RAFAEL DA SILVA TEIXEIRA

ABOVE AND BELOW GROUND PLANT INPUTS AND SOIL ORGANIC MATTER CYCLING IN AN EUCALYPT PLANTATION IN THE CERRADO BIOME

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

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BIOGRAFIA

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SUMÁRIO

ABSTRACT

TEIXEIRA, Rafael da Silva, D.Sc., Universidade Federal de Viçosa, September, 2017. **Above and below ground plant inputs and soil organic matter cycling in an eucalypt plantation in the cerrado biome.** Adviser: Ivo Ribeiro da Silva. Co-adviser: Emanuelle Mercês Barros Soares.

Soil organic matter (SOM) plays key roles on high productive agrosystems, further may offer an alternative to reduce soil CO_2-C emissions and improve soil C sequestration. In Brazil, most of the Cerrado (Brazilian Savannah) were initially converted to pastures using unsustainable practices, which promoted soil degradation, soil organic carbon (SOC) stocks losses and increase in GHG emissions. Thus, the conservation land-management systems that favor the input of aboveground and belowground plant residue-C to soil may reduce the impacts caused by land-use change. Because eucalypt trees are fast-growing, they are attractive for C sequestration (aboveground and belowground) and subsequent C input to the soil. So, eucalypt plantation may sequester C in compartments with different timescales: *i)* Plant biomass and *ii)* Soil organic matter (SOM). So, this thesis aimed to study the C and N dynamics, focusing in the processes that underline CO_2-C emissions in on eucalypt plantation since the land-use change following a pasture, until 4-years-old. We report our research in three chapters, aimed at understanding some research gaps. In the first chapter we analyzed the changes in C and N stocks, CO_2-C and CH_4-C fluxes in Cerrado, pasture (cultivated for 34-years following the clearing of the Cerrado) and eucalypt (cultivated for 4 years following the pasture). The soil surface CO_2-C, CH_4-C fluxes and also CO_2-C concentration along the vertical soil profile were measured in different seasons (Wet and Dry) over three years. It was also determined the C and N stocks associated to the particulate organic matter (POM) and mineral-associated organic matter (MAOM). Variation in natural abundance of ^{13}C ($\delta^{13}C$) was used to partition the SOM in old (Cerrado- or pasture-derived) and their replacement by the new input C (eucalypt-derived). It was observed that the wet season had the strongest influence on soil surface CO_2-C and CH_4-C fluxes, and CO_2-C concentration at soil depths for the different land uses. The soil under eucalypt plantation emitted ~70% more CO_2-C than those under Cerrado and pasture after 40-months of eucalypt planting, while the pasture soil emitted more CH_4-C to the atmosphere than those under Cerrado and eucalypt in Sep 2012, Jan 2013 and Oct

2015. The old MAOM-C losses in deep soil layers were not compensated by the new eucalypt C inputs, resulting in net soil C losses. Nevertheless, no differences were detected to POM-C and -N in the soil (0.0-1.0 m), perhaps indicating a recovery in SOM in eucalypt stands at a more advanced stand age. In the second chapter, we investigated de dynamics of CO_2-C components in soil surface and soil profile, also tracking the influence of eucalypt root growth (especially fine roots) on these processes. Due historical use was possible partition the soil surface CO_2-C flux and the CO_2-C concentration in depth in CO_2-C plant-derived and CO_2-C soil-derived. In addition, the root priming effect was calculated. The evaluations were carried out in six seasons: 3, 7, 15, 19, 31 and 40-month-old eucalypt. After the implantation of eucalypt forests there was an increase in soil surface CO_2-C flux along plant growth (4.33 kg ha^{-1} h^{-1} in 40 month-old eucalypt). The root growth contributes greatly to the soil surface CO_2-C flux (correlated at $p<0.01$; $r: 0.61$) promoting the surface RPE over time (correlated at $p<0.01$; $r: 0.63$). The moisture has greater influence in the decomposition of litterfall (correlated at $p<0.01$; $r: 0.70$) and root respiration and/or rhizodeposition decomposition (correlated at $p<0.01$; $r: 0.79$). Finally, in the third chapter we accessed the biomass C storage (Leaves, branches, barks, woods, fine roots, medium roots and coarse roots) and C storage in different SOM pools (POM and MAOM) over time. Eucalypt forest at 36-months-old allocated 72.01 Mg ha^{-1} of C, with 41.5% being directed to the roots (29.92 Mg ha^{-1} of C). After 49-months of planting there were mineralization in POM-, MAOM-Cerrado and Pasture, providing an estimated N mineralization of 0.535 Mg ha^{-1} in the 0.0-1.0-m layer. In contrast, the root-derived C imputed to soil was more efficient in soil organic matter formation (58% higher) than the litterfall- + root-derived C imputed to soil. After 49-months of eucalypt planting the forest was not a potential sequestration of C (ΔC_{Soil}: -2.22 Mg ha^{-1}) to 0.0-1.0 m soil layer. However, studies with longer time scales are required for completeness of information about potential of CO_2-C sequestering by eucalypt forest.

RESUMO

TEIXEIRA, Rafael da Silva, D.Sc., Universidade Federal de Viçosa, setembro de 2017. **Aporte de planta abaixo e acima do solo e ciclagem da matéria orgânica do solo em plantios de eucalipto no bioma cerrado.** Orientador: Ivo Ribeiro da Silva. Coorientadora: Emanuelle Mercês Barros Soares.

Matéria orgânica do solo (MOS) desempenha papel chave em agroecossitemas de alta produtividade, além de oferecer uma alternativa para reduzir as emissões de C-CO_2 e aumentar o sequestro de C no solo. No Brasil, a maioria do Cerrado brasileiro foi inicialmente convertida em pastagens manejadas inapropriadamente, o qual promoveu a degradação do solo, perdas dos estoques de carbono orgânico do solo (COS) e aumentos nas emissões dos GEE. Assim, a conversão do uso do solo pode favorecer o aporte de C-resíduos de plantas sobre e abaixo do solo podendo reduzir os impactos causados pela mudança de uso do solo. Devido às árvores de eucalipto serem de rápido crescimento, elas são atrativas para o sequestro de C no solo (sobre e abaixo do solo) e subsequente aporte de C para o solo. Então, plantios de eucalipto podem sequestrar C em compartimentos com diferentes escalas de tempo: *i)* Biomassa da planta e *ii)* Matéria orgânica do solo. Desta forma, esta Tese objetivou estudar a dinâmica de C e N, focando nos processos que envolvem as emissões de C-CO_2 em plantios de eucalipto em substituição a pastagens, até os 4 anos de idade. Nós realizamos três capítulos, objetivando entender algumas lacunas na pesquisa. No primeiro capítulo analisamos as mudanças nos estoques de C e N, fluxos de C-CO_2 e C-CH_4 no Cerrado, pastagem (cultivada durante 34 anos após a implantação no Cerrado) e eucalipto (cultivado por 4 anos após a implantação na pastagem). Os fluxos superficiais do solo de C-CO_2, C-CH_4 e as concentrações de C-CO_2 ao longo do perfil do solo foram mensuradas em diferentes épocas (chuvosa e seca) durante três anos. Foram também determinados os estoques de C e N associados à matéria orgânica particulada (MOP) e aquela associada aos minerais do solo (MOAM). A variação na abundância natural do ^{13}C ($\delta^{13}C$) foi utilizada para particionar a MOS antiga (derivado do Cerrado ou pastagem) e sua substituição pelo aporte do C novo (derivado do eucalipto). Foi observado forte influência da época chuvosa para os fluxos superficiais de C-CO_2 e C-CH_4, e concentrações de C-CO_2 nas profundidades do solo para os diferentes usos. O solo sob plantações de eucalipto emitiu ~70% mais C-CO_2 do que aqueles com Cerrado e pastagem após 40 meses do plantio do eucalipto, enquanto o solo de pastagem emitiu mais C-CH_4 para a atmosfera do que aqueles sob Cerrado e eucalipto in Set 2012, Jan 2013 e Out 2015. As perdas de C-MOAM antigo nas camadas mais profundas do solo não foram compensadas pelo aporte do C derivado do eucalipto, resultando em perdas líquidas de C do solo. Mesmo assim, não foram detectadas diferenças para o C e N-MOP no solo (0.0-1.0 m), talvez indicando recuperação da MOS nos plantios de eucalipto em idades mais avançadas. No segundo capítulo, nós investigamos a dinâmica dos componentes do C-CO_2 na superfície e no perfil do solo, também rastreando a influência do crescimento das raízes do eucalipto (especialmente raízes finas) para este processo. Devido ao histórico de uso foi possível particionar os fluxos superficiais de C-CO_2 e as concentrações de C-CO_2 em profundidade em C-CO_2 derivado da planta e C-CO_2 derivado do solo. Além disso, o efeito priming rizosférico (EPR) foi calculado. As avaliações foram realizadas em seis épocas: 3, 7, 15, 19, 31 e 40 meses de idade do eucalipto. Após a implantação das florestas de eucalipto houve um aumento nos fluxos superficiais de C-CO_2 ao longo do crescimento da planta (4,33 kg ha^{-1} h^{-1} aos 40 meses de idade). O crescimento das raízes contribuiu grandemente para os fluxos superficiais de C-CO_2 (correlacionados a $p<0,01$; $r:0,61$) promovendo o EPR superficial ao longo do tempo (correlacionado a $p<0,01$; $r:0,63$). A umidade teve grande influência na decomposição do litter (correlacionado a $p<0,01$; $r:0,70$) e respiração de raiz e/ou decomposição de rizodepositos (correlacionados a $p<0,01$; $r:0,79$). Finalmente, no terceiro capítulo nós acessamos a estocagem de C na biomassa do eucalipto (folhas, galhos, casacas, tronco, raízes finas, raízes médias e raízes grossas) e nos compartimentos da MOS (MOP e MOAM) ao longo do tempo. Florestas de eucalipto aos 36 meses de idade alocou 72,01 Mg ha^{-1} de C, com 41,5% sendo direcionado para as raízes (29,92 Mg ha^{-1} de C). Após 49 meses do plantio houve mineralização na MOP-, MOAM-Cerrado e Pastagem, promovendo uma mineralização estimada de N de 0,535 Mg ha^{-1} na camada de 0,0-1,0 m. Em contrapartida, o C aportado ao solo pelas raízes foi mais eficiente em formar MOS (58% maior) em relação ao C derivado do litter + raízes. Após 49 meses do plantio do eucalipto a floresta não apresentou potencial de sequestro de C no solo (ΔC_{Solo}: -2,22 Mg ha^{-1}) para camada de 0,0-1,0 m. Entretanto, estudos com uma escala de tempo mais longa são requeridos para complementar informações sobre o potencial das florestas de eucalipto em sequestrar C-CO_2 no solo.

I. GENERAL INTRODUCTION

There are growing concerned regarding climate changes since increases in greenhouse gas (GHG), mainly CO_2, CH_4 and N_2O emissions to atmosphere have been detected (IPCC, 2014). The soil organic matter (SOM) constitutes the largest reservoir of terrestrial C (Epron et al., 2006), and is considered an effective sink to the C sequestration (IPCC, 2014; Arneth et al., 2017). The more conservation land-management agrosystems that favor the plant residue-C inputs to the soil can improve the soil organic carbon (SOC) storage.

On other hand, agrosystems when improperly managed may affect the microbial activity and stimulate SOM decomposition (Don et al., 2011; Beniston et al., 2014). In Brazil, most of the Cerrado (Brazilian Savannah) were initially converted to pastures using unsustainable practices, which promoted soil degradation, SOC stocks losses and increase in GHG emissions (Bustamante et al., 2006; Lal., 2008). Thus, the conservation land-management systems that favor the input of aboveground and belowground plant residue-C to soil may reduce the impacts caused by land-use change.

Fast-growing forest species, like *Eucalyptus* sp., may be used as potential CO_2-C sequesters in its biomass (aboveground and belowground), with subsequent C input to the soil. However, the rate at which C accumulates in soil depends on the a balance between the C inputs and losses, due to soil respiration (CO_2-C efflux). Soil respiration comprises three main components: (*i*) Autotrophic respiration from roots (Ar); (*ii*) Heterotrophic respiration due to the breakdown of soil organic matter (Hr_{SOM}) and *(iii)* Heterotrophic respiration due to the breakdown of new C input from rhizodepositions and litterfall (Hr_{NewC}).

Further, the Hr_{NewC} rhizosphere C inputs can stimulate or slow down native SOM decomposition, speeding up or retarding CO_2-C flux (Cheng and Kuzyakov, 2005; Cheng et al., 2014; Kumar et al., 2016). This process is so-called Rhizosphere Priming Effect (RPE), but the actual mechanisms underlying RPE still remain unclear (Cheng and Kuzyakov, 2005). Thus, the root growth influence the C storage in depth, since root-released substrates may stimulates microbial growth (Ewing et al., 2006; Schmidt et al., 2011).

The eucalypt forests can sequester C in compartments with different timescales: *i)* Plant biomass ($\Delta C_{biomass}$) and *ii)* Soil organic matter (ΔC_{Soil}). The allocation to plant biomass is a C sequestration process considered short-term, while allocation to SOM may increase C sequestration in the long-term.

The $\Delta C_{biomass}$ is the net primary production (*NPP*), consisting of aboveground net primary production sum (leaves, branches, bark, wood, stump and litterfall; *ANPP*) and belowground net primary production (fine roots, medium roots and coarse roots; *BNPP*). The ΔC_{Soil} is the difference between input C (*Net-C-litterfall* + *Net-C-root*) and soil C output (*SOC decomposition*).

So, this thesis aimed to study the C and N dynamics, focusing in the processes that underline CO_2-C emissions in on eucalypt plantation since the land-use change following a pasture, until 4-years-old. We report our research in three chapters, aimed at understanding some research gaps. In the first chapter we analyzed the changes in C and N stocks, CO_2-C and CH_4-C fluxes in Cerrado, pasture and eucalypt. In the second chapter, we investigated de dynamics of CO_2-C components in soil surface and soil profile, also tracking the influence of eucalypt root growth (especially fine roots) on these processes. Finally, in the third chapter we accessed the biomass C storage and C storage in different SOM pools over time. So, our findings provide valuable information for estimating potential C sequestration in eucalypt plantations and for determining rational forest management practices to help to mitigate climate change.

2. REFERENCES

Arneth, A.; Sitch, S.; Pongratz, J.; Stocker, B.D.; Ciais, P.; Poulter, B.; Bayer, A.D.; Bondeau, A.; Calle, L.; Chini, L.P.; Gasser, T.; Fader, M.; Friedlingstein, P.; Kato, E.; Li, W.; Lindeskog, M.; Nabel, J.E.M.S.; Pugh, T.A.M.; Robertson, E.; Viovy, N.; Yue, C.; Zaehle, S. 2017. Historical carbon dioxide emissions caused by land-use changes are possibly larger than assumed. Nature Geoscience, vol. 10.

Beniston, J.W.; DuPont, S.T.; Glover, J.D.; Lal, R.; Dungait, J.A.J. 2014. Soil organic carbon dynamics 75 years after land-use change in perennial grassland and annual wheat agricultural systems. Biogeochemistry, 120:37-49.

Bustamante, M.M.C.; Corbeels, M.; Scopel, E.; Roscoe, R. 2006.Soil carbon and sequestration potential in the Cerrado Region of Brazil. In: Lal, R.; Cerri, C.C.; Bernoux, M.; Etchevers, J.; Cerri, C.E.P. Carbon sequestration in soils of Latin America. New York, Haworth, p.285-304.

Cheng, W.X.; Kuzyakov, Y. 2005. Root effects on soil organic matter decomposition. In: Zobel, R.W., Wright, S.F. (Eds.), Roots and Soil Management: Interactions between Roots and the Soil, Agronomy Monograph No. 48. ASA-CSSA-SSSA, Madison, WI, p.119-143.

Cheng, W.X.; Parton, W.J.; Gonzalez-Meler, M.A.; Phillips, R.; Asao, S.; McNickle, G.G.; Brzostek, E.; Jastrow, J.D. 2014. Tansley review: synthesis and modeling perspectives of rhizosphere priming. New Phytologist, 201:31-44.

Don, A.; Schumacher, J.; Freibauer, A. 2011. Impact of tropical land-use change on soil organic carbon stocks – A meta-analys. Global Change Biology, 17:1658-1670.

Epron, D.; Bosc, A.; Bonal, D.; Freycon, V. 2006. Spatial variation of soil respiration across a topographic gradient in a tropical rain forest in French Guiana. Journal of Tropical Ecology, 22:565-574.

Ewing, S.A.; Sanderman, J.; Baisden, W.T.; Wang, Y.; Amundson, R. 2006. Role of large-scale soil structure in organic carbon turnover: evidence from California grassland soils. Journal of Geophysical Research, 111:G03012.

IPCC. 2014. Climate Change 2014 Mitigation of Climate Change: Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Kumar, A.; Kuzyakov, Y., Pausch, J. 2016. Maize rhizosphere priming: field estimates using ^{13}C natural abundance. Plant Soil, 409:87-97.

Lal, R. 2008. Sequestration of atmospheric CO_2 into global carbon pool. Energy & Environmental Science, 1:86-100.

Schmidt, M.W.; Torn, M.S.; Abiven, S.; Dittmar, T.; Guggenberger, G.; Janssens, I.A.; Kleber, M.; Kögel-Knabner, I.; Lehmann, J.; Manning, D.A.C.; Nannipieri, P.; Rasse, D.P.; Weiner, S.; Trumbore, S.E. 2011. Persistence of soil organic matter as an ecosystem property. Nature, 478:49-56.

II. CHAPTER 1

Land use change with pasture and short-rotation eucalypt impacts soil CO_2 and CH_4 fluxes and organic C stocks in the Cerrado biome

ABSTRACT

Soil organic matter (SOM) plays key roles on high productive agrosystems, so the rapid expansion of short-rotation eucalypt plantations in low SOM sandy soils may offer an alternative to reduce soil CO_2 emissions and improve soil C sequestration. Because eucalypt trees are fast-growing, they are attractive for C sequestration (aboveground and belowground) and subsequent C input to the soil. The objectives of this study were *i)* to quantify changes in C and N stocks in physically-separated organic matter fractions for pasture soils converted to short-rotation eucalypt; *ii)* to estimate the turnover of C in different SOM fractions following conversion of the native Cerrado vegetation (Brazilian Savannah) to pasture and then the pasture to eucalypt plantation. Therefore, our study focused on soils under native Cerrado vegetation, an adjacent planted pasture (cultivated for 34-years following the clearing of the Cerrado) and eucalypt stand (cultivated for 4 years) established in place of the 34-year-old pasture field. The soil surface CO_2-C, CH_4-C fluxes and also CO_2-C concentration along the vertical soil profile were measured in different seasons (Wet and Dry) over three years. It was also determined the C and N stocks associated to the particulate organic matter (POM) and mineral-associated organic matter (MAOM). Variation in natural abundance of ^{13}C ($\delta^{13}C$) was used to partition the SOM in old (Cerrado- or pasture-derived) and their replacement by the new input C (eucalypt-derived). It was observed that the wet season had the strongest influence on soil surface CO_2-C and CH_4-C fluxes, and CO_2-C concentration at soil depths for the different land uses. The soil under eucalypt plantation emitted ~70% more CO_2-C than those under Cerrado and pasture after 40-months of eucalypt planting, while the pasture soil emitted more CH_4-C to the atmosphere than those under Cerrado and eucalypt in Sep 2012, Jan 2013 and Oct 2015. The old MAOM-C losses in deep soil layers were not compensated by the new eucalypt C inputs, resulting in net soil C losses. Nevertheless, no differences were detected to POM-C and -N in the soil (0.0-1.0-m), perhaps indicating a recovery in SOM in eucalypt stands at a more advanced stand age.

Key-words: Particulate organic matter-POM, Mineral-associated organic matter-MAOM, Tropical soils.

1. INTRODUCTION

Currently, there are emerging concerns regarding climate changes as a result of increasing atmosphere greenhouse gases (GHG), mainly CO_2, CH_4 and N_2O (IPCC, 2014). Although the amounts of methane CH_4 and N_2O gases emitted into the atmosphere are much smaller, the global warming potential of these gases is 23 and 296 times greater than that of CO_2 (IPCC, 2014). Soil organic matter (SOM) is an important terrestrial C pool and its increase has been proposed as an alternative to improve CO_2-C sequestration (IPCC, 2014; Arneth et al., 2017). Soil organic carbon (SOC) storage reflects the net balance between C inputs (mainly aboveground and/or belowground plant residues) and microbial decompositions (output as CO_2 and CH_4) (Alberti et al., 2015).

The major process governing SOM output is decomposition. Biotic drivers like plant properties (i.g. cellulose and lignin content, nutrient levels and plant components) as well as soil microbial diversity (i.g. abundance of bacteria, fungi, actinomycetes) and abiotic drivers (i.g. soil temperature and soil moisture) affect the decomposition processes on soil. Thus, changes in C inputs and soil environment imposed by land-use changes may affect the microbial communities and stimulate the decomposition process and negatively affect SOC storage (Don et al., 2011; Beniston et al., 2014).

Savannah vegetation contributes with 20-30% of global primary production. The Brazilian Savannah covers about 200 Mha (approximately 23% of Brazil`s surface) (Bustamante et al., 2006; Lal, 2008). However, most of the Brazilian Savannah (Cerrado) were initially converted to pastures, which currently show some degree of degradation, SOC losses and increase in greenhouse gas emissions (Bustamante et al., 2006; Lal., 2008). The adoption of conservation land-management systems that favor the aboveground and belowground plant residue-derived C transfer and stabilization into soils may reduce the impacts caused by the land-use change. Thus, fast-growing forests like those of *Eucalyptus* sp. have a large capacity for CO_2-C fixation in their biomass (aboveground and belowground), which may subsequently be soil C inputs.

Short-rotation eucalypt plantations account for approximately 20 million hectares worldwide (Iglesias-Trabado and Wilstermann, 2008) of which approximately 5.6 million ha are in Brazil (IBA, 2016). The area under eucalypt in Brazil is fast expanding mainly in the Cerrado biome where plantations have been established in areas previously under poorly managed and degraded pastures, mostly often on soils which have lost a significant part of their original SOM content (Pegoraro et al., 2011; Vergutz, 2010). Such pasture substitution by planted eucalypt stands could be an opportunity to restore, at least partially, the original SOC stocks and contribute to reduce GHG in the atmosphere. However, studies accessing the effect of land-use changes involving eucalypt plantations in the short-term remain scarce.

The SOM has a heterogeneous composition and its constituents interact among them and with the soil mineral matrix, resulting in distinct fractions and turnover rates (Lehman and Kleber, 2015; Kabiri et al., 2015). Particulate organic material (POM) in soil is mainly associated with macroaggregates (Six et al., 2002; Plante et al., 2006) and it has been shown to be more sensitive to microbial degradation and thus more affected by tillage practices (Kabiri et al., 2015). The similarity in chemical composition of POM to plant materials is related with its greater lability (Grandy et al., 2007), which open the possibility of using it as an early indicator of shifts in SOC as a result of land-use changes (Pikul et al., 2007). Meanwhile, mineral-associated organic matter (MAOM) is considered a less labile fraction that is physically and chemically protected and stabilized (Mazzilli et al., 2015).

Besides the changes in SOM fractions, soil CO_2-C and CH_4-C flux measures could be useful for detecting short-term changes in SOM dynamics. Therefore, CO_2-C and CH_4-C flux measurement, and SOM physical fractionation techniques in combination with natural abundance of stable C isotopes constitute a powerful approach to study the effects of long-term land-use changes (Beniston et al., 2014; Wang et al., 2015), and their potential in SOM cycling in eucalypt plantations has already been demonstrated (Epron et al., 2015).

We hypothesized that the replacement of the native savannah vegetation by a planted pasture about three decades ago led to the reduction in SOM content, but the more recent substitution of the pasture by a eucalypt plantation would reduce soil CO_2-C and CH_4-C emissions and favor the recovery of more labile and more stable

SOM fractions. Our objectives were *i)* to evaluate the soil surface CO_2-C and CH_4-C fluxes, and the CO_2-C concentrations in depth for soils under Cerrado, planted pasture and eucalypt stand; *ii)* to evaluate changes in C and N stocks in physically-separated SOM fractions in soils under Cerrado, planted pasture and eucalypt stand.

2. MATERIALS AND METHODS

Experimental setup

The experimental site is located in Três Lagoas county, Mato Grosso do Sul state, Brazil (20° 53' 36.61 "S 51° 54' 25.67" W, 384 m elevation) (Figure 1). The soil is a Typic Haplustox (Soil Survey Staff, 2014) or Latossolo Vermelho Amarelo (Embrapa, 2013) and the main characteristics are described in Table 1. The relief is gently undulating, averaging less than 5% slope. The climate is Aw type according to Köppen`s classification, with rainy, hot summers (October to March) and dry, mild winters (April to September).

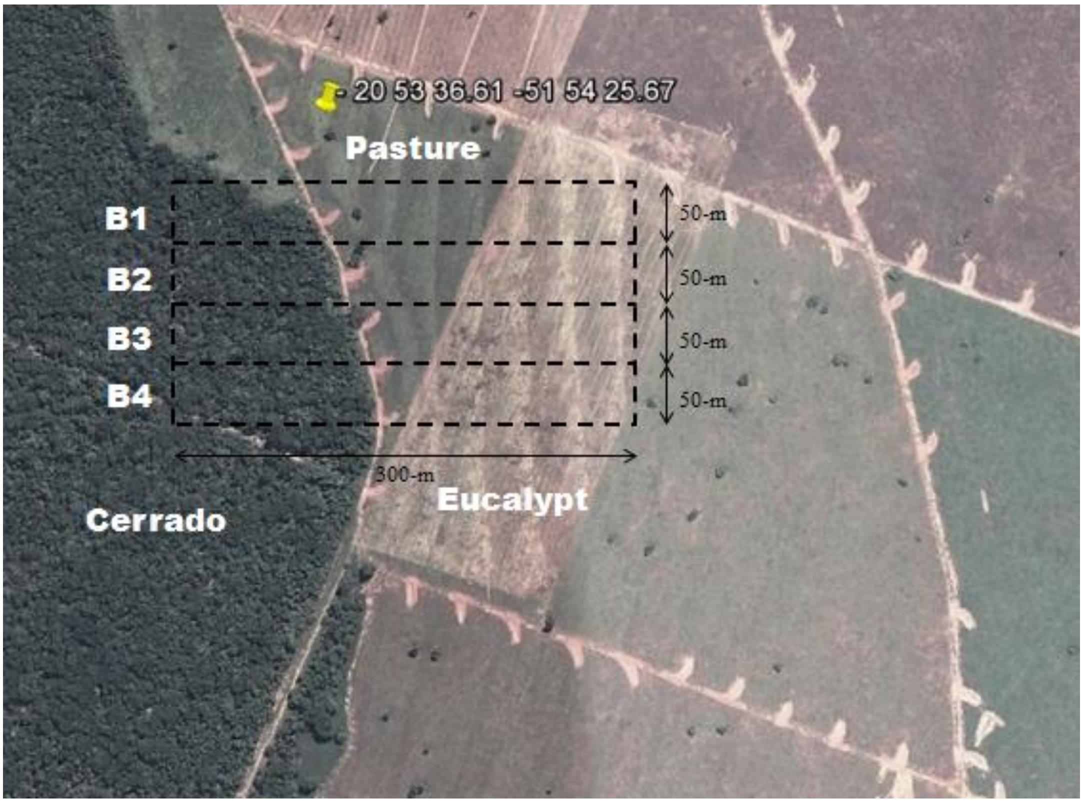

Figure 1. Geographic location of the study site before the eucalypt planting illustrating the delimitation of blocks, in Três Lagoas, Mato Grosso do Sul state, Brazil.

