of the studied oxide-rich soil led to an intense transformation, selectively preserving stable organic matter. The small amount of organic material stored as occluded light fraction, as well as its stability, suggests that this is not an important or manageable sink for sequestration of atmospheric  $\text{CO}_2$ .

**Index terms:** soil organic matter, density fractionation,  $^{13}\text{C}$ -CPMAS-NMR,  $^{13}\text{C}/^{12}\text{C}$  isotopic ratio.

---

<sup>(1)</sup> Recebido para publicação em abril de 2003 e aprovado em agosto de 2004.

<sup>(2)</sup> Pesquisador da Embrapa Agropecuária Oeste. Caixa Postal 661, CEP 79804-970 Dourados (MS), Brasil. E-mail: roscoe@cpao.embrapa.br

<sup>(3)</sup> Ph.D, Associate Professor of the Laboratory of Soil Science and Geology, Department of Environmental Sciences, Wageningen University, P.O. Box 37, 6700 AA, Wageningen, The Netherlands.

<sup>(4)</sup> BSc, Technician of the Laboratory of Soil Science and Geology, Department of Environmental Sciences, Wageningen University, P.O. Box 37, 6700 AA, Wageningen, The Netherlands.

**RESUMO:** *TRANSFORMAÇÕES NA FRAÇÃO LEVE OCLUSA DA MATÉRIA ORGÂNICA EM UM LATOSSOLO ARGILOSO; EVIDÊNCIA A PARTIR DO  $^{13}\text{C}$ -RMN-PCAMR E ABUNDÂNCIA NATURAL DE  $^{13}\text{C}$*

*O presente estudo tomou, como referência, a hipótese de que, durante a oclusão dentro de agregados granulares de solos ricos em óxidos, a fração leve da matéria orgânica do solo passa por um intenso processo de decomposição, decorrente do lento processo de formação desses agregados ou da digestão no trato digestivo da meso e macrofauna. O objetivo deste trabalho foi comparar a dinâmica e a composição química das frações leves (livre e oclusa) de um solo sob vegetação de cerrado e uma pastagem (*Brachiaria spp.*) adjacente, para elucidar as transformações durante o processo de oclusão da fração leve em agregados de um Oxissol argiloso. As técnicas de Ressonância Magnética Nuclear do  $^{13}\text{C}$ , com Polarização Cruzada e Ângulo Mágico de Rotação ( $^{13}\text{C}$ -RMN-PCAMR), e de análise da relação isotópica  $^{13}\text{C}/^{12}\text{C}$  para acessar, respectivamente, a composição e dinâmica dos materiais orgânicos presentes. A fração leve oclusa apresentou um tempo de ciclagem bem maior, quando comparada à fração leve livre e a pesada. A matéria orgânica nessa fração também apresentou maior grau de decomposição. Segundo os resultados, o processo de oclusão de matéria orgânica nas típicas estruturas "granulares fortes muito pequenas" dos solos ricos em óxidos levou esse material a sofrer uma intensa transformação, preservando seletivamente matéria orgânica estável. As pequenas quantidades de carbono acumuladas na fração leve oclusa, assim como sua estabilidade, indicaram não representar essa fração importante e manejável sumidouro de  $\text{CO}_2$  atmosférico.*

*Termos de indexação: matéria orgânica do solo, fracionamento dissimétrico,  $^{13}\text{C}$ -RMN-PCAMR, relação isotópica  $^{13}\text{C}/^{12}\text{C}$ .*

## INTRODUCTION

Although soil organic matter (SOM) represents only a small portion of the total soil matrix, it is a very active component linked to fundamental soil processes, such as biological activity, nutrient cycling, and aggregation (Christensen, 2001). In addition, soils hold about three times more carbon than the atmosphere, representing an important sink of  $\text{CO}_2$  (IPCC, 2000). The large amounts of  $\text{CO}_2$  emitted to the atmosphere by human activity have the potential of increasing global temperature. Understanding the dynamics of SOM may help in designing land-use systems to maintain carbon in the soil, which will contribute to an improvement of soil quality and mitigation of global warming.

SOM is composed of all sorts of organic material derived from plants and animals, in different stages of decomposition and degree of association with the mineral matrix. SOM can be free or only weakly associated to soil particles (*free light fraction* – F-LF), or strongly associated to mineral particles as *organo-mineral complexes* (OMC) (Golchin et al., 1997; Christensen, 2001). These complexes are called *primary*, when formed by direct interaction between primary mineral particles and organic compounds. At a higher hierarchical level, primary complexes may bind together into secondary complexes or aggregates. In this process of aggregation, free

organic material can be encapsulated, forming the *intra-aggregate particulate organic matter* or *occluded light fraction* (O-LF). The dynamics of these SOM pools is usually different. The most labile fraction is the F-LF, where, apart from environmental conditions, only the intrinsic recalcitrance of its compounds will determine the dynamics of the fraction. For O-LF, the occlusion inside aggregates limits the access of decomposers (micro-organisms) and extra-cellular enzymes. In addition to these two mechanisms, the heavy fraction (HF) is also protected by direct complexation with clay particles. Therefore, the turnover rate of the three fractions is expected to decrease in the order: F-LF > O-LF > HF (Golchin et al., 1997; Christensen, 2001).

