

University of São Paulo
"Luiz de Queiroz" College of Agriculture

Soil organic matter dynamics in pasture-sugarcane land use conversions in south-central Brazil

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Thesis presented to obtain the degree of Doctor in
Science. Area: Soil and Plant Nutrition

Piracicaba
2017

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Brazil

versão revisada de acordo com a resolução CoPGr 6018 de 2011

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To those full of potential that will die in Brazilian slums without a single opportunity in life
I DEDICATE

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“The ultimate measure of a man is not where he stands in moments of comfort and convenience, but where he stands at times of challenge and controversy.”

Martin Luther King Jr.

“Even though I walk through the valley of deep darkness, I will fear no evil, for You are with me.”

Psalm 23:4

“The aesthetics of nature extend well beyond our primitive ability to write equations.”

Arthur Stewart

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RESUMO

Dinâmica da matéria orgânica do solo na conversão pastagem - cana-de-açúcar no Centro-Sul do Brasil

Alterações na matéria orgânica do solo (MOS) associadas à mudança de uso da terra (MUT) estão entre as principais fontes de incerteza em avaliações do ciclo de vida dos biocombustíveis. No Brasil, atualmente o maior produtor de cana-de-açúcar e o segundo maior produtor de bioetanol do mundo, os possíveis efeitos negativos da MUT geram questionamentos sobre a efetividade do bioetanol como uma opção sustentável. Grande parte da expansão da cana-de-açúcar ocorre em áreas de pastagem. Nesse sentido, conduziu-se um estudo no Centro-Sul do Brasil, a maior região produtora de cana-de-açúcar do mundo, objetivando-se avaliar os efeitos da MUT vegetação nativa - pastagem - cana-de-açúcar na MOS. A principal hipótese é que a conversão de pastagens degradadas para o cultivo da cana-de-açúcar melhore a qualidade e a quantidade da MOS. A conversão da vegetação nativa para pastagem induz significativas perdas de C no solo ($1,01 \text{ Mg ha}^{-1} \text{ ano}^{-1}$). Já a conversão dessas pastagens para cana-de-açúcar associa-se a ganhos de C, a uma taxa de $1,97 \text{ Mg ha}^{-1} \text{ ano}^{-1}$ até 1m de profundidade. Além disso, avaliações da MOS restritas a camadas superficiais relacionam-se a indesejáveis vieses em estudos de MUT. A principal diferença na composição molecular da MOS após a conversão de pastagens para cana-de-açúcar é o aumento na contribuição de formas menos estáveis de C, aspecto associado a alta adição de material vegetal ao solo em áreas de cana-de-açúcar sem queima. A conversão da vegetação nativa para pastagem diminui o C lábil (CL), bem como o índice de manejo de C (IMC), enquanto a conversão da pastagem para a cana-de-açúcar aumenta o IMC de acordo com todos os métodos avaliados. Nesse sentido, o método utilizado para quantificar o CL e o IMC é determinante ao se inferir sobre os efeitos da MUT na MOS. O modelo DayCent estimou que a conversão vegetação nativa-pastagem associa-se a perdas de C no solo de $0,34 \pm 0,03 \text{ Mg ha}^{-1} \text{ ano}^{-1}$, enquanto a conversão pastagem - cana-de-açúcar associa-se a ganhos de C a $0,16 \pm 0,04 \text{ Mg ha}^{-1} \text{ ano}^{-1}$ na camada de 0-0,3 m. Além disso, simulações mostraram decréscimos de $0,19 \pm 0,04 \text{ Mg ha}^{-1} \text{ ano}^{-1}$ do C do solo nas áreas de cana-de-açúcar com remoção de palha para produção de etanol de segunda geração (2G). No entanto, a adoção de algumas práticas de manejo podem mitigar essas perdas, com destaque para a aplicação de vinhaça e torta de filtro ($+0,14 \pm 0,03 \text{ Mg C ha}^{-1} \text{ ano}^{-1}$). Nosso estudo mostrou que a conversão de pastagens para cana-de-açúcar apresenta efeitos positivos na qualidade e na quantidade da MOS, favorecendo o balanço de C do etanol brasileiro. Nossos resultados endossam o potencial da cana-de-açúcar em recuperar, parcialmente, os estoques de C em pastagens degradadas. No entanto, esses ganhos são altamente dependentes da alta adição de resíduos vegetais nas áreas de cana-de-açúcar, e a remoção de palha para produção de etanol 2G poderá afetar a MOS em áreas de expansão. Por fim, com base na disponibilidade de áreas e nos efeitos positivos sobre a MOS, meios para estimular a expansão da cana-de-açúcar em áreas de pastagem degradadas no Brasil devam ser considerados.

Palavras-chave: Expansão da cana-de-açúcar; Biocombustíveis; Estoques de C no solo; Particionamento de C; Qualidade da matéria orgânica do solo

ABSTRACT

Soil organic matter dynamics in pasture-sugarcane land use conversions in south-central Brazil

Land use change (LUC) induces modifications on soil organic matter (SOM), which is one of the main source of uncertainty in life cycle assessments of biofuels. In Brazil, currently the world largest producer of sugarcane and second biggest producer of bioethanol, the potential negative effects of LUC has raised doubts about the sugarcane ethanol as a sustainable option. Recently, most of sugarcane expansion has been placed over extensive pastures. Therefore, we conducted a field study within the south-central Brazil, the largest sugarcane-producing region in the world, to evaluate the effects of the most common LUC sequence in sugarcane expansion areas (i.e., conversions from native vegetation to pasture and from pasture to sugarcane) on SOM. Our main hypothesis is that the conversion of degraded pastures to unburnt sugarcane enhance SOM quantity and quality from sites in Brazil. Long-term conversion from native vegetation to pasture induced significant C stock losses ($1.01 \text{ Mg ha}^{-1} \text{ yr}^{-1}$). In contrast, the conversion from pasture to sugarcane increased C stocks at a rate of $1.97 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ down to 0-1.0 m depth. In addition, our findings indicated that SOM assessments restricted to the surface soil layers can generate bias in studies regarding LUC. The main difference in SOM molecular composition undergoing the conversion pasture-sugarcane was the notably higher contribution from compounds associated to fresh litter inputs in sugarcane areas, probably related to the high litter input in sugarcane fields under green management in Brazil. The conversion of areas under native vegetation to pasture decreases both the labile C (LC) and the C management index (CMI), whilst the conversion of pasture to sugarcane increased the CMI according to all evaluated methods. Additionally, the method used to quantify LC and CMI is critical to infer about the LUC effects on SOM. The DayCent model estimated that the conversion native vegetation-pasture caused C losses of $0.34 \pm 0.03 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, whilst the conversion pasture-sugarcane resulted in C gains of $0.16 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ down to 0.3 m depth. Moreover, simulations showed C decreases of $0.19 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ in sugarcane areas with straw removal for second-generation (2G) ethanol production. However, our analysis suggested that adoption of some best management practices can mitigate these losses, highlighting the application of organic amendments ($+0.14 \pm 0.03 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$). Overall, our study showed that the conversion of pastures to sugarcane has positive effects on SOM quantity and quality, increasing the C savings of Brazilian sugarcane ethanol. Moreover, our findings endorse the potential of sugarcane production to partially recover SOM in degraded pastures. However, most of these gains greatly depends on the high litter input in sugarcane fields under green management, and straw removal for 2G ethanol production is likely to potentially affect SOM in areas of sugarcane expansion in Brazil. Finally, based on land availability and positive effects on SOM, we believe that stakeholders involved with the governance of bioethanol expansion should consider ways to incentivize sugarcane expansion on degraded pastures in Brazil.

Keywords: Sugarcane expansion; Biofuels; Soil C stocks; C-partitioning; Soil organic matter quality

1. GENERAL INTRODUCTION

As population grows, humans have increasingly distorted the C cycle. Our two main influences in the C cycle have been (i) changes in land use, and (ii) fossil fuels combustion (Janzen, 2004); with crop-based biofuels playing a key role in both processes. Biofuels are critical for environmental security and climate change mitigation. Future projections suggest that 30% of the world's fuel supply might be bio-based by 2050 (Macedo *et al.*, 2015). However, despite being promoted as clean energy alternatives, environmental issues related to land use change (LUC) has raised controversies about the sustainability of crop-based biofuels. The relevance of LUC has been emphasized by several authors, especially in relation to political decisions made for increasing biofuel production (Hudiburg *et al.*, 2016, Lapola *et al.*, 2010).

The negative effects of LUC brought out concerns about the efficiency of the sugarcane ethanol as a climate change mitigation option (Fargione *et al.*, 2008, Mello *et al.*, 2014). However, sugarcane ethanol shows the largest average net GHG mitigation compared to other first-generation ethanol feedstocks (Renouf *et al.*, 2008). Nowadays, Brazil is considered to have developed the world's first sustainable biofuel economy and in many respects is the biofuel industry leader (Souza *et al.*, 2014). This reputation is largely based on its sugarcane industry.

Recently, most of sugarcane expansion has been placed over extensive pastures in Brazil (Fig. 1). The replacement of pastures, besides being the main current strategy for sugarcane expansion, is the most likely future scenario for cropped-area increments in Brazil. Government policies for the sustainable intensification of Brazilian livestock production can effectively make large amounts of land available to establishment of crops (ABC Brazil, 2012). Since deforestation is no longer a feasible option (more effective law enforcement and market regulation), the land sparing by livestock intensification is expected to be the main approach for the sugarcane projected expansion in Brazil in the next years.

LUC induces modifications on soil organic matter (SOM), which is one of the main source of uncertainty in life cycle assessments of biofuels (Qin *et al.*, 2016). The C stored in the soil, which globally is more than three times the amount of C in the atmosphere (Batjes, 1996), plays a key role in C cycling (Cotrufo *et al.*, 2011, Janzen, 2004). The influence that LUC has on SOM is a key component of assessing sustainability within a biofuels context. Some previous studies have indicated that biofuels crop expansion may result in SOM losses, which is particularly troubling from a climate change perspective, since biofuels are supposed to be a mitigation option. Moreover, besides being a source for increased biogenic CO₂ emissions, decreases in the quantity and quality of SOM can reduce agricultural productivity and food

security, particularly in tropical regions (Lal, 2006). Finally, this C balance greatly depend on the previous land use replaced, and the replacement of degraded lands (as most of Brazilian pastures) with biofuel crops may result in a positive C balance (Gelfand *et al.*, 2013, Gollany *et al.*, 2015).

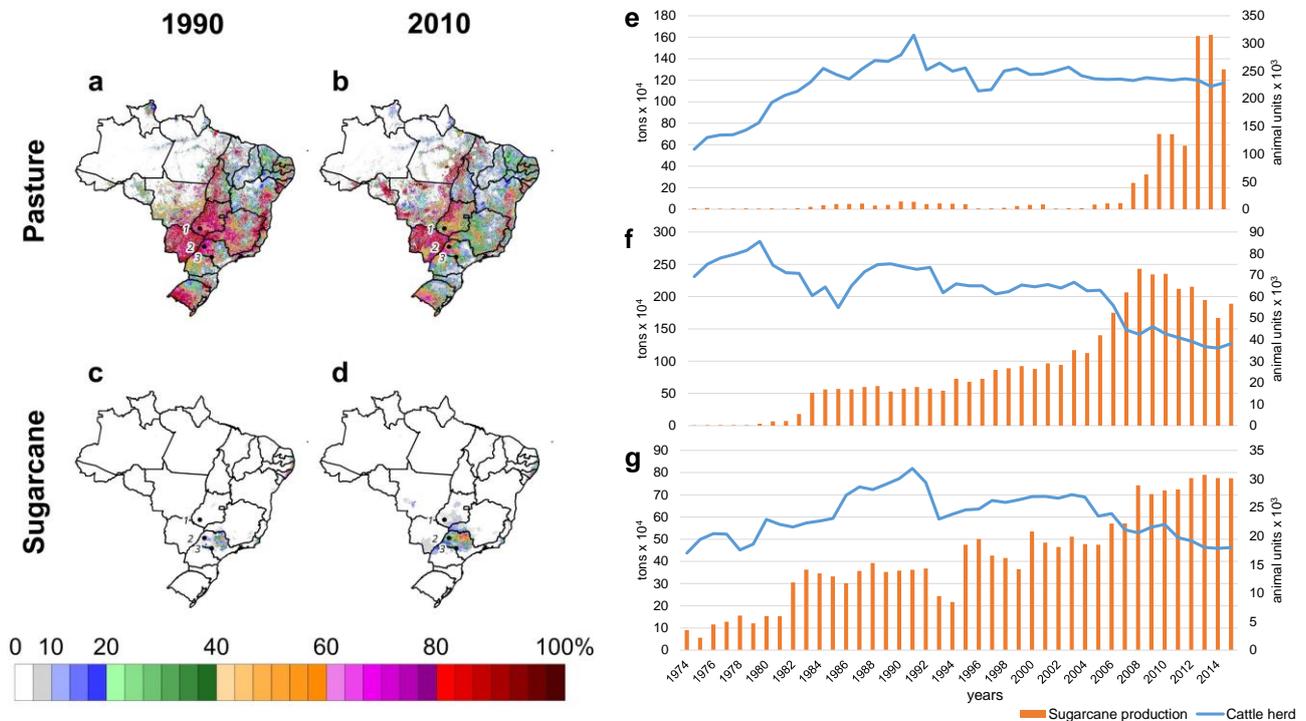


Figure 1. Pasture and sugarcane land-use change patterns in Brazil in the last years. **a-d:** Most of recent sugarcane expansion has been placed over extensive pastures (Dias *et al.*, 2016). **e-g:** Over the last years, there is a tendency of contraction in the cattle herd followed by an increment on sugarcane production, mainly in the municipalities surrounding our study sites Lat_21S (Region 2, **f**) and Lat_23S (Region 3, **g**) (IPEA, 2017).

Therefore, we conducted a field study within the south-central Brazil (Fig. 1), the largest sugarcane-producing region in the world, to evaluate the effects of the most common LUC sequence in sugarcane expansion areas (i.e., conversions from native vegetation to pasture and from pasture to sugarcane) on SOM. Our main hypothesis is that the conversion of degraded pastures to unburnt sugarcane enhance the SOM quantity and quality from sites in Brazil. Specifically, we aimed to (i) quantify the soil C and N stocks, and isotopic composition in native vegetation, pasture and sugarcane areas down to 1.0 m depth; (ii) evaluate the SOM molecular composition and how it shifts in sites undergoing LUC; (iii) assess alterations on labile C (LC) contents and C management index (CMI) in areas of sugarcane expansion, and evaluated the sensitivity of different methods commonly used to quantify LC and CMI; and (iv) predict the impact of unburnt sugarcane expansion into pasture areas, as well as to evaluate the effect of different management practices, such as straw removal, no-tillage and application of organic amendments on long-term SOM changes in sugarcane areas in Brazil using the DayCent model.

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2. SOIL CARBON CHANGES IN AREAS UNDERGOING EXPANSION OF SUGARCANE INTO PASTURES IN SOUTH-CENTRAL BRAZIL

ABSTRACT

In Brazil, the recent sugarcane expansion for ethanol production has been predominantly on areas previously used as pasture. Losses of C and N induced by land use change raise controversies about the environmental suitability of biofuel production. Therefore, we conducted a field study within the largest sugarcane-producing region of Brazil to evaluate the effects of the primary land use change (LUC) sequence in sugarcane expansion areas (i.e., native vegetation to pasture to sugarcane), on C and N dynamics in the top 1 meter soil layer. The LUC sequences caused substantial but varying changes in soil C and N stocks in areas undergoing expansion of sugarcane in south-central Brazil. The increase of C stocks in areas converted from pasture to sugarcane cultivation was $1.97 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, in contrast to conversion of native vegetation to pasture, which decreased soil C stocks by $1.01 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ for 0-1.0 m soil layer. The use of ^{13}C measurements to partition soil C sources showed that the greater C stocks in sugarcane areas compared to pasture was due to the reduction on the rates of native-C losses and increased accrual of modern-C comparing to pasture. Finally, the inclusion of deeper soil layers, at least down to 1.0 m depth, is essential to assess the impacts of LUC on C balances in agricultural areas.

Keywords: Land use change; Soil organic matter; C and N isotopes; Sugarcane ethanol; Biofuels

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2.1. Introduction

The ethanol derived from 1 ha of sugarcane avoids the emission of about $14 \text{ Mg CO}_2 \text{ eq yr}^{-1}$ relative to the use of fossil fuels (Betts, 2011). When compared to other “first-generation” ethanol feedstocks such as corn, sugarcane is the most effective in mitigating greenhouse gases

(GHG) emissions (Renouf *et al.*, 2008). As a result, the global demand for sugarcane ethanol is rising (Goldemberg *et al.*, 2014).

In Brazil, the largest producer of sugarcane ethanol in the world, the area cropped to sugarcane is expanding and the most pervasive scenario of land use change (LUC) is the conversion of pastures into sugarcane fields (Goldemberg *et al.*, 2014, Lapola *et al.*, 2014). Replacing pastures with sugarcane fields has been deemed an agronomically-feasible strategy for sugarcane expansion in Brazil. According to the Brazilian sugarcane Agroecological Zoning (Manzatto *et al.*, 2009), there are 64 Mha suitable for sugarcane production, of which 34 Mha correspond to abandoned areas or degraded pastures (Walter *et al.*, 2014). In addition, current government policies to enhance livestock productivity (ABC Brazil, 2012) could release grazed pasture area to sugarcane production without reducing food production or pressuring natural ecosystems (Goldemberg *et al.*, 2014).

Land use change induces modifications of soil organic matter (SOM), which is one of the main sources of uncertainty in life cycle assessments of biofuels (Qin *et al.*, 2016). The C stored in the soil, which globally is more than three times the amount of C in the atmosphere (Batjes, 1996), plays a key role in GHG dynamics (Cotrufo *et al.*, 2011, Janzen, 2004). Several studies have demonstrated that the conversion from natural ecosystems to croplands or pastures decreases soil C stocks (Assad *et al.*, 2013, Don *et al.*, 2011, Eclesia *et al.*, 2012), raising controversies about the environmental suitability of biofuels crops (Lapola *et al.*, 2010). On the other hand, some studies have reported no net loss or even increases in soil C stocks with the replacement of natural vegetation to pastures in Brazil (Braz *et al.*, 2013, Maia *et al.*, 2009).

Isotopic techniques have been applied in studies of C dynamics, where forests with C₃ photosynthetic pathway plants are replaced by C₄ plants such as tropical forages and sugarcane (Assad *et al.*, 2013, Franco *et al.*, 2015, Rossi *et al.*, 2013), to determine the soil C origin using $\delta^{13}\text{C}$ values. The determination of original C losses from natural vegetation and its replacement by the C derived from pasture or sugarcane is critical to understand the complex dynamics of C in soils following LUC. Although interpretation of $\delta^{15}\text{N}$ as a measure of impacts of LUC on N cycling and SOM dynamics in tropical agroecosystems is much more complex, some general inferences can be made (Lerch *et al.*, 2011).

In a recent study, the conversion of pasture to sugarcane was found to produce a net C emission of $1.3 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ over 20 years (considering the 0-0.3 m soil layer), primarily due to the loss of SOM from C₄-cycle plants (Franco *et al.*, 2015). However, the influence of LUC for sugarcane expansion on SOM dynamics and C-origin in deeper soil layers remains unclear.

Therefore, we conducted a field study within the largest sugarcane-producing region of Brazil to evaluate the effects of the most common LUC sequence in sugarcane expansion areas (i.e., conversions from native vegetation to pasture and from pasture to sugarcane), on C and N losses and inputs for the 0-1.0 m soil depth. Specifically, our aims were: i) to compare C and N stocks among different land uses; ii) to assess SOM alterations induced by conversions of native vegetation into pastures and then from pastures into sugarcane using C-partitioning, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values; and iii) to evaluate the rates of C stocks changes in pasture and sugarcane soils through the soil profile.

2.2. Material and Methods

2.2.1. Description of study sites

The study sites were located in three strategic and representative locations in the south-central Brazil, the main sugarcane producing region of the world (Fig. 1). The first site, Lat_17S, is located in Jataí, southwestern region of Goiás state (Lat.: 17°56'16"S; Long.: 51°38'31"W) with a mean altitude of 800 m and a predominance of clayey Acrudox soils (USDA, 2014). The climate classification is Awa type (Köppen) mesothermal tropical, with a mean annual temperature of 24.0 °C and an annual precipitation of 1,600 mm, with a dry season in the winter (May to September). The second site, Lat_21S, is located in Valparaíso, west region of São Paulo state (Lat.: 21°14'48"S; Long.: 50°47'04"W) with a mean altitude of 425 m and predominance of loamy Hapludalf soils (USDA, 2014). The climate classification is Aw type (Köppen classification) humid tropical, with rains concentrated in the summer (October to April) and a dry season in the winter (April to September). The area has a mean annual temperature of 23.4 °C and an annual precipitation of 1,240 mm. The third site, Lat_23S, is located in Ipaussu, south-central region of the São Paulo state (Lat.: 23°05'08" S; Long.: 49°37'52" W), with a mean altitude of 630 m and predominance of clayey Hapludox soils (USDA, 2014). The climate classification is Cwa type (Köppen) tropical, with rains concentrated in the summer (October to April) and a dry season in winter (May to September). The annual mean temperature is 21.7 °C and the annual precipitation is 1,470 mm. More information about soil parent material and soil classification is available in Cherubin *et al.* (2015).

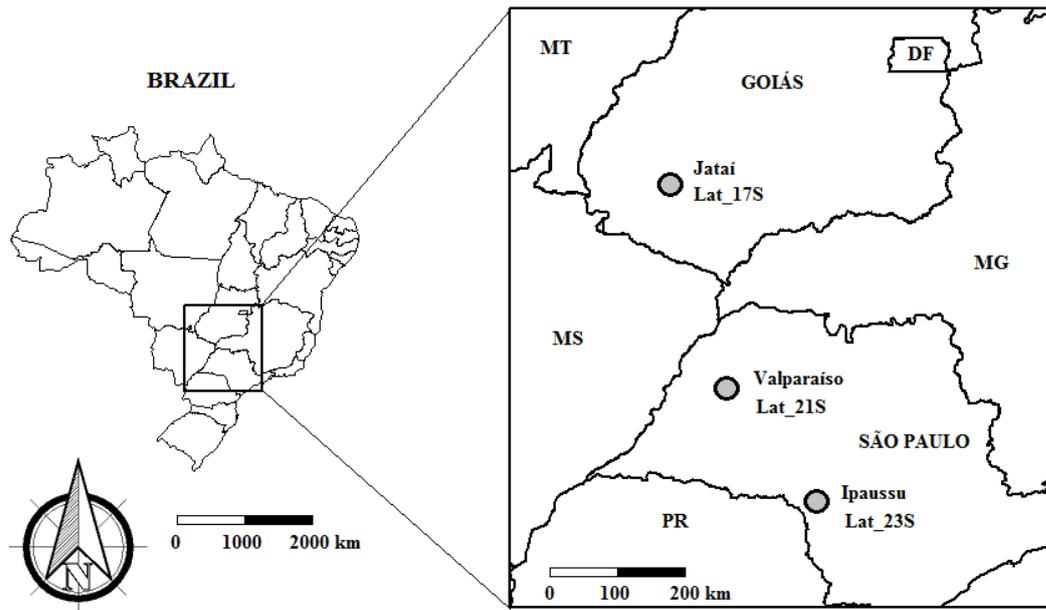


Figure 1. Geographic location of the study sites in south-central Brazil (Cherubin *et al.*, 2015).

2.2.2. Land use change sequence and sampling design

In this study, we used a chronosequence approach, comprising three land uses in each of the three sites: native vegetation (NV), pasture (PA) and sugarcane (SG), representing the most common LUC sequence in south-central Brazil. Chronosequences were used because there are no long-term experiments that represent this LUC sequence. The three land uses are located adjacent to each other, minimizing differences in climatic, topographic and soil characteristics. The general description of each land use site are showed in Table 1.

Soil sampling was carried out in January 2014. The sampling design in the three land uses in each study site was a sampling grid with nine points, 50 meters away from each other. In the three first soil layers (0-0.1, 0.1-0.2, 0.2-0.3 m), the samples were collected from the sidewall of pits (0.6 x 0.6 x 0.3 m). Deeper soil layers (0.3-0.5, 0.5-0.7, 0.7-0.9, 0.9-1.0 m) were sampled using a Dutch auger. Litter samples were taken at these same points. In each land use, one pit (2.0 x 2.0 x 1.0 m) was opened to collect undisturbed soil samples using Kopeck rings at 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5, 0.5-0.7, 0.7-0.9, 0.9-1.0 m soil layers, with three replications at each layer, to determine soil bulk density, which was used to calculate C and N stocks. For more information about LUC sequence and sampling, see Cherubin *et al.* (2015).

Table 1. Historical characterization and brief description of studied sites in south-central Brazil.

Site	Land use	Description ^a
Lat_17S	Native vegetation	Cerradão forest formation, Cerrado biome, characterized by sclerophyllous and xeromorphic species. The vegetation is dense compared to the Cerrado <i>stricto sensu</i> (savana).
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Brachiaria</i> and supports 1.5 AU ha ⁻¹ full year.
	Sugarcane	Conversion from pasture at 2009. Cultivar RB855453 with mean yield of 81.5 ton ha ⁻¹ . Conventional tillage procedures and chemical fertilization. At the sampling time, sugarcane was in the third ratoon cropping of its cycle. Sugarcane is mechanically harvested without burning since its implantation.
Lat_21S	Native vegetation	The local vegetation is seasonal semideciduous forest, Atlantic forest biome, in which a portion of the trees defoliates during the dry season.
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Brachiaria</i> and supports 2 AU ha ⁻¹ full year. Annually 120 kg ha ⁻¹ of the fertilizer formulation 20:5:19 are applied.
	Sugarcane	Conversion from pasture at 2010. Cultivar SP791011 with a mean yield of 80 ton ha ⁻¹ . Conventional tillage procedures and chemical fertilization + vinasse application. At the sampling time sugarcane was in the fourth ratoon cropping of its cycle. Mechanically harvested without burning since its implantation.
Lat_23S	Native vegetation	The local vegetation is seasonal semideciduous forest, Atlantic forest biome, in which a portion of the trees defoliates during the dry season.
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Cynodon spp.</i> and supports 1 AU ha ⁻¹ full year.
	Sugarcane	Conversion from pasture at 1990. Cultivar CTC6 with a mean yield of 85 ton ha ⁻¹ . Conventional tillage procedures and chemical fertilization + vinasse and filtercake application. At the sampling time sugarcane was in the fifth ratoon of its cycle. Pre-harvest burning between 1990 and 2002. Since 2013, 50% of straw has been removed for energy production.

^a For further details about land use description, see Cherubin *et al.* (2015). AU: animal units.

2.2.3. Carbon and nitrogen stocks and isotopic abundance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in soil

Soil C and N content and isotope composition of C and N were determined using an elemental analyzer and mass spectrometer (Thermo Finnigan Delta Plus Spectrometer, Waltham-USA). Results were expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) using PDB-Vienna as reference for ^{13}C levels and air composition for ^{15}N .

The equivalent soil mass technique, which adjusts for different soil mass differences between land uses, was applied to calculate C and N stocks, as described in Equation 1 (Ellert & Bettany, 1995):

$$C = \sum_{i=1}^{n-1} (C_{Ti} \cdot M_{Ti}) + \left[M_{Tn} - \left(\sum_{i=1}^n M_{Ti} - \sum_{i=1}^n M_{Si} \right) \right] C_{Tn} \quad (1)$$

where C = total soil C stocks on a mass equivalent basis for the soil profile under land use change, C_{Ti} = carbon content (Mg C Mg soil^{-1}) in each layer i above the deepest layer n , M_{Ti} = soil mass (Mg ha^{-1}) in each layer i above the deepest layer n , M_{Tn} = soil mass (Mg ha^{-1}) in the deepest layer of the soil profile under changed land use, C_{Tn} = carbon content (Mg C Mg soil^{-1}) in the deepest layer of the soil profile under changed land use and M_{Si} = soil mass (Mg ha^{-1}) in each layer i under native vegetation. The same procedure was performed to calculate N stocks considering equivalent soil mass among the three land uses.

The conversion factors (CF) for the conversion from native vegetation to pasture and then to sugarcane (NV-PA-SG) was calculated considering the changes on soil C stocks, with the native vegetation as reference, as described in Equation 2:

$$CF = CM / CNV \quad (2)$$

where CF = conversion factor; CM = soil C stocks in modern land uses (PA or SG) (Mg ha^{-1}); CNV = soil C stocks in NV (Mg ha^{-1}).

The rate of C stock change associated with both LUCs (NV-PA and PA-SG) was calculated considering the difference in C stocks between the current and the previous land use, as described in Equation 3:

$$\Delta C_{LUC} = \frac{C_c - C_p}{t_{LUC}} \quad (3)$$

where ΔC_{LUC} = rate of C stocks change after land use change ($\text{Mg ha}^{-1} \text{ yr}^{-1}$); C_c = soil C stocks in the current land use (Mg ha^{-1}); C_p = soil C stocks in the previous land use (Mg ha^{-1}); t_{LUC} = time since land use conversion (years).

Using $\delta^{13}\text{C}$ of soil and litter samples, we estimated the proportion of C from native vegetation (NC) and the proportion of modern carbon (MC) in the soils of pasture and sugarcane areas according to the following isotopic dilution equation (Equation 4):

$$MC = \frac{\delta^{13}C_{soilM} - \delta^{13}C_{ltNV}}{\delta^{13}C_{ltM} - \delta^{13}C_{ltNV}} \times 100 \quad (4)$$

where MC = proportion of modern carbon (%); $\delta^{13}C_{soilM}$ = C isotopic composition of SOM (‰); $\delta^{13}C_{ltNV}$ = C isotopic composition of the native vegetation litter (‰); $\delta^{13}C_{ltM}$ = the carbon isotopic composition of the modern litter (‰). The NC was estimated as $100 - MC$.

