ALINE DE ALMEIDA VASCONCELOS

EMISSÕES DE CO_2, PARTICIONAMENTO DA RESPIRAÇÃO E QUALIDADE DA MATÉRIA ORGÂNICA EM SOLOS SOB CULTIVO DE EUCALIPTO NO CERRADO

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Solos e Nutrição de Plantas, para obtenção do título de *Doctor Scientiae*.

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Ivo Ribeiro da Silva
(Orientador)

Aos meus pais, Gildete de Almeida Vasconcelos e Audênio Barros de Vasconcelos.

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Com amor, dedico.

"Rir muito e com frequência; ganhar o respeito de pessoas inteligentes e o afeto das crianças; merecer a consideração de críticos honestos e suportar a traição de falsos amigos; apreciar a beleza, encontrar o melhor nos outros; deixar o mundo um pouco melhor, seja por uma saudável criança, um canteiro de jardim ou uma redimia condição social; saber que ao menos uma vida respirou mais fácil porque você viveu. Isso é ter tido sucesso"

Ralph Waldo Emerson

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BIOGRAFIA

Aline de Almeida Vasconcelos, filha de Audênio Barros de Vasconcelos e Gildete de Almeida Vasconcelos, nasceu em 05 de outubro de 1983 no município de Aracaju, Sergipe.

Em 2002, concluiu o ensino médio no Colégio de Ciências Pura e Aplicada (CCPA) em Aracaju, SE.

Graduou-se em Engenharia agronômica pela Universidade Federal de Viçosa (2003-2008) e em julho de 2010 obteve o título de Mestre em Solos e Nutrição de Plantas pela mesma instituição. Em agosto de 2010 ingressou no doutorado no Programa de Pós-graduação em Solos e Nutrição - UFV; participou do programa Doutorado Sanduiche na Iowa State University -ISU por nove meses, e submeteu-se à defesa de tese em agosto de 2014.

SUMÁRIO

RESUMO

VASCONCELOS, Aline de Almeida, D.Sc., Universidade Federal de Viçosa, agosto de 2014. **Emissões de CO_2, particionamento da respiração e qualidade da matéria orgânica em solos sob cultivo de eucalipto no Cerrado**. Orientador: Ivo Ribeiro da Silva. Coorientador: Emanuelle Mercês Barros Soares.

O Cerrado brasileiro contribuí com 20-30 % da produção primária global, e a conversão de áreas nativas com o corte e queima de vegetação seguida do cultivo do solo resulta em mudanças na dinâmica da matéria orgânica do solo (MOS), com alterações nas emissões dos gases causadores de efeito estufa (CO_2) da biosfera para a atmosfera e na composição da MOS. Sistemas de cultivo conservacionistas que privilegiam o aporte de resíduos ao solo e menor revolvimento deste podem mitigar a redução nos conteúdos de C do solo e ocasionar mudanças na qualidade da MOS causada pela substituição dos sistemas naturais. Plantações de eucalipto estão inseridas neste contexto, como potenciais sequestradoras de C da atmosfera, pela expressiva produção de biomassa vegetal em curto espaço de tempo. No entanto, pouco se sabe sobre as emissões de CO_2 por florestas plantadas de eucalipto. Desta forma, este trabalho objetivou avaliar o potencial das florestas plantadas de eucalipto na emissão de CO_2, estimar a contribuição relativa da respiração das raízes e da MOS no CO_2 emitido pelo componente solo utilizando a composição isotópica do carbono do CO_2 e determinar a variabilidade sazonal das emissões dos gases CO_2 no capitulo 1 e determinar as alterações moleculares da matéria orgânica do solo pela adição de resíduo de eucalipto por meio da técnica de Ressonância Magnética Nuclear (RMN) no Capitulo II. Para isso, no experimento do Capitulo I foram feitas determinações dos fluxos CO_2 do solo, e da sua composição isotópica em floresta adulta de eucalipto, mantendo como referência uma de cerrado nativo adjacente. Foram instalados encaixe de câmaras para monitoramento dos fluxos de CO_2 em superfície e razão isotópica do CO_2 dos diversos compartimentos *in situ.* Além de instalados tubos de coleta de ar em profundidade nas camadas 0 - 10; 10-20; 20-40; 40-60 e 60 - 100 cm, distribuídos na linha e entrelinha, todas as determinações foram realizadas por meio do parelho cavity ringdown laser spectroscopy (CRDS). A umidade e a temperatura do solo foram medidas por ocasião das coletas nas época seca e época chuvosa. O cálculo utilizado para particionamento da respiração foi o proposto por Millard et al. (2010). No experimento do Capítulo II, foram extraídos a matéria orgânica associada aos minerais (MAM) de três solos com diferenças texturais, teores de Fe e teores iniciais de carbono (C) da região de Cerrado provenientes de experimento de incubação com resíduo de plantas de eucalipto enriquecido com ^{13}C e ^{15}N para avaliação das alterações da composição molecular via ^{13}C e ^{15}N NMR multiple cross polarization (MultCP). As maiores emissões de CO_2 foram encontradas na época chuvosa, sendo que o eucalipto apresentou menores fluxos que a mata nativa na época chuvosa e não diferiu na época seca. Na condição seca, o eucalipto possui menor respiração heterotrófica, enquanto que na época chuvosa não ocorreu diferença. As concentrações de CO_2 em profundidade entre os usos diferiram nas camadas mais profundas. Em relação a qualidade da MOS por meio da ^{13}C RMN, observou-se maior predominância do grupo O-alkyl C (28%) e que a estabilização da MAM do solo parece estar mais relacionada as características mineralogia e amorficidade dos óxidos de Fe e Al do que ao teor de Fe, textura e déficit de saturação de C. A adição do resíduo vegetal reduziu a aromaticidade em 29%, principalmente pela redução dos compostos aromáticos não protonados. Observou-se por ^{15}N NMR a predominância de compostos amida e essa foi maior nos solos com maior nos solos com maiores teores iniciais de C e não houve padrão em relação as outras características do solo avaliadas.

ABSTRACT

VASCONCELOS, Aline de Almeida, D.Sc., Universidade Federal de Viçosa August, 2014. **CO_2 emissions, respiration partioning and organic matters quality in soils under eucalyptus cultivation in the Cerrado**. Adviser: Ivo Ribeiro da Silva. Co-adviser: Emanuelle Mercês Barros Soares.

The Brazilian Cerrado contributes 20-30% of global primary production and the conversion of native forests by cutting and burning vegetation followed by the cultivation of the soil results in changes in the soil organic matter dynamics (SOM), with changes greenhouse gases emissions (CO_2) from the biosphere to atmosphere and SOM composition. Conservation cultivation systems that favor the vegetal residue inputs to the soil and soil tillage reduction could mitigate the losses of C content in soil, and SOM quality modifications, caused by the natural systems substitution. Eucalyptus plantations is inserted in this context, as potential sequester of C from the atmosphere, by the significant biomass production in a short time. However, information about the CO_2 emissions and SOM compositions by planted eucalyptus forests is still incipient. Thus, firstly, this study aimed at evaluating the potential of eucalyptus planted forests in surface soil CO_2 emissions, estimating the relative contribution of root and SOM respiration in the CO_2 emitted by soil component using the isotopic composition of carbon and determining the CO_2 emissions seasonal variability. Secondly, determining the soil organic matter molecular changes by adding eucalyptus residue by Nuclear Magnetic Resonance technique (NMR). For this, in Chapter I experiment was evaluated CO_2 soil surface emissions, and its isotopic composition in adult eucalypt forest, and a native vegetation as reference. Chambers were installed for CO_2 effluxes monitoring in soil surface and CO_2 isotope ratio of different compartments in situ. Besides tubes of depth were installed into the 0-10; 10-20; 20-40; 40-60 and 60-100 cm layers to collect air, it was distributed in row and interrow, all determinations were performed using cavity ringdown spectroscopy (CRDS). The soil moisture and temperature were measured during both dry and rainy season. The calculation used to soil partitioning respiration was proposed by Millard et al. (2010). In Chapter II experiment, organic matter associated with minerals (MAOM) were extracted from three different representative tropical soil with different textural soil, Fe and Al content and initial carbon (C) located in Cerrado region. These soil were incubated with enriched eucalyptus plant residues ^{13}C and ^{15}N to assess the changes in the molecular composition by ^{13}C and ^{15}N NMR multiple cross polarization (MultCP). The greater CO$_2$ emissions were in the rainy season, and the eucalyptus had lower efflux then native forest in the rainy season and did not differ in the dry season. In dry condition, eucalyptus has a lower heterotrophic respiration, while in the rainy season there was no difference. The CO$_2$ concentrations in depth were higher in native vegetation than eucalypt in high soil moisture condition. Regarding the SOM quality by ^{13}C NMR, there was a higher prevalence of group O-alkyl C (28%) and the stabilization of MAOM, and seems to be more related to mineralogy characteristics and amorphicity of Fe and Al oxides than the Fe and Al content, texture and saturation deficit C. The addition of plant residue reduced the aromaticity by 29%, mainly by reducing aromatics not protonated. The predominant compound-N was the amide and its contribution was higher in soils with higher initial C content, but was it was not influenced by the soil characteristics evaluated.

INTRODUÇÃO GERAL

Desde o final dos anos sessenta, extensas áreas de vegetação nativa do Cerrado (savana) na região central do Brasil foram convertidas para diversos usos do solo, o que resultou em alteração na quantidade e qualidade da matéria orgânica do solo - MOS (Bernoux et al., 2008, Batlle-Bayer et al., 2010), a maior parte destas áreas foi convertida em pastagens plantadas (Rada, 2013; Lapola et al., 2013), que por sua vez, estão sendo rapidamente substituídas por culturas anuais (Lapola et al., 2013), e, mais recentemente, em plantações de eucalipto de curta rotação (ABRAF 2013).

Devido às demandas oriundas da indústria de móveis, celulose e papel, e setor de energia impulsionando a necessidade de matéria prima, o cultivo de eucalipto é uma importante atividade econômica no Brasil, e ocupa uma área de aproximadamente 7 milhões de hectares (ABRAF, 2013). Esta espécie se destaca em razão do rápido crescimento, e em solos altamente intemperizados e de baixa fertilidade, seu crescimento está diretamente correlacionado com o teor de MOS (Menezes, 2005).

Estudos nos MOS nos estados de Minas Gerais (Lima et al., 2006; 2009; Pulronik et al., 2009; Vergutz et al., 2010; Leite et al., 2010), Bahia (Silva, 2008), Espírito Santo (Pegoraro, 2007) e Rio Grande do Sul (Soares, 2009) têm atestado o potencial das florestas de eucalipto em sequestrar o CO_2 atmosférico na biomassa e na MOS. A avaliação do impacto do cultivo de eucalipto na MOS no bioma Cerrado com grande sazonalidade das condições ambientais, as quais apresentam chuvas no verão e períodos secos durante o inverno, com altas temperaturas na maior parte do ano, as quais favorecem a decomposição da MOS (Powers et al., 2009), consistem em importante cenário para estudos de MOS. Os impactos podem ser medidos além da quantificação dos estoques, pelo monitoramento do CO_2 liberado.

Quantificar fluxos de CO_2 do solo em campo pode originar informações importantes quanto a sustentabilidade a respeito de manejo e mudanças no uso do solo. No entanto, a medição apenas do fluxo de CO_2 pode não elucidar se os solos são fontes ou drenos de CO_2 atmosférico, pois tanto as fontes heterotróficas como autotróficas são contabilizadas (Hanson, et al, 2000; Kuzyakov, 2006). O particionamento das várias fontes da respiração no solo pode ser realizado usando o método de exclusão de raiz, anelamento, integração de componentes baseada na quantidade de massa de matéria seca e uso das diferenças na composição isotópica (Hanson et al 2000).

Dessas, o particionamento usando a composição isotópica tem a vantagem de não causar grandes distúrbios e consequentes superestimações, além de não ser necessário matar a planta (Hanson et al 2000; Kuzyakov et al 2006).

Em adição, estudos que avaliam as transformações da MOS qualitativamente são importantes para tentar entender melhor os processos de formação e estabilização em ambientes distintos, de forma a tentar inferir que fatores influenciam prioritariamente em sua qualidade. Certamente, as maiores respirações heterotróficas possuem relação com o tipo de C que está no solo. A composição da MOS em áreas cultivadas com eucalipto avaliada varia com os solos de distintas texturas (Zinn et al., 2002, Soares et al. 2013). Outros fatores que causam alteração da qualidade da MOS em áreas cultivadas com eucalipto dependem, entre outros, do percentual de transferência de C do resíduo para a MOS, que por sua vez, parece ser influenciada pelo teor inicial de C no solo, ou seja, o déficit de saturação, e ressaltado como um possível limitador da estocagem de C (Six. et al., 2002; Stewart et al., 2007; 2008; West and Six, 2007; Feng et al., 2013; Poirier et al., 2014; Wiesmeier et al., 2014). Por outro lado estudos recentes têm enfatizado, que a composição da fração argila parece ser importante no processo de estabilização (Vogel et al., 2014).

Assim, a presente tese é apresentada em capítulos. O primeiro é referente ao estudo de emissões sazonais de CO_2 e particionameto da respiração em solo sob povoamento de eucalipto, usando mata nativa de cerrado adjacente como referência. O segundo capítulo é um estudo da qualidade da MOS sob influência do teor inicial de C (déficit de saturação de C), teor de silte mais argila, e teores de óxidos de Al e Fe na fração da matéria orgânica associada ao mineral de solos de cerrado após incubação com e sem resíduo de eucalipto duplamente marcado com ^{13}C e ^{15}N, avaliada por meio da técnica espectroscópica de Ressonância Magnética Nuclear.