The process of occlusion may strongly affect the composition and dynamics of organic material in the light fraction. Changes in the composition of SOM during occlusion may provide important clues about the process of aggregation. According to Golchin et al. (1997), occlusion may preserve relatively easily decomposable organic material in places inaccessible to microbiota. However, these authors studied mainly oxide-poor soils, where blocky structures dominate. In these soils, relatively high portions of the total carbon (up to 31 %) may be found in the O-LF (Roscoe & Machado, 2002). In oxide-rich soils, this amount is about 1 % of the total carbon, which is probably related to their peculiar structures.

Currently, two major hypotheses are used to explain the formation and stabilisation of the typical "strong very fine granular" structure observed in Oxisols. The physical-chemical hypothesis states that this structure derives from high contents of Fe and Al oxo-hydroxides in combination with a long period of soil formation (Resende et al., 1997). The combination of strong binding agents with stress caused by repeated cycles of wetting and drying for long periods of time, would create very stable spherical structures (the most geometrically stable solid, due to its low volume to surface ratio). Alternatively, according to the biogenic theory, the granular structures would be a product of biological activity, especially termites. 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 up (in the subsoil) and transporting (to the surface) very stable spherical aggregates, with a mean diameter of 0.6 mm. The physical and biological processes are not conflicting and may happen simultaneously, but the biogenic process should be much more rapid than the physical one. Both processes would probably cause occlusion of organic materials in an advanced stage of decomposition. Since the physical-chemical process is very slow, the occlusion of the organic compounds would not happen abruptly. Similarly, in biogenic aggregates the entrapped material would have passed through the gut of macro- or meso-fauna. This could cause the decomposition of more available organic materials, leaving behind more recalcitrant compounds. Therefore, both processes could accumulate relatively more recalcitrant material within the granular aggregates of oxide-rich soils.

In contrast to the general preservation of light fraction material in large aggregates of soils with blocky structures, we hypothesise that the light fraction occluded in aggregates of oxide-rich soils may have undergone significant decomposition, either due to the slow process of aggregate formation and stabilisation or to digestion in the macro- and meso-fauna guts. This process would favour the accumulation of recalcitrant materials inside aggregates. The aim of this study was to compare the dynamics and the chemical composition of free and occluded light fraction organic matter in a natural cerrado vegetation (woodland savannah) and a nearby pasture (*Brachiaria* spp.) to elucidate the transformations during the occlusion process within aggregates.

## MATERIAL AND METHODS

Two representative sites were selected in a homogeneous dystrophic Red Latosol (Typic Haplustox) soil unit at the research station of

Embrapa-CPMS, Sete Lagoas (MG), Brazil (19 °2 ' S; 44 ° 10 ' W; 730 m). The mean annual temperature is 22.1 °C, and annual precipitation is 1,340 mm, with a dry season from April to September. Site 1 was a reserve of native "cerrado sensu-stricto" vegetation (woodland savannah), dominated by C<sub>3</sub>-plants. Site 2 was an adjacent cultivated pasture, where the native Cerrado was replaced by *Brachiaria* spp. 23 years before sampling. Duplicate profiles were described at each site and sampled down to 1m depth (Roscoe et al., 2001). For this study, composite samples were taken from the upper horizon of each profile, in three of the pit walls (Table 1). The four samples were used for density fractionation and isotopic analysis, while the two samples from each plot were combined for NMR analysis.

SOM was separated by density fractionation (Roscoe et al., 2001). Air-dried soil samples (20 g of soil < 2 mm) of the A<sub>b</sub> (Site 1) and A<sub>p</sub> horizons (Site 2) were fractionated in triplicate. The light-fraction was separated by flotation using a NaI solution (1.7 g cm<sup>-3</sup>) before (*free light fraction* – F-LF) and after (*occluded light fraction* – O-LF) ultrasonic dispersion (260–270 J mL<sup>-1</sup>). The ultrasonic equipment was calibrated using the calorimetric method according to Roscoe et al. (2000). The fraction with a density above 1.7 g cm<sup>-3</sup> constituted the *heavy fraction* – HF.

Organic Carbon (OC) was analysed for all the samples and fractions in an Interscience Elemental Analyser EA1180. <sup>13</sup>C abundance was determined after the conversion of total C to CO<sub>2</sub>, purified by CuO and Ag, in a VG/SIRA 9 Mass Spectrometer at the Centre for Isotope Research at the University of Groningen, Netherlands. Results were expressed as δ<sup>13</sup>C (‰).