The term “modern” refers to pasture and sugarcane land uses. The closeness between the carbon isotopic composition of pasture litter and sugarcane straw makes impossible the distinction of these sources in the sugarcane areas using an isotope mixing model (Phillips & Gregg, 2003). Thereby, the most feasible approach was to group the sugarcane straw and the pasture litter as the same source. Therefore, in sugarcane areas we assumed that the $\delta^{13}C_{ltM}$ is the mean of sugarcane $\delta^{13}\text{C}$ straw and pasture $\delta^{13}\text{C}$ litter, with values of -11.78, -12.77 and -12.42 ‰ for Lat_17S, Lat_21S and Lat_23S sites, respectively.

2.2.4. Data analysis

Comparisons between land uses (NV, PA and SG) were carried out for C stocks, N stocks and C-origin using one-way ANOVA with land use type as the main factor and sites considered as blocks and treated as a random variable. The normality distribution of the data was confirmed by Shapiro-Wilk test. The means between land uses were compared (considering each site as a block) by Tukey test ($p < 0.05$) using Statistical Analysis System – SAS v.9.3 (SAS Inc, Cary, USA).

2.3. Results

2.3.1. Carbon and nitrogen stocks

There were significant alterations of C and N stocks due to LUC (Table 2). In Lat_17S and Lat_21S, the LUC caused similar alterations on C stocks: conversions from NV to PA decreased C stocks; in contrast, conversion from PA to SG tended to increase C stocks. These C changes were uniformly observed throughout soil profiles. At Lat_23S site, the C stock in NV (212 Mg ha^{-1} for 0-1.0 m) was the highest for all land uses. While PA tended to show higher C stocks than SG for 0-0.3 and 0-0.5 m layers, total C stocks to 1 m depth were higher under SG. Overall, the C stocks in these three sites were higher for SG when compared to PA for the 0-1.0 m soil layer. At the regional scale, NV had higher C stocks than PA and SG for all depths evaluated. Furthermore, PA and SG had no differences for C stocks for 0-0.3 and 0-0.5 m layers. However, SG fields had 17% higher C stocks compared to PA for 0-1.0 m layer (Table 2). Regarding N stocks, values in NV areas were higher than PA and SG fields for all depths assessed, which did not differ from each other (Table 2).

Table 2. C stocks and N stocks (Mg ha^{-1}) in three soil depths (0-0.3, 0-0.5 and 0-1.0 m) of areas under different land uses - native vegetation (NV), pasture (PA), sugarcane (SG) - in three sites (Lat_17S, Lat_21S, Lat_23S) and regional scale in south-central Brazil.

Site	Land Use	C stocks	N stocks	C stocks	N stocks	C stocks	N stocks
		Mg ha ⁻¹					
		0-0.3 m		0-0.5 m		0-1.0 m	
Lat_17S	NV	49.1±3.5 ^a	3.0±0.3	64.9±5.1	4.2±0.3	94.3±4.3	5.8±0.6
	PA	37.17±3.2	1.7±0.2	46.4±3.9	2.3±0.2	72.3±5.1	3.8±0.2
	SG	38.15±2.1	2.3±0.2	52.3±2.9	3.0±0.3	91.0±5.4	5.4±0.5
Lat_21S	NV	48.6±3.2	4.7±0.5	57.7±5.8	5.5±0.6	82.7±8.7	7.4±0.4
	PA	37.2±2.8	2.6±0.2	47.6±3.6	3.1±0.3	72.4±4.1	4.7±0.2
	SG	40.1±3.1	3.1±0.3	51.6±4.8	3.7±0.3	76.6±4.7	5.6±0.6
Lat_23S	NV	89.9±8.5	7.4±0.5	123.1±10.4	9.7±1.0	212.0±16.2	14.8±1.1
	PA	76.9±6.7	6.1±0.4	96.9±8.2	7.9±0.6	141.9±11.4	10.8±0.8
	SG	60.5±4.2	4.5±0.3	85.6±6.6	6.0±0.4	167.2±12.2	10.6±0.7
Regional	NV	62.5a ^b	5.2a	81.9a	6.4a	129.7a	9.3a
	PA	50.4b	3.5b	63.6b	4.4b	95.5c	6.4b
	SG	46.5b	3.3b	63.2b	4.2b	111.6b	7.2b

^a Standard deviation from the mean values (n=9, except to regional scale). ^b Letters represent statistically significant differences between land uses in the regional scale (considering each site as a block, n =27), according the Tukey test (5%).

2.3.2. Conversion factor (CF) and rates of carbon stock changes

The CF values were less than 1.0 for all soil depths, indicating that the LUC sequence caused C stocks losses at all sites (Fig. 2). For the 0-0.3 layer, the C stock reductions ranged from 14.4% (Lat_23S) to 24.3% (Lat_17S) for the conversion NV-PA, and ranged from 16.2% (Lat_21S) to 32.6% (Lat_23S) for the complete LUC sequence (NV-PA-SG). At the regional scale, the CF values were 0.81 and 0.74 for the 0-0.3 m soil layer for NV-PA and NV-PA-SG, respectively (Fig. 2). For the 0-0.5 m soil layer, CF values were 0.78 and 0.77 for the conversions NV-PA and NV-PA-SG, respectively. Overall, the conversions NV-PA and NV-PA-SG, when calculated to 1 m depth, showed CF values of 0.74 and 0.86, respectively (Fig. 2).

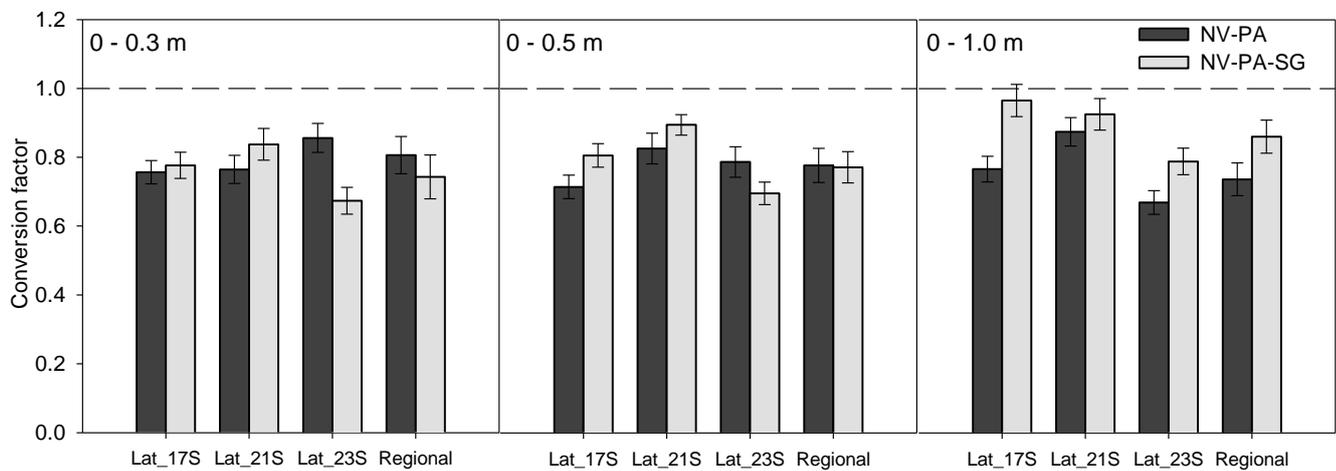


Figure 2. Conversion factors derived for changes in the C stocks associated with the conversion from native vegetation to pastures (NV to PA) and conversion from native vegetation to pasture and then conversion from pastures to sugarcane (NV to PA to SG) for three soil depths (0-0.3, 0-0.5 and 0-1.0 m) in three sites (Lat_17S, Lat_21S, Lat_23S) and regional scale in south-central Brazil. The baseline are the C stocks in NV. Bars represent the standard deviation from the mean values (n=9), except to regional scale, which each site was considered as a block (n=27).

The conversion NV-PA led to significant depletion of C stocks, with loss rates of 0.36, 0.34 and 0.38 Mg ha⁻¹ yr⁻¹ in Lat_17, Lat_21S and Lat_23S, respectively; or an average of 0.36 Mg ha⁻¹ yr⁻¹ at the regional scale for the 0-0.3 m soil layer (Fig. 3). In this same layer, excepting Lat_23S, the LUC PA-SG showed positive rates of C stock change. At the regional scale, the conversion PA-SG increased C stocks at a rate of 0.12 Mg ha⁻¹ yr⁻¹ for the 0-0.3 m layer (Fig. 3). For the 0-0.5 m layer, the rates followed similar patterns to those observed for the 0-0.3 m layer (Fig. 3), which the conversion from NV-PA decreased C stocks by 0.54 Mg ha⁻¹ yr⁻¹ at regional scale. In contrast, SG replacing PA increased soil C stocks at a rate of 0.56 Mg ha⁻¹ yr⁻¹.

The LUC NV-PA induced significant decreases in C stocks in the 0-1.0 m layer at all sites, with the highest rate of 2.06 Mg ha⁻¹ yr⁻¹ at Lat_23S (Fig. 3). On the other hand, the LUC

PA-SG increased C stocks for all sites. At the regional scale, the conversion NV-PA promoted C stocks depletion of $1.01 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, while the SG cultivation replacing PA areas led to increases of $1.97 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ on C stocks for 0-1.0 m soil layer.

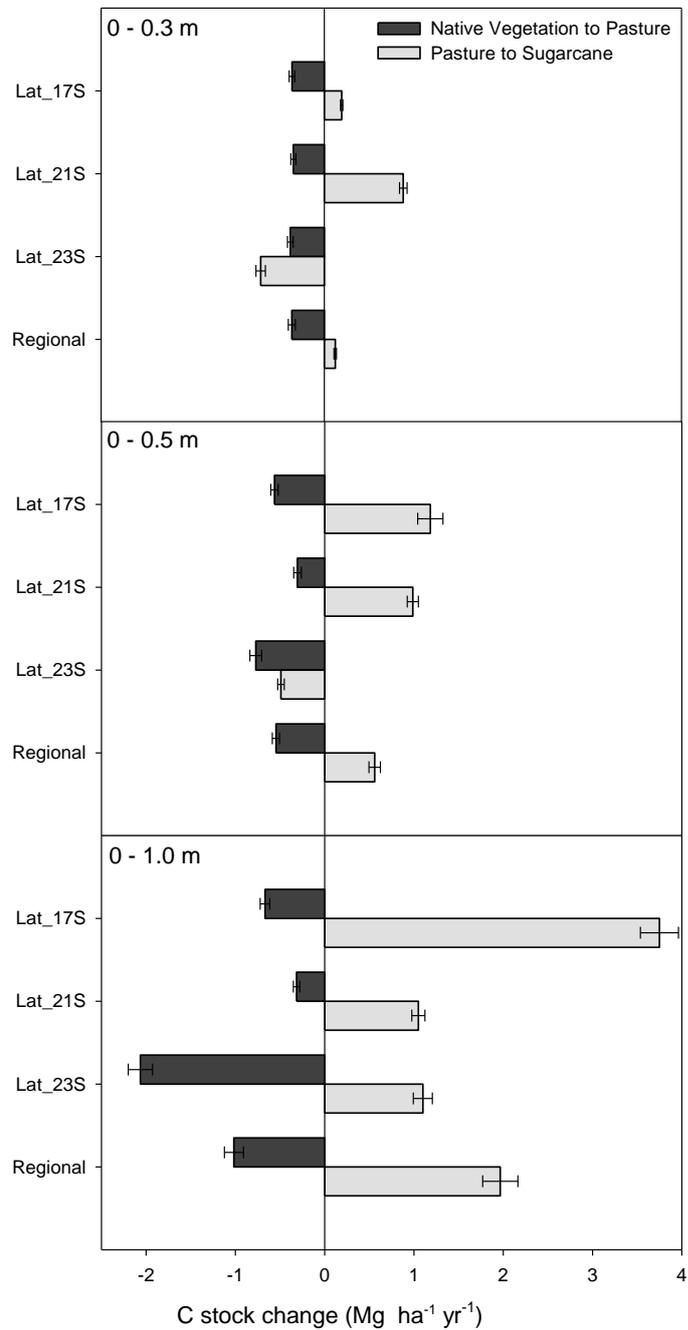


Figure 3. Rates of soil carbon stock change ($\text{Mg ha}^{-1} \text{ yr}^{-1}$) associated with conversions from native vegetation to pasture and conversions from pasture to sugarcane for three soil depths (0-0.3, 0-0.5 and 0-1.0 m) in three sites (Lat_17S, Lat_21S, Lat_23S) and in regional scale in south-central Brazil. Bars represent the standard deviation from the mean values ($n=9$), except to regional scale, which each site was considered as a block ($n=27$).

2.3.3. C and N isotopes abundance and C partitioning

Native vegetation soils showed typical values of $\delta^{13}\text{C}$ for areas with C3 plants (Fig. 4). An increase in ^{13}C values with depth was observed in all sites, especially at Lat_17S, where there was an increase of 3 ‰ between the surface (0-0.1 m) and deepest (0.9-1.0 m) soil layers. The LUC from natural ecosystems to agricultural lands (PA and SG) affected significantly soil $\delta^{13}\text{C}$ values (Fig. 4). The conversion NV-PA increased the $\delta^{13}\text{C}$ in the soils in all sites evaluated, especially for surface soil layers. Below 0.5 m, the $\delta^{13}\text{C}$ values decreased in PA, but still remained higher than those observed in NV soils. The conversion PA-SG further increased $\delta^{13}\text{C}$ values in the soil. However, the $\delta^{13}\text{C}$ values observed in SG did show a notable decrease in depth as observed in PA soils (Fig.4).

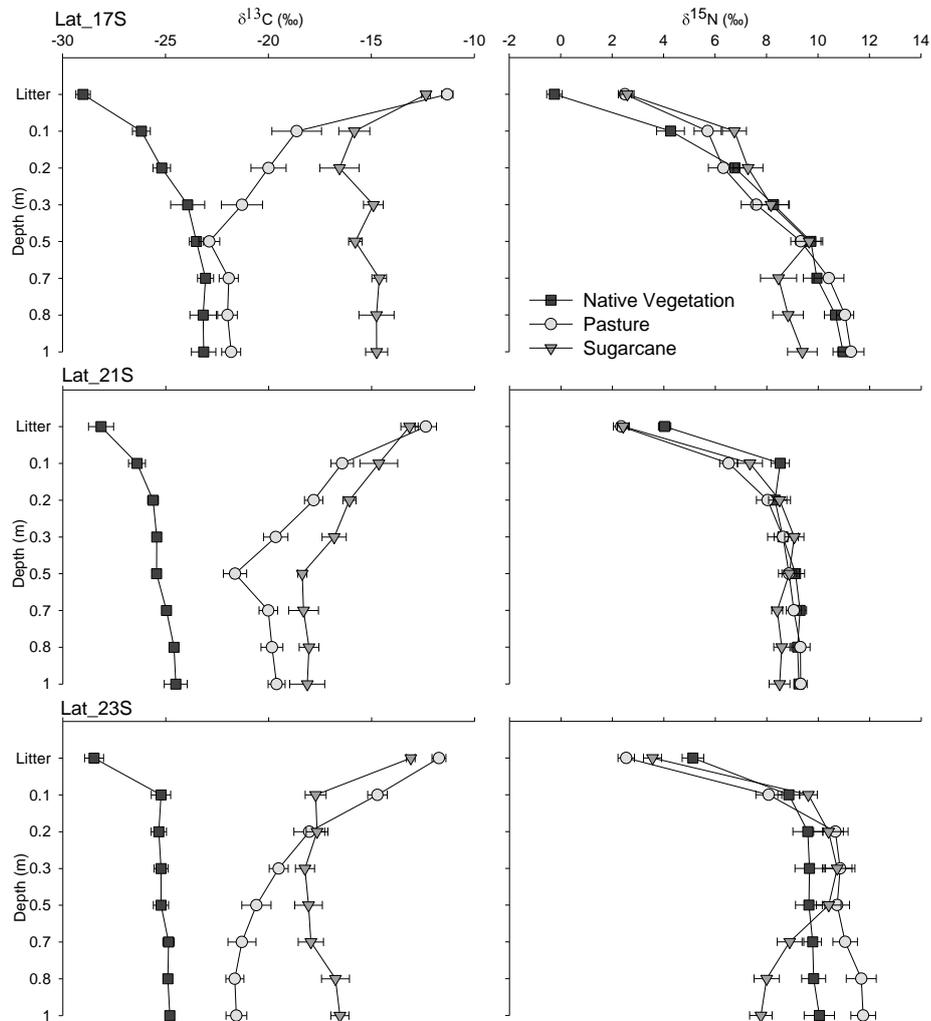


Figure 4. C and N isotopic composition (‰) of litter and soil of areas under different land uses (Native Vegetation – Pasture – Sugarcane) in three sites (Lat_17S, Lat_21S, Lat_23S) in south-central Brazil. Bars represent the standard deviation from the mean values (n=9).

The $\delta^{15}\text{N}$ values for surface soil layers were similar for all land uses in all sites evaluated (Fig. 4). The cropping of SG in these areas revealed a tendency to increase $\delta^{15}\text{N}$ in surface soil layers compared to PA. In NV and PA soils, there were a trend of increasing $\delta^{15}\text{N}$ with depth, with higher values under PA soils. Sugarcane cultivation also induced a slightly increasing trend of $\delta^{15}\text{N}$ values down to 0.5 m layer; but a significant decrease in $\delta^{15}\text{N}$ values was observed for deeper layers.

Native-C stocks were notably higher than modern-C stocks in both PA and SG regardless of site and soil layer (Table 3). The LUC PA-SG did not promote significant changes of soil native-C and modern-C stocks down to 0.5 m. However, this land use conversion increased modern-C stocks for the full 0-1.0 m soil depth (Table 3). In pastures, the average contribution of modern-C was $\sim 30\%$ for surface soil layers, decreasing to less than 20% for deeper layers (Fig. 5). Under SG fields was observed a similar pattern for the surface soil layers, with an average contribution from modern-C of 32%. However, the LUC PA-SG increased the modern-C contribution for soil layers below 0.5 m, with a more homogeneous distribution of the modern-C stocks through the soil down to 1.0 m depth (Fig. 5).

Table 3. Origin of C stocks (Mg ha^{-1}) in areas of south-central Brazil undergoing the conversion of pastures to sugarcane.

	Lat_17S		Lat_21S		Lat_23S		Regional	
	Pasture	Sugarcane	Pasture	Sugarcane	Pasture	Sugarcane	Pasture	Sugarcane
C-origin ^a	Carbon stock (Mg ha^{-1})							
	0-0.3 m							
Native-C	28.6 \pm 2.4 ^b	25.8 \pm 1.3	27.5 \pm 1.9	28.2 \pm 2.2	54.1 \pm 5.7	44.8 \pm 3.6	36.5Aa ^c	32.9Aa
Modern-C	8.6 \pm 1.3	12.4 \pm 0.9	9.7 \pm 0.9	12.5 \pm 1.0	22.8 \pm 3.3	15.8 \pm 1.8	13.7Ba	13.6Ba
	0-0.5 m							
Native-C	36.3 \pm 4.0	35.3 \pm 1.8	36.2 \pm 2.6	36.4 \pm 2.8	70.2 \pm 5.0	63.4 \pm 4.7	47.6Aa	45.1Aa
Modern-C	10.1 \pm 1.3	17.0 \pm 1.2	11.4 \pm 1.1	15.1 \pm 1.2	26.7 \pm 2.5	22.1 \pm 2	16.1Ba	18.1Ba
	0-1.0 m							
Native-C	57.7 \pm 4.8	60.5 \pm 3.8	55.9 \pm 2.9	55.3 \pm 4.3	113.4 \pm 9.8	118.7 \pm 9.1	75.7Aa	78.2Aa
Modern-C	14.5 \pm 1.9	30.5 \pm 1.7	16.6 \pm 1.3	21.2 \pm 2.5	28.5 \pm 2.1	48.4 \pm 3.3	19.9Bb	33.4Ba

^a Native-C: C from native vegetation. Modern-C: C from pasture or from pasture and sugarcane (in sugarcane soils).

^b Standard deviation from the mean values (n=9, except to regional scale). ^c Capital letters represent statistically significant differences between C-origin in a land use and small letters represent statistically significant differences between land uses in the regional scale (considering each site as a block, n =27), according the Tukey test (5%).

2.4. Discussion

2.4.1. Carbon and nitrogen stocks

Land use change (LUC) caused substantial changes in C and N stocks in soils of areas undergoing expansion of sugarcane in south-central Brazil (Table 2). At all sites evaluated, C and N stocks were higher in NV. Lower C stocks under PA soils compared to NV are consistent with other studies in tropical soils (Assad *et al.*, 2013, Don *et al.*, 2011).

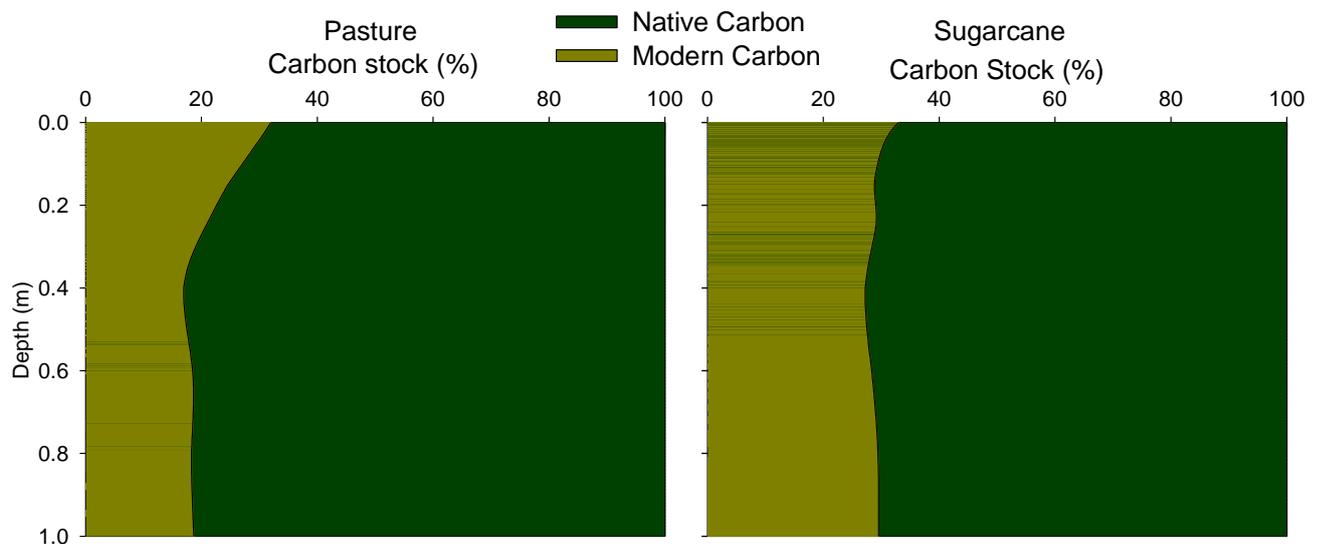


Figure 5. Carbon partitioning (%) in areas under different land uses (Pasture and Sugarcane) in south-central Brazil. Native Carbon: C from native vegetation. Modern Carbon: C from pasture or from pasture and sugarcane (in sugarcane areas). n = 27.

For the 0-1.0 m layer, soil C stocks in SG were higher than in PA at our sites (Table 2). In Brazil, recent studies have reported higher soil C stocks under PA compared to SG (Franco *et al.*, 2015, Mello *et al.*, 2014, Rossi *et al.*, 2013). However, Rossi *et al.* (2013) assessed the soil only down to 0.6 m depth on sites with pre-harvest burning of sugarcane, while Franco *et al.* (2015) sampled the soil only down to 0.3 m, factors that may explain these differences. Also in our study, there was little difference between pasture and sugarcane down to 0.5 m depth; it was only with the inclusion of soil C stocks down to 1 m that total C under SG was greater than for PA, particularly for two of the sites (Lat_17S and Lat_23S). Similar to this study, Mello *et al.* (2014) also considered C stocks down to 1.0 m depth. However, of the 57 sites where these authors evaluated the LUC PA-SG, the sugarcane was still harvested with burning at 18 sites and at 32 sites the pre-harvest burning had been stopped for three years or less before the sampling. Sugarcane areas in Lat_17S and Lat_21S have been harvested without burning since their establishment, while in Lat_23S this practice was banned 12 years before sampling (Table 1). The

green management of sugarcane (harvest without burning) has led to increases in C stocks (Cerri *et al.*, 2011). Nowadays, almost all sugarcane in south-central Brazil is harvested green (UNICA, 2015).

Soil nitrogen stocks in PA and SG areas were lower than N stocks in areas under NV. The conversion of natural ecosystems to agricultural land decreases N stocks (Franco *et al.*, 2015, Yan *et al.*, 2012). Due to the lack of N inputs by fertilizer and continuous N exportation by cattle grazing, lower N stocks are common in Brazilian pastures and this trend is closely linked with the declines in pasture productivity (Boddey *et al.*, 2004). At the Lat_23S site, the low N stocks values in SG may be associated with pre-harvest burning of sugarcane in this area in the past, as also reported by Anaya and Huber-Sannwald (2015).

Studies have revealed the positive correlation between N and C stocks in soils worldwide (Anaya & Huber-Sannwald, 2015, Cherubin *et al.*, 2015). Nitrogen limitation is one of the mainly factors constraining C sequestration (Wieder *et al.*, 2015). Therefore, the C accumulation in soils under PA and SG probably is negative affected by the lower N stocks following land use change.

2.4.2. Land use conversion factor

Our findings indicated that the conversion NV-PA decreased C stocks by 19, 22 and 26%, for 0-0.3, 0-0.5 and 0-1.0 m layers, respectively (Fig. 2). Similar losses were reported by Don *et al.* (2011) and Assad *et al.* (2013), who observed decreases in C stocks of about 12 and 16% for the 0-0.3 m layer for NV-PA conversions in tropical soils. In this case, the C losses can be attributed to both deforestation and biomass burning effects, and subsequent processes of soil degradation in pasture lands (Maia *et al.*, 2009).

As reported for C and N stocks, our conversion factors showed distinct patterns when different depths were considered. For example, if our assessment were restricted to the 0-0.3 and 0-0.5 m layers, we would have concluded that the conversion NV-PA-SG caused greater C losses compared to conversion NV-PA in areas of south-central Brazil (Fig. 2). However, the assessment for the full 0-1.0 m layer revealed a distinct pattern; while the conversion NV-PA caused losses of ~26% in C stocks, the conversion NV-PA-SG was associated with a smaller decrease (~14%). This variation throughout the soil profile may be related to different factors, such as: i) C stocks in topsoil are more sensitive to LUC and other perturbations than subsoil; ii) soil layers below 0.3 m depth were less affected by conversion NV-PA-SG due to C incorporation by tillage in the conversion of pasture to sugarcane; iii) tillage may even increase

subsoil C stocks in croplands due to C-rich topsoil being mixed with deeper layers (Hughes *et al.*, 2000). Therefore, as stressed by Baker *et al.* (2007) and Qin *et al.* (2016), the sampled depth can limit the inferences about the LUC effects on C balance.

Our findings suggest that the introduction of sugarcane in pasture areas has the potential to partially offset the C debt resulting from the conversion of natural vegetation to pastures. Therefore, the replacement of lands with previous low C stocks, as in most of Brazilian pastures, with high productivity bioenergy crops such as sugarcane, may result in a positive soil C balance, increasing the carbon savings of Brazilian sugarcane ethanol.

2.4.3. Rates of carbon stock changes

The LUC NV-PA led to negative rates of carbon stock change, with average values of 0.36, 0.54 and 1.01 Mg C ha⁻¹ yr⁻¹, for 0-0.3, 0-0.5 and 0-1.0 m soil layers, respectively (Fig. 3). Assessing C stock changes in the 0-0.3 m soil layer associated with the LUC NV-PA, Maia *et al.* (2009) and Franco *et al.* (2015) found losses of C at rates of 0.28 and 0.40 Mg ha⁻¹ yr⁻¹, respectively. Global study (Guo & Gifford, 2002) and studies in Brazil (Braz *et al.*, 2013, Maia *et al.*, 2009) show that pasturelands have potential to accumulate C in soil. However, these studies evaluated well-managed pastures, a quite different scenario from those commonly observed in Brazilian pastures. Although management improvements such as liming and fertilization could increase primary productivity and, therefore, C stocks on degraded pastures, the large area occupied by degraded pastures and some intrinsic features of Brazilian livestock production make it unfeasible to adopt these practices on all these areas. Thus, the conversion of a portion of the pasture areas into sugarcane, besides avoiding deforestation (Lapola *et al.*, 2014), could promote soil C sequestration.

The pasture-sugarcane LUC showed increases in C stocks in all evaluated depths in sites Lat_17S and Lat_21S (Fig. 3), where sugarcane was recently introduced and has never been harvested with burning. The increase in soil C stocks in sugarcane systems under green management is mainly related to the large input of organic material (Cerri *et al.*, 2010). There is an annual input of 10–20 Mg of dry matter (5–10 Mg C) left on the soil surface, comprised of dry sugarcane leaves and tops.

The average increase of C stocks in areas undergoing the LUC PA-SG in south-central Brazil was 1.97 Mg ha⁻¹ yr⁻¹ for the 0-1.0 m depth (Fig. 3). This mean value is higher than the C accumulation rate of 1.5 Mg ha⁻¹ yr⁻¹ for the first 0.3 m depth with the conversion of burned to unburned sugarcane in the same region (Cerri *et al.*, 2011, Galdos *et al.*, 2010). Similarly, as the

abolition of burning brought a paradigm shift in C dynamics of sugarcane areas, the introduction of sugarcane into pastures with low C contents can be accompanied by significant changes in the C balance of Brazilian sugarcane ethanol.