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SURFACE TOTAL CO2 FLUX IN A TROPICAL CERRADO SOIL UNDER SHORT-ROTATION EUCALYPT AND IN NATIVE VEGETATION: AUTOTROPHIC AND HETEROTROPHIC OF CONTRIBUTION TO SOIL

ABSTRACT

The CO2 flux in soils is resulted from autotrophic and heterotrophic activity contribution. Although important and useful information can be analyzed from this parameter, the measurement of just this characteristic might not elucidate whether the soil will be the source or sink for atmospheric CO_2. In this context, the objective of this study was to quantify the total CO_2 soil emissions and the partition of total CO_2 fluxes in soils under Eucalyptus plantations and under native savanna vegetation (Cerrado) evaluating the autotrophic and heterotrophic components contribution in these ecosystems. The experiment was conducted in two sites at tropical woody Savanna ecosystem (Cerradão) in Mato Grosso do Sul, Brazil and the measurements were performed in September (dry season) 2012 and January (rainy season) 2013. The C-CO_2 efflux soil respiration surface was performed by closed chamber system and C-CO_2 concentration along the soil profile by PVC access tubes. The roots were exposed and the CO_2 from root respiration was measured in situ. All CO_2 and isotopic determinations were obtained by cavity ring down spectrometer. The soil under Eucalyptus plantation had lower total (autotrophic+heterotrophic) surface CO_2 emissions compared to soil under Cerrado at the rainy season, and no differences were observed between land uses at the dry season. Higher correlation was observed between the total CO_2 soil surface emissions, compared to temperature, and these fluxes are lower at the dry season for both vegetation types. The partitioning from CO_2 total soil surface based on C isotopic composition ($\delta^{13}C$-CO_2) shows that at the drier season the soil under Cerrado had proportionally greater contribution from

heterotrophic respiration/SOM decomposition (59%) compared to the soil under Eucalypt (27%), while at the rainy season the opposite was observed. The CO_2 concentrations at different soil layers were higher at the rainy season and mostly from autotrophic (root respiration) contribution, while at the dry season, they were more derived from SOM decomposition. At the dry season, the δ13C-CO_2 values indicate an increased contribution from eucalypt root activity (respiration) on deeper soil layers, as an adaptation to the reduced water availability at the superficial layers. The Eucalypt cultivation emits less CO_2 than the native forest and CO_2 emissions is directed by soil moisture

INTRODUCTION

Since the late sixties, extensive areas of native Cerrado vegetation (savanna) in central Brazil have been converted to diverse land uses and this has altered C cycling and soil C stocks (Bernoux et al., 2008; Batlle-Bayer et al., 2010). Most of this land was converted to planted exotic pastures (Rada, 2013), which in turn are being rapidly replaced by annual crops as well as short-rotation eucalypt plantations in more recent years (ABRAF, 2013).

Studies have reported high biomass production in a short time span, large shoot and root litter inputs by short-rotation eucalypt stands (Gatto et al., 2010; Leite et al., 2010; Stape et al., 2010; Silva et al., 2012). Thus, in most instance these planted forests have the potential to recover soil C stocks (Pulrolnik et al., 2009; Maquere et al., 2008), specially in soils under degraded pasture land, with low initial soil C (Lima et al., 2006). Carbon accumulation in soil is a balance of the input from plants and losses due to microorganism activity as CO_2. The CO_2 emission rates become more relevant under tropical conditions where high temperature and soil moisture favor the SOM decomposition (Powers et al., 2009). Research carried out in the African Congo showed that in the first year after converting savanna to short-rotation eucalypt, the release of CO_2 by the soil was 662 g m^{-2} (Nouvellon et al., 2008). Organic residual biomass management in the replanted field after harvest have a strong relationship between CO_2 efflux in the seasons, and after two years cumulative CO_2 release from treatments such as removing total residues, stem wood harvest, and double slash was 1.83, 2.98 and 3.57 kg m^{-2}, respectively (Versini et al., 2013). Data regarding to CO_2 emissions in Brazilian savanna soils under eucalypt are still incipient. Soil CO_2 efflux of 1265 g m^{-2} $year^{-1}$ at *Eucalyptus grandis* plantation located in a site at an Atlantic forest and savanna transitional biome in Southeastern Brazil has been reposted (Campoe et al. 2012).

Despite important information can be depicted from soil CO_2 fluxes, measurement of only total CO_2 efflux from soil cannot elucidate whether soils are a source or sink for atmospheric CO_2 because both autotrophic and heterotrophic sources are accounted (Hanson, et al., 2000; Kuzyakov, 2006). In a given site CO_2 measured at soil surface originated from several sources can be grouped in order to refine total CO_2 efflux from the soil, including: root-derived CO_2, SOM-derived CO_2, rhizosphere respiration, heterotrophic microbial respiration (respiration by heterotrophs), and respiration by autotrophs (Kuzyakov et al 2006). The partitioning of these several sources, though, can be achieved applying root exclusion, component integration and isotopic approached (Hanson et al 2000).

^{13}C natural abundance techniques have an edge over other techniques because of the need of low to no soil disturbance (Hanson et al., 2000; Kuzyakov et al., 2006). Millard et al. (2010) distinguished autotrophic and heterotrophic soil respiration in a forest soil by exploring small differences in isotopic values between root-derived CO_2 and SOM-derived $CO_{2.}$ The authors found that 61% of total respiration in Kanuka forest (*Kunzea ericoides*) was from heterotrophic respiration, however, they could not separate litter contribution from root, because those components showed similar isotopic composition. Normally, CO_2 fluxes decrease under dry conditions (Davidson, el al., 2000; Wang and Fang, 2009), with lowered activity of plants (Flanagan et al., 2002) and soil microorganisms (Austin et al., 2004; Carbone et al., 2011). Root and microbial respiration sources respond differently in timing and magnitude to both seasonal and episodic soil moisture (Carbone et al., 2011).

Fest et al. (2009) showed greater temporal variability relation between CO_2 soil emissions (325 mg CO_2 m^{-2} h^{-1}) and soil temperature and, litter temperature, and soil moisture through multiple linear regression after first rainfall event, in eucalypt successional mixed-age forest with and without presence of *Acacia dealbata*, localized in Australia.

Another factor that may influence the fluxes of CO_2 is the amount and type of plant material input, which in turn can affect microbial activity (Barreto et al., 2008). Differences are also observed among plant growing season when the root contribution to total soil respiration is commonly higher than in dormant season (Hanson et al., 2000). Those contributions were investigated using an stable isotope approach (Amundson at al. 1998; Hanson et al., 2000; Millard et al., 2007; Millard et al. 2010).

In addition to understanding C cycling under natural conditions, it is important to evaluate CO_2 fluxes and their source partitioning in order to develop and validate more sustainable land management that promote the increase of SOM. However, studies about partitioning surface soil CO_2 into components and their magnitude in eucalypt plantation under tropical condition is still incipient in the Cerrado biome in Brazil. Thus, the objective with this work was to quantify total soil CO_2 emissions and the partition total CO_2 fluxes in autotrophic and heterotrophic components in a planted eucalypt stand and a reference soil under native savanna vegetation. Evaluations were carried out for both dry and rainy seasons.

MATERIAL AND METHODS

Two sites were selected for sampling in a tropical woody savanna ecosystem, nationally known as Cerrado with predominant species as *Tapirira guianensis* Aubl.; *Cupania vernalis* Camb.; *Hymenea stigonocarpa* Mart. ex Hayne; *Dipteryx alata* Vog. (lat. 21°00’14”S; long. 52°21’53” W, elevation 130 m) in Mato Grosso do Sul, Brazil. Sites consisted in a first rotation six-years-old clonal hybrid eucalypt (*E. grandis* x *E. urophylla*) plantation, and an adjacent native savanna vegetation area. Eucalypt seedlings were planted in a 3 x 3 m spacing after the grass had been killed with non-selective herbicide (glyphosate). Lime and fertilization were performed according to soil analysis and expected stemwood yield. Average eucalypt stemwood growth rate for the period of the experiment was 38 m^3 ha^{-1} $year^{-1}$. The original savanna vegetation was clear-cut and used for pasturing (*Uroclhoa spp*) 30 years before eucalypt had been cultivated. The soil is classified as a rhodic, clay-loam Oxisol (US Soil Taxonomy) and the relief is gently with slopes averaging less than 2%. According to the Koppen system, the climate is classified as Aw (hot and rainy summers, from October to March, and warm and dry winters, from April to September (Figure 1.)

An eucalypt stand of approximately 150 ha was chosen in the region based on the need of a homogeneous adult stand that was planted in C-C_4 richer SOM in order to allow the use of variations in ^{13}C natural abundance to partition CO_2 sources. Besides, an adjacent stand with native savanna vegetation area were used as reference. Four replicates 10 x 30 m plots were delimited randomly in each area. Soil field capacity was determined at 0.147 m^3 m^{-3}.

In each of plots rectangular metal chambers bases (0.5 x 1.0 m) were inserted up to 5 cm into the soil in the following situations: row and inter-row position (only in the eucalypt stand). Evaluations started in September 2012, at the end of the dry season.

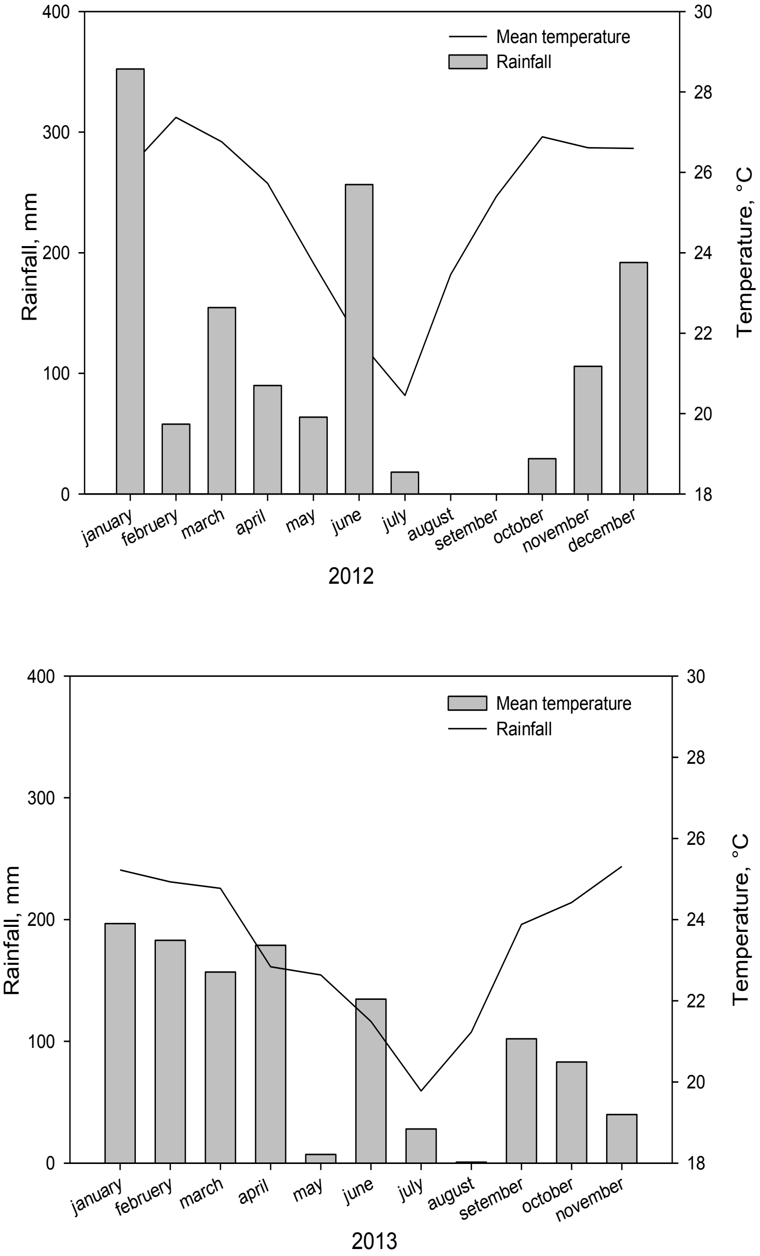

Figure 1. Monthly mean temperature and rainfall during the evaluation period.

The measurements were performed in September (dry season) 2012 and in January (rainy season) 2013. Rainfall in those months were 18.00 and 154.43 mm, respectively. For determining soil surface respiration we used a static closed chamber system (Davidson et al., 2002) equipped with a polybutyl septum to allow air samples collection with a syringe equipped with a three-way valve along a time series (0, 15, 30 and 60 min) after chamber closure. Following each measurement, the flux was calculated using a fit of linear regression between time and CO_2 concentration. The air inside the chamber during the accumulation and collection period was constantly homogenized by four slow speed coolers. Concurrently, soil temperature and moisture to a depth of 5 cm and air temperature were measured with a portable system (EC-5; Decagon Devices Inc., Pullman, WA).

To measure root respired CO_2 *in situ*, a soil trench was hand excavated to expose the roots. A medium-sized root (about 5 mm diameter) was inserted in a PVC chamber (4 cm in diameter and 40 cm length and 0.51 cm^3 volume) without dissecting it from the root system and then tightly sealed. Root respired $C\text{-}CO_2$ was allowed to accumulate for 1 hour and then its isotopic composition ($\delta\ ^{13}C$) was determined using a CRDS system. Biomass of roots were determined for row and inter-row positions along the soil profile through manual excavation of trenches up to 2 m deep, in both dry and rainy seasons.

After having surface CO_2, the isotopic composition of $C\text{-}CO_2$ in the soil was measured after removing all roots by hand, also soil samples were collected beside each measurement chamber in the experimental plot. All fine and medium-sized roots were removed by hand, and then incubated the root-free soil under controlled conditions (25 ± 1^{o} C) to determinate isotopic composition of $C\text{-}CO_2$ released.

The total $C\text{-}CO_2$ and isotopic composition were determined in a CRDS (G2131-i Picarro, Sunnyvale, CA) and solid (soil, roots, litter) samples were analyzed in an isotope ratio mass spectrometer (20 20 GLS, Sercon, Crewe, UK). The isotopic composition of gaseous and solid samples were expressed in delta values ($\delta^{13}C$) in parts per mill (‰), taking as reference the PDB international standard.

The heterotrophic respiration (fR_{het}) contribution to soil respiration was calculated following the isotopic approach according to Millard et al. (2010):

$$fR_{het} = 1 – [(\delta^{13}CR_{field} – d13CR_{root\ free})/ (\delta^{13}CR_{root} – \delta\ ^{13}CR_{root\ free})] \quad \text{eq. (1)}$$

Where: $\delta^{13}CR_{field}$ was $\delta^{13}C$- CO_2 soil value obtained in field; $\delta^{13}CR_{root}$ was $\delta^{13}C$- CO_2 root incubation value obtained in field; and, $\delta\ ^{13}CR_{root\ free}$ soil root free value obtained in laboratory incubation.