For the estimation of carbon derived from each vegetation, we used a mass balance equation (1), according to Balesdent & Mariotti (1996):

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

where C<sub>T</sub> is the total soil organic carbon, C<sub>C3</sub> is the soil organic carbon derived from C3 vegetation (cerrado), C<sub>C4</sub> is the soil organic carbon derived from C4 vegetation (pasture), δ<sup>13</sup>C<sub>T</sub> is the δ<sup>13</sup>C of total soil organic carbon, δ<sup>13</sup>C<sub>C3</sub> is the δ<sup>13</sup>C of soil organic carbon derived from C3 vegetation (cerrado), and δ<sup>13</sup>C<sub>C4</sub> is the δ<sup>13</sup>C of soil organic carbon derived from C4 vegetation (pasture).

The reference values for C<sub>3</sub>-derived carbon were those observed for the cerrado plot in each fraction. The reference δ<sup>13</sup>C value for the C<sub>4</sub>-derived carbon (C from pasture) was calculated for each organic C fraction using the δ<sup>13</sup>C of the pasture litter (-13.52 ± 0.07‰) and the same δ<sup>13</sup>C proportional discrimination (D) during decomposition as observed for the C<sub>3</sub> reference (Roscoe et al., 2001) by equation (2):

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

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

The Nuclear Magnetic Resonance of the  $^{13}\text{C}$ , with Cross Polarisation and Magic Angle Spinning ( $^{13}\text{C}$ -CPMAS-NMR) was performed at the Wageningen NMR Centre in a Bruker AMX 300 spectrometer operating at a  $^1\text{H}$  frequency of 300 MHz. The MAS probe is a 7 mm o.d. double bearing zirconia spinners with Kei F cap. The samples were spun at 5 kHz. In order to improve the spectra quantification, the optimal contact time for each sample was obtained using variable contact time (VCT) pulse sequence as described by Conte et al. (1997) and Van Lagen & de Jager (2004). Cross polarization was achieved using contact times ranging from 0.1 to 7 ms. The acquisition time was 33 ms, and fully relaxed spectra were obtained by a recycle time of 1 s. An exponential function using a line broadening of 50 Hz was applied prior to Fourier transformation. By custom, NMR spectra is given in parts per million (ppm) of chemical shift, which is the difference between the resonance frequency of the proton being observed and that of tetramethylsilane (TMS). Chemical shift (in ppm) is obtained by dividing the shift downfield from TMS (in Hz) by the total spectrometer frequency (in MHz). As for the semi-quantitative interpretation, the spectra were subdivided into: alkyl C (0–46 ppm), O-alkyl C (46–110 ppm), aromatic C (110–160 ppm), and carbonylic C (160–210 ppm). Based on the semi-quantification, three indices were calculated: *aromaticity* (aromatic C divided by alkyl + O-alkyl + carbonylic C); *hydrophobicity* (aromatic + alkyl C divided by carbonylic + O-alkyl C); and the ratio *alkyl/O-alkyl*.

Qualitative interpretation of the spectra was performed based on publications by Preston et al. (1989), Wershaw et al. (1996), Kögel-Knabner (1997), Mahieu et al. (1999), and Nierop et al. (1999). The spectra was subdivided into: alkyl C (0–46 ppm), O-alkyl C (46–110 ppm), aryl C (110–140 ppm), O-aryl C (140–160 ppm) and aldehyde/ketone (160–210 ppm).

## RESULTS AND DISCUSSION

### Carbon origin and distribution

The total C content in the upper horizon of the pasture was about half of that found in cerrado (Table 1), which may be explained by the difference in depth of the upper horizon. For the density fractions (Table 2), the largest difference was observed in the F-LF, followed by the HF. The O-LF represents only a small portion (~ 1 %) of total C in both cases, which is in agreement with data reported by Golchin et al. (1995) and Freixo et al. (2002a,b).

The difference in depth does not allow comparisons of C distribution among the fractions between pasture and cerrado. However, in this study, cerrado was taken only as a reference to  $\delta^{13}\text{C}$ , which was used in replacement calculations (Equation 1).

Due to the introduction of C4 grass material,  $\delta^{13}\text{C}$  values were always higher in the fractions from pasture than in those from cerrado (Table 2) (Balesdent & Mariotti, 1996). Compared to the light fractions, a marked increase in  $\delta^{13}\text{C}$  values was observed in the HF (Table 2), which is attributed to isotopic discrimination during decomposition (Balesdent & Mariotti, 1996).