A few caveats should be made in regard to our findings. First, tillage operations during sugarcane replanting increase soil C losses and reduced the potential of soil C sequestration in areas of sugarcane cropping. It has been reported that up to 3.5 Mg CO₂ ha⁻¹ could be released during soil preparation in sugarcane fields (Silva-Olaya *et al.*, 2013). In addition, the temporal dynamics of soil C stocks are influenced by antecedent C stocks, soil texture, mineral fertilizer and organic material application (Galdos *et al.*, 2010). The rates of C change were calculated using the most common approach, which is an arithmetic average. It did not consider the temporal or spatial variation of the dataset. Our sampling design does not allow us to do so, but we suggest that future research should take that into account. Our results are promising; however, we must be cautious in stating that soil C sequestration occurs at high rates and during long periods in sugarcane fields converted from pasture areas.

2.4.4. C and N isotopes abundance and C partitioning

Soils under NV showed typical values of $\delta^{13}\text{C}$ for areas of Cerrado and Atlantic Forest biome in Brazil (Assad *et al.*, 2013). In addition, the increase of $\delta^{13}\text{C}$ values with depth is a typical pattern of undisturbed soils under pure C3 or C4 vegetation (Marin-Spiotta *et al.*, 2009). This $\delta^{13}\text{C}$ increase in depth can be mainly attributed to: i) isotopic discrimination during decomposition; ii) differences in decomposition rates of organic components with different $\delta^{13}\text{C}$ signatures and; iii) differences in $\delta^{13}\text{C}$ values between aboveground litter and roots (Wynn *et al.*, 2006).

The LUC NV-PA increased the $\delta^{13}\text{C}$ in all evaluated sites, similar to finding in other conversions of natural ecosystems to pastures in tropical soils (Assad *et al.*, 2013, Yonekura *et al.*, 2012). This shows that the C losses (native-C) in areas undergoing LUC are partially offset by the C input from the current land uses (modern-C), especially in surface soil layers. The soil C derived from C4 grasses has a higher $\delta^{13}\text{C}$ value, such that converted pasture soils showed intermediate values between the two sources (Bernoux *et al.*, 1998).

The LUC PA-SG further increased the $\delta^{13}\text{C}$ values. Rossi *et al.* (2013) found a similar pattern, with increasing of $\delta^{13}\text{C}$ in soils undergoing the conversion NV-PA-SG. Again, this demonstrates the potential of the SG to add C into the soil, including deeper layers, as shown by changes in $\delta^{13}\text{C}$ even to 1.0 m depth. In Lat_23S, SG soils had lower $\delta^{13}\text{C}$ values compared to PA soil for the 0-0.1 m soil layer. As mentioned above, SG in this area was managed with pre-harvest

burning (Table 1). The $\delta^{13}\text{C}$ values of C4 charcoal derived from natural burns were depleted by 8 ‰ (Krull *et al.*, 2003).

In areas of NV and PA the $\delta^{15}\text{N}$ values increased with depth. The values decreased rapidly down to 0.3 m depth and thereafter the trends were less noticeable. This pattern can be associated with different factors, such as i) addition of fresh organic material on the surface, considering that plant tissue tends to be $\delta^{15}\text{N}$ depleted and; ii) successive microbial decomposition of N-containing substrates results in the progressive increase in $\delta^{15}\text{N}$ (Högberg, 1997).

The increment of $\delta^{15}\text{N}$ values observed for superficial layer of SG soils can be related to an increased degree of decomposition of SOM in SG areas (Franco *et al.*, 2015). However, this pattern was observed even in areas where sugarcane cultivation began recently (Lat_17S). It is possible that tillage and addition of N fertilizers were associated with $\delta^{15}\text{N}$ enrichment in these areas, by increasing N losses by nitrification, denitrification and NH_3 volatilization (Högberg, 1997). In addition, the decreasing trend in $\delta^{15}\text{N}$ values below 0.5 m is another aspect that suggests fresh plant material input over the depth in SG areas. Roots tends to be more $\delta^{15}\text{N}$ depleted compared to other plant components (Liao *et al.*, 2006).

The native-C stocks were higher than modern-C stocks (Table 3), representing more than 60% of C stocks in PA and SG areas in south-central Brazil (Fig. 5). This results showed that, even in long-term conversions (>30 years), the C from NV plays an important role in the SOM dynamics. In addition, because derives from less recently added material, it is possible that native-C might represent a relatively stable C pool (Yonekura *et al.*, 2012), such that microbial respiration is mainly associated with materials recently added. Evaluating PA and/or SG areas in south-central Brazil, some authors have found a higher contribution of modern-C to soil C stocks (Assad *et al.*, 2013, Franco *et al.*, 2015, Rossi *et al.*, 2013). However, these authors focused on surface soil layers. In Indonesia, Yonekura *et al.* (2012) found that in pastures established in 1983 the native-C represented 59% of the C stocks down to 1.0 m depth.

The C partitioning showed that increases in C stocks in areas undergoing the LUC PA-SG were due to the reduction on the rates of native-C losses and increases in modern-C stocks (Table 3). The partially maintenance of native-C stocks suggests that soil disturbance, with consequent incorporating of fresh material and the addition of fertilizers, did not cause losses of more stable forms of C (native-C) due to a priming effect (Kuzyakov *et al.*, 2000). This is a potential concern when less managed lands (e.g. pastures) are converted to more intensive uses (e.g. sugarcane cultivation). Furthermore, despite the lower contribution when compared to

native C-stocks, the increments on the modern-C pool (Table 3) were directly responsible for increases in soil C stocks in areas undergoing the land use change PA-SG.

2.4.5. How much does assessed soil depth impact inferred C balance in sugarcane areas?

Studies have showed that about $3.5 \text{ Mg CO}_2 \text{ ha}^{-1}$ could be released during soil preparation in sugarcane fields (Silva-Olaya *et al.*, 2013). Furthermore, the inventory-based balance accounting for N_2O , CH_4 and CO_2 emissions from diesel fuel use, N fertilizer use and other emissions from sugarcane production in south-central Brazil is about $3.1 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ (Galdos *et al.*, 2010). Thus, in a period of 20 years (with replanting every six years), all GHG emissions during sugarcane production will account for about $76 \text{ Mg ha}^{-1} \text{ CO}_2$. Using the sequestration rate of $0.44 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$ to areas undergoing LUC PA-SG calculated for 0-0.3 m soil layer, would result in a net C debit of $67.2 \text{ Mg ha}^{-1} \text{ CO}_2$ associated with sugarcane production over a period of 20 years.

Considering the 0-0.5 m soil layer, the sequestration rate was $2.05 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$, which decreases the C debt to $34.93 \text{ Mg CO}_2 \text{ ha}^{-1}$. However, when considered the increment in C stocks for the 0-1.0 m soil layer in areas undergoing LUC PA-SG, the sequestration rate was $7.22 \text{ Mg CO}_2 \text{ ha}^{-1} \text{ yr}^{-1}$. In this case, contrary to inferences based on the 0-0.3 or 0-0.5 m layers, the sugarcane production in areas previously used as pastures in our study showed a C saving of $68.47 \text{ Mg CO}_2 \text{ ha}^{-1}$ over a period of 20 years. Although simplistic, this approach is important because it's an overview of the C balance associated with LUC and sugarcane cropping in south-central Brazil, and reiterates the need for the inclusion of deeper layers in studies to assessing the impacts of LUC on C balances in agricultural areas (Baker *et al.*, 2007, Qin *et al.*, 2016).

2.5. Conclusions

Land use change causes substantial changes on C and N stocks in soils of areas undergoing expansion of sugarcane in south-central Brazil. The conversion of natural ecosystems to agricultural land decreases N stocks, with similar trends in pastures and sugarcane areas. Overall, long-term conversion from native vegetation to pasture induced significant C stock losses ($1.01 \text{ Mg ha}^{-1} \text{ yr}^{-1}$) for the 0-1.0 m soil layer. In contrast, the conversion from pasture to sugarcane increased C stocks at a rate of $1.97 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. The C-partitioning showed that the gain in C stocks in sugarcane areas was determined by i) the reduction on the rates of native-C

losses and; ii) increasing the amount of modern-C comparing to pasture. In addition, our findings indicated that SOM assessments restricted to the surface soil layers can generate bias in studies regarding LUC. The inclusion of deeper soil layers, at least down to 1.0 m depth, is essential for assessing the impacts of LUC on C balances in agricultural areas.

Assuming that the projected expansion for sugarcane areas (6.4 Mha) by 2021 will follow the same pattern observed in recent years, when 73% of the new areas were established over pasture (Goldemberg *et al.*, 2014), an additional 4.67 Mha of pasture will be converted to sugarcane in Brazil. In this scenario, based on our results, we projected a soil sequestration of 358 Tg CO₂ in these new sugarcane areas over the next 20 years. Thus, our findings endorse the potential of sugarcane production to recover C stocks in pasture areas with previous low C levels, increasing the carbon savings of Brazilian sugarcane ethanol.

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3. MOLECULAR CHARACTERIZATION OF SOIL ORGANIC MATTER FROM NATIVE VEGETATION-PASTURE-SUGARCANE TRANSITIONS IN BRAZIL

ABSTRACT

Replacing pastures (PA) with sugarcane (SG) has been deemed an agronomically feasible strategy for sugarcane expansion in Brazil. However, there are some uncertainties about the environmental impacts regarding this land use change (LUC), mainly related to soil organic matter (SOM), a key factor of environmental sustainability of Brazilian ethanol. LUC-related losses of SOM can overcome the C savings from biofuels. The molecular composition of SOM was evaluated to understand the C dynamics regarding LUC from PA to SG, using native vegetation (NV) as reference. Our study areas were located in the south-central region of Brazil. Soil sampling was performed at three depths (0-0.1 m, 0.2-0.3 m and 0.9-1 m) in three representative sites with known LUC history and management practices since 1970. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) was chosen to study SOM chemistry. Content and isotopic composition of soil organic C and N were also determined. The LUC caused decreases on C and N contents and on $\delta^{13}\text{C}$ isotopic values. Depth was the major factor that influenced SOM composition, while the influence of LUC was mainly evident in surface soils and diminished rapidly with depth. The main difference in SOM composition undergoing the conversion PA-SG was a higher contribution fresh plant materials in SG areas, probably related to the high litter input in sugarcane fields under green management in Brazil.

Keywords: Land use change; Soil carbon; Pyrolysis-GC/MS; Biofuels

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3.1. Introduction

The land use change (LUC) induced alterations in biomass and soil organic matter (SOM) contents are the major uncertainty in accounting schemes and in life cycle assessments of tropical agricultural products (Don *et al.*, 2011). In Brazil, the most pervasive scenario of

sugarcane expansion is into areas previously used as pasture (Goldemberg *et al.*, 2014). The conversion of pasturelands to sugarcane for bioenergy production may cause either a depletion of soil C stocks (Mello *et al.*, 2014) or could sequester atmospheric C into the soil (Oliveira *et al.*, 2016). Despite these efforts to clarify the effects of LUC on soil C stocks in sugarcane expansion areas, there is no qualitative data on SOM composition upon LUC from pasture to sugarcane in Brazil.

The SOM molecular composition is a key factor in understanding soil C dynamics because soil-atmosphere C fluxes are largely determined by SOM stability. In addition to environmental conditions, SOM stability is depending on the chemistry of the input material (plant/litter chemistry, charring) and decomposition processes, all of which leave a chemical fingerprint on SOM. Therefore, combining qualitative (chemical composition of SOM) with quantitative (C stocks) data is considered a key factor in understanding the fate of soil C upon LUC. Several techniques have been applied to study the chemical composition of SOM. Analytical techniques include (i) spectroscopic techniques, such as infrared (IR) spectroscopy and solid-state ^{13}C nuclear magnetic resonance (^{13}C NMR) (Derenne & Nguyen Tu, 2014) and (ii) pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) (Derenne & Quénéa, 2015). Whereas IR and ^{13}C NMR provide information on the environment of carbon atoms (functional groups), detailed structural information (molecules) is obtained by Py-GC/MS (Derenne & Nguyen Tu, 2014, Derenne & Quénéa, 2015). Py-GC/MS allows detailed comparison of SOM produced under different environments and land uses (Buurman & Roscoe, 2011, Carr *et al.*, 2013, Schellekens *et al.*, 2013).

It has been shown that LUC alters the quantity and quality of litter inputs and interfere in the dynamics of SOM decomposition, both being aspects that influence C stocks. In France, the conversion of pasture to continuous crop rotation (wheat, barley and maize) did not promote remarkable shifts on SOM composition, assessed by a Py-GC/MS study (Rumpel *et al.*, 2009). In Canada, the conversion of agricultural land to short rotation coppice systems with hybrid poplar (*Populus* spp.) for bioenergy production caused significant changes in the composition of SOM, which were mainly associated to shifts in litter inputs and fungal communities (Yannikos *et al.*, 2014).

Studies about the effect of LUC on SOM composition using Py-GC/MS for tropical areas are scarce. In Brazil, we can highlight the studies of Buurman and Roscoe (2011) and de Assis *et al.* (2012), although these researches did not encompass sugarcane fields or crops related to bioenergy production. Thus, the effects of sugarcane expansion on SOM composition are still unknown. The objectives of this study are to evaluate the molecular composition of SOM from

three different locations in Brazil and to assess how it shifts upon a change from pasture to sugarcane using native vegetation as a reference.

3.2. Material and Methods

3.2.1. Description of study sites

The study sites were located in three strategic and representative locations in the south-central, main sugarcane-producing region of Brazil (Fig. 1). The study sites are: 1) Lat_17S: located in Jataí county, southwestern region of the Goiás state (Lat.: 17°56'16"S; Long.: 51°38'31"W) with a mean altitude of 800 m and predominance of Acrudox sandy clay soils (USDA, 2014). The climate classification is Awa type (Köppen) mesothermal tropical, with a mean annual temperature of 24.0 °C and an annual precipitation of 1,600 mm, with a dry season in the winter (May to September); 2) Lat_21S: located in Valparaíso county, western São Paulo state (Lat.: 21°14'48"S; Long.: 50°47'04"W) with a mean altitude of 425 m and predominance of Hapludalf sandy loam soils (USDA, 2014). The climate classification is Aw type (Köppen classification) humid tropical, with rains concentrated in the summer (October to April) and a dry season in the winter (April to September). The area has a mean annual temperature of 23.4 °C and an annual precipitation of 1,240 mm; 3) Lat_23S: located in Ipaussu county, south-central São Paulo state (Lat.: 23°05'08" S; Long.: 49°37'52" W), with a mean altitude of 630 m and predominance of Hapludox clay soils (USDA, 2014). The climate classification is Cwa type (Köppen) tropical, with rains concentrated in the summer (October to April) and a dry season in winter (May to September). The annual mean temperature is 21.7 °C and the annual precipitation is 1,470 mm. For information about geology and soil characterization see Cherubin *et al.* (2015).

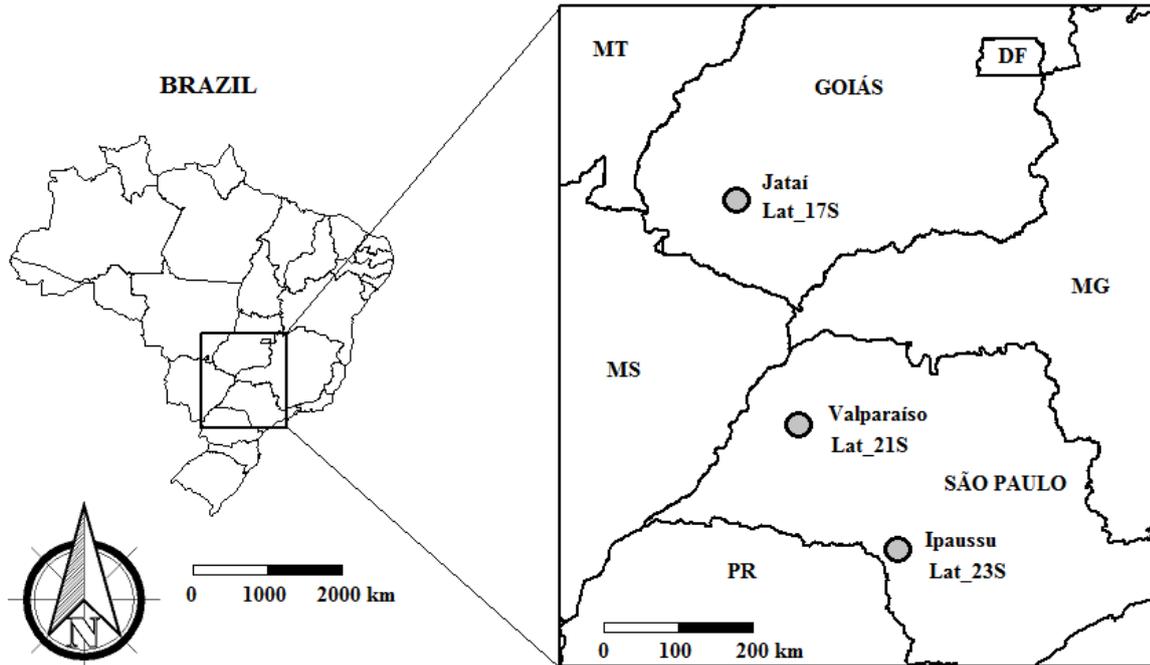


Figure 1. Geographic location of the study sites in south-central Brazil (Cherubin et al., 2015).

3.2.2. Land use change sequence and sampling design

In this study we used a synchronic approach (chronosequence) because there are no long-term experiments that represent the situation in the region studied. To do so, each of three studied sites (Lat_17S; Lat_21S; Lat_23S) comprised three land uses: native vegetation (NV), pasture (PA) and sugarcane (SG), representing one of the most common LUC sequence in the south-central region of Brazil. In each area, the three land uses are located in adjacent plots, minimizing the effects of climatic, topographic and soil variations. The general description of each land use site are shown in Table 1. Each study site was composed of a sampling grid with nine points, 50 m apart. The samples were collected using an auger, at seven depths: 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5, 0.5-0.7, 0.7-0.9, 0.9-1.0 m. For Py-GC/MS analysis, the three samples on diagonal position in the sampling grid were chosen at 0-0.1 m and 0.2-0.3 m depth (Fig. 2), while for 0.9-1.0 m layer one sample was analyzed per land use. These depths are considered to represent soil conditions at the surface, subsurface and deeper soil layers.

Table 1. Land use history and brief description of the studied sites in south-central Brazil.

Site	Land use	Description ^a
Lat_17S	Native vegetation	Cerradão forest formation, Cerrado biome, characterized by sclerophyllous and xeromorphic species. The vegetation is dense compared to the Cerrado <i>stricto sensu</i> (savana).
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Brachiaria</i> and supports 1.5 AU ha ⁻¹ full year.
	Sugarcane	Conversion from pasture at 2009. Cultivar RB855453 with mean yield of 81.5 ton ha ⁻¹ . Conventional tillage procedures and chemical fertilization. At the sampling time, sugarcane was in the third ratoon cropping of its cycle. Sugarcane is mechanically harvested without burning since its implantation.
Lat_21S	Native vegetation	The local vegetation is seasonal semideciduous forest, Atlantic forest biome, in which a portion of the trees defoliates during the dry season.
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Brachiaria</i> and supports 2 AU ha ⁻¹ full year. Annually 120 kg ha ⁻¹ of the fertilizer formulation 20:5:19 are applied.
	Sugarcane	Conversion from pasture at 2010. Cultivar SP791011 with a mean yield of 80 ton ha ⁻¹ . Conventional tillage procedures and chemical fertilization + vinasse application. At the sampling time sugarcane was in the fourth ratoon cropping of its cycle. Mechanically harvested without burning since its implantation.
Lat_23S	Native vegetation	The local vegetation is seasonal semideciduous forest, Atlantic forest biome, in which a portion of the trees defoliates during the dry season.
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Cynodon spp.</i> and supports 1 AU ha ⁻¹ full year.
	Sugarcane	Conversion from pasture at 1990. Cultivar CTC6 with a mean yield of 85 ton ha ⁻¹ . Conventional tillage procedures and chemical fertilization + vinasse and filtercake application. At the sampling time sugarcane was in the fifth ratoon of its cycle. Pre-harvest burning between 1990 and 2002. Since 2013, 50% of straw has been removed for energy production.

^a For further details about land use description, see Cherubin *et al.* (2015).

3.2.3. Soil organic matter extraction

Purification of SOM is a regular step in studies using pyrolysis techniques and avoids that reactive minerals act as catalysts during pyrolysis (Miltner & Zech, 1997). From the possibilities to isolate OM from soils, we chose alkaline extraction with 0.1 M NaOH, being equivalent to the exhaustively studied humic acids. Although it has been shown that the major part of SOM is extractable with NaOH (Marques *et al.*, 2015, von Lützow *et al.*, 2007), we stress that the un-extractable SOM and/or large part of the mineral associated SOM is not represented here. However, the NaOH-extractable SOM is expected to provide representative results to compare the molecular composition of SOM in the studied samples.

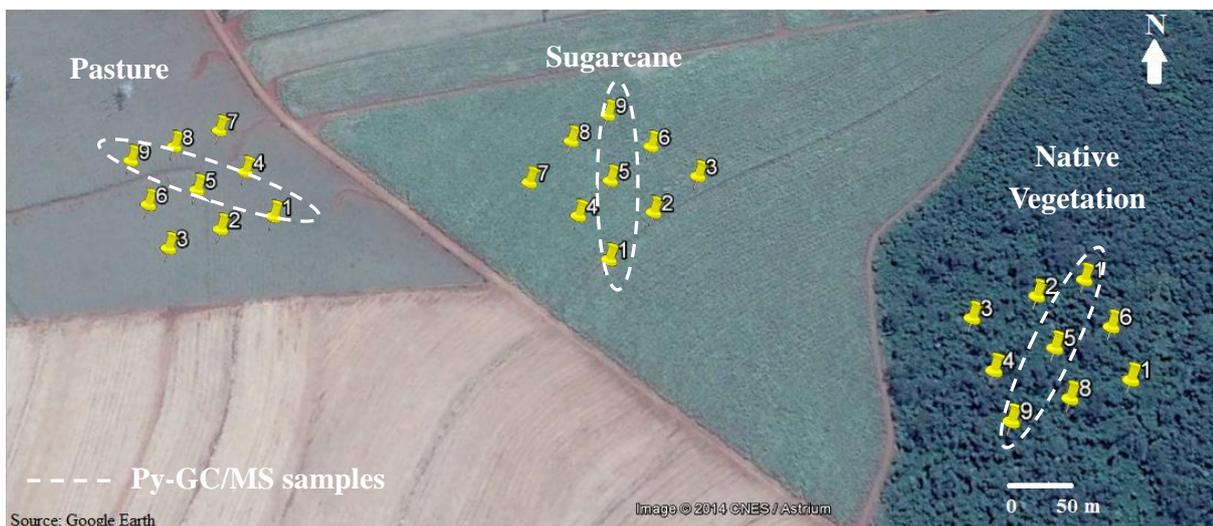


Figure 2. Example of sampling design (Lat_23S). Samples analyzed with Py-GC/MS are indicated by circles.

For SOM extraction, 5 g of soil (< 2 mm) were shaken with 25 mL of NaOH (0.1 M) for 18 h. The mixture was centrifuged for 30 min at 4000 rpm and the supernatant was collected. The extraction was repeated twice and the extracts were combined. The extracts were acidified to pH 1-2 with 1M HF/HCl (3:1) and shaken for 18 hours to remove minerals. It appeared that mineral material was still present in the extracts, which is probably related to the high clay content of Oxisols (Schaefer, 2001). Therefore, the extracts were re-treated with 1M HF/HCl (3:1) to remove these minerals. The extracts were then dialyzed against distilled water through cellulose membranes with a pore diameter of 6,000-8,000 Dalton. Finally, the extracts were freeze-dried and homogenized.

3.2.4. C and N elemental analysis and isotopic composition

Content and isotopic composition of soil C and N were determined by using a Thermo Finnigan Delta Plus spectrometer (Waltham, USA) at the Center of Nuclear Energy in Agriculture, University of São Paulo (CENA-USP) in Piracicaba, Brazil. Isotopic composition was expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) using Vienna-PDB as reference for ^{13}C levels and air composition for ^{15}N levels.

3.2.5. Pyrolysis-GC/MS

Pyrolysis was performed at the department of soil science from ESALQ/USP (Piracicaba, Brazil) using a single shot PY-3030S pyrolyser (Frontier Laboratories, Fukushima, Japan) coupled to a GCMS-QP2010 (Shimadzu, Kyoto, Japan). The pyrolysis temperature was set at 600 °C (+/-0.1 °C); Helium was used as carrier gas at a constant flow of 34.5 mL min⁻¹. The injection temperature of the GC (split 1:20) and the GC/MS interface were set at 320 °C. The GC oven was heated from 50 to 320 °C (held 10 min) at 7 °C min⁻¹. The GC instrument was equipped with a UltraAlloy-5 column (Frontier Laboratories LTD.), length 30 m, thickness 0.25 mm, diameter 0.25 µm. The MS was scanning in the range of m/z 45–600. Pyrolysis products were identified using the NIST '14 mass spectral library.

All 63 samples were analyzed except for two samples from 0.9–1.0 m depth (PA from Lat_17S and NV from Lat_21S) that did not have enough material for analysis. Four representative samples were analyzed in duplicate, replicate pyrograms perfectly agreed ($r^2 > 0.97$). An image of some representative pyrograms is given in Fig. 3. Altogether about 300 pyrolysis products were recognized, 123 of which were quantified. All dominant products were selected for quantification. In addition to the dominant products, lignin phenols, levoglucosan, n-alkanes and n-alkenes, and PAHs were selected for quantification because they provide useful information on source, decay and burning. The products were grouped according to probable origin and chemical similarity into a number of source groups: n-alkanes, n-alkenes, fatty acids, polyaromatic hydrocarbons (PAHs), aromatics, phenols, lignin products, N containing compounds, polysaccharides and benzofurans. Quantification was based on the peak area of characteristic ions (m/z values) for each pyrolysis product. All quantification was checked manually. For each sample, the sum of the quantified peak areas was set at 100% and relative amounts were calculated with respect to this. The resulting data set is thus semi-quantitative and provides relative abundances. The quantitative character of Py-GC/MS results is further hindered

by the fact that different compounds do not have the same detection threshold (MS response factors). Therefore, comparison of numerical values of (groups of) pyrolysis products within a sample is difficult, but it allows us to assess the variations within a set of samples (Jacob *et al.*, 2007, Schellekens *et al.*, 2015). Table 2 lists the number of quantified compounds by chemical group.

3.2.6. Statistical analysis

A difficulty with the interpretation of pyrolysis results is that several processes may influence the abundance of a single pyrolysis product. This problem is enlarged in case the data are simplified to groups when this grouping is based on chemical similarity (e.g., carbohydrates, N containing compounds, etc.) instead of on the behavior of products in the samples. Another problem with grouping of pyrolysis products in the comparison of their abundance in samples is that the abundance of products that contribute to the group may highly differ; the group may thus more closely reflect a single product instead. Thus, although many studies simplify pyrolysis data to chemical groups to compare environmental samples (de Assis *et al.*, 2012, Rumpel *et al.*, 2009), information (variation) may be lost and the group may not be representative for the individual products. Therefore, we use the composition of pyrolysates in terms of chemical groups only to provide a general indication of the chemical composition of the studied soils, while we chose factor analysis to investigate the differences between the samples.

Factor analysis shows trends, the abundance of a compound not affecting the results. The factor analysis was applied to all 123 quantified pyrolysis products using Statistica[®] Version 6 (StatSoft Inc., Tulsa).

Table 2. Quantified pyrolysis products.