The obtained $\delta^{13}C$ of C-CO_2 values without $\delta^{13}C$- $\delta^{13}C$-CO_2 atmospheric interference were corrected contribution using the approach proposed by Miller & Tans (2003).

To measure C-CO_2 concentration along the soil profile PVC (polyvinyl chloride) access tubes (50 mm in diameter and 110 cm in length) have been installed in the native savanna area, and in the row and inter-row positions in the eucalypt plantation. This tubes were sectioned and partitioned in the 0-10; 10-20; 20-40; 40-60; 60-100 cm layers, with no link among them. Lateral 10 mm holes allowed the equilibration of the soil atmosphere of distinct depths with that inside the tube. The gas inside the tube at each soil layer has been sampled with permanent polyethylene capillary tubes connected straight to a cavity ring down spectrometer (CRDS; details in appendix). Tubes were installed in soil holes carefully, handed drilled to ensure tightness. The access tubes were set up one month before starting the measurements, in order to allow equilibration from the initial soil disturbance.

Mean comparisons of C-CO_2 efflux, temperature and soil moisture, heterotrophic respiration proportion, CO_2 concentration and δ^{13}C-CO_2 with soil depth between land uses and seasons were compared using F test, and their relationship was tested using simple linear correlation ($p < 0,05$). Statistical analysis were performed using the Sisvar 5.3 and Statistic 7.0 packages.

RESULTS AND DISCUSSION

Seasonal variation of total C-CO_2 effluxes at soil surface were seven times higher in the rainy season than in the dry season (Figure 2). Because of the biological nature of the processes involved in root respiration and SOM decomposition, studies have pointed out the important role of soil temperature and moisture variations on soil CO_2 emissions (Wang et al., 2008; Craine et al., 2011). In the current study the high correlations (94 and 97 %) were observed between soil total C-CO_2 flux and soil moisture, while temperature explained 89 and 80 % ($p < 0.05$) of the variation in soil CO_2 fluxes in the Cerrado and eucalyptus sites, respectively.

In the current study, the soil moisture of soil surface in the rainy season was 81 % higher than the soil in dry season (Figure 1, b). The higher positive correlations between soil moisture and total CO_2 fluxes at soil surface can be explained microbial activity, which is affected by changes in the availability of soil moisture (Orchard and Cook, 2003).

The soil average temperatures were different between seasons; however, they showed low fluctuation (4°C) (Figure 2), which made its effect less pronounced on soil C-CO_2 eflux. Soil temperature was high in rainy season (Figure 2, B), and, the rain was expected in the summer with high air temperature while in the winter there was low air temperature. There was greater soil temperature response to CO_2 efflux in temperate conditions because of the bigger air temperature amplitude during the year and consequently in soil temperature (Hashimoto et al. 2007).

The lowest total soil C-CO_2 fluxes in the rainy season were 109 and 302 mg h^{-1} m^{-2}, respectively, for the Cerrado vegetation and eucalypt plantation (Figure 2). These results could be because soil moisture under eucalypt was higher than under savanna, which may lead to a decrease of CO_2 diffusivity, as 63% of eucalyptus total porosity filled by water, while the Cerrado soil had 43% of pore space water-filled in the rainy season (Figure 2).

Contrasting to the others studies, it was found that savanna soils usually have higher moisture than adjacent eucalyptus plantations (Ngouabi, 2001), in this research, this effect was the opposite and may have contributed to reduce soil surface total CO_2 efflux at the savanna site. Temperature and moisture variations most possibly have also affected plant growth and respiration (Flanagan & Johnson, 2005), which in turn may have altered soil surface total CO_2 efflux. Total soil respiration in a transitional forest at the northwestern Brazil showed an optimum response to soil moisture with the maximum respiration rates at the 12 % soil moisture (Valentini el at., 2008). In the current study, the savanna soil moisture was similar (14 %) to that reported by Valentini et al. (2008), while soil under eucalypt plantation was 18% (0.20 and 0.26 cm^3 cm^{-3} water content).

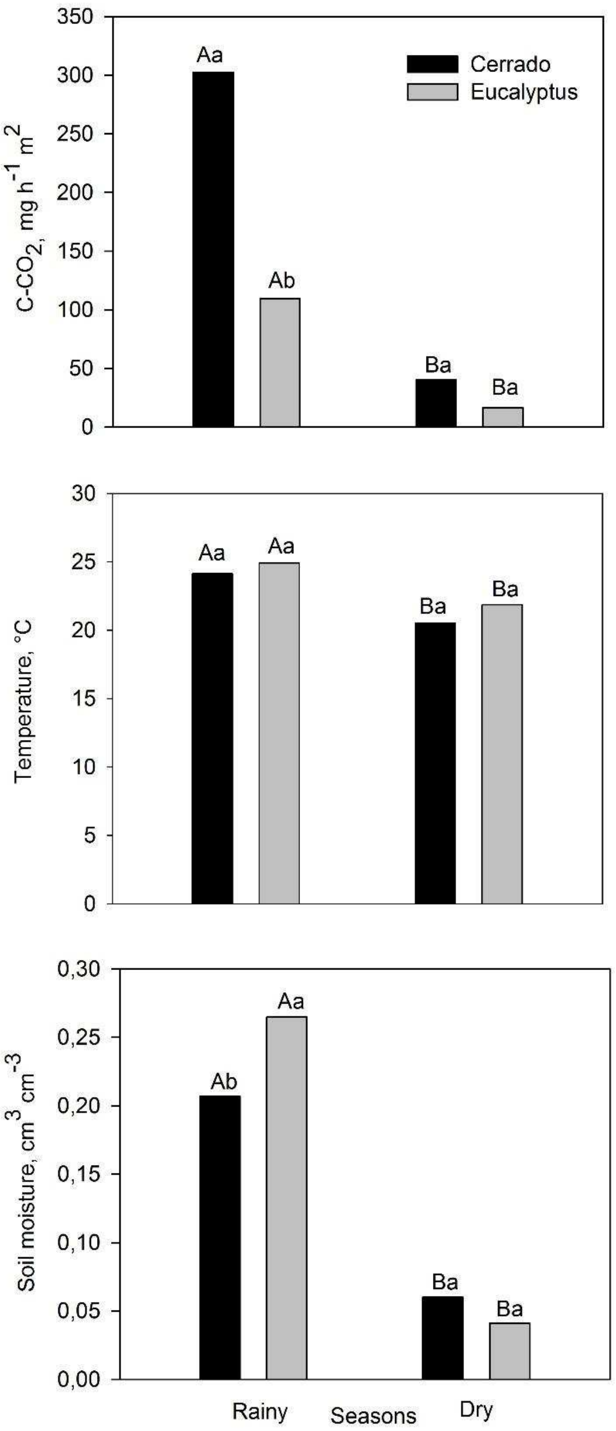

Figure 2. Soil surface total C-CO_2 efflux (mg h^{-1} m^{-2}), soil temperature (°C) and soil moisture (m^3 m^{-3}) in the native or eucalypt stand, in the rainy and dry seasons. Uppercase letters compare season (rainy and dry) within each land use (Cerrado and eucalyptus); lowercase letters compare land uses within the same season. Statistical significance determined at $F < 0.05$.

Another factor that may interfere in the soil total surface C-CO_2 efflux is the organic matter quality (lability) interacting with the soil moisture (Craine et al. 2001). These authors reported relationships between soil moisture and the temperature sensitivity of labile soil organic carbon decomposition, observing different SOM decomposition patterns at uplands and downlands. In the former, the more recalcitrant organic matter is more decomposed when there is an intermediate soil moisture, while in the later, the biochemically recalcitrant organic matter decomposition continues to increase with increasing soil moisture. These results suggest that alterations of soil moisture for some specific soils have the potential to alter the temperature sensitivity of some SOM to decomposition in different ways and this could amplify or dampen direct effects of temperature on the decompositions process.

The distinct effects of temperature and moisture on total soil respiration rates occur mainly because plants and microorganisms respond differently in the changing in the soil moisture due to respiratory enzymatic activity, substrate supply, and, or, osmolality differences (He et al., 1997; Davidson at al., 2006).

The type of vegetation and consequently the amount and quality of litter added to the environment are different and it had a significant effect on total surface soil respiration (Figure 2). Natural biomes presents higher substrate availability compared to cultivated forests (Martin et al., 2011) and it may have contributed to the greater soil total surface CO_2 efflux in the native site, especially in the rainy season. Lower concentration of polysaccharides was found in eucalypt litter compared to Cerrado' s vegetation soil (Zinn et al., 2002) and from planted pasture (Pegoraro et al., 2011)

The main contributor in the native Cerrado soil total surface CO_2 eflux was from autotrophic origin (root respiration) (Figure 3), being 6 % in the rainy season, derived from the heterotrophic microbial decomposition of SOM (18 mg h^{-1} m^{-2}), while in the soil under eucalypt it was 18% (17 mg h^{-1} m^{-2}) (Figure 3). The larger contribution of root respiration occurred during the wet season, when heterotrophic microorganism respiration was proportionally smaller, may be due to the greater forest photosynthesis and substrate availability to roots in this season. A great substrate supplying was observed in semiarid grassland in China (Yhan et al., 2010), positive correlation between soil respiration and growing season, which corroborates what was observed in this study and also helps understand the results found by Binkley et al. (2006), who demonstrated a decrease of 16-24 % of total soil respiration after girdling of eucalypt stem due to the interruption of the substrate flow from shoot to roots through the phloem.

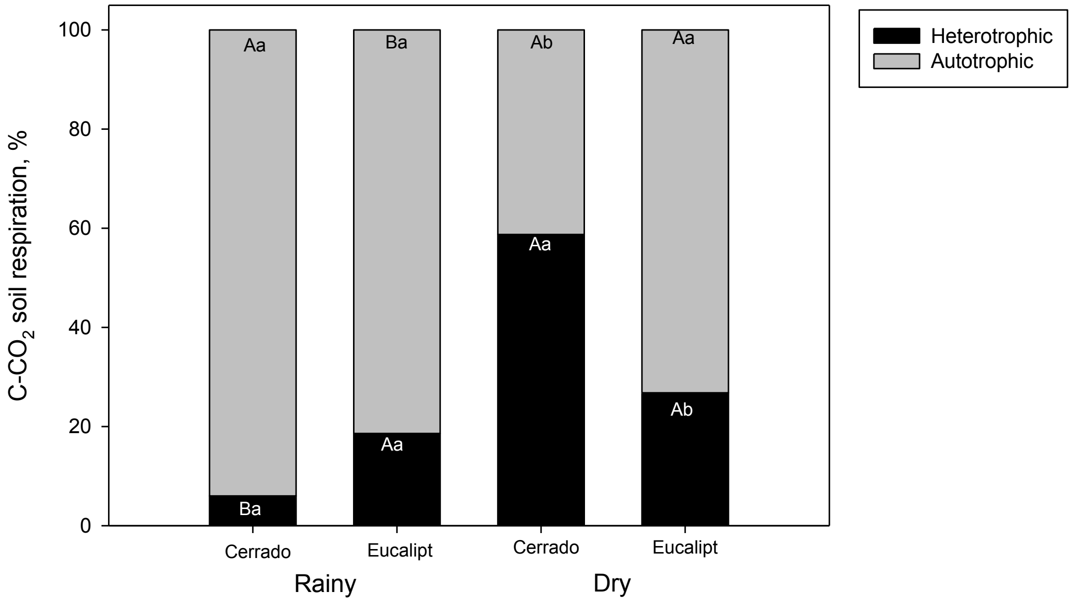

Figure 3. Relative contribution of heterotrophic and autotrophic respiration to total soil surface C-CO_2 flux in Cerrado region. Uppercase letters compare seasons within each land use (Cerrado and eucalypt); lowercase letters compare land use within each season. Statistical significance determined at F < 0.05.

The contribution of heterotrophic respiration at the dry season for the total surface CO_2 flux was higher in the soil under Cerrado vegetation than that under eucalyptus, although there was no significant differences ($p < 0.05$) between seasons at the eucalyptus site. Vargas et al. (2006) observed, at the beginning, a strong seasonal pattern of fine roots and rhizomorph lengths, with declining of fine roots in the dry season.

Soil CO_2 efflux positively relates with the amount of fine roots and this can be related to higher autotrophic respiration contribution in the rainy season. Besides in the dry season occur higher root biomass proportion caused by root elongation looking for water (Gonçalves, 1994; Neves, 2000), the rainy season have showed higher root biomass (Figure 4).

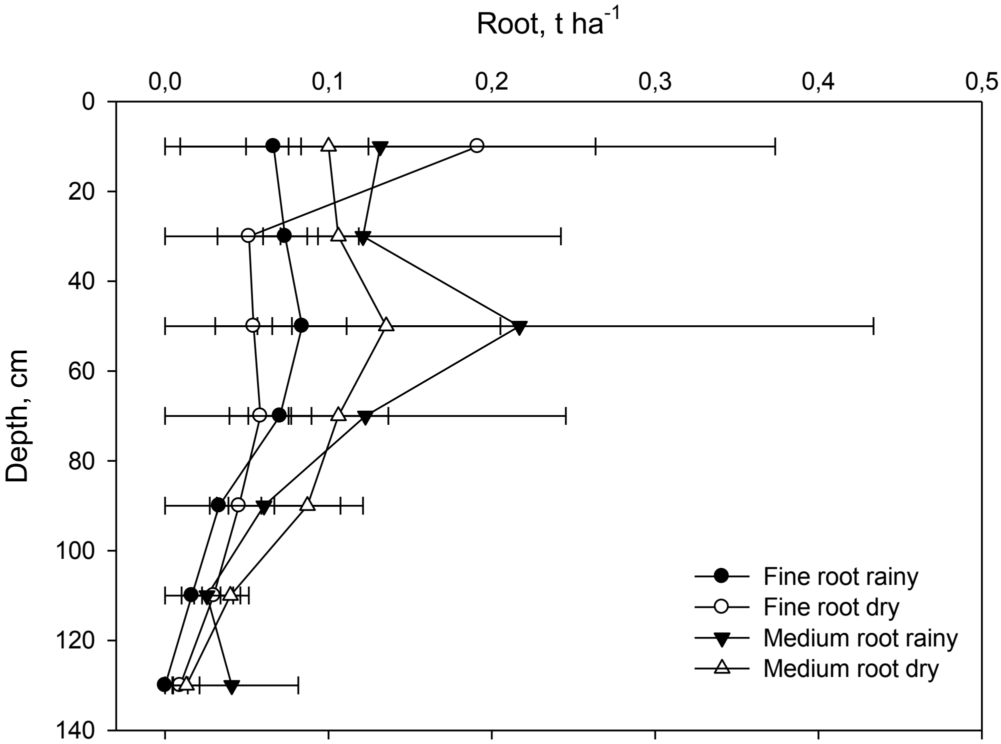

Figure 4 .Eucalypt root mass distribution in soil profile in the dry and rainy season. Statistical significance determined at $F < 0.05$ (standard error bars, n= 4).