Table 1. Chemical and physical soil characteristics in different land-use (Cerrado, Pasture and Eucalypt)

[†]Land-use	Depth	pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H + Al	T	P-Rem	Mn	Fe	Sand	Silt	Clay	D_{Soil}
	... m ...	H_2O	... mg dm^{-3} ...		.... $cmol_c$ dm^{-3}					…….. mg L^{-1}			……..... %			kg dm^{-3}
Cerrado	0.0 - 0.1	4,48	7,80	44,00	0,71	0,36	0,70	4,00	5,18	43,90	24,70	79,60	78,30	5,50	16,20	1,48
	0.1 - 0.2	4,38	2,70	32,00	0,29	0,20	0,70	3,50	4,07	44,40	9,40	53,80	79,40	3,70	16,90	1,50
	0.2 - 0.4	4,17	3,50	36,00	0,20	0,12	1,00	3,50	3,91	40,00	5,60	31,60	71,40	7,00	21,60	1,47
	0.4 - 0.6	4,27	3,10	31,00	0,16	0,10	1,00	3,30	3,64	43,00	6,00	33,80	74,30	6,70	18,90	1,46
	0.6 - 1.0	4,17	3,40	19,00	0,12	0,07	0,90	2,30	2,54	39,40	3,50	36,00	71,90	5,70	22,40	1,50
Pasture	0.0 - 0.1	5,10	7,00	32,00	0,73	0,41	0,20	2,50	3,72	45,40	27,40	29,00	77,00	5,60	17,40	1,50
	0.1 - 0.2	5,23	2,60	21,00	0,60	0,20	0,20	2,60	3,45	47,50	18,40	44,40	76,90	4,60	18,50	1,71
	0.2 - 0.4	5,00	4,10	22,00	0,37	0,09	0,40	1,80	2,32	44,40	10,00	34,10	74,10	7,50	18,40	1,61
	0.4 - 0.6	4,74	2,40	6,00	0,37	0,07	0,90	2,10	2,56	39,10	8,30	41,80	69,40	7,30	23,30	1,58
	0.6 - 1.0	4,74	0,50	3,00	0,27	0,06	0,70	2,50	2,84	31,60	7,80	41,30	67,30	10,40	22,30	1,57
[*]Eucalypt	0.0 - 0.1	5,10	20,90	24,00	0,65	0,36	0,40	2,30	3,37	51,30	30,20	33,20	79,30	6,70	14,00	1,50
	0.1 - 0.2	5,23	5,80	9,00	0,49	0,22	0,10	2,10	2,83	47,00	25,60	42,90	74,00	7,90	18,20	1,56
	0.2 - 0.4	4,75	127,70	69,00	0,85	0,16	0,40	2,80	3,99	52,40	20,80	42,40	73,80	7,10	19,10	1,52
	0.4 - 0.6	4,60	17,40	16,00	0,38	0,12	0,40	1,50	2,04	38,90	10,50	34,00	71,20	7,90	20,80	1,50
	0.6 - 1.0	4,44	2,20	3,00	0,33	0,12	0,40	1,70	2,16	36,50	5,30	25,20	71,30	7,50	21,30	1,48

[†]Soil samples collected in June 2012. pH in water (L:S 2,5 L kg^{-1}); exchangeable Ca^{2+}, Mg^{2+}, Al^{3+} in a 1 mol L^{-1} KCl soil extract; available P, K, Fe and Mn extracted by Mehlich-1; H + Al extracted by Calcium acetate 0.5 mol L^{-1} - pH 7.0; Cation exchange capacity (CEC) measured with 0,5 mol L^{-1} calcium acetate at pH 7,0; P-rem: Equilibrium phosphorus (Alvarez V. et al., 2000); Particle size analysis with the pipette method (Ruiz et al., 2005); D_{Soil}: Soil bulk density; [*]Weighted average [(1/3*PL) + (2/3*BPL)] between planting row (PR) and between planting row (BPR) after soil mechanical preparation and fertilization for eucalypt planting.

The study was developed based on a land-use change chronosequence (Figure 2; Supplementary material 1) and there were three distinct land uses: native Cerrado vegetation, a planted pasture field (cultivated for 34 years following the clearing of the native Cerrado) and a clonal eucalypt stand (cultivated for 4-years in part of the pasture field). The native vegetation has a number of species, but is mainly composed of *Hymenea stigonocarpa, Cupania vernalis* and *Tapirira guianensis*. The pasture (*Urochloa decumbens*) was established in 1982 and cultivated for 34 years with a low input grazing system with average 1 animal unit ha^{-1}.

In May 2012 part of the pasture field was killed using glyphosate and then used for establishing the eucalypt stand in a 3.6 x 2.4 m spacing (1157 plants ha^{-1}). Fertilizer was applied four times over the study time: The first, at planting was used (350 kg ha^{-1} of NPK 06-30-06 + 0.4% Cu + 0.4% Zn) along the subsoiled and furrows, three other times applied was by plane at 6-month-old (300 kg ha^{-1} of NPK 18-00-18 + 0.7% B), at 12-month-old (300 kg ha^{-1} of NPK 10-00-30 + 0.7% B), at 24-month-old (100 kg ha^{-1} of NPK 11-52-00). 1500 kg ha^{-1} of lime was broadcast before planting. Chemicals were applied in the first year to control leaf-cutting ants (sulfluramid) and weeds (ghyphosate).

In each land use we established four 300 x 50 m strips taking into account the slope of the field. Following, for each land use we set up four 50 x 50 m plots within the previously marked strips. This approach was used as a compromise for our inability to fully randomize the land use treatments. One disadvantage of such approach is the lack of randomization of the each land-use strip and they were used as replicates. Treatments were compared using a completely randomized block design with four replicates.

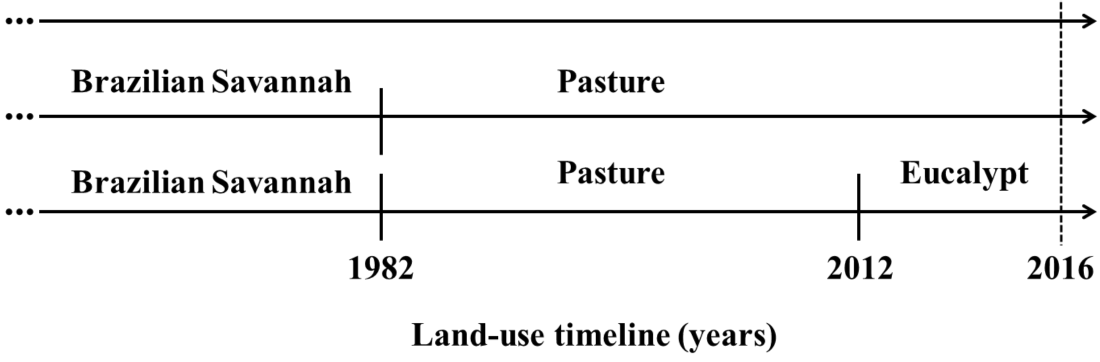

Figure 2. Land-use change chronosequence scheme.

Soil surface CO_2-C and CH_4-C flux and CO_2-C concentration in depth

In order to access the CO_2 and CH_4 emissions in different land-use, measures of soil surface CO_2-C and CH_4-C fluxes, and CO_2-C concentration in depths along the soil profile were taken over time. The soil surface CO_2-C flux was monitored using static PVC chambers (0.30-m height x 0.4-m diameter) installed in Cerrado, pasture and eucalypt sites (Supplementary material 2) in six gases sampling (Sept 2012, Jan 2013, Sept 2013, Jan 2014, Jan 2015 and Oct 2015). In the eucalypt stand the static PVC chambers were installed at the planting row (PR) and between planting rows (BPR) after the eucalypt planting. Mobile caps fitted with rubber septum on top were placed on top of the chambers used to concentrate the gaseous atmosphere. Gas samples were collected with plastic syringes (60-mL) equipped with a 3-way valve at 0, 10, 20 and 40-min. After closing the chambers at the moment of the sampling, the soil surface temperature and soil surface moisture were measured on top soil (0.00-0.05 m soil layer) using a EC-5 sensor (Decagon Devices Inc., Pullman, WA) (Supplementary material 3).

For the gas sampling in depth it was installed in Cerrado, pasture and eucalypt (PR and BPR) one PVC pipe (1.0-m height x 0.025-m diameter) with compartments in the layers of 0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-1.0 m (Supplementary material 2). Gas samples were collected with plastic syringes (60-mL). Six gas sampling were performed: Sept 2012, Jan 2013, Sept 2013, Jan 2014, Jan 2015 and Oct 2015. The average monthly precipitation (Figure 3a) and average monthly temperature (Figure 3b) were monitored over the experimental period by a micrometeorological station set up on site (2012, 2013, 2014 and 2015).

The syringes were taken to the lab and CO2 and CH4 were measured within two weeks after sampling. The CO_2-C and CH_4-C concentrations (CO_2-C conc. and CH_4-C conc.; μmol mol^{-1}) were determined using a ring-down resonant cavity spectrometer (CRDS, G2131-i, Picarro, Sunnyvale, CA).

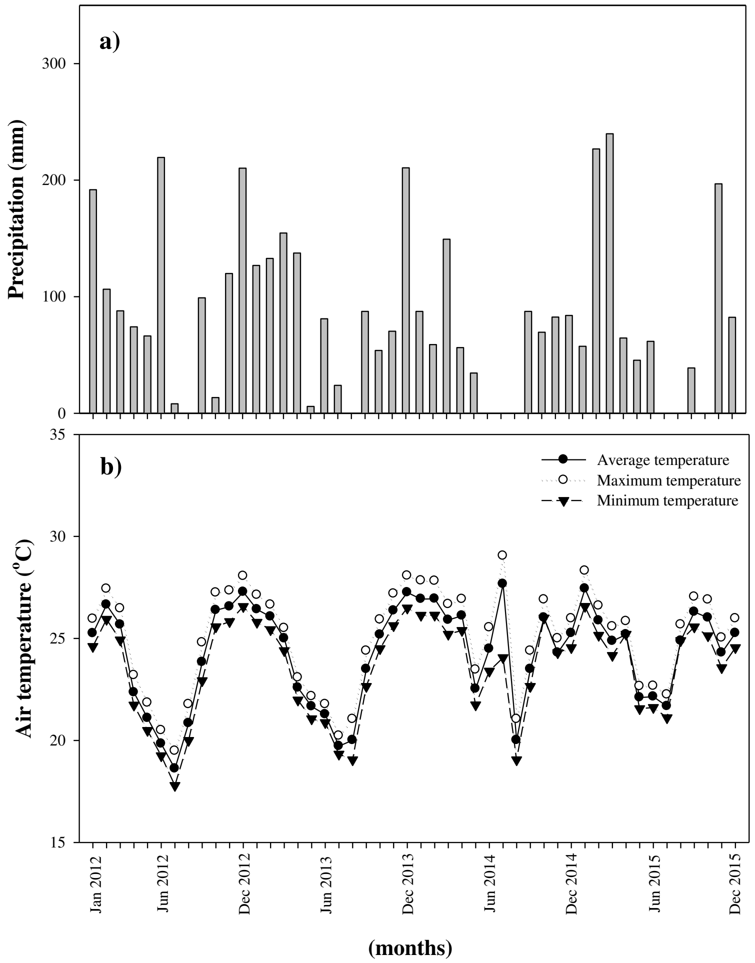

Figure 3. Average monthly precipitation (mm; a), average monthly temperature (°C; b), maximum monthly temperature (°C; b) and minimum monthly temperature (°C; b) from January 2012 (Jan 2012) to December 2015 (Dec 2015).

The soil surface CO_2-C and CH_4-C fluxes were calculated based on the variation of the CO_2-C conc. and CH_4-C conc. over time, according to Eq. 1 (Smith and Conen, 2004):

$$\textit{Soil surface } CO_2\textit{-C or } CH_4\textit{-C flux} = [(\Delta Q/\Delta t) \times M \times P \times V] / R \times T \times A \qquad \textit{Eq. 1}$$

Where, *Soil surface* CO_2*-C or* CH_4*-Cflux* is CO_2-C or CH_4-C flux (kg ha^{-1} h^{-1}); ($\Delta Q/\Delta t$) is the variation in CO_2-C conc. or CH_4-C conc. over time; M is the molar mass of C; P is the pressure inside the chamber, assumed to be 1 atmosphere (atm); V is the chamber volume (L); R is the universal gas constant (0.0821 L atm K^{-1} mol^{-1}); T is the temperature of the atmosphere (K); A is the chamber area (m^2).

Due to the initial mechanical preparation in the area for eucalypt planting, the calculations to the *Soil surface CO_2-C and CH_4-C flux* in eucalypt plantations were carried out according to Eq. 2:

$$\textit{Soil surface } CO_2\textit{-C or } CH_4\textit{-C flux} = (1/3 \times \text{CO}_2\text{-C or CH}_4\text{-C flux}_{PR}) + (2/3 \times \text{CO}_2\text{-C or CH}_4\text{-C flux}_{BPR}) \quad \textit{Eq. 2}$$

Where, *Soil surface CO_2-C or CH_4-Cflux* is the CO_2-C or CH_4-C flux (kg ha^{-1} h^{-1}); the *1/3* represents an area proportion under influence of the initial mechanical preparation in PR and *2/3* represents an area proportion of the BPR of eucalypt plantation.

The CO_2-C conc. and CH_4-C conc. in depth for each soil layer (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-1.0 m) was expressed according to Eq. 3:

$$CO_2\textit{-C or } CH_4\textit{-Cconc.} = [(\text{CO}_2\text{-C } \textit{or } CH_4\textit{-Cconc.}_{PR}) + (\text{CO}_2\text{-C } \textit{or } CH_4\textit{-Cconc.}_{BPR})] / 2 \quad \textit{Eq. 3}$$

Where, *CO_2-C or CH_4-Cconc.* is the CO_2-C or CH_4-Cconc. (µmol mol^{-1} ha^{-1}).

Soil sampling and analysis

Soil sampling was performed in 2016 (Table 1) accounting 34-years and 4-years of cultivation time for pasture and eucalypt, respectively. In the eucalypt stand the soil samples were collected in the PR and BPR (to be more representative). The soil samples were collected in depth: 0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m. The soils were taken to the laboratory, passed in a 2-mm sieve and air dried.

Soil organic matter fractions were separated in particulate fraction (POM) and fraction associated with minerals (mineral associated with organic matter, MAOM) according to Cambardella and Elliott (1992). Sub-samples of soil were milled and analyzed for C, N and $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$ ‰ values) in an elemental CN analyzer coupled to an isotope ratio mass spectrometer (IRMS, ANCA GSL 20-20, Sercon, Crewe, UK). The organic C associated with organic material retained in the 53 µm sieve corresponds to POM-C and that associated with the silt + clay minerals fraction correspond to MAOM-C.

Because the distinct plants involved in the land-use changes have different photosynthetic systems (Cerrado and eucalypt - C_3 plants and pasture - C_4 plants) it was possible to identify the C derived from the prior land-use. To do so, the C partitioning was performed based on the $\delta^{13}C$ values of pasture C inputs. The measured $\delta^{13}C$ values were used to calculate the proportion of C derived from pasture (f_{new}) in pasture or eucalypt land-use, by using a mass balance equation (Del Galdo et al., 2003, Cotrufo et al. 2011).

$$f_{new} = (\delta^{13} C_{Soil} - \delta^{13} C_{Old}) / (\delta^{13} C_{Veg.} - \delta^{13} C_{Old}) \qquad Eq.\ 4$$

Where $\delta^{13}C_{Soil}$ is $\delta^{13}C$ of the soil organic matter of the pasture or eucalypt, $\delta^{13}C_{Old}$ is the $\delta^{13}C$ of the soil organic matter of the Cerrado, and $\delta^{13}C_{Veg}$ is the $\delta^{13}C$ of the *Brachiaria brizantha* (-13.00 ‰), according to Cerri and Volkoff (1991).

Knowing the f_{new} values for the new C, the soil organic C concentrations (% C), soil depth (D, m), area of study plot (A, ha) and soil bulk density (σ, kg m^{-3}), *Pasture-C* amounts (Mg ha^{-1}) were computed for the soil samples as follows (Eq. 5):

$$Pasture\text{-}C = f_{new} .\%\ C.(A.D.\ \sigma)/1000\ \ (Mg\ ha^{-1}) \qquad Eq.\ 5$$

In the eucalypt plantations the calculations were carried out according to Eq. 6:

$$Pasture\text{-}C_{euc.} = (1/3.Pasture\text{-}C_{PR}) + (2/3.Pasture\text{-}C_{BPR}) \qquad Eq.\ 6$$

Where, *Pasture-*$C_{euc.}$ is the Pasture-C in eucalypt plantations (Mg ha^{-1}); The *1/3* and *2/3* represent the area proportioned of the planting row (*PR*) and between planting row (*BPR*) of eucalypt.

Then, the C_3-$C_{Cerrado\ (C)\ or\ Cerrado\ +\ Eucalypt\ (C\ +\ Euc)}$ were calculated using a mass balance equation (Eq. 7):

$$TOC_{POM\ and\ MAOM} = Pasture\text{-}C_{Pasture\ or\ Eucalypt} + C_3\text{-}C_{C\ or\ C\ +\ Euc} \qquad Eq.\ 7$$

Where, $TOC_{POM\ and\ MAOM}$ is total soil organic carbon in POM and MAOM fractions; $Pasture\text{-}C_{Pasture\ or\ Eucalypt}$ is the Pasture-C partitioned in the pasture or in the eucalypt sites; $C_3\text{-}C_{C\ or\ C\ +\ Euc}$ is the C_3-C partitioned in the pasture or eucalypt.

In order to detect the land-use change we calculated the total change in C stocks (Eq. 8) and the C loss derived from prior use (Cerrado or Pasture) according to Eq. 9.

$$C\text{-}Change_{\ Pasture\ or\ Eucalypt} = C_{\ Pasture\ or\ Eucalypt} - C_{\ Cerrado\ or\ Pasture} \qquad Eq.\ 8$$

$$Last\ use\text{-}C_{Pasture\ or\ Eucalypt} = last\ use\text{-}derived\ C_{\ Pasture\ or\ Eucalypt} - last\ use\text{-}derived\ C_{Cerrado\ or\ Pasture} \qquad Eq.\ 9$$

Where, $C\text{-}Change_{\ Pasture\ or\ Eucalypt}$ is the C change following the replacement of pasture or eucalypt; $C_{\ Pasture\ or\ Eucalypt}$ is the C in current use in pasture and eucalypt; $C_{\ Cerrado\ or\ Pasture}$ is the C in previous use, Cerrado and pasture, respectively; $Last\ use\text{-}C_{Pasture\ or\ Eucalypt}$ is the Last use-C change after planting pasture or eucalypt; $last\ use\text{-}derived\ C_{\ Pasture\ or\ Eucalypt}$ is the Cerrado-derived C in actual pasture area and pasture-derived C in actual eucalypt area; $last\ use\text{-}derived\ C_{Cerrado\ or\ Pasture}$ is the C in Cerrado and pasture-derived C in current pasture area.

Statistical analysis

Repeated-measures ANOVA analysis was used to assess the differences in analyzed variables. Tukey's test was performed to compare means for different land-uses ($\alpha=0.05$). Statistical analyzes were performed using the software package SISVAR (Ferreira, 2008).

3. RESULTS

Soil surface CO_2-C and CH_4-C flux and CO_2-C in depth

The soil surface CO_2-C flux changed according to the seasons (Figure 4a). Greater emissions were observed in Oct 2015, with the eucalypt soil reaching 4.33 kg ha^{-1} h^{-1} of soil surface CO_2-C flux. In the other sampling seasons (Sep 2012-to-Jan 2014) no differences were detectable among land-uses (Figure 4a).

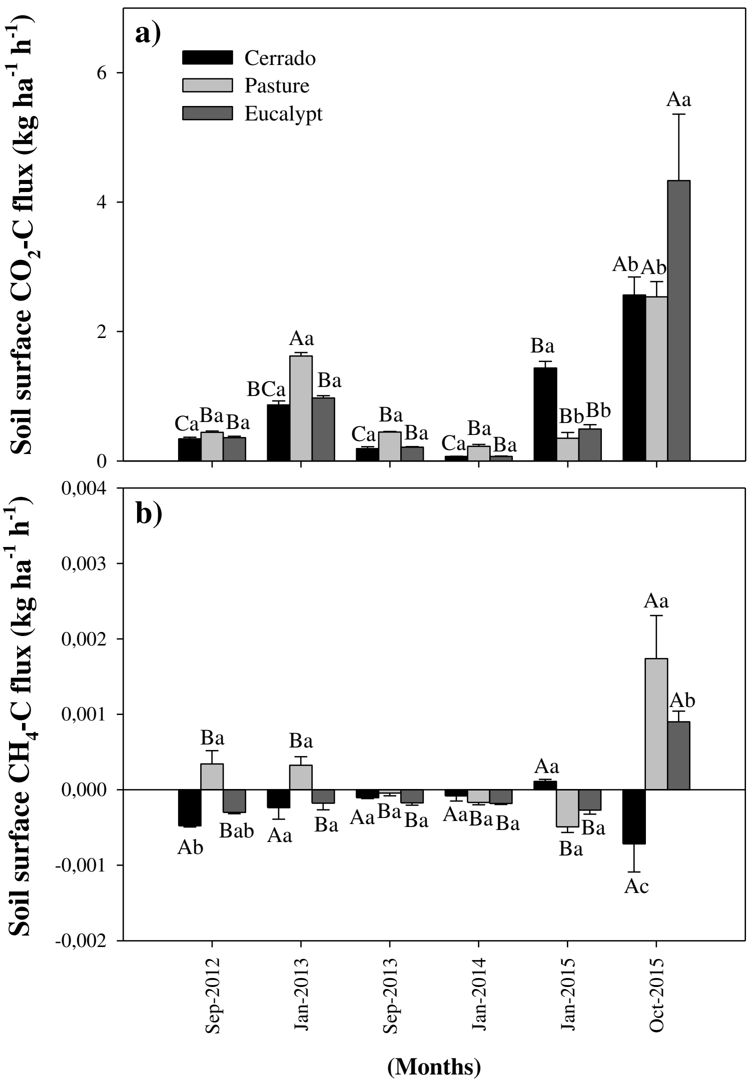

Figure 4. Soil surface CO_2-C flux (kg ha^{-1} h^{-1}; a) and Soil surface CH_4-C flux (kg ha^{-1} h^{-1}; b) in Cerrado, pasture and eucalypt in different seasons measured over 40-months. Different capital letters denote significant differences among seasons, while different lowercase letters denote significant differences among the land-uses within each season by the Tukey's test ($\alpha=0.05$). Vertical bars denote the standard error of the mean (n=4).

However, the pasture soil had the greater emission of soil surface CH_4-C flux (0.0017387 kg ha^{-1} h^{-1}), while in the Cerrado soil had a soil surface CH_4-C influx of -0.000715 kg ha^{-1} h^{-1}. The pasture soil exhibited an emitting behavior of CH_4-C in half of the gas samplings (Sep 2012, Jan 2013 and Oct 2015) (Figure 4b).

The CO_2-C in depth showed elevated concentrations in Jan 2013 to all soil layers analyzed (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6 and 0.6-1.0 m; Figures 5a-o). In soil surface layer (0.0-0.1 m) the Cerrado had the greater CO_2-C concentrations in Jan 2013 (7891.10 µmol mol^{-1}) and Jan 2015 (6565.23 µmol mol^{-1}). On the other hand, in soil subsurface layers (0.1-0.2 to 0.6-1.0 m) the pasture showed the greatest CO_2-C concentrations in Jan 2013 (Figures 5g-j). The CO_2-C concentration increased along the soil profile reaching the highest CO_2-C concentration in the 0.6-1.0-m soil layer of pasture (40,257.20 µmol mol^{-1}; Figure 5j).

Soil organic matter fractions-C and -N with the land-use change

No differences were observed in POM-C and POM-N stocks among different land-uses in all soil layers (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6 and 0.6-1.0 m; Table 2). On other hand, the MAOM-C showed lower stocks in eucalypt, mainly in soil subsurface (0.0-0.1-to-0.6-1.0 m; Table 2). No significant differences in C-Change POM and C-Change MAOM were detectable between pasture and eucalypt for all soil layers studied (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6 and 0.6-1.0 m; Figures 6a, b, e and f). However, the C-Change POM and C-Change MAOM on pasture had increase trends in soil profile (C loss in depth). The grater C-Change MAOM on pasture occurred in the 0.6-1.0-m soil layer (2.10 Mg ha^{-1}; Figure 6e). While the C-Change POM in eucalypt soil had positive values in the 0.4-0.6 and 0.6-1.0-m soil layers (0.20 and 0.46 Mg ha^{-1}), which signals increase of eucalypt-derived C in depth (Figure 6b).

Strong differences were detectable to POM-$\delta^{13}C$ and MAOM-$\delta^{13}C$ which allowed partition the derived C from the previous use (Last use-C) in both land-uses (pasture and eucalypt; Table 2). The negatives values of Last use-C represented last use-derived C loss in pasture and eucalypt. Higher Last use-C loss was detectable in soil subsurface (0.0-0.1-to-0.6-1.0 m) on pasture than eucalypt (Figures 6g-h).

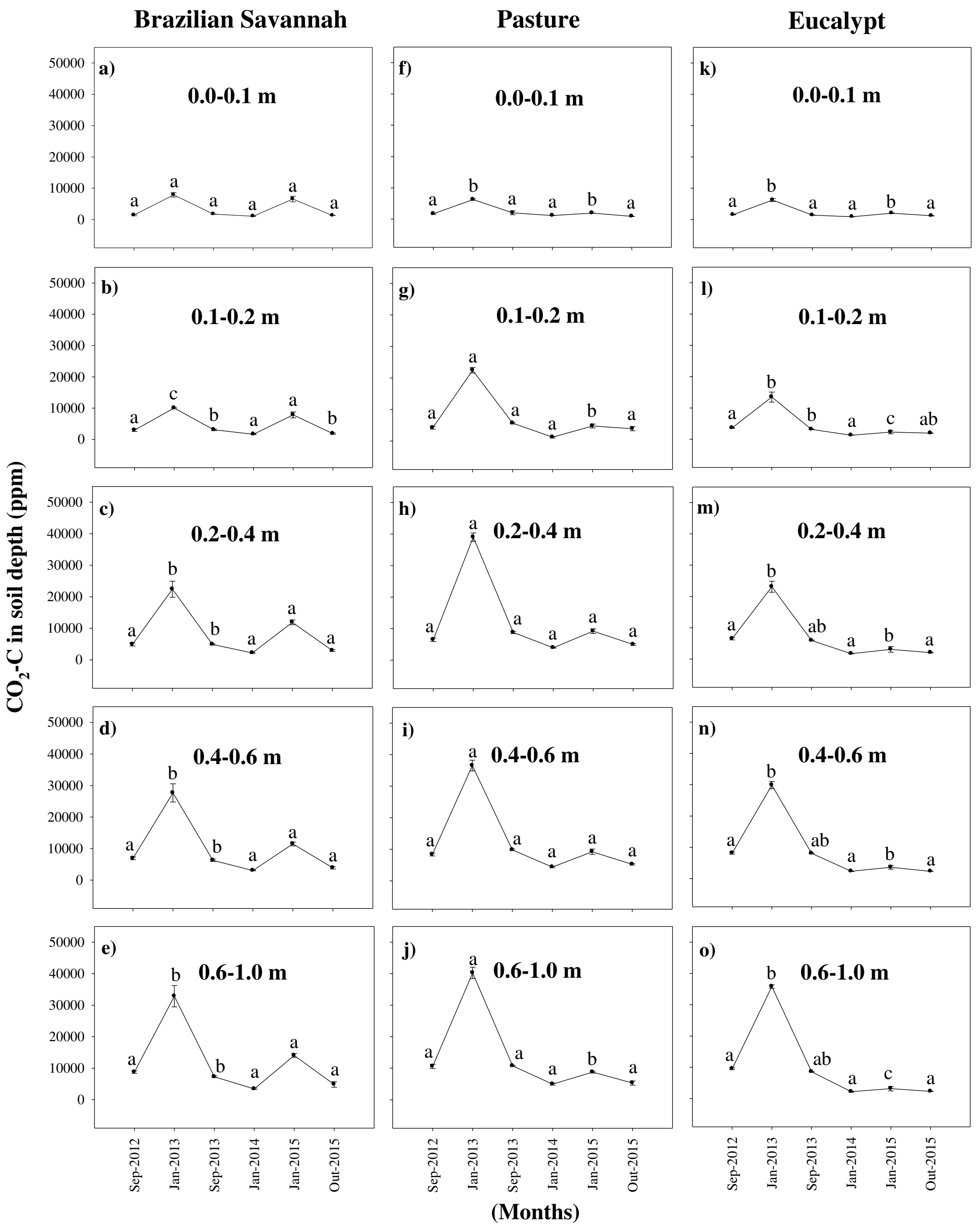

Figure 5. Concentration of CO_2-C (μmol mol^{-1}) in soil layers (0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0-m) along different seasons to Cerrado (a-e), Pasture (f-j) and Eucalypt (k-o). Different letters denote significant differences among land-uses within each season by the Tukey's test ($\alpha = 0.05$). Vertical bars denote the standard error of the mean (n=4).