Name	Code	m/z^a	RT ^b	Name	Code	m/z^a	RT ^b
Aromatics				N compounds			
benzene	Ar1	78	1.842	Pyridine	N1	52+79	2.375
indane	Ar2	117+118	6.567	Pyrrole	N2	67	2.425
indene	Ar3	115+116	6.958	Acetamide	N3	59	2.692
toluene	Ar4	91+92	2.583	methyl-1 <i>H</i> -pyrrole	N4	80+81	3.358
n-Alkanes				methyl-1 <i>H</i> -pyrrole	N5	80+81	3.483
C ₉₋₃₅ <i>n</i> -alkanes	C:0	57+71	-	Benzonitrile	N6	76+103	5.767
n-Alkenes				1 <i>H</i> -pyrrole-2-carboxaldehyde	N7	94+95	6.183
C ₉₋₂₈ <i>n</i> -alkenes	C:1	57+71	-	2,5-pyrrolidinedione	N8	99+56	8.675
Lignin phenols				benzeneacetonitrile	N9	90+117	8.842
4-vinylphenol	Lg1	91+120	10.408	Indole	N10	90+117	11.958
4-acetylphenol	Lg2	121+136	14.783	3-hydroxybenzonitrile	N11	119+64	13.267
guaiacol	Lg3	109+124	7.833	1 <i>H</i> -isoindole-1,3(2 <i>H</i>)-dione	N12	76+104	15.083
4-methylguaiacol	Lg4	123+138	9.925	1,2,3,4- <i>HH</i> -isoquinolin-1,3-dione	N13	89+118	17.108
4-ethylguaiacol	Lg5	137+152	11.650	Diketodipyrrole	N14	93+186	19.267
4-vinylguaiacol	Lg6	135+150	12.358	Diketopiperazine	N15	70+154	22.492
4-formylguaiacol	Lg7	151+152	13.967	Diketopiperazine	N16	70+194	22.675
4-(prop-2-enyl)guaiacol, <i>trans</i>	Lg8	164	14.858	Hexadeceneamide	N17	59+72	25.742
4-acetylguaiacol	Lg9	151+166	15.543	9-octadeceneamide	N18	59+72	27.992
vanillic acid	Lg10	153+168	17.075	Octadecaneamide	N19	59+72	28.242
syringol	Lg11	139+154	13.042	C ₁₄ alkylnitrile	N20	110+124	22.000
4-methylsyringol	Lg12	153+168	14.808	C ₁₆ alkylnitrile	N21	110+124	24.775
4-ethylsyringol	Lg13	167+182	16.200	C _{16:1} alkylnitrile	N22	122+136	24.467
4-vinylsyringol	Lg14	165+180	16.867	Benzofurans			
4-(prop-2-enyl)syringol, <i>trans</i>	Lg15	194	19.092	Benzofuran	Bf1	89+118	6.000
4-acetylsyringol	Lg16	181+196	19.625	Methylbenzofuran	Bf2	131+132	8.217
ferulic acid methyl ester	Lg17	145+208	21.208	Dibenzofuran	Bf3	139+168	16.092
Polyaromatics (PAHs)				Dibenzofuranol	Bf4	184+128	21.350
naphthalene	PA1	128	9.792	Dibenzofuranol	Bf5	184+128	21.683
unidentified PAH	PA2	102+130	9.817	Phenols			
methylnaphthalene	PA3	141+142	11.975	Phenol	Ph1	66+94	5.667
methylnaphthalene	PA4	141+142	12.308	Methylphenol	Ph2	107	7.517
biphenyl	PA5	154	13.583	C ₂ phenol	Ph3	107	9.375
C ₂ naphthalene	PA6	141+156	14.392	Catechol	Ph4	64+110	10.108
methylbiphenyl	PA7	168+167	15.650	3-methoxy-5-methylphenol	Ph5	109+138	12.442
fluorene	PA8	165+166	17.225	Fatty acids			
phenanthrene	PA9	178	20.400	palmitic acid (C ₁₆)	F16	60+73	22.825
anthracene	PA10	178	20.542	stearic acid (C ₁₈)	F18	60+73	25.475
methylphenanthrene	PA12	192+191	22.450	Carbohydrates			
2-phenylnaphthalene	PA13	204+202	23.158	2-furaldehyde	Ps1	95+96	3.275
pyrene	PA14	202	24.417	5-methyl-2-furaldehyde	Ps2	109+110	5.350
fluoranthene	PA15	202	25.133	2 <i>H</i> -pyran-2-one	Ps3	68+96	5.517
retene	PA16	219+234	27.742	4-hydroxy-5,6-dihydro-(2 <i>H</i>)-pyran-2-one	Ps4	58+114	6.008
perylene	PA17	252+250	33.333	Levoglucosan	Ps5	60+73	15.592

^a: Mass fragments used for quantification. ^b: Retention time (min).

3.3. Results and Discussion

3.3.1. Soil C and N contents and isotopic composition

Considering each site as a block (regional scale), NV showed larger contents of soil C compared to PA and SG at all assessed depths (Table 3), except at 0.9-1.0 m depth (no significant difference). N contents followed similar trends, although differences were statistically significant ($p < 0.05$) only at 0-0.1 m depth (Table 3). This is in agreement with a loss of soil C upon the conversion from NV to PA in tropical areas (Assad *et al.*, 2013, Don *et al.*, 2011).

The areas under NV showed lower values of $\delta^{13}\text{C}$ at all evaluated depths (Table 3). The conversion of NV to PA caused enrichment on $\delta^{13}\text{C}$, a common trend when areas with predominance of C3 plants (NV) are converted to C4 plants (PA) (Assad *et al.*, 2013). For the PA-SG transition, we observed a tendency of increase $\delta^{13}\text{C}$ values, although differences were statistically significant ($p < 0.05$) only at 0.9-1.0 m depth (Table 3), driven by the greater contribution of C from C4 plants (PA and SG) in SG areas. The LUC did not cause notable changes on $\delta^{15}\text{N}$ values. For more detail on content and isotopic composition of C and N of soil in these areas, see Oliveira *et al.* (2016).

3.3.2. General composition of NaOH extractable SOM pyrolysates

Irrespective of site or land use, the pyrograms showed a typical SOM signature, representing a mixture of compounds from plant biopolymers, microbial material and black carbon (BC). Pyrolysis products from plant biopolymers include n-alkane/alkene pairs from cutan and suberan (Nierop *et al.*, 2001), levosugars from cellulose and (di)methoxyphenols from lignin (Ralph & Hatfield, 1991); microbial material results in nitrogen containing compounds and carbohydrates upon pyrolysis (Derenne & Quénéa, 2015), and polyaromatic hydrocarbons (PAHs) are pyrolysis products from BC (González-Pérez *et al.*, 2014). Qualitative comparisons between the mass spectra of different land uses revealed contrasting molecular signatures (Fig. 3).

Table 3. Total organic carbon (TOC), total nitrogen (TN) and isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of bulk soil in areas under different land uses in south-central Brazil.

Site	Land Use	TOC (g kg^{-1})		TN (g kg^{-1})		^{13}C (δ ‰)		^{15}N (δ ‰)		TOC (g kg^{-1})		TN (g kg^{-1})		^{13}C (δ ‰)		^{15}N (δ ‰)	
		0-0.10 m				0.2-0.3 m				0.9-1.0 m							
Lat_17S	NV	22.1±2.2*	1.4±0.1	-26.2±0.4	4.3±0.3	9.9±0.9	0.6±0.1	-24.5±0.8	8.3±0.6	4.3±0.5	0.3±0.0	-23.2±0.6	11.0±0.4				
	PA	9.5±0.7	0.5±0.0	-18.62±1.2	5.7±0.6	7.1±0.8	0.3±0.0	-21.3±1.0	7.6±0.8	2.3±0.3	0.2±0.0	-21.8±0.5	11.3±0.5				
	SG	10.4±1.1	0.7±0.0	-15.82±0.8	6.8±0.7	10.3±1.0	0.6±0.1	-14.9±0.5	8.2±0.7	5.0±0.5	0.4±0.0	-14.7±0.5	9.4±0.8				
Lat_21S	NV	18.9±2.7	1.9±0.3	-26.42±0.4	8.5±0.8	6.8±0.4	0.6±0.1	-25.4±0.2	8.6±0.3	2.4±0.4	0.4±0.0	-24.5±0.5	8.3±0.4				
	PA	12.7±1.8	1.0±0.1	-14.7±0.9	6.5±0.4	6.3±0.5	0.5±0.0	-16.8±0.6	8.6±1.0	2.8±0.6	0.4±0.0	-18.1±0.9	9.3±0.8				
	SG	11.5±1.8	0.7±0.1	-16.4±0.6	7.3±0.9	6.6±0.6	0.6±0.1	-19.7±0.6	9.1±0.4	2.3±0.2	0.4±0.0	-19.6±0.4	8.5±0.4				
Lat_23S	NV	42.4±5.0	3.3±0.3	-25.2±0.5	8.9±0.5	20.9±1.6	1.7±0.1	-25.2±0.3	9.7±0.6	11.2±1.9	0.9±0.1	-24.8±0.2	10.0±0.9				
	PA	33.3±2.2	2.4±0.3	-14.7±0.5	8.1±1.0	14.1±1.7	1.2±0.1	-19.5±0.5	11.7±0.6	7.7±1.1	0.7±0.0	-21.6±0.5	10.7±0.8				
	SG	19.5±2.4	1.5±0.2	-17.7±1.1	9.6±0.7	15.7±1.4	1.2±0.1	-18.2±0.5	10.7±0.7	11.3±1.6	0.7±0.1	-16.5±0.4	7.8±0.7				
Regional	NV	27.8±4.2a	2.2±0.5a	-25.9±2.1b	7.2±1.3a	12.5±2.1a	1.0±0.4a	-25.1±2.1b	8.9±1.2a	6.0±0.8a	0.5±0.1a	-24.2±1.4c	9.8±1.1a				
	PA	18.5±3.1b	1.3±0.4b	-16.0±0.9a	6.8±1.1a	9.2±1.1b	0.7±0.3a	-19.2±1.5a	9.3±1.0a	4.2±0.5b	0.4±0.1a	-20.5±1.1b	10.4±1.0a				
	SG	13.8±2.5c	1.0±0.5b	-16.7±1.8a	7.9±1.1a	10.9±1.2b	0.8±0.3a	-17.6±1.3a	9.3±1.6a	6.2±1.0a	0.5±0.1a	-17.0±1.2a	8.6±0.9a				

NV: Native vegetation; PA: Pasture; SG: Sugarcane. *: Standard deviation. $n=9$ and $n=27$, to sites and to regional, respectively. Letters represent statistically significant differences between land uses in the regional scale (considering each site as a block), according to the Tukey test (5 %).

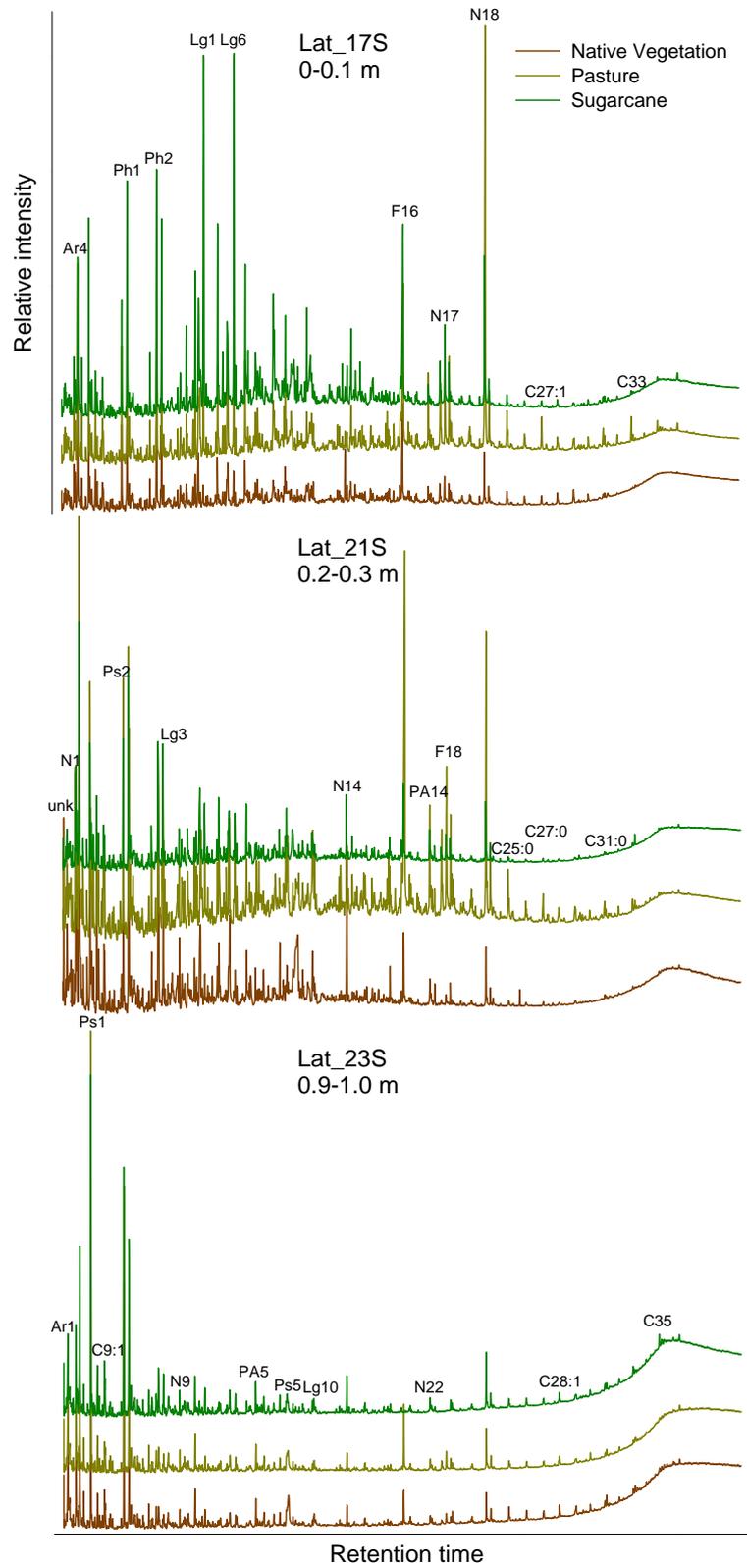


Figure 3. Examples of representative pyrograms of SOM extracts from areas under different land uses in south-central Brazil. See Table 2 for codes.

The relative abundance of groups of quantified pyrolysis products is given in Table 4. We highlight the high contribution from N containing compounds and carbohydrates, in agreement with strong decomposition and a relatively high contribution from microbial material, common under tropical conditions (Buurman & Roscoe, 2011). A contribution of 2–3 % PAHs and the presence of 4 and 5-ring PAHs (PA14, PA15, PA17; Table 2) is unequivocal evidence for burnt material (González-Pérez *et al.*, 2014), and generally agrees with values found in other Brazilian Oxisols (de Assis *et al.*, 2012, Marques *et al.*, 2015).

In the deepest samples (0.9-1.0 m) there is a tendency of increasing contribution from carbohydrates and PAHs, and less contribution from products derived from plant biopolymers (lignin phenols and n-alkanes and n-alkenes). The increase of carbohydrates suggests that the SOM is mostly microbial at this depth, while the higher contribution from PAHs probably reflects the fact that charred material relatively accumulates due to selective decomposition. These changes are in agreement with an increase in the degree of decomposition of SOM with depth, according to Rumpel and Kögel-Knabner (2011).

3.3.3. Factor analysis applied to the total pyrolysis data set

In factor analysis of the total data set (PCA_{All}), more than 50% of the variation was explained by the first two factors; factors 1 (F1) and factor 2 (F2) explained 30.4 % and 22.3 %, respectively. Because each of the subsequent factors explained less than 10% of the total variability, the discussion will be limited only to the first two factors. On F1, highest positive scores were generally found in the sequence SG > PA > NV, except for some PA samples. Within each soil profile, surface samples (0-0.1 m) showed more positive scores than the corresponding subsurface samples (0.2-0.3 m) and deepest samples (0.9-1 m; Fig. 4a). All lignin phenols and phenols showed high positive loadings, while particularly aliphatics (n-alkanes and n-alkenes) and PAHs showed high negative loadings on F1 (Fig. 4b). F1 is therefore interpreted to reflect decomposition, negative values corresponding to more recalcitrant (relatively difficult to decompose) plant biopolymers (aliphatics; (Tegelaar *et al.*, 1989) and BC (PAHs; (González-Pérez *et al.*, 2014), and positive values to relatively fresh plant material (lignin phenols; (Klotzbücher *et al.*, 2011).

Table 4. Relative abundance of groups of pyrolysis products in NaOH extractable SOM from areas under different land use in south-central Brazil.

Groups	----- 0-0.1 m -----			----- 0.2-0.3 m -----			----- 0.9-1.0 m -----		
	NV	PA	SG	NV	PA	SG	NV	PA	SG
Relative contribution (%)									
N compounds	26.2±2.9 ^a	20.2±4.6	17.4±3.5	28.3±2.5	26.0±5.7	25.0±2.2	18.1±4.3	19.5±4.4	22.9±3.7
Phenols	15.8±2.0	16.6±1.6	19.2±1.7	11.8±3.0	11.2±1.6	15.1±3.6	7.2±1.2	9.8±0.4	12.6±1.3
Lignin	13.1±3.6	22.3±7.3	29.3±7.0	6.3±2.0	9.0±1.7	10.4±2.8	2.3±0.6	4.3±1.4	6.8±0.5
Carbohydrates	13.1±1.8	12.2±2.8	13.1±0.9	17.4±2.9	16.1±3.8	16.5±2.8	40.0±4.7	32.1±4.2	23.6±5.4
Aromatics	12.3±0.8	10.6±1.7	8.9±2.2	15.7±2.2	13.6±1.3	16.0±2.3	14.4±3.7	13.5±3.2	16.0±3.2
<i>n</i> -Alkanes	9.1±2.4	7.6±1.3	4.0±1.0	8.6±2.9	9.2±2.6	6.5±2.4	5.3±1.1	6.9±1.3	5.4±0.7
<i>n</i> -Alkenes	5.2±1.5	5.1±1.1	2.9±0.6	5.7±2.4	5.9±0.7	4.2±1.8	5.0±1.4	4.0±0.3	4.5±0.3
Fatty acids	2.5±1.4	2.8±1.2	3.0±1.2	2.7±1.9	6.1±2.1	2.5±1.4	4.3±1.6	4.8±1.8	2.9±0.7
PAHs	2.1±0.5	1.6±0.6	1.6±0.4	2.8±0.6	2.3±0.8	3.0±0.6	3.6±1.9	4.3±0.6	4.6±2.0
Benzofurans	0.7±0.1	0.6±0.1	0.6±0.1	0.8±0.2	0.6±0.1	0.9±0.2	0.8±0.3	0.9±0.2	1.0±0.1

NV: Native vegetation; PA: Pasture; SG: Sugarcane. ^a: Standard deviation. *n*=9. For 0.9-1.0 m, *n*=2, 2 and 3, to NV, PA, SG, respectively.

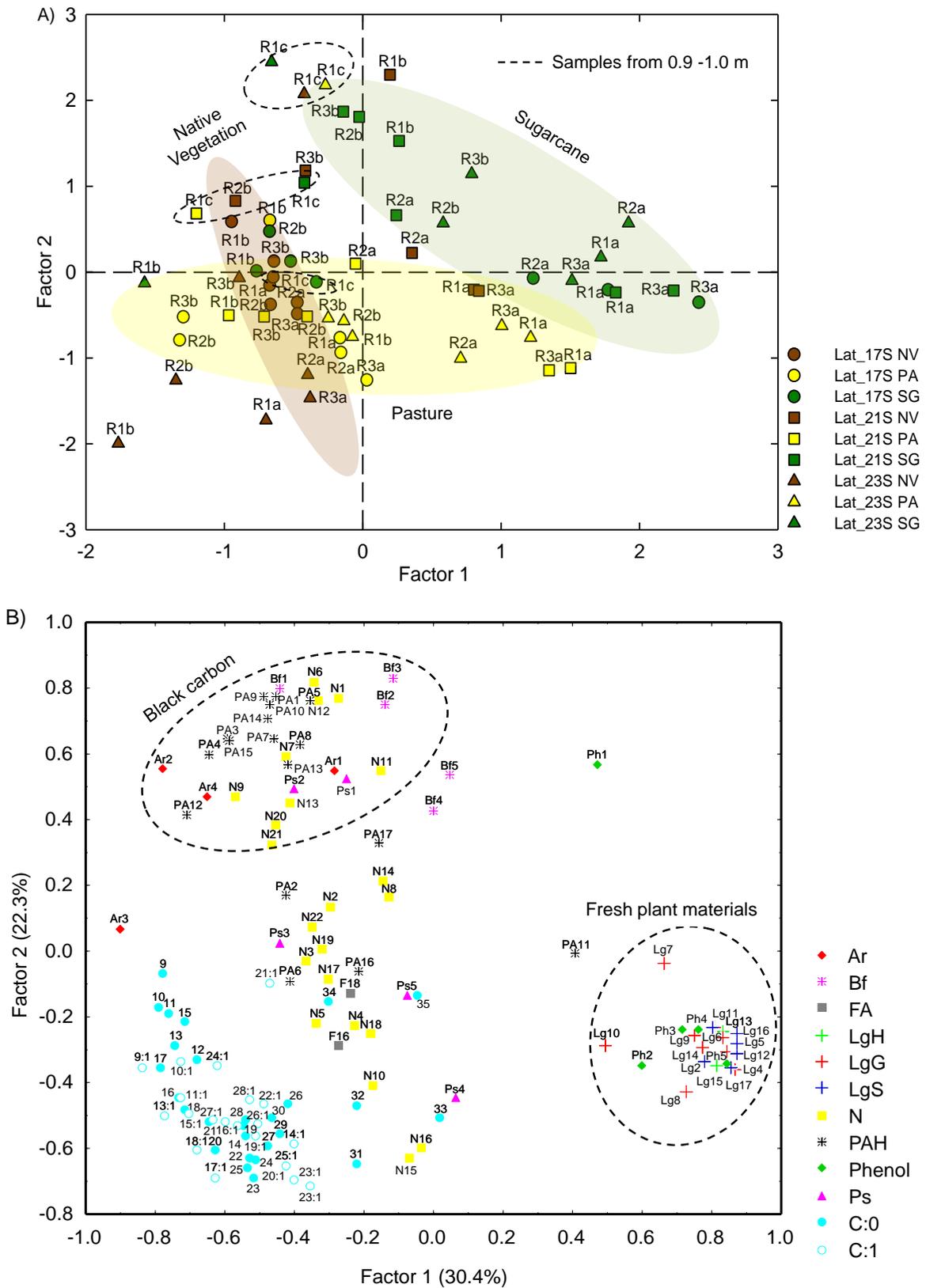


Figure 4. Scores (A) and loadings (B) on F1F2 projections of factor analysis applied to all SOM extracts from areas under different land uses in south-central Brazil. NV: Native Vegetation, PA: Pasture, SG: Sugarcane. R1, R2 and R3: replicates. a, b and c: samples from 0-0.1 m, 0.2-0.3 m and 0.9-1.0 m, respectively. Ar = aromatic, Bf = benzofuran, FA= fatty acid, Lg H = p-hydroxyphenyl lignin, Lg G = guaiacyl lignin, Lg S = syringyl lignin, PA = poliaromatics, Ps = polysaccharide, C:0 = alkane, C:1 = alkene, N =nitrogen containing compound. See Table 2 for codes.

Although the sequence SG > PA > NV may suggest that F1 reflects vegetation differences (NV having a higher contribution from woody tissue reflected by aliphatics from suberan and PAHs from charcoal), various observations support the interpretation of F1 to reflect decomposition: i) the negative loadings of nearly all N compounds, indicative of microbial SOM (Derenne & Quénéa, 2015), ii) the depth trend on F1, surface samples having (larger) positive scores, iii) the decreasing C content in the surface samples for NV > PA > SG.

The higher contribution from fresh organic matter (lignin phenols) in soils under SG is assigned as reflecting the continuous incorporation of plant material in agricultural soils due to tillage (Schellekens *et al.*, 2013), while in PA it agrees with the continuous root input in grassland soils (Fisher *et al.*, 1994). The fact that in NV i) surface samples showed much less positive loadings and in most cases even negative ones on F1 and ii) the difference between surface and subsurface samples was much smaller on F1, reflects that under NV decomposition is slower compared to agricultural land uses, in agreement with Yassir and Buurman (2012).

F2 is interpreted to reflect the contribution from BC (i.e., fire frequency), because F2 clearly separated the aliphatic (negative loadings) from PAH (positive loadings) products. Others products that showed high positive loadings on F2 are also indicative of burnt material and include benzonitrile (N6; (Song & Peng, 2010), benzofurans (Bf1–Bf3), pyridine (N1) and most aromatics (González-Pérez *et al.*, 2014). On F2, the deepest samples (0.9-1.0 m) all showed positive scores, except for very low negatives ones from Lat_17S (Fig. 4a). All the deepest samples were clustered on F2 according to site, independent of land use, with highest positive scores for Lat_23S, less positive scores for Lat_21S and neutral scores for Lat_17S (Fig. 4a). This clustering according to location on F2 indicates that at this depth a change in land use did not influence SOM composition. The separation of location on F2 for the deepest samples suggests a difference in past fire frequency, probably related to changing climatic conditions, in agreement with long-term wildfire frequency events (Mayle *et al.*, 2000).

The factor analysis applied to all samples showed which aspect had the major effects on soil chemistry: land use, depth or site. It appeared that depth + land-use had the major effect on SOM decomposition (F1), while depth + site mainly determined the contribution from burnt material (F2). The decomposition effect reflected by F1 is largely a short term effect, because it was related to the presence of fresh litter. The difference in fire intensity between sites presumably is more a long term effect and less related to land use. In order to eliminate the depth effect and obtain more detailed information on the effect of LUC, a separate factor analysis was applied to surface (0-0.1 m) and subsurface (0.2-0.3 m) samples.

3.3.4. Factor analysis applied to surface samples (0-0.1 m)

The factor analysis at 0-0.1 m depth shows that for each site samples from different land use were separated by F1 in the sequence NV-PA-SG, with highest negative scores for NV and strongest positive scores for SG samples (Fig. 5a). The PA samples were scattered along F1, but considering each site separately, it showed scores in between NV and SG. Samples from the same site generally formed clear clusters in F1-F2 projection, indicating that the results are statistically substantiated and can be assumed to reflect the chemical variation between land uses (Fig. 5a). The samples from Lat_21S are an exception, and deviate from the other two samples from the same site (NV-R2, PA-R2 and SG-R2). These deviating samples showed lower lignin content and higher contribution from PAHs compared to its replicas, suggesting that this deviation was caused by burning. The deviation found from pyrolysis data was also evident in C and N content (lower values; not shown), in agreement with a loss of SOM upon burning (Carr *et al.*, 2013), and with lower values of $\delta^{13}\text{C}$ and especially $\delta^{15}\text{N}$ isotopic composition that also corresponds to an increase of BC (Bird & Ascough, 2012). The fire fingerprint in these samples is probably very local, in the following we therefore use the scores of the other two samples to represent the surface samples from Lat_21S. The distribution of pyrolysis products in the F1-F2 projection (loadings; Fig. 5b) is similar to that of PCA_{all} (Fig. 4b), and the interpretation of the factors remains the same. Thus, F1 reflects decomposition with positive values reflecting fresh material and negative ones more recalcitrant material, while F2 reflects burning, with positive values indicating a higher contribution from BC.

This interpretation of the factors is further supported by the distribution of compounds within each group, although these observations were also present in PCA_{all}, they were clearer in the loadings of PCA_{0-0.1}. First, within the lignin group, syringyl moieties generally showed higher positive loadings compared to guaiacyl moieties; in addition, moieties with a C3 alkyl side chain, indicative of intact lignin, showed higher positive loadings while oxygenated side chains (degraded lignin) showed lower loading (vanillic acid, Lg10) (Schellekens *et al.*, 2012). Second, the n-alkanes and n-alkenes clearly showed an increase in chain length on F1 and decrease on F2, reflecting chain length shortening upon burning (F2; (González-Pérez *et al.*, 2014) and decomposition (F1; (Buurman & Roscoe, 2011). Third, within the products indicative of BC (positive on F2, negative on F1), the benzofurans showed the lowest scores, while the aromatics showed the highest negative loadings. This also reflects a degradation gradient on F1, benzofurans being easier degradable than PAHs, and aromatics being degradation products of PAHs (Marques *et al.*, 2015).

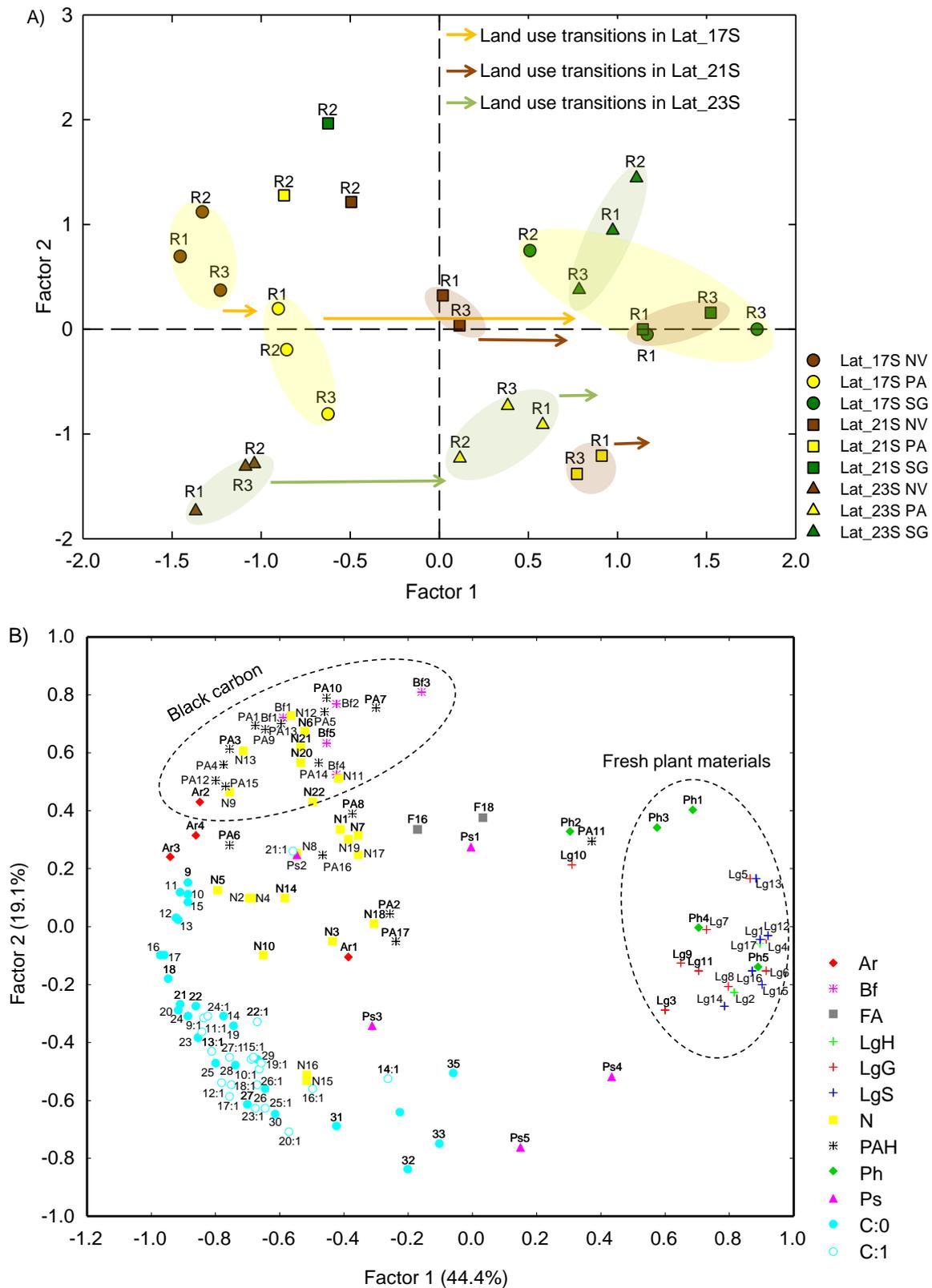


Figure 5. Scores (A) and loadings (B) on F1F2 projections of factor analysis applied to SOM extracts from 0-0.1 m soil depth from areas under different land uses in south-central Brazil. NV: Native Vegetation, PA: Pasture, SG: Sugarcane. R1, R2 and R3: replicates. Arrows: land use transitions on F1. Ar = aromatic, Bf = benzofuran, FA= fatty acid, Lg H = p-hydroxyphenyl lignin, Lg G = guaiacyl lignin, Lg S = syringyl lignin, PA = poliaromatics, Ps = polysaccharide, C:0 = alkane, C:1 = alkene, N =nitrogen containing compound. See Table 2 for codes.