Our results diverge from the ones found by Butler et al (2012), which showed a smaller heterotrophic respiration in the dry season, most likely due to the different approaches used in its estimative. These authors used the root exclusion method to partition soil respiration, which could underestimate the heterotrophic respiration by the decrease of microorganism activity, considering that root exclusion method cannot estimate exudates contribution (Kuzyakov et al., 2006; Hanson et al., 2000).

Along the soil profile there is a gradient of increasing concentration of CO_2 with the depth (Figure 4). There is also substantial differences between land uses in the rainy season, but this effect was restricted only for the deeper soil layer, while in the dry season there was difference on CO_2 concentrations from 50 cm downward (Figure 4). Higher CO_2 concentrations in the rainy season are caused by higher production and reduced diffusivity of CO_2 generated at deeper soil layers to the soil surface caused by water-filled pores a finding also backed by a study in a tropical forest in Thailand (Hashimoto et al., 2007)

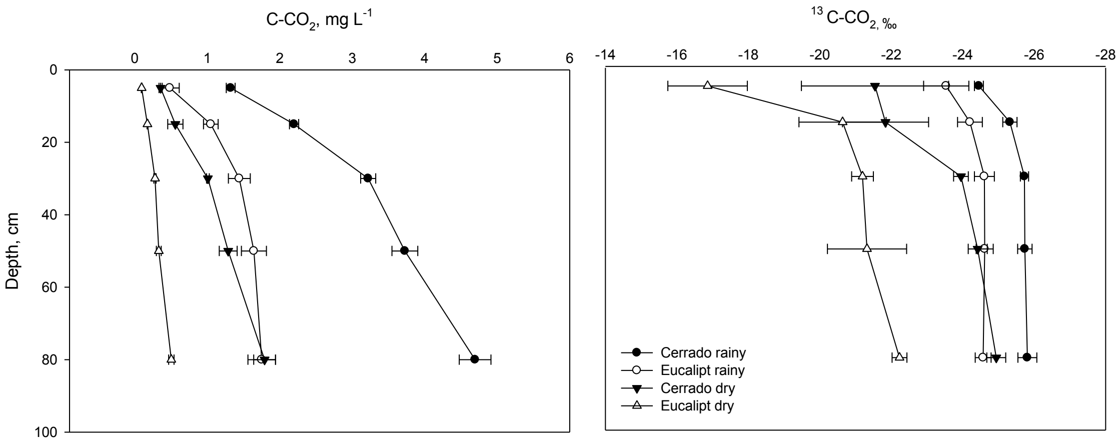

Figure 5. C-CO_2 concentration (mg L^{-}) and its isotopic composition (δ^{13}C-CO_2) at different soil depths, for profiles under Cerrado or planted eucalypt, in the dry and rainy season. Statistical significance determined at $F < 0.05$ (standard error bars, n= 4).

Another factor suggests that the increased production of CO_2 in the rainy season is of autotrophic origin are the more negative values of δ^{13}C-CO_2 which decrease toward values characteristic (- 24 to -30 ‰) of plants that have C-C3 photosynthetic routes (Farquhar et al., 1989) like eucalypt (Machado et al., 2011) and woody Cerrado’ s plants (Wilcke and Lillenfein, 2004). Such results highlight the importance of root respiration, and respiration of freshly deposited root exudates to the total soil CO_2 production. Furthermore, in the dry season such trend for less negative $\delta^{13}C-CO_2$ values along the soil profile supports a high activity of roots at deep soil layers, which is less reduced in the Cerrado site as compared to that under eucalypt, a reflex of a better adaptation of Cerrado plants to the low soil moisture content, typical of the dry season in the Cerrado biome area. Nonetheless, despite the low CO_2 concentrations along the soil profile in the eucalypt site (Figure 5), there is a clear trend for less negative $\delta^{13}C-CO_2$ values especially below the more superficial soil layer pointing to an active root system in such deeper soil layers, where moisture conditions are more favorable to plant growth.

A greater variation in CO_2 concentrations and isotopic composition ($\delta^{13}C-CO_2$) in the soil under eucalyptus, a reflect the previous land use (planted C-C4 grass) resulting in less negative values particularly in the dry season (Figure 5) when plant growth is more limited than microbial activity and thus the greater relative contribution of heterotrophic sources (SOM decomposition) to CO_2 production.

CONCLUSIONS

The soil moisture is major factor to CO_2 emissions in the Cerrado region and greater soil moisture is favorable to autotrophic respiration.

Eucalypt cultivation emits less CO_2 than the native forest in the rainy season, however in the dry season differences were not seen.

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MOLECULAR COMPOSITION OF SOIL ORGANIC MATTER IN DISTINCT TROPICAL SOILS TREATED WITH DOUBLE LABELLED EUCALYPT RESIDUES ASSESSED BY QUANTITATIVE SOLID-STATE ^{13}C- AND ^{15}N-NMR

ABSTRACT

Identifying and quantifying the SOM components are the prerequisites to understanding the mechanisms involved in its stabilization. Quantification of composition is a central aspect of solid-state NMR characterization of complex materials and natural organic matter. The objective of this study was to evaluate the influence of initial soil C (C saturation deficit), clay + silt content, and Al and Fe oxides levels on the major molecular alterations of physically separated mineral associated organic matter (MAOM). It enables NMR to provide accurate functional-group concentrations and aromatic-carbon fractions. In this study, a new method was used, the multi-CP pulse sequence; it yields quantitative solid-state magic-angle spinning (MAS) ^{13}C NMR spectra of organic materials with good signal-to-noise ratios. This technique was used to evaluate the alterations in the molecular composition of soil organic matter associated with mineral in soils with distinct textures, iron content and saturation deficit C. These soils were previously incubated with eucalypt residues. Soil was submitted to a physical fractionation. In general, the ^{13}C-NMR recognizable groups in plant materials were alkyl, carbohydrates, aromatics and carboxylic organic compounds in all residue components. The molecular nature of the MAOM fraction C under native conditions were primarily O-alkyl C (28 %), lipids alkyl-CH_2 (15.5 %), COO groups (13 %), aromatics protonated C (12 %), OCH_3 (10.2 %) NCH (8.4 %), aromatic protonated C (6 %), phenolic C (5.8 %), alkyl-CH_3 (5.6 %) and C=O (1.9 %). Dipolar dephasing technique indicated the presence of significant fractions of condensed aromatic C, possibly inherited from charred materials derived from natural and anthropic fires in the region. The eucalypt residue addition caused an aromaticity average decrease (29%) after one year of incubation. The texture itself had little influence on molecular composition of MAOM C, but clay mineralogy influenced some organic groups. *^{15}N NMR* of the eucalypt residue showed signals moistly intense assigned to free amino groups and all N composts present in the eucalypt residues were found in the MAOM fraction as well.

INTRODUCTION

Eucalypt plantation is an important economic activity in Brazil, where short-rotation stands are currently found in approximately 7 million ha (ABRAF, 2013), being the largest planted eucalypt forest in the world. Eucalypt plants are renowned for their rapid growth, but in highly weathered, low fertility tropical soils their growth is positively related to soil organic matter (SOM) content (Menezes, 2005), being relevante because influences on their chemistry, physics and biology (Nambiar, 1996; Garay et al., 2004; Lal et al., 2005; Franchini et al., 2007). So, maintaining SOM is therefore important to sustain adequate yields.

However, under high temperature and moisture conditions most of the time in the year is not trivial. Protective mechanism against degradation and losses must be very efficient. SOM was believed to be stabilized by mechanisms associated to chemical/colloidal protection, physical protection and inherent structural resistance to degradation with the formation of recalcitrant SOM compounds (Stevenson, 1994; Six et al., 2002).

In more recent years, however, there is growing evidence that sorptive protection of organic compounds by clay-sized minerals plays a major role in stabilizing SOM (Eusterhues et al., 2003; Kleber and Johnson, 2008; Stockman et al., 2013; Jones and Singh, 2014; Whalen et al., 2014). Pedogenic oxides offer sorptive surfaces to SOM and they also contribute to protection of SOM through effects on soil microaggregation (Six et al., 2002; Lalonde et al., 2012; Wagai & Mayer, 2007; Cotrufo et al., 2014). Nonetheless, the increased specific surface area promoted by the oxides in tropical soils is no guarantee of increased stable C accumulation (Thaymuang et al., 2014).

Even in highly weathered tropical soils, there is large variations in clay content and the proportion of Al/Fe oxides. Instance, Marques et al. (2004) reported that soils varied from loamy sands to clays, with Al (14–439 g kg^{-1} Al_2O_3), Fe (10–331 g kg^{-1} Fe_2O_3), and contents closely following the clay contents. The mineral matrix has strong interaction with SOM due to bonding between surface metals and acidic organic ligands (Feller et al., 1997; Guggenberger & Kaiser, 2003; Zinn et al., 2005).

The importance of clay minerals on SOM stabilization is highlighted by Zinn et al. (2002) who found more significant SOM losses in soils with lower clay content in the Brazilian Cerrado region and in southern Brazil SOM stocks was found positively related with the clay content, but not to litter input or quality (Sausen et al., 2014). Dick et al. (2005) characterized and quantified the SOM stocks from six Ferralsol profiles under native vegetation, taking into consideration textural and mineralogical characteristics and local environmental conditions (tropical and subtropical soil). They reported that SOM in those environments is stabilized mainly through organo-mineral interactions with the Fe- and Al-oxides and kaolinite, the main clay fraction components.

Knowledge about litter composition effects on its degradation are key factor to understand the transfer of plant-derived compounds to more stable soil organic carbon and nitrogen (Prescot, 2010; Cotrufo et al., 2014). Afforestation cause chemical alterations in the SOM stocks (Zinn et al., 2002) and quality. For example, Soares et al. (2013) found greater contribution of nonpolar alkyl C groups in humic acid and fulvic acid from eucalypt plantation when compared with native vegetation. On average, eucalypt cultivation decreased humic substances aromaticity when compared to humic substances from the native vegetation soils. It is not clear, however, how the mineralogy of silt and clay fractions would alter the amount and the quality of organic matter in organo-mineral fractions. There is growing body of evidence that the formation of the organo-mineral components by microbial derived products play an essential role in C stabilization, especially when there is ample supply of labile C sources (Cotrufo et al., 2013, Feng et al., 2014; Luce et al., 2014). It is expected then that microbial synthesis may lead to changes in SOM molecular composition, such as the increase in alkyl- and methyl-C content of soil observed during decomposition (Hopkins et al., 1997).

Last but not least, transfer of plant-derived C to the more stabilized organic matter fractions seems to depend on the initial soil C content, in which soils with high initial SOM status have a lower C stabilization efficiency compared with low SOM containing soils. Accordingly, soils with C contents much below their potential sequestration capacity (larger C saturation deficit) may have a larger C stabilization, with less input C being lost as CO_2 to the atmosphere (Six et al., 2002; Stewart et al., 2007; 2008; West and Six, 2007; Feng et al., 2013; Poirier et al., 2014; Wiesmeier et al., 2014). Besides, Stewart et al. (2009) observed that the carbon saturation deficit influenced the transfer of C added as wheat residue to chemically and biochemically protected mineral fractions of distinct soils. The decomposition pattern of ^{13}C-labelled wheat roots was horizon specific and resulted in SOM of different quality.

Identifying and quantifying the SOM components are the prerequisites to understanding the mechanisms involved in its stabilization. In this study, a recently developed method was used, the multi-CP pulse sequence (Johnson and Schmidt-Rohr, 2014) to obtain quantitative solid-state magic-angle spinning (MAS) ^{13}C NMR spectra of organic materials with good signal-to-noise ratios. According to the authors this method achieves long (>10 ms) cross polarization (CP) from ^{1}H without significant magnetization losses due to relaxation and with a moderate duty cycle of the radio-frequency irradiation, by multiple 1 ms CP periods alternating with ^{1}H spin–lattice relaxation periods that repolarize the protons.

This study aimed at quantifying the influence of initial soil C (C saturation deficit), clay + silt content, and Al and Fe oxides levels on the major molecular alterations of physically separated mineral associated OM (MAM) following addition of double labelled (^{13}C and ^{15}N) eucalypt litter in highly weathered soils employing ^{13}C and ^{15}N NMR spectroscopy.

MATERIAL AND METHODS

Studied soils

This study involved a qualitative and quantitative assessment of physically separated SOM associated with minerals fraction C from soils that received application of eucalypt residues. It was used eucalypt residue obtained from hybrid clonal eucalypt (*Eucalyptus urophylla* x *E. grandis*) seedlings that had been double labelled with ^{13}C and ^{15}N following the procedures of Machado et al. (2009) for ^{13}C, and Eagle et al. (2002) for ^{15}N. The characteristics of the eucalypt residue individual components are shown in Table 1.

Table 1. Chemical characteristics of individual components of double-labeled eucalypt residues.

Residue component	C	H	N	δ $^{13}C_{V\text{-}PDB}$	^{15}N
	------------------- %-------------------			---- ‰----	-- atom % --
Leaf	46.23	6.17	1.833	342	76.245
Root	45.8	6.06	0.920	398	66.743
Wood	46.84	6.85	0.200	313	70.662
Bark	44.01	5.90	0.470	320	81.387
Branch	44.1	6.12	0.763	349	72.331

For the incubation experiment it was selected representative tropical soil located in two main eucalypt growing regions in the Brazilian Cerrado, namely Minas Gerais and Mato Grosso do Sul states. These soils were collected under native vegetation (Cerrado) in Patos de Minas, MG (18°33'17.73"S. 46°30'3.38" W), São Gotardo, MG (19°37'15.19"S 46°5'11.12" W) and Três Lagoas, MS (20°53'42.75"S 51°54'29.57"W).