Table 2. POM-C, POM-N, POM-δ^{13}C, MAOM-C, MAOM-N and MAOM-δ^{13}C in different soil layers (0.0-0.1, 0.1-02, 0.2-0.4, 0.4-0.6, 0.6-1.0 and 0.0-1.0 m) in the Cerrado, Pasture and Eucalypt

Land-use	Depth (m)	POM-C	POM-N	POM-δ^{13}C	MAOM-C	MAOM-N	MAOM-δ^{13}C
		.... Mg ha^{-1}		.... ‰	.... Mg ha^{-1}		.... ‰ ....
Cerrado	0.0-0.1	2.05 ± (0.19) a	0.08 ± (0.01) a	-26.03 ± (0.20) c	10.96 ± (1.30) a	1.09 ± (0.10) a	-25.40 ± (0.14) c
	0.1-0.2	1.14 ± (0.07) a	0.04 ± (0.00) a	-25.94 ± (0.34) b	9.39 ± (0.54) a	0.99 ± (0.08) a	-24.81 ± (0.06) b
	0.2-0.4	1.62 ± (0.20) a	0.06 ± (0.01) a	-25.97 ± (0.29) b	13.00 ± (0.74) a	1.59 ± (0.13) a	-24.10 ± (0.29) b
	0.4-0.6	1.45 ± (0.60) a	0.05 ± (0.02) a	-25.01 ± (0.44) b	8.83 ± (0.26) a	1.31 ± (0.09) a	-23.42 ± (0.22) b
	0.6-1.0	1.56 ± (0.32) a	0.06 ± (0.01) a	-24.09 ± (0.66) b	14.22 ± (0.72) a	2.67 ± (0.03) a	-22.96 ± (0.22) b
	0.0-1.0	7.82 ± (0.61) a	0.29 ± (0.01) a	n.d.	56.39 ± (2.85) a	7.64 ± (0.40) a	n.d.
Pasture	0.0-0.1	2.05 ± (0.64) a	0.05 ± (0.01) a	-14.97 ± (0.56) a	10.61 ± (2.20) a	1.06 ± (0.21) a	-15.11 ± (0.23) a
	0.1-0.2	1.03 ± (0.13) a	0.03 ± (0.00) a	-16.73 ± (1.40) a	8.18 ± (0.71) ab	0.91 ± (0.06) a	-16.34 ± (0.25) a
	0.2-0.4	1.48 ± (0.56) a	0.04 ± (0.01) b	-18.65 ± (1.99) a	11.94 ± (0.99) ab	1.46 ± (0.07) a	-17.18 ± (0.37) a
	0.4-0.6	0.62 ± (0.07) a	0.02 ± (0.00) a	-18.86 ± (0.27) a	7.92 ± (0.79) a	1.38 ± (0.11) a	-17.56 ± (0.25) a
	0.6-1.0	1.02 ± (0.08) a	0.03 ± (0.00) a	-16.83 ± (0.32) a	12.11 ± (0.83) a	2.21 ± (0.06) ab	-17.68 ± (0.48) a
	0.0-1.0	6.19 ± (1.37) a	0.16 ± (0.02) b	n.d.	50.05 ± (2.79) a	7.01 ± (0.36) a	n.d.
Eucalypt	0.0-0.1	1.18 ± (0.17) a	0.05 ± (0.01) a	-24.17 ± (0.56) b	6.66 ± (0.53) a	0.81 ± (0.13) a	-16.90 ± (0.47) b
	0.1-0.2	0.86 ± (0.09) a	0.03 ± (0.01) a	-24.36 ± (0.46) b	6.30 ± (0.31) b	0.77 ± (0.13) a	-16.74 ± (0.35) a
	0.2-0.4	1.15 ± (0.14) a	0.04 ± (0.01) b	-24.99 ± (0.74) b	9.75 ± (0.51) b	1.39 ± (0.17) a	-16.86 ± (0.31) a
	0.4-0.6	0.82 ± (0.07) a	0.02 ± (0.00) a	-25.84 ± (0.83) b	8.05 ± (1.50) a	1.29 ± (0.21) a	-17.98 ±(1.01) a
	0.6-1.0	1.48 ± (0.15) a	0.04 ± (0.01) a	-25.50 ± (0.64) b	9.71 ± (0.28) b	1.98 ± (0.27) b	-17.39 ± (0.35) a
	0.0-1.0	5.49 ± (0.39) a	0.17 ± (0.03) b	n.d.	40.47 ± (2.45) b	6.24 ± (0.85) a	n.d.

Significant differences among land-uses within each soil layer are indicated by different letters (Tukey's test, *α=0.05*). n.d.: Not determined. Values between parenthesis followed denote the standard error of the mean (n=4).

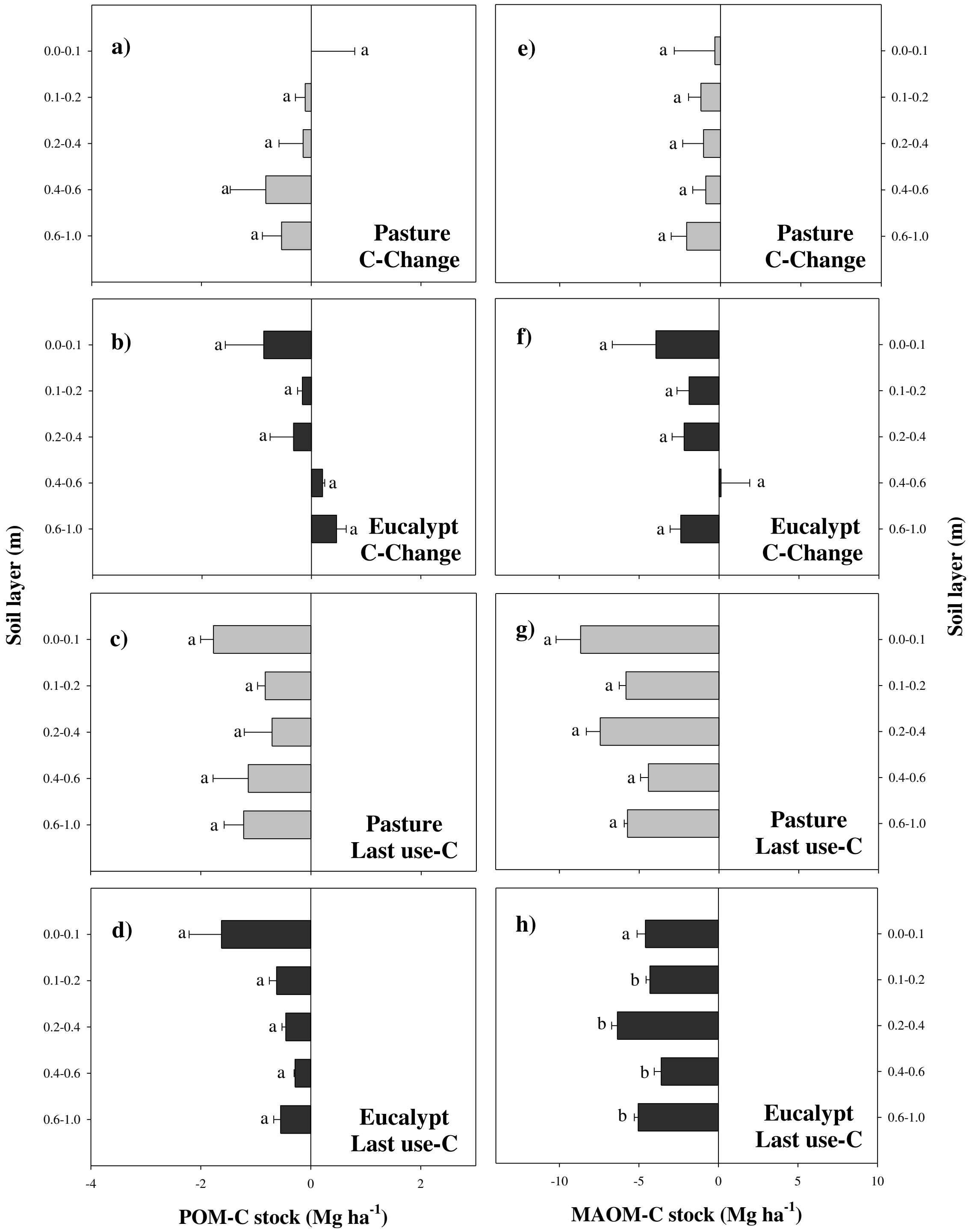

Figure 6. C-Change (Mg ha^{-1}) and Last use-C (Mg ha^{-1}) of the POM and MAOM in different soil layers (0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m) to Pasture (a, c, e and g) and Eucalypt (b, d, f and h). Different letters denote significant differences between pasture and eucalypt within each soil layer by the Tukey's test (*α=0.05*). Vertical bars denote the standard error of the mean (n=4).

4. DISCUSSION

CO_2-C and CH_4-C emissions

The CO_2-C and CH_4-C emissions are early indicators of potential shifts in SOC storage. Many studies have reported the influence of temperature and moisture in CO_2-C and CH_4-C emissions (Xu et al., 2001; Wang et al., 2014). In our study, strong influence was found for seasons with higher rainfall (Figure 3) over the study period (Figures 4 and 5). In general, higher soil water availability by precipitation stimulated the microbial activity with consequent decomposition process. Differences along the land-uses were observed in the last samplings (Jan 2015 and Oct 2015), in which the eucalypt showed the highest CO_2-C emission (4.33 kg ha^{-1} h^{-1}; Figure 4a).

The highest eucalypt CO_2-C emission in Oct 2015 seems to be related with surface processes (like litterfall depositions and decomposition, root growth and respiration), since the CO_2-C in depth has not shown similar trends (Figures 5k-o). The litterfall deposition in eucalypt forest until the Oct 2015 accounted to 18.77 ± 0.53 Mg ha^{-1}, and the microbial decomposition and nutrient release of litter may have stimulated the surface root exploration (mainly fine roots) for nutrients acquisition. Together, these factors provide strong influence in soil surface CO_2-C flux, resulting of litterfall decomposition as well as SOM being primed in the possible co-metabolism effect (*"Priming effect"*; Kuzyakov, 2010). Furthermore, root respiration is one of the major contributors to total CO_2-C emissions (Hopkins et al., 2013), which may had great influence in eucalypt forest.

The dynamics of CO_2-C conc. in depth not necessary denote similar behavior with the soil surface CO_2-C flux, since that the soil surface layer have the highest influence on soil surface CO_2-C flux (Luther-Mosebach et al., 2016; Nan et al., 2016). However, the CO_2-C conc. in soil profile increases in the soil deep layer under all land-use (Figures 5a-o). Soil properties as soil porosity, soil tortuosity, soil bulk density, soil texture and moisture affect the soil gas diffusivity (Pingintha et al., 2010; Goutal et al., 2012). Consequently, slower vertical transport is observed from soil deep layers to soil surface.

No strong differences were detected in CO_2-C conc. in depth among the land-uses (Figures 5a-o). However, the soil sampling in Jan 2013 was characterized by the highest CO_2-C conc. in depth, and it coincided with the largest precipitation along the experimental period (Figure 3a). Probably, the water available in soil profile stimulated the microbial activity and CO_2-C production (Xu et al., 2001; Wang et al., 2014).

In most of gas sampling campaigns we observed soil surface CH_4-C influx (i.e. negative soil surface CH_4-C flux), except in Sep 2012, Jan 2013 and Oct 2015 at which the pasture showed soil surface CH_4-C efflux (i.e. positive soil surface CH_4-C flux). Chamberlain et al. (2017) studying subtropical pastures in south Florida for three wet-dry seasonal cycles observed strong CH_4 sources emitting up to 0.027 kg ha^{-1} h^{-1} of CH_4-C. However, water retention practices did not explain the majority of pasture CH_4 emissions. Furthermore, also in eucalypt plantation it was observed soil surface CH_4-C efflux in Oct 2015. Many studies mention different and unclear effect of vegetation types on soil surface CH_4-C flux. However, soil surface CH_4-C influx have been observed in many forest types studies (Saggar et al., 2008; Fest et al., 2009; Wang et al., 2013). The uptake of CH_4-C occurs by methanotrophic bacteria that oxidize CH_4 and use it as a source of C and energy. The CH_4 consumption by methanotrophic bacteria is intensified in well-aerated soils conditions (Boeckx et al., 1997) like in our study site.

In Oct 2015 high soil surface CH_4-C fluxes were observed along soil surface CO_2-C flux (Figures 4a-b). The highest soil surface CO_2-C flux drives O_2 consumption and may lead to formation of anaerobic microsites, which will result in CH_4 production (Verchot et al., 2000; Wang et al., 2013). So, these closely related processes may explain greatest emission of soil surface CO_2-C and CH_4-C flux in eucalypt plantation in Oct 2015.

Such results indicate that planted eucalypt forests may act as strong sinks for atmospheric C-CH_4 and help mitigate the negative effects of such a potent greenhouse gas.

Organic matter-C and -N dynamics after land-use change

The SOM fractions following land-use change are affected mainly by soil disturbance (i.e. machinery operations for site preparation) and changes in the amount and quality of C inputs (aboveground and belowground). Species characteristics regulate soil C stocks by C allocation in above and belowground (roots and rhizodepositions), and also its release through soil respiration (De Deyn et al., 2008). Meanwhile, the litterfall decomposition is largely controlled by litterfall quality (Hobbie et al., 2007), which in turn may have strong influence on soil C stocks.

We found that after 34-years of conversion from Cerrado to pasture there were not POM-C and MAOM-C stocks changes (Table 2). Similar results were observed to POM-N and MAOM-N stocks. However a larger loss of Cerrado-derived MAOM-C was observed (Figure 6g), and it was counter-balanced by a greater contribution of pasture-derived C to this fraction (Figure 6e).

The MAOM fraction is considered as more stable organic fraction, since complex interactions between organic compounds and the mineral matrix restricts the microbial accessibility and leads to slower decomposition (Mazzilli et al., 2015). The root system of a pasture under moderate grazing has high belowground biomass production and turnover (Chen et al., 2015). Also, the grazing can stimulate the fine and shallow roots (Derner et al., 2006). The roots growth and activity of the pasture could be favor rhizodepositions, which could then lead to SOM mineralization (Rhizosphere Priming Effect - RPE) (Cheng and Kuzyakov, 2005), whereas that the physical proximity of roots to soil minerals could be preferentially stabilizing the root-derived C in comparison with shoot-derived C (Rasse et al., 2005). Furthermore, Eclesia et al. (2012) studying conversion of native forest to pasture in South America highlighted the importance of pasture age to the soil C storage, which supports our results of great pasture-derived POM-C and MAOM-C contribution after 34-years of Cerrado to pasture conversion.

Conversely, after 4-years of conversion from pasture to eucalypt plantation there were significant MAOM-C stocks losses, mainly in soil subsurface (0.1-0.2 to 0.6-1.0 m; Table 2). While POM-N and MAOM-N stocks showed no change after 4-years of eucalypt planting (Table 2). Despite the higher sensitivity of POM to tillage practices due to be within macro-aggregates or inter-aggregate pores (Kabiri et al., 2015; Liao et al., 2006), no differences in POM-C stocks after 4-years of eucalypt planting can its location related to the increase of eucalypt C input. Consistent with our results, Mao et al. (2010) studying afforestation of agricultural lands with poplar in semi-arid region, also detected no changes in POM-C and POM-N after 10-years of afforestation. In our site study, the lack of differences in POM-C and POM-N may have occurred by the increase in eucalypt litterfall (18.77 ± 0.53 Mg ha^{-1}) and root system inputs, which offset the C losses by initial soil disturbance (Figures 6b and d) (Chang et al., 2011; Gartzia-Bengoetxea et al., 2009; Mao et al., 2010). The coupling trends of POM-C and POM-N is related by potentially mineralizable-N with the POM-C contents (Bu et al., 2015), demonstrating that POM plays a vital role in soil N mineralization under land-use change.

In other hand, MAOM-C in soil profile (0.0-1.0 m) losses were detectable after 4-year of eucalypt planting (Table 2). Possibly, the root growth and rhizodepositions were not enough to compensate for C losses in deep soil layers. The initial soil preparation can disturb soil structure, modify microclimate and enhance aeration (La Scala et al., 2005). Also, disruption of soil aggregates may provide labile organic compounds to microbial demand (La Scala et al., 2008). This fact may lead to greater CO_2-C and thus reduction soil C stocks in the early years after the soil disturbance (Wang et al., 2016). These negative impacts were mitigated by the litter C inputs in the more superficial soil layers.

Li et al. (2012) in meta-analysis approach with afforestation under cropland and pasture reported significant C and N stock increases 30 and 50-years after afforestation. Additionally, Eclesia et al. (2012) found that MAOM-C recovery demands longer-term to recover comparatively to the POM-C when transitioning between tree- and grass-dominated (grazed) ecosystems in South America. So, in our study the MAOM-C recovery in soil subsurface was not achieved until the current stand age (4-years-old).

We hypothesize the eucalypt forest as a potential land-use to recovery the SOC storage in former pasture fields. However, the “land-use time” is an important driver involving the SOC storage in eucalypt forest (Li et al., 2012), besides of a combination of past land-use history, environmental factors (soils and climate), plantation productivity and management practices (Cook et al., 2016). Although, after 4-years of eucalypt planting there was not a recovery in more stable soil organic matter fraction (MAOM), studies have been shown that in the long-term forest soils that are far from being C-saturated and will indeed sequester C (Garcia-franco et al., 2014).

5. CONCLUSIONS

Our study demonstrates the strong influence of the wet season in soil surface CO_2-C and CH_4-C flux, and also in CO_2-C concentration in depth for soil under distinct land uses. The eucalypt soil (4-years-old) emitted ~70% more CO_2-C than those under Cerrado and pasture (34-years-old). While the pasture showed as source of CH_4-C to atmosphere.

The MAOM-C losses in soil deep layers due to the initial pasture-eucalypt land-use transition (i.e. initial soil disturbance, changes in amount and quality of C input) was not compensated by new eucalypt C input (Rhizodepositions). Despite this, C and N associated with more sensitive organic matter fraction (POM) did recover to soil layer until 1 m deep, indicating possible recovery MAOM-C in older age of eucalypt plantation.

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7. REFERENCES

Alberti, G.; Vicca, S.; Inglima, I.; Belelli-Marchesini, L.; Genesio, L.; Miglietta, F.; Marjanovic, H.; Martinez, C.; Matteucci, G.; D'Andrea, E.; Peressotti, A.; Petrella, F.; Rodeghiero, M.; Cotrufo, M.F. 2015. Soil C:N stoichiometry controls carbon sink partitioning between above-ground tree biomass and soil organic matter in high fertility forests. iForest, 8:195-206.

Alvarez, V.H.; Novais, R.F.; Dias, L.E.; Oliveira, J.A. 2000. Determinação e uso do fósforo remanescente. Boletim Informativo da Sociedade Brasileira de Ciência do Solo. 25:27-32.

Arneth, A.; Sitch, S.; Pongratz, J.; Stocker, B.D.; Ciais, P.; Poulter, B.; Bayer, A.D.; Bondeau, A.; Calle, L.; Chini, L.P.; Gasser, T.; Fader, M.; Friedlingstein, P.; Kato, E.; Li, W.; Lindeskog, M.; Nabel, J.E.M.S.; Pugh, T.A.M.; Robertson, E.; Viovy, N.; Yue, C.; Zaehle, S. 2017. Historical carbon dioxide emissions caused by land-use changes are possibly larger than assumed. Nature Geoscience, vol. 10.

Beniston, J.W.; DuPont, S.T.; Glover, J.D.; Lal, R.; Dungait, J.A.J. 2014. Soil organic carbon dynamics 75 years after land-use change in perennial grassland and annual wheat agricultural systems. Biogeochemistry, 120:37-49.

Bustamante, M.M.C.; Corbeels, M.; Scopel, E.; Roscoe, R. 2006.Soil carbon and sequestration potential in the Cerrado Region of Brazil. In: Lal, R.; Cerri, C.C.; Bernoux, M.; Etchevers, J.; Cerri, C.E.P. Carbon sequestration in soils of Latin America. New York, Haworth, p.285-304.

Cambardella, C. A.; Elliott, E. T. 1992. Participate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal, 56:777-783.

Cerri, C.C.; Volkoff, F. 1991.Nature and behaviour of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus. Forest Ecology and Management, 38:247-257.

Chang, R.Y.; Fu, B.J.; Liu, G.H.; Liu, S.G. 2011.Soil carbon sequestration potential for “Grain for Green” Project in Loess Plateau, China. Environmental Management, 48:1158-1172.

Chamberlain, S.D.; Groffman, P.M.; Boughton, E.H.; Gomez-Casanovas, N.; DeLucia, E.H.; Bernacchi, C.J.; Sparks, J.P. 2017. The impact of water management practices on subtropical pasture methane emissions and ecosystem service payments. Ecological Applications, 27(4):1199-1209.

Cotrufo, M.F.; Alberti, G.; Inglima, I.; Marjanovi, H.; LeCain, D.; Zaldei, A.; Peressotti, A.; Miglietta, F. 2011. Decreased summer drought affects plant productivity and soil carbon dynamics in a Mediterranean woodland. Biogeosciences, 8:2729-2739.

De Deyn, G.B.; Cornelissen, J.H.C.; Bardgett, R.D. 2008. Plant functional traits and soilcarbon sequestration in contrasting biomes. Ecology Letters, 11:516-531.

Del Galdo, I.; Six, J.; Peressotti, A.; Cotrufo, M.F. 2003.Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes. Global Change Biology, 9(8):1204-1213.

Derner, J. D.; Boutton, T. W.; Briske, D. D. 2006.Grazing and ecosystem carbon storage in the North American Great Plains. Plant Soil, 280:77-90.

Empresa Brasileira de Pesquisa Agropecuária - EMBRAPA. 2013. Sistema brasileiro de classificação de solos. 3.ed. Brasília, p.353.

Epron, D.; Mouanda, C.; Mareschal, L.; Koutika, L. 2015. Impacts of organic residue management on the soil C dynamics in an tropical eucalypt plantation on a nutrient-poor sandy soil after three rotations. Soil Biology and Biochemistry, 85:183-189.

Ferreira, D.F. 2008. SISVAR: um programa para análises e ensino de estatística. Revista Symposium, 6:36-41.

Fest, B.J.; Livesley, S.J.; Drösler, M.; Gorsel, E.; Arndt, S.K. 2009. Soil-atmosphere greenhouse gas exchange in a cool, temperate Eucalyptus delegatensis forest in South-eastern Australia. Agricultural and Forest Meteorology . 149:393-406.

Gartzia-Bengoetxea, N.; González-Arias, A.; Merino, A.; de Arano, I.M. 2009.Soil organic matter in soil physical fractions in adjacente semi-natural and cultivated stands in temperate Atlantic forests. Soil Biology and Biochemistry, 41:1674-1683.

Grandy, A.S.; Neff, J.C.; Weintraub, M.N.; 2007. Carbon structure and enzyme activities in alpine and forest ecosystems. Soil Biology and Biochemistry, 39:2701-2711.

Hobbie, S.; Ogdahl, M.; Chorover, J.; Chadwick, O.; Oleksyn, J.; Zytkowiak, R.; Reich, P. 2007. Tree species effects on soil organic matter dynamics: the role of soil cation composition. Ecosystems, 10:999-1018.

Hopkins, F.; Gonzalez-Meler, M.A.; Flower, C.E.; Lynch, D.J.; Czimczik, C.; Tang, J.; Subke, J. 2013. Ecosystem-level controls on root-rhizosphere respiration. New Phytologist, 199:339-351.

Iglesias-Trabado, G.; Wilstermann, D. 2008. Eucalyptus Universalis. Global Cultivated Eucalypt Forests Map 2008 Version 1.0.1. http://www.gitforestry.com/downloads/GIT_Forestry_Global_Eucalyptus_Map_2009_Brochure_ENG.pdf(accessed 08.02.17).

Industria Brasileira de árvores (IBA). Anuário estatístico do IBA – ano base 2016. Brasília, Brasil.

IPCC. 2014. Climate Change 2014 Mitigation of Climate Change: Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Kabiri, V.; Raiesi, F.; Ghazavi, M.A. 2015. Six years of different tillage systems affected aggregate-associated SOM in a semi-arid loam soil from Central Iran. Soil Tillage Research, 154:114-125.

Kuzyakov, Y. 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology and Biochemistry, 42:1363-1371.

La Scala, N.; Lopes, A.; Panosso, A.R.; Câmara, F.T.; Pereira, G.T. 2005. Soil CO2 efflux following rotary tillage of a tropical soil. Soil Tillage Research, 84:222-225.

La Scala, N.; Lopes, A.; Spokas, K.; Bolonhezi, D.; Archer, D.W.; Reicosky, D.C. 2008. Short-term temporal changes of soil carbon losses after tillage described by a first-order decay model. Soil Tillage Research, 99:108-118.

Lal, R. 2008. Sequestration of atmospheric CO2 into global carbon pool. Energy and Environmental Science, 1:86-100.

Lehmann, J.; Kleber, M. 2015. The contentious nature of soil organic matter. Nature, 528: Dec.

Li, D.; Niu, S.; Luo, Y, 2012. Global patterns of the dynamics of soil carbon and nitrogen stocks following afforestation: a meta-analysis. New Phytologist, 195:172-181.

Luther-Mosebach, J.; Kalinski, K.; Gröngröft, A.; Eschenbach, A. 2016. CO_2 fluxes in subtropical dryland soils - a comparison of the gradient and the closed chamber method. Journal of Plant Nutrition and Soil Science, 000:1-10.

Mazzilli, S.R.; Kemanian, A.R.; Ernst, O.R.; Jackson, R.B.; Piñeiro, G. 2015. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. Soil Biology and Biochemistry, 85:22-30.

Nan, W.; Yue, S.; Li, S.; Huang, H.; Shen, Y. 2016. The factors related to carbon dioxide effluxes and production in the soil profiles of rain-fed maize fields. Agriculture, Ecosystems and Environment, 216:177-187.

Pegoraro, R.F.; Silva, I.R.; Novais, R.F.; Barros, N.F.; Fonseca, S.; Dambroz, C.S. 2011. Estoques de carbono e nitrogênio nas frações da matéria orgânica em argissolo sob eucalipto e pastagem. Ciência Florestal, 21:261-273.

Pikul, J.; Shannon, O.; Michael, E.; Walter, R. 2007. Particulate organic matter and water-stable aggregation of soil under contrasting management. Soil Science Society of America Journal, 71:766-776.

Pingintha, N.; Leclerc, M.Y.; Beasley JR, J.P.; Zhang, G.; Senthong, C. 2010.Assessment of the soil CO_2 gradient method for soil CO_2 efflux measurements: comparison of six models in the calculation of the relative gas diffusion coeficiente. Tellus, 62B:47-58.