Replacements of C from cerrado by C from pasture, 23 years after conversion, were much smaller for the O-LF than for the F-LF (Table 2). Golchin et al. (1997) reported the same behaviour for Mollisols and Alfisols. The present soil, however, shows less replacement in the Occluded-LF than in the HF, which has not been reported for other soils (Roscoe et al., 2001). This suggests that the occluded material is more effectively protected from influx of fresh organic material than the bulk material associated with the mineral matrix. This may happen because the material bound to mineral particles is on the surface of the granular structure and relatively accessible to microbiota. Baldock et al. (1992) postulated that some easily decomposable

**Table 1. Properties of the topsoils of a Typic Haplustox (Latossolo Vermelho distrófico típico under cerrado and pasture**

Site	Depth	Clay	Organic C	Total N	C:N Ratio	Clay mineralogy <sup>(1)</sup>	
						Ka	Gb
	cm		g kg <sup>-1</sup>				
Cerrado 1	0–3	870	64.7	5.9	11	++++	+
Cerrado 2	0–2	870	61.4	6.1	10	++++	+
Pasture 1	0–8	880	32.3	2.6	12	++++	+
Pasture 2	0–10	840	34.9	2.4	15	++++	+

<sup>(1)</sup> Ka – kaolinite and Gb – gibbsite.



**Table 2. Distribution of organic C and  $\delta^{13}\text{C}$  in the different density fractions: free light fraction (F-LF), occluded light fraction (O-LF), and heavy fraction (HF). The bottom row show the replacement of C (range and mean) from the “cerrado” vegetation (C3) by the C from pasture (C4), after 23 years of pasture**

Site	F-LF		O-LF		HF	
	OC	$\delta^{13}\text{C}$	OC	$\delta^{13}\text{C}$	OC	$\delta^{13}\text{C}$
	g kg <sup>-1</sup>		g kg <sup>-1</sup>		g kg <sup>-1</sup>	
Cerrado 1	24.8	-27.1	0.6	-28.1	39.1	-25.1
Cerrado 2	20.9	-26.6	1.0	-27.4	39.3	-24.7
Pasture 1	2.2	-21.9	0.3	-25.1	27.8	-20.7
Pasture 2	3.6	-19.0	0.8	-24.9	29.3	-19.1
Replacement (%)	41-61 (51)		19 (19)		24-44 (34)	

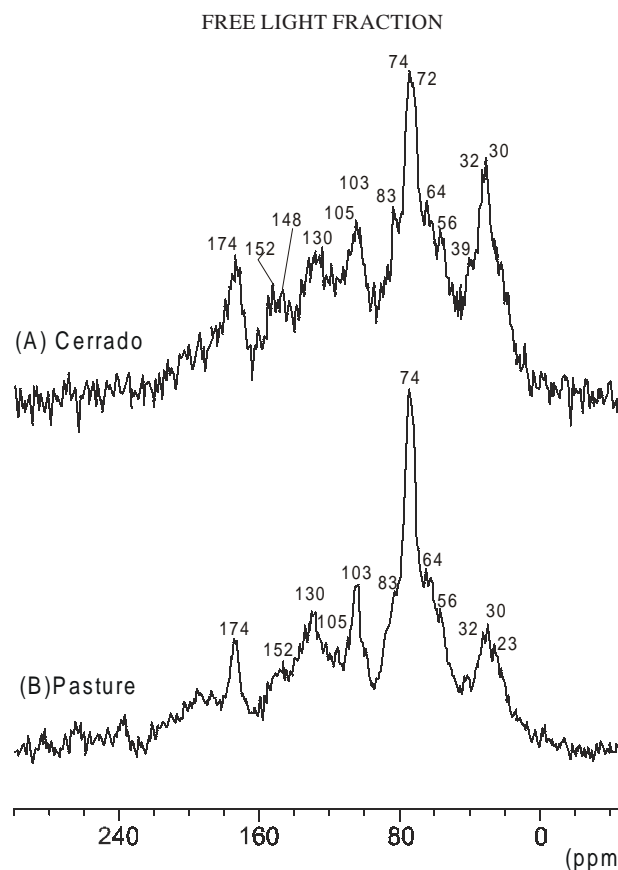
material accumulates in the clay-size fraction by adsorption. This material is weakly retained by soil particles and available for the microbiota, which would explain the lesser accessibility of the O-LF, compared to the HF.