The soils were chosen because of their distinct characteristics regarding SOM content, clay + silt content, and Fe and Al oxides content. Both A (0-10 cm) and B_w (60-100 cm) horizons were sampled in order to obtain soil samples with high initial (low saturation deficit) and low initial C content (high saturation deficit). Their mineralogical and chemical composition are summarized below (Table 2). Soil texture was determined by the pipette method; C and N content and isotopic composition was obtained on a continuous flow isotope ratio mass spectrometer – IRMS (20–20 ANCA GSL, Sercon, Crewe, UK). The ^{13}C results were expressed in a per mil basis in comparison to the PDB international standard, whereas ^{15}N data are expressed in atom %. Clay mineralogy was determined by powder X-Ray diffraction (XRD) in a multifunctional Panalytical X’Pert Pro PW 3040/60 diffractometer equipped with a 1800 W, 60 kV cobalt tube in the range of 10 to 50 °2θ in 0.0167 °2θ at 1 step s^{-1} at 40 kV and 40 mA and equipped with an X’Celerator detector.

The content of Fe in more crystalline oxides (Fed) was estimated by extraction with sodium bicarbonate–citrate–dithionite (Mehra and Jackson, 1960) and that in poorly crystalline oxides (Feo) was determined by the ammonium oxalate method at clay fraction (Schwertmann, 1964).

Table 2. Soil class, chemical, and physical characteristics of the three soils, at two sampling depths, before incubation with eucalypt residues

Soil Classification[a]	Layer	Mineralogical composition [b]	C	$\delta\ ^{13}C$	N	$\delta\ ^{15}N_{air}$	Silt	Clay	Al_o	Fe_o	Al_d	Fe_d	Fe_o/Fe_d	Al_o/Al_d
	cm		g kg^{-1}	‰	g kg^{-1}	‰	------g kg^{-1} -----		------------g kg^{-1} ------------------				-------%------	
					São Gotardo, MG									
Xanthic Haplustox/Yellow Latosol	0-10	kt, Hm, Gb, An	16.2	-12.3	0.9	6.4	86.6	517.6	4.7	1.2	7.9	22.0	5.4	59.2
	60-100		9.8	-12.0	0.6	15.2	44.4	562.7	4.0	1.0	6.4	20.0	4.9	62.0
					Patos de Minas, MG									
Rhodic Haplustox/ Red Latosol	0-10	kt,Hm, Gt, Gb, AN, Mh	37.7	-23.4	3.6	13.9	205.1	599.5	6.4	6.1	20.3	172.5	3.5	31.7
	60-100		9.8	-15.0	0.7	16.4	202.2	585.8	4.7	5.8	21.8	181.9	3.2	21.5
					Três Lagoas, MS									
Typic Haplustox/Red-Yellow Latosol	0-10	kt, Hm, Gt	15.9	-23.6	1.2	15.7	60.0	360.0	2.8	2.1	6.1	68.2	3.1	45.2
	60-100		2.9	-18.6	0.2	8.9	72.1	375.0	1.8	1.0	5.5	58.8	1.7	32.8

[a]USDA soil taxonomy/ Brazilian soil system classification; [b] Kt: kaolinite, Hm: hematite, Gt: goethite, Gb: gibbsite, An: anatase, Mh: maghemite; Al_o and Fe_o: Acid ammonium oxalate extraction, Al_d and Fe_d: citrate-bicarbonate-dithionite extraction.

Eucalypt residue and treatments

The amount of eucalypt residue added into the soil was equivalent to 80 t ha^{-1}, and the individual plant components were combined in the same proportion found in the plant at harvest (44 % leaf, 18.94 % root, 7.10% bark, 7.06 % branch and 16.91 % of stemwood). The resulting residue (< 2 mm) was rehydrated and then mixed with 20 g of each of soil moisture (at 80% of the field capacity) in 0.3 L glass jars. The jars were closed and accommodated in an incubation room at dark and temperature kept at 25° ± 1°C for one year. During the incubation period the jars were open daily in the first week and then every other week to allow atmosphere renovation. Small plastic tubes filled with water were kept inside the jars in order to maintain humidity the soil + residue mixture. Elapsed the incubation time, soil samples were collected and physical fractionation of the soil was carried out (Cambardella and Elliot, 1992) in order to obtain the MAOM – mineral associated organic matter and the POM-particulate organic matter. In this study it was reported only on the MAOM fraction because it contained the more stabilized organic matter. After separation from the POM, the MAOM was treated 10 times with 10% (w/w) HF to eliminate paramagnetic ions (Gonçalves et al., 2003; Soares et al., 2013). The C and N contents, and ^{13}C and ^{15}N abundance values before and after HF treatment were determined by isotope ratio mass spectrometry (20–20; Anca-GLS. Sercon. Crewe. UK) and expressed in a per mil basis (‰) in comparison to their respective international standards (V-PDB for C and atmospheric N_2 for N) (Table 3).

The 12 treatments in table 3 resulted from the combination of the three soil types (Clay, Clay and Sand), with Low, Intermediate or High Iron content (LFe, IFe, HFe), with low initial C (LIC) or high initial C (HIC). These soils received no eucalypt residue (-Res) or were incubated with double-labelled eucalypt residue (+Res). The experiment was run in a completely randomized design, with three repetitions, totaling 36 experimental units.

Table 3. Elemental and isotopic composition of soil organic matter associated to minerals (MAOM) in the three soils used in the incubation study.

Soil	C	N	δ ^{13}C	^{15}N	C/N
	-------%---------		---‰---	-- atom %--	
Clay_LFe_-Res_HIC	2.5	0.14	-12	0.37	17.7
Clay_LFe_+Res_HIC	3.2	0.18	66	8.74	18.4
Clay_LFe_-Res_LIC	1.6	0.10	-13	0.37	15.9
Clay_LFe_+Res_LIC	2.4	0.13	105	14.08	18.8
Clay_HFe_-Res_HIC	4.1	0.40	-23	0.37	10.7
Clay_HFe_+Res_HIC	4.5	0.42	18	4.24	11.0
Clay_HFe_-Res_LIC	1.1	0.07	-14	0.37	15.1
Clay_HFe_+Res_LIC	1.7	0.11	119	14.62	16.6
Sand_IFe_-Res_HIC	4.1	0.30	-24	0.37	12.9
Sand_IFe_+Res_HIC	4.6	0.36	26	8.58	13.2
Sand_IFe_-Res_LIC	0.6	0.05	-19	0.37	13.0
Sand_IFe_+Res_LIC	1.7	0.09	211	19.59	19.4

LFe: Low Iron content; HFe: High Iron content; IFe; Intermediate Iron content +Res: with plant residue; -Res: without residue plant; HIC: High amount of initial carbon; LIC: Low initial carbon.

NMR experiments

For most of the ^{13}C NMR experiments, the MAOM samples (composited from the three repetitions) were packed in 4-mm rotors and measured using a Bruker Biospin DSX 400 spectrometer operating at a ^{13}C frequency of 14 kHz, in a double-resonance magic-angle spinning (MAS) probehead, ^{13}C chemical shifts were referenced to glycine.

The new multiple-CP (multi-CP) pulse sequence proposed by Johnson and Schmidt-Rohr (2014) was used. This technique yields quantitative cross polarization (CP) spectra of organic materials. ^{1}H T_1 relaxation restores ^{1}H magnetization between repeated CP periods. The corresponding subspectro with signals of nonprotonated C and mobile groups such as rotating CH_3 was obtained by ^{13}C CP/TOSS combined with 40 μs dipolar dephasing.

For quantifying the solid-state ^{13}C NMR data in terms of the molecular composition of MAOM, the spectra were divided into eight ranges whose areas were determined by integration. Generally, the assignments are as follow (Mao et al., 2000; Soares et al., 2013) (Table 4):

Table 4: Assignment of ^{13}C NMR signals in MAOM

Functional Group	**NMR experiment**	**Chemical shift (ppm)**
Nonpolar alkyl	Multi-CP /MAS and 1H inversion recovery experiment	0-46
OCH_3 groups	Multi-CP /MAS and multi-CP /MAS dipolar dephasing	48-65
NCH	Multi-CP /MAS and multi-CP /MAS dipolar dephasing	48-65
Anomeric and carbohydrate C	Multi-CP /MAS	65-115
Nonpolar nonprotonated aromatics	Multi-CP /MAS and multi-CP /MAS dipolar dephasing	115-154
Protonated nonpolar aromatics	Multi-CP /MAS and multi-CP /MAS dipolar dephasing	115-154
Phenolics (aromatic C-O)	Multi-CP /MAS and multi-CP /MAS dipolar dephasing	145-161
COO and NC=O	Multi-CP /MAS	161–188
Aldehyde C	Multi-CP /MAS	188-220
Aromaticity and alkyl/O-alkyl (A/O-A)	Calculated using chemical shift ration	115 to 161/0 to 115 and 0 to 46/65-115

For ^{15}N NMR spectra were measured using a Bruker DSX 400 spectrometer at 7 kHz, using magic-angle spinning (MAS) of 4-mm rotors in double- and triple-resonance probe heads. ^{15}N chemical shifts were referenced to proline.

To plot a spectrum a normalization was performed using the C/N content, the number of scans and C/N isotope ratio (^{13}C / ^{12}C or ^{15}N/^{14}N) of each of MAOM sample and to determined amount of each carbon component was quantified by integrating the area under each peak.

RESULTS AND DISCUSSION

The molecular composition of the eucalypt residue

The individual plant components that were in the eucalypt residue applied to the soils presented similar molecular composition. In general, the ^{13}C-NMR recognizable groups were alkyl, carbohydrates, aromatics and carboxylic organic compounds in all residue components. However, there were different peaks intensities, thus a different proportion of organic molecules among them (Figure 1). The spectra obtained are typical for plant components and the general pattern is similar to those obtained by others authors for plant materials (Knicker et al 1995; Hopkins et al 1997; Conte et al., 2010; Strukelj et al., 2013; Johnson and Schmidt-Rohr, 2014).

Leaves showed the greater intensity at the alkyl C region (0-46 ppm) with peaks centered at 23, 30 and 33 ppm, while the wood component had the lowest contribution in this region with one peak at 21 ppm (CH_3) (Figure 1. A and D). The bark presented different resonance peaks in this region, as found by Dick-Pérez et al. (2011) (Figure 1. B). These authors attributed the signals to glycoproteins in plant cell wall in *Arabidopsis.* The branch and root were mostly two peaks centered at 21 and 29 ppm (Figure 1, C and E). Conte et al. (2010) evaluated eucalypt leaf materials and attributed the peaks centered at 26, 30 and 32 ppm to linear methylene ($-CH_2-$) chains. Belonging to lipids, cutin-like structures and others aliphatic bio-moieties resonating at 39 ppm and 42 ppm can be assigned to secondary methylene C (-CH-) and to fully substituted C, respectively. They can be originated from chlorophyll-like structures or from molecules belonging to eucalypt oil (a complex mixture of terpenoids) (Conte et al., 2010).

The region from 46 to 60 ppm can be attributed to nitrogen compounds and methoxyl groups of lignin (Conte et al., 2010). In the current study, resonance peaks were centered in 54, 56 and 57 ppm, depending on the plant component.

It was observed an overall larger contribution for the carbohydrate region (60-90 ppm), mainly for the peak centered around 74 ppm (OCH groups). Cellulose and hemicellulose are the main contributors in this region. All plant components showed resonances at 63 and 65 ppm (cellulose), as well as other peaks at 83 and 89 ppm (cellulose), which were less intense for leaf residue.

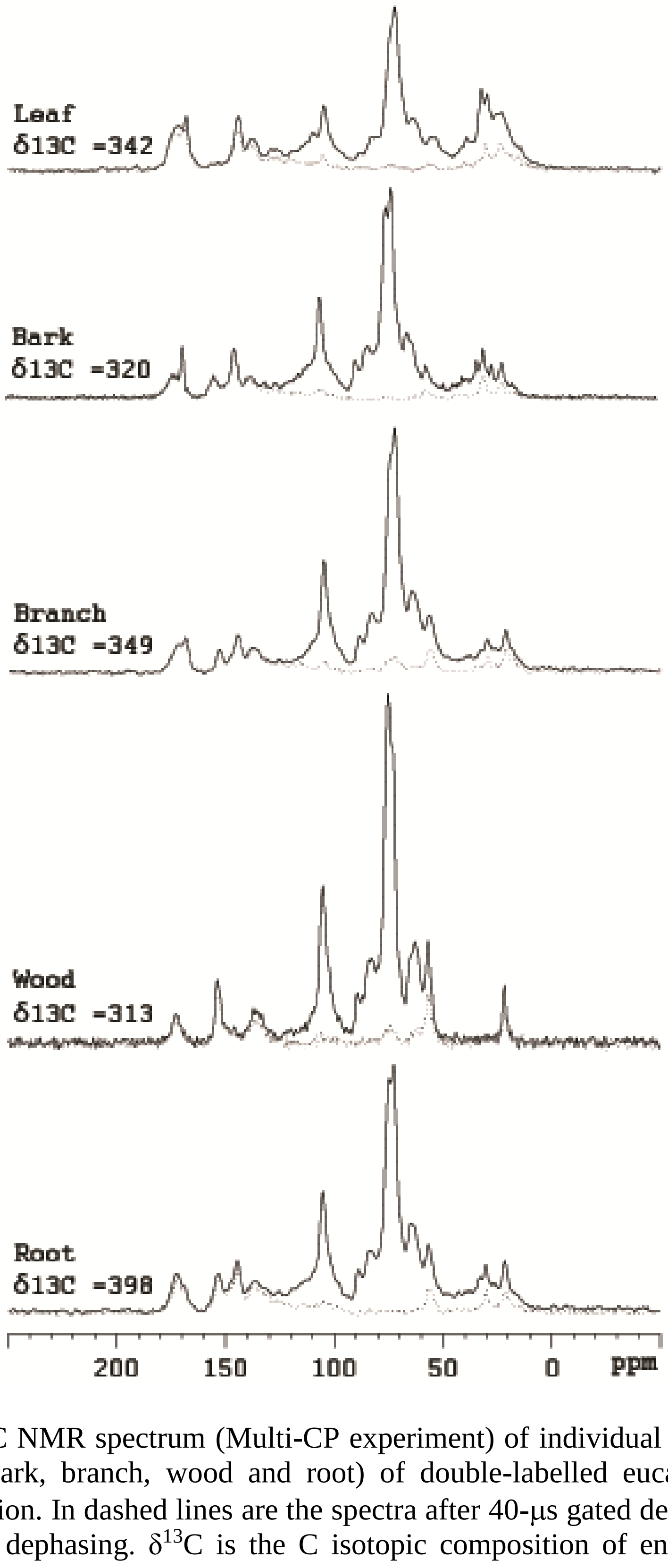

Figure 1. Full ^{13}C NMR spectrum (Multi-CP experiment) of individual components (leaf, bark, branch, wood and root) of double-labelled eucalypt before incubation. In dashed lines are the spectra after 40-μs gated decoupling for dipolar dephasing. δ^{13}C is the C isotopic composition of enriched plant components relative to the PDB international standard.