The factor scores clearly showed the transition from NV-PA-SG within each site on F1 (Fig. 5a), which reflects the contribution from relatively fresh plant materials to SOM in PA and particularly SG in surface samples, as was explained for PCA_{all}. Contrarily, several studies have reported a decrease of lignin moieties when native vegetation or pasture was converted to cropland (e.g. (Nierop *et al.*, 2001), (Rumpel *et al.*, 2009) and linked this tendency to less litter inputs in areas under agricultural use. However, all studied SG areas are under green management, i.e., harvesting system without burning SG (Table 1). The high litter input in sugarcane areas under green management in Brazil (Cerri *et al.*, 2010) probably contributed to the dominance of fresh litter in the molecular signature of SOM in SG areas.

In addition, the disruption of aggregates by soil tillage in SG may have enhanced the microbial decomposition of more stable compounds. In Galicia (Spain), the SOM loss because of land use change from native vegetation to agricultural use mainly corresponded to secondary and recalcitrant SOM fractions, such as N-compounds and aliphatics, respectively (Verde *et al.*, 2008). Furthermore, comparing to pastures, the decomposition under natural ecosystems appears to be efficient and keeps pace with litter production, as was also observed by Yassir and Buurman (2012) under tropical conditions.

For the SG areas, the interpretation of F2 is in agreement with the slash and burn practices in the past (Table 1), SG samples from Lat_23S showing positive scores on F2 while the SG samples from the other locations have near neutral scores on F1 (except for the deviating R2 samples; Fig. 5a). It is further observed that at Lat_23S both NV and PA showed a low contribution from BC compared to SG, in agreement with the burning practice in SG areas from Lat_23S until 2003.

3.3.5. Factor analysis applied to subsurface samples (0.2-0.3 m)

At 0.2-0.3 m depth, factor analysis (PCA_{0.2-0.3}; Fig.6) showed that differences between land use decreased while the similarity within sites increased compared to that of the surface samples (PCA_{0-0.1}), indicating a decrease of the influence of LUC on SOM chemistry with depth (Fig. 6a). This is probably because deeper material has a longer mean residence time and has an increasing contribution from microbial input, being less influenced by environmental changes such as LUC (Rumpel & Kögel-Knabner, 2011); comparing the change in chemical composition between land uses at 0-0.1 m and 0.2-0.3 m should therefore correspond to the time passed since LUC.

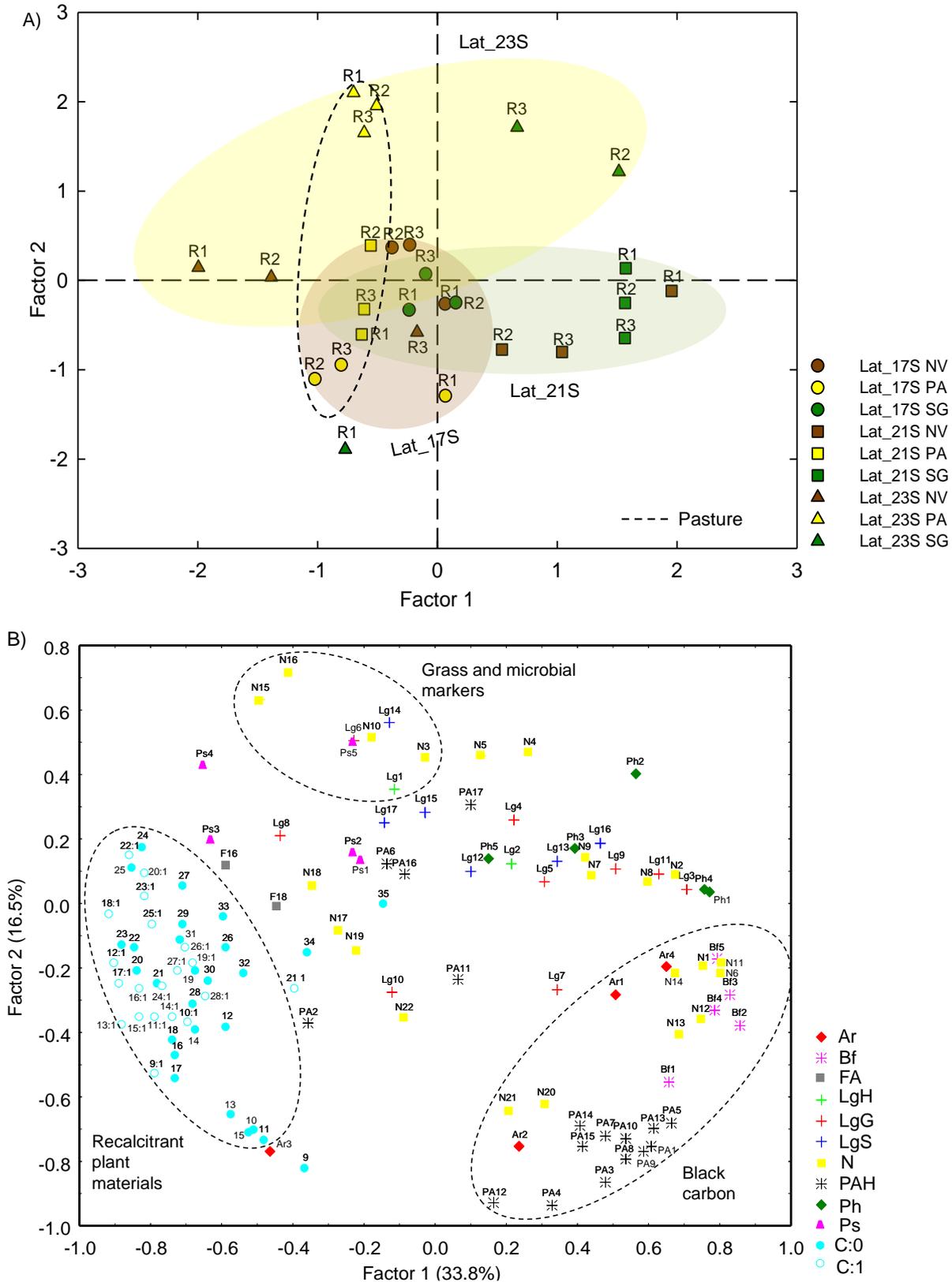


Figure 6. Scores (A) and loadings (B) on F1F2 projections of factor analysis applied to SOM extracts from 0.2-0.3 m soil depth from areas under different land uses in south-central Brazil. NV: Native Vegetation, PA: Pasture, SG: Sugarcane. R1, R2 and R3: replicates. Ar = aromatic, Bf = benzofuran, FA= fatty acid, Lg H = p-hydroxyphenyl lignin, Lg G = guaiacyl lignin, Lg S = syringyl lignin, PA = poliaromatics, Ps = polysaccharide, C:0 = alkane, C:1 = alkene, N =nitrogen containing compound. See Table 2 for codes.

Similar to the surface samples, the subsurface samples from the same site generally formed clear clusters in F1-F2 projection, except for sample R1 under SG from Lat_23S (Fig. 6a). We assume that this deviating sample is caused by very local differences, and in the following only the other two SG samples from Lat_23S will be used for interpretation.

Contrarily to the surface samples that were mainly separated by land use in F1-F2 projection, the factor scores of the subsurface samples showed clusters according to site. This depth effect was stronger for the deepest samples from 0.9-1.0 m depth (PCA_{all}), and reflects the decreasing influence of land use with depth and an increase of environmental factors on the chemical composition of SOM (Schmidt *et al.*, 2011).

There is a clear difference in the distribution of pyrolysis products compared to the surface samples (PCA_{0-0.1}; Fig. 5b). While in the surface samples F1 separated lignin phenols from more recalcitrant compounds (aliphatics and PAHs), the most evident chemical shift in PCA_{0.2-0.3} separated aliphatics (negative loadings) from benzofurans, some N containing compounds (N1, N6, N11-N14), phenols, some guaiacyl lignin moieties and PAHs (positive loadings; Fig. 6b). The lower loadings of lignin phenols at 0.2-0.3 m is probably related to the ploughing (SG) and rooting (PA) depth, decreasing fresh litter inputs.

On F2, compounds with negative loadings relate to the influence of BC (PAHs), meanwhile those positive loadings are associated with fresh non-woody litter, indicated by 4-vinylphenol (Lg1) and 4-vinylguaiacol (Lg6) that may indicate grass (Rumpel *et al.*, 2009), and is probably related to the continuous input of fresh root material in pasture. In addition, high positive loadings were found for some N compounds such as diketopiperazine (N15 and N16) and acetamide (N3) that may originate from microbial material (Stankiewicz *et al.*, 1996).

3.3.6. Correlations among factor scores and SOM attributes

Correlations among factor scores and content and isotopic composition of C and N support the interpretation of the factors (Table 5). The interpretation of the factors for the different PCAs is provided in Table 6. In PCA_{all} and PCA_{0-0.1}, F1 was positively correlated to $\delta^{13}\text{C}$ values ($r=0.45$ and 0.70 , respectively). In both cases, high positive values on F1 were related to fresh litter input, strongly decreasing with depth (PCA_{all}); while from PCA_{0-0.1} the influence of agriculture was clearly indicated by the higher scores of SG compared to PA (Table 6). Both sugarcane and grasses cultivated in south-central Brazil are C4 plants, with $\delta^{13}\text{C}$ values greater than C3 plants predominant in NV areas. The higher scores for SG compared to PA in combination with the positive correlation of F1 with $\delta^{13}\text{C}$, suggests that the SOM fingerprint

under SG reflects a relatively low mean residence time C source, probably reflected by the high litter input in SG.

F2 was negatively correlated to TOC and TN in PCA_{all} and PCA_{0-0.1}, to TOC in PCA_{0.2-0.3}, and negatively correlated to TN in PCA_{0.2-0.3} (Table 5). In all cases this was related to the contribution from BC, with a higher contribution from BC associated with lower TOC and TN contents (Table 6). Carr *et al.* (2013) also observed that pyrolysates enriched in PAHs were associated with relatively low organic matter content in surface soils from South Africa. This consistent tendency suggests that frequent burning in the past causes lowering of C stocks (Oliveira *et al.*, 2016).

Table 5. Pearson's correlation coefficients between soil attributes - total carbon (TC), total nitrogen (TN) and isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) - and factors from factor analysis applied to pyrolysis products from soil samples from areas under different land use in south-central Brazil.

	PCA _{All} ¹			
	TOC (g kg ⁻¹)	¹³ C (δ ‰)	TN (g kg ⁻¹)	¹⁵ N (δ ‰)
F1	0.16	0.45*	0.17	-0.20
F2	-0.44*	0.03	-0.39*	0.18
	PCA _{0-0.1}			
	TOC (g kg ⁻¹)	¹³ C (δ ‰)	TN (g kg ⁻¹)	¹⁵ N (δ ‰)
F1	-0.27	0.71*	-0.17	0.08
F2	-0.51*	-0.09	-0.46*	0.02
	PCA _{0.2-0.3}			
	TOC (g kg ⁻¹)	¹³ C (δ ‰)	TN (g kg ⁻¹)	¹⁵ N (δ ‰)
F1	-0.38**	0.02	-0.24	-0.10
F2	0.31	0.16	0.40*	0.77*

¹: PCA_{All}: factors from PCA of all samples; n=61. PCA_{0-0.1}: factors from PCA of 0-0.1 m samples; n=27. PCA_{0.2-0.3}, factors from PCA of 0.2-0.3 m samples; n=27. *: Pearson's correlation coefficients significant (p<0.005). **: Pearson's correlation coefficients significant (p<0.01).

The agreement between TN and TOC (PCA_{all}; PCA_{0-0.1}) is explained by their parallel origin from organic matter. Finally, the $\delta^{15}\text{N}$ values showed a strong positive correlation with F2 from PCA_{0.2-0.3} ($r = 0.77$), reflecting the contribution from microbial material that is known to cause higher $\delta^{15}\text{N}$ values (Högberg, 1997). There was no significant (positive) correlation between N containing pyrolysis products and TN (Carr *et al.*, 2013, Schellekens *et al.*, 2014).

As discussed in Section 3.3.3, Table 6 reinforces that depth is the major factor that influenced SOM composition, and the influence of land use was only dominant in surface soils and diminished rapidly with depth. The influence of site was becoming more important with

depth, which suggests that microbial processes and burning were site characteristic instead of related to land use. Differences in shifts from NV to PA to SG between sites could not be related to its LUC history. A higher number of sites must be compared to understand such differences.

Table 6. Scheme of factor analysis interpretation.

		PCA _{all}		PCA _{0-0.1}		PCA _{0.2-0.3}	
		F1 (30.4%)	F2 (22.3%)	F1 (44.4%)	F2 (19.1 %)	F1 (33.8%)	F2 (16.5%)
Scores	Positive	Surface ¹ SG	Deeper	SG	NV (Lat_17S) SG (Lat_23S)	Lat_21S	PA and SG (Lat_23S)
	Negative	Subsurface, deeper NV, PA	Surface	NV	PA NV (Lat_23S)	Lat_17S Lat_23S	No clear separation
Loadings	Positive	Fresh plant material	Charred material	Fresh plant material	Charred material	Charred material	Fresh plant and microbial material
	Negative	Charred and recalcitrant material	Fresh plant and recalcitrant materials	Charred and recalcitrant material	Recalcitrant plant materials	Recalcitrant plant material	Charred and recalcitrant material

¹: Surface: samples from 0-0.1 m. Subsurface: samples from 0.2-0.3 m. Deeper: Samples from 0.9-1.0 m. NV: native vegetation. PA: pasture. SG: sugarcane.

3.4. Conclusions

The use of Py-GC/MS on NaOH extractable SOM provided us detailed information on shifts in the molecular composition of SOM in areas of sugarcane expansion. Depth was the major factor that influenced SOM composition. The effect of LUC was mainly observed in surface samples. The chemical differences between native vegetation, pasture and sugarcane decreased with depth and LUC did not alter the SOM composition at 0.9-1.0 m depth. At 0.9-1.0 m depth, the chemistry was determined by site, suggesting a climatic/edaphic control.

The effect of LUC in the surface soil layer was clear at all study sites, and showed a larger contribution from more stable compounds (aliphatics and polyaromatics) under native vegetation compared to areas under sugarcane that had a higher contribution from compounds related to fresh plant materials (lignin moieties and phenols). The main difference in SOM composition undergoing the conversion pasture-sugarcane was the notably higher contribution from compounds associated to fresh litter inputs in the soil surface (0-0.1 m depth); this is probably related to the high litter input in sugarcane fields under green management in Brazil (Cerri *et al.*, 2010).

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4. ASSESSING LABILE ORGANIC CARBON IN SOILS UNDERGOING LAND USE CHANGE IN BRAZIL: A COMPARISON OF APPROACHES

ABSTRACT

Labile organic C (LC) and C management index (CMI) can be useful indicators of alterations of soil organic matter (SOM) in areas undergoing land use change (LUC) for biofuels production. However, there is no consensus on which methodology is best suited for quantifying LC and CMI. The main goal of this study was to assess alterations on LC contents and CMI values in sites undergoing the LUC transition native vegetation (NV)-pasture (PA)-sugarcane (SC) in south-central Brazil, and evaluated the sensitivity of different methods commonly used to assess LC and CMI, in order to select a best-suited method to quantify these indicators in tropical regions. The conversion NV-PA decreased the LC and CMI, whilst the conversion of PA-SC tended to increase the CMI. Accordingly, cropping sugarcane in areas previously used as pastures, as currently has been observed in Brazil, enhance SOM quality. The methodology used to quantify the LC and the CMI is critical to infer about LUC effects. Both methods proposed by Blair *et al.* (1995) and Diekow *et al.* (2005) were highly sensitive to the conversions evaluated in this research. However, Diekow *et al.* (2005) is the most suitable method to estimate the LC and CMI in sites undergoing LUC in Brazil, since the approach of Blair *et al.* (1995) notably overestimates these SOM quality indicators. We reiterate that the SOM changes are well expressed by the total soil organic C in areas undergoing LUC and, integrated approaches, such as the CMI, are suitable to evaluate the effects of LUC on SOM.

Keywords: Soil organic matter; C management index; Particulate organic C; Easily oxidizable C; Sensitivity index; Sugarcane ethanol

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4.1. Introduction

Soil organic matter (SOM) is a key component of terrestrial ecosystems and its abundance and composition have important effects on processes that occur in the system (Batjes,

1996). Besides being a source for increased biogenic CO₂ emissions, decreases in the quantity and quality of SOM can reduce agricultural productivity and food security, particularly in tropical regions (Lal, 2006). Recent insights on SOM turnover assert that virtually all organic compounds can be decomposed in soil, regardless of intrinsic molecular “recalcitrance” (Lehmann & Kleber, 2015). However, organic compounds with more simple structures, such as polysaccharides, lipids and proteins, are more prone to decomposition and to stimulate biological activity when compared to other organic compounds comprising SOM (Wang *et al.*, 2015).

Soil labile organic carbon (LC) can be defined as the SOM fraction which fuels the soil food web and therefore greatly influences nutrient cycles and many biologically related soil properties (Zak *et al.*, 1994). There are several techniques for LC assessment, which include procedures based on soil biological activity, chemical oxidation and physical fractionation (von Lützow *et al.*, 2007). Chemical-based methods, such as the use of potassium permanganate (KMnO₄) (Blair *et al.*, 1995, Lefroy *et al.*, 1993) and potassium dichromate (K₂Cr₂O₇) (Chan *et al.*, 2001), have been proposed. These oxidizing reagents are assumed to mimic the enzymatic breakdown of SOM, which is also largely an oxidative process. Physical fractionation of SOM has been used for LC assessment, generally based on particle size and degree of association with the soil mineral fraction, as a measure of its bioavailability (Cambardella & Elliott, 1992).

To evaluate the effects of management and land use on LC and total soil organic carbon (SOC) in an integrated approach, Blair *et al.* (1995) proposed the carbon management index (CMI). Subsequently, the CMI has been extensively used as an indicator of soil quality in response to soil management changes (Benbi *et al.*, 2015, Vieira *et al.*, 2007). The CMI expresses the soil quality in terms of increments in the SOC and in the proportion of LC compared to a reference soil, generally under native vegetation, which arbitrarily has a CMI of 100.

In Brazil, the largest producer of sugarcane ethanol of the world, the area cropped to sugarcane is expanding and the most common type of land use change (LUC) is the conversion of pastures into sugarcane (Dias *et al.*, 2016, Lapola *et al.*, 2014). LUC induces modifications on SOM, which is one of the main source of uncertainty in life cycle assessments of biofuels (Qin *et al.*, 2016). Accordingly, the LC and the CMI can be useful indicators of alterations of SOM in sites under LUC, and allow possible inferences on the sustainability of sugarcane cropping in areas previously under pastures. However, there is no consensus on which of the methods mentioned above is best suited for quantifying LC and calculating CMI in land use conversions. Thus, this study aimed to assess alterations on LC contents and CMI values in sites undergoing the LUC transition of native vegetation-pasture-sugarcane in south-central Brazil. Moreover, we

evaluated the sensitivity of different methods commonly used to assess LC and CMI in tropical regions, in order to select a best-suited method to quantify these indicators in tropical regions.

4.2. Material and Methods

4.2.1. Description of study sites

The study sites were located in three strategic and representative locations in the south-central, main sugarcane-producing region of Brazil. The climate at all three sites has rainfall concentrated in the spring and summer (October–April), while the dry season is in the autumn and winter (May–September). The soils are typical of the Brazilian tropical region, well drained and highly weathered, with a predominance of 1:1 clay mineral kaolinite, Fe oxides (goethite and hematite), and Al oxide (gibbsite) in the clay-size fraction. The soil classification (USDA, 2014), as well as the Köppen climate classification, are presented in Table 1. For details about geology and soil characterization, see Cherubin *et al.* (2015).

4.2.2. Land use change sequence and soil sampling

In this study, we used a chronosequence approach because there are no long-term experiments that represent this LUC sequence in the studied region. To do so, each of the three studied sites (Lat_17S; Lat_21S; Lat_23S) comprised three land uses: native vegetation (NV), pasture (PA) and sugarcane (SC), representing one of the most common LUC sequence in the south-central region of Brazil. In each site, the three land uses are located in adjacent plots, minimizing the effects of climatic, topographic and soil variations. The general description of each site is shown in Table 1. Each land use was composed of a sampling grid with nine points, 50 m apart (~ 4 ha). The samples were collected using an auger, at seven depths: 0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.5, 0.5-0.7, 0.7-0.9, 0.9-1.0 m. For LC analysis, the three samples on the diagonal position of the sampling grid were chosen at 0-0.1 m, 0.2-0.3 m, 0.3-0.5 and 0.9-1.0 m depth (Fig. 1). Based on our previous studies (Oliveira *et al.*, 2016a, Oliveira *et al.*, 2016b), these sampling points and depths are considered to fully represent the main effects of LUC on SOM in these sites in a cost-effective way.

Table 1. Land use history and brief description of the studied sites in south-central Brazil.

Site	Land use	Description ^a
Lat_17S 17°56'16"S; 51°38'31"W clayey Acrudox soils Awa (Köppen)	Native vegetation	Cerradão forest formation, Cerrado biome, characterized by sclerophyllous and xeromorphic species. The vegetation is dense compared to the Cerrado <i>stricto sensu</i> (savana).
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Brachiaria</i> and supports 1.5 AU ha ⁻¹ full year.
	Sugarcane	Conversion from pasture at 2009. Cultivar RB855453 with mean yield of 81.5 Mg ha ⁻¹ . Conventional tillage procedures and chemical fertilization. At the sampling time, sugarcane was in the third ratoon cropping of its cycle. Sugarcane is mechanically harvested without burning since its implantation.
Lat_21S 21°14'48"S; 50°47'04"W loamy Hapludalf soils Aw (Köppen)	Native vegetation	The local vegetation is seasonal semi-deciduous forest, Atlantic forest biome, in which a portion of the trees defoliates during the dry season.
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Brachiaria</i> and supports 2 AU ha ⁻¹ full year. Annually 120 kg ha ⁻¹ of the fertilizer formulation 20:5:19 are applied.
	Sugarcane	Conversion from pasture at 2010. Cultivar SP791011 with a mean yield of 80 Mg ha ⁻¹ . Conventional tillage procedures and chemical fertilization + vinasse application. At the sampling time sugarcane was in the fourth ratoon cropping of its cycle. Mechanically harvested without burning since its implantation.
Lat_23S 23°05'08" S; 49°37'52" W clayey Hapludox soils Cwa (Köppen)	Native vegetation	The local vegetation is seasonal semi-deciduous forest, Atlantic forest biome, in which a portion of the trees defoliates during the dry season.
	Pasture	Conversion from native vegetation at 1980. Composed by tropical grasses of the genus <i>Cynodon spp.</i> and supports 1 AU ha ⁻¹ full year.
	Sugarcane	Conversion from pasture at 1990. Cultivar CTC6 with a mean yield of 85 Mg ha ⁻¹ . Conventional tillage procedures and chemical fertilization + vinasse and filtercake application. At the sampling time sugarcane was in the fifth ratoon of its cycle. Pre-harvest burning between 1990 and 2002. Since 2013, 50% of straw has been removed for energy production.

AU: animal unit. For further details about land use description, see Cherubin *et al.* (2015).

4.2.3. Methods to quantify the labile C

Although the principles of chemical oxidation and physical fractionation as an indicator of biological lability are completely different, both approaches have been promoted for measurement of the LC fraction of SOM. Accordingly, we assessed LC using five different approaches. For all analyses, soil samples were first air dried, mixed and passed through a 2mm sieve. Soil subsamples were ground and sieved through a 100 mesh (0.149 mm) sieve.

Method of Blair *et al.* (1995) (Bl): Briefly, about 1 g of soil (<0.149 mm) was weighed into plastic screw top centrifuge tubes and 25 mL 333 mM L⁻¹ KMnO₄ were added to each tube. All tubes were tightly sealed, tumbled for 1 h (60 rpm) and centrifuged for 5 min at 2000 rpm. The supernatant was subsequently diluted with deionized water (1:250), and the KMnO₄ consumed was determined by colorimetry (565 nm). The amount of LC was calculated from the change in the concentration of KMnO₄ when compared with the blank samples.

Method of Shang and Tiessen (1997) (Sg): same described above (Blair *et al.*, 1995), with exception of the KMnO₄ concentration, which was 10 times lower, in this case 33 mM L⁻¹.

Method of Chan *et al.* (2001) (Cn): About 0.5 g of soil (<0.149 mm) was weighed into an Erlenmeyer flask and 10 mL of 0.167 mol L⁻¹ K₂Cr₂O₇ was added, followed by 5 mL of concentrated sulfuric acid. After the reaction (30 min), the excess dichromate was determined by titrating against 0.5 mols L⁻¹ FeSO₄. The amount of dichromate consumed by the soil was used to calculate the amount of LC based on the theoretical value of 1.0 mL of 0.167 mol L⁻¹ K₂Cr₂O₇ oxidizing 3 mg of SOC.

Method Alternative (Al): We tested an alternative methodology using an adaptation of Chan *et al.* (2001), which we added only 2.5 mL of sulfuric acid instead of 5.0 mL.

Method of Diekow *et al.* (2005) (Dk): Based on physical fractionation of SOM (Cambardella & Elliott, 1992, Christensen, 1992), Diekow *et al.* (2005) suggested the use of the particulate organic carbon (POC) as a measurement of LC. In our study, about 20 g of soil (<2 mm) were weighed into a 100 mL flask, adding 70 mL of deionized water. The sample was then treated with ultrasound for 15 min and transferred to a 200 mesh (0.074 mm) sieve, where it was washed with deionized water. The fraction retained on the 200 mesh was transferred to a crucible, where the organic and the mineral fractions were separated by flotation in deionized water (Christensen, 1992). The C content in the light coarse fraction of POC was determined by dry combustion method in an elemental analyzer and assumed to encompass the LC. Despite based on the suggestions of Diekow *et al.* (2005), our research employed a different approach to physical fractionation of SOM (Christensen, 1992).

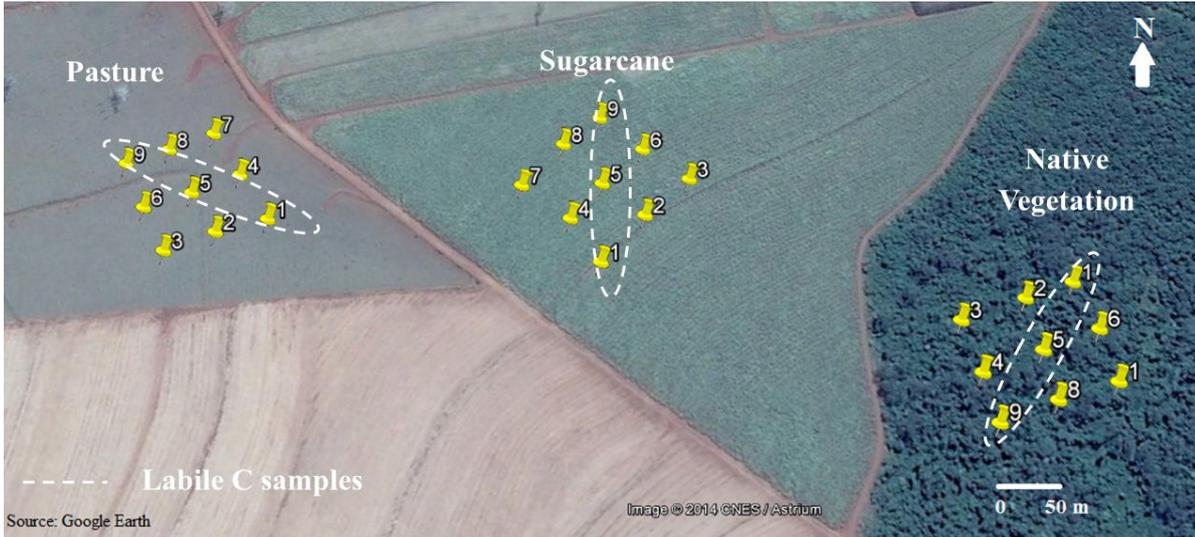


Figure 1. Example of sampling design (Lat_23S). Samples used in the labile C assessments are indicated by circles.

4.2.4. Carbon Management Index and sensitivity index assessment

The C management index (CMI) originally proposed by (Blair *et al.*, 1995) was calculated assuming LC values determined from each of the above methodologies. The non-labile organic carbon (NLC), equivalent to the residual SOC not quantified as LC, was estimated by difference (NLC = SOC – LC). SOC was assessed by dry combustion method in an elemental analyzer. The CMI was calculated using the follow equation (1):

$$\text{CMI} = \text{CPI} \times \text{LI} \times 100 \quad (1)$$

where CPI (carbon pool index) = total soil organic C of a land use (pasture or sugarcane) (g kg^{-1}) / total soil organic C of native vegetation (g kg^{-1}); LI (lability index) = soil C lability of a land use (pasture or sugarcane) / soil C lability of native vegetation; lability = labile C (g kg^{-1}) / non-labile C (g kg^{-1}).