### Qualitative interpretation of NMR spectra

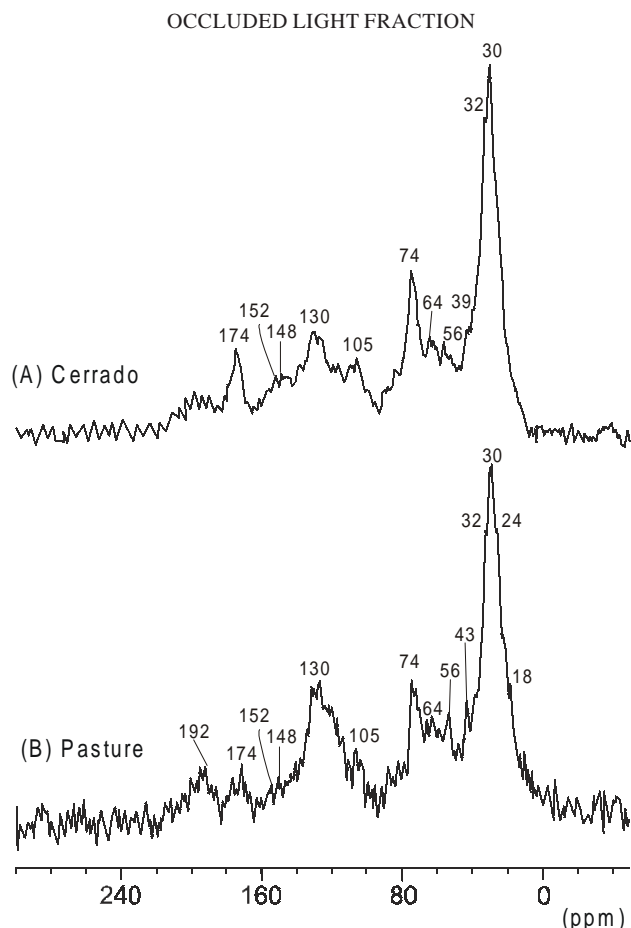
In the alkyl region (0–46 ppm), the F-LF and O-LF fractions of both cerrado and pasture had a main NMR signal around 30 ppm (Figures 1 and 2). Less clear signals were observed at 18, 23, 24, 39, and 43 ppm. The signal at 30 ppm is attributed to methylene groups in long chain aliphatic compounds (Mahieu et al., 1999; Nierop et al., 1999). Among them, Nierop et al. (1999) emphasise the importance of the hydrolysable polymers cutin and suberin, and the non-hydrolysable cutan and suberan. These polymers are very recalcitrant and may show relative accumulation upon decomposition of the bulk OM (Preston et al., 1989; Nierop et al., 1999). That signal was more intense in the O-LF than in the F-LF, suggesting a higher degree of decomposition for the occluded fraction.

In the O-alkyl region (46–110 ppm), the most intensive signal was around 74 ppm (Figures 1 and 2). Other important peaks appeared at 56, 64, 72, 103, and 105 ppm. According to Kögel-Knabner (1997), the O-alkyl range can be divided into three groups. Signals between 45 and 60 ppm are attributed to methoxyl groups, to C<sub>6</sub> of carbohydrates and sugars, and to C<sub>a</sub> of most amino-acids. The range from 60 to 90 ppm includes signals of carbohydrate-derived structures (C<sub>2</sub> and C<sub>3</sub>) in hexoses, of C<sub>a</sub> of some amino-acids, and of high alcohols. The 90–110 ppm range includes signals from anomeric C of carbohydrates, and C<sub>2</sub> and C<sub>6</sub> of syringyl units of lignin. About the same ranges were reported by Wershaw et al. (1996), who also emphasised the difference between the 62–80 ppm and the 95–105 ppm ranges. While the first one is

attributed to a variety of aliphatic alcohols, the second represents only anomeric C from carbohydrate structures. Based on those assignments, we attributed the chemical shift at 56 ppm to lignin-derived methoxyl-C; the peaks at 64, 72, and 74 were attributed to carbohydrate-



**Figure 1.  $^{13}\text{C}$ -CPMAS-NMR of the free light fraction (F-LF) of SOM from samples in the (A) cerrado and (B) pasture plots.**



**Figure 2.**  $^{13}\text{C}$ -CPMAS-NMR of the occluded light fraction (O-LF) of SOM from samples in the (A) cerrado and (B) pasture plots.

derived structures ( $\text{C}_2$  and  $\text{C}_3$ ) in hexoses,  $\text{C}_\alpha$  of some amino-acids, and high alcohols; and peaks at 103 and 105 ppm were assigned to anomeric C of carbohydrates. The 105 ppm peak may also be due to units of lignin or tannins, which usually appears at 106 ppm (Nierop et al., 1999). The intensity of carbohydrate derived peaks in this region was lower in O-LF than in F-LF, suggesting a higher degree of decomposition in the former.

The main peaks observed in the aryl region (110–140 ppm) were around 130 ppm. The 130 ppm chemical shift is typical of C-substituted aromatic carbon, especially from lignin (Wershaw et al. 1996; Kögel-Knabner, 1997; Nierop et al., 1999) and from charred material, as black carbon (Skjemstad et al., 1996, 1999). In the O-aryl region (140–160 ppm), the signal at 148 ppm can be attributed to O-substituted (OH and OMe groups) aromatic carbons of guaiacol and that at 151–153 ppm may represent aromatic carbons connected to methoxy groups in syringol units (Nierop et al., 1999). Those peaks were more clearly separated in the Cerrado than in the grassland samples (Figure 1).