Plate, A.F.; Conast, R.T.; Stewart, C.E.; Paustian, K.; Six, J. 2006.Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. Soil Science Society of America, 70:287-296.

Rasse, D.; Rumpel, C.; Dignac, M.-F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil, 269:341-356.

Ruiz, H. A. 2005. Incremento da exatidão da análise granulométrica do solo por meio da coleta da suspensão (silte+argila). Revista Brasileira de Ciência do Solo, 29: 297-300.

Six, J.; Conant, R.T.; Paul, E.A.; Paustian, K. 2002. Stabilization mechanisms of organic matter: Implications for C-saturation of soils. Plant and Soil, 241:155-176.

Smith, K.A.; Conen, F. 2004. Measurement of Trace Gases, I: Gas analysis, Chamber Methods, and Related Procedures. In: Soil and Environmental Analysis: Modern Instrumental Techniques. 3 ed. New York, p.576.

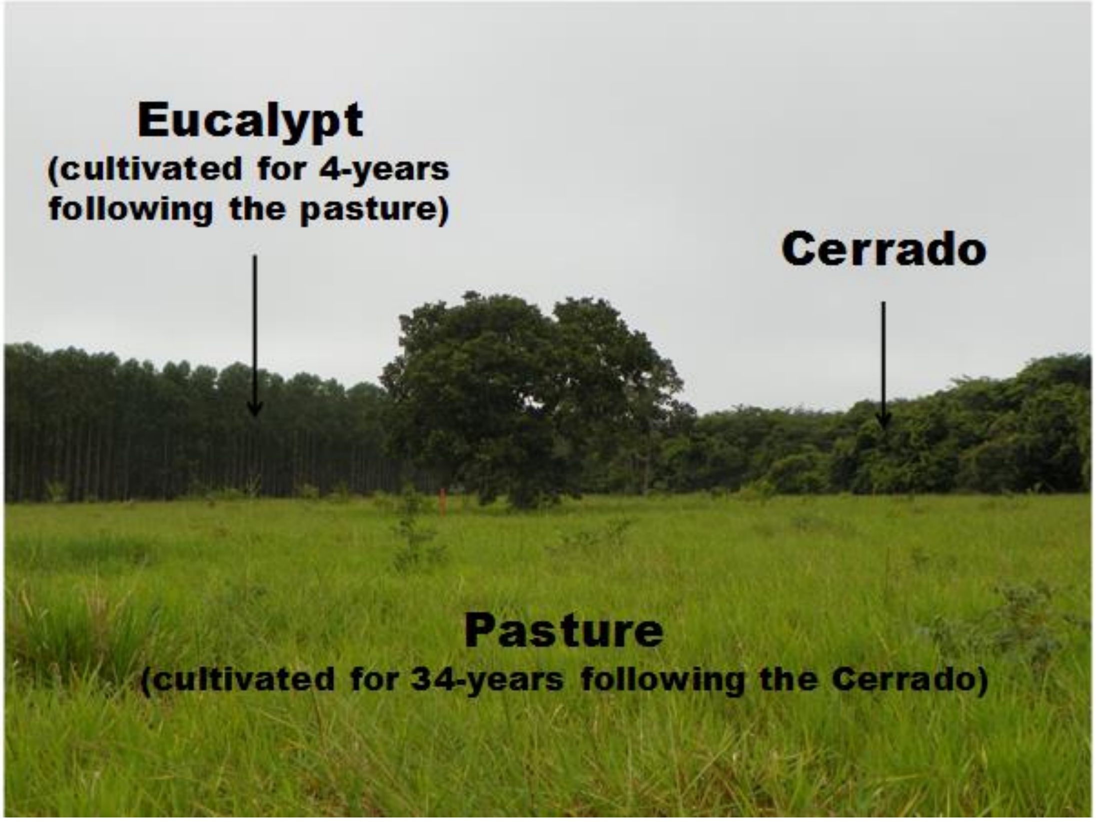
Soil Survey Staff. 2014. Keys to soil taxonomy. USDA-Natural Resources Conservation Service: Washington.

Vergütz, L.; Novais, R.F.; Silva, I.R.; Barros, N.F.; Nunes, T.N.; Piau, A.A.M. 2010. Mudanças na matéria orgânica do solo causadas pelo tempo de adoção de um sistema agrossilvopastoril com eucalipto. Revista Brasileira de Ciência do Solo, 34:43-57.

Wang, X.; Liu, L.; Piao, S.; Janssens, I.A.; Tang, J.; Liu, W.; Chi, Y.; Wang, J.; Xu, S. 2014. Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. Global Change Biology, 20:3229-3237.

Xu, M.; Qi, Y. 2001.Soil-surface CO2 efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California. Global Change Biology, 7(6):667-677.

8. SUPPLEMENTARY MATERIAL



Supplementary material 1. Illustrative picture of the current land uses in the experimental site in Três Lagoas, Mato Grosso do Sul state, Brazil.

Supplementary material 2. Static PVC chambers (0.30-m height x 0.4-m diameter) used to measure soil surface CO_2-C and CH_4-C fluxes in the Cerrado (c), pasture (d) and eucalypt (a) and PVC (1.0-m height x 0.025-m diameter) access tube with subdivisions used to collect soil air in the layers 0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-1.0 m (b and e).

Supplementary material 3. Soil surface temperature (^{o}C) and soil surface moisture (m^3m^{-3}) for different land-uses (Cerrado, Pasture and Eucalypt) and sampling campaigns

Land-use	.... *Soil surface temperature (^{o}C).........................					
	Sep 2012	Jan 2013	Sep 2013	Jan 2014	Jan 2015	Oct 2016
Cerrado	24,73 (±0.18)	26,07 (±0.81)	22,17 (±0.71)	30,83 (±0.33)	26,67 (±0.25)	25,33 (±0.48)
Pasture	26,57 (±2.07)	31,67 (±1.03)	23,13 (±0.79)	29,20 (±0.83)	25,33 (±0.25)	27,33 (±0.58)
Eucalypt	33,09 (±0.73)	26,50 (±0.34)	23,39 (±1.11)	28,95 (±0.95)	25,86 (±0.50)	25,31 (±0.41)
	.... *Soil surface moisture ($m^3\ m^{-3}$).........................					
	Sep 2012	Jan 2013	Sep 2013	Jan 2014	Jan 2015	Oct 2016
Cerrado	2,58 (±0.14)	10,78 (±1.41)	5,63 (±0.15)	5,70 (±0.32)	2,35 (±0.00)	6,68 (±0.96)
Pasture	1,64 (±0.15)	10,17 (±0.04)	7,94 (±0.20)	7,94 (±0.65)	5,97 (±0.03)	9,00 (±0.75)
Eucalypt	1,27 (±0.12)	12,12 (±0.42)	5,92 (±0.09)	4,67 (±0.22)	1,34 (±0.08)	8,08 (±0.36)

*Measured on the top soil (0.00-0.05 m soil layer). Values between parenthesis followed denote the standard error of the mean (n=4).

III.CHAPTER 2

Above and belowground eucalypt litter inputs alter surface and deep plant- and soil-derived CO_2-C levels in a tropical soil

ABSTRACT

The early discussion about crescent atmospheric concentrations CO_2, have directed the attention to potential sink of CO_2-C played by the soils. Forest soils are important components of the terrestrial ecosystem that store C. So, understanding soil C dynamics and CO_2-C flux in forest soils is of utmost importance since it plays major influence on the global C cycle and regulation atmospheric CO_2 concentration. In this study, we investigated the dynamics of soil surface CO_2-C flux and CO_2-C in depth of young eucalyptus plantation (hybrid *E. grandis x E. urophylla*). Due historical use was possible partition the soil surface CO_2-C flux and the CO_2-C concentration in depth in CO_2-C plant-derived (root respiration + rhizodeposition decomposition) and CO_2-C soil-derived (soil organic matter decomposition). In addition, the root priming effect was calculated. The evaluations were carried out in six seasons: 3, 7, 15, 19, 31 and 40-month-old eucalypt. After the implantation of eucalypt forests there was an increase in soil surface CO_2-C flux along plant growth (4.33 kg ha^{-1} h^{-1} in 40 month-old eucalypt). The root growth contributes greatly to the soil surface CO_2-C flux (correlated at $p<0.01$; $r:0.61$) promoting the surface RPE over time (correlated at $p<0.01$; $r:0.63$). The moisture has greater influence in the decomposition of litterfall (correlated at $p<0.01$; $r:0.70$) and root respiration and/or rhizodeposition decomposition (correlated at $p<0.01$; $r:0.79$).

Key-words: CO_2-δ^{13}C, CO_2-C plant-derived, Root priming effect, Fine root-C

1. INTRODUCTION

The early discussion about crescent atmospheric CO_2 concentrations has directing the attention to potential sink of CO_2-C played by the soils (IPCC, 2014). Globally, soils contain more than two thirds of the total carbon found in terrestrial ecosystems (Amundson, 2001). The understanding of soil C dynamics and CO_2-C flux is of utmost importance since it plays major influence on the global C cycle and in the regulation atmospheric CO_2 concentration and climate change.

Forest soils are important components of the terrestrial ecosystem that store C. Brazilian territory has around 0.84 % of its area covered by planted forests, where Eucalypt forest plantation covering approximately 5.6 million hectares (IBA, 2016). During their growth, eucalypt tree deposits shoot litter-C (Litterfall: leaves, branches, flowers, seeds, barks and fruits) aboveground and root litter-C (Rhizodepositions: lysates, mucilage, exudates, secretions and rhizodebris) belowground.

However, the rate at which C accumulates in soil is a balance between the C inputs and losses due soil respiration (CO_2-C efflux). Soil respiration comprises three main components: (*i*) Autotrophic respiration from roots (Ar); (*ii*) Heterotrophic respiration due the breakdown of soil organic matter – SOM (Hr_{SOM}) and *(iii)* Heterotrophic respiration due the breakdown new C input from rhizodepositions and litterfall (Hr_{NewC}). However, in field conditions the most of Hr_{NewC} is undetectable thus, it is quantified with Ar. When able, the partition soil respiration is important for understanding of the factors that drive the soil organic matter turnover (Millard et al., 2007).

Further Hr_{NewC}, the rhizospheric processes can stimulate or inhibit native SOM decomposition, releasing or retarding CO_2-C flux (Cheng and Kuzyakov, 2005; Cheng et al., 2014). This process is so-called rhizosphere priming effect (RPE), but the actual mechanisms underlying RPE still remain unclear (Cheng and Kuzyakov, 2005). One mechanism proposed to explain the positive RPE (increased native SOM decomposition) relates to root-released exudates, stimulating microbial growth, leading to extracellular enzyme production and enhanced decomposition of native SOM (Zhu and Cheng, 2011; Phillips et al., 2011). This fact mentioned occurs due to the increase in the plant nutrients demand (mainly N), which stimulates gross N mineralization by native SOM decomposition from microorganisms (Koranda et al., 2011; Phillips et al., 2011).

The roots fulfill an important role of C cycling and CO_2-C flux, mainly fine roots (Ø< 2.0 mm) that play a key role in nutrient cycle and soil C sequestration (Chang et al., 2012; Upson and Burgess, 2013). The relevance of fine roots occurs due it has faster turnovers, as well as higher metabolic activity compared to others order-roots (McCormack et al., 2013). Besides, studies showed positive correlations between spatial heterogeneity of soil organic carbon (SOC) and the vertical distribution of fine roots (Beniston et al., 2014). Also, another positive correlation was fine root-C with soil C across deep soil layer (Liao et al., 2014).

Due to many physical and chemical processes that inhibit SOC decomposition by microorganisms at depth, the deep soil carbon (DSC) is considered a "stable pool" (Kuzyakov, 2010; Schmidt et al., 2011; Bernal et al., 2016). The mechanisms behind the persistence of DSC still remain unclear (Schmidt et al., 2011). However, it is known that the persistence of DSC can change with root growth in the soil profile, as root-released substrates stimulate microbial growth (Ewing et al., 2006; Schmidt et al., 2011). These mentioned factors may influence the dynamics of CO_2-C in the deep soil layer. However, how the eucalypt root growth affects the DSC dynamic sand CO_2-C release following its establishment in and old pasture field remains unclear.

In this study, we investigated de dynamics of soil surface CO_2-C flux and CO_2-C in vertical soil profile in a young eucalypt plantation. Our specific objectives were (*i*) to estimate overall emissions and the components of soil surface CO_2-C flux over time after eucalypt planting, also (*ii*) detect the influence of eucalypt root growth (especially fine root) on soil surface CO_2-C flux and CO_2-C concentration in depth.

2. MATERIALS AND METHODS

Experimental setup

The study was developed in eucalypt plantations (hybrid *E. grandis x E. urophylla*) planted in June 2012 according to material and methods section of Chapter 1. The treatments were established before eucalypt planting according to the two eucalypt-derived C inputs that would be fed to the soil: *(i)* eucalypt with root litter and shoot litter (*+RL/+SL*) also *(ii)* eucalypt only with shoot litter (*-RL/+SL*). For the exclusion of root litter (root debris and rhizodepositions) in the *-RL/+SL* treatment, a PVC pipe (1.0-m height x 0.4-m diameter) was installed in the planting row (PR) and between planting row (BPR) of the eucalypt plantation. Thus, the two treatments were arranged completely randomized block design (36 x 24-m each block) with four replicates.

Soil sampling and analysis

Soil sampling was performed at *+RL/+SL* and *-RL/+SL* installed in PR and BPR of eucalypt plantation in three different eucalypt age: 0, 19 and 31-month-old (Jun 2012, Jan 2014 and Jan 2015). Soil sampling of the pasture area fragment was performed in the same seasons. All soil samples were collected in depth: 0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m. The soils were taken to the laboratory, passed in the 2-mm sieve and air dried.

Soil sub-samples from each sampling season were milled and analyzed for C and $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$ ‰ values) in an elemental CN analyzer coupled to an isotope mass spectrometer (IRMS, ANCA GSL 20-20, Sercon, Crewe, UK).

Soil surface CO_2-C flux and CO_2-C concentration in depth

The soil surface CO_2-C flux was monitored by static PVC chambers (0.30-m height x 0.4-m diameter), installed in the PR and BPR after the eucalypt planting. Mobile caps fitted with rubber septum on top, were used to concentrate the gaseous atmosphere. Gas samples were collected with plastic syringes (60-mL) equipped with a 3-way valves at 0, 10, 20 and 40-min. After closing the chambers at the moment of the sampling, the soil surface temperature and humidity were measured using a EC-5 sensor (Decagon Devices Inc., Pullman, WA).

For the gas sampling in depth it was installed in eucalypt plantation (PR and BPR) a PVC pipe (1.0 x 0.025-m) with compartments in the layers of 0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-1.0 m. Gas samples (instantaneous) was collected with plastic syringes (60-mL). Six gas sampling were performed: Sept 2012, Jan 2013, Sept 2013, Jan 2014, Jan 2015 and Oct 2015 (3, 7, 15, 19, 31 and 40-month-old eucalypt).

The syringes were taken to the lab and CO_2 and CH_4 were measured within two weeks after samplings. The CO_2-C concentrations (CO_2-C conc.; ppm) and CO_2-$\delta^{13}C$ (‰; in relation to the PDB standard) were determined using a ring-down resonant cavity spectrometer (CRDS, G2131-i, Picarro, Sunnyvale, CA).

The soil surface CO_2-C flux was calculated based on the variation of the CO_2-C conc. over time, according to Eq. 1 (Smith and Conen, 2004):

$$Soil\ surface\ CO_2\text{-}C\ flux = [(\Delta Q/\Delta t) \times M \times P \times V] / R \times T \times A \qquad Eq.\ 1$$

Where, *Soil surface CO_2-C flux* is CO_2-C flux (kg ha^{-1} h^{-1}); ($\Delta Q/\Delta t$) is the variation in CO_2-C conc. over time; *M* is the molar mass of C; *P* is the pressure inside the chamber, assumed to be 1 atmosphere (atm); *V* is the chamber volume (L); *T* is the temperature of the atmosphere (K); *R* is the universal gas constant (0.0821 L atm K^{-1} mol^{-1}).

The calculations to the *Soil surface CO_2-C flux* in eucalypt plantations (*+RL/+SL* and *-RL/+SL*) were carried out according to Eq. 2, due to the initial mechanical preparation in the area for eucalypt planting.

$$Soil\ surface\ CO_2\text{-}C\ flux_{+RL/+SL\ \mathrm{or}\ -RL/+SL} = (1/3 \times \mathrm{CO_2\text{-}C\ flux}_{PR}) + (2/3 \times \mathrm{CO_2\text{-}C\ flux}_{BPR})\ Eq.\ 2$$

Where, *Soil surface CO_2-C $flux_{+RL/+SL\ \mathrm{or}-RL/+SL}$* is the CO_2-C flux in *+RL/+SL* or-*RL/+SL* (kg ha^{-1} h^{-1}); the *1/3* represents an area proportion under influence of the initial mechanical preparation in PR, and *2/3* represents an area proportion of the BPR of eucalypt plantation.

The Keeling plot method (Keeling, 1958), was used to determine the CO_2-$\delta^{13}C$ of the soil surface CO_2-C fluxes. The CO_2-C conc. in depth for each soil layer (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6, 0.6-1.0 m) was expressed according to Eq. 3:

$$CO_2\text{-}C\ conc._{+RL/+SL\ \mathrm{or}\ -RL/+SL} = [(\mathrm{CO_2\text{-}C}\ conc._{PR}) + (\mathrm{CO_2\text{-}C}\ conc._{BPR})] / 2\ \ Eq.\ 3$$

Where, *CO_2-C $conc._{+RL/+SL\ \mathrm{or}\ -RL/+SL}$* is the CO_2-C conc.*+RL/+SL* or *-RL/+SL* (µmol mol^{-1}).

Soil incubation trial and partitioning of CO_2-C

Since our study site presents a use history of pasture (C_4 plant; $\delta^{13}C_{PDB}$: -13.00 ‰; Cerri and Volkoff, 1991), and eucalypt being a C_3 plant (average root litter and shoot litter, $\delta^{13}C_{PDB}$: -27.29 ‰), it was possible to partition the soil surface CO_2-C flux, and CO_2-C conc. in CO_2-C eucalypt- and soil-derived.

It was necessary to obtain soil-derived CO_2-δ^{13}C (‰) free roots ($CO_2\text{-}\delta^{13}C_{Soil\ free\ roots}$), in soil pasture reference, for the partitioning of soil CO_2-C flux and CO_2-C conc. in depth. Subsamples of soil (20-g) of each soil sampling season (0, 19 and 31-month-old eucalypt), in soil pasture reference were incubated in glass pots (500-mL) with septum in the cap. Soil moisture was adjusted to 80% of water holding capacity and the atmospheric temperature adjusted to ±25°C. Gas samples were collected with syringes (60-mL) at 0, 60, 120 and 180-min.

The CO_2-C conc. and CO_2-δ^{13}C were determined using a ring-down resonant cavity spectrometer (CRDS, G2131-i, Picarro, Sunnyvale, CA) and expressed in μmol mol^{-1} and ‰ (in relation to the PDB standard), respectively. The soil surface CO_2-C flux was calculated by variation of the CO_2-C conc. over time, according to Eq.1 and the CO_2-δ^{13}C was calculated according to Keeling plot method (Keeling, 1958) (Supplementary material 1).

The CO_2-C partitioning was performed according to Millard et al. (2010) following the Eq.4:

$$CO_2\text{-}C_{SD} = 1 - [(CO_2\text{-}\delta^{13}C_{Total} - CO_2\text{-}\delta^{13}C_{Soil\ free\ roots})/(\delta^{13}C_{Eucalypt} - CO_2\text{-}\delta^{13}C_{Sfree\ roots})$$

Eq. 4

Where, $CO_2\text{-}C_{SD}$ is the CO_2-C soil-derived flux or conc.; $CO_2\text{-}\delta^{13}C_{Total}$ is the CO_2-δ^{13}C from CO_2-C flux or conc. in *+RL/+SL* and *-RL/+SL*; $CO_2\text{-}\delta^{13}C_{Soil\ free\ roots}$ is the CO_2-δ^{13}C from CO_2-C flux of soil free roots; $\delta^{13}C_{Eucalypt}$ is the $\delta^{13}C_{PDB}$ of eucalypt plant (average root litter and shoot litter, $\delta^{13}C_{PDB}$: -27.29 ‰).

It was used the CO_2-$\delta^{13}C_{Soil\ free\ roots}$ of soil sampling season 0-month-old eucalypt to partition the gases sampling season 3, 7 and 15-month-old eucalypt. Soil sampling season 19-month-old eucalypt was used to partition the gases sampling season 19-month-old eucalypt. Also, sampling season 31-month-old eucalypt was used to partition the gases sampling season 31 and 40-month-old eucalypt. Only the CO_2-$\delta^{13}C_{Soil\ free\ roots}$ equivalent the 0.0-0.1 m soil layer were used to partitioning calculation in soil surface CO_2-C flux. While the CO_2-$\delta^{13}C_{Soil\ free\ roots}$ of each soil layer (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6 and 0.6-1.0 m) were used to partitioning calculation in CO_2-C conc. in depth.

Root priming effect (RPE)

We considered *Root priming effect*, the induction in SOM decomposition provided by the root system growth. However, due the methodology used to access this effect, indirect effect will be accounted join (not only those restricted to the rhizosphere soil), like as: increase in the diversity and amount of non-rhizosphere soil fauna and microorganism. The RPE on surface or in depth (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6 and 0.6-1.0 m) was calculated according to Eq. 5:

$$RPE = [(CO_2\text{-}Cflux/conc._{+RL/+SL} - CO_2\text{-}Cflux/conc._{-RL/+SL})/\ CO_2\text{-}C\ flux/conc._{-RL/+SL}] \times 100 \quad Eq.\ 5$$

Where, *RPE* is the Root priming effect (%); $CO_2\text{-}Cflux/conc._{+RL/+SL}$ is the soil surface CO_2-C flux or CO_2-C conc. in depth in *+RL/+SL*; $CO_2\text{-}Cflux/conc._{-RL/+SL}$ is the soil surface CO_2-C flux or CO_2-C conc. in depth in *-RL/+SL.*

Root and litterfall inputs

The measurements in eucalypt roots were performed during three seasons: 12, 24 and 36-month-old eucalypt. After the inventory of the plantations (Supplementary material 2), 12 medium-trees were selected. Of these, the roots were collected in ¼ of the area occupied by tree (including PR and BPR), in each soil layer: 0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6; 0.6-1.0; 1.0-1.2 and 1.2-1.4 m. Fine roots (Ø<2-mm), medium roots (2<Ø>10-mm) and coarse roots (Ø>10-mm) were separated, washed and weighed. Litterfall traps (27.5-m^2) were installed in each experimental block when the eucalypt was 12-month-old. The litterfall were collected and weighed monthly until 40-month-old eucalypt.

The subsamples of root component and litterfall were brought to the laboratory and dried at 60°C to constant weight. The total C and N of each plant component were determined in an elemental C-N analyzer coupled to an isotope mass spectrometer (IRMS, ANCA GSL 20-20, Sercon, Crewe, UK) (Supplementary material 3 and 4). The fine root-C stocks were expressed in accumulated values in

0.0-1.0 m soil layer. While, the litterfall-C stocks were expressed in accumulated values over five seasons: 0, 15, 19, 31 and 40-month-old eucalypt.

The parameter CO_2-C Plant/Fine root-C was used as metabolized root-derived C measure: *Ra* +Rh_{NewC} (kg ha^{-1} h^{-1} or µmol mol^{-1}) per unit of fine root-C stocks (Mg ha^{-1}); We also consider it like a proxy for root activity since it correlates with root respiration and rhizodeposition process. While CO_2-C Plant/Litterfall-C was used like a metabolized litterfall-derived C measure: Rh_{NewC} (kg ha^{-1} h^{-1}) per unit of litterfall-C stocks (Mg ha^{-1}).

Statistical analysis

Repeated-measures ANOVA analysis was used to assess the differences in soil surface CO_2-C flux (kg ha^{-1} h^{-1}), CO_2-C conc. in depth (µmol mol^{-1}) and RPE (%) in different eucalypt ages. Tukey's test was performed to separate means if differences were significant ($\alpha=0.05$). Statistical analyzes were performed using the SISVAR package.

The inputs of Fine root-C (0.0-1.0 m layer, Mg ha^{-1}) and Shoot litter deposition-C (Mg ha^{-1}) over time were, also assessed through regression analysis. The overall correlation analysis were assessed only in *+RL/+SL* treatment. Statistical regression and correlation analyzes were performed using the SIGMAPLOT 11.0 statistical software package (Systat Software Inc., Chicago, IL, USA).

3. RESULTS

Total organic carbon and nitrogen

It was not observed differences in TOC and TN stocks (*+RL/+SL* or *-RL/+SL*) after 31-month eucalypt planting in surface soil layer (0.0-0.1 m; Table 1) and soil profile (0.0-1.0 m; Table 1). Furthermore, no differences in TOC and TN stocks were detectable between *+RL/+SL* and *-RL/+SL* (Table 1).

Table 1. Total organic carbon (Mg ha^{-1}) and Total nitrogen (Mg ha^{-1}) along different soil layer (0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m) in eucalypt plantation with root litter and shoot litter (*+RL/+SL*) and without root litter and with shoot litter (*-RL/+SL*) along different eucalypt age (0, 19 and 31-month-old eucalypt)

	Total organic carbon (Mg ha^{-1})					
Depth	………………..... Month-old eucalypt					
(m)	.... 0		.... 19		.... 31	
	+RL/+SL	-RL/+SL	+RL/+SL	-RL/+SL	+RL/+SL	-RL/+SL
0 - 0.1	8.95 (± 0.06) Aa	8.95 (± 0.06) Aa	9.03 (± 0.40) Aa	8.48 (± 0.46) Aa	7.80 (± 0.72) Aa	7.28 (± 0.38) Aa
0.1 - 0.2	7.49 (± 0.30) Aa	7.49 (± 0.30) ABa	8.63 (± 0.54) Aa	8.37 (± 0.88) Aa	6.92 (± 0.30) Ba	6.19 (± 0.20) Ba
0.2 - 0.4	11.83 (± 0.61) Aa	11.83 (± 0.61) Aa	12.81 (± 0.70) Aa	13.15 (± 1.24) Aa	10.03 (± 0.35) Aa	10.44 (± 0.41) Aa
0.4 - 0.6	8.63 (± 0.62) Aa	8.63 (± 0.62) Aa	10.18 (± 0.71) Aa	10.66 (± 0.78) Aa	7.15 (± 0.12) Aa	7.47 (± 0.81) Aa
0.6 - 1.0	11.48 (± 0.37) Ba	11.48 (± 0.37) Aa	19.11 (± 0.83) Aa	13.07 (± 0.31) Ab	11.13 (± 0.88) Ba	12.95 (± 1.10) Aa
0.0 - 1.0	48.38 (± 1.67) Ba	48.38 (± 1.67) ABa	59.77 (± 2.56) Aa	53.73 (± 1.49) Ab	43.02 (± 0.99) Ba	44.33 (± 1.93) Ba

	Total nitrogen (Mg ha^{-1})					
Depth	………………..... Month-old eucalypt					
(m)	.... 0		.... 19		.... 31	
	+RL/+SL	-RL/+SL	+RL/+SL	-RL/+SL	+RL/+SL	-RL/+SL
0 - 0.1	0.75 (± 0.01) Aa	0.75 (± 0.01) Aa	0.74 (± 0.04) Aa	0.70 (± 0.03) Aa	0.63 (± 0.11) Aa	0.61 (± 0.04) Aa
0.1 - 0.2	0.75 (± 0.10) Aa	0.75 (± 0.10) Aa	0.71 (± 0.04) Aa	0.69 (± 0.09) Aa	0.59 (± 0.05) Aa	0.52 (± 0.03) Aa
0.2 - 0.4	1.06 (± 0.05) Aa	1.06 (± 0.05) Aa	1.03 (± 0.06) Aa	1.03 (± 0.11) Aa	0.85 (± 0.06) Aa	0.87 (± 0.05) Ba
0.4 - 0.6	0.78 (± 0.07) Aa	0.78 (± 0.07) Aa	0.83 (± 0.05) Aa	0.77 (± 0.03) Aa	0.63 (± 0.04) Aa	0.63 (± 0.06) Aa
0.6 - 1.0	0.84 (± 0.12) Ba	0.84 (± 0.12) Aa	1.43 (± 0.12) Aa	1.15 (± 0.01) Aa	1.00 (± 0.05) ABa	1.15 (± 0.08) Aa
0.0 - 1.0	4.18 (± 0.13) Aa	4.18 (± 0.13) Aa	4.74 (± 0.28) Aa	4.34 (± 0.20) Aa	3.70 (± 0.25) Aa	3.77 (± 0.12) Aa

Significant differences between eucalypt age are indicated by different capital letters (Tukey's test; $\alpha=0.05$), while significant differences between +RL/+SL and -RL/+SL are indicated by different lowercase letter (Tukey's test; $\alpha=0.05$). Values between parenthesis followed denote the standard error of the mean (n=4).