The sensitivity index (SI) of the LC for both land use conversions (NV-PA and PA-SC) was calculated using the equation 2 (Banger *et al.*, 2010, Sheng *et al.*, 2015). The same procedure was used to test the sensitivity of CMI.

$$\text{SI} = \frac{|\text{LCc} - \text{LCp}|}{\text{LCp}} \quad (2)$$

where LCc: labile organic C in the current land use (g kg^{-1}); LCp: labile organic C in the previous land use (g kg^{-1}).

4.2.5. Data analysis

Comparisons among land uses (NV, PA and SC) were carried out for LC quantified by different methodologies using one-way ANOVA with land use type as the main factor and sites considered as blocks and treated as a random variable. Data transformations were not necessary to meet the assumptions of ANOVA. The means between land uses were compared (considering each site as a block) by Tukey test ($p < 0.05$). For the sensitivity index, statistically significant differences ($p < 0.05$) were determined by the non-overlap of the upper and lower limits of the averages at the 85% confidence intervals (Payton *et al.*, 2000). All analyses were conducted using the software R, version 3.2.2 (R-Core-Team, 2015).

4.3. Results

4.3.1. Effects of land use change on labile C and C management index

The conversion of NV to PA decreased LC quantified by all methodologies used for the 0-0.1 m soil depth (Table 2). The LC on SC areas did not differ statistically from those under PA, unlike SOC (0-0.1 m) which was greater in PA. For the 0.2-0.3 soil layer, NV soil also had the highest LC content accessed by different methodologies (Table 2). However, SC areas had higher LC contents than PA areas using the Dk and Al methodologies.

At 0.5-0.7 m soil layer, the conversion NV-PA did not decrease the LC assessed by the Sg method. In addition, only the LC quantified by Al differed statistically between NV and SC (Table 2). For soil samples from the 0.9-1.0 m layer, except for Sg methodology, the conversion NV-PA decreased LC contents in all other tested methods. At this depth, the LC contents in SC areas did not differ from NV in any of the methods. For both 0.5-0.7 and 0.9-1.0 m soil layers, only the Dk method showed higher LC contents in SC areas compared to PA (Table 2).

The conversion of NV to PA decreased the CMI at all depths and with all methods (Fig. 2). Overall, areas under SC had higher values for CMI compared to PA and these differences were accentuated with increasing depth, regardless of the method used. In soil samples from PA at 0-0.1 m layer, we highlight the low CMI values according the Bl and Dk methods; whereas in

soil samples from SC at 0.9-1.0 m depth, the high CMI values stand out, with values greater than 80 for all methods used, except the Cn method (Fig. 2).

Table 2: Total soil organic C (SOC) and labile C assessed by different methodologies – Blair *et al.* (1995) (Bl); Shang and Tiessen (1997) (Sg); Chan *et al.* (2001) (Cn); Diekow *et al.* (2005) (Dk); alternative methodology (Al) - at four depths (0-0.1, 0.2-0.3, 0.5-0.7, 0.9-1.0 m) in soils of sites under different land uses in south-central Brazil.

	NV	PA	SC	NV	PA	SC
	Carbon (g kg ⁻¹) 0-0.1 m			Carbon (g kg ⁻¹) 0.2-0.3 m		
SOC ¹	28.62±1.9 ^a	18.06±0.8 ^b	15.62±1.2 ^c	12.49±1.1 ^a	9.21±0.7 ^b	10.33±0.8 ^b
Bl	16.35±1.9 ^a	9.19±1.1 ^b	9.40±1.0 ^b	7.33±0.9 ^a	4.84±0.6 ^b	5.82±0.7 ^{ab}
Sg	1.92±0.2 ^a	1.40±0.1 ^b	1.31±0.1 ^b	0.86±<0.1 ^a	0.66±<0.1 ^b	0.69±<0.1 ^b
Cn	10.66±0.9 ^a	7.25±0.5 ^b	6.98±1.1 ^b	5.90±0.7 ^a	4.20±0.5 ^b	4.54±0.6 ^b
Dk	4.93±0.4 ^a	2.98±0.4 ^b	2.84±0.3 ^b	1.35±0.1 ^a	0.88±<0.1 ^b	1.19±0.1 ^a
Al	6.32±0.7 ^a	3.94±0.4 ^b	4.29±0.5 ^b	2.62±0.2 ^a	1.76±0.1 ^c	2.22±0.1 ^b
	Carbon (g kg ⁻¹) 0.5-0.7 m			Carbon (g kg ⁻¹) 0.9-1.0 m		
SOC	9.00±0.9 ^a	6.34±0.5 ^b	8.00±0.9 ^{ab}	8.00±0.5 ^a	4.55±0.3 ^b	5.71±0.5 ^b
Bl	5.36±0.6 ^a	3.35±0.4 ^b	4.47±0.8 ^{ab}	4.22±0.4 ^a	2.17±0.1 ^b	3.55±0.3 ^{ab}
Sg	0.51±<0.1 ^a	0.40±<0.1 ^a	0.44±<0.1 ^a	0.83±0.1 ^a	0.64±<0.1 ^a	0.77±<0.1 ^a
Cn	3.52±0.3 ^a	2.64±0.3 ^b	3.03±0.2 ^{ab}	3.15±0.4 ^a	1.82±0.1 ^b	2.46±0.2 ^{ab}
Dk	0.46±<0.1 ^a	0.30±<0.1 ^b	0.42±<0.1 ^a	0.48±<0.1 ^a	0.29±<0.1 ^b	0.45±<0.1 ^a
Al	1.68±0.1 ^a	1.31±<0.1 ^b	1.38±0.1 ^b	1.42±0.1 ^a	1.17±<0.1 ^b	1.33±0.1 ^{ab}

¹: Oliveira *et al.* (2016a). NV: Native vegetation; PA: Pasture; SC: Sugarcane. Letters represent statistically significant differences between land uses (considering each site as a block, $n = 9$), according the Tukey test (5 %).

4.3.2. Comparing different approaches to assess labile C and C management index

The methods differed notably regarding the percentage of SOC quantified as LC (Table 3), highlighting the large fraction of C quantified as LC by the Bl and Cn methodologies. Furthermore, with exception of Bl, Cn and Dk at 0-0.1 m, and Sg and Al at 0.9-1.0 m layer, the methodologies yielded the same percentage of SOC as LC regardless of land use. The LC contents assessed by all tested methodologies showed positive correlations with SOC (Table 4), especially Bl ($r=0.97$; $p<0.01$). Moreover, the methodologies used for LC quantification were significantly correlated with each other, with few exceptions (Table 4).

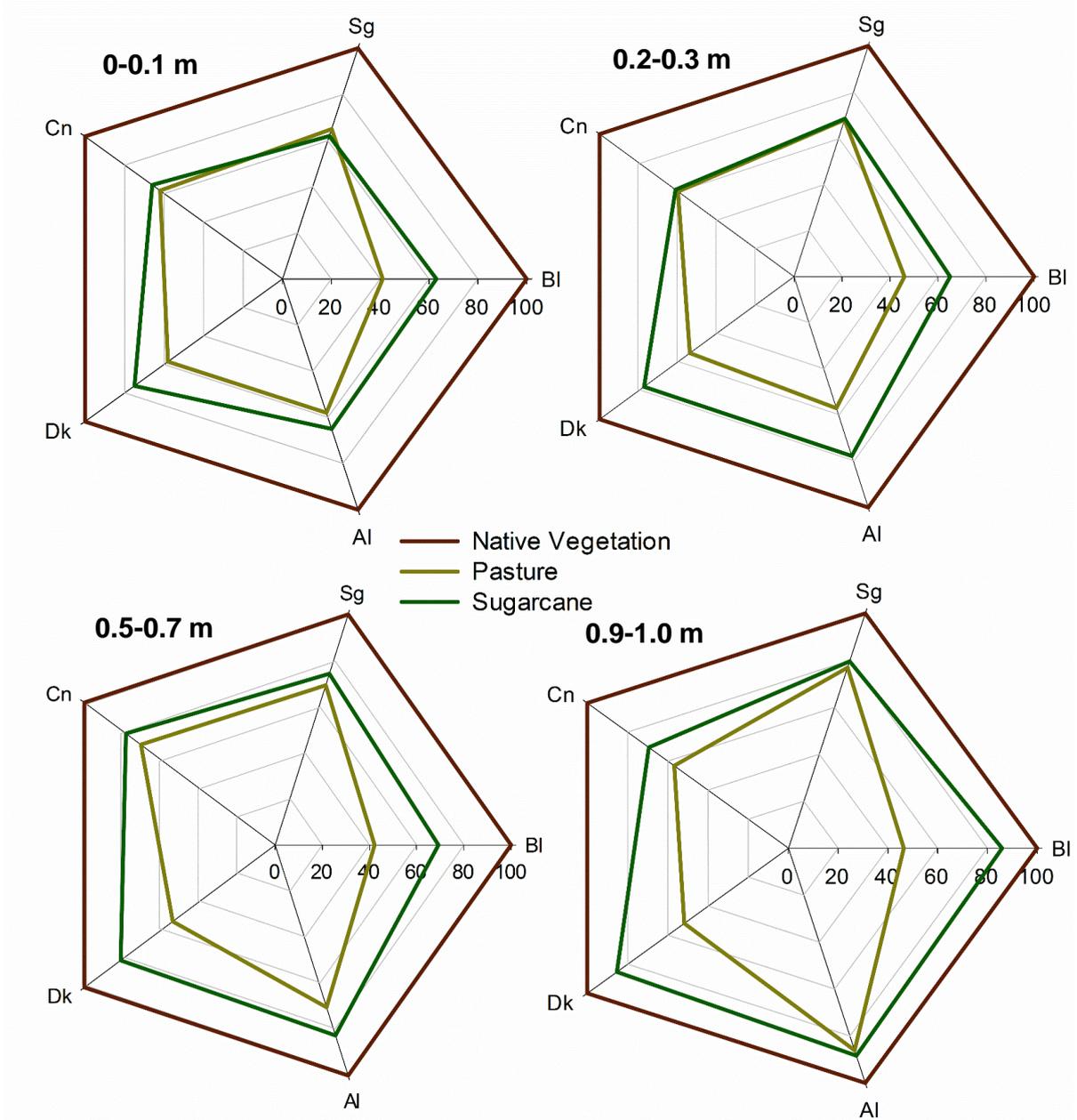


Figure 2. Carbon Management Index assessed by different methodologies – Blair *et al.* (1995) (BI); Shang and Tiessen (1997) (Sg); Chan *et al.* (2001) (Cn); Diekow *et al.* (2005) (Dk); alternative methodology (Al) – at four depths (0-0.1, 0.2-0.3, 0.5-0.7, 0.9-1.0 m) in soils of sites under different land uses in south-central Brazil.

Table 3. Percentage of total soil organic C (SOC) quantified as labile C according to different methodologies – Blair *et al.* (1995) (Bl); Shang and Tiessen (1997) (Sg); Chan *et al.* (2001) (Cn); Diekow *et al.* (2005) (Dk); alternative methodology (Al) – at four depths (0-0.1, 0.2-0.3, 0.5-0.7, 0.9-1.0 m) in soils of sites under different land uses in south-central Brazil.

	NV	PA	SC	NV	PA	SC
	% of SOC ¹ 0-0.1 m			% of SOC 0.2-0.3 m		
Bl	59.84±6.1 ^b	58.51±3.9 ^b	65.44±7.3 ^a	61.19±8.1 ^a	53.37±6.5 ^a	58.96±5.1 ^a
Sg	7.31±1.2 ^a	8.80±0.9 ^a	8.85±1.1 ^a	7.01±1.3 ^a	7.36±1.1 ^a	6.95±1.2 ^a
Cn	40.14±2.5 ^b	43.90±3.2 ^{ab}	47.37±2.9 ^a	48.10±4.7 ^a	46.48±1.9 ^a	44.38±1.5 ^a
Dk	16.80±2.1 ^b	15.93±1.8 ^b	22.82±1.9 ^a	9.97±1.5 ^a	8.74±1.5 ^a	10.64±1.9 ^a
Al	20.64±2.5 ^a	22.02±1.8 ^a	25.04±2.7 ^a	21.05±2.6 ^a	19.37±1.5 ^a	22.50±2.4 ^a
	% of SOC 0.5-0.7 m			% of SOC 0.9-1.0 m		
Bl	61.90±8.1 ^a	55.61±7.1 ^a	55.91±4.9 ^a	61.55±7.1 ^a	61.54±8.4 ^a	58.24±5.7 ^a
Sg	6.27±0.8 ^a	6.62±1.0 ^a	6.01±0.4 ^a	11.40±1.3 ^b	16.18±2.1 ^a	12.03±0.9 ^b
Cn	35.41±3.2 ^a	37.32±3.8 ^a	35.22±2.9 ^a	44.79±4.1 ^a	48.71±3.9 ^a	43.79±3.1 ^a
Dk	5.58±0.7 ^a	4.78±1.1 ^a	5.76±1.0 ^a	10.21±1.2 ^a	9.53±1.3 ^a	10.44±1.4 ^a
Al	18.43±1.9 ^a	19.56±2.2 ^a	17.42±3.1 ^a	16.95±2.1 ^b	26.57±2.5 ^a	19.97±2.3 ^b

¹: % of SOC = (LC/SOC)*100. NV: Native vegetation; PA: Pasture; SC: Sugarcane. Letters represent statistically significant differences between land uses (considering each site as a block, n = 9), according the Tukey test (5 %).

Table 4. Pearson's correlation coefficients between total soil organic C (SOC) and labile C assessed by different methodologies – Blair *et al.* (1995) (Bl); Shang and Tiessen (1997) (Sg); Chan *et al.* (2001) (Cn); Diekow *et al.* (2005) (Dk); alternative methodology (Al) – in soils of sites under different land uses in south-central Brazil.

	SOC	Bl	Sg	Cn	Dk	Al
SOC	1	0.97 ^{***}	0.25 [*]	0.33 [*]	0.53 ^{**}	0.22 [*]
Bl		1	0.31 ^{**}	0.39 ^{n.s.}	0.40 ^{***}	0.29 ^{**}
Sg			1	0.84 ^{***}	0.80 ^{***}	0.56 ^{**}
Cn				1	0.85 ^{***}	0.29 ^{n.s.}
Dk					1	0.73 ^{***}
Al						1

*: p<0.1. **: p<0.05. ***: p<0.01. n.s.: non-significant correlation. n=108.

Compared to other methods used to quantify the LC fraction, the Bl and Dk methodologies tended to be more sensitive regarding the conversion NV-PA in all layers assessed but not differed in sensitivity comparing to SOC at 0-0.1 m and 0.9-1.0 m soil layers (Fig. 3). Regarding the conversion PA-SC, the C fraction quantified by Dk exhibited notably higher sensitivity compared to SOC and other LC methodologies, whilst Al also showed good sensitivity, but restricted to the two top layers (Fig. 3).

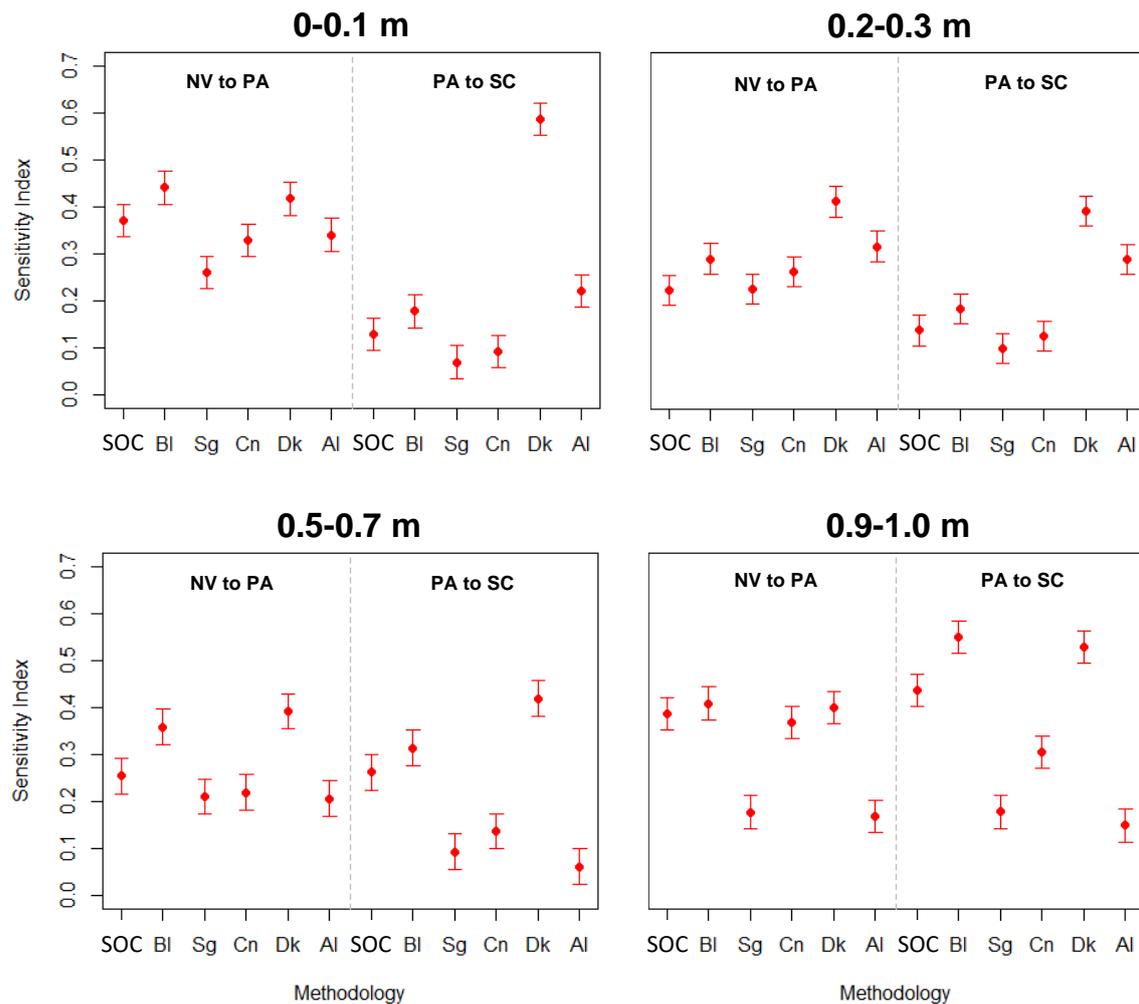


Figure 3. Sensitivity Index of total soil organic C (SOC) and labile C assessed by different methodologies – Blair *et al.* (1995) (Bl); Shang and Tiessen (1997) (Sg); Chan *et al.* (2001) (Cn); Diekow *et al.* (2005) (Dk); alternative methodology (Al) – for the conversion of native vegetation to pasture (NV to PA) and pasture to sugarcane (PA to SC) at four soil depths (0-0.1, 0.2-0.3, 0.5-0.7, 0.9-1.0 m) in sites of south-central Brazil. Bars mean the confidence intervals of mean values (85%). $n=9$.

Comparing the SI of the different methodologies for CMI calculation, Bl and Dk yielded CMI values more sensitive to the conversion NV-PA for all depths evaluated, not differing from each other (Fig. 4). Regarding the conversion PA-SC, the SI's for the CMI from Bl and Dk remained higher than the others methodologies, with Dk more sensitive to this conversion at 0-0.1 m and 0.2-0.3 m layer (Fig. 4). The alternative methodology also showed good sensitivity at 0-0.1 and 0.2-0.3 m soil layers. Overall, the deeper soil layers (0.5-0.7 and 0.9-1.0 m) were as sensitive to the conversion NV-PA-SC as shallow soil layers (0-0.1 and 0.2-0.3 m).

4.4. Discussion

4.4.1. Land use change and labile C contents by different methods

Overall, the conversion of native vegetation (NV) to pasture (PA) decreased the labile fractions of soil organic matter (SOM) according to all the methods used (Table 2). In theory, the labile organic carbon (LC) quantified by chemical oxidation is comprised of amino acids, simple carbohydrates, a fraction of microbial biomass, and other simple organic compounds (Zou *et al.*, 2005); meanwhile the LC quantified as the light fraction of particulate organic C mainly consists of partially decomposed plant and animal residues, root fragments and fungal hyphae (Skjemstad *et al.*, 2006). In common, these SOM fractions depend strongly on the C input to the soil (Vieira *et al.*, 2007), an aspect which certainly is associated to the shifts in the LC regarding the land use change (LUC) in the evaluated sites.

We suggest that the higher LC contents in sugarcane (SC) when comparing to PA are related to the high C input in sugarcane areas under green management (Cerri *et al.*, 2011). In Brazil, Blair *et al.* (1998) reported no significant change in total soil organic carbon (SOC), but a significant increase in the LC (C oxidized by KMnO_4 333 mM L^{-1}) in sugarcane areas without pre-harvest burning. More recently, Thorburn *et al.* (2012) linked the increments on LC (quantified by several methodologies) in sugarcane areas in Australia to the high C input by harvest residues, assigning part of this increases to the high concentration of soluble C in sugarcane straw. Besides the C input via root deposition and bioturbation (Rumpel & Kögel-Knabner, 2011), the translocation of soluble forms of C and the residue incorporation by tillage can be associated with increases on LC at deeper soil layers under SC.

Pasture areas had CMI values much smaller than those in NV (Fig. 2), showing that the conversion NV-PA substantially reduces the quantity and quality of C inputs, as well as the C lability, as also reported by Srivastava *et al.* (2016). SOC and the C lability directly influence soil physical, chemical and biological attributes as well as the self-organization capacity of soils (Blair *et al.*, 1995, Smith *et al.*, 2015). In addition, labile fractions of SOM are associated with nutrient mineralization and can make an important contribution to nutrient availability, nutrient cycling and biomass production. In Brazil, Moraes Sá *et al.* (2014) found a strong relation among SOM contents, labile fractions, and the grain yield of wheat and soybean.

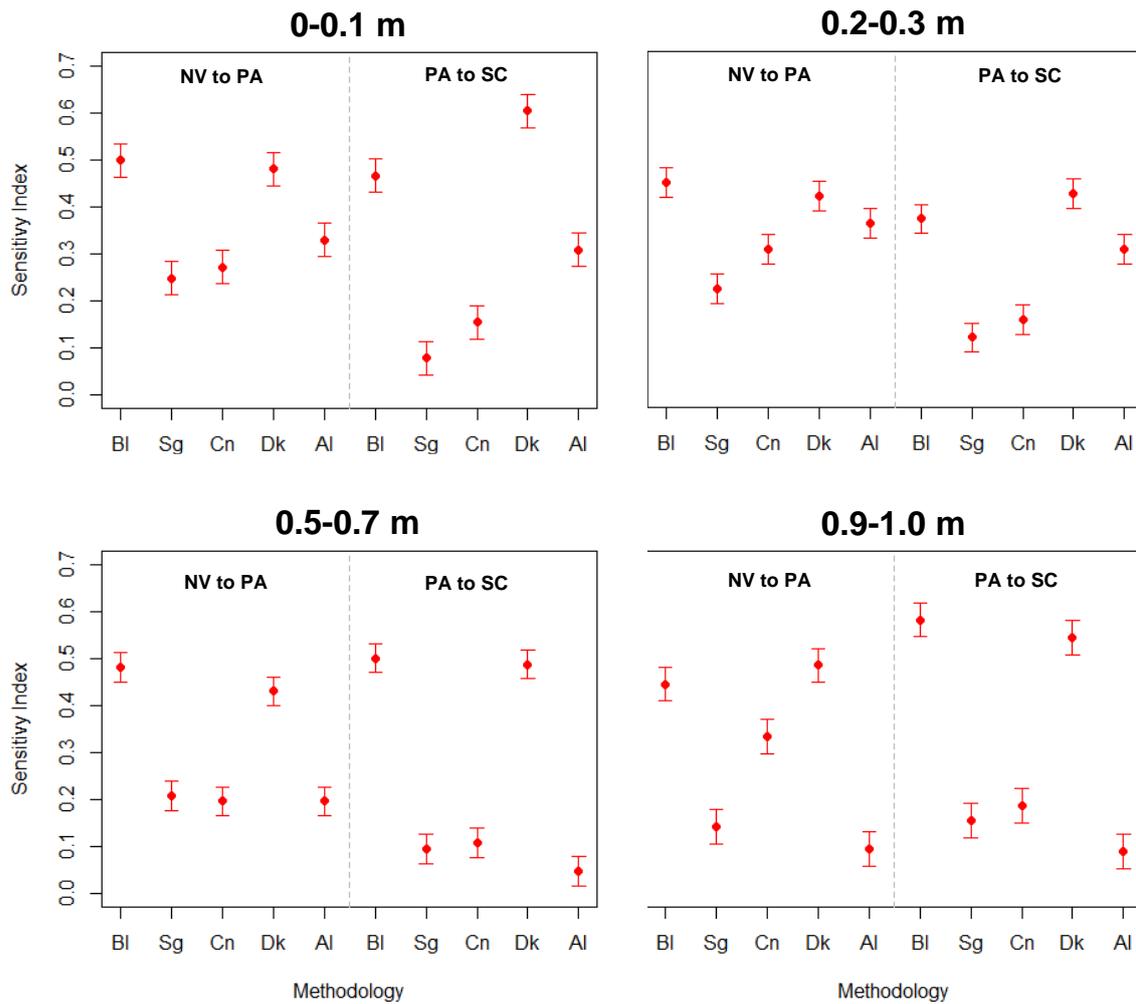


Figure 4. Sensitivity Index of the carbon management index (CMI) calculated by different methodologies – Blair *et al.* (1995) (Bl); Shang and Tiessen (1997) (Sg); Chan *et al.* (2001) (Cn); Diekow *et al.* (2005) (Dk); alternative methodology (Al) – for the conversion of native vegetation to pasture (NV to PA) and pasture to sugarcane (PA to SC) at four soil depths (0-0.1, 0.2-0.3, 0.5-0.7, 0.9-1.0 m) in sites of south-central Brazil. Bars mean the confidence intervals of mean values (85%). $n=9$.

The conversion PA-SC tended to increase CMI, mainly for soil layers below 0.2 m. Comparing crop systems to pasture areas in Northwest Himalayas, Verma and Sharma (2007) observed higher CMI values in pasture areas, linking these differences to the effects of soil tillage in crop areas. In our study, even with the periodic plowing in SC (Table 1), the conversion PA-SC increased CMI. In a recent study in these sites, Oliveira *et al.* (2016a) observed increases in the C stocks for the conversion PA-SC and associated this increment mainly to inputs of modern C (C from C4 plants) at deeper soil layers. These evidences suggest that the cropping of sugarcane in areas previously used as pastures might enhance the quantity and quality of SOM in sites of south-central Brazil. However, the CMI values in SC areas remained below 100 in all method tested (Fig. 2), showing that even improving soil quality relative to the pastures studied, sugarcane cultivation does not have the same potential of the native vegetation to maintain the lability of

the SOM and the soil quality. Finally, we highlight that the method used to quantify the LC and the CMI is critical to infer about the LUC effects on SOM.

4.4.2. Choosing the most suitable method to assess labile C in areas under land use change

The methods used differed notably regarding the fraction of SOC quantified as LC (Table 3), suggesting that different methods quantifying different fractions of SOM, including C compounds less/or not readily available to the soil microorganisms (Benbi *et al.*, 2015). The methodology proposed by Blair *et al.* (1995) (Bl) showed the higher amounts, yielding up to 65% of SOC as LC (Table 3), similar to the quantified amount in soils of Australia (Skjemstad *et al.*, 2006). The LC content in soils is quite variable, but values above 60% of SOC are clearly overestimations (von Lützow *et al.*, 2007). In this sense, our results reiterated that the methodology proposed by Blair *et al.* (1995) overestimates the labile fraction of SOM in tropical soils, as also suggested by Shang and Tiessen (1997) and Diekow *et al.* (2005).

The KMnO_4 efficiently oxidizes lignin, although it has little effect on several SOM components that are widely recognized as easily degraded by soil microorganisms, e.g. structural carbohydrates, sugars and amino acids (Suárez-Abelenda *et al.*, 2014). Accordingly, the high amount of lignin materials in the SOM of these sites (Oliveira *et al.*, 2016b) may have contributed to overestimated the LC assessed by Blair *et al.* (1995). The methodology proposed by Chan *et al.* (2001) (Cn) also quantified high proportions of SOC as LC (~40%). In this sense, the adaptation proposed in this research is a conveniently alternative to LC quantification using $\text{K}_2\text{Cr}_2\text{O}_7$.

Despite the high amount of C quantified as LC, Bl was quite similar to the methodology proposed by Diekow *et al.* (2005) (Dk), with both showing higher sensitivity of LC in sites undergoing LUC. Some studies concluded that the C fraction assessed by Bl can be as sensitive to management effects as the SOC, the microbial biomass, hot-water soluble C, and the LC assessed by Dk (Culman *et al.*, 2010, Wang *et al.*, 2014). However, despite the sensitivity of this indicator in our study, we suggested that the methodology proposed by Blair *et al.* (1995) may not be a reliable measure of LC in tropical areas, because the high amount of C quantified as LC.

In this sense, is possible that the methodology proposed by Diekow *et al.* (2005) is more feasible to the quantification of LC and assessment of CMI in tropical regions. The particulate C represents the youngest and most biologically SOM, such as particles of fresh or partially decomposed plant residues and microbial tissues (Skjemstad *et al.*, 2006). As a result, different studies have demonstrated the higher sensitivity of LC quantified by Dk to management practices

compared to other indicators such as SOC, microbial biomass and the LC quantified by Bl (Banger *et al.*, 2010, Yang *et al.*, 2012). Vieira *et al.* (2007) suggested that CMI calculated using the particulate C was a sensitive method for assessing the capacity of management systems to promote soil quality because of its close correlation with soil physical, chemical, and biological attributes. Additionally, we believe that the methodology suggested by Diekow *et al.* (2005) is most compatible with the new insights about the nature of SOM (Lehmann & Kleber, 2015).