A peak centered at 105 was observed in all plant components, and is attributed to carbohydrates contribution as C1 of cellobiose (Rumpel at al., 2004; Dick-Pérez et al. 2011; Strukelj et al 2013). The greatest intensity was observed in the wood material and the least in the leaf component.

The results demonstrated a variation of aromatic peaks chemical shifts and intensities with plant component. Leaf material showed peaks at 127, 138, 145 ppm; Branches material showed peaks at 137,144 and 153 ppm, and in wood they appeared at 135, 145 and 153 ppm; In the bark material they were centered at 124, 130, 136, 144 and 154 ppm, whereas the root component showed many peaks (125, 131, 136, 144, 152 ppm).

Aromatic C resonates from 115-161 ppm and the main peaks reported on literature are at 130, 144 and 154 ppm, all attributed to lignin components, p-hydroxyphenol derivative structures are assumed to give a signal around 130 ppm, whereas O-aryl C from guaiacyl and syringyl units may give resonance at 137, 144, and 154 ppm (Roscoe et al., 2004.). Strukelj et al. (2013) had studied the chemical transformations in downed logs and snags of mixed boreal species during decomposition and attributed the resonances at 113, 122, 134, 149, and 152 ppm to guaiacyl units of lignin from coniferous leaf, while deciduous plant spectra showed resonances at 136 and 154 ppm attributed to lignin syringyl units. However, Conte et al (2010) found syringyl (137 and 144 ppm) in *eucalyptus*, while 144 and 154 ppm in *Pinus* and 137 ppm in *Cupressus sempervierens*.

Conte et al. (2010) also attributed contribution of resin materials in this region. According to their interpretation, the differences between pinus and eucalypt leaves in 120-106 ppm region was the presence of acid resins, which are more abundant in pinus than in eucalypt leaf. Nierop et al. (1999) highlights the importance of the hydrolysable polymers cutin and suberin, and the non-hydrolisable cutan and suberan. These polymers are very recalcitrant and may show relative accumulation upon decomposition of the bulk OM (Nierop et al., 1999).

In the carboxyl region (161-188 ppm), representing COO and NCO (amide), it was observed one peak at 171 ppm in root and wood material, and 168 ppm in branch material, while two peaks appeared in the bark (169 and 171 ppm) and in the leaf (169 and 171 ppm) material as shown in Conte et al. (2010). This contribution was mostly from aldehyde because there was not differences between dashed and no lines from indicating ketone. Mao at al. (2006) found more keto groups than aldehydes in intact pine wood. It was observed no resonances in the carbonyl region (188-220 ppm).

The molecular nature of the MAOM fraction C

The major NMR-identifiable components in soil organic matter associated to minerals (MAOM) were primarily O-alkyl C (28%), lipids alkyl-CH_2 (15.5 %), COO groups (13 %), aromatics noprotonated C (12 %), OCH_3(10.2 %) NCH (8.4 %), aromatic protonated C (6%), phenolic C (5.8 %), alkyl-CH_3 (5.6%) and C=O (1.9 %) (Figures 2 to 4).

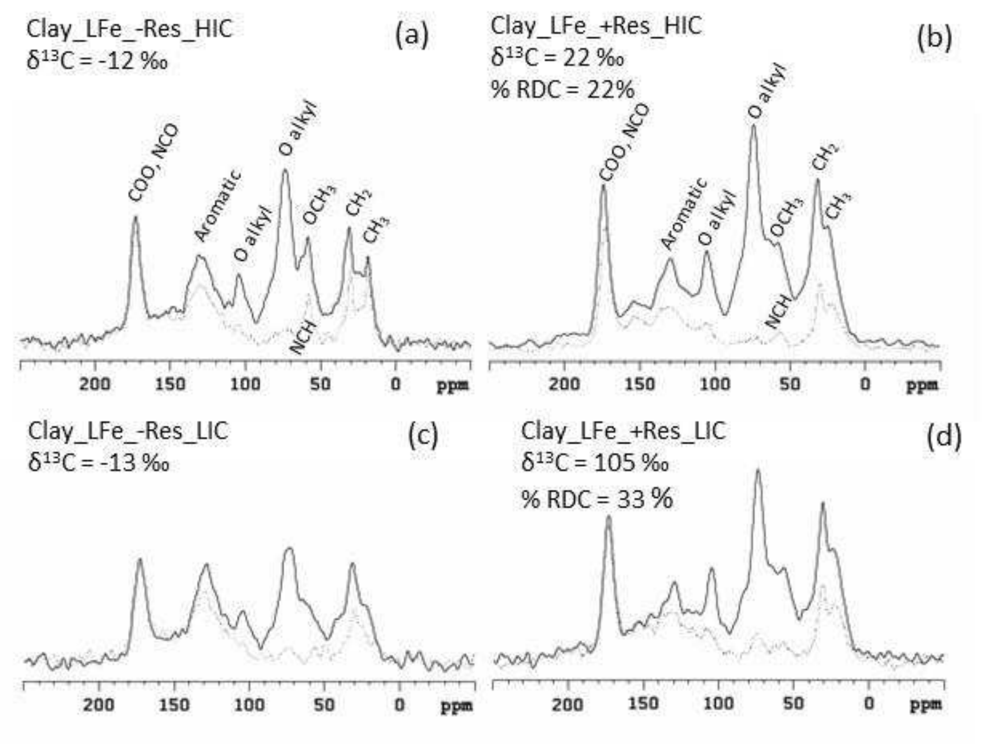

Figure 2. Full ^{13}C NMR spectrum (Multi-CP experiment) of MAOM fraction of soils with variable textures, iron contents, and initial soil C contents, in the absence and in the presence of eucalypt residue. Soils with Clayey texture, Low iron content, High Initial Carbon content (a, b); Soils with Low Initial C content without eucalypt residue incorporation (c, d) and with eucalypt residues incorporation (b, d); RDC is the proportion of residue-derived C (%) in the MAOM fraction. Dashed lines represent spectra after 40-μs gated decoupling for dipolar dephasing.

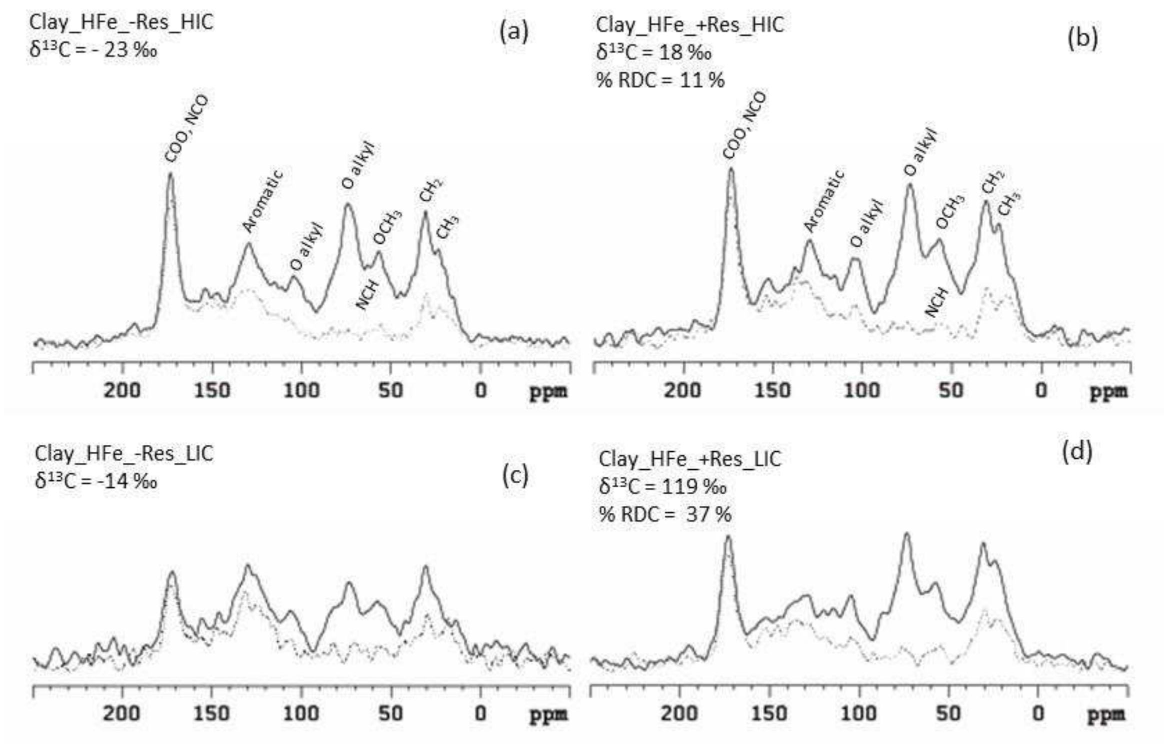

Figure 3. Full ^{13}C NMR spectrum (Multi-CP experiment) of MAOM fraction of soils with variable textures, iron contents, and initial soil C contents, in the absence and in the presence of eucalypt residue. Soils with Clayey texture, High iron content, High Initial Carbon content (a, b); Soils with Low Initial C content without eucalypt residue incorporation (c, d) and with eucalypt residues incorporation (b, d); RDC is the proportion of residue-derived C (%) in the MAOM fraction. Dashed lines represent spectra after 40-μs gated decoupling for dipolar dephasing.

The nonpolar alkyl source can be attributed to fatty acids (Stevenson, 1994) and poly(methylene) highly stable crystals of polymethylene (Hu et al., 2000; Mao et al., 2007). The resonance from 0 to 22 ppm and from 22 to 46 ppm can be assigned to CH_3 and CH_2, respectively (Johnson and Schmidt-Roh, 2014). The presence of poly(methylene) in the present study was confirmed through a ^{1}H inversion recovery experiment, after the indicated recovery delays at 26 ms, only the poly(methylene) remains inverted (Figure 5). A second region, 48-65 ppm, can be attributed to NCH groups representing proteins (Mao et al., 2001). Although amide is readily degradable, it could be protected through encapsulation into hydrophobic sites of organic matter and through organo-mineral interaction (Dieckow at al 2005). In this region theres is an overlapping of NCH and OCH_3 groups. Using dipolar-dephasing experiments, the peak assigned to OCH_3 groups still shows in all spectra due to the CH_3 mobility and the relatively large distance of the OCH_3 carbon from other protons; correspondingly, it is suppressed in the short-CP/MAS spectrum (Mao et al., 2001). OCH_3 is characteristic of lignin contribution and its presence can also be verified by the presence of bands resonating in 55, 155 and 110 ppm (Mao et al., 2008). Mao et al. (2001) observed strong cross-peaks between OCH_3 carbons and aromatic protons, showing that the OCH_3 groups are connected directly with aromatic rings near protonated aryl carbons, in a lignin-like structure. This group was increased after residue incubation because lignin was present in the eucalypt residue and may have been selectively preserved (Cotrufo et al., 2013)

O-alkyl C groups resonate from 65 to 115 ppm and normally are the most abundant group found in OM in NMR spectra (Wang et al., 2004; Dieckow et al., 2005; Zinn et al., 2005, Fang et al., 2010). Those O alkyl are typical of carbohydrates and can be traced to degradation of plant material, root exsudation or microorganism activity (Stevenson, 1994). Carbohydrates play a major role source of energy for microbial in soils, and are important in soil aggregation (Martins el al., 2012)

Aromatic C groups resonating at 115-154 ppm was observed in the spectra, and to distinguish between protonated and non-protonated (dashed lines in Figures from 2 to 4) carbons, it was used dipolar dephasing by gated decoupling based on Multi-CP NMR (Johnson and Schmidt-Roh, 2014). In the current study, no protonated aromatic C group had greater contribution than the protonated one, probably because the presence of substantial amounts of charred material, most likely derived from burning of the Cerrado, which takes place virtually every year under natural conditions. This signal can be derived from condensed aromatic structures like black carbon (Mao et al., 2007; Brewer et al., 2009).The presence of black fragments in an optical transmission photomicrograph associated with a prominent aromatic C signal at 130 ppm in the NMR spectrum observed by Dieckow et al. (2005) was taken as evidence for the occurrence of materials derived from charcoal in SOM of a southern Brazil sub-tropical soil. The aromatic signal remaining around 130 ppm can also be assigned to contributions from highly modified lignin molecules.