The carbonylic region (carbonyl + aldehyde + ketone) was dominated by the resonance at 174 ppm (Figures 1 and 2). This peak is assigned to carbonyl groups of aliphatic acids, benzene-carboxylic acids, esters, and amides (Wershaw et al., 1996; Mahieu et al., 1999).

Evidence of lignin transformation was obtained by comparing the peaks at 56, 130, 148, and 152. The 130 ppm peak was an important signal in all spectra (Figures 1 and 2). However, the peak at 130 ppm has the interference of black carbon aromatic units, which appear in the same region (Skjemstad et al., 1996, 1999) and can not be used as diagnostic for lignin alone. The 148 ppm and 152 ppm peaks are more specific for lignin compounds. The peaks were clearer in cerrado samples (Figure 1) and decreased in intensity from F-LF (Figure 1a) to O-FL (Figure 1b), indicating demethoxylation in the latter. The observed chemical shift at 56 ppm is unambiguously attributed to methoxyl C in lignin (Kögel-Knabner, 1997; Nierop et al., 1999). This signal appeared as a clear peak in the spectrum of cerrado F-LF (Figure 1a) and as a shoulder in pasture F-LF (Figure 1b). However, the signal was less evident in the occluded fractions (Figure 2). The trends observed for the 56, 148, and 152 ppm peaks suggested more oxidised lignin monomer or losses of this compound in the O-LF (Nierop et al., 1999).

### Semi-quantitative interpretation of NMR spectra

Quantification of  $^{13}\text{C}$ -CPMAS-NMR spectra is usually problematic since cross polarisation and signal decay do not follow the same temporary pattern for all carbon groups. Using variable contact times (VCT) allows estimating the maximum signal intensity of each group (Conte et al., 1997; Van Lagen & de Jager, 2004). Therefore, VCT experiments were used to quantify CPMAS signals. Nevertheless, some other limitations of the method do not allow an absolute quantification of the groups, such as the invisibility of quaternary C in aromatic structures (Skjemstad et al., 1996, 1999). For this reason, the method was considered a semi-quantitative approach.

In general, there were only small differences between the  $^{13}\text{C}$ -CPMAS-NMR spectra of the same fractions in pasture and cerrado (Table 3). F-LF in cerrado seems to be more oxidised than in pasture, as evidenced by a trend of higher values of alkyl C and lower values of O-alkyl C and carbonylic C than in pasture. This is also visible in the hydrophobicity and the Alkyl/O-alkyl ratios. The F-LF in cerrado is apparently more hydrophobic and the aliphatic chains are more decomposed than in pasture. This difference could be attributed to a higher degree of decomposition of the fraction in cerrado when compared to pasture. It is important to notice that the sampled layer in pasture (0–10 cm) is deeper than

in cerrado (0-3 cm), which would imply more recalcitrant material in pasture, since the degree of decomposition tends to increase with depth. Part of the difference, however, could also be attributed to contrasting composition of litter material derived from the two vegetations. While grasses dominate the pasture, the cerrado *sensu-stricto* is a woody vegetation (Roscoe et al., 2001). The latter can provide more lignin-rich material and also larger amounts of long chain aliphatic compounds (e.g. suberin and cutin) than the grass material. The lignin effect is illustrated by the presence of the 148 ppm and 152 ppm peaks in cerrado and their virtual absence in pasture. It is also interesting to notice that SOM input, as well as the process of decomposition, is notably different in cerrado and pasture. In cerrado, most C input happens on the soil surface, via above ground litter deposition. In pasture, a large part of SOM originates from root deposition, and, in cerrado, large part of the decomposition takes place in the litter layer, before entering the soil. In pasture, as belowground litter deposition can be particularly intense, more easily decomposable material will be found in the light fractions.

For both plots, there is evidence for a higher degree of decomposition in O-LF than in F-LF. O-alkyl groups strongly decreased, and alkyl groups increased in the occluded light fraction, which suggests a loss of more easily decomposable compounds (e.g. carbohydrates) and a relative accumulation of long chain aliphatic materials (Golchin et al., 1997; Christensen, 2001). Christensen (2001) postulated that aromatic compounds can also accumulate in the first stages of litter decomposition. A small increase in aromatics was observed for pasture (Table 3), but not for cerrado. The disagreement with Christensen (2001) might be attributed to the low sensitivity of the  $^{13}\text{C}$ -CPMAS-NMR technique to quaternary C in condensed aromatic structures (Conte et al., 1997; Skjemstad et al., 1996, 1999).