The sensitivity indices did not differ or were smaller than the SI of SOC for some of the methodologies evaluated (Fig. 3). Additionally, with a couple exceptions, the methodologies quantified a similar percentage of SOC as LC across the land uses (Table 3) and correlated significantly with SOC (Table 4). Despite still widely used, we need to be cautious about concluding that fractions of SOM assessed by partial chemical oxidation (or even by physical fractionation) are inherently more sensitive to LUC than the bulk measurements of SOC in tropical regions. In India, no single labile fraction of C assessed by chemical oxidation could be used as a more sensitive indicator of land-use induced changes on SOM (Benbi *et al.*, 2015). These approaches may be good indicators of SOM changes in areas under different management practices, whereas with the large alterations due to land use change, the SOM changes may be equally well expressed by the SOC or integrated approaches, as the CMI.

The CMI showed itself as a useful tool for evaluate the land use effects on SOM dynamics in tropical regions, as also reported by Srivastava *et al.* (2016). However, the methodology used to quantify the LC was critical to the CMI values and to the sensitivity (SI) of this indicator regarding the LUC (Fig. 4). According to SI, both Bl and Dk methodologies can be used to quantify the LC and to calculate the CMI in tropical sites under LUC. However, the methodology proposed by Blair *et al.* (1995) clearly overestimated the LC (Table 3). In this sense, we suggest that the methodology proposed by Diekow *et al.* (2005) is the most suitable to estimations about the labile pool of SOM and to calculation of the CMI in sites undergoing LUC.

The alternative methodology (Al) proposed in this study for the quantification of LC was sensitive to the conversion NV-PA-SC to soil samples from shallow layers (0-0.1 and 0.2-0.3). Therefore, we suggest this methodology as an alternative to scenarios where the quantification of LC by Diekow *et al.* (2005) is not possible (e.g. elemental analyzer not available). We reiterate that the methodology proposed did not proved to be sensitive to alterations on SOM that occurred below the plow soil layer (0.3 m).

4.4.3. Labile C alterations at deeper soil layers

LC and CMI alterations showed that deeper soil layers (0.5-0.7 and 0.9-1.0 m) are as sensitive to the conversion NV-PA-SC as shallow soil layers (0-0.1 and 0.2-0.3 m). Recent studies have focused on the SOM lability in subsoil and its dynamic response to land use and management practices (Rumpel & Kögel-Knabner, 2011, Sheng *et al.*, 2015). Just as observed in our study, these studies found that alterations on SOM lability regarding the LUC were not restricted to shallow soil layers. Subsoil (>0.3 m) SOM accretion, turnover and stabilization are key knowledge gaps on SOM research (Paustian *et al.*, 2016). In China, Sheng *et al.* (2015) found that the alterations in C input (mainly through fine root biomass) and in microorganism activity were the dominant factors leading to the loss of LC from subsoil after land use change.

Furthermore, the sensitivity of some methodologies to LUC were different comparing shallow and deeper soil layers (e.g. the methodology proposed in our study was sensitive to alterations on LC up to 0.3 m, but seemed not to respond to shifts in deeper soil layers). Therefore, is essential the inclusion of deeper soil layers in studies regarding LUC, as well as the choice of the most sensitive and suitable indicators to evaluate the shifts on SOM in these layers.

4.5. Conclusions

The conversion of areas under native vegetation to pasture decreases both the LC and the CMI down to 1.0 m soil depth, whilst the conversion of pasture to sugarcane increased the CMI according to all evaluated methods, mainly below 0.2 m depth. These evidences suggest that cropping sugarcane in areas previously used as pastures, as currently has been observed in Brazil, enhances the SOM quality.

The method used to quantify LC and CMI is critical to infer about the LUC effects on SOM. Both methodologies proposed by Blair *et al.* (1995) and Diekow *et al.* (2005) were highly sensitive to the conversions evaluated in this research. However, Diekow *et al.* (2005) is the most suitable to estimate the SOM labile pool and to calculate the CMI in sites undergoing LUC in Brazil, since the approach of Blair *et al.* (1995) notably overestimates these indicators. Finally, we reiterate that the SOM changes are often well expressed by the SOC in areas undergoing LUC. In this sense, integrated approaches, such as the CMI, are quite suitable to evaluate the effects of LUC on SOM.

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5. PREDICTING SOIL C CHANGES OVER SUGARCANE EXPANSION IN BRAZIL USING THE DAYCENT MODEL

ABSTRACT

In recent years, the increase in Brazilian ethanol production has been based on expansion of sugarcane cropped area, mainly by the land use change (LUC) pasture-sugarcane. However, second generation (2G) cellulosic-derived ethanol supplies are likely to increase dramatically in the next years in Brazil. Both these management changes potentially affect soil C (SOC) changes and may have a significant impact on the greenhouse gases balance of Brazilian ethanol. To evaluate these impacts, we used the Daycent model to predict the influence of the LUC native vegetation (NV) - pasture (PA) - sugarcane (SG), as well as to evaluate the effect of different management practices (straw removal, no-tillage and application of organic amendments) on long-term SOC changes in sugarcane areas in Brazil. The DayCent model estimated that the conversion of NV-PA caused SOC losses of $0.34 \pm 0.03 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, whilst the conversion PA-SG resulted in SOC gains of $0.16 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. Moreover, simulations showed SOC losses of $0.19 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ in SG areas in Brazil with straw removal. However, our analysis suggested that adoption of some best management practices can mitigate these losses, highlighting the application of organic amendments ($+0.14 \pm 0.03 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$). Based on the commitments made by Brazilian government in the UNFCCC, we estimated the ethanol production needed to meet the domestic demand by 2030. If the increase in ethanol production was based on the expansion of sugarcane area on degraded pasture land, the model predicted a SOC accretion of 144 Tg from 2020-2050, whilst increased ethanol production based on straw removal as a cellulosic feedstock was predicted to decrease SOC by 50 Tg over the same 30 year period.

Keywords: Land use change; Soil organic matter; Straw removal; Biofuels; Second generation ethanol; Best management practices

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5.1. Introduction

Bioenergy is critical for environmental security and climate change mitigation. Future projections suggest that 30% of the world's fuel supply might be bio-based by 2050 (Macedo *et al.*, 2015). However, the C balance in the agricultural phase still raises uncertainties about the environmental feasibility of biofuels expansion. Land use change (LUC) due to biofuel crop establishment may be associated with soil C (SOC) losses that negatively impact the biofuel's greenhouse gases (GHG) balance (Fargione *et al.*, 2008, Mello *et al.*, 2014). The relevance of LUC has been emphasized by several authors, especially in relation to political decisions made for increasing biofuel production (Hudiburg *et al.*, 2016, Lapola *et al.*, 2010).

In Brazil, the negative effects of LUC brought out concerns about the efficiency of the sugarcane ethanol as a climate change mitigation option (Fargione *et al.*, 2008, Lapola *et al.*, 2010). However, sugarcane ethanol shows the largest average net GHG mitigation (including LUC effects) compared to other first-generation ethanol feedstocks (Renouf *et al.*, 2008). Nowadays, Brazil is considered to have developed the world's first sustainable biofuel economy and in many respects is the biofuel industry leader (Souza *et al.*, 2014). This reputation is largely based on its sugarcane industry.

Between 2004 and 2012, Brazil's GDP increased by 32% (IPEA, 2016), while GHG emissions decreased by 52% (MCTI, 2014), breaking the link between economic growth and GHG emissions. Despite these advances, the Brazilian government announced ambitious goals in the last UNFCCC: reduce GHG emissions by 43% below 2005 levels by 2030 (iNDC Brazil, 2015). To do so, among other strategies, the government established that the sugarcane contribution to the energy supply in Brazil by 2030 must be around 16%. Meeting this mandate probably will require a substantial increase in sugarcane production area.

Previous studies using the Century model evaluated the effects of green harvest management (GM - harvest without burning) and organic amendments on SOC changes in sugarcane areas in Brazil (Brandani *et al.*, 2015, Galdos *et al.*, 2009). As concluded by these studies, the high crop residue inputs in areas under GM is the main factor associated with increments on SOC in sugarcane areas in Brazil. However, the sugarcane residues have become an attractive source of biomass for bioelectricity and second generation (2G) ethanol production in Brazil (Walter & Ensinas, 2010). Crop residue removal is associated with decreases on SOC (Wilhelm *et al.*, 2007, Wortmann *et al.*, 2010), but the adoption of some best management practices can mitigate these losses (Paustian *et al.*, 2016).

In recent years, almost all the sugarcane expansion in Brazil has been done under pasture areas (Dias *et al.*, 2016). Using the Century model, Silva-Olaya *et al.* (2016) studied the

impact of LUC from native vegetation and pasture to sugarcane cultivation on SOC dynamics in Brazil. The site-level data used in Silva-Olaya *et al.* (2016) were those reported by Mello *et al.* (2014), where most of sugarcane areas were either still harvested with burning or this practice had just been stopped for three years or less before the sampling time. In this sense, the longer-term effects of the conversion pasture-sugarcane on SOC remain unclear for areas under GM. Moreover, there are not published papers on the effects of straw removal on SOC in sugarcane areas in Brazil. Simulation models provide a feasible and cost effective option to predict the long-term potential impacts of LUC and management practices on SOC. Furthermore, predictions on SOC changes are a useful tool to encourage decision makers and planners to develop sustainable land use strategies and soil management systems in areas to biofuel production (Campbell & Paustian, 2015). In this study, we used the Daycent model to predict the impact of unburnt sugarcane expansion into pasture areas, as well as to evaluate the effect of different management practices, such as straw removal, no-tillage and application of organic amendments (vinasse and filter cake), on long-term SOC changes in sugarcane areas in Brazil.

5.2. Material and Methods

5.2.1. Description of study sites

For the field data used in this research (Cherubin *et al.*, 2015, Oliveira *et al.*, 2016), we sampled three land uses - native vegetation (NV), pasture (PA) and sugarcane (SG) - at sites across south-central Brazil, the largest sugarcane region in the world, accounting for 93.4% of Brazilian ethanol production (UNICA, 2015). The climate at all the sites has rainfall concentrated in the spring and summer (October–April), while the dry season is in the autumn and winter (May–September). The soils are typical of the Brazilian tropical region, well-drained and highly weathered, with a predominance of kaolinite, Fe oxides (goethite and hematite), and Al oxide (gibbsite) in the clay-size fraction.

The first site, Lat_17S, is located in Jataí, southwestern region of Goiás state (Lat.: 17°56'16"S; Long.: 51°38'31"W) with a mean altitude of 800 m and a predominance of clayey Acrudox soils (USDA, 2014). The climate classification is Awa type (Köppen) mesothermal tropical, with a mean annual temperature of 24.0 °C and an annual precipitation of 1,600 mm. The second site, Lat_21S, is located in Valparaíso, west region of São Paulo state (Lat.: 21°14'48"S; Long.: 50°47'04"W) with a mean altitude of 425 m and predominance of loamy Hapludalf soils (USDA, 2014). The climate classification is Aw type (Köppen) humid tropical.

The area has a mean annual temperature of 23.4 °C and an annual precipitation of 1,240 mm. The third site, Lat_23S, is located in Ipaussu, south-central region of the São Paulo state (Lat.: 23°05'08" S; Long.: 49°37'52" W), with a mean altitude of 630 m and predominance of clayey Hapludox soils (USDA, 2014). The climate classification is Cwa type (Köppen) tropical. The annual mean temperature is 21.7 °C and the annual precipitation is 1,470 mm. A general description of each land use is showed in Fig 1. For more information about soil parent material and soil classification, LUC sequence, sampling and laboratory procedures, see Cherubin *et al.* (2015) and Oliveira *et al.* (2016).

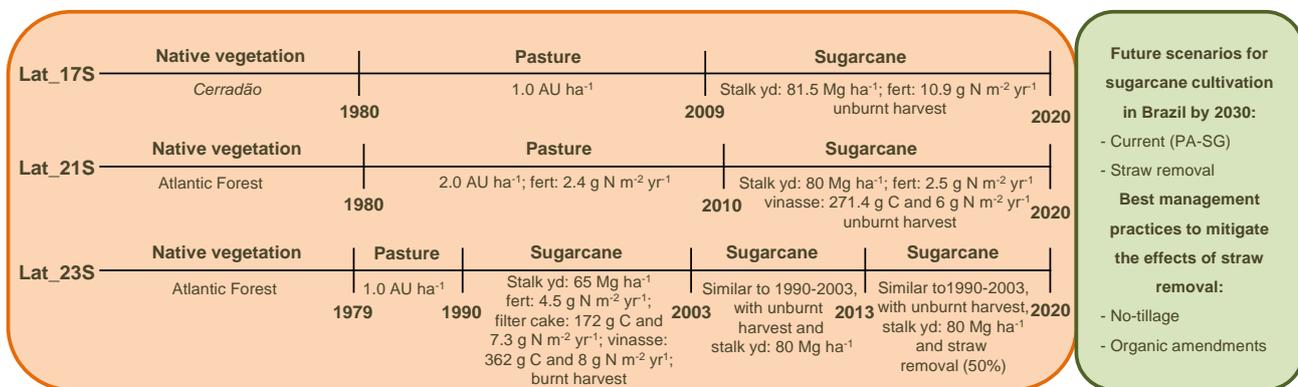


Figure 1. Land use transitions and brief description of the management practices of studied sites in south-central Brazil and future scenarios to sugarcane cultivation in Brazil. Stalk yd: stalk yield. Fert: fertilizers applied. AU: animal units. PA-SG: pasture to sugarcane land use conversion.

5.2.2. The DayCent Model

We used the most recent version of DayCent model (DD14centEVI) to simulate changes in soil organic matter (SOM) dynamics in areas under LUC to sugarcane expansion in Brazil. DayCent (Del Grosso *et al.*, 2001, Parton *et al.*, 1998) is a modified, daily time step version of the biogeochemical ecosystem Century model (Parton *et al.*, 1987). Both Century and DayCent simulates fluxes of C and N between the atmosphere, vegetation, and soil, including the dynamics of multiple C and N soil organic matter pools, but DayCent also includes other processes such as greenhouse gases emissions.

In DayCent, phenology, net primary productivity, shoot:root ratio, and the C:N ratio of biomass in plant components are species-specific. Moreover, the model calculates potential plant growth as a function of water, light, and soil temperature and limits actual plant growth based on specific plant nutrient requirements. The type and timing of each management event can be specified, including tillage, fertilization, organic matter addition, harvest, burning, and grazing

intensity. Litter decomposition and SOM turnover are determined by the amount and quality of residue returned to the soil, the size of the SOM pools, and temperature and water controls (Del Grosso *et al.*, 2001). These aspects allow DayCent to generate accurate simulations for multiple vegetation types under a wide range of management practices at diverse sites, which make the model particularly useful for simulating land use change. Accordingly, DayCent has been used and validated across a range of land use and management scenarios (Del Grosso *et al.*, 2009, Duval *et al.*, 2013, Hudiburg *et al.*, 2016). The Century model was widely used for simulations in pastures (Cerri *et al.*, 2007, Cerri *et al.*, 2004) and sugarcane areas (Brandani *et al.*, 2015, Galdos *et al.*, 2009, Galdos *et al.*, 2010, Silva-Olaya *et al.*, 2016) in Brazil. However, there is no published research using the DayCent model for simulations in Brazil so far.

5.2.3. Modeling procedures

The DayCent model requires input of climate and soil data. In this study, we used climate data (daily maximum and minimum average temperature and precipitation) from 1901 to 2015, provided by MsTMIP (Wei *et al.*, 2014). We opted to use this gridded global product because it is the only long-term and daily weather data available for these sites. Other weather data available for Brazil (e.g. INMET, Cepagri) were restricted to more recent periods or in a monthly basis. The site-specific soil attributes used to initialize the model are available in Cherubin *et al.* (2015).

To initialize the model prior to simulating forest clearing and pasture establishment, we used the forest submodel to estimate equilibrium SOM levels and plant productivity under native forest conditions, over a 7000-year simulation period. Two kinds of native vegetation were simulated using the parameterization developed by Silva-Olaya *et al.* (2016): Cerradão Forest (Lat_17S) and Atlantic Forest (Lat_21S and Lat_23S) (Fig. 1). In our simulations, the main difference between these forest types is the N input by biological fixation in Cerradão Forest (Bustamante *et al.*, 2012). The disturbances on these areas were fire events and tree mortality (Cerri *et al.*, 2004). After simulating the equilibrium condition in native vegetation, the model was set to simulate the deforestation process following the slash and burn procedure. Those events were parameterized using similar calibration procedures as those developed by Cerri *et al.* (2004) for the Century model.

As for most pastures in Brazil, the pastures evaluated in our assessment are to some degree degraded and do not achieve the level of productivity characteristic of well-managed pastures. To simulate this condition, we adjusted the potential aboveground production, based

on the biomass production for degraded pastures in Brazil reported by Lilienfein and Wilcke (2003). Regarding the grazing management, since the areas presented different stocking rates (Fig. 1), we specified different levels of grazing according to the options currently available in the model. We assumed continuous grazing through all the year, including the dry season period.

The simulations for sugarcane areas were done using parameterization for the sugarcane crop developed by Galdos *et al.* (2009), Galdos *et al.* (2010), Silva-Olaya *et al.* (2016) and Campbell (2015). The potential biomass production was adjusted in order to match the field data for south-central Brazil (UNICA, 2015), assuming the biomass partitioning develop for sugarcane by Galdos *et al.* (2010). Sugarcane renovation was performed every 6 years and the tillage operations (plowing, disking and subsoiling) were simulated using the intensive default tillage parameters specified at the model. Organic amendments (vinasse and filter cake) are currently applied on two of our sites (Lat_21S and Lat_23S). The composition of the filter cake used in this study was 228 g C kg⁻¹, 12 g N kg⁻¹, and 160 g lignin kg⁻¹ (Galdos *et al.*, 2009). The composition of vinasse used was based on the analysis reported by Prado *et al.* (2013), with 11.56 g C L⁻¹ and 0.42 g N L⁻¹.

Currently, all the sites evaluated are under GM. However, in Lat_23S, the sugarcane was harvested with burning during a 13 years period (Fig. 1). In this specific case, we used the parameters for burning events developed by Galdos *et al.* (2009), in which 85% of the dry matter of the trash (leaves and tops) is removed by the fire, and 80% of the N in the residue material is lost to the atmosphere. For the GM, the model was set to remove 99% of aboveground biomass, with 94% of dry matter in tops and leaves and 1% of stalks returned to the system as litter after the harvest (rates reported by mills in Brazil).

5.2.4. Model outputs and statistical analysis

Usually, DayCent model is set up for simulations of SOM dynamics in the top 0.2 m soil depth (Parton *et al.*, 1998). For this study, DayCent was parametrized to simulate SOM dynamics to a depth of 0.3 m, by decreasing the decay rate of all SOM pools by 15% (W. Parton and M. Hartman, pers. comm.). Simulation output variables evaluated were total soil C and N stocks, and natural isotopic abundance of ¹³C. The proportion of soil C derived from native vegetation (native-C) or from pasture and sugarcane (modern-C) was calculated with the equations for soil C partitioning proposed by Bernoux *et al.* (1998) using the simulated δ¹³C values. The rates of soil C change associated with land use/management shifts are the average of the three sites (n=3).

Statistical analyses of model results were done in accordance with tests proposed by Smith *et al.* (1997) to assess goodness-of-fit of the DayCent model to the measured C stocks, N stocks and soil C partitioning. The statistical metrics were: correlation coefficient (r), root mean square error (RMSE), mean difference (M), relative error (E) and lack of fit (LOFIT).

5.2.5. Future scenarios

Based on feasible management strategies for future sugarcane cultivation in Brazil, we simulated the soil C changes in areas of sugarcane under five management scenarios:

Scenario I: green management, without burning or straw removal (GM)

Scenario II: Straw removal

Scenario III: Straw removal with no-tillage

Scenario IV: Straw removal with organic amendments

Scenario V: Straw removal with no-tillage and organic amendments

We assumed a maximum rate of 75% of straw removal for sugarcane areas in Brazil, as reported by Cardoso *et al.* (2013). No-tillage operations were simulated using the default files available in the DayCent model. The organic amendments were vinasse and filter cake, applied in rates commonly used in sugarcane areas in Brazil, i.e., 200 m³ ha⁻¹ yr⁻¹ and 25 Mg ha⁻¹ yr⁻¹, respectively (Prado *et al.*, 2013). Moreover, based on the commitments made by Brazilian government in the UNFCCC (iNDC Brazil, 2015), we estimated the sugarcane area expansion according to the projected increased ethanol production needed to meet the domestic demand by 2030 in two scenarios: with and without the contribution of 2G technologies to ethanol production. To reach the estimated production of ethanol in 2030, we assumed linear rates of increment in planted area. Using these two scenarios of expansion and the simulated rates of soil C change under different management practices, we estimated soil C changes in sugarcane areas in Brazil over the next decades, without assuming any biophysical or economic basis for expansion allocation across south-central Brazil.

5.3. Results

5.3.1. Model performance

The DayCent model estimates were consistent with the field-observed SOM changes in areas undergoing LUC for sugarcane expansion in Brazil (Table 1, Fig. 2). The measured and simulated SOC were well correlated ($r=0.98$; $p<0.05$), with the model underestimating the SOC by 2.1 ± 4.6 , 4.8 ± 1.3 and $2.7\pm 8.6\%$ in NV, PA and SG areas, respectively (Table 1). Despite the correlation between the measured and simulated values ($r=0.91$; $p<0.05$), the model showed a tendency to overestimate the N stocks in these areas, with values $23.4\pm 18.2\%$ greater than the measured N stocks in PA and SG areas (Table 1, Fig. 2). The DayCent model also accurately simulated our measured results for C-partitioning, with simulated soil native-C underestimated by $7.7\pm 2.1\%$ and the simulated soil modern-C $9.7\pm 19.7\%$ greater than the measured values.

Table 1. Measured and simulated C, N, native-C and modern-C soil stocks (Mg ha^{-1}) at 0-0.3 m layer of areas under different land uses - native vegetation (NV), pasture (PA), sugarcane (SG) - in three sites (Lat_17S, Lat_21S, Lat_23S) of south-central Brazil.

	Soil C stocks (Mg ha^{-1})		Soil N stocks (Mg ha^{-1})		Native-C (Mg ha^{-1})		Modern-C (Mg ha^{-1})	
	Measur.	Simul.	Measur.	Simul.	Measur.	Simul.	Measur.	Simul.
Lat_17S								
NV	49.1 ± 3.5^a	50.9	3.0 ± 0.3	3.5	49.1 ± 3.5	50.9	-	-
PA	37.2 ± 3.2	38.7	1.7 ± 0.2	2.7	28.6 ± 2.4	27.4	8.6 ± 1.3	11.3
SG	38.2 ± 2.1	39.0	2.3 ± 0.2	3.0	25.8 ± 1.3	25.8	12.4 ± 1.0	13.2
Lat_21S								
NV	48.6 ± 3.2	47.4	4.7 ± 0.6	3.9	48.6 ± 3.2	47.4	-	-
PA	37.2 ± 2.8	36.5	2.6 ± 0.2	3.0	27.5 ± 1.9	26.6	9.7 ± 0.9	9.9
SG	40.1 ± 3.1	37.5	3.1 ± 0.3	3.3	28.2 ± 4.3	26.5	12.5 ± 1.0	11.1
Lat_23S								
NV	89.9 ± 8.5	86.2	7.4 ± 0.5	7.9	89.9 ± 8.5	86.2	-	-
PA	76.9 ± 6.7	74.2	6.1 ± 0.4	7.0	54.1 ± 5.7	49.4	22.8 ± 3.3	24.8
SG	60.5 ± 4.2	68.5	4.5 ± 0.3	7.1	44.8 ± 3.6	44.9	15.8 ± 1.8	23.6

^a: Standard deviation from the mean values (n=9).

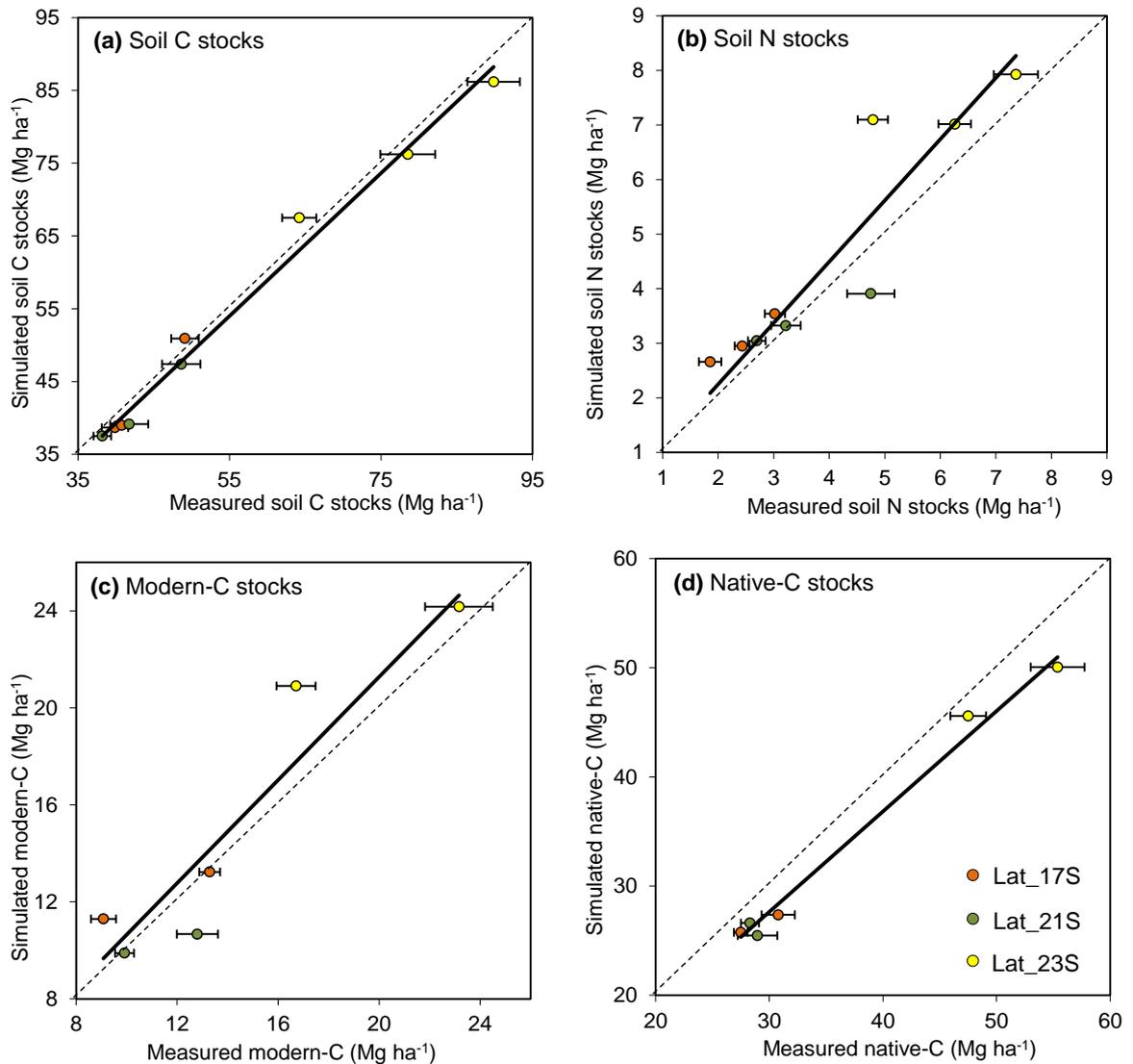


Figure 2. Measured versus simulated soil C stocks (a), soil N stocks (b), modern-C stocks (c) and native-C stocks (d) of areas under native vegetation, pasture and sugarcane cropping in south-central Brazil. Bars represent the standard deviation from the mean values. $n=9$.

Goodness-of-fit measures show that the DayCent model represented well the changes of SOM for the NV-PA-SG conversions evaluated. With exception of the N stocks, values for RMSE indicated a small difference between measured and simulated values (Table 2). Values for M and E showed an absence of significant bias in the simulated soil C and N stocks, and C-partitioning. However, LOFIT pointed to lack of fit between the measured and simulated N stocks and soil C-partitioning (Table 2).

Table 2. Statistical tests applied for the validation between measured and simulated values of soil C and N stocks and C-partitioning (native-C and modern-C) of areas under native vegetation, pasture and sugarcane cropping in south-central Brazil.

Statistical test	Soil C stocks	Soil N stocks	Native-C stocks	Modern-C stocks
r = Correlation Coefficient	0.98	0.91	0.99	0.94
F = $((n-2) r^2) / (1-r^2)$	509.97	39.17	351.00	28.06
F-value at (P=0.05)	5.59	5.59	7.71	7.71
RMSE = Root mean squared error of model	4.17%	23.73%	8.81%	15.27%
RMSE (95% Confidence Limit)	10.36%	16.17%	9.99%	12.95%
M = Mean Difference	0.95	-0.57	2.93	-0.86
t = Student's t of M	1.31	1.97	5.92	0.96
t-value (Critical at 2.5% - Two-tailed)	2.36	2.36	2.78	2.78
E = Relative Error	1.74	-14.02	8.04	-6.11
E (95% Confidence Limit). = +/-	9.63	15.17	9.23	11.72
LOFIT = Lack of Fit	418.85	74.48	555.47	252.24
F = MSLOFIT/MSE ^a	2.17	29.60	11.56	15.95
F (Critical at 5%)	2.19	2.19	2.65	2.65

^a: MS: Mean squared. MSE: Mean squared error.