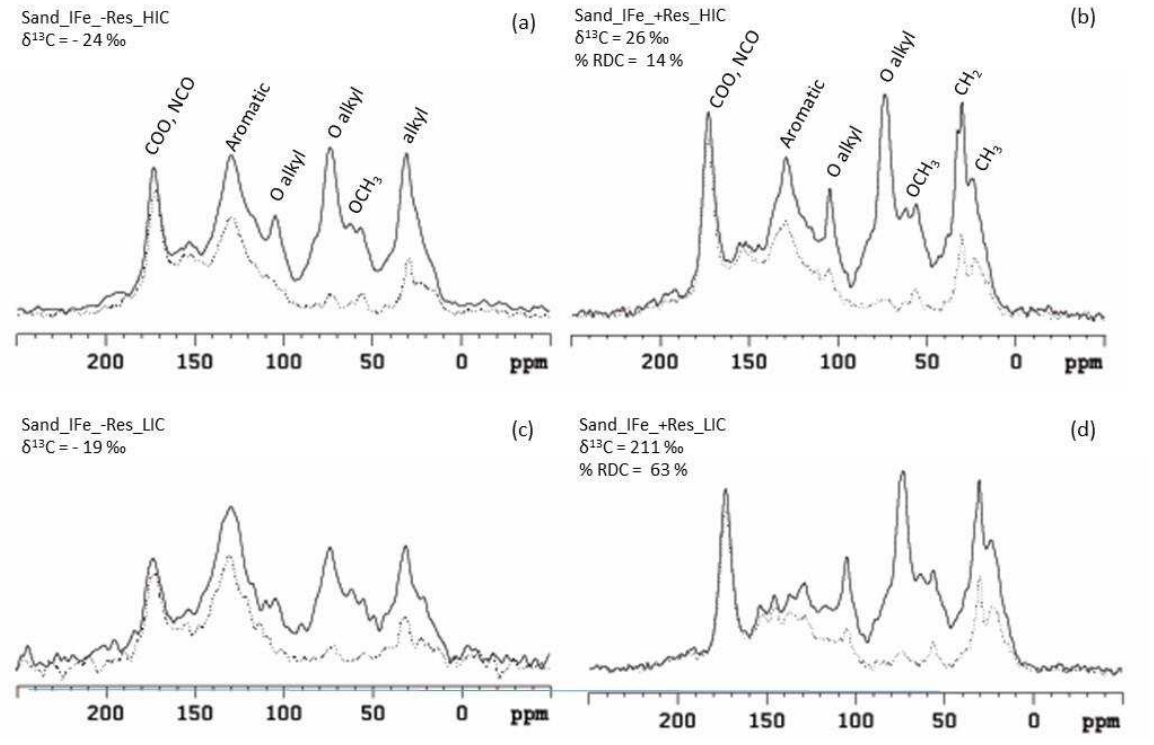

Figure 4. Full ^{13}C NMR spectrum (Multi-CP experiment) of MAOM fraction of soils with variable textures, iron contents, and initial soil C contents, in the absence and in the presence of eucalypt residue. Soils with Sandy texture, Intermediate iron content, High Initial Carbon content (a, b); Soils with Low Initial C content without eucalypt residue incorporation (c, d) and with eucalypt residues incorporation (b, d); RDC is the proportion of residue-derived C (%) in the MAOM fraction. Dashed lines represent spectra after 40-μs gated decoupling for dipolar dephasing.

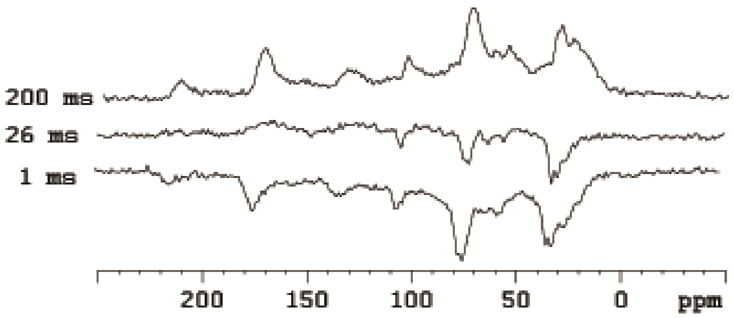

Figure 5. Full ^{13}C NMR detected ^{1}H inversion recovery of the MAOM fraction from the sandy soil with intermediate iron content, high initial carbon content, and with eucalypt residue incorporation.

The aromaticity (aromatic carbon fraction) was accurately determined based on Multi-CP NMR. The determination of relative intensity of functional groups of SOM has been most frequently carried out using CP techniques (Bayer et al., 2000; González-Perez et al., 2003; Alcântara et al., 2004; Rumpel et al., 2004; Dick et al., 2005; Dieckow et al., 2005, 2009). However, if SOM samples are from soils under distinct environments and have distinct characteristics, in particular high aromaticity, the use of ^{13}C CP/MAS as a quantitative tool may prove inadequate (Preston 1996; Mao et al., 2000; Conte et al., 2004; Skjemstad et al., 1996, 1999; Smernik and Oades 2000; Mao et al., 2002; Keeler and Maciel, 2003; Knicker et al., 2005; Nelson and Baldock, 2005; Kaal et al., 2008). Soares et al. (2013) found that for soils in long-term under eucalypt cultivation, where there is substantial amounts of black carbon the DP approach was more adequate and that CP NMR experiments resulted in an underestimation in 30 % of some aromatic C groups.

Overall, we found greater contribution of alkyl, O- alkyl and metoxyl in the MAOM fraction, irrespective of the initial soil C level (soil horizon). Additionally, the protonated aromatic group in soils with higher initial carbon content did not change their contribution after residue addition, while in soils with low initial C content (higher carbon saturation deficit, or the subsoil MAOM), the relative contribution of aromatics groups was reduced.

The molecular nature of the MAOM fraction C under native vegetation

Difference in Alquil/O alquil ratio of MAOM were not detected among sites under natural vegetation, this value was ranged at 0.5 or 0.6. The soil with lower clay content showed the higher aromaticity and lower carbohydrates contribution in both layers, surface and depth, 50.8 and 56.4 %, respectively. Possibly a greater recalcitrance of aromatic compounds allowed this greater aromaticity in response to less protection of SOM caused by the interaction with the soil texture (Jindaluang, et al., 2013).

On average, layers with lower C (47%) content or lower depth showed higher aromaticity than high C (37%) because the greater presence of noprotonet aromatic proportion compared with another compounds (Table 5). Dick et al. (2005) found similar results to Cerrado site. These author observed proportions of aromatic C and of phenolic C increase with depth, whereas methoxyl/N-alkyl C, carboxyl C and carbonyl C do not vary considerably. They attributed the organo-mineral interactions as mostly C stabilization factor.

Rumpel et al. (2009) has synthesized in reviews, that difference in the properties and dynamics between C deep and C surface may be explained by the more presence of amorphous Fe and Al oxides for OM stabilization in deeper, more C microorganism derived in deep than surface soil.

Alterations in the MAOM fraction caused by eucalypt residue addition

Generally, the aromaticity of the MAOM showed an average decrease of 29% after one year of incubation of soils with eucalyptus residues (Table 5). A decrease of aromaticity in areas cultived with eucalypt in Brazil was also observed by Zinn et al. (2002) and Soares et al. (2013), in humic substances, when comparing them to native cerrado conditions. Zinn et al. (2002) attributed the aromaticity decrease after eucalypt cultivation to input of oil and waxes from litter. Interestingly, in the current study, it was observed that direct contribution of signals corresponding to the aliphatic region at 0-46 ppm from residue component spectra is only minor (Figure 1). Although, aliphatic signals from eucalypt residues are low, the increase after eucalypt residue incubation were observed, suggesting that they can be derived from microorganism metabolites (Kiem and Kogel-Knabner et al., 2003) and are probably preserved by mineral interactions (von Lützow et al., 2008; Mikutta and Kaiser, 2011, Cotrufo et al., 2013). Baumann et al. (2013) investigated decomposition of root material and a mixture of soil and wheat residues along 36 months in three depths soils and demonstrated an increase of alkyl/O-alkyl ratio, and lignin content. The authors pointed that this ratio usually increases during leaf litter decomposition outside the mineral soil due to the loss of polysaccharides and selective preservation of plant-derived alkyl compounds and/or neoformation of microbial-derived alkyl decomposition products.

Alterations in MAOM molecular nature as affected by the C saturation deficit

Major changes in the organic components occurred in soils with greater transfer from C residue to the MAOM (Stewart et al., 2009) and the higher transfer occurred in the soils with high C saturation deficit (low initial soil C). The results based on MAOM-C stable isotope composition indicate that an average of 12% of MAOM-C was derived from eucalypt residue in the surface low C saturation deficit soil, while in the soil with low initial C this amount was 44% (information presented on Figures 2 to 4).

The rate of organic C mineralization was found to be dependent on the status of pre-existing soil organic C (Kimetu et al., 2009). The authors proposed that a C limit (saturation) may have led to reduced protection of the added OM, which was hence mineralized to a large extent. This could indicate reduced stabilization efficiency in soils containing already large amounts of SOC (Stewart et al., 2006; Wiesmeier et al., 2014).

Alterations in MAOM molecular nature as affected by soil texture and Fe and Al content

The amount of Fe and Al in soils influenced the O-alkyl, carboxylic and phenolic groups of the MAOM. O-Alkyl was 71% ($p < 0.05$) positively correlated with Fe_o/Fe_d ratio, while, phenolic C was 86 % ($p < 0.05$) negatively correlated with Fe_o/Fe_d and Al_o/Al_d and carboxylic group C was positively correlated (65 and 63 %, respectively) with the content of Fe_o and Al_d, (Table 5).

Mikutta et al. (2006) had studied the quantitative contribution of recalcitrant and mineral-protected OM to stable OM in soils of varying mineralogical composition from acid subsoil horizons and found a great stabilization of SOM by interaction with poorly crystalline minerals. In this study results indicate a greater preservation of more labile organic C structures (O-Alkyl C) in soils with a higher proportion of poorly crystalline Fe- and Al-oxides, with none to negative effect of poorly crystalline oxides on preservation of phenolic C appear to be in agreement with the findings of Mikuta's group since they demonstrated that a substantial amount (13%) of O-alkyl C, but only small amounts of lignin phenols were found associated to poorly crystalline minerals.

Kleber et al. (2005) worked with different ratios of Fe and Al content as predictors of C stabilization and their results suggest a contribution of hydroxylated surfaces to the protection of SOM by oxalate-extractable Fe and Al. Also, SOM stabilization has been attributed to clay minerals with capacity to adsorb SOM (Mikutta at al 2006; Bruun et al, 2010; Schneider et al, 2010). The effect of hydrous Fe oxides on SOC stabilization varies with clay mineralogy (Saidy et al., 2010).The authors found that presence of goethite associated with kaolinity reduced mineralization of added C. In their study, the presence of goethite had reduced 11 % of C mineralized accumulated because almost doubled its specific surface.

Carboxylic and phenolic group binds to goethite by forming strong surface complexes with preferential sorption sites, similar to mouths of micropores (Kaiser and Guggenberger, 2007). In the present study, the soil showed less phenolic contribution in the absence of this mineral, according to X-Ray diffraction (Table 1).

Bruun et al. (2010) also suggest that free oxides could play an important role in SOM stabilization by complexation. In their study, it was observed an inverse correlation between Ald + Fed and soil respiration rate.

Table 5. ^{13}C Multi-CP/MAS NMR functional groups in soil organic matter associated to mineral from soils under Cerrado with distinct clay and Fe and Al content, and initial soil C incubated with eucalypt residues

	C=O	COO,NCO	Phenolic	Aromatic		O alkyl	OCH$_3$	NCH	Nonpolar alkyl		Aromaticity[a]	A/O-A[b]
Chemical shift range (ppm)				noprot	prot				CH$_2$	CH$_3$		
	220-188	188-161	161-145	154-115	154-115	115-65	65-48	65-48	46-22	22-0		
Soil	%											
Clay_LFe_-Res_HIC	1.9	12.6	4.7	11.4	4.8	32.5	11.2	8.1	13.6	5.9	29.3	0.6
Clay_LFe_+Res_HIC	1.4	12.1	4.6	7.2	6.3	33.5	11.7	10.4	16.8	5.8	23.1	0.5
Clay_LFe_-Res_LIC	0.7	13.4	4.7	16.3	5.3	30.1	8.8	7.1	15.7	4.4	39.8	0.5
Clay_LFe_+Res_LIC	2.4	12.1	4.7	9.7	3.7	31.9	11.3	9.4	16.8	6.6	23.8	0.5
Clay_HFe_-Res_HIC	2.1	14.8	6.2	10.4	6.3	28.4	10.7	8.8	15.1	5.5	33.4	0.5
Clay_HFe_+Res_HIC	2.4	14.0	5.9	10.1	5.8	28	11.1	9.5	15.8	6.1	30.9	0.6
Clay_HFe_-Res_LIC	1.9	13.0	6.3	16.4	6.3	24.2	10.0	7.8	15.2	6.3	45.7	0.6
Clay_HFe_+Res_LIC	1.8	13.2	6.0	9.8	5.5	28.7	10.8	8.7	16.4	7.2	29.7	0.6
Sand_IFe_-Res_HIC	1.6	12.7	7.0	14.8	8.1	24.1	8.7	7.4	14.6	4.1	50.8	0.6
Sand_IFe_+Res_HIC	2.2	13.4	6.3	12.2	7.0	28.0	9.6	8.2	15.9	4.7	38.4	0.5
Sand_IFe_-Res_LIC	2.5	12.3	6.5	18.5	7.7	24.4	8.5	7.0	13.3	4.8	56.4	0.6
Sand_IFe_+Res_LIC	2.4	13.4	6.2	9.60	4.9	30.0	10.1	8.7	16.7	6.1	28.9	0.6

* [a] Aromaticity= (115 to 161 ppm range)/(0 to 115 ppm range); [b] A/O-A=(0 to 46 ppm)/(65-115 ppm).