### Aggregate dynamics and implications

The isotopic study showed that occluded light organic material (O-LF) inside the granular structure of the studied Oxisol had a decreased accessibility (Table 2). At the same time, the O-LF showed characteristics of organic materials in advanced stage of decomposition. These results suggest that, during occlusion, decomposition continues, thereby causing selective preservation of recalcitrant compounds. As there is no addition of fresh organic matter, the material inside aggregates remain rather recalcitrant. This is in agreement with the theories of aggregation of oxide-rich soils, either the physical-chemical (Resende et al., 1997) or the biogenic (Jungerius et al., 1999). The results also support the idea that these aggregates have a relatively slow turnover, since their probable processes of formation would demand quite long periods of time to produce the present abundance of aggregates in the sampled Oxisols.

It was observed that only around 1 % of the total carbon present in the soil was in the O-LF (Table 2), which is in agreement with the literature (Roscoe & Machado, 2002). The results suggest that the material occluded inside the typical granular structure of Oxisols is not an efficient or manageable pool for storing carbon to mitigate global warming. The fraction apparently retains carbon over a long-term period, but the processes of occlusion in those typical structures can barely be affected by human induced changes.

Roscoe & Machado (2002) suggested that these granular structures would be the building blocks of larger blocky structures, especially in the upper horizons of Oxisols. In that case, another level of aggregation is added and light fractions may be occluded at various hierarchical levels of structure. Further studies are needed to clarify the possible importance of higher levels of organisation of soil particles on carbon dynamics in Oxisols.

**Table 3. Proportions of the different groups (%) present in the  $^{13}\text{C}$ -CPMAS-NMR spectra, calculated after VCT experiments (Conte et al., 1997), and the derived indices *aromaticity*, *hydrophobicity*, and *alkyl/O-alkyl* ratio, in the different density fractions: free light fraction (F-LF), occluded light fraction (O-LF), and heavy fraction (HF)**

Site	Fraction	Alkyl (0-46 ppm)	O-alkyl (46-110 ppm)	Aromatic (110-160 ppm)	Carbonylic (160-210 ppm)	Aroma- ticity	Hydro- phobicity	Alkyl/O-alkyl
%								
Cerrado	F-LF	28.5	43.6	19.5	8.5	0.24	0.92	0.65
	O-LF	44.9	31.0	16.3	7.8	0.19	1.58	1.45
Pasture	F-LF	23.9	49.4	17.0	9.8	0.20	0.69	0.48
	O-LF	43.8	32.0	16.7	7.5	0.20	1.54	1.37

## CONCLUSIONS

1. Our results confirm that processes of soil organic matter occlusion in the typical "very fine strong granular" structure from the studied oxide-rich soil led to an intense transformation, selectively accumulating organic material chemically (high amounts of recalcitrant compounds) and dynamically (low turnover) stable.

2. The small amount of organic material stored as occluded light fraction, as well as its stability, suggests that this is not an important or manageable sink for sequestration of atmospheric CO<sub>2</sub>.

## ACKNOWLEDGEMENTS

The authors acknowledge the permission of the Wageningen NMR Centre for the use of the Bruker-AMX300 spectrometer. The authors also acknowledge Dr. Carlos Alberto Vasconcelos from *Embrapa Maize and Sorghum* for helping with field work and discussions. This study was supported by the Brazilian Financial Agency CAPES.