Soil surface CO_2-C flux

The *+RL/+SL* presented the largest soil surface CO_2-C flux in the 40-month-old eucalypt (4.33 kg ha^{-1} h^{-1}, Figure 1a), with the CO_2-C soil-derived flux contributing the greater part (2.66 kg ha^{-1} h^{-1}; Figure 1b). However, for the *-RL/+SL* the largest soil surface CO_2-C flux was observed at 7-month-old eucalypt (2.74 kg ha^{-1} h^{-1}; Figure 1a). Also, this age showed the largest contribution from CO_2-C plant-derived flux (2.43 kg ha^{-1} h^{-1}; Figure 1c).

In the 40-month-old eucalypt the *-RL/+SL* showed low emissions of CO_2-C soil-derived flux (0.27 kg ha^{-1} h^{-1}; Figure 1c), while the emission of CO_2-C plant-derived flux was 1.24 kg ha^{-1} h^{-1}.

Surface RPE was positive in all gas sampling seasons. The highest effect was observed at 40-month-old eucalypt (880%; Figure 1d).

The C input aboveground (Litterfall-C, Mg ha^{-1}) and C allocation in belowground by fine roots (Fine root-C, 0.0-1.0 m, Mg ha^{-1}) had sigmoid behavior over eucalypt ages ($R^2=0.973$ and $R^2=0.982$, respectively, Figures 1e-f). For the *+RL/+SL* and *-RL/+SL* there was at 7-month-old eucalypt a C input by litterfall-C of 0.22 Mg ha^{-1} reaching 8.64 Mg ha^{-1} at 40-month-old eucalypt (Figure 1f). While the fine root at 40-month-old eucalypt had 3.27 Mg ha^{-1}immobilized on fine roots (Figure 1e).

Statistical correlation analysis showed that, in eucalyptus growth there was an increase in total CO_2-C total flux ($r=0.61^{**}$), CO_2-C soil-derived flux ($r=0.49^{**}$), CO_2-C plant-derived flux ($r=0.67^{**}$), Fine root-C ($r=0.96^{**}$), Litterfall-C ($r=0.95^{**}$) and RPE ($r=0.63^{**}$). However, with eucalypt growth there was a decrease in CO_2-C plant/Fine root-C ($r=-\ 0.38^{o}$; Table 2).

Soil surface moisture influenced only the CO_2-C plant/Fine root-C ($r=0.79^{**}$) and CO_2-C plant/Litterfall-C ($r=0.70^{**}$; Table 2). The increase of the C inputs derived from fine roots and litterfall stimulated the emissions of CO_2-C soil-derived flux ($r=0.50^{*}$ and $r=0.59^{**}$, respectively). The Fine root-C and Litterfall-C inputs provided a positive RPE ($r=0.58^{**}$ and $r=0.65^{**}$, respectively; Table 2).

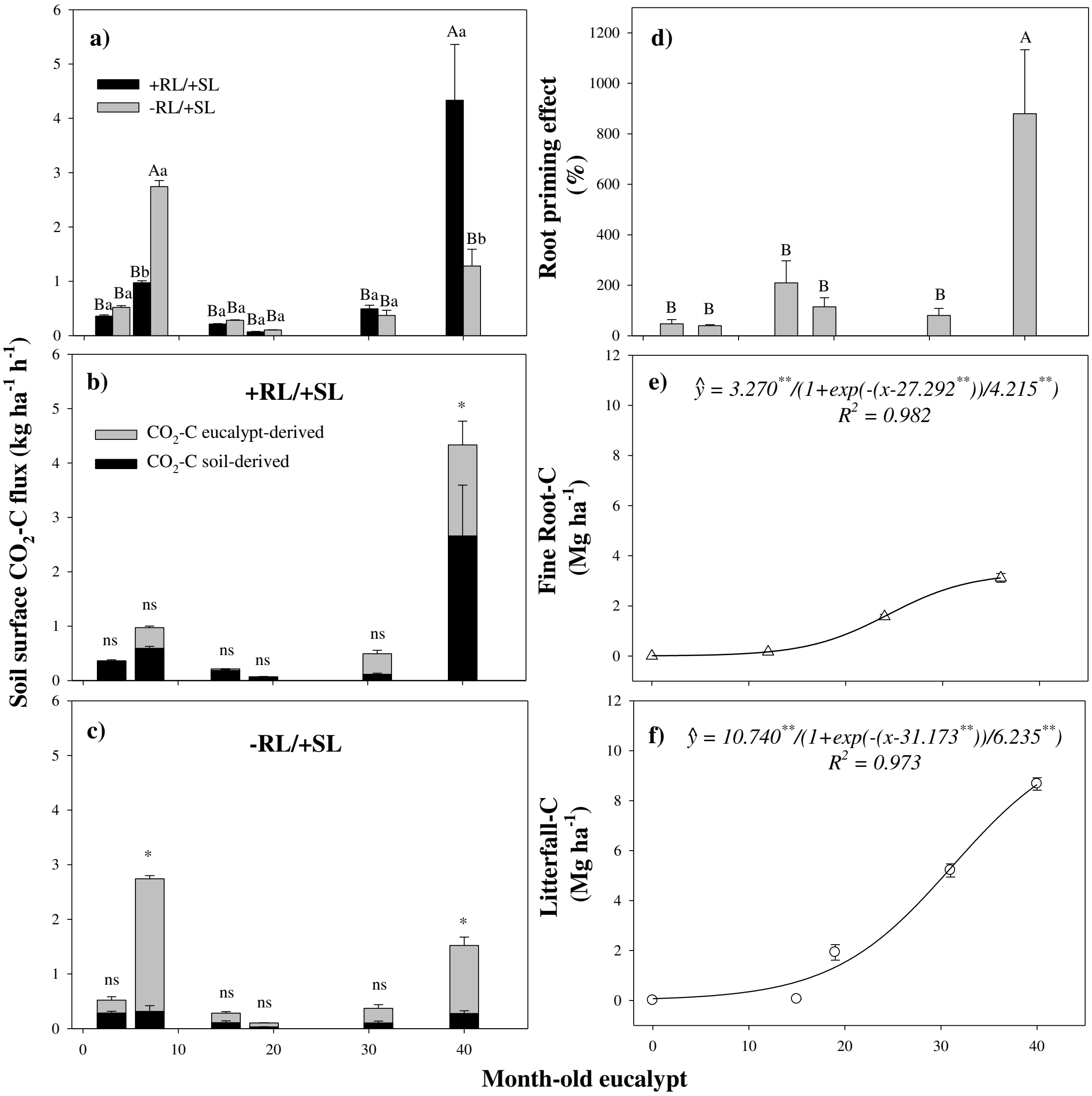


Figure 1. Total soil surface CO_2-C flux (kg ha^{-1} h^{-1}, a); CO_2-C eucalypt-derived flux (kg ha^{-1} h^{-1}, b-c) and CO_2-C soil-derived flux (kg ha^{-1} h^{-1}, b-c) in eucalypt plantation with root litter and shoot litter (*+RL/+SL*) and without root litter and with shoot litter (*-RL/+SL*) along different eucalypt ages. Root priming effect (%, d); Fine root-C (0.0-1.0 m, Mg ha^{-1}, e) and Litterfall-C (Mg ha^{-1}, f) over different eucalypt ages (3, 7, 15, 19, 31 and 40-month-old eucalypt). Different capital letters denote significant differences over eucalypt ages, while different lowercase letters denote significant differences between *+RL/+SL* and *-RL/+SL* to each eucalypt age by the Tukey's test ($\alpha=0.05$). $^{(*)}$ indicate significant differences between CO_2-C eucalypt- and soil-derived flux by the Tukey's test ($\alpha=0.05$). $^{(ns)}$ indicate no significant. Vertical bars denote the standard error of the mean (n=4).

Table 2. The coefficients of the relationships of Pearson (*r*) between Eucalypt age (months); Soil temperature (^{o}C, Temp); Soil humidity (m^3 m^{-3}, Hum); CO_2-C Soil-derived flux (kg ha^{-1} h^{-1}); CO_2-C Plant-derived flux (kg ha^{-1} h^{-1}); Fine root-C (0.0-1.0 m, Mg ha^{-1}); Litterfall-C (Mg ha^{-1}); CO_2-C Plant/Fine root-C; CO_2-C Plant/Litterfall-C; Root priming effect (RPE; %)

	Temp	Hum	CO_2-C Total flux	CO_2-C Soil derived flux	CO_2-C Plant derived flux	Fine Root-C	Litterfall-C	CO_2-C Plant/ Fine root-C	CO_2-C Plant/ Litterfall-C	RPE
Eucalypt age	-0.47*	-0.06^{ns}	0.61**	0.49**	0.67**	0.96**	0.95**	-0.38^{o}	-0.30^{ns}	0.63**
Temp		-0.39^{o}	-0.21^{ns}	-0.13^{ns}	-0.30^{ns}	-0.33^{ns}	-0.34^{ns}	-0.08^{ns}	-0.07^{ns}	-0.29^{ns}
Hum			0.33^{ns}	0.32^{ns}	0.28^{ns}	-0.15^{ns}	-0.04^{ns}	0.79**	0.70**	0.19^{ns}
CO_2-C Total flux				0.96**	0.87**	0.62**	0.71**	0.01^{ns}	0.05^{ns}	0.92**
CO_2-C Soil derived flux					0.69**	0.50*	0.59**	0.01^{ns}	0.03^{ns}	0.86**
CO_2-C Plant derived flux						0.69**	0.76**	0.03^{ns}	-0.08^{ns}	0.83**
Fine root-C							0.98**	-0.34^{o}	-0.27^{ns}	0.58**
Litterfall-C								-0.31^{ns}	-0.24^{ns}	0.65**
CO_2-C Plant/Fine root-C									0.90**	-0.20^{ns}
CO_2-C Plant/Litterfall-C										-0.14^{ns}

^{o}, *, ** indicate significant coefficients at $\alpha=0.1$; *0.05* and *0.01* by the t test, respectively. ^{ns} indicate no significant.

CO_2-C conc. in depth

The *+RL/+SL* and *-RL/+SL* presented the highest CO_2-C conc. at 7-month-old eucalypt, with CO_2-C conc. increases in depth (Figures 2a-j). There were higher contributions of CO_2-C soil-derived for all soil layers (0.0-0.1, 0.1-0.2, 0.2-0.4, 0.4-0.6 and 0.6-1.0 m; Figures 2a-j) over time.

No similar results were observed to RPE in soil layers along eucalypt growth. In the soil layers 0.0-0.1 and 0.1-0.2 m there was positive and higher RPE at 7-month-old eucalypt (128.7 and 142.0%, respectively; Figures 2k-1). While in the 0.2-0.4, 0.4-0.6 and 0.6-1.0 m soil layers the highest RPE in depth occurred at 40-month-old eucalypt (36.7, 73.1 and 69.9%, respectively; Figures 2m-o).

Negative statistical correlation were detected between the eucalypt age and CO_2-C total ($r=-0.47^{**}$), CO_2-C plant-derived conc. ($r=-0.30^{**}$), CO_2-C soil-derived conc. ($r=-0.48^{**}$), Fine root-C ($r=-0.83^{**}$) and CO_2-C Plant/Fine root-C ($r=-0.17^{o}$) (Table 3). While the depth statistical correlated with CO_2-C total ($r=0.35^{**}$), CO_2-C plant-derived conc. ($r=0.19^{*}$), CO_2-C soil-derived conc. ($r=0.36^{**}$), RPE ($r=-0.24^{**}$), Fine root-C ($r=0.19^{*}$) and CO_2-C Plant/Fine root-C ($r=0.26^{**}$) (Table 3).

The CO_2-C Total and CO_2-C soil-derived conc. correlated negatively with Fine Root-C ($r=-0.36^{**}$) and Fine Root-C:N ($r=-0.47^{**}$; Table 3). The RPE did not present correlations with CO_2-C Plant/Fine Root-C ($r=-0.06^{ns}$) and Fine Root-C:N ($r=0.09^{ns}$).

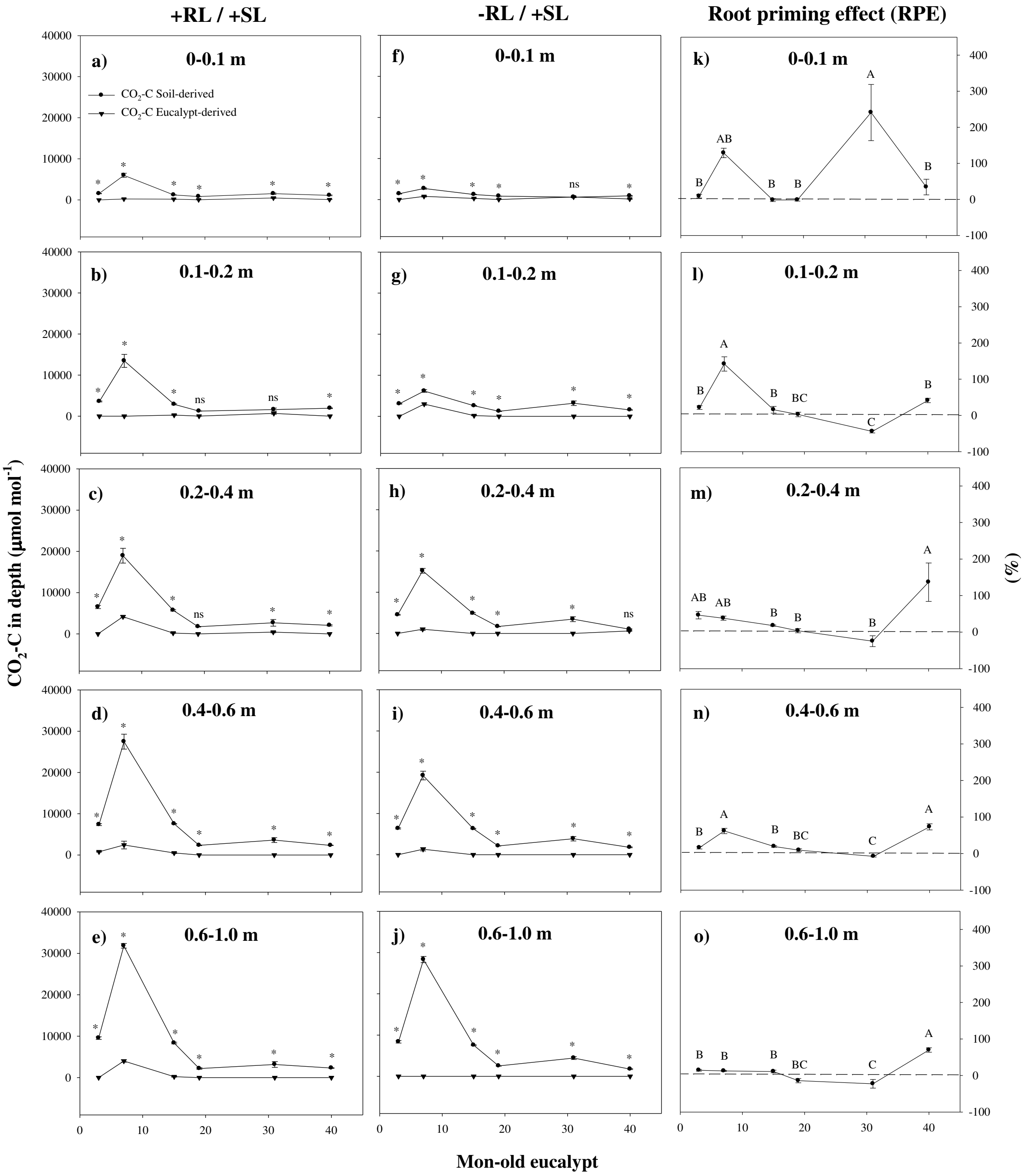


Figure 2. CO_2-C plant-derived and CO_2-Csoil-derived (µmol mol^{-1}) over different soil layers (0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m) also different eucalypt ages (3, 7, 15, 19, 31 and 40-month-old eucalypt). Eucalypt plantation with root litter and shoot litter (*+RL/+SL*, a-e); without root litter and with shoot litter (*-RL/+SL*, f-j) and Root priming effect (RPE; %, k-o). $^{(*)}$ indicate significant differences by the Tukey's test ($\alpha=0.05$) between CO_2-C eucalypt or soil-derived within each eucalypt age. $^{(ns)}$ Indicate no significant differences. Different letter denote statistical differences between eucalypt ages. Vertical bars denote the standard error of the mean (n=4).

Table 3. The coefficients of the relationships of Pearson (*r*) between Eucalypt ages (months); CO_2-C Total concentration (µmol mol^{-1}); CO_2-C plant-derived conc. (µmol mol^{-1}); CO_2-C soil-derived conc. (µmol mol^{-1}); Root priming effect (RPE, %); Fine root-C (Mg ha^{-1}); CO_2-C Plant/Fine root-C (µmol mol^{-1} Mg^{-1} ha^{-1}) and Fine root-C:N

	Depth	CO_2-C Total flux	CO_2-C Plant-derived flux	CO_2-C Soil-derived flux	RPE	Fine root-C	CO_2-C Plant/ Fine root-C	Fine root-C:N
Eucalypt age	0.00^{ns}	-0.47^{**}	-0.30^{**}	-0.48^{**}	0.09^{ns}	0.83^{**}	-0.17^{o}	1.00^{**}
Depth		0.35^{**}	0.19^{*}	0.36^{**}	-0.24^{**}	0.19^{*}	0.26^{**}	0.00^{ns}
CO_2-C Total flux			0.83^{**}	1.00^{**}	0.07^{ns}	-0.36^{**}	0.64^{**}	-0.47^{**}
CO_2-C Plant-derived flux				0.78^{**}	-0.01^{ns}	-0.25^{**}	0.59^{**}	-0.30^{**}
CO_2-C Soil-derived flux					0.08^{ns}	-0.36^{**}	0.63^{**}	-0.48^{**}
RPE						0.12^{ns}	-0.06^{ns}	0.09^{ns}
Fine root-C							-0.14^{ns}	0.83^{**}
CO_2-C Plant/Fine root-C								-0.17^{o}

^{o}, *, **: indicate significant coefficients at *α=0.1; 0.05* and 0.01 by the t test, respectively. $^{(ns)}$ indicate no significant.

4. DISCUSSION

Soil CO_2-C components dynamics

The soil surface CO_2-C flux in forest ecosystems represents the net process in soil profile with different contributions around soil layers, which depend of abiotic drivers (i.e. temperature, moisture and available nutrients) (Xu et al., 2001; Mo et al., 2008; Wang et al., 2014). They influence root growth and activity, litterfall deposition, as well as soil microbiological activity. Furthermore, soil diffusion properties as water content, soil porosity, soil bulk density and soil gas diffusivity can influence the CO_2-C flux in soil profile to the surface (Pinginthaet al., 2010).

After eucalypt planting, the soil surface CO_2-C flux showed increasing along eucalypt growth (*+RL/+SL*; Figure 1a and Table 2). The increases in soil surface CO_2-C flux soil occur by increasing in C input by root biomass (manly fine roots) in depth (Figure 1e; Table 2) and litterfall (Figure 1f; Table 2). The root exploration increased the root respiration and rhizodepositions decomposition (Figure 1b; Table 3) (Nouvellon et al., 2008; Paterson and Sim, 2013; Zhu et al., 2014). Furthermore, the growth of eucalypt trees promoted an increase in the deposition of litterfall aboveground (Litterfall-C; Figure 1f; Table 2), which through litterfall decomposition contributes to the CO_2-C plant-derived flux.

Litterfall decomposition-derived CO_2-C contributes between 5 and 45% to total of soil surface CO_2-C emissions in temperate forests (Borken and Beese, 2005; Vose and Bolstad, 2007), in tropical forest these values may be higher. Leitner et al., (2016) studying the removed litter in beech forest and its influence in soil surface CO_2-C flux, verified contribution to total CO_2-C flux ranged from 15.6 to 46.1% when the litter was present, which can be explained by the active decomposition of litter material, that is rich in readily available C and nutrients.

Soil moisture and temperature are important abiotic factors in the decomposition activity of soil microorganisms, with the consequent release of CO_2-C (Moyano et al., 2013, Zhu et al., 2013, Zhu et al. ., 2014). In our study, we observed no significant correlations ($p \leq 0.1$) of the soil surface temperature (0.0-0.1 m) with the soil surface CO_2-C flux, while the soil surface moisture correlated positively with CO_2-C plant/Fine root-C ($r=0.79^{**}$) and CO_2-C plant/Litterfall-C ($r=0.70^{**}$) (Table 2).

The microbial activity can be altered by shifts in water content that affect solute and oxygen diffusion, thereby changing substrate supply and decomposition rates (Davidson et al., 2006). Thus, with higher soil moisture, there is greater root activity for water absorption in the soil and consequently higher rhizodeposition rates (Davidson et al., 1995; Zhu et al., 2013). These factors together may have contributed to the correlation between moisture and CO_2-C plant/Fine root-C and CO_2-C plant/Litterfall-C. The lack of correlation between soil surface temperatures with soil surface CO_2-C flux may be correlated with the low air temperature variation in the region, while the precipitation (rain event) showed a greater variation in the experimental period (see Material and methods section of Chapter 1).

Despite studies in different conditions report the major influence of surface soil layer to soil surface CO_2-C flux (Davidson et al., 2006; Luther-Mosebach et al., 2016; Nan et al., 2016), the investigation of vertical distribution CO_2-C is important as a predictor of futures scenarios in soil surface CO_2-C flux. We observed increase in CO_2-C conc. in depth (Figures 2a-e; Table 3) along the eucalypt grow. In addition, the abiotic factors indirectly influencing the CO_2-C production. There is a vertical root growth exploring deeper soil layers, which affect the processes around of CO_2-C production in soil profile (Lloyd et al. 2016).

The litter manipulation (Root litter and Shoot litter) appears to be an influence of roots on the decomposition of litterfall (Figures 1b-c). In the treatment *-RL/+SL*, there was a peak of litterfall decomposition (CO_2-C plant-derived flux) at 7-month-old eucalypt (2.43 kg ha^{-1} h^{-1}; Figure 1c). However, the peak was not observed in *+RL/+SL*. At 7-month-old eucalypt, there was deposition of 0.21 Mg ha^{-1} litterfall-C (Figure 1f); together with a higher precipitation period and temperature. These abiotic factors may have favored this high litterfall decomposition (Figure 1c).

The higher precipitation period at 7-month-old eucalypt (see Material and methods section of Chapter 1) influenced the process around CO_2-C production in soil deep layers. However, because the early age of eucalypt root system (7-month-old eucalypt) probably the major contribution was from the soil microbial activity.

In addition to this fact, the first broadcast fertilization was carried out one month before (6-month-old eucalypt; see Material and Methods section of Chapter 1). Which it may also has led to a greater availability of nutrients and a higher microbial population stimulated by the availability of nutrients. However, when the roots are present, seems to be an inhibition in the litterfall decomposition. Another hypothesis would be that, the presence of roots allowed the absorption of a large part of the nutrients applied by the broadcast fertilization one month before, therefore, providing slight influence by nutrient availability on the soil microbial activity.

The higher precipitation period at 7-month-old eucalypt (see Material and Methods section of Chapter 1) influenced the process around CO_2-C production in soil deep layers. However, because the early age of eucalypt root system (7-month-old eucalypt), probably the major contribution was from the soil microbial activity.

Root priming effect and eucalypt root growth

In our study site, after 31-month of eucalypt planting no loses of TOC and TN were observed by root growth (Table 1). Thus, the experimental period (31-month-old eucalypt) was not possible to detect losses in total deep soil carbon provide by root-released substrates stimulating microbial growth (Ewing et al., 2006; Schmidt et al., 2011). However, the increase in root biomass provided a surface RPE of 880% in 40-month-old eucalypt (Figure 1d; Table 2). Recently, studies have reported RPE from -50% to +400% (Zhu and Cheng, 2011; Cheng et al., 2014). Although, the RPE observed in our study is much higher than those currently reported, the authors emphasize the unclear real mechanisms involving this effect.

Lloyd et al. (2016) observed similar increases in RPE over plant growth, and concluded that the mainly drivers of these processes were: *(i)*increases in rate of photosynthesis and rhizodeposition; *(ii)*increases in microbial and plant competition for N and others nutrients and *(iii)*Supply energy demand by rhizodeposition for the microbes to obtain nutrients from SOM mineralization.

The RPE in depth along eucalypt growth showed variations (Figures 2k-o; Table 3). The highest positive RPE at 7-month-old eucalypt on surface soil layers (0.0-0.1 and 0.1-0.2 m) suggest an intense root exploration in the first months of eucalypt age (Supplementary Material 3). However, at 40-month-old eucalypt, the RPE in depth (all soil layers), had positive values with little variation. This fact occurred due to a more homogeneity of root distribution (Supplementary Material 3).

We observed an increase in Fine root-C:N correlated with eucalypt growth, and decrease in the metabolized fine roots-C (Table 3). Fine roots have greater tissue N concentrations, and faster respiration rates than higher order roots (Burton et al., 2012, Jia et al., 2013). The increase of N fine root concentration promotes more actively growing meristems, more active synthesis, maintenance of storage and enzymatic proteins that stimulate respiration (Oren et al., 2001, Throop et al., 2004).

Thus, the root system growth, mainly fine roots of the eucalypt, influences the CO_2-C conc. dynamics in deep layers. Although, there are still no significant changes in DSC (Table 1). The soil CO_2-C components measures may be a useful predictor to future changes in DSC and highlight the processes that occur in soil.

5. CONCLUSIONS

Summarizing, after the implantation of eucalypt forests there are increases in soil surface CO_2-C flux with plant growth. The root growth and root activity contributes greatly to the soil surface CO_2-C and the CO_2-C in depth, promoting surface Root Priming Effect over time. The moisture has greater influence in the litterfall decomposition and root respiration and/or rhizodeposition decomposition of eucalypt forests.

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7. REFERENCES

Alvarez, V.H.; Novais, R.F.; Dias, L.E.; Oliveira, J.A. 2000. Determinação e uso do fósforo remanescente. Boletim Informativo da Sociedade Brasileira de Ciência do Solo. 25:27-32.

Amundson, R. 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences, 29:535-562.

Beniston, J.W.; DuPont, S.T.; Glover, J.D.; Lal, R.; Dungait, J.A.J. 2014. Soil organic carbon dynamics 75 years after land-use change in perennial grassland and annual wheat agricultural systems. Biogeochemistry, 120:37-49.

Bernal, B.; McKinley, D.C.; Hungate, B.A.; White, P.M.; Mozdzer, T.J.; Megonigal, J.P. 2016. Limits to soil carbon stability; Deep, ancient soil carbono decomposition stimulated by new labile organic inputs. Soil Biology and Biochemistry, 98:85-94.

Borken, W.; Beese, F. 2005. Soil respiration in pure and mixed stands of European beech and Norway spruce following removal of organic horizons. Canadian Journal of Forest Research, 35:2756-2764.