Table 6. Correlation between soil characteristics and ^{13}C Multi-CP/MAS NMR functional groups in soil organic matter associated to mineral from soils under Cerrado with distinct clay and Fe and Al content, and initial soil C incubated with eucalypt residues

Functional groups	C=O	COO,NCO	Phenolic	Aromatic		O alkyl	OCH3	NCH	Nonpolar alkyl	
				noprot	prot				CH2	CH3
Chemical shift range (ppm)	220-188	188-161	161-145	154-115	154-115	115-65	65-48	65-48	46-22	22-0
Soil characteristics						%				
Fed	0.094 (p=0.77)	0.56 (p=0.061)	0.23 (p=0.482)	-0.14 (p=0.66)	-0.03(p=0.92)	-0.25 (p=0.44)	0.35 (p=0.26)	0.23 (p=0.48)	0.092 (p=0.78)	0.55 (p=0.064)
Ald	-0.13 (p=0.69)	0.48 p=0.11)	-0.30 (p=0.34)	-0.39 (p=0.20)	-0.26 (p=0.4)	0.24 (p=0.44)	**0.63* (p=0.027)**	0.48 (p=0.11)	0.13 (p=0.689)	0.41 (p=0.19)
Feo	0.16 (p=0.625)	**0.65* (p=0.022)**	0.39 (p=0.21)	-0.11 (p=0.74)	0.11 (p=0.74)	-0.37 (p=0.24)	0.25 (p=0.43)	0.18 (p=0.58)	0.06 (p=0.85)	0.41 (p=0.18)
Alo	-0.13 (p=0.69)	0.48(p=0.11)	-0.30 (p=0.34)	-0.40 (p=0.20)	-0.24 (p=0.41)	0.25 (p=0.44)	0.63 (p=0.027)	0.48 (p= 0.11)	0.13 (p=0.69)	0.41 (p=0.19)
Feo/Fed	-0.53 (p=0.075)	-0.25 (p=0.42)	**-0.87* (p=0.00)**	-0.35 (p=0.26)	-0.46 (p=0.13)	**0.72* (p=0.09)**	0.54 (p=0.07)	0.48 (p=0.231)	0.16 (p=0.61)	0.11 (p=0.74)
Alo/Alo	-0.43 (p=0.168)	-0.46 (p= 0.134)	**-0.75* (p=0.005)**	-0.15 (p=0.637)	-0.32 (p=0.313)	0.65 (p=0.22)	0.13 (p=0.691)	0.11 (p=0.72)	0.08 (p=0.81)	-0.32 (p=0.31)

* Significant correlation ($p < 0.05$).

Overall, the texture itself had little influence on molecular composition of MAOM C. Only the CH_3 was 63% ($p<0.05$) positively correlated with soil texture (Table 5), so that this group was more abundant in soils with higher amount of silt + clay. Beyond its intrinsic recalcitrant, this group is protected from microbial degradation by bonding with the mineral matrix (Eusterhues el al 2003; Kleber et al 2007). Jindaluang et al. (2013) looked for the influence of texture and mineral clay on the SOM composition by diffuse reflectance infrared Fourier transformed (DRIFT) spectroscopy and found opposite results to the present study; these authors observed one other hand that a decrease in the clay content resulted in an increase in the proportion of aliphatics, on the other hand the aromatic-C and amide N decreased.

Although many studies have found a positive relationship between SOM stabilization and clay content (Wiseman et al., 2006), other studies highlight the importance of other factors beyond simple clay content such as the clay mineralogy (von Lützow et al., 2006; Saidy et al., 2012; Pronk el al., 2013 Jindaluang et al., (2013), specific surface area (Eusterhues et al., 2005), and previously adsorbed organic compounds (Mikutta and Klaus Kaiser, 2001, Vogel et al., 2014). Even clay specific surface area may not be an indicator for C stabilization since only about 19% of clay surface was found covered with (old) organic C, and that input of litter led the new C being adsorbed preferentially to hot spots already occupied by old C (Vogel et al., 2014). In the face of such findings it is not clear, however, why soils with high initial soil C do not show a greater stabilization of the newly added C, as in the present study.

^{15}N NMR experiments

Multi-CP ^{15}N NMR spectroscopy applied to eucalypt residues and MAOM of soils showed peptides and proteinaceous constituents (Figure 6 and 7). No signals could be detected in the sample without labelled residue addition to the soil because the low content of ^{15}N (spectra not shown). Most of soil N is found in organic forms and it has been primarily identified by acid hydrolysis (Stevenson, 1994). ^{15}N NMR spectroscopy can be used as an alternative noninvasive method for soil samples or soil fractions (Kogel-Knabner, 1997).

The leaf component of the eucalypt residue showed signals more intense assigned to free amino groups at 33 ppm, arginine at 75 and 85 ppm (Dicosty et al., 2003; Smernik and Baldock, 2005), a large peak from amide-N at 120 ppm (Smernik and Baldock, 2005) and histidine at 174 ppm (Dicosty et al, 2003; Hong et al, 2001) than those in the bark and root components. These results contrast to those found by Smernik and Baldock (2005), who found that their amine participation in roots was more abundant than in eucalypt leaves.

All N composts present in the eucalypt residues were also found in the MAOM fraction. The magnitude of peaks varied according to the soil type. Amide-N was the main peak observed in all soils, similarly to previously results with SOM and ^{15}N NMR (Mahieu et al., 1996; Hopkins et al., 1997; Schmidt et al., 1997; Knicker et al., 2002; Dieckow et al.,2005). Strong organo-mineral interaction between mineral surface and proteinaceous material may lead to the protection of organic N (Kleber et al., 2007).

The soil with more oxides content showed a smaller proteinaceous peak. Among our soils with high initial C content the intensity of this peak increased as the clay content decreased, but there were no significant relationships with soil characteristics (Figure 7). According to Pronk et al., (2013) soil N can be strongly bound to phylosilicate minerals and may not have any effect by metal oxides, corroborating the lower protection of proteinaceous compounds in soils that are richer in Fe and Al oxides.

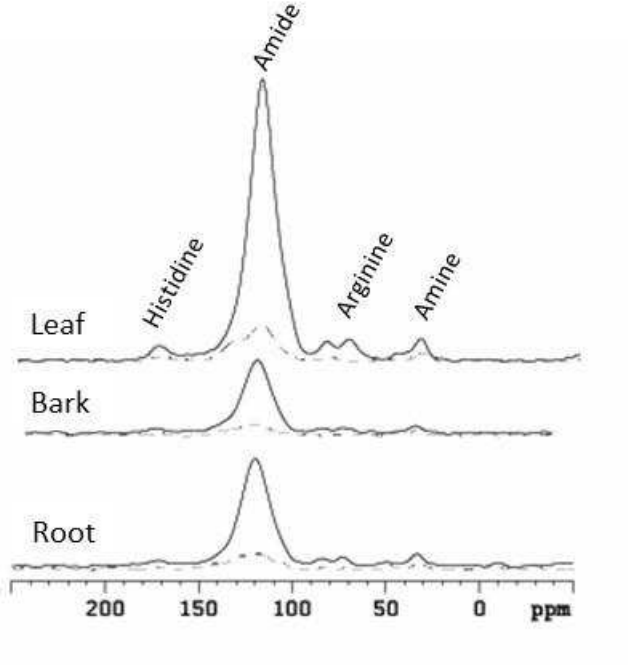

Figure 6. Full ^{15}N NMR spectrum (MultCP experiment) of double-labelled eucalypt residue individual components (leaf, bark, branch, wood and root) before incubation. Dashed lines represent spectra after 55-μs gated decoupling for dipolar dephasing

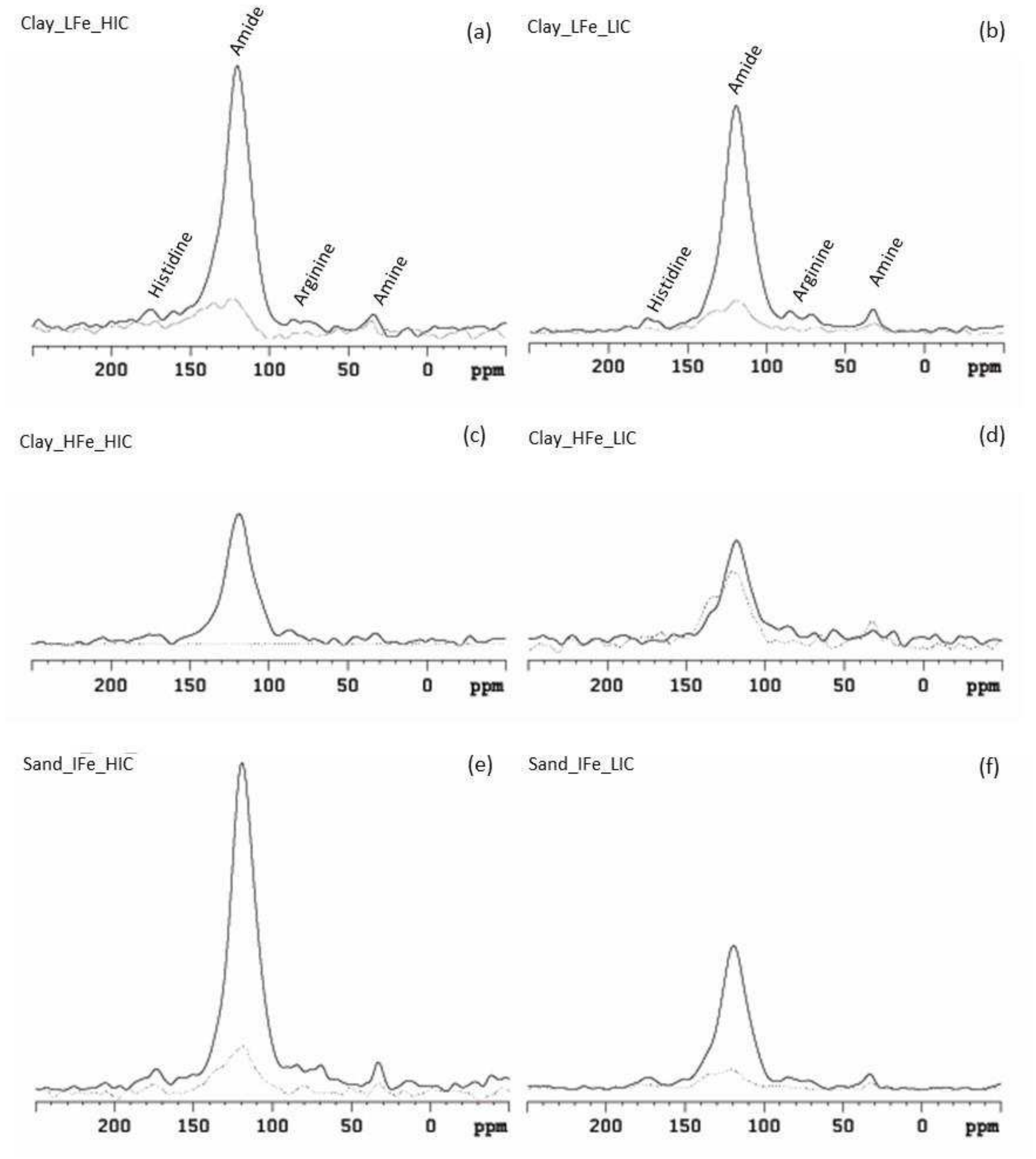

Figure 7. Full ^{15}N NMR spectrum (MultCP experiment) of soil organic matter associated organic matter associated to mineral after eucalypt residues incorporation from Clayey texture, with Low iron content (a, b); Clayey texture, with High iron content (c, d) Sandy texture, with Intermediate iron content (e, f). High carbon content initial (left column) and Low carbon (right column). In dashed lines spectrum after 40-μs gated decoupling for dipolar dephasing.

CONCLUSIONS

The functional groups predominant in the MAOM fraction appears to be related with the mineralogy and amorphous oxides of iron and aluminum than the soil texture and amount of oxides and the soil.

The group with the highest prevalence was the O-alkyl C (28%). Initially the existing C appears to be present in the charred materials which had relative contribution decreased by the addition of C derived from the residue of C, which contributed to the reduction the aromaticity of 29%.

The predominant compound-N was the amide and its contribution was higher in soils with higher initial C content, but was not influenced by the soil characteristics evaluated.

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APÊNDICE

Figura 1**.** Câmaras fechadas para avaliação do fluxo de CO_2 confeccionadas em placas de PVC ou aço galvanizado dotadas de coolers para homogeneização do ar interno(a) e aparelho CRDS conectados ao conversor de 12V para 127V e bateria veicular como de energia (b).

Figure 1. CO_2 efflux measuring chamber was made either in PVC or galvanized steel, it equipped with coolers for homogenization of the internal air (angel) and CRDS apparatus connected to 12V converter for 127V and car battery as energy source.

Figura 2**.** Tubos de acesso em profundidade foram confeccionados usando-se tubos de PVC de 50 mm de diâmetro seccionados no comprimento contemplando a camada de avaliação desejada com furos para equilíbrio da atmosfera do ar entre o tubo e solo (a). O isolamento entre cada camada foi realizado usando-se tarugos de náilon de 50 mm de diâmetro e o delineamento foi realizado em torno mecânico de forma que que o encaixe fosse perfeito (b). Tubo de acesso e em profundidade montado, para fixação do tarugo ao tubo de PVC foram utilizados rebites (c). Detalhe do tubo de acesso instalado no solo com a camada sob superfície com o cap de proteção (d) e sem o cap com as cinco mangueiras de cinco mm conectadas individualmente em cada profundidade (e).

Figure 2**.** Depth access tubes were made using PVC tubes of 50 mm diameter; it was sectioned covering layer with holes to keep the atmosphere air equilibrium between the tube and soil (a). The insulation between each layer was carried out using nylon billet 50 mm in diameter and the design was carried out in the lathe so that the fit were perfect (b). Access tube mounted, for fixing the billet to the PVC sections were used rivets (c). Details of the access tube installed on the ground with the surface layer under the protective cap (d) and without the cap with five hoses of 5 mm connected individually at each depth (e).

Figura 3. Mensuração da composição isotópica do CO_2 respirado pela raiz *in situ* usando-se tubo de PVC de 40 mm, na qual a raiz passa por uma borracha cilíndrica sem que ocorra rompimento da mesma com total vedação (a) e com cap dotado de septo (b) na qual foi conectado a agulha vinda do CRDS. A composição heterotrófica do CO_2 oriundo da respiração heterotrófica foi obtida por e meio da incubação do solo livre de raiz em potes de vidros e tampas dotadas de septo (c) com a coleta realizada por seringa de 60 mL e mensuradas no aparelho CRDS (d).

Figure 3. Isotopic composition measuring of CO2 root respiration in situ using 40 mm PVC tube, which passes the root through a cylindrical rubber without disruption and overall sealing (a) and equipped with a cap septum (b) where CRDS was connected. The composition of CO2 from heterotrophic respiration was obtained by means of incubation root free soil in pots of glass and equipped with septum lids (c) carried out by collecting 60 ml syringe and measured in CRDS (d).