5.3.2. Long-term SOC changes undergoing NV-PA-SG conversions in Brazil

The DayCent model estimated that the conversion of NV-PA is associated with SOC losses of 0.34 ± 0.03 Mg C ha⁻¹ yr⁻¹ in areas of south-central Brazil (Fig. 3). After the conversion of these pastures to sugarcane under GM, we observed the partial recovery of the SOC, at a rate of 0.16 ± 0.04 Mg C ha⁻¹ yr⁻¹. We did not include the SOC changes for Lat_23S between 1990-2003, when the sugarcane was harvested with burning (Fig. 1). In this case, the SOC losses simulated by the Daycent model were 1.04 Mg C ha⁻¹ yr⁻¹ (Fig. 3c). Moreover, the simulated SOC losses in the year right after sugarcane crop renovation were 1.14 ± 0.46 Mg C ha⁻¹ (Fig. 3). Normalizing the SOC values relative to those under native vegetation (NV=100) at each site, we observed that the simulated SOC changes after LUC showed a very similar pattern across sites, with a consistent SOC loss after the LUC NV-PA and SOC increases within the transition PA-SG (Fig. 3d). By 2050, under the current management practices, the SOC in SG areas were predicted to be $86.1 \pm 2.8\%$ of those observed in NV.

The C-partitioning using the simulated $\delta^{13}\text{C}$ values of SOM also showed a clear pattern in areas undergoing the LUC NV to PA to SG in Brazil (Fig. 3). In PA areas, native-C losses were 0.93 ± 0.41 Mg C ha⁻¹ yr⁻¹, coupled with modern-C gains of 0.48 ± 0.20 Mg C ha⁻¹ yr⁻¹. For SG, native-C losses and modern-C increases were 0.39 ± 0.17 and 0.56 ± 0.22 Mg C ha⁻¹ yr⁻¹, respectively (Fig. 3).

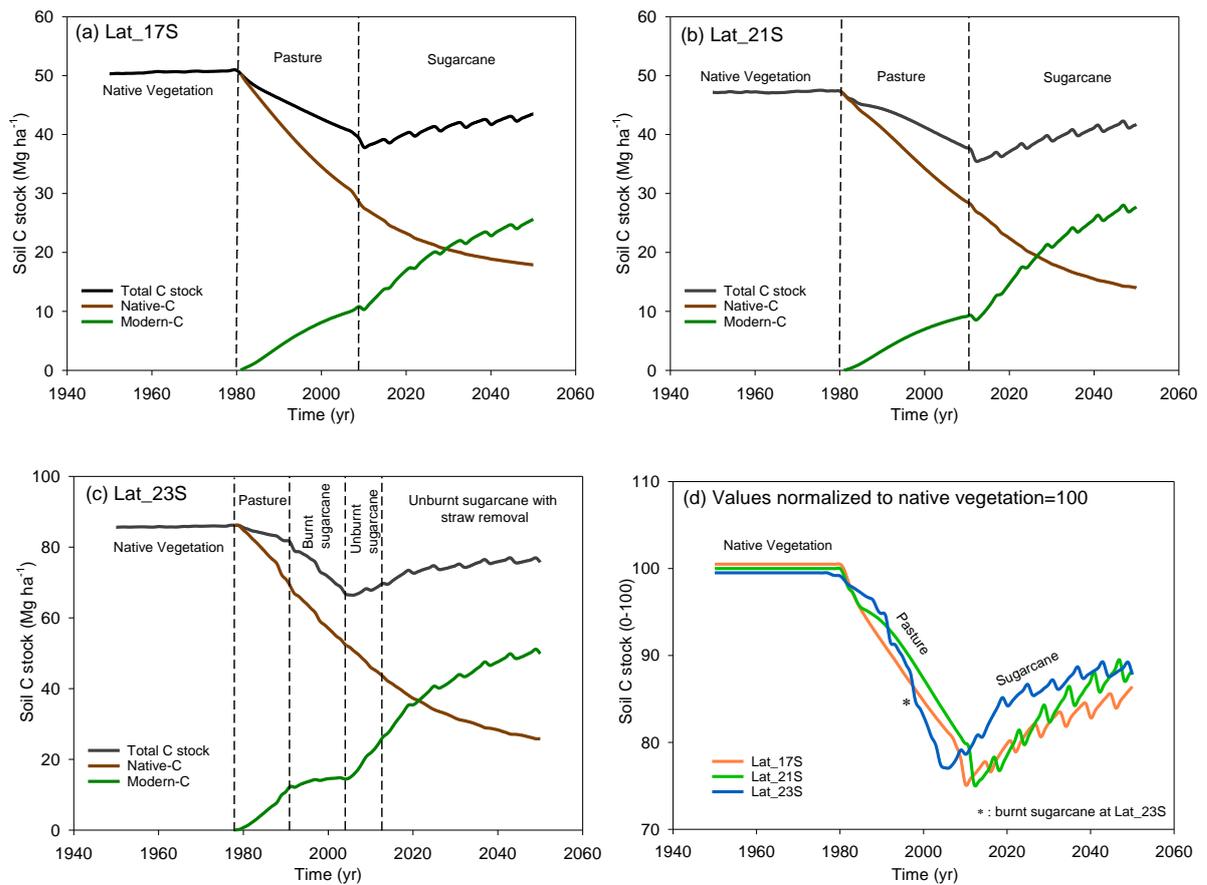


Figure 3. Long-term simulations of soil C stocks and C partitioning at 0-0.3 m layer of three sites - Lat_17S (a), Lat_21S (b), Lat_23S (c) - undergoing the land use transition native vegetation-pasture-sugarcane in south-central Brazil. (d): Values normalized considering the C stocks in native vegetation areas equal to 100.

5.3.3. Predicted effects of straw removal on SOC in sugarcane areas in Brazil

Straw management is a major issue affecting long-term SOC maintenance under sugarcane in Brazil (Fig. 4). The DayCent model suggested that GM would promote increased SOC, whilst straw removal can notably reduce SOC in sugarcane areas (Fig. 4). However, adoption of best management practices can mitigate the negative effects of straw removal, highlighting the application of organic amendments, which in our simulations showed similar results to areas under GM (Fig. 4).

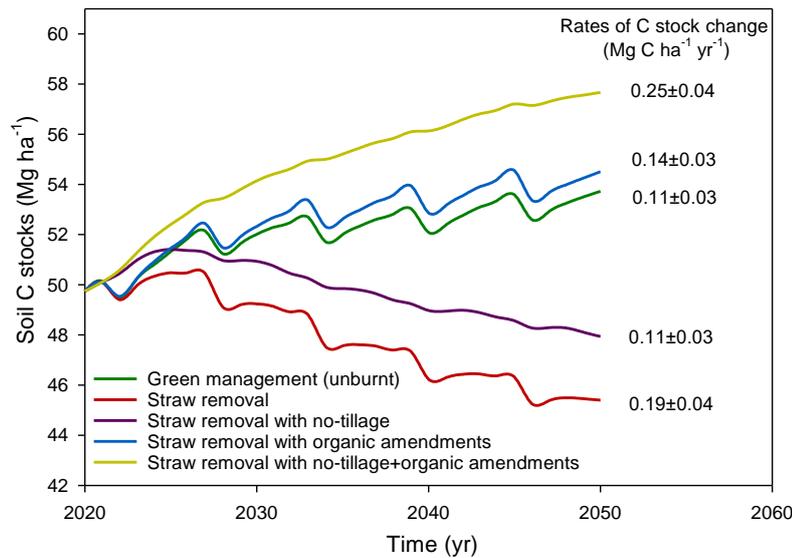


Figure 4. Simulated soil C stocks at 0-0.3 m layer in sugarcane areas under green management, straw removal and best management practices in Brazil. The reference for the rates of C stock change is the soil C stock in areas under green management by 2020. $n=3$.

The implementation of 2G technologies in Brazil will drastically alter the land demand for sugarcane production in the next decades. Without 2G ethanol contribution, we estimated an expansion of sugarcane planted area of 56.4% to meet the domestic ethanol demand by 2030, based on the commitments made by Brazilian government in the UNFCCC (iNDC Brazil, 2015). The contribution of 2G technologies can notably decrease the land demand for sugarcane ethanol production (Table 3). However, estimated SOC changes in a scenario where the increase in ethanol production is based on the expansion of sugarcane into pastures areas pointed to gains in C-savings of Brazilian ethanol, whilst the straw removal can affect negatively the C balance by decreasing the SOC in 50 Tg between 2020-2050 in sugarcane areas in Brazil (Table 3).

5.4. Discussion

The DayCent model reliably reflected the main trends of SOC changes undergoing the LUC NV-PA-SG in our sites. Using the Century model, Galdos *et al.* (2009), Brandani *et al.* (2015), Silva-Olaya *et al.* (2016) successfully simulated SOC changes in sugarcane areas in Brazil. Moreover, Duval *et al.* (2013) concluded that the DayCent model performed well for simulating SOC changes undergoing the conversion pasture-energy cane in US.

Table 3. Sugarcane cultivated area and soil C changes associated with the projected increase on ethanol production to meet the domestic demand in Brazil by 2030 in different scenarios.

Estimative	Value
Energy consumption in Brazil by 2030 ^a	3529 TWh
Sugarcane contribution to the energy supply by 2030 ^b	16%
Ethanol contribution in the energy supply by sugarcane ^c	88%
Ethanol yield by 2030 ^d	10000 L ha ⁻¹ yr ⁻¹
Ethanol yield by 2030 including 2G technology ^e	15000 L ha ⁻¹ yr ⁻¹
Expected Brazilian ethanol production by 2030 ^f	84.3 billions of L
Expected sugarcane cultivated area by 2030 ^g	8.4 Million ha
Expected sugarcane cultivated area by 2030 (with 2G) ^g	5.6 Million ha
Soil C changes in sugarcane areas between 2020-2050 with the increase on ethanol production based on LUC to pastures	144 Tg C
Soil C changes in sugarcane areas between 2020-2050 with the increase on ethanol production based on 2G ethanol (straw removal)	-50 Tg C

^a: Bronzatti and Iarozinski Neto (2008)

^b: iNDC Brazil (2015)

^c: 12% to bioelectricity from sugarcane bagasse burning (Kutas, 2016).

^d: Based on the increments on ethanol yield in the last years (Goldemberg & Guardabassi, 2010).

^e: Assuming that 2G technologies will increase the sugarcane ethanol yield about 50% (Kutas, 2016).

^f: Based on the sugarcane ethanol contribution in the energy supply by 2030 (iNDC Brazil, 2015).

^g: Only for ethanol production. Currently, 59.4% of the sugarcane cultivated area is harvested to ethanol production, whilst the remaining (40.6%) is used by sugar industry (UNICA, 2015).

Despite the absence of significant bias (Table 2), the model appeared to overestimate N stocks, mainly in PA and SG areas (Table 1). Conant *et al.* (2005) observed that the DayCent model overestimated N stocks for half of pastures evaluated in sites from US, UK and Canada. However, due to the rapid N transformations in a warm and humid environment, we must take into account the possibility of N losses during the sampling, transport, and initial processing of the soil samples, which could have contributed to the discrepancies between measured and modeled soil N stocks in our study.

For the C-partitioning some lack of fit between the measured and simulated values were observed (Table 2), mainly related with the disagreement between the measured and simulated modern-C stocks in SG areas from Lat_23S (Table 1). At this site, sugarcane was harvested with burning between 1990-2003 (Fig. 1). Burning events cause shifts in $\delta^{13}\text{C}$ values of C4-derived charcoal (Krull *et al.*, 2003), which certainly interfere in the C-partitioning. Effects of pyrogenic C

on estimates of SOM partitioning is not accounted for in the DayCent model estimates. Nevertheless, the RMSE values indicated that the simulated C-partitioning still fell within the 95% confidence interval for the whole dataset (Table 2).

Overall, despite the disagreements discussed above, our DayCent simulations matched the direction of the main SOM shifts undergoing the LUC NV-PA-SG for all sites evaluated, even for N stocks and C-partitioning. Smith *et al.* (2012) showed that widely used process-based models (including Century) simulated values in the same uncertainty range as estimates derived from field experiments in areas for biofuels production. Moreover, DayCent is the most comprehensive of the process-based models when it comes to C dynamics representing plant and soil interactions (Robertson *et al.*, 2015).

DayCent model predicted SOC losses of 0.34 ± 0.03 Mg C ha⁻¹ yr⁻¹ in the transition NV-PA (Fig. 3). Assessing SOC changes associated with the LUC NV-PA in Brazil, Maia *et al.* (2009) and Franco *et al.* (2015) found losses of SOC at rates of 0.28 and 0.40 Mg ha⁻¹ yr⁻¹, respectively. These SOC losses can be attributed to both deforestation and biomass burning effects, and subsequent processes of soil degradation in pasture areas (Maia *et al.*, 2009). After the LUC PA-SG (under GM), the simulations showed increments on SOC at a rate of 0.16 ± 0.04 Mg C ha⁻¹ yr⁻¹ until 2050. This result matched the previous rate (0.12 ± 0.03 Mg ha⁻¹ yr⁻¹) obtained in our field-scale assessment (Oliveira *et al.*, 2016). In US, positive SOC changes were predicted when pastures were converted to energy cane (Duval *et al.*, 2013) or Miscanthus production (Dunn *et al.*, 2013). Moreover, Galdos *et al.* (2009) projected SOC gains of 0.23 Mg ha⁻¹ yr⁻¹ in Century simulations for SG areas under green harvest management (GM) in Brazil.

The overall trend of increase on SOC in areas under GM is mainly related to the large input of organic material by sugarcane crop residues. In our study, the simulated C-partitioning suggested that the high input of crop residues in SG areas under GM is associated with a positive C balance, with the losses of native-C lower than the gains of modern-C, the opposite of PA areas. In Lat_23S, when the SG was harvest with burning (1990-2003), a drop in SOC was observed (Fig. 3), as reported in other simulation studies (Brandani *et al.*, 2015, Galdos *et al.*, 2009). As a consequence, the conversion PA-SG with pre-harvest burning is associated with SOC losses (Mello *et al.*, 2014). However, nowadays almost all SG plantations in Brazil are green harvested (UNICA, 2015).

Sugarcane is usually replanted every sixth year. Under conventional tillage, the whole replant area is disturbed using plowing, disking and, commonly, subsoiling. Our simulations showed that tillage operations caused a SOC loss of 1.14 ± 0.42 Mg ha⁻¹ in the year right after sugarcane replanting, in agreement with previous studies in Brazil (Figueiredo *et al.*, 2015, Silva-

Olaya *et al.*, 2013). The C-partitioning showed that most of the C from sugarcane (modern-C) from the previous five-year production period can be lost during the replanting period (Fig. 3). Such SOC losses are comparable with the GHG emissions from sugarcane burning estimated by Bordonal *et al.* (2012). In this sense, we suggest the adoption of management systems involving “less aggressive” tillage operations, in order to decrease the SOC losses in SG areas under GM in Brazil.

Nowadays, more than 60% of Brazilian pastures are in some degree of degradation (Andrade *et al.*, 2014). The replacement of degraded lands (with low soil C stocks) with high productivity energy crops may result in a positive soil C balance and additional C savings for biofuels (Gelfand *et al.*, 2013, Gollany *et al.*, 2015). Our simulations showed that the replacement of pastures with sugarcane is associated with SOC gains, which partially offset the C debt resulting from the conversion of natural vegetation to pastures. One of the potential consequences of such LUC is the migration of livestock to other regions, increasing deforestation (Lapola *et al.*, 2010). This indirect LUC, although very controversial, is now seen to have far less impact than previously thought (Macedo *et al.*, 2015). Currently government actions to improve pasture conditions (ABC Brazil, 2012), along with livestock production intensification, can effectively make large amounts of land available for alternative uses in Brazil. In this sense, we estimated a SOC accretion of 144 Tg if the projected increments on ethanol production were based on expansion of sugarcane into pasture areas in the next years in Brazil (Table 3).

DayCent simulations showed SOC losses of $0.19 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ in SG areas in Brazil with straw removal (Fig. 4). The harvest of crop residues is associated with potential environmental impacts, highlighting SOC losses (Wilhelm *et al.*, 2007, Wortmann *et al.*, 2010), since crop residues are a key component for SOC accretion (Paustian *et al.*, 2016). Modelled SOC losses associated with straw removal in sweet sorghum showed that these emissions could eliminate all GHG mitigation benefits of bioethanol compared with gasoline (Wortmann *et al.*, 2010). Using DayCent simulations, Miner *et al.* (2013) concluded that all stover is needed to be left in the field to maintain SOC levels in wheat, corn and grain sorghum areas in US. Moreover, studies from Americas (Gollany *et al.*, 2015), US (Wilhelm *et al.*, 2007) and Australia (Zhao *et al.*, 2015) suggested that the SOC losses are the main constraint regarding the straw removal in agricultural areas for biofuels production. In this sense, the straw removal in SG areas in Brazil might be beneficial from an energy security point of view as more ethanol (or electricity) will be produced, but not necessarily will result in the higher C savings because the potential SOC losses associated (Fig. 4).

In Brazil, the development of technologies for 2G ethanol production have been moving at a slower pace than other places for many reasons but, now, seem to be accelerating. Currently, Brazil has two commercial 2G ethanol mills in operation, three demo mills and 20 projects in the pipeline (Kutas, 2016). Moreover, substantial investment by the private sector and government is a strong market signal that sugarcane 2G ethanol supplies are likely to increase dramatically in the next years. In addition, straw removal in SG areas is also happening to support electricity production (Walter & Ensinas, 2010), such as currently in Lat_23S site. Thereby, straw removal is likely to become a common practice in Brazilian sugarcane areas soon and management practices must be proposed in order to mitigate the negative effects of the straw removal on SOC.

The adoption of no-tillage in SG areas with straw removal can decrease the rates of SOC losses comparing with areas under straw removal only (Fig. 4). In Brazil, SOC gains have been reported in sugarcane areas under no-tillage (Segnini *et al.*, 2013). Century simulations showed that the adoption of no-tillage reduces the losses or even result in SOC gains in corn areas with stover harvest to ethanol production in US (Sheehan *et al.*, 2003). However, in our study, DayCent simulations showed positive SOC changes only when another source of C (vinasse and filter cake) was added (Fig. 4). According to Century simulations, vinasse and filter cake application were predicted to increase SOC in sugarcane areas in Brazil (Brandani *et al.*, 2015). Filter cake and vinasse are produced in large quantities by the sugar-alcohol agroindustry. Moreover, the vertical integration of the sugarcane industry in Brazil makes the distribution of these sub-products easier because of shorter distances from the refinery to the field. In this sense, filter cake and vinasse applied to the soil is a practice widely used in SG areas in Brazil (Prado *et al.*, 2013) and, as we observed, can have a prominent role on SOC dynamics in SG areas with straw removal. Despite its benefits, the adoption of no-tillage is not common in SG areas in Brazil. However, in a scenario with straw removal and possible SOC losses, we need to consider no-tillage during sugarcane crop renovation, mainly if it is combined with other best management practices, such as vinasse and filter cake application (Fig. 4). Lastly, we must mention that soil C accretion is finite and, under the same management practices and C inputs, the soil C stocks in these areas are expected to reach a new equilibrium over the next decades.

Without the contribution of 2G ethanol, we projected that the sugarcane area in Brazil is expected to expand by 3.04 Mha by 2030. With the fully implementation of 2G ethanol production in the next years, we projected an expansion on SG area of only 0.23 Mha to meet domestic ethanol demands by 2030 (Table 3). LUC projections based on feedstock demands are a quite complex task and inherently uncertain. Moreover, the possible inclusion of 2G ethanol in

the Brazilian energy supply in the next years increases the uncertainty about the land required for sugarcane production. Similarly uncertain are the spatial extrapolations about SOC, since C dynamics are known to be highly dependent on environmental characteristics and local management factors. In this sense, despite the limitations discussed above, the data presented in the Table 3 aim to show the likely direction and relative magnitudes of land conversions and SOC changes related in two feasible scenarios of SG expansion in Brazil. Moreover, our projections raised concerns about the sustainability of straw removal in SG areas. The SOC changes could be greater or less than estimated here, but our research can be a starting point for development of management strategies to mitigate possible SOC losses regarding 2G ethanol production in Brazil.

Based on land availability and positive effects on C savings of sugarcane ethanol, we believe that stakeholders involved with the governance of bioethanol expansion should consider ways to incentivize sugarcane expansion on degraded pastures in Brazil. Moreover, we are sure that 2G technology will increase notably the energy output from sugarcane, but inferences about the net mitigation potential of 2G ethanol from sugarcane will require analysis of the entire biofuel life cycle, in which possible SOC losses should be taken into account. In this sense, field studies about the environmental suitability of straw removal in sugarcane areas are mandatory before using crop residues as a source of biomass for large-scale ethanol production in Brazil. Finally, the time horizon is quite relevant when evaluating soil C dynamics in agricultural areas (e.g.: time since adoption of the GM system has great impact on the potential increase on SOC in sugarcane areas). However, extensive field measurements and data collection is costly or impossible, and thus simulation models can help researchers to expand short-term field research to longer scenarios where field measurements are difficult to conduct. Our results supported that DayCent model can complement and extend the applicability of information collected in field studies (Campbell & Paustian, 2015, Robertson *et al.*, 2015) and may be applied to obtain credible long-term assessments of sugarcane production effects on SOC in tropical regions.

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6. FINAL REMARKS

Land use change (LUC) has been reported as the most contentious issue related to biofuels sustainability (Hudiburg *et al.*, 2016, Lapola *et al.*, 2010). Some previous studies have indicated that biofuels crop expansion may result in soil organic matter (SOM) losses, which is particularly troubling from a climate change perspective, since biofuels are supposed to be a mitigation option (Lapola *et al.*, 2010, Qin *et al.*, 2016). In Brazil, currently the world largest producer of sugarcane and second biggest producer of bioethanol, the potential negative effects of LUC has raised doubts about the sugarcane ethanol as a sustainable option (Fargione *et al.*, 2008, Lapola *et al.*, 2010, Mello *et al.*, 2014). In this sense, our study provided science-based and insightful informations regarding the SOM changes related to sugarcane expansion over pasture areas in Brazil (Fig. 1).

In Chapter II (Oliveira *et al.*, 2016a), we concluded that LUC causes substantial changes on SOM contents in areas undergoing sugarcane expansion in Brazil. The conversion of natural ecosystems to agricultural land decreases N stocks, with similar trends in pastures and sugarcane areas. Overall, long-term conversion from native vegetation to pasture induced significant C stock losses ($1.01 \text{ Mg ha}^{-1} \text{ yr}^{-1}$). In contrast, the conversion from pasture to sugarcane increased C stocks at a rate of $1.97 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ down to 0-1.0 m depth (Fig. 1). The C-partitioning showed that the gain in C stocks in sugarcane areas was determined by i) the reduction on the rates of native-C losses and; ii) increasing the amount of modern-C comparing to pasture. In addition, our findings indicated that SOM assessments restricted to the surface soil layers can generate bias in studies regarding LUC.

In the Chapter III (Oliveira *et al.*, 2016b), the use of pyrolysis-gas chromatograph-mass spectrometry provided detailed information on shifts in the molecular composition of SOM in areas of sugarcane expansion. Depth is a major factor that influenced SOM composition in these sites. At 0.9-1.0 m depth, the SOM composition was determined by site, suggesting a climatic/edaphic control. Conversely, the effects of LUC in surface soil layer was clear at all study sites, and showed a larger contribution from more stable compounds (aliphatics and polyaromatics) under native vegetation compared to areas under sugarcane that had a higher contribution from compounds related to fresh plant materials (lignin moieties and phenols) (Fig. 1). The main difference in SOM composition undergoing the conversion pasture-sugarcane was the notably higher contribution from compounds associated to fresh litter inputs in sugarcane areas, probably related to the high litter input in sugarcane fields under green management in Brazil (Cerri *et al.*, 2010).

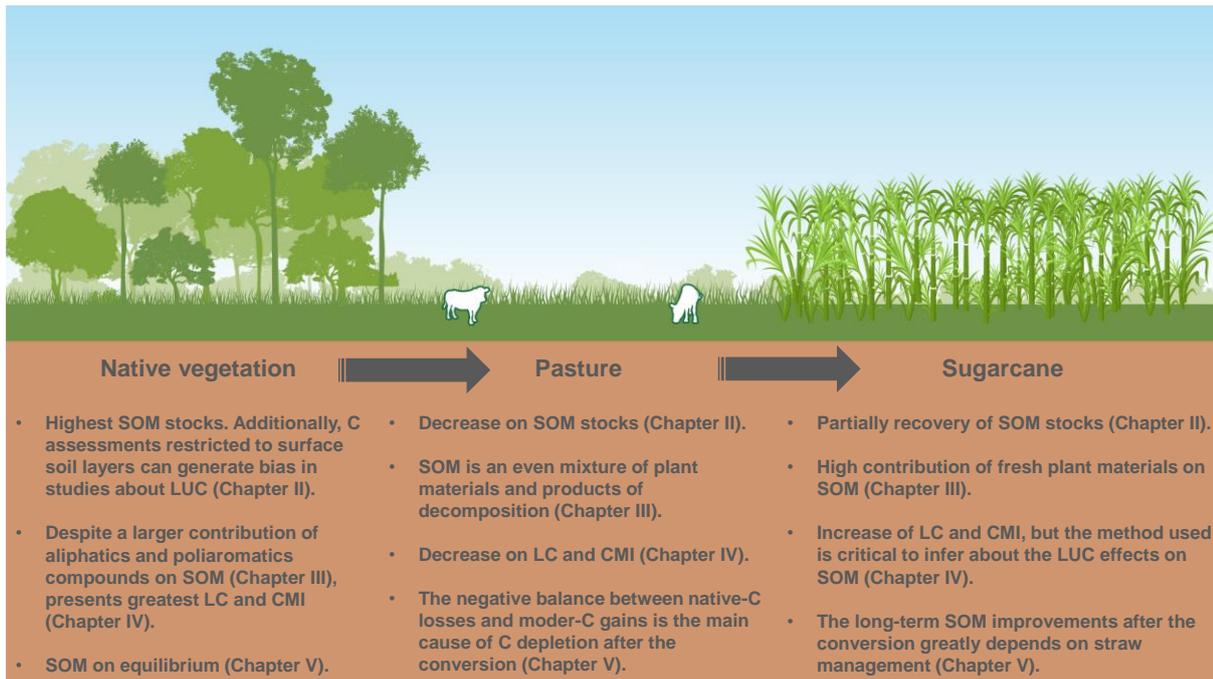


Figure 1. Soil organic matter dynamics in pasture-sugarcane land use conversions in south-central Brazil: overview and main finds. SOM: soil organic matter. LUC: land use change. LC: labile C. CMI: C management index.

In Chapter IV (Oliveira *et al.*, 2017a), the conversion of areas under native vegetation to pasture decreases both the labile C (LC) and the C management index (CMI) down to 1.0 m soil depth, whilst the conversion of pasture to sugarcane increased the CMI according to all evaluated methods, mainly below 0.2 m depth (Fig. 1). These evidences suggest that cropping sugarcane in areas previously used as pastures enhances the SOM quality. However, the method used to quantify LC and CMI is critical to infer about the LUC effects on SOM. Both methodologies proposed by Blair *et al.* (1995) and Diekow *et al.* (2005) were highly sensitive to the conversions evaluated in this research. However, Diekow *et al.* (2005) is the most suitable methodology to estimate the SOM labile pool and to calculate the CMI in sites undergoing LUC in Brazil, since the approach of Blair *et al.* (1995) notably overestimates these indicators. Finally, we reiterate that the SOM changes are well expressed by the total C content in areas undergoing LUC and, integrated approaches, such as the CMI, are suitable to evaluate the effects of LUC on SOM.

In Chapter V (Oliveira *et al.*, 2017b), our results supported that DayCent model can complement and extend the applicability of information collected in field studies (Campbell & Paustian, 2015, Robertson *et al.*, 2015) and may be applied to obtain credible long-term assessments of sugarcane production effects on SOM in tropical regions. The DayCent model estimated that the conversion NV-PA caused C losses of $0.34 \pm 0.03 \text{ Mg ha}^{-1} \text{ yr}^{-1}$, whilst the conversion PA-SG resulted in C gains of $0.16 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ down to 0.3 m depth. Moreover, simulations showed C decreases of $0.19 \pm 0.04 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ in SG areas with straw removal for

second-generation (2G) ethanol production (Fig. 1). However, our analysis suggested that adoption of some best management practices can mitigate these losses, highlighting the application of organic amendments ($+0.14 \pm 0.03 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$). Based on the commitments made by Brazilian government in the UNFCCC (iNDC Brazil, 2015), we estimated the ethanol production needed to meet the domestic demand by 2030. If the increase in ethanol production was based on the expansion of sugarcane area on degraded pasture land, the model predicted a C accretion of 144 Tg from 2020-2050, whilst increased ethanol production based on straw removal as a cellulosic feedstock was predicted to decrease C by 50 Tg over the same 30 year period.

Overall, our study showed that the conversion of pastures to sugarcane has positive effects on SOM quantity and quality (Fig. 1), increasing the carbon savings of Brazilian sugarcane ethanol. Moreover, our findings endorse the potential of sugarcane production to partially recover SOM in degraded pastures. However, most of these gains greatly depends on the high litter input in sugarcane fields under green management. Therefore, straw removal for 2G ethanol production is likely to potentially affect SOM in areas of sugarcane expansion in Brazil. One of the potential consequences of the LUC pasture-sugarcane is the migration of livestock to other regions, increasing deforestation (Lapola *et al.*, 2010). This indirect LUC, although very controversial, is now seen to have far less impact than previously thought (Macedo *et al.*, 2015). In this sense, based on land availability and positive effects on SOM, we believe that stakeholders involved with the governance of bioethanol expansion should consider ways to incentivize sugarcane expansion on degraded pastures in Brazil.

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