Burton, A.J.; Jarvey, J.C.; Jarvi, M.P.; Zak, D.R.; Pregitzer, K.S. 2012. Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. Global Change Biology, 18:258-266.

Cerri, C.C.; Volkoff, F. 1991.Nature and behaviour of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus. Forest Ecology and Management, 38:247-257.

Chang, R.; Fu, B.; Liu, G.; Yao, X.; Wang, S. 2012. Effects of soil physicochemical properties and stand age on fine root biomass and vertical distribution of plantation forests in the Loess Plateau of China. Ecological Research, 27:827-836.

Cheng, W.X.; Kuzyakov, Y. 2005. Root effects on soil organic matter decomposition. In: Zobel, R.W.; Wright, S.F. (Eds.), Roots and Soil Management: Interactions between Roots and the Soil, Agronomy Monograph No. 48. ASA-CSSA-SSSA, Madison, WI, pp. 119-143.

Cheng, W.X.; Parton, W.J.; Gonzalez-Meler, M.A.; Phillips, R.; Asao, S.; McNickle, G.G.; Brzostek, E.; Jastrow, J.D. 2014. Tansley review: synthesis and modeling perspectives of rhizosphere priming. New Phytologist, 201:31-44.

Davidson, E.A.; Savage, K.E.; Trumbore, S.E.; Borken, W. 2006.Vertical partitioning of CO_2 production within a temperate forest soil. Global Change Biology, 12(6):944- 956.

Davidson, E.A.; Trumbore, S.E. 1995.Gas diffusivity and production of CO_2 in deep soils of the eastern Amazon. Tellus, 47B:550-565.

Ewing, S.A.; Sanderman, J.; Baisden, W.T.; Wang, Y.; Amundson, R. 2006. Role of large-scale soil structure in organic carbon turnover: evidence from California grassland soils. J. Geophys. Research-Biogeosciences, 111(G3):G03012.

Industria Brasileira de árvores (IBA). Anuário estatístico do IBA – ano base 2016. Brasília, Brasil.

IPCC (2014). Climate Change 2014 Mitigation of Climate Change: Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Jia, S.; McLaughlin, N.B.; Gu, J.; Li, X.; Wang, Z. 2013. Relationships between root respiration rate and root morphology, chemistry and anatomy in *Larix gmelinii* and *Fraxinus mandshurica*. Tree Physiology, 33:579-589.

Keeling, C. D. 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. Geochimica et Cosmochimica Acta, 13:322-334.

Koranda, M.; Schnecker, J.; Kaiser, C.; Fuchslueger, L.; Kitzler, B.; Stange, C.L.; Sessitsch, A.; Zechmeister-Boltenstern, S.; Richter, A. 2011. Microbial processes and community composition in the rhizosphere of European beechdthe influence of plant C exudates. Soil Biology and Biochemistry, 43:551-558.

Kuzyakov, Y. 2010. Priming effects: interactions between living and dead organic matter. Soil Biology and Biochemistry, 42:1363-1371.

Leitner, S.; Sae-Tun, O.; Kranzinger, L.; Zechmeister-Boltenstern, S.; Zimmermann, M. 2016. Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest. Plant Soil, 403:455-469.

Lloyd, D.A.; Ritz, K.; Paterson, E.; Kirk, G.J.D. 2016.Effects of soil type and composition of rhizodeposits on rhizosphere priming phenomena.Soil Biology and Biochemistry, 103:512-521.

Luther-Mosebach, J.; Kalinski, K.; Gröngröft, A.; Eschenbach, A. 2016. CO_2 fluxes in subtropical dryland soils - a comparison of the gradiente and the closed chamber method. Journal of Plant Nutrition and Soil Science, 000:1-10.

McCormack, M.L.; Eissenstat, D.M.; Prasad, A.M.; Smithwick, E.A. 2013. Regional scale patterns of fine root lifespan and turnover under current and future climate. Global Change Biology, 19:1697-1708.

Millard, P.; Midwood, A.J.; Hunt, J.E.; Barbour, M.M.; Whitehead, D. 2010.Quantifying the contribution of soil organic matter turnover to forest soil respiration, using natural abundance $\delta^{13}C$. Soil Biology and Biochemistry, 42:935-943.

Millard, P.; Sommerkorn, M.; Grelet, G.-A. 2007. Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. New Phytologist, 175:11-28.

Mo, J.M.; Zhang, W.; Zhu, W.X.; Gundersen, P.; Fang, Y.T., et al.2008. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biology 14(2):403-412.

Moyano, F.E.; Manzoni, S.; Chenu, C. 2013. Response of soil heterotrophic respiration to moisture availability: an exploration of processes and model. Soil Biology and Biochemistry, 59:72-85.

Nan, W.; Yue, S.; Li, S.; Huang, H.; Shen, Y. 2016. The factors related to carbon dioxide effluxes and production in the soil profiles of rain-fed maize fields. Agriculture, Ecosystems and Environment, 216:177-187.

Nouvellon, Y.; Epron, D.; Kinana, A.; Hamel, O.; Mabiala, A.; D'Annunzio, R.; Deleporte, P.; Saint-Andre, L.; Marsden, C.; Roupsard, O.; Bouillet, J.; Laclau, J. 2008. Soil CO_2 effluxes, soil carbon balance, and early tree growth following avannah afforestation in Congo: Comparison of two site preparation treatments. Forest Ecology and Management, 255:1926-1936.

Oren, R.; Ellsworth, D.S.; Johnsen, K.H.; Phillips, N.; Ewers, B.E.; Maier, C.; Schãfer, K.V.R.; McCarthy, H.; Hendrey, G.; McNulty, S.G.; Katul, G.G. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature, 411:469-472.

Paterson, E.; Sim, A. 2013. Soil-specific response functions of organic matter mineralization to the availability of labile carbon. Global Change Biology, 19:1562-1571.

Phillips, R.P.; Finzi, A.C.; Bernhardt, E.S. 2011. Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under elevated long-term CO_2 fumigation. Ecology Letters, 14:187-194.

Pingintha, N.; Leclerc, M.Y.; Beasley JR, J.P.; Zhang, G.; Senthong, C. 2010.Assessment of the soil CO_2 gradient method for soil CO_2 efflux measurements: comparison of six models in the calculation of the relative gas diffusion coeficiente. Tellus, 62B:47-58.

Ruiz, H. A. 2005. Incremento da exatidão da análise granulométrica do solo por meio da coleta da suspensão (silte+argila). Revista Brasileira de Ciência do Solo, 29: 297-300.

Schmidt, M.W.; Torn, M.S.; Abiven, S.; Dittmar, T.; Guggenberger, G.; Janssens, I.A.; Kleber, M.; K€ogel-Knabner, I.; Lehmann, J.; Manning, D.A.C.; Nannipieri, P.; Rasse, D.P.; Weiner, S.; Trumbore, S.E. 2011. Persistence of soil organic matter as an ecosystem property. Nature, 478:49-56.

Soil Survey Staff. 2014. Keys to soil taxonomy. USDA-Natural Resources Conservation Service: Washington.

Throop, H.L.; Holland, E.A.; Parton, W.J.; Ojima, D.S.; Keough, C.A. 2004. Effects of nitrogen deposition and insect herbivory on patterns of ecosystem-level carbon and nitrogen dynamics: results from the CENTURY model. Global Change Biology, 10:1092-1105.

Upson, M.A.; Burgess, P.J. 2013. Soil organic carbon and root distribution in a temperate arable agroforestry system. Plant Soil, 373:43-58.

Vose, J.M; Bolstad, P.V. 2007. Biotic and abiotic factors regulating forest floor CO_2 flux across a range of forest age classes in the southern Appalachians. Pedobiologia, 50:577-587.

Wang, X.; Liu, L.; Piao, S.; Janssens, I.A.; Tang, J.; Liu, W.; Chi, Y.; Wang, J.; Xu, S. 2014. Soil respiration under climate warming: differential response of heterotrophic and autotrophic respiration. Global Change Biology, 20:3229-3237.

Xu, M.; Qi, Y. 2001. Soil-surface CO_2 efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California. Global Change Biology 7(6):667-677.

Zhu, B.; Cheng, W. 2013.Impacts of dryingewetting cycles on rhizosphere respiration and soil organic matter decomposition. Soil Biology and Biochemistry, 63:89-96.

Zhu, B.; Cheng, W.X. 2011. Rhizosphere priming effect increases the temperature sensitivity of soil organic matter decomposition. Global Change Biology, 17: 2172-2183.

Zhu, B.; Gutknecht, J.L.M.; Herman, D.J.; Keck, D.C.; Firestone, M.K.; Cheng, W. 2014. Rhizosphere priming effects on soil carbon and nitrogen Mineralization. Soil Biology and Biochemistry, 76:183-192.

8. SUPPLEMENTARY MATERIAL

Supplementary material 1. Soil surface CO_2-C flux (mg $ha^{-1}Mg^{-1}$) and CO_2-$\delta^{13}C$ (‰) of the pasture soil reference free roots in different soil layers (0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0-m) along different eucalypt age (0, 19 and 31-mon-old eucalypt)

	Eucalypt age		
	.... (month-old eucalypt)		
	0	19	31
Depth	CO_2-C flux		
...... (m)	............................(mg h^{-1} Mg^{-1})............................		
0 - 0.1	2.73 (± 0.33)	2.54 (± 0.45)	1.97 (± 0.11)
0.1 - 0.2	1.33 (± 0.13)	1.61 (± 0.09)	2.07 (± 0.62)
0.2 - 0.4	2.09 (± 0.16)	2.75 (± 0.18)	0.62 (± 0.13)
0.4 - 0.6	2.31 (± 0.46)	1.32 (± 0.04)	0.85 (± 0.11)
0.6 - 1.0	1.31 (± 0.10)	1.35 (± 0.09)	1.78 (± 0.44)
Depth	CO_2-$\delta^{13}C$		
...... (m)	.... (‰)		
0 - 0.1	-16.29 (± 0.12)	-16.68 (± 0.49)	-16.35 (± 0.13)
0.1 - 0.2	-18.13 (± 0.33)	-18.07 (± 0.82)	-18.72 (± 0.42)
0.2 - 0.4	-20.49 (± 1.47)	-20.28 (± 0.50)	-19.70 (± 0.55)
0.4 - 0.6	-19.36 (± 0.18)	-21.74 (± 0.74)	-21.93 (± 0.22)
0.6 - 1.0	-19.55 (± 0.17)	-22.18 (± 0.42)	-23.52 (± 0.31)

Values between parenthesis followed denote the standard error of the mean (n=4).

Supplementary material 2. Plant height (m), Chest height circumference (CHC, m) and wood (m^3 ha^{-1}) in different eucalypt age (12, 24 and 36-month-old eucalypt)

Eucalypt age (mon-old eucalypt)	Plant height ... m ...	CHC[*] ... m ...	volume of wood[**] ...... m^3 ha^{-1}......
12	6.67 (±0.35)	0.19 (±0.001)	8.59 (±0.22)
24	14.99 (±0.13)	0.36 (±0.004)	79.48 (±2.03)
36	18.06 (±0.09)	0.41 (±0.004)	120.63 (±3.03)

[*] CHC (1.30-m aboveground); [**] considering a eucalypt stand of 1157 plants ha^{-1}. Values between parenthesis followed denote the standard error of the mean (n=4).

Supplementary material 3. Fine roots-C (Ø< 2 mm, Mg ha^{-1}), Medium roots-C (2 <Ø> 10 mm, Mg ha^{-1}) and Coarse roots-C (Ø> 10 mm, Mg ha^{-1}) in PR and BPR of the eucalypt plantation along different soil layer (0.2; 0.2-0.4; 0.4-0.6; 0.6-0.8; 0.8-1.0; 1.0-1.2 and 1.2-1.4-m) and along different eucalypt age (12, 24 and 36-month-old eucalypt). Fine root-C/N, Medium root-C/N and Coarse root-C/N along different eucalypt age (12, 24 and 36-month-old eucalypt)

Depth (m)	PR			BPR		
	Fine root-C (Ø<2-mm, Mg ha^{-1})					
	……… Eucalypt age (month-old eucalypt) ………					
	12	24	36	12	24	36
0-0.2	0.031 (± 0.007)	0.241 (±0.022)	0.399 (±0.087)	0.029 (±0.005)	0.225 (±0.024)	0.201 (±0.024)
0.2-0.4	0.027 (±0.002)	0.262 (±0.026)	0.505 (±0.108)	0.028 (±0.003)	0.105 (±0.019)	0.135 (±0.013)
0.4-0.6	0.027 (±0.003)	0.239 (±0.032)	0.505 (±0.057)	0.024 (±0.002)	0.123 (±0.014)	0.208 (±0.028)
0.6-0.8	0.000 (±0.000)	0.093 (±0.030)	0.440 (±0.037)	0.000 (±0.000)	0.096 (±0.003)	0.198 (±0.021)
0.8-1.0	0.000 (±0.000)	0.095 (±0.016)	0.273 (±0.024)	0.000 (±0.000)	0.000 (±0.000)	0.069 (±0.023)
1.0-1.2	0.000 (±0.000)	0.067 (±0.024)	0.117 (±0.021)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
1.2-1.4	0.000 (±0.000)	0.029 (±0.001)	0.064 (±0.012)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
C:N	71.72	91.84	139.58	71.72	91.84	139.58
Depth (m)	Medium root-C (2<Ø>10-mm, Mg ha^{-1})					
	……… Eucalypt age (month-old eucalypt) ………					
	12	24	36	12	24	36
0-0.2	0.043 (±0.009)	0.367 (±0.072)	0.317 (±0.032)	0.051 (±0.013)	0.172 (±0.010)	0.000 (±0.000)
0.2-0.4	0.056 (±0.010)	0.487 (±0.054)	1.011 (±0.204)	0.036 (±0.004)	0.127 (±0.017)	0.169 (±0.037)
0.4-0.6	0.041 (± 0.006)	0.577 (±0.117)	1.338 (±0.236)	0.038 (±0.004)	0.105 (±0.010)	0.193 (±0.057)
0.6-0.8	0.000 (±0.000)	0.469 (±0.130)	1.312 (±0.252)	0.000 (±0.000)	0.117 (±0.013)	0.063 (±0.026)
0.8-1.0	0.000 (±0.000)	0.352 (±0.018)	0.682 (±0.101)	0.000 (±0.000)	0.000 (±0.000)	0.081 (±0.021)
1.0-1.2	0.000 (±0.000)	0.175 (±0.008)	0.301 (±0.036)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
1.2-1.4	0.000 (±0.000)	0.132 (±0.017)	0.152 (±0.032)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
C:N	75.15	132.75	192.37	75.15	132.75	192.37
Depth (m)	Coarse root-C (Ø>10-mm, Mg ha^{-1})					
	……… Eucalypt age (month-old eucalypt) ………					
	12	24	36	12	24	36
0-0.2	0.123 (±0.020)	0.748 (±0.063)	1.140 (±0.224)	0.060 (±0.015)	0.000 (±0.000)	0.000 (±0.000)
0.2-0.4	0.153 (±0.028)	3.45 (±0.393)	7.244 (±0.931)	0.130 (±0.033)	0.229 (±0.019)	0.439 (±0.001)
0.4-0.6	0.133 (±0.023)	2.859 (±0.285)	7.554 (±0.820)	0.084 (±0.017)	0.162 (±0.017)	0.000 (±0.000)
0.6-0.8	0.000 (±0.000)	2.268 (±0.193)	3.076 (±0.310)	0.000 (±0.000)	0.000 (±0.000)	0.193 (±0.002)
0.8-1.0	0.000 (±0.000)	1.011 (±0.351)	1.062 (±0.053)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
1.0-1.2	0.000 (±0.000)	0.811 (±0.134)	0.474 (±0.107)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
1.2-1.4	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)	0.000 (±0.000)
C:N	93.03	138.29	230.36	93.03	138.29	230.36

Values between parenthesis followed denote the standard error of the mean (n=4).

Supplementary material 4. Sigmoid model fitted to Fine root-C (Mg ha^{-1}) in PR and BPR of the eucalypt plantation along different soil layer (0.2; 0.2-0.4; 0.4-0.6; 0.6-0.8; 0.8-1.0; 1.0-1.2 and 1.2-1.4-m). Linear model fitted to Fine root-C/N in 0.0-0.1 to 0.6-1.0-m

	.... PR	
Depth (m)	*Sigmoid model fitted*	R^2
0-0.2	$y = 0.4125^{**}/(1+exp(-(x-25.5592^{**})/4.2237^{o}))$	*0.81*
0.2-0.4	$y = 0.5274^{**}/(1+exp(-(x-27.0345^{**})/4.1620^{ns}))$	*0.82*
0.4-0.6	$y = 0.5378^{**}/(1+exp(-(x-27.9732^{**})/4.4011^{o}))$	*0.93*
0.6-0.8	$y = 0.4404^{**}/(1+exp(-(x-28.2528^{ns})/0.9594^{ns}))$	*0.95*
0.8-1.0	$y = 0.2730^{**}/(1+exp(-(x-27.5921^{ns})/0.9433^{ns}))$	*0.95*
1.0-1.2	$y = 0.1174^{**}/(1+exp(-(x-26.7290^{ns})/0.9598^{ns}))$	*0.77*
1.2-1.4	$y = 0.0646^{**}/(1+exp(-(x-27.2130^{ns})/0.9459^{ns}))$	*0.86*

	.... BPR	
Depth (m)	*Sigmoid model fitted*	R^2
0-0.2	$y = 0.2130^{**}/(1+exp(-(x-15.9208^{**})/0.4997^{ns}))$	*0.9117*
0.2-0.4	$y = 0.1381^{**}/(1+exp(-(x-21.3812^{**})/4.7288^{**}))$	*0.8795*
0.4-0.6	$y = 0.2215^{**}/(1+exp(-(x-25.7884^{**})/5.1482^{**}))$	*0.9004*
0.6-0.8	$y = 0.1984^{**}/(1+exp(-(x-27.0426^{ns})/0.8860^{ns}))$	*0.951*
0.8-1.0	$y = 0.1341^{ns}/(1+exp(-(x-39.9427^{**})/0.9238^{ns}))$	*0.7001*
1.0-1.2	*No roots*	-
1.2-1.4	*No roots*	-

	.... Fine root-C/N	
Depth (m)	*Linear model fitted*	R^2
0.0-0.1 to 0.6-1.0	$7.1156^{*} + 3.3497^{**}x$	*0.9711*

^{o}, *, ** indicate significant coefficients at $p < 0.1$; *0.05* and *0.01* by the t test, respectively. ^{ns} indicate no significant.

IV.CHAPTER 3

Carbon fixation, cycling, and storage in a young *Eucalyptus* plantation on a tropical soil

ABSTRACT

Great attention has been given to global emissions of CO_2-C, since it is one of responsible gases of greenhouse effect. So, the role of forest ecosystem in the global C balance is critical, since forests may sequester C in compartments with different timescales: *i)* Plant biomass and *ii)* Soil organic matter (SOM). Due the great representability of planted forests of eucalypt worldwide (approximately 20 million ha) and in Brazil (5.6 million ha), they have been thought as an alternative for carbon sequestration strategy. We hypothesized that with the increase of eucalypt C biomass storage, the eucalypt-derived C in soil would increase significantly over time, promoting the replacement of the existing C pools. For this, we evaluated the stored eucalypt C biomass (Leaves, branches, barks, woods, fine roots, medium roots and coarse roots) and the stored soil C (particulate organic matter – POM and mineral associated organic matter – MAOM) over eucalypt age. We set up the experiment in an area with a pasture historical use. Eucalypt forest at 36-months-old allocated 72.01 Mg ha^{-1} of C (*NPP*), with 41.5% being directed to the roots (*BNPP*: 29.92 Mg ha^{-1} of C). After 49-months of planting there were mineralization in POM-, MAOM-Cerrado and Pasture, providing an estimated N mineralization of 0.535 Mg ha^{-1} in the 0.0-1.0-m layer. In contrast, the root-derived C imputed to soil was more efficient in soil organic matter formation (58% higher) than the litterfall- + root-derived C imputed to soil. After 49-months of eucalypt planting the forest was not a potential sequestration of C (ΔC_{Soil}: -2.22 Mg ha^{-1}). However, studies with longer time scales are required for completeness of information about potential of CO_2-C sequestering by eucalypt forest.

Key-words: δ^{13}C-SOM, Rhizosphere priming effect, Net primary production-C and Mineral associated organic matter-MAOM.

1. INTRODUCTION

Great attention has been given to global emissions of CO_2-C, since it is one of responsible gases of greenhouse effect (IPCC, 2014). In this context, soils are important components of global C stores, containing about two and a half times as much carbon as is found in vegetation (Batjes, 1998; Field and Raupach, 2004). Thus, the uses of recommended management practices that minimize C losses and/or promote C sequestration have been studied (Fekete et al., 2014; Epron et al., 2015).

The role of forest soils in global C balance is critical, although they cover less than one-third of the earth's land surface, they provide 52-72% of global net primary biomass production (NPP) (Melillo et al., 1993; Roy et al., 2001; FAO, 2010). Therefore, they contain approximately 80% of aboveground carbon pools (FAO, 2005). In the meantime, the area covered by forest plantations is increasing (FAO, 2010), which partly compensates for gross deforestation emissions (IPCC, 2014). Species of eucalypt account for a large portion of these plantations with approximately 20 million hectares worldwide (Iglesias-Trabado and Wilstermann, 2008) and 5.6 million hectares in Brazil (IBA, 2016). Eucalypt plantations have historically provided pulpwood, charcoal, and firewood. They have begun to expand to a carbon sequestration strategy recently.

Eucalypt forests may store C in compartments with different timescales: *i)* Plant biomass and *ii)* Soil organic matter (SOM). The allocation to plant biomass is a C sequestration process considered short-term, while an allocation to SOM increases C sequestration in long-term.

The net ecosystem productions (NEP) are very useful in C sequestration studies, and have been based on a full ecosystem mass balance (Randerson et al., 2002). Therefore, *NEP* is defined as the difference between ecosystem level gross photosynthetic gain of C (gross primary production, *GPP*) and ecosystem respiratory losses (R_{eco}). Alternatively, we can express the NEP as: NEP = $\Delta C_{biomass}$+ ΔC_{Soil} (Campbell et al. 2004), where $\Delta C_{biomass}$ is the net change in plant biomass, and ΔC_{Soil} is the net change in soil organic C (SOC) stocks. The $\Delta C_{biomass}$ is considered all net primary production (*NPP*), consisting of the sum of the aboveground net primary production (Leaves, branch, bark, wood, stump and litterfall, *ANPP*) and belowground net primary production (Fine roots, medium roots and coarse roots, *BNPP*). While the ΔC_{Soil} is the difference between Input C (*Net-C-litterfall* + *Net-C-root*) and Output soil C (*SOC decomposition*). The ΔC_{Soil} also provides interesting information, for soils at steady-state ($\Delta C_{soil} = 0$). The C input to soil is the amount of C that replaces SOC decomposition, thus becoming a measure for SOC turnover. For soils which are net C sinks ($\Delta C_{soil} > 0$), the Input of C to soil exceeds SOC mineralization and a fraction of it enlarges the SOC pool, leading to soil C sequestration.

There are different tracer methods to estimate *Net-C-litterfall* and *Net-C-root*, such as continuous labeling, pulse labeling and ^{13}C natural abundance (Kuzyakov, 2010). When the use of labeling methods is not possible, the growing of C_3 plants ($\delta^{13}C$ of approximately -27‰) in soil with organic matter derived from C_4 plants ($\delta^{13}C$ of approximately -12‰) or *vice versa* has been useful tool for access (or to track) the plant-derived organic matter.

Due to the physical proximity of roots and soil minerals, root-derived C appears to be preferentially stabilized when compared with shoot-derived C (Rasse et al., 2005). Besides, biomass root-derived C rizhodepositions (exudates, secretions, mucilage and lysates) represent an important pool for the input C to the soil (Kuzyakov & Jones, 2006), stimulating the microbiological diversity in the rhizosphere (Kuzyakov, 2002). Close interaction between plant and microbes in the rizhosphere reflect in SOM decomposition. Trees likely mine nutrients from SOM by stimulating microbes to produce extracellular enzymes through so-called priming effects (Cheng et al., 2014).

In addition, the differentiation of soil organic matter pools is important when considering the forest management and offers clues for more sustainable management. The organic particulate matter (POM) is the most sensitive to land-use changes (Cambardella and Elliot, 1992; Poeplau and Don, 2013; Kabiri et al., 2015), while the mineral associated organic matter (MAOM) is the most stable compartment in SOM (Mazzilli et al., 2015).

We hypothesized that with C biomass storage, the eucalypt-derived C in soil would increase significantly over time, promoting the replacement of the existing C pools. Our findings should provide valuable information for estimating potential C sequestration in eucalypt forest to determining rational forest management practices to mitigate climate change.

2. MATERIALS AND METHODS

Experimental setup

The study was developed in eucalypt plantations (hybrid *E. grandis x E. urophylla*) planted in June 2012 according to material and methods section of Chapter 1. The treatments were established before eucalypt planting according to the three eucalypt-derived C inputs that would be fed to the soil: *(i)* eucalypt with root litter and shoot litter (*+RL/+SL*); *(ii)* eucalypt only with shoot litter (*-RL/+SL*) and eucalypt only with root litter (*+RL/-SL*). For the root litter (root debris and rhizodepositions) exclusion in the *-RL/+SL*, a PVC pipe (1.0-m height x 0.4-m diameter) was installed in the planting row (PR) and between planting row (BPR) of the eucalypt plantation. While for the shoot exclusion a Litterfall traps (27.5-m^2) were installed in each experimental block when the eucalypt was 12-month-old (Jun 2013). Thus, the three treatments were arranged completely randomized block design (36 x 24-m each block) with four replicates.

Soil sampling and analysis

Soil sampling was performed at *+RL/+SL*, *-RL/+SL* *+RL/-SL* and installed in PR and BPR of eucalypt plantation (be more representative) in four different eucalypt ages: 0, 19, 31 and 49-month-old eucalypt (Jun 2012, Jan 2014, Jan 2015 and Jul 2016). Soil sampling in area fragment of the pasture was performed in the same seasons. The soil samples were collected in depth: 0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m. The soils were taken to the laboratory, passed in the 2-mm sieve and air dried.

Soil organic matter fractions were separated in particulate fraction (POM) and fraction associated with minerals (mineral associated with organic matter, MAOM) according to Cambardella and Elliott (1992). Sub-samples of soil from each sampling season were milled and analyzed for C, N and $^{13}C/^{12}C$ ratio (expressed as $\delta^{13}C$ ‰ values) in an elemental CN analyzer coupled to an isotope mass spectrometer (IRMS, ANCA GSL 20-20, Sercon, Crewe, UK). The organic C associated with organic material retained in the 53 μm sieve corresponds to POM-C and that associated with the silt + clay minerals fraction correspond to MAOM-C.

The soil before eucalypt planting had a historical use of the C_3 plants (Cerrado: ~ - 27.00 ‰) and C_4 plants (Pasture: ~ -13.00 ‰). The C partitioning was performed based on the $\delta^{13}C$ values of pasture, since that eucalypt and Cerrado had a close $\delta^{13}C$ signature. The measured $\delta^{13}C$ values were used to calculate the proportion of pasture-derived C (f_{new}), by using a mass balance equation (Del Galdo et al., 2003, Cotrufo et al. 2011; Equation 1).

$$f_{new} = (\delta^{13}C_{Soil} - \delta^{13}C_{Old}) / (\delta^{13}C_{Veg} - \delta^{13}C_{Old}) \qquad Eq.\ 1$$

Where $\delta^{13}C_{Soil}$ is δ^{13}C of the soil organic matter of the pasture, *+RL/+SL*, *-RL/+SL* and *+RL/-SL*; $\delta^{13}C_{Old}$ is the δ^{13}C of the soil organic matter of the Cerrado, and $\delta^{13}C_{Veg}$ is the δ^{13}C of the *Brachiaria brizantha* (-13.00 ‰; Cerri and Volkoff, 1991).