## LITERATURE CITED

- BALESDENT, J. & MARIOTTI, A. Measurement of soil organic matter turnover using <sup>13</sup>C natural abundance. In: BOUTTON, T.W. & YAMASAKI S., eds. Mass spectrometry of soil. New York, Marcel Dekker, 1996. p.47-82.
- BALDOCK, J.A.; OADES, J.M.; WATERS, A.G.; PENG, X.; VASSALLO, A.M. & WILSON, M.A. Aspects of the chemical structure of soil organic materials as revealed by solid-state <sup>13</sup>C NMR spectroscopy. *Biogeochem.*, 16:1-42, 1992.
- CHRISTENSEN, B. Physical fractionation of soil and structural and functional complexity in organic matter turnover. *Eur. J. Soil Sci.*, 52:345-353, 2001.
- CONTE, P.; PICCOLO, A.; van LAGEN, B.; BUURMAN, P. & DE JAGER, P.A. Qualitative aspects of solid-state <sup>13</sup>C-NMR spectra of humic substances from soils of volcanic systems. *Geoderma*, 80:327-338, 1997.
- FREIXO, A.A.; CANELLAS, L.P. & MACHADO, P.L.O.A. Propriedades espectrais da matéria orgânica leve-livre e intra-agregados de dois Latossolos sob plantio direto e preparo convencional. *R. Bras. Ci. Solo*, 26:445-453, 2002a.
- FREIXO, A.A.; MACHADO, P.L.O.A.; GUIMARÃES, C.M.; SILVA, C.A. & FADIGAS, F.S. Estoques de carbono e nitrogênio e distribuição de frações orgânicas de Latossolo do Cerrado sob diferentes sistemas de cultivo. *R. Bras. Ci. Solo*, 26:425-434, 2002b.
- GOLCHIN, A.; BALDOCK, J.A. & OADES, J.M. A model linking organic matter decomposition, chemistry, and aggregate dynamics. In: LAL, R.; KIMBLE, J.M.; FOLLETT, R.F. & STEWART, B.A., eds. Soil processes and the carbon cycle. Boca Raton, CRC Press, 1997. p.245-266. (Advances in Soil Science)
- GOLCHIN, A.; OADES, J.M.; SKJEMSTAD, J.O. & CLARKE, P. Structural and dynamic properties of soil organic matter as reflected by <sup>13</sup>C natural abundance, pyrolysis mass spectrometry and solid-state <sup>13</sup>C NMR spectroscopy in density fractions of an Oxisol under forest and pasture. *Aust. J. Soil Res.*, 33:59-76, 1995.
- INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE – IPCC. Land use, land-use change, and forestry: special report of the IPCC. Cambridge, Cambridge University Press, 2000. 375p.
- JUNGERIUS, P.D.; van den ANCKER, J.A.M. & MUCHER, H.J. The contribution of termites to the microgranular structure of soils on the Uasin Gishu Plateau, Kenya. *Catena*, 34:349-363, 1999.
- KÖGEL-KNABNER, I. <sup>13</sup>C and <sup>15</sup>N NMR spectroscopy as a tool in soil organic matter studies. *Geoderma*, 80:243-270, 1997.
- MAHIEU, N.; POWLSON, D.S. & RANDALL, E.W. Statistical analysis of published carbon-13 CPMAS NMR spectra of soil organic matter. *Soil Sci. Soc. Am. J.*, 63:307-319, 1999.
- NIEROP, K.G.J.; BUURMAN, P. & LEEUW, J.W. Effect of vegetation on chemical composition of H horizons in incipient podzols as characterized by <sup>13</sup>C NMR and pyrolysis-GC/MS. *Geoderma*, 90:111-129, 1999.
- PRESTON, C.M.; AXELSON, D.E.; LÉVESQUE, M.; MATHUR, S.P.; DINEL, H. & DUDLEY, R.L. Carbon-13 NMR and chemical characterization of particle-size separates of peat differing in degree of decomposition. *Org. Geochem.*, 14:393-403, 1989.
- RESENDE, M.; CURI, N.; REZENDE, S.B. & CORRÊA, G.F. *Pedologia: base para distinção de ambientes*. 2.ed. Viçosa, Universidade Federal de Viçosa, NEPUT, 1997. 367p.
- ROSCOE, R.; BUURMAN, P. & VELTHORST, E.J. Disruption of soil aggregate by different amounts of ultrasonic energy in SOM fractionation of a clay Latosol: carbon, nitrogen, and d<sup>13</sup>C distribution in particle-size fractions. *Eur. J. Soil Sci.*, 51:445-454, 2000.
- ROSCOE, R.; BUURMAN, P.; VELTHORST, E.J. & VASCONCELLOS, C.A. Soil organic matter dynamics in density and particle-size fractions as revealed by the <sup>13</sup>C/<sup>12</sup>C isotopic ratio in a Cerrado's Oxisol. *Geoderma*, 104:185-202, 2001.
- ROSCOE, R. & MACHADO, P.L.O.A. Fracionamento físico do solo em estudos da matéria orgânica. Dourados, Embrapa Agropecuária Oeste, 2002. 86p.
- SKJEMSTAD, J.O.; CLARKE, P.; TAYLOR, J.A.; OADES, J.M. & McCLURE, S.G. The chemistry and nature of protected carbon in soil. *Aust. J. Soil Res.*, 34:251-271, 1996.
- SKJEMSTAD, J.O.; TAYLOR, J.A. & SMERNIK, R.J. Estimation of charcoal (char) in soils. *Comm. Soil Sci. Plant Anal.*, 30:2283-2298, 1999.
- van LAGEN, B. & DE JAGER, P.A. Improving quantification of <sup>13</sup>C-CPMAS-NMR by steady state and well-defined data processing in variable contact time experiments. *Fres. Environ. Bull.*, 2004. (In press)
- WERSHAW, R.; LEENHEER, J.A.; KENNEDY, K.R. & NOYES, T.I. Use of <sup>13</sup>C NMR and FTIR for elucidation of degradation pathways during natural litter decomposition and composting: I. Early stage leaf degradation. *Soil Sci.*, 161:667-679, 1996.