Knowing the f_{new} values for the new C, the soil organic C concentrations (% C), soil layer (L, m), and area of study plot (A, ha) and soil bulk density (D_{Soil}, kg m^{-3}), *Pasture-C* amounts (Mg ha^{-1}) were computed for the soil samples as follows (Eq. 2):

$$Pasture\text{-}C = f_{new}\,.\%\,C.(A.L.D_{Soil})/1000 \qquad Eq.\ 2$$

In the eucalypt plantations (*+RL/+SL*, *-RL/+SL* and *+RL/-SL*), the calculations were carried out to Eq. 3:

$$Pasture\text{-}C_{+RL/+SL,\ -RL/+SL\ \mathrm{and}\ +RL/-SL} = (1/3.Pasture\text{-}C_{PR}) + (2/3.Pasture\text{-}C_{BPR}) \qquad Eq.\ 3$$

Where, $Pasture\text{-}C_{+RL/+SL,\ -RL/+SL\ \mathrm{and}\ +RL/-SL}$ is the *Pasture-C* in *+RL/+SL*, *-RL/+SL* and *+RL/-SL* (Mg ha^{-1}); the *1/3* represents an area proportion under influence of the initial mechanical preparation in PR and *2/3* represents an area proportion of the BPR of eucalypt plantation.

Then, the C_3-$C_{Ce\ or\ Ce\ +\ Euc}$ were calculated using a mass balance equation (Eq. 4):

$$SOC = Pasture\text{-}C_{Pasture,\ +RL/+SL,\ -RL/+SL\ \mathrm{and}\ +RL/-SL} + C_3\text{-}C_{Ce\ or\ Ce\ +\ (+RL/+SL,\ -RL/+SL\ \mathrm{and}\ +RL/-SL)} \qquad Eq.\ 4$$

Where, *SOC* is soil organic carbon; $Pasture\text{-}C_{Pasture,\ +RL/+SL,\ -RL/+SL\ \mathrm{and}\ +RL/-SL}$ is the Pasture-C partitioned in the pasture, *+RL/+SL*, *-RL/+SL* and *+RL/-SL*; $C_3\text{-}C_{Ce\ or}$

$_{Ce + (+RL/+SL, -RL/+SL \text{ and } +RL/-SL)}$ is the C_3-C partitioned in the pasture, *+RL/+SL*, *-RL/+SL* and *+RL/-SL*.

For the distinction between the Cerrado-C and the Eucalypt-C (*+RL/+SL*, *-RL/+SL* and *+RL/-SL*) we accepted the assumption that the organic matter decomposition rate derived of Cerrado in eucalypt plantations was similar those observed in the pasture (*Urochloa decumbens* cultivated for 30-years). Thus, with the Pasture-C values over the sampling season within the pasture use, it was possible to determine the Cerrado-derived over time. So, the Eucalypt-C (*+RL/+SL*, *-RL/+SL* and *+RL/-SL*) was calculated following the Eq. 5:

$$Eucalypt\text{-}C = C_3\text{-}C_{+RL/+SL,\ -RL/+SL \text{ and } +RL/-SL} - (Cerrado\text{-}C_{\ Pasture}) \qquad Eq.\ 5$$

Where, $C_3\text{-}C_{+RL/+SL,\ -RL/+SL \text{ and } +RL/-SL}$ is the C_3-C in the *+RL/+SL*, *-RL/+SL* and *+RL/-SL* for each sampling season; $Cerrado\text{-}C_{\ Pasture}$ is the Cerrado-derived C in Pasture.

Tree measures

The measurements in eucalypt trees were performed during three seasons: 12, 24 and 36-months-old eucalypt. After the inventory of the plantations (Supplementary material 1), 12-medium trees were selected. Of these, biomass of leaves, branches, barks and woods were separated and weighed. The stump values were obtained considering a eucalypt cutting height of 0.05 m aboveground. The trees circumference with and without bark was measured in different height: 0, 1, 2, 3, 4, 5, 10, 15, 25, 35, 45, 55, 65, 75, 85 and 95% of the height of the trees. The wood volume of each plant was calculated according to the area of section and length of the stem, as described in Mülleret al. (2009). The tip values were obtained by extrapolating the trunk volume (wood + bark) to the remaining 95-100% of the tree height. The roots were collected in ¼ occupied by tree (including PR and BPR). So, fine roots (Ø<2-mm), medium roots (2<Ø>10-mm) and coarse roots (Ø>10-mm) were separated, washed and weighed. Litterfall traps (27.5-m^2) were installed in each experimental block when the eucalypt was 12-month-old (Jun 2013). The litterfall were collected and weighed monthly until Jul 2016. In addition, samples of the above-ground litter (litterfall debris and dead wood in different degradation stage) were collected using a rectangular frame (0.5-m^2) in each experimental block in three distinct seasons: Jan 2015, Oct 2015 and Jul 2016.

The vegetable subsamples of each plant component were brought to the laboratory and dried at 60^{o}C to constant weight. The total C and N of vegetable subsamples was determined by elemental C-N analyzer coupled to an isotope mass spectrometer (IRMS, ANCA GSL 20-20, Sercon, Crewe, UK).

Net primary production (NPP) and soil C sequestration (ΔC_{Soil})

The Net primary production and Soil C sequestration were calculated only for the treatment *+RL/+SL*. Net primary production was obtained on the basis of C as the sum of aboveground net primary production (ANPP) and belowground net primary production (BNPP). The ANPP was defined as the sum of the plant components: leaves, branches, barks, woods, tips, stumps and litterfall. The BNPP was defined as the sum of fine, medium and coarse roots.

The Soil C sequestration in 49-months-old eucalypt was obtained by the difference of Soil C inputs and the Soil C outputs, according to Eq. 6:

$$\Delta C_{Soil} = \textit{Net-Input soil C} - \textit{Net-Output soil C} \qquad \textit{Eq. 6}$$

Where, *Net-Input soil C* is the POM-C-Eucalypt + MAOM-C-Eucalypt; *Net-Output soil C* is the difference between POM-C-Pasture and -Cerrado + MAOM-C-Pasture and -Cerrado in age of 49-months-old eucalypt and POM-C-Pasture and -Cerrado + MAOM-C-Pasture and -Cerrado in 0-months-old eucalypt.

The Organic matter formation efficient (OMFE) to different C inputs (Litterfall + Rhizodebris + Rhizodepositions; Litterfall and Rhizodebris + Rhizodepositions) were calculated according to Eq. 7:

$$OMFE = \textit{POM-C-Eucalypt} + \textit{MAOM-C-Eucalypt} / \textit{C input}_{\textit{Litterfall and/or Rhizodebris + Rhizodepositions}} \qquad \textit{Eq. 7}$$

Where, *OMFE* is Organic matter formation efficient; *POM-C-Eucalypt + MAOM-C-Eucalypt* is the POM-C + MAOM-C in +RL/+SL, +RL/-SL or -RL/+SL; *C input* $_{\textit{Litterfall and/or Rhizodebris + Rhizodepositions}}$ is the C input derived from Litterfall + Rhizodebris + Rhizodepositions, Litterfall or Rhizodebris + Rhizodepositions. The

Rhizodebris was estimated following turnover approach (Total fine root turnover in 3-months; Jourdan et al., 2008) in sigmoidal model adjusted in Figure 4a; The Rhizodepositions was estimated following the average measured exudation rate to forest species (Brzostek et al., 2013).

Estimated N mineralization

An estimated N mineralization after 49-months of eucalypt planting was calculated to the different soil organic matter fraction (POM and MAOM), from Cerrado and Pasture, according to Eq. 8 and 9:

$$N\ miner. = \Delta C_{MOP\ or\ MAOM} / C{:}N\text{-}MOP\ or\ MAOM \qquad Eq.8$$

$$\Delta C_{MOP\ or\ MAOM} = C\text{-}MOP\ or\ MAOM_{49\text{-}month\text{-}old} - C\text{-}MOP\ or\ MAOM_{Before\ eucalypt\ planting} \qquad Eq.\ 9$$

Where, *N miner.* is an estimated N mineralization after 49 months of eucalypt planting; $C\text{-}MOP\ or\ MAOM_{49\text{-}month\text{-}old}$ is the POM-C or MAOM-C after 49 months of eucalypt planting; $C\text{-}MOP\ or\ MAOM_{Before\ eucalypt\ planting}$ is the POM-C or MAOM-C before the eucalypt planting.

Statistical analysis

The changes over time in POM-C, MOAM-C and Total-C derived from Cerrado, Pasture and Eucalypt in eucalypt plantations (*+RL/+SL*, *-RL/+SL* and *+RL/-SL*) were assessed through regression analysis. In addition, the inputs of root carbon stocks in plant biomass (fine root, medium root and coarse root), Dry matter stocks, carbon and nitrogen stocks in litterfall and above-ground litter over time were also assessed through regression analysis. Statistical analyzes were performed using the SIGMA PLOTR 11.0 package (SystatR Software, San Jose, CA, USA).

Analysis of variance (ANOVA) was used to assess the differences in DM (Mg ha^{-1}), C (Mg ha^{-1}) and C:N in different eucalypt age for each plant compartment. Tukey's test was performed to separate means if differences were significant ($\alpha=0.05$). Furthermore, the OMFE were checked to different C inputs (ANOVA; Tukey's test, $\alpha=0.10$).

3. RESULTS

C and N allocation in forest biomass

In all seasons of tree measures (12, 24 and 36-months-old eucalypt) there was increases of the C allocated in all compartments of the plant biomass (Table 1). At 36-months-old eucalypt the C:N ratio of leaves, branches, barks, woods, tips, stumps, fine root, medium roots and coarse roots increased over time (Table 1). There was an increase in *BNPP: ANPP* rate over time, as well as it was detected 72.01 Mg ha^{-1} of C in NPP at 36-months-old eucalypt (Table 1).

There was a linear deposition of litterfall, while the remainder above-ground litter showed quadratic behavior (Figures 1d-f). At 36-months-old eucalypt the average net litterfall decomposition was 4.07 Mg ha^{-1} of C and the average net mineralization was 0.067 Mg ha^{-1} of N (Figures 1e and f). The root carbon stocks in 0.0-1.0-m layer to fine, medium and coarse roots assumed sigmoid behavior ($R^2=0.982$, $R^2=0.947$ and $R^2=0.974$, respectively). The proportions of fine, medium and coarse roots at 36-months-old eucalypt were 10.4, 18.8 and 70.8% respectively (Figures 1a-c).

Table 1. Dry matter (DM, Mg ha^{-1}), C (Mg ha^{-1}) and C:N of the leave, branch, bark, wood, tip, stump, litterfall, fine root, medium root, coarse root, Aboveground net primary production (ANPP), Belowground net primary production (BNPP) and Net primary production (NPP)

	.... Months-old eucalypt								
	.... 12			.... 24			.... 36		
	DM (Mg ha^{-1})	C (Mg ha^{-1})	C:N	DM (Mg ha^{-1})	C (Mg ha^{-1})	C:N	DM (Mg ha^{-1})	C (Mg ha^{-1})	C:N
Aboveground									
Leaves	0.05 (±0.00) c	0.02 (±0.00) c	17.96 (±0.00) c	4.21 (±0.36) b	1.89 (±0.16) b	16.80 (±0.00) b	5.60 (±0.22) a	2.54 (±0.10) a	21.95 (±0.00) a
Branch	0.17 (±0.01) b	0.06 (±0.00)	103.11 (±0.00) c	14.72 (±0.47) a	5.86 (±0.18) a	193.82 (±0.00) b	14.52 (±0.54) a	5.80 (±0.21) a	223.84 (±0.00) a
Bark	0.06 (±0.00) c	0.02 (±0.00) c	70.90 (±0.00) c	3.43 (±0.12) b	1.27 (±0.04) b	107.57 (±0.00) b	5.78 (±0.19) a	2.21 (±0.07) a	119.66 (±0.00) a
wood	0.11 (±0.00) c	0.04 (±0.00) c	175.41 (±0.00) c	31.21 (±1.00) b	12.92 (±0.41) b	301.43 (±0.00) b	56.77 (±1.37) a	23.39 (±0.56) a	391.55 (±0.00) a
Tip	-	-	-	0.37 (±0.01) a	0.15 (±0.00) a	264.90 (±2.29) a	0.29 (±0.00) b	0.12 (±0.00) b	327.06 (±4.32) b
Stump	0.002 (±0.00) c	0.0008 (±0.00) c	175.41 (±0.00) c	0.35 (±0.01) b	0.14 (±0.00) b	259.38 (±0.53) b	0.52 (±0.01) a	0.21 (±0.00) a	327.35 (±0.87) a
Litterfall*	5.62 (±0.00) c	2.60 (±0.00) c	52.85 (±0.00) b	11.25 (±0.00) b	5.21 (±0.00) b	52.95 (±0.00) a	16.87 (±0.00) a	7.82 (±0.00) a	52.95 (±0.00) a
ANPP	6.01 (±0.01) c	2.76 (±0.01) c	53.39 (±0.15) c	65.55 (±0.80) b	27.45 (±0.34) b	92.71 (±2.80) b	100.37 (±1.21) a	42.09 (±0.50) a	114.29 (±0.71) a
*Belowground***									
Fine roots	0.43 (±0.03) c	0.17 (±0.01) c	71.722 (±0.000) c	4.07 (±0.21) b	1.58 (±0.08) b	91.85 (±0.00) b	7.92 (±0.44) a	3.12 (±0.17) a	139.58 (±0.00) a
Medium roots	0.68 (±0.07) c	0.27 (±0.02) c	75.158 (±0.000) c	7.86 (±0.78) b	3.08 (±0.30) b	132.76 (±0.00) b	13.33 (±1.28) a	5.62 (±0.54) a	192.38 (±0.00) a
Coarse roots	1.69 (±0.24) c	0.68 (±0.10) c	93.032 (±0.000) c	28.65 (±0.70) b	11.54 (±0.28) b	138.30 (±0.00) b	50.39 (±3.80) a	21.18 (±1.59) a	230.36 (±0.00) a
BNPP	2.81 (±0.19) c	1.12 (±0.08) c	84.339 (±0.925) c	40.58 (±1.54) b	16.21 (±0.61) b	130.82 (±0.21) b	71.65 (±2.95) a	29.92 (±1.25) a	208.35 (±1.74) a
BNPP:ANPP	0.46 (±0.03) c	0.40 (±0.02) c	-	0.61 (±0.02) b	0.59 (±0.02) b	-	0.71 (±0.02) a	0.71 (±0.02) a	-
NPP	8.82 (±0.20) c	3.88 (±0.08) c	59.72 (±0.36) c	106.13 (±1.56) b	43.66 (±0.64) b	103.91 (±2.09) b	172.02 (±3.72) a	72.01 (±1.57) a	140.68 (±1.08) a

ANPP: Aboveground net primary production; BNPP: Belowground net primary production; NPP: Net primary production. [*]: Assessed by linear model adjusted in Figure 4 e; [**]: Roots in 0.0-1.0 m layer. Significant differences are indicated by different letters (Tukey's test, *α=0.05*). Values between parenthesis followed denote the standard error of the mean (n=4).

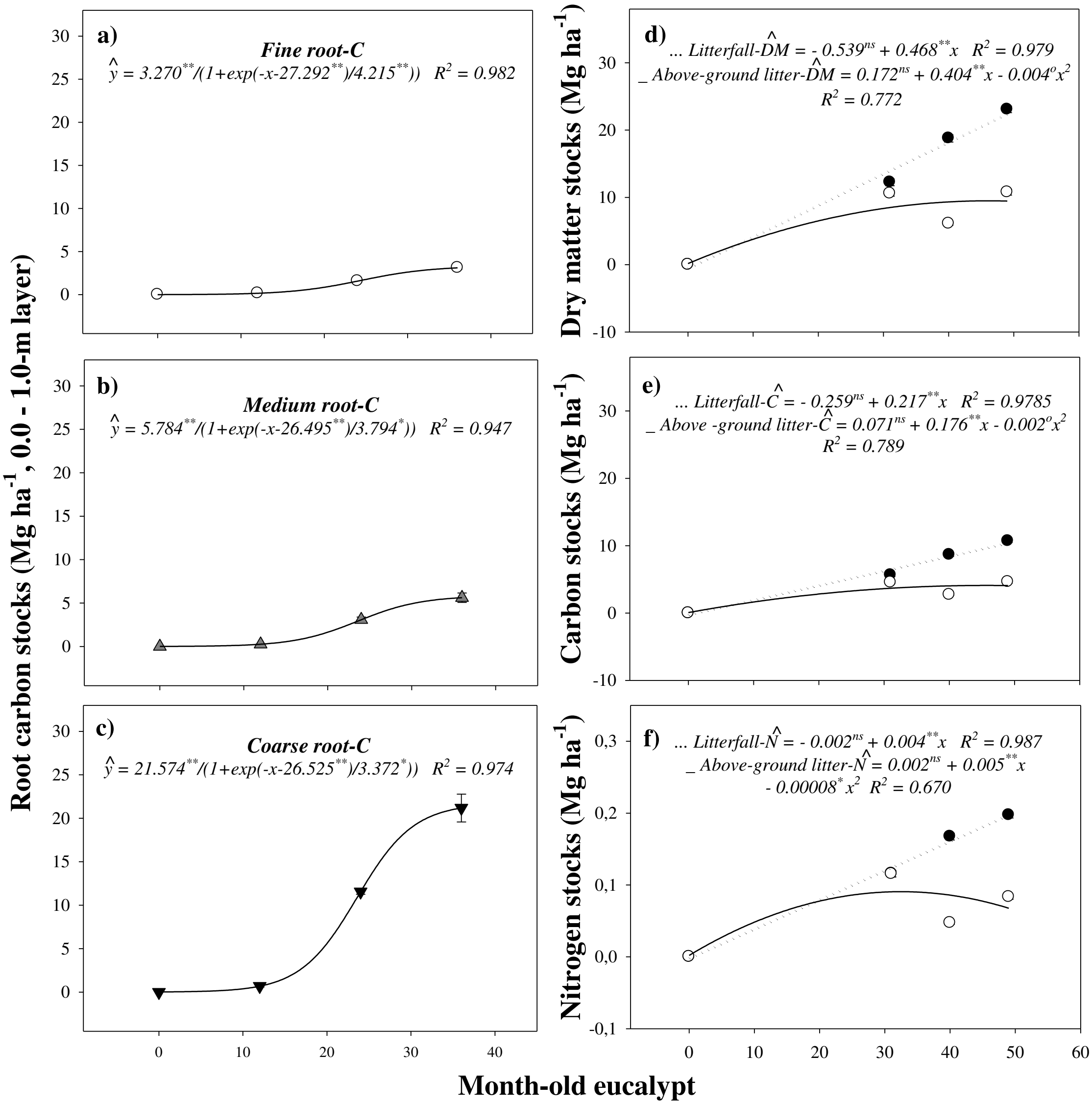

Figure 1. Root carbon stocks (Mg ha^{-1}, 0.0-1.0 m layer) of fine root, medium root and coarse root in different eucalypt ages (0, 12, 24 and 36-months-old). Dry matter (Mg ha^{-1}), Carbon stocks (Mg ha^{-1}) and Nitrogen stocks (Mg ha^{-1}) of litterfall and above-ground litter in different eucalypt ages (0, 19, 31 and 49-months-old). Linear and quadratic equation were adjusted. Vertical bars represent stand errors (n=4).

Dynamics of soil organic matter fractions

The POM-C, MAOM-C and Total-C derived from Cerrado, Pasture and Eucalypt were expressed as values accumulated in the 0.0-1.0 m soil layer (Figure 2). The POM-C showed similar behavior when the root litter was present (*+RL/+SL and +RL/-SL*) and absent (*-RL/+SL*) (Figures 2a, d and g). Linear model were adjusted to POM-C-Eucalypt (+RL/+SL: $R^2=0.753$; -RL/+SL: $R^2=0.514$ and +RL/-SL: $R^2=0.347$), POM-C-Pasture (+RL/+SL: $R^2=0.171$; -RL/+SL: $R^2=0.325$ and +RL/-SL: $R^2=0.290$) and POM-C-Cerrado (+RL/+SL: $R^2=0.307$; -RL/+SL: $R^2=0.126$ and +RL/-SL: $R^2=0.391$) (Figures 2a, d and g).

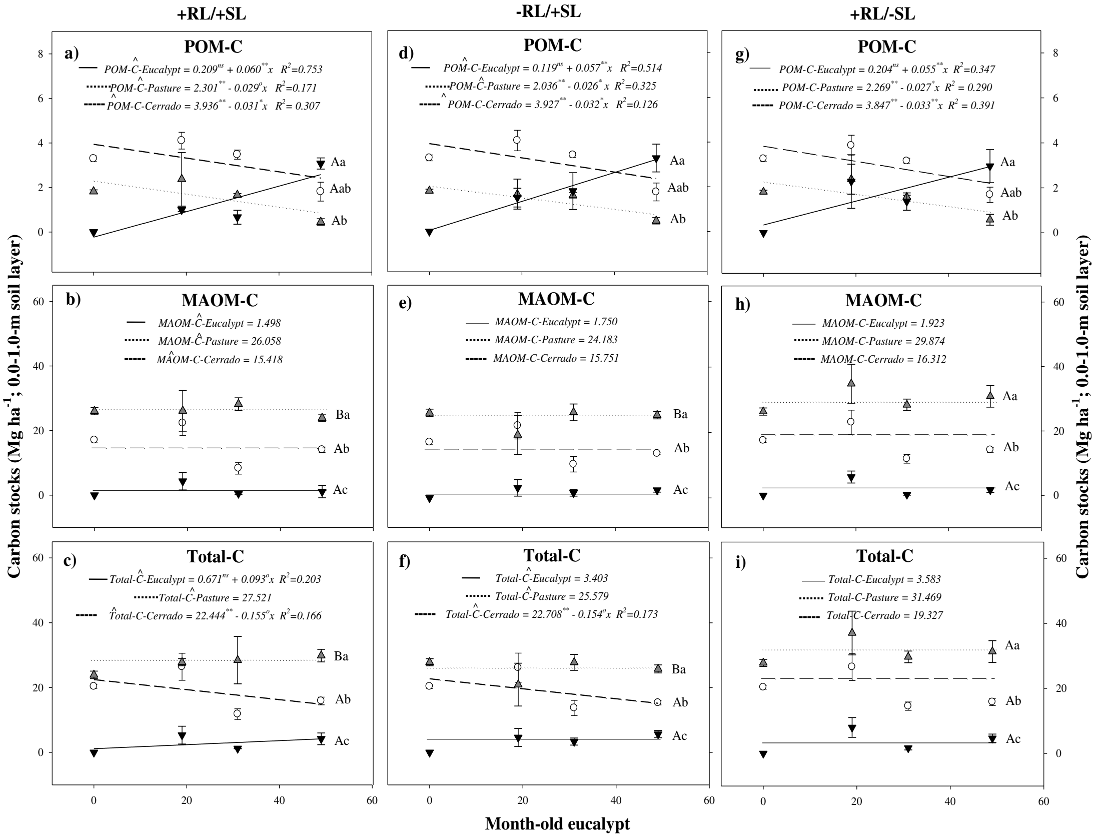

Figure 2. Carbon stocks (Mg ha^{-1}, 0.0-1.0 m layer) of POM-C, MAOM-C and Total-C derived from Cerrado, Pasture and Eucalypt in different eucalypt ages (0, 19, 31 and 49-months-old). Linear equation was adjusted. Significant differences between *+RL/+SL*, *-RL/+SL* and *+RL/-SL* are indicated by different capital letter (Tukey's test; $\alpha=0.05$), while significant differences among land-uses are indicated by different lowercase letter (Tukey's test; $\alpha=0.05$). Vertical bars denote the standard error of the mean (n=4).

The MAOM-C fraction showed no change trends over time for Eucalypt-C, Pasture-C and Cerrado-C (Figures 2b, e and h). Changes in Total-C-Eucalypt was detectable only on +RL/+SL litter manipulation (*Linear model,* $R^2=0.203$). While the Total-C-Cerrado had a linear decrease trends in both litter manipulation (+RL/+SL: $R^2=0.166$ and -RL/+SL: $R^2=0.173$).

The eucalypt plantation up to 49-months-old incorporated 5.72 Mg ha^{-1} of C (POM-C + MAOM-C) in soil profile (1.0 m; Table 2). However, there was a loss of 7.94 Mg ha^{-1} of C (POM-C + MAOM-C) derived from Pasture and Cerrado soil organic matter decomposition. So, in the eucalypt plantation up to 49-months-old C there was not C sequestration in soil, in which reached an estimated of -2.22 Mg ha^{-1} of C (Table 2).

Table 2. Input Soil C (Mg ha^{-1}), Output Soil C (Mg ha^{-1}) and Net soil C sequestration (ΔC_{Soil}, Mg ha^{-1}) in 49-months-old eucalypt

	.... C (Mg ha^{-1}) ....
I. Input Soil C	
Aboveground	
Litterfall	10,63
*Net-POM-C**	0,21
Net-MAOM-C*	0,29
OMFELitterfall	0,05
Belowground	
Rhizodebris	23,61
Rhiodepositions	8,74
*Net-POM-C**	0,26
Net-MAOM-C*	0,56
OMFERhiodebris + Rhiodepositions	0,03
Sub-total	1,31
II. $_{Output}$ Soil C	
SOC decomposition+	5,58
Sub-total	5,58
Δ*CSoil* (I - II)	-4,27

POM: Particulate organic matter and MAOM: Mineral associated organic matter. Values between parenthesis followed denote the standard error of the mean (n=4).

No differences was observed to POM-C derived from eucalypt in +RL/+SL, +RL/-SL and -RL/+SL to 0.0-0.2 m soil layer (Table 3). However, the +RL/-SL provided 186 % more MAOM-C than -RL/+SL ($p \leq 0.10$). Thereby, the root-derived C (Rhizodebris + Rhizodepositions) was 58% ($p \leq 0.10$) more efficient in organic matter formation (OMFE) than root-derived C + litterfall-derived C (Litterfall + Rhizodebris + Rhizodepositions) to 0.0-0.2 m soil layer (Table 3).

Table 3. Organic matter formation efficient (OMFE) to different input soil C (Litterfall and/or Rhizodebris + Rhizodepositions) in 0.0-0.2 m soil layer

Organic matter formation efficient	Values
Litterfall + (Rhizodebris +Rhizodepositions)(Mg ha^{-1})	19.72 (± 0.48)
POM-C* (Mg ha^{-1})	1.23 (± 0.12) a
MAOM-C* (Mg ha^{-1})	0.71 (± 0.36) ab
OMFE $_{Litterfall+ Rhizodebris + Rhizodepositions}$ (%)	0.10 (± 0.02) b
Litterfall^{o} (Mg ha^{-1})	10.70 (± 0.21)
POM-C** (Mg ha^{-1})	1.10 (± 0.34) a
MAOM-C** (Mg ha^{-1})	0.43 (± 0.16) b
OMFE $_{Litterfall}$ (%)	0.14 (± 0.03) ab
Rhizodebris† (Mg ha^{-1})	6.73 (± 0.26)
Rhizodepositions†† (Mg ha^{-1})	2.29 (± 0.09)
POM-C*** (Mg ha^{-1})	0.87 (± 0.20) a
MAOM-C*** (Mg ha^{-1})	1.26 (± 0.57) a
OMFE $_{Rhizodebris + Rhizodepositions}$ (%)	0.24 (± 0.07) a

OMFE: (POM-C + MAOM-C) / Litterfall and/or Rhizodebris + Rhizodepositions; *: Assessed by *+RL/+SL*; **: Assessed by *-RL/+SL*; ***: Assessed by *+RL/-SL;* ^{o}: Measured values; †: Assessed by turnover estimation (Total fine root turnover in 3-months; Jourdan et al., 2008) in sigmoidal model adjusted in Figure 4a (Rhiodebris); ††: Average measured exudation rate to forest species (Brzostek et al., 2013). Significant differences to different C input (Litterfall + Rhizodebris + Rhizodepositions; Litterfall and Rhizodebris + Rhizodepositions) are indicated by different letters (Tukey's test, $\alpha=0.10$). Values between parenthesis followed denote the standard error of the mean (n=4).

Estimated N mineralization

After 49-months of eucalypt planting the total accumulation of Eucalypt-C was 6.57 Mg ha^{-1} (0.0-1.0 m; Table 4), since the soil surface layer (0.0-0.1 m) represented 20% the total contribution. On the other hand, we observed estimated N mineralization of 158 kg ha^{-1} in soil surface layer (0.0-0.1 m). Meanwhile, an overall N mineralization of 535 kg ha^{-1} was estimated to 0.0-1.0 m.

Table 4. Relative C change (ΔC, Mg ha^{-1}), C:N and estimated N mineralization (*N-miner.*; Mg ha^{-1}) in POM and MOAM derived from Brazilian Savannah, Pasture and Eucalypt to different soil layer (0.0-0.1; 0.1-0.2; 0.2-0.4; 0.4-0.6 and 0.6-1.0 m) after 49 months of eucalypt planting

Depth (m)	Land-use	POM			MAOM		
		ΔC ... Mg ha^{-1}...	C:N*	*N-miner.* ... Mg ha^{-1}...	ΔC ... Mg ha^{-1}...	C:N*	*N-miner.* ... Mg ha^{-1}...
0.0-0.1	Cerrado	-0.193 (±0.025)	25.850 (±1.846)	0.008 (±0.001)	-0.916 (±0.287)	11.201 (±0.098)	0.082 (±0.027)
	Pasture	-0.686 (±0.251)	37.267 (±2.699)	0.018 (±0.007)	-0.500 (±0.575)	10.453 (±0.183)	0.050 (±0.056)
	Eucalypt	0.755 (±0.177)	-	-	0.619 (±0.292)	-	-
0.1-0.2	Cerrado	-0.104 (±0.116)	26.834 (±1.128)	0.003 (±0.004)	0.175 (±0.203)	9.079 (±1.573)	-0.022 (±0.025)
	Pasture	-0.278 (±0.060)	36.967 (±2.201)	0.008 (±0.002)	-0.686 (±0.304)	9.939 (±1.286)	0.087 (±0.052)
	Eucalypt	0.471 (±0.152)	-	-	0.094 (±0.094)	-	-
0.2-0.4	Cerrado	-0.116 (±0.391)	27.611 (±0.509)	0.005 (±0.014)	-0.553 (±0.582)	11.327 (±0.121)	0.048 (±0.051)
	Pasture	-0.271 (±0.059)	36.667 (±1.843)	0.007 (±0.001)	-0.486 (±0.214)	11.541 (±0.422)	0.044 (±0.020)
	Eucalypt	0.501 (±0.193)	-	-	0.000 (±0.000)	-	-
0.4-0.6	Cerrado	-0.296 (±0.066)	27.305 (±0.770)	0.011 (±0.002)	-1.139 (±0.385)	11.310 (±1.807)	0.094 (±0.024)
	Pasture	-0.156 (±0.068)	36.367 (±1.717)	0.004 (±0.002)	-0.426 (±0.501)	9.730 (±1.434)	0.021 (±0.059)
	Eucalypt	0.491 (±0.026)	-	-	1.768 (±1.768)	-	-
0.6-1.0	Cerrado	-0.769 (±0.036)	28.014 (±0.980)	0.028 (±0.002)	-0.567 (±0.346)	19.551 (±7.881)	0.039 (±0.032)
	Pasture	0.024 (±0.116)	36.068 (±1.870)	-0.001 (±0.003)	-0.002 (±0.333)	10.758 (±0.469)	0.001 (±0.033)
	Eucalypt	1.010 (±0.101)	-	-	0.013 (±0.013)	-	-
0.0-1.0	Cerrado	-1,580 (±0.530)	-	0.054 (±0.012)	-2.500 (±0.920)	-	0.242 (±0.062)
	Pasture	-1.210 (±0.150)	-	0.036 (±0.005)	-1.620 (±2.110)	-	0.204 (±0.025)
	Eucalypt	3.230 (±0.250)	-	-	1.370 (±0.740)	-	-

*Cerrado and Pasture C:N values were obtained from Cerrado and Pasture after eucalypt planting. Values between parenthesis followed denote the standard error of the mean (n=4).

4. DISCUSSION

Eucalypt forest ecosystem as sink of CO_2-C

Forest ecosystem as sink of CO_2-C has been strongly studied recently (Fekete et al., 2014, Alberti et al., 2015, Epron et al., 2015); however more accurate measurements, considering the two compartments of the ecosystem are necessary. Our study evaluated the two compartments of C allocation in the ecosystem (*shorter term* and *long term*): Plant biomass and Soil.

During 36-months of eucalypt planting it was possible to observe increments in C allocation over all aboveground and belowground compartments of the plant (Table 1; Figure 3). Similarly, the *BNPP:ANPP* increased over time, demonstrating a higher C allocation to the roots. This fact is important for the storage of soil C, since several studies have suggested that the majority of SOC is derived from belowground inputs, with aboveground litter inputs having a limited influence on SOC storage (Rasse et al., 2005; Schmidt et al., 2011).

Furthermore, our findings demonstrated that root-derived C was 58% more efficient in organic matter formation (OMFE) than root-derived C + litterfall-derived C (Litterfall + Rhizodebris + Rhizodepositions) to 0.0-0.2 m soil layer (Table 3). Recent meta-analysis study compiled 10 experimental sites demonstrating that belowground inputs C retained in SOM was around 45%, while the aboveground inputs C retained in SOM was only 8% (Jackson et al., 2017). The higher root OMFE is related due same root characteristics: *i)* the roots have more lignified compounds than litterfall materials (Puget and Drinkwater, 2001; Lu et al., 2003); *ii)* the rhiodepositions activity promotes large C input (easy degradation compounds), which are readily sorbed to mineral surfaces; *iii)* Furthermore, the rhiodepositions are widely used by soil microorganisms (source energy), contributing to the incorporation of microbial-derived compounds in the more stable soil fractions (Cotrufo et al., 2013).

After 36-months-old eucalypt, 70.8% of the C allocated belowground derived from coarse roots, while only 10.4% derived from fine roots (Table 1; Figure 3). Although of the fine roots contribute with only 10.4% of the C belowground, it is well known that they present intense activity and turnover in the soil, been the main drivers of rhizodepositions. Thus, they contribute significantly to the storage of soil C (Rumpel and Kögel-Knabner, 2011; Tefs and Gleixner, 2012; Clemmensen et al., 2013). Santos et al. (2016) studying the dynamics of fine roots in northern temperate and boreal forest, verified a fast turnover of residues, being attributed to low C:N initial and lignin: N rates.

The forest eucalypt ecosystem allocated 72.01 Mg C ha^{-1} in the plant biomass at 36-month-old eucalypt (*NPP*; Table 1 and Figure 3). At the ecosystem scale, variation in global plant productivity across ecosystems has been often related to environmental factors (Reichstein et al., 2007), but also to N availability (Zha et al., 2013, Epon et al., 2013). Thus, N again assumes a primordial role in the *NPP* of forest ecosystems (Alberti et al., 2015).

The N mineralized from litterfall in 36-month-old eucalypt (0.067 Mg ha^{-1} of N; Figure 1f) may have been reallocated to the total of 0.511 Mg ha^{-1} of N in the plant (Table 1). When we consider the N input by fertilization (see Material and Methods section of Chapter 1), we found an input of 0.116 Mg ha^{-1}, lower than N stored by the plant. This fact suggests that there is considerable mining of SOM for the plant by the acquisition of N, as previously mentioned.

Dynamics of soil organic matter in eucalypt forest

In forest plantations is well known the increase capacity of soil carbon sequestration due to high aboveground productivity, recalcitrant C inputs, and deep rooting systems (Don et al., 2010; Laganiére et al., 2010). This fact has been observed especially in eucalypt forest plantations which presents rapid growth and high-efficiency in the use of sources (Stape et al., 2010; Silva et al., 2012).

However, the net balance of the SOC in eucalypt forest plantations does not only depend of the input plant-derived C biomass quantity. Many interactions plant-microbes-SOM occurs belowground intermediated by rhizodepositions. Throughout the plants growth, scavenge nutrients from the soil solution is likely to provide diminishing returns over time if nutrients become locked-up in slow turnover pools as forests mature (Johnson, 2006). This has led to view that in addition to scavenging for nutrients, mature trees likely mine nutrients from SOM by stimulation of microbes to produce extracellular enzymes through priming effects directing the storage of SOC (Cheng et al., 2014).

In our study, until 49-months-old eucalypt (+RL/+SL) there was strong C turnover derived from “old SOM” (Cerrado- and Pasture-derived C) (Table 2 and Figure 3). The decomposition of Cerrado- and Pasture-derived C promoted an estimated overall N mineralization (0.0-1.0 m) of 0.535 Mg ha^{-1} (Table 4), in which probably has been stimulating by root growing (Johnson, 2006; Cheng et al., 2014).

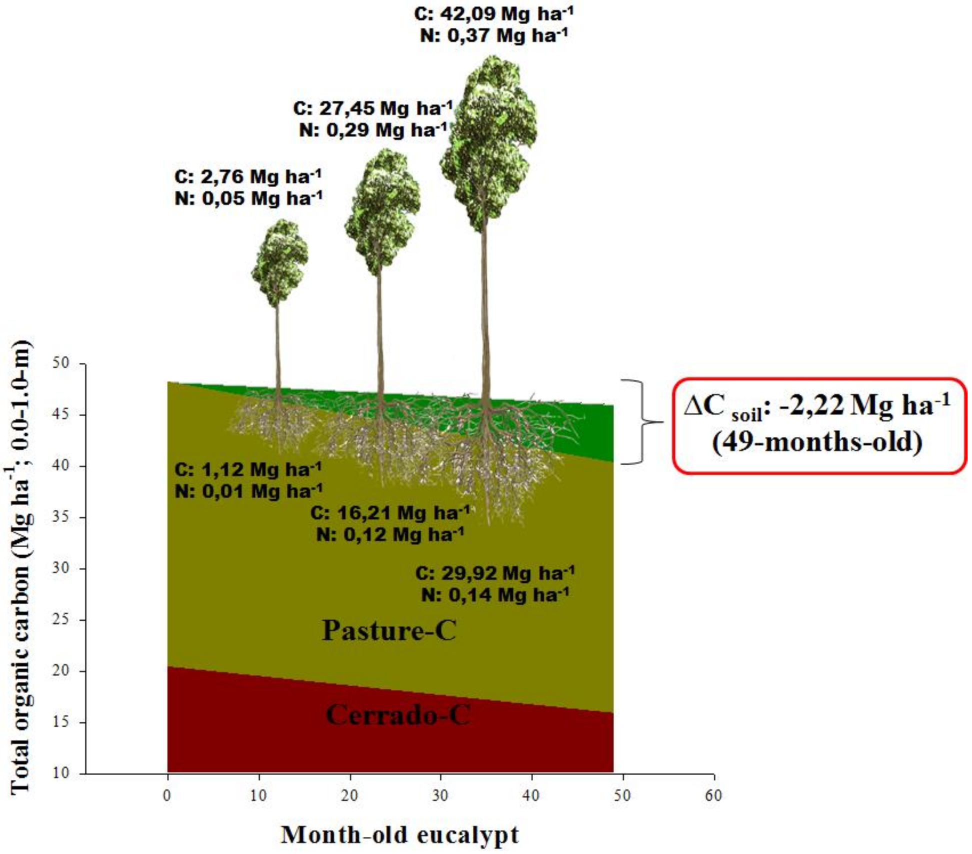

Figure 3. C allocation in eucalypt biomass and soil to different eucalypt ages.

Our findings showed an estimated overall N mineralization (0.0-1.0 m) of 0.535 Mg ha^{-1} no statistical difference in *POM-C*, *MAOM-C* and *Total-C* to the different C inputs (+RL/+SL, +RL/-SL and -RL/+SL) until the 49-months-old eucalypt (Figure 2, a-i). The soil C, mainly MAOM-C, has been reported as more stable organic fraction (Mazzilli et al., 2015), and less sensitive to the soil changes than CO_2-C flux. So, changes in C stokes can be hardly detected in C stocks, and demands long-term to recovery the C storages (Eclesia et al., 2012).

The potential sequestration of CO_2-C to forest has been reported in others studies (Don et al., 2010; Laganiére et al., 2010). The eucalypt plantation until 49-months-old eucalypt (+RL/+SL), incorporated 5.72 Mg ha^{-1} of C (POM-C and MAOM-C) to soil profile (0.0-1.0 m). Despite this, there was not enough to sequester C in the soil (ΔC_{Soil}: -2.22 Mg ha^{-1}; Table 3). However, the study period does not correspond to the current periods of the commercial cycle yet (± 6.0 years). Thus, studies with longer time scale are required to completeness of information about potential of CO_2-C sequestering by eucalypt forest.

5. CONCLUSIONS

To sum up, Forest eucalypt at 36-months-old allocated 72.01 Mg ha^{-1} of C (*NPP*), with 41.5% being directed to the roots (*BNPP*: 29.92 Mg ha^{-1} of C). The eucalypt growing promoted a mineralization in POM-, MAOM-Cerrado and Pasture, providing an estimated N mineralization of 0.535 Mg ha^{-1} in the 0.0-1.0-m layer (after 49-months of planting). In contrast, the root-derived C imputed to soil was more efficient in soil organic matter formation (58% higher) than the litterfall- + root-derived C imputed to soil.

After 49-months of eucalypt planting the forest was not a potential sequestration of C (ΔC_{Soil}: -2.22 Mg ha^{-1}). However, the study period does not correspond to the current periods of the commercial cycle yet (± 6.0 years). Thus, studies with longer time scales are required for completeness of information about potential of CO_2-C sequestering by eucalypt forest.

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7. REFERENCES

Alberti, G.; Vicca, S.; Inglima, I.; Belelli-Marchesini, L.; Genesio, L.; Miglietta, F.; Marjanovic, H.; Martinez, C.; Matteucci, G.; D'Andrea, E.; Peressotti, A.; Petrella, F.; Rodeghiero, M.; Cotrufo, M.F. 2015. Soil C:N stoichiometry controls carbon sink partitioning between above-ground tree biomass and soil organic matter in high fertility forests. iForest, 8:195-206.

Alvarez, V.H., Novais, R.F., Dias, L.E., Oliveira, J.A. 2000. Determinação e uso do fósforo remanescente. B. Inf. Soc. Bras. Ci. Solo. 25, 27-32.

Batjes, N.H., 1998. Mitigation of atmospheric CO2 concentrations by increased carbon sequestration in the soil. Biology and Fertility of Soils, 27:230-235.

Brzostek, E.R.; Greco, A.; Drake, J.E.; Finzi, A.C. 2013. Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. Biogeochemistry,115:65-76.

Cambardella, C. A.; Elliott, E. T. 1992. Particulate soil organic-matter changes across a grassland cultivation sequence. Soil Science Society of America Journal, 56:777-783.

Campbell. J.L.; Sun, O.J.; Law, B.E. 2004.Disturbance and net ecosystem production across three climatically distinct forest landscapes. Global Biogeochemical Cycles 18: 1-11.

Cerri, C.C.; Volkoff, F. 1991.Nature and behaviour of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus.For. Ecol. Manag., 38:247-257.

Cheng, W.X., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R.P., Asao, S., McNickle, G., Brzostek, E., Jastrow, J.D., 2014. Synthesis and modeling perspectives of rhizosphere priming. New Phytologist, 201:31-44.

Clemmensen, K.; Bahr, A.; Ovaskainen, O.; Dahlberg, A.; Ekblad, A.; Wallander, H.; Stenlid, J.; Finlay, R.D.; Wardle, D.A.; Lindahl, B.D. 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science, 339:1615-1618.

Cotrufo, M.F.; Alberti, G.; Inglima, I.; Marjanovi, H.; LeCain, D.; Zaldei, A.; Peressotti, A.; Miglietta, F. 2011. Decreased summer drought affects plant productivity and soil carbon dynamics in a Mediterranean woodland. Biogeosciences 8: 2729-2739.

Cotrufo, M.F.; Wallenstein, M.D.; Boot, C.M.; Denef, K.; Paul, E. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology, doi: 10.1111/gcb.12113.

Del Galdo, I.; Six, J.; Peressotti, A.; Cotrufo, M.F. 2003.Assessing the impact of land-use change on soil C sequestration in agricultural soils by means of organic matter fractionation and stable C isotopes. Global Change Biology, 9(8):1204-1213.

Don, A.; Schumacher, J.; Freibauer, A. 2010.Impact of tropical land-use change on soil organic carbon stocks - a meta-analysis. Global Change Biol, 17:1658-1670.

Epron, D.; Mouanda, C.; Mareschal, L.; Koutika, L. 2015. Impacts of organic residue management on the soil C dynamics in an tropical eucalypt plantation on a nutrient-poor sandy soil after three rotations. Soil Biology & Biochemistry 85:183-189.

Epron, D.; Nouvellon, Y.; Mareschal, L.; Moreira, R.M.; Koutika, L.; Geneste, B.; Delgado-Rojas, J.S.; Laclau, J.; Sola, G.; Gonçalves, J.L.M.; Bouillet, J. 2013. Partitioning ofnetprimaryproductionin Eucalyptus and Acacia stands andin mixed-species plantations:Twocase-studiesincontrastingtropicalenvironments. Forest Ecology and Management. 301:102-111.

FAO, 2005. Global Forest Resource Assessment: Progress Towards Sustainable Forest Management. FAO. FAO Forestry Paper, Rome, 147.

FAO, 2010. Global Forest Resources Assessment 2010: Main Report. Roma, Italy. Fontaine, S.; Bardoux, G.; Abbadie, L.; Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters, 7:314-320.

Fekete, I.; Kotroczó, Z.; Varga, C.; Nagy, P.T.; Várbíró, G.; Bowden, R.D.; Tóth, J.A.; Lajtha, K. 2014. Alterations in forest detritus inputs influence soil carbono concentration and soil respiration in a Central-European deciduous forest.Soil Biology & Biochemistry 74:106-114.

Field, C.B.; Raupach, M.R. (Eds.), 2004. The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World. Island Press, Washington, D.C., 529 pp.

Fontaine, S.; Bardoux, G.; Abbadie, L.; Mariotti, A. 2004. Carbon input to soil may decrease soil carbon content. Ecology Letters ,7:314-320.

Fontaine, S.; Mariotti, A.; Abbadie, L. 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology and Biochemistry, 35:837-843.

Hobbie, S.E.; Oleksyn, J.; Eissenstat, D.M.; Reich, P.B. 2010. Fine root decomposition rates do not mirror those of leaf litter among temperate tree species. Oecologia, 162: 505-513.

Industria Brasileira de árvores (IBA). Anuário estatístico do IBA – ano base 2016. Brasília, Brasil.

Iglesias-Trabado, G.; Wilstermann, D. 2008. Eucalyptus Universalis. Global Cultivated Eucalypt Forests Map 2008 Version 1.0.1. http://www.gitforestry.com/downloads/GIT_Forestry_Global_Eucalyptus_Map_2009_Brochure_ENG.pdf(accessed 08.02.17).

IPCC (2014) Climate Change 2014 Mitigation of Climate Change: Working Group III Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Jackson, R.B.; Lajtha, K.; Crow, S.E.; Hugelius, G.; Kramer, M.G.; Piñeiro, G. 2017. The Ecology of Soil Carbon: Pools, Vulnerabilities, and Biotic and Abiotic Controls. Annual Review of Ecology, Evolution, and Systematics, 48:419-45.

Johnson, D.W., 2006. Progressive N limitation in forests: review and implications for long-term responses to elevated CO2. Ecology 87:64-75.

Jourdan, C.; Silva, E.V.; Gonçalves, J.L.M.; Ranger, J.; Moreira, R.M.; Laclau, J.P. 2008. Fine root production and turnover in Brazilian Eucalyptus plantations under contrasting nitrogen fertilization regimes. Forest Ecology and Management, 256(3):396-404.

Kabiri, V., Raiesi, F., Ghazavi, M.A., 2015. Six years of different tillage systems affected aggregate-associated SOM in a semi-arid loam soil from Central Iran. Soil Till. Res. 154, 114–125.

Kuzyakov, Y. 2010. Priming effects: Interactions between living and dead organic matter. Soil Biology & Biochemistry, 42:1363-1371.

Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sci., 165(4): 382-396.

Kuzyakov, Y.; Jones, D.L. 2006.Glucose uptake by maize roots and its transformation in the rhizosphere. Soil Biology and Biochemistry, 38:851–860.

Laganiére, J.; Angers, D.A.; Paré, D. 2010. Carbon accumulation in agricultural soils after afforestation: a meta-analysis. Global Change Biol., 16:439-453.

Lehmann, J.; Kleber, M. 2015.The contentious nature of soil organic matter.Nature, 528:60-68.

Lu, Y.; Watanabe, A.; Kimura, M. 2003. Carbon dynamics of rhizo deposits, root- and shoot residues in a rice soil. Soil Biology and Biochemistry, 35:1223-1230.

Mazzilli, S.R., Kemanian, A.R., Ernst, O.R., Jackson, R.B., Piñeiro, G., 2015. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. Soil Biol. Biochem. 85, 22–30.

Melillo, J.M.; Mcguire, A.D.; Kicklighter, D.W.; Moore, B.I.I.; Vorosmarty, C.J.; Schloss, A.L. 1993. Global climate change and terrestrial net primary production. Nature, 363:234-240.

Müller, M. D.; Fernandes, E. N.; Castro, C. R. T.; Paciullo, D. S. C.; Alves, F. F. 2009. Estimativa de acumulo de biomassa e carbono e sistema agrossilvipastoril na zona da mata mineira. Pesquisa Florestal Brasileira, 60:11-17.

Poeplau, C.; Don, A. 2013. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma, 192:189-201.

Puget, P.; Drinkwater, L. E. 2001. Short-term dynamics of root- and shoot-derived carbono from a leguminous green manure. Soil Science Society of America Journal, v. 65, 3: 771-779.

Randerson, J.T.; Chapin, F.S.; Harden, J.W.; Neff, J.C.; Harmon, M.E. 2002. Net ecosystem production: A comprehensive measure of net carbon accumulation by ecosystems. Ecological Applications, 12:937-947.

Rasse, D.; Rumpel, C.; Dignac, M.-F. 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil, 269:341–356.

Rasse, D.P.,Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as

Reichstein, M.; Papale, D.; Valentini, R.; Aubinet, M.; Bernhofer, C.; Knohl, A.; Laurila, T.; Lindroth, A.; Moors, E.; Pilegaard, K.; Seufert, G. 2007. Determinants of terrestrial ecosystem carbon balance inferred from European eddy covariance flux sites. Geophysical Research Letters, 34(1):1-5.

Roy, J.; Saugier, B.; Mooney, H.A. (Eds.). 2001. Terrestrial Global Productivity: Past, Present, and Future. Academic Press, San Diego, California, USA.

Ruiz, H. A. 2005. Incremento da exatidão da análise granulométrica do solo por meio da coleta da suspensão (silte+argila). Rev. Bras. Ciênc. Solo. 29:297-300.

Rumpel, C.; Kögel-Knabner, I. 2011.Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant Soil, 338:143-158.

Santos, F.; Nadelhoffer, K.; Bird, J.A. 2016.Rapid fine root C and N mineralization in a northern temperate forest soil. Biogeochemistry, 128:187-200.

Schmidt, M.W.I.; Torn, M.S.; Abiven, S.; Dittmar, T.; Guggenberger, G.; Janssens, I.A.; Kleber, M.; Kogel-Knabner, I.; Lehmann, J.; Manning, D.A.C.; Nannipieri, P.; Rasse, D.P.; Weiner, S.; Trumbore, S.E. 2011. Persistence of soil organic matter as an ecosystem property. Nature, 478:49-56.

Soil Survey Staff. 2014. Keys to soil taxonomy. USDA-Natural Resources Conservation Service: Washington.

Silva, V E. A.; Silva, C. A., Silva, I. R.; Marques, J. J. G. S. M.; Araujo, E. F.; Carvalho, S. A.; Silva, S. H. G.; Curi, N. 2012. Frações de carbono em topossequências de solos sob eucalipto com diferentes históricos de uso. Revista Brasileira de Ciência do Solo, 36(1): 1167-1178.

Stape, J. L., Binkley, D.; Ryan, M. G. 2010. The Brazil Eucalyptus Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. Foresrt. Ecology Management, 259(9):1684-1694.

Tefs, C.; Gleixner, G. 2012. Importance of root derived carbon for soil organic matter storage in a temperate old-growth beech forest-evidence from C, N and 14C content. For Ecol Manag, 263:131-137.

Zha, T.S.; Barr, A.G.; Bernier, P.Y.; Lavigne, M.B.; Trofymow, J.A.; Amiro, B.D.; Arain, M.A.; Bhatti, J.S.; Black, T.A.; Margolis, H.A.; McCaughey, J.H.; Xing, Z.S.; Van Rees, K.C.J.; Coursolle, C. 2013. Gross and aboveground net primary production at Canadian forest carbon flux sites. Agricultural and Forest Meteorology, 174-175:54-64.

8. SUPPLEMENTARY MATERIAL

Supplementary material 1. Plant height (m), CHC (m) and volume of wood (m^3 ha^{-1}) in different eucalypt age (12, 24 and 36 months). Values followed represent stand error

Eucalypt age	Plant height	CHC[*]	volume of wood[**]
... (Months) ...	... m ...	... m ...	...... m^3 ha^{-1}......
12	6.67 (±0.35)	0.19 (±0.001)	8.59 (±0.22)
24	14.99 (±0.13)	0.36 (±0.004)	79.48 (±2.03)
36	18.06 (±0.09)	0.41 (±0.004)	120.63 (±3.03)

[*] Chest height circumference (1.30 m aboveground); [**] considering an eucalypt stand of 1157 plants ha^{-1}

V. FINAL CONSIDERATIONS

Our main objective in this thesis study was to investigate the C and N dynamic associated with different soil organic matter pools in a replacement of the native savannah vegetation by three decades planted pasture and the most recent substitution of the pasture by a eucalypt plantation. Once, soil organic matter of eucalypt plantations plays an important role in C storage, being considered a strong sink of CO_2.

In conclusion, our study demonstrated strong influence of wet season on soil surface CO_2-C and CH_4-C flux, also in CO_2-C concentration in depth to different land-use. However, safe predictions only may be achieved with systemic monitoring over time, needing in this way more long-term studying involving land-use changes.

We hypothesized eucalypt forest how a potential land-use to recovery the SOC storage; however that was not achieved until the stand age (4-years-old). The MAOM-C decreased in soil deep layers due the initial pasture-eucalypt land-use transition, in which it was not compensated by new eucalypt C input (Rhizodepositions) until 4-years-old. Despite, C and N associated with more sensitive organic matter fraction (POM) was recovered to soil layer until 1-m.

After implantation of eucalypt forests, there was increase in soil surface CO_2-C flux with plant growth. The roots growth and activity contributes greatly to soil surface CO_2-C, promoting surface RPE over time. The moisture has greater influence in the litter and/or rhizodeposition decomposition and root respiration of eucalypt forests.

Forest eucalypt at 36-months-old allocated 72.01 Mg C ha^{-1} (*NPP*), with 41.5% being directed to the roots. After 49-months of planting, there was estimated N mineralization of 0.535 Mg N ha^{-1} in the 0.0-1.0-m layer.

After 49-months of eucalypt planting the forest was not a potential sequestration of C (ΔC_{Soil}: -2.22 Mg ha^{-1}). However, the study period does not correspond to the current periods of the cycle commercial yet (± 6.0 years). Thus, longer time scales are required to the completeness of information about potential of CO_2-C sequestering by eucalypt